Molecular and Translational Medicine

Series Editors: William B. Coleman · Gregory J. Tsongalis

Yuzhuo Wang
Dong Lin
Peter W. Gout *Editors*

Patient-Derived Xenograft Models of Human Cancer

Molecular and Translational Medicine

Series editors

William B. Coleman
Dept of Pathology and Lab Medicine
Univ of N.Carolina School of Medicine
Chapel Hill, North Carolina
USA

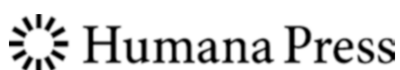
Gregory J. Tsongalis
Dept of Pathology and Lab Medicine
Dartmouth-Hitchcock Medical Center
Lebanon, New Hampshire
USA

As we enter into this new era of molecular medicine with an expanding body of knowledge related to the molecular pathogenesis of human disease and an increasing recognition of the practical implications for improved diagnostics and treatment, there is a need for new resources to inform basic scientists and clinical practitioners of the emerging concepts, useful applications, and continuing challenges related to molecular medicine and personalized treatment of complex human diseases. This series of resource/reference books entitled Molecular and Translational Medicine is primarily concerned with the molecular pathogenesis of major human diseases and disease processes, presented in the context of molecular pathology, with implications for translational molecular medicine and personalized patient care.

More information about this series at http://www.springer.com/series/8176

Yuzhuo Wang • Dong Lin • Peter W. Gout
Editors

Patient-Derived Xenograft Models of Human Cancer



Editors
Yuzhuo Wang
Department of Urologic Sciences
University of British Columbia
Vancouver, BC, Canada

Department of Experimental Therapeutics
BC Cancer Research Centre
Vancouver, BC, Canada

Vancouver Prostate Centre
Vancouver, BC, Canada

Dong Lin
Department of Urologic Sciences
University of British Columbia
Vancouver, BC, Canada

Department of Experimental Therapeutics
BC Cancer Research Centre
Vancouver, BC, Canada

Vancouver Prostate Centre
Vancouver, BC, Canada

Peter W. Gout
Department of Experimental Therapeutics
BC Cancer Research Centre
Vancouver, BC, Canada

Living Tumor Laboratory
BC Cancer Agency/Vancouver
Prostate Centre
Vancouver, BC, Canada

ISSN 2197-7852 ISSN 2197-7860 (electronic)
Molecular and Translational Medicine
ISBN 978-3-319-55824-0 ISBN 978-3-319-55825-7 (eBook)
DOI 10.1007/978-3-319-55825-7

Library of Congress Control Number: 2017943865

© Springer International Publishing AG 2017
This work is subject to copyright. All rights are reserved by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.
The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.
The publisher, the authors and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, express or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Printed on acid-free paper

This Humana Press imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Preface

Although successes have been achieved in cancer treatment, more effective therapies, specifically targeting molecular alterations that drive carcinogenesis and tumor progression, are urgently needed. In developing anticancer therapeutics, reliable cancer models for testing the efficacies of potential drugs are of paramount importance. Cultures of human cancer cells and cell line-based xenograft rodent models, which were commonly used in the past decades for drug screening, proved to be inadequate as they led to severe discrepancies between preclinical and clinical drug efficacies. Consequently, clinically more relevant cancer models, closely mimicking the cancer patient's clinical condition and response to treatment, are of critical importance to the development of superior anticancer therapeutics and regimens in the fight against the disease.

In the last decade, it was recognized that the structure and microenvironment of tumors are important factors influencing the growth and development of cancers. Efforts have therefore been made to devise cancer models in which these elements are preserved as much as possible. This led to the development of patient-derived xenograft (PDX) models of various cancer types, based on direct implantation of patients' cancer tissues into immunodeficient mouse hosts. The PDXs have been found to retain major genetic and histopathological characteristics of the original malignancies, including tumor heterogeneity and tumor tissue architecture. They therefore resemble patients' malignancies more closely than cancer models based on grafting of suspended cultured cancer cells and have been shown to be useful for studies of development of metastatic ability and therapy resistance, preclinical drug efficacy testing, development of personalized cancer therapy regimens, identification of potential biomarkers, and prediction of patient outcomes. They are expected to play an increasingly important role in translational cancer research.

As there have been major advances in the development and application of PDX cancer models, this book has been written to provide a concise yet comprehensive summary of the current status of the field and is aimed at guiding preclinical and possibly clinical applications, as well as stimulating investigative efforts. Accordingly, this book consists of four parts. The first part (Chap. 1) reviews the history of PDX models; the second part (Chaps. 2–4) focuses on the methodology used to establish models and their characterization; the third part (Chaps. 5–12) discusses applications of PDX models, including their use in studies of cancer heterogeneity, cancer stem cells, cancer metastasis, drug resistance, biomarker

development, preclinical drug screening, and personalized cancer therapy; the fourth part (Chap. 13) discusses the limitations of PDX models and future directions.

We, at the Living Tumor Laboratory (www.livingtumorlab.com), hope that this book will serve as a useful resource for researchers and clinicians dealing with, or interested in, the use of PDX models in cancer research. We expect that this book will propagate innovative concepts and prompt the development of groundbreaking technological solutions in the field.

Finally, we very much appreciate the contributions of our coauthors. This book could not have become a reality without their expertise, hard work, and unselfish input. Special thanks also go to Colton Coreschi, Hruska Richard, and Dhanapal Palanisamy at Springer for organizing and coordinating the efforts that led to the final publication.

Vancouver, Canada

Yuzhuo Wang, Ph.D.
Dong Lin, M.D., Ph.D.
Peter W. Gout, Ph.D.

Contents

1 **Patient-Derived Tumor Xenografts: Historical Background** 1
Dong Lin, Xinya Wang, Peter W. Gout, and Yuzhuo Wang

2 **Towards Best Practice in Establishing Patient-Derived Xenografts** 11
Gail P. Risbridger and Mitchell G. Lawrence

3 **Fidelity and Stability of PDX Models** 29
Gang Li

4 **Humanized Mouse and Rat PDX Cancer Models** 43
Fallon K. Noto and Tseten Yeshi

5 **Studies of Cancer Heterogeneity Using PDX Models** 59
Danyi Wen, Feifei Zhang, and Yuan Long

6 **The Plasticity of Stem-Like States in Patient-Derived Tumor Xenografts** 71
Alastair H. Davies, Fraser Johnson, Kirsi Ketola, and Amina Zoubeidi

7 **Modeling Cancer Metastasis** 93
Nora M. Navone and Estefania Labanca

8 **Modeling Drug Resistance in PDX Models** 115
Erin L. Stewart and Ming Sound Tsao

9 **Using PDX for Biomarker Development** 127
Werner J. Struss and Peter C. Black

10 **Patient-Derived Xenografts as Cancer Models for Preclinical Drug Screening** 141
Juliet A. Williams

11 **First-Generation Tumor Xenografts: A Link Between Patient-Derived Xenograft Models and Clinical Disease** 155
Xin Dong, Peter W. Gout, Lu Yi, Yinhuai Wang, Yong Xu, and Kuo Yang

12 Application of PDX Cancer Models in Co-clinical Trials and Personalized/Precision Medicine 177
Hongwei Cheng, Zongwen Liu, Hui Xue, Peter W. Gout, and Hong Shan

13 Prospectives 193
Dong Lin, Yuzhuo Wang, and Peter W. Gout

Index 201

List of Contributors

Peter C. Black Vancouver Prostate Centre, Vancouver, BC, Canada

Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada

Hongwei Cheng Living Tumor Laboratory, BC Cancer Agency/Vancouver Prostate Centre, Vancouver, BC, Canada

Alastair H. Davies Vancouver Prostate Centre, Vancouver, BC, Canada

Department of Urologic Sciences, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada

Xin Dong Living Tumor Laboratory, BC Cancer Agency/Vancouver Prostate Centre, Vancouver, BC, Canada

Peter W. Gout Living Tumor Laboratory, BC Cancer Agency/Vancouver Prostate Centre, Vancouver, BC, Canada

Department of Experimental Therapeutics, BC Cancer Research Centre, Vancouver, BC, Canada

Fraser Johnson Vancouver Prostate Centre, Vancouver, BC, Canada

Department of Urologic Sciences, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada

Kirsi Ketola Vancouver Prostate Centre, Vancouver, BC, Canada

Department of Urologic Sciences, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada

Estefania Labanca Department of Genitourinary Medical Oncology, The University of Texas-MD Anderson Cancer Center, Houston, TX, USA

Mitchell G. Lawrence Prostate Research Group, Cancer Program - Biomedicine Discovery Institute, Department of Anatomy and Developmental Biology, Monash Partners Comprehensive Cancer Consortium, Monash University, Clayton, VIC, Australia

Gang Li Ignyta, Inc., San Diego, CA, USA

Dong Lin Vancouver Prostate Centre, Vancouver, BC, Canada

Department of Urologic Sciences, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada

Department of Experimental Therapeutics, BC Cancer Research Centre, Vancouver, BC, Canada

Zongwen Liu Department of Radiotherapy, The Second Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China

Yuan Long Shanghai LIDE Biotech, Co. Ltd., Pudong, People's Republic of China

Nora M. Navone Department of Genitourinary Medical Oncology, The University of Texas-MD Anderson Cancer Center, Houston, TX, USA

Fallon K. Noto Hera BioLabs, Inc., Lexington, KY, USA

Gail P. Risbridger Prostate Research Group, Cancer Program - Biomedicine Discovery Institute, Department of Anatomy and Developmental Biology, Monash Partners Comprehensive Cancer Consortium, Monash University, Clayton, VIC, Australia

Prostate Cancer Translational Research Program, Cancer Research Division, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

Hong Shan The 5th Affiliated Hospital, Sun Yat-Sen University, Zhuhai, China

Erin L. Stewart Princess Margaret Cancer Research Tower, Toronto, ON, Canada

Werner J. Struss Vancouver Prostate Centre, Vancouver, BC, Canada

Department of Urologic Sciences, University of British Columbia, Vancouver, BC, Canada

Ming Sound Tsao Princess Margaret Cancer Research Tower, Toronto, ON, Canada

Xinya Wang Vancouver Prostate Centre, Vancouver, BC, Canada

Yinhuai Wang Department of Urology, The Second Xiangya Hospital, Zhongnan University, Changsha, China

Yuzhuo Wang Vancouver Prostate Centre, Vancouver, BC, Canada

Department of Urologic Sciences, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada

Department of Experimental Therapeutics, BC Cancer Research Centre, Vancouver, BC, Canada

Danyi Wen Shanghai LIDE Biotech, Co. Ltd., Pudong, People's Republic of China

Juliet A. Williams Oncology Disease Area, Novartis Institutes for Biomedical Research, Cambridge, MA, USA

Yong Xu Tianjin Institute of Urology, Tianjin Medical University, Tianjin, China

Hui Xue Living Tumor Laboratory, BC Cancer Agency/Vancouver Prostate Centre, Vancouver, BC, Canada

Kuo Yang Tianjin Institute of Urology, Tianjin Medical University, Tianjin, China

Tseten Yeshi Hera BioLabs, Inc., Lexington, KY, USA

Lu Yi Department of Urology, The Second Xiangya Hospital, Zhongnan University, Changsha, China

Feifei Zhang Shanghai LIDE Biotech, Co. Ltd., Pudong, People's Republic of China

Amina Zoubeidi Vancouver Prostate Centre, Vancouver, BC, Canada

Department of Urologic Sciences, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada

Patient-Derived Tumor Xenografts: Historical Background

1

Dong Lin, Xinya Wang, Peter W. Gout, and Yuzhuo Wang

Abbreviations

NOD	Non-obese diabetic
PDX	Patient-derived xenograft
SCID	Severe combined immunodeficiency

A Point on Terminology

Given the extensive history of cancer, the history of patient-derived xenograft (PDX) models is also difficult to fully recapitulate. In particular, this task is complicated by the irregularity with which PDX models were designated. The current use of "PDX cancer models" is a relatively recent addition to the lexicon. However, the general concept of PDX models—i.e., the transplantation of human cancers into animal models—can be found throughout the chronicles of cancer research. However, it wasn't until the discovery of host immunity and its crucial role in graft

D. Lin (✉) • Y. Wang
Vancouver Prostate Centre, Vancouver, BC, Canada

Department of Urologic Sciences, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada

Department of Experimental Therapeutics, BC Cancer Research Centre, Vancouver, BC, Canada
e-mail: dlin@bccrc.ca

X. Wang
Vancouver Prostate Centre, Vancouver, BC, Canada

P.W. Gout
Living Tumor Laboratory, BC Cancer Agency/Vancouver Prostate Centre, Vancouver, BC, Canada

Department of Experimental Therapeutics, BC Cancer Research Centre, Vancouver, BC, Canada

© Springer International Publishing AG 2017
Y. Wang et al. (eds.), *Patient-Derived Xenograft Models of Human Cancer*, Molecular and Translational Medicine, DOI 10.1007/978-3-319-55825-7_1

survival that the idea of serial transplantation could be realized. Therefore, in order to better reflect the nature of its history, the term "human tumor models" will be used in place of "PDX models," except when appropriate. Finally, it is important to also mention the existence of the other labels that have been used, including, but not limited to, "human tumor xenografts," "xenopatients," "heterotransplant tumor models," "heterotransplanted human tumors," and "transplantable tumor models."

A Brief Historical Context

Concurrent with the initial observation of cancer emerged a need to recapitulate tumors for further observation and experimentation. The idea of what is now known to be a PDX tumor model is composed of two general concepts: "xenotransplantation" (i.e., the transplantation of tissue into a foreign species) and the general use of animals for medical research. Although PDX technology may seem to be a more recent innovation, recapitulating a human cancer within an animal model has been a long-standing goal of cancer research. Such attempts at producing a human cancer model have been underway for centuries, beginning with the first recorded experiment in 1775 by the French surgeon Peyrilhe, who injected extracts of a human breast cancer into a dog [1]. Although Peyrilhe's experiment ultimately failed ("At length my maid, disgusted by the stench of the ulcer, and softened by the cries of the animal, put an end to his life, and thus prevented my observing the ultimate effects of this disease"), similar efforts to transplant human tumors into various animal models have since been continuously in progress.

Early Attempts

In an effort to learn about the origin of cancer, early scientists attempted to induce spontaneous tumor formation by a method termed "cancer genesis." They employed a variety of methods to "irritate" or "misplace" cells, with the ultimate goal of generating cancers in otherwise healthy tissue [1]. Although the majority of these experiments ended in failure, these attempts eventually culminated in the successful transplantation of human cancer into animals [2].

Following Peyrilhe's attempt in 1775, pioneering oncologists began to conduct experiments in order to determine the transmissibility of cancer. These experiments were largely motivated by a sense of curiosity, in that scientists were initially interested in transplanting cancers into animals for purposes of observing the pathology of this otherwise unknown disease. However, more than a century was to pass before a consistent method of tumor transplantation could be achieved. Among the many initial attempts were those made by Dupuytren, Langenbeck, and Lebert and Follin. In 1807, Dupuytren attempted a variety of methods in order to transplant a human cancer into an animal, including "[feeding] animals with cancerous material, [introducing] it into the abdomen, [injecting] cancer juice into the peritoneal cavity and the veins, and [inoculating] the pus of an ulcerated cancer" [1]. In 1840, Langenbeck attempted a similar method by injecting a dog with fluid from a human medullary carcinoma of

the humerus. However, in Langenbeck's case, several round nodules were found in the lung 2 months following the inoculation [1]. Then, in 1851, Lebert and Follin injected emulsified mammary cancer into the jugular vein of a dog. Although the animal died 15 days later, the autopsy found nodules along the wall of the heart [1]. Unfortunately, in both cases, it was unknown whether the observed nodules had occurred as a direct result of successful tumor engraftment or was due to spontaneous tumor formation. With regard to Langenbeck's experiment, the pathologist Virchow had observed the nodules to more closely resemble spontaneous cancer of dog rather than human origin [1, 3]. From there, scientists began to slowly make incremental advancements toward the generation of human cancer models. Of particular note, in 1938, Greene reported the successful engraftment of human uterine adenoma and adenocarcinoma in rabbits, which was then serially transplanted for several subsequent generations [4]. Furthermore, Greene later followed with the successful transplantation of human tumors into the eye of a guinea pig, rabbit, and mouse [4–8].

Concurrent with attempts to transplant human cancers, scientists also began to examine the morphology of the graft and surrounding tissue. In 1890, Klebs studied fragments of a human carcinoma engrafted into the peritoneal cavities of white rats and found the epithelial constituents of the original graft to have disappeared by the third day following engraftment [1, 3]. Jensen also monitored the outcome of transplanted tumors and observed stromal changes and the disappearance of connective tissue cells within the central region of the original graft [1]. Additionally, Jensen reported the formation of many blood vessels and the appearance of fibroblasts around the tumor and within the margin [1].

Finally, scientists looked toward refining the transplantation technique and streamlining the procedure. In order to do so, a variety of factors were taken into evaluation, including engraftment technique, dosage (e.g., type and origin of the inoculate, frequency, and location), site of engraftment, and environmental conditions (e.g., temperature and environment). Furthermore, many experiments were also conducted in order to examine transplantation efficiency in different hosts, by implanting tumors into rats, the cheek pouch of hamsters, dogs, and chicken embryos [1, 9–13].

In 1912, Murphy reported the successful serial growth of the Jensen rat sarcoma in a chicken embryo [5, 14, 15]. However, much to his surprise, attempts to further propagate the tumor into adult chickens were not found to be similarly successful. With regard to this differential transplantation success between the chick embryo and the adult chicken, Murphy hypothesized that "[the embryo] either provides a food substance utilizable by these tissues, which is lacking in the adult, or else lacks a defensive mechanism against such an invasion, which is possessed by the adult" [16]. Although we now know the reason to be the latter (i.e., host immunity), this experiment (alongside several others—including another of Murphy's involving the transplant of Rous chicken sarcoma into a chicken embryo) had several important implications [16]. Most importantly, these observations helped to guide researchers toward careful considerations of the animal host. Namely, questions arose that would later crystallize into (and/or were resolved by) theories regarding host immunity and graft rejection.

One scientist in particular, who made significant contributions toward the development of human tumor models (and ultimately PDX models), was Dr. Helene Wallace Toolan. From the Sloan-Kettering Institute for Cancer Research, Toolan was highly prolific during the 1950s in her research into the transplantation of human tumors into laboratory animals, particularly with regards to methodology and technique. Most significantly, in 1951, on the basis of the results from Murphy [16], Toolan devised a protocol for the irradiation of rats and mice prior to transplantation [17]. Observing positive evidence of successful engraftment, Toolan proceeded to successfully transfer the tumors for several generations using X-irradiated and/or cortisone-treated rats and hamsters [18]. Ninety out of the 101 human tumors were reported to successfully survive and proliferate in the treated hosts, thus demonstrating the feasibility and efficacy of Toolan's method [14, 18, 19].

By addressing the observations and speculations of Murphy, Toolan had effectively demonstrated the essential role of host immunity in transplantation success. Accordingly, the use of chicken embryos and the cheek pouch of hamsters (an immunologically privileged site) was beneficial in the sense that the pretreatment of conditioning agents was not necessary [2]. However, a comparative study examining the transplantation of human tumors into animals and eggs and as a tissue culture demonstrated the conditioned animal to be the most effective host for establishing and maintaining tumor growth [20]. According to the study, certain characteristics (such as the rate of growth) can vary depending on the medium in which the human tumors are grown. For example, the human epidermoid carcinoma designated H. Ep. #3 was reported to be highly virulent and metastatic when implanted into the animal or onto the egg but was observed to be "delicate" in tissue culture [20]. Conversely, H. Ep. #1 and H. Ep. #2 were found to grow relatively slowly within the conditioned hamster or rat, or on the egg, but grew in "gallon lots" as a tissue culture. However, when transplanted back from the tissue culture into an animal host, the cancers were found to revert back to a slower and more indolent pace of proliferation [20].

Despite the documented success of using a conditioned animal host for the transplantation of human tumors, there were several caveats to preclude their continued use. For the most part, this was due to a shift in the intended application of human tumor models. As society's knowledge and understanding of cancer progressed, scientists began to consider the possibility of using human tumor models as a platform for screening possible anticancer agents. As a result, the preconditioning of animal hosts did not provide the ideal experimental conditions. Palm et al. emphasized the importance of minimizing the influence of conditioning agents from the experimental design and cautioned against possible "ambiguous and/or misleading information" resulting from the conditioning agents rather than the experimental agent [21]. Furthermore, Palm et al. expressed concerns with regards to "spurious antitumor effects caused by less than optimum host conditioning," which he thought would conceal and/or dilute the effects of the potential therapeutic agents. Thus, it became imperative to find an alternate method of subduing host immunity.

For the most part, in the initial years following the first successful transplantation of human tumors into laboratory animals, mice were largely neglected as hosts in favor of rats and hamsters. However, the rationale for the use of mice as experimental models had existed since the 1900s. According to Woglom, the use of mice was motivated by "the ease with which [mice] can be procured and the cheapness and facility with which they can be can kept under observation." Furthermore, Woglom asserted that the "subcutaneous location of its tumors [makes] for early and ready recognition" [1]. Therefore, by acknowledging and recognizing the benefits associated with mouse models, Gallily and Woolley looked toward the production of mouse-grown human tumor models for the evaluation of "anticancer drugs, chemicals, and regimes" [4]. These models were produced via the serial transfer of H. Ep. #3 into treated and/or conditioned mice. Additionally, Gallily and Woolley investigated the heterotransplantability of the H. Ep. #3 tumor by testing different inoculation sites, which included subcutaneous, intramuscular, and intraperitoneal sites. As a result, the experiments conducted by Gallily and Woolley formed an important component of early efforts toward producing what would eventually be recognized as a PDX collection.

Establishing PDX Cancer Models Using Immune-Deficient Mice

However, the early success rate of PDX establishment is extremely low, which is largely due to the rejection of grafts by the host immune system. Although some methods such as X-ray irradiation and thymectomy have been used to suppress the host immune system [22–24], advances in the development of PDXs largely benefited from the generation of immune-deficient hosts (mice).

The first breakthrough was the development of nude mice. In 1962, Isaacson and Cattanach were the first to report a mutant mouse (BALB/c nu/nu) suitable for xenografting of human cancer tissue [25]. These mice have the *Foxn1* mutation, are athymic, and therefore lack the functional T cells [26]. However, natural killer (NK) cell activity is slightly increased, and the humoral antibody response system is only partially impaired in these mice. A number of PDX cancer models were established by grafting various types of cancer tissues into nude mice [24, 27, 28]. Nude mice are still commonly used as hosts for xenotransplantation of human tumors. Although the take rate of immortalized cell lines in nude mice ranges from 50 to 100%, the take rate of tumor tissue implants is generally low and varies largely among tumors of different origins.

In 1983, Bosma et al. reported the severe combined immunodeficiency (scid) mutant CB17 mice [29]. The mice that were homozygous for the mutant $Prkdc^{scid}$ (protein kinase, DNA activated, catalytic polypeptide) were designated C.B-17 scid. These mice are deficient for both T- and B-lymphocytes and therefore were recognized as valuable tools for studying immune and hematological disorders and engraftment of human cancer tissues [30–35].

Further crossing of SCID mice with the non-obese diabetic (NOD) strain led to the development of NOD-SCID mice [36], which lack both T- and B-lymphocytes.

Importantly, these mice showed apparent decreased NK cell activity and innate immunity, which is one of the main obstacles to the successful engraftment of human cancer cells [37]. In addition, these mice lack "leakiness" (the spontaneous generation of mouse T and B cells during aging) associated with the SCID mice. In view of this, many groups then shifted to the use of NOD-SCID mice for PDX model establishment and successfully established a number of panels of PDX models from various types of tumor [38–41].

Most recently, three different murine strains with IL-2 receptor (IL-2R) γ-chain deficiency have been developed, e.g., NOD-*scid* $IL2r\gamma^{null}$ (NSG) and NOD-$Rag1^{null}$ $IL2r\gamma^{null}$ (NRG) and BALB/c-$Rag2^{null}$ $IL2r\gamma^{null}$ (BRG) mice [42]. NSG mice completely lack the *Il2rg* gene and are therefore deficient in IL-2R, while NOG and BRG mice express a truncated IL-2R, which can still bind cytokines. The mice bearing *Il2rg*-targeted mutations demonstrate severe impairments in T- and B-cell development and function and importantly lack NK cells [43–45]. These have become common strains used for xenografting and have increased rates of engraftment.

In the last several decades, with the wide usage of immune-deficient mice, a large number of PDX tumor models were successfully established from various types of cancer. These models retain the histopathological, molecular characteristics and drug responses of their parental tumors [46–49]. Importantly, a panel of PDX models can recapitulate tumor heterogeneity that cell line systems fail to capture and have demonstrated potential predictive power of clinical trial response at the population level. In view of this, the PDX models provide a powerful tool and have been widely used for studying cancer biology, assisting personalized cancer therapy and drug screening in a preclinical setting.

In the following chapters, we mainly focus on the PDX tumor models developed with immune-deficient mice. The details of development, features, and applications of these new generation PDX models are reviewed.

Acknowledgments We thank all past and current members at the Living Tumor Laboratory (www.livingtumorlab.com) for their original work, thoughts, and suggestions. This study was supported by Dr. Yuzhuo Wang's grants from the Canadian Institutes of Health Research, Terry Fox Research Institute, BC Cancer Foundation, Prostate Cancer Canada, and Princess Margaret Hold'em for Life.

References

1. Woglom WH. The study of experimental cancer, a review, vol. 1. New York: Columbia University Press; 1913.
2. Woolley GW. Discussion of part VI*. Ann N Y Acad Sci. 1958;76(3):821–5. doi:10.1111/j.1749-6632.1958.tb54899.x.
3. Takayama S, Woolley GW. Successful intramuscular growth of human tumor in conditioned mice*. Ann N Y Acad Sci. 1958;76(3):797–811. doi:10.1111/j.1749-6632.1958.tb54897.x.
4. Gallily R, Woolley GW. The human tumor in the mouse*. Ann N Y Acad Sci. 1958;76(3):791–6. doi:10.1111/j.1749-6632.1958.tb54896.x.

5. Greene HSN. Heterologous transplantation of human and other mammalian tumors. Science. 1938;88(2285):357–8. doi:10.1126/science.88.2285.357.
6. Greene HSN. Heterologous transplantation of mammalian tumors. J Exp Med. 1941;73(4):475–86. doi:10.1084/jem.73.4.475.
7. Greene HSN. The heterologous transplantation of mouse tumors induced in vitro. Cancer Res. 1946;6(8):396–402.
8. Greene HSN. The use of the mouse eye in transplantation experiments. Cancer Res. 1947;7(8):491–501.
9. Adams RA, Flowers A, Sundeen R, Merk LP. Chemotherapy and immunotherapy of three human lymphomas serially transplantable in the neonatal Syrian hamster. Cancer. 1972;29(2):524–33.
10. Cobb LM. Metastatic spread of human tumour implanted into thymectomized, antithymocyte serum treated hamsters. Br J Cancer. 1972;26(3):183–9.
11. Cobb LM. The hamster as a host for the growth and study of human tumor cell populations. Cancer Res. 1974;34(5):958–63.
12. Korngold L, Lipari R. Tissue antigens of human tumors grown in rats, hamsters, and eggs. Cancer Res. 1955;15(3):159–61.
13. Smith GM. The effect of cytotoxic agents on human tumours transplanted to the hamster cheek pouch. Br J Cancer. 1969;23(1):78–87.
14. Greene HSN, Lund PK. The heterologous transplantation of human cancers. Cancer Res. 1944;4(6):352–63.
15. Greene HSN, Saxton JA. Uterine adenomata in the rabbit. J Exp Med. 1938;67(5):691–708. doi:10.1084/jem.67.5.691.
16. Murphy JB. Transplantability of malignant tumors to the embryos of a foreign species. J Am Med Assoc. 1912;LIX(11):874–5. doi:10.1001/jama.1912.04270090118016.
17. Toolan HW. Successful subcutaneous growth and transplantation of human tumors in X-irradiated laboratory animals. Proc Soc Exp Biol Med. 1951;77(3):572–8.
18. Toolan HW. Transplantable human neoplasms maintained in cortisone-treated laboratory animals: H.S. No. 1; H.Ep. No. 1; H.Ep. No. 2; H.Ep. No. 3; and H.Emb.Rh. No. 1. Cancer Res. 1954;14(9):660–6.
19. Toolan HW. Permanently transplantable human tumors maintained in conditioned heterologous hosts: H. Chon. #1, H. Ep. #4, and H. Ad. #1. Cancer Res. 1957;17(5):418–20.
20. Toolan HW. The transplantable human tumor*. Ann N Y Acad Sci. 1958;76(3):733–41. doi:10.1111/j.1749-6632.1958.tb54891.x.
21. Palm JE, Teller MN, Merker PC, Woolley GW. Host conditioning in experimental chemotherapy*. Ann N Y Acad Sci. 1958;76(3):812–20. doi:10.1111/j.1749-6632.1958.tb54898.x.
22. Clemmesen J. On transplantation of tumor cells to normal and pre-irradiated heterologous organisms. Cancer Res. 1937;29(2):313–32.
23. Grogan JB, Hardy JD. Increased survival of xenogeneic tumor in thymectomized hosts. J Surg Res. 1968;8(1):7–9.
24. Rygaard J, Povlsen CO. Heterotransplantation of a human malignant tumour to “Nude” mice. Acta pathologica et microbiologica Scandinavica. 1969;77(4):758–60.
25. Isaacson JH, Cattanach BM. Mouse News Lett. 1962;27:31.
26. Pantelouris EM. Absence of thymus in a mouse mutant. Nature. 1968;217(5126):370–1.
27. Merenda C, Sordat B, Mach JP, Carrel S. Human endometrial carcinomas serially transplanted in nude mice and established in continuous cell lines. Int J Cancer. 1975;16(4):559–70.
28. Shimosato Y, Kameya T, Nagai K, Hirohashi S, Koide T, Hayashi H, et al. Transplantation of human tumors in nude mice. J Natl Cancer Inst. 1976;56(6):1251–60.
29. Bosma GC, Custer RP, Bosma MJ. A severe combined immunodeficiency mutation in the mouse. Nature. 1983;301(5900):527–30.
30. Angevin E, Glukhova L, Pavon C, Chassevent A, Terrier-Lacombe MJ, Goguel AF, et al. Human renal cell carcinoma xenografts in SCID mice: tumorigenicity correlates with a poor clinical prognosis. Lab Investig. 1999;79(7):879–88.

31. Huynh H, Soo KC, Chow PK, Panasci L, Tran E. Xenografts of human hepatocellular carcinoma: a useful model for testing drugs. Clin Cancer Res. 2006;12(14 Pt 1):4306–14. doi:10.1158/1078-0432.CCR-05-2568.
32. Loukopoulos P, Kanetaka K, Takamura M, Shibata T, Sakamoto M, Hirohashi S. Orthotopic transplantation models of pancreatic adenocarcinoma derived from cell lines and primary tumors and displaying varying metastatic activity. Pancreas. 2004;29(3):193–203.
33. Rubio-Viqueira B, Jimeno A, Cusatis G, Zhang X, Iacobuzio-Donahue C, Karikari C, et al. An in vivo platform for translational drug development in pancreatic cancer. Clin Cancer Res. 2006;12(15):4652–61. doi:10.1158/1078-0432.CCR-06-0113.
34. Visonneau S, Cesano A, Torosian MH, Santoli D. Cell therapy of a highly invasive human breast carcinoma implanted in immunodeficient (SCID) mice. Clin Cancer Res. 1997;3(9):1491–500.
35. Xu Y, Silver DF, Yang NP, Oflazoglu E, Hempling RE, Piver MS, et al. Characterization of human ovarian carcinomas in a SCID mouse model. Gynecol Oncol. 1999;72(2):161–70. doi:10.1006/gyno.1998.5238.
36. Shultz LD, Schweitzer PA, Christianson SW, Gott B, Schweitzer IB, Tennent B, et al. Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. J Immunol. 1995;154(1):180–91.
37. Christianson SW, Greiner DL, Schweitzer IB, Gott B, Beamer GL, Schweitzer PA, et al. Role of natural killer cells on engraftment of human lymphoid cells and on metastasis of human T-lymphoblastoid leukemia cells in C57BL/6J-scid mice and in C57BL/6J-scid bg mice. Cell Immunol. 1996;171(2):186–99. doi:10.1006/cimm.1996.0193.
38. Beckhove P, Schutz F, Diel IJ, Solomayer EF, Bastert G, Foerster J, et al. Efficient engraftment of human primary breast cancer transplants in nonconditioned NOD/Scid mice. Int J Cancer. 2003;105(4):444–53. doi:10.1002/ijc.11125.
39. Cutz JC, Guan J, Bayani J, Yoshimoto M, Xue H, Sutcliffe M, et al. Establishment in severe combined immunodeficiency mice of subrenal capsule xenografts and transplantable tumor lines from a variety of primary human lung cancers: potential models for studying tumor progression-related changes. Clin Cancer Res. 2006;12(13):4043–54. doi:10.1158/1078-0432.CCR-06-0252.
40. Press JZ, Kenyon JA, Xue H, Miller MA, De Luca A, Miller DM, et al. Xenografts of primary human gynecological tumors grown under the renal capsule of NOD/SCID mice show genetic stability during serial transplantation and respond to cytotoxic chemotherapy. Gynecol Oncol. 2008;110(2):256–64. doi:10.1016/j.ygyno.2008.03.011.
41. Wang Y, Xue H, Cutz JC, Bayani J, Mawji NR, Chen WG, et al. An orthotopic metastatic prostate cancer model in SCID mice via grafting of a transplantable human prostate tumor line. Lab Investig. 2005;85(11):1392–404.
42. Shultz LD, Brehm MA, Garcia-Martinez JV, Greiner DL. Humanized mice for immune system investigation: progress, promise and challenges. Nat Rev Immunol. 2012;12(11):786–98. doi:10.1038/nri3311.
43. Cao X, Shores EW, Hu-Li J, Anver MR, Kelsall BL, Russell SM, et al. Defective lymphoid development in mice lacking expression of the common cytokine receptor gamma chain. Immunity. 1995;2(3):223–38.
44. DiSanto JP, Muller W, Guy-Grand D, Fischer A, Rajewsky K. Lymphoid development in mice with a targeted deletion of the interleukin 2 receptor gamma chain. Proc Natl Acad Sci U S A. 1995;92(2):377–81.
45. Ohbo K, Suda T, Hashiyama M, Mantani A, Ikebe M, Miyakawa K, et al. Modulation of hematopoiesis in mice with a truncated mutant of the interleukin-2 receptor gamma chain. Blood. 1996;87(3):956–67.
46. Hidalgo M, Amant F, Biankin AV, Budinská E, Byrne AT, Caldas C, et al. Patient-derived xenograft models: an emerging platform for translational cancer research. Cancer Discov. 2014;4:998–1013. doi:10.1158/2159-8290.CD-14-0001.

47. John JTA, Aik Choon T, Colin DW, Antonio J, Stephen L, Todd MP, et al. Patient-derived tumour xenografts as models for oncology drug development. Nat Rev Clin Oncol. 2012;9:338–50. doi:10.1038/nrclinonc.2012.61.
48. Rosfjord E, Lucas J, Li G, Gerber HP. Advances in patient-derived tumor xenografts: from target identification to predicting clinical response rates in oncology. Biochem Pharmacol. 2014;91(2):135–43. doi:10.1016/j.bcp.2014.06.008.
49. Siolas D, Hannon GJ, Despina S, Gregory JH. Patient-derived tumor xenografts: transforming clinical samples into mouse models. Cancer Res. 2013;73:5315–9. doi:10.1158/0008-5472.can-13-1069.

2 Towards Best Practice in Establishing Patient-Derived Xenografts

Gail P. Risbridger and Mitchell G. Lawrence

Abbreviations

NOD-SCID	Non-obese diabetic severe combined immunodeficient mice
CTC	Circulating tumour cell
NK cell	Natural killer cell
NSG	NOD-SCID interleukin-2 receptor gamma chain null mice
PAS	PDX Authentication System
PDX	Patient-derived xenograft
SCID	Severe combined immunodeficient mice
SNP	Single nucleotide polymorphism
STR	Short tandem repeat
TURP	Transurethral resection of the prostate

G.P. Risbridger
Prostate Research Group, Cancer Program - Biomedicine Discovery Institute, Department of Anatomy and Developmental Biology, Monash Partners Comprehensive Cancer Consortium, Monash University, Clayton, VIC 3000, Australia

Prostate Cancer Translational Research Program, Cancer Research Division, Peter MacCallum Cancer Centre, Melbourne, VIC 3800, Australia

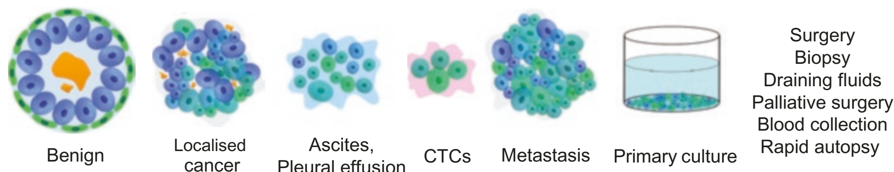
Sir Peter MacCallum Department of Oncology, The University of Melbourne, Melbourne, VIC 3010, Australia

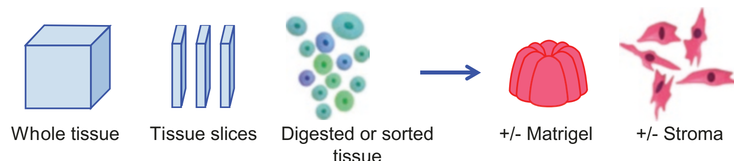
M.G. Lawrence (✉)
Prostate Research Group, Cancer Program - Biomedicine Discovery Institute, Department of Anatomy and Developmental Biology, Monash Partners Comprehensive Cancer Consortium, Monash University, Clayton, VIC 3800, Australia
e-mail: mitchell.lawrence@monash.edu

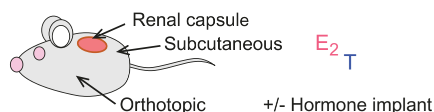
© Springer International Publishing AG 2017
Y. Wang et al. (eds.), *Patient-Derived Xenograft Models of Human Cancer*,
Molecular and Translational Medicine, DOI 10.1007/978-3-319-55825-7_2

Introduction

For decades, cancer researchers have attempted to grow samples of patient tumours as xenografts. Initially, there was limited success, and only a small number of patient-derived xenografts (PDXs) could be established, mostly from particularly aggressive tumours. Nevertheless, through a combination of serendipity and careful trial and error, the methods for establishing successful PDXs have gradually improved. Most tumour types can now be grown as PDXs, and large consortia are developing extensive collections of PDXs [1–3]. Yet, there is still room for improvement. Some tumour types still have low engraftment rates and are under-represented in PDX collections. The difficulty in establishing PDXs also means that they are beyond the resources of many laboratories, which may limit the use of PDXs in preclinical cancer research and narrow the spectrum of tumours represented by PDX models. Therefore, this chapter will focus on the methods for establishing PDXs of solid tumours. In particular, it will address four critical aspects of PDX protocols: collection of viable patient tissue, preparation of tissue for xenografting, choice of host mice and authentication of established grafts (Fig. 2.1).







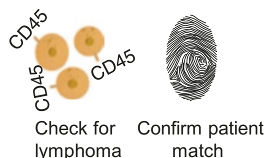


Fig. 2.1 Overview of the main steps in establishing PDXs. Step 1: High-quality patient specimens are obtained from various stages of cancer progression, from benign tissue to metastases, using a variety of collection methods. Step 2: Patient specimens are carefully prepared for grafting as either whole pieces of tissue, tissue slices, digested cells or sorted cells, with or without the addition of Matrigel or stroma. Step 3: Specimens are engrafted into the chosen strain of immunocompromised mice at the subcutaneous, subrenal capsule or orthotopic site. Hormone implants are used for PDXs of hormone-dependent cancers. Step 4: PDXs are validated to confirm that they are not contaminated with lymphoma cells and match the original patient specimen

Continued optimisation of each of these steps will maximise the likelihood of establishing successful xenografts from patient specimens.

Primary Versus Serially Transplantable PDXs

Xenografts can be derived from various sources of cancer cells; however, this chapter will specifically focus on patient-derived xenografts, sometimes also referred to as tumour grafts or patient-derived tumour xenografts. We define PDXs as those that are established from patient tissue but not from immortalised cell lines. We will also discuss xenografts from near-patient samples, such as organoids. PDXs can be further divided into primary and serially transplantable models, which have different advantages and disadvantages.

Primary PDXs, also known as first-generation PDXs, are clinical specimens that are grown in host mice for only one generation [4–7]. Depending on the experiment, this generation can last a few months, which is sufficient time for preclinical testing of candidate therapeutics [4]. Primary PDXs have high take rates, because most tissues grow *in vivo* for at least one generation, assuming that the samples are of high quality and the xenografting conditions are optimal [5]. Thus, primary PDXs provide an opportunity to maximise the utility of specimens that may not produce serially transplantable PDXs, which is particularly important for rare tumours [8]. Another advantage of primary PDXs is that they maintain the complex pathology of the original samples. Benign, premalignant and malignant cells can co-exist within a single graft, just as they do in patient tissue [7, 9, 10]. Other cell types that are retained include fibroblasts, smooth muscle and endothelial cells [7, 11]. These cell types are gradually overtaken by cancer cells and recruited mouse stroma after serial transplantation [12]. Therefore, there are several advantages to only growing patient tissues in host mice for one generation as primary PDXs.

The disadvantages of primary PDXs are offset by the benefits of serially transplantable PDXs. Primary PDX experiments require ongoing access to fresh patient specimens, whereas serially transplantable PDXs are actively growing tumours that can be regrafted into new host mice for multiple generations [13]. Thus, they provide a continuing source of tissue for numerous experiments. Over several generations, cancer cells become the most prevalent cell type within serially transplantable PDXs, so their pathology becomes more similar to metastatic than localised tumours. Nevertheless, the grafts are populated by mouse stroma, so serially transplantable PDXs are more complex models compared to *in vitro* monocultures of cancer cells. Furthermore, like *in vitro* cell cultures, serially transplantable PDXs can be cryopreserved and shared between laboratories [1, 12, 13]. Collectively, these features make serially transplantable PDXs valuable preclinical models to study tumour biology and test novel therapeutics.

One of the main limitations of serially transplantable PDXs is that some tumour types are difficult to grow. Some cancers, such as melanoma, readily establish serially transplantable PDXs, while others, such as prostate and oestrogen receptor-positive breast cancer, have much lower rates of success [7, 14–16]. There are several interrelated explanations for why some tumours are easier to establish as serially transplantable PDXs than others. One factor is the origin and availability of

tumour tissue. The surgical procedures used to remove tumours can affect the quality and viability of samples as well as the time taken to transport them to the laboratory [8]. Moreover, different patterns of early diagnosis and clinical practice between tumour types mean that samples may be available from different stages of cancer progression. Another factor influencing PDX success rates is the ability of each tumour to adapt to growing in the mouse host. Some tumours may be more sensitive to xenografting conditions, including the methods used to prepare the grafts and the choice of mouse strain. Finally, the success rate of establishing PDXs might simply reflect the aggressiveness of the cancer type and the individual patient specimen [17–19]. This particular variable is beyond a researcher's control; however, many other factors can be optimised to maximise the likelihood that tumours will produce serially transplantable PDXs. Therefore, the following sections will discuss sources of tumour tissue, preparation of grafts and choice of mouse strain, because the methods of establishing PDXs may underpin their eventual success.

Methods for Generating Patient-Derived Xenografts

Sources of Tissue for Patient-Derived Xenografts

Xenografting is a challenging technique from the very first step of the process—collecting high-quality patient specimens. The sources of patient tissue determine the take rate of PDXs and the scientific questions they can be used to investigate. This section will address the benefits, limitations and applications of different sources of tissue spanning disease progression, from non-malignant samples to metastatic cancer specimens.

Non-Malignant Tissue

Non-malignant tissues are often overlooked as samples for establishing PDXs. However, they can be used to optimise xenografting techniques, study angiogenesis and the interactions between epithelium and stroma, investigate the normal physiological responses of tissues to treatment, compare the features of patient-matched benign and malignant tissue and identify cancer cells of origin [6, 10, 20–24]. Non-malignant tissue is often dissected from the same surgical specimen as the tumour by sampling regions that are distant from known tumour foci [10, 23, 25]. Other possible sources of non-malignant tissue include prophylactic surgeries, such as mastectomies and oophorectomies from women with pathogenic *BRCA1* or *BRCA2* mutations, or procedures for benign conditions, such as transurethral resection of the prostate (TURP) for treating benign prostatic hyperplasia [8, 24, 26].

Depending on the experiment and tumour type, thorough histopathology may be required to confirm that these specimens are truly non-malignant. Furthermore, some cancers are thought to exert a field effect on surrounding tissue, so non-malignant samples may be best defined as "benign" or "morphologically normal", rather than "normal" [27, 28]. Notwithstanding this limitation, non-malignant samples are still useful because they often have high take rates as primary PDXs

[10, 25]. Anecdotally, benign epithelial cells often persist in primary PDXs, even when cancer cells fail to grow. However, unlike tumours, non-malignant tissues do not produce serially transplantable PDXs. Collectively, this means that non-malignant specimens are a convenient source of tissue for short-term PDX experiments as long as their histopathology is carefully reviewed.

Localised Tumour Tissue

Localised tumours are a common source of tissue for PDXs, because surgery with curative intent is standard practice for treating many cancers. These specimens can provide large amounts of tumour tissue from each patient, and it is sometimes possible to obtain locally advanced cancer from surrounding lymph nodes. Regions of tumour tissue can be dissected from surgical specimens with a scalpel or biopsy needle, ideally avoiding benign or necrotic tissue [5, 29]. Whichever method is used, it is essential that the overall architecture of the specimen is preserved for routine pathology reporting of surgical margins, tumour differentiation and histology [5]. PDXs can also be established from primary tumours after patients have received neoadjuvant treatments [30, 31]. In cases where surgery is not performed, tissue can be obtained from biopsies, including fine needle aspirates [10, 13, 32, 33]. Thus, for many cancers localised tumours are a widely available source of tissue for xenografting.

PDXs of primary tumour have many applications. Large biobanks of PDXs have been established from primary tumours of numerous cancers [1–3, 34]. Genomic analyses of these large cohorts have shown that they approximate the inter-patient diversity of tumours in the clinic [1, 2]. Thus, they provide comprehensive preclinical platforms for drug screening. Serially transplantable PDXs of primary cancers are also useful for studying tumour biology. For example, some laboratories have grown PDXs under selective pressure to create models of therapy resistance [13], while others have established PDXs from matched localised and metastatic tumours from the same patient [1, 32]. Therefore, even though PDXs of primary tumours usually do not represent lethal disease, they are invaluable models for cancer research.

Metastatic Tumour Tissue

PDXs of metastatic tumours provide models of the most aggressive stages of cancer progression, including therapy resistance [14, 35]. This makes PDXs of metastatic tumours ideal for studying mechanisms of drug resistance and for testing the efficacy of novel therapeutics. For many cancers, however, there is limited access to metastatic tumours compared to localised disease. Patients with some cancers rarely undergo surgical resection of metastases and are instead treated with radiotherapy or systemic therapies like chemotherapy. Nevertheless, once the logistical and ethical challenges are overcome, it is still possible to obtain metastatic samples from patients during treatment or after death.

PDXs can be established from numerous sources of metastatic tumour cells from patients who are still undergoing treatment. For example, several laboratories have generated PDXs from surgically resected liver metastases of colon cancer [34, 36–38].

Surgery is less commonly performed on metastases of many other cancers; however, other types of samples are sometimes available. This includes malignant ascites or pleural effusions, from which cancer cells can be isolated and injected or grafted into mice [17, 39–42]. Metastatic tissue can also be obtained from palliative surgeries, which are sometimes used to alleviate pain or repair fractures due to bone or spinal metastases [43–45]. Biopsies are another common source of metastatic tumour tissue [46, 47]. They are usually performed during the course of patient treatment but are sometimes undertaken specifically to obtain tissue for research or clinical trials, which carries a small risk of complications to the patient [48–50]. This means that it is sometimes possible to plan the timing of biopsies and even use them to obtain serial samples from patients [49]. However, the limitations of biopsies include the small amounts of tissue they provide and the inability to sample some metastatic sites. Nevertheless, along with surgical resections, ascites and pleural effusions, biopsies are an essential source of metastatic tumour cells for establishing PDXs.

Certain limitations of collecting samples from living patients are overcome with rapid autopsy programmes. Rapid autopsy, also known as warm or immediate autopsy, involves the collection of tumour tissue within a few hours of a patient's death [51]. The speed of this process is important for maintaining high-quality, viable tumour tissue before autolysis occurs. This creates logistic challenges, so rapid autopsy programmes typically involve team members from clinical care, palliative care, funeral services, forensic medicine, tissue banking and cancer research [14, 52]. Most rapid autopsy protocols use imaging and clinical notes to identify the locations of metastases. These sites are then reviewed macroscopically during dissection to avoid any necrotic tissue [14, 52]. Despite concerns about the viability of rapid autopsy samples, serially transplantable PDXs have been established from melanoma, rhabdomyosarcoma and breast, pancreatic, prostate and ovarian cancers [14, 35, 51–53]. The reported success rate for generating PDXs varies between tumour types, from 5% for prostate cancer to 100% for melanoma [14, 52].

There are benefits and limitations to rapid autopsy programmes and the samples they provide. One of the benefits of rapid autopsy is that it enables extensive sampling of multiple metastatic sites, including those that cannot be accessed before death [14]. This is particularly useful for studying intra-patient tumour heterogeneity [54–56]. Furthermore, compared to biopsies, rapid autopsies can provide greater amounts of tissue from more sites and without patient discomfort or the risk of complications. Yet, rapid autopsies are not routine. This makes them a low-throughput source of metastatic tissue for xenografting, especially compared to biopsies. Therefore, rapid autopsy programmes are often used for detailed studies of carefully selected patient cohorts, where samples are gradually accumulated over time. Overall, this means that rapid autopsies and other sources of metastatic tissue are complementary methods of collecting patient samples for xenografting.

Circulating Tumour Cells

Circulating tumour cells (CTCs) are an emerging source of cancer cells for PDXs. Successful PDXs have been established from breast, prostate and small-cell lung cancer CTCs directly implanted into immunocompromised host mice [57–59].

PDXs have also been generated from CTCs that were cultured as organoids before engraftment (see Section “Sorted or Cultured Cancer Cells”) [60–62]. CTCs have yielded tumours when directly injected into subcutaneous and bone sites, which is often cited as evidence that CTCs contain a subpopulation of metastasis-initiating cells [57, 58, 63]. Not surprisingly, samples with greater numbers of CTCs have higher take rates when xenografted [57, 58]. This could be due to the increased likelihood that some of the CTCs within a sample will be tumourigenic, as well as the association between high CTC counts and aggressive tumours. Since the process of collecting CTCs from blood or “liquid biopsies” is non-invasive, it might be possible to obtain serial samples to establish PDXs from different stages of disease progression, such as before and after therapeutic resistance. Therefore, CTCs are likely to become increasingly popular samples for establishing PDXs.

Sorted or Cultured Cancer Cells

PDXs are usually established from samples of intact or digested patient tissue; however, they can also be grown from tumour cells that are preselected using sorting or primary culture. Cell sorting is used to enrich defined populations of cancer cells based on their expression of cell surface antigens or phenotype. Common techniques include flow cytometry, magnetic bead separation and differential attachment to coated plates. Xenografts of sorted cells have primarily been used to study cancer-repopulating cells [37, 64, 65]. The frequency of cancer-repopulating cells can be calculated by decreasing numbers of sorted cells to establish a tumour [66]. The other common method of preselecting cancer cells, primary cell culture, is also based on the premise that only a subpopulation of cancer cells may have the potential to form tumours. In this way, *in vitro* cell culture can be used to select patient specimens that are more likely to establish successful PDXs. These primary cultures are increasingly being established as organoids rather than adherent or suspension cultures [60–62, 67]. This method is particularly useful for samples with low take rates, because it is easier to monitor their growth *in vitro* and then subsequently graft the cells into host mice. This also provides matched *in vitro* and *in vivo* models to study tumour biology and drug responses in various contexts. An unresolved question is whether there are differences in PDXs established from pieces of tissue compared to cultured cells, other than the obvious lack of human stroma in early generations. Nevertheless, preselecting cancer cells through sorting or primary culture provides researchers with a way to control the success rate of PDXs.

Preparation of Fresh Tissue for Xenografting

After obtaining high-quality patient specimens, the next important step in establishing successful PDXs is to carefully prepare the tissues for xenografting. The standard procedure for many tumours is to either graft whole pieces of tissue or enzymatically digested tissue as quickly as possible into the host mouse [68]. However, additional processing steps may improve the take rate of tissues that are difficult to grow as PDXs. One approach is to use a tissue slicer to cut thin and

precise samples, typically about 300 μm thick [69, 70]. Once grafted, these thin slices may be more highly oxygenated than larger pieces of tissue, potentially increasing their survival during the time it takes them to become vascularised. Slicing is also useful for specimens with a heterogeneous composition of cancer and benign regions, because it is possible to compare paired slices and assess the tumour content in fixed slices [69].

Other methods of preparing tissue aim to enrich the graft microenvironment. For example, grafts are often embedded in Matrigel, which provides growth factors and extracellular matrix to encourage the growth of the patient tissue and host vasculature [17, 23, 71]. Matrigel is also useful for binding together dispersed cells or fragile pieces of tissues. The development of biomimetic scaffolds by tissue engineers may provide alternatives to Matrigel where the composition and stiffness of the matrix is customised to match the patient tissue [72]. Another way of providing a supportive microenvironment is to add stroma to grafts; however, the source of stroma is critical. The take rate of primary prostate PDXs is increased when they are recombined with mesenchyme from embryonic or neonatal mice [5, 7, 9, 73]. Similarly, the growth and vascularisation of primary breast cancer PDXs is improved by co-implanting mesenchymal stromal cells [17]. In contrast, immortalised human fibroblasts from normal breast tissue had no effect on the take rate of primary breast cancer PDXs and actually decreased their serial transplantability [42]. These studies emphasise that the graft microenvironment, and the method of preparing tissue in general, can be critical in establishing PDXs.

Influence of the Mouse Host on Patient-Derived Xenografts

Some of the most dramatic improvements in PDX protocols have been due to changes in the mouse host. This section will discuss the importance of systemic features of host mice, in particular their immunocompetence and circulating steroid levels, as well as the local features of the graft site.

Systemic Features of the Mouse Host: Strains and Steroids

It is essential to use immunocompromised host mice to avoid rejection of PDXs. Early methods of supressing the host immune system included X-ray irradiation and thymectomy [74, 75]. The subsequent discovery of athymic nude mice (*nu*/*nu*), which lack functional T cells, foreshadowed the use of increasingly immunocompromised mouse strains for xenografting [76]. Many laboratories then shifted to using severe combined immune-deficient (SCID) mice, which are deficient in both T and B cells [77]. Non-obese diabetic SCID (NOD-SCID) mice then became more popular for xenografting, because they avoid the leaky phenotype of SCID mice and also have impaired natural killer (NK) cell function [78, 79]. To further abrogate the host immune response in these strains, some laboratories pretreated mice with etoposide, an immunosuppressant, a few days prior to grafting [39, 80]. It was subsequently shown, however, that etoposide decreased the take rate and growth of breast cancer PDXs in thoracic fat pads [39].

Most recently, NOD-SCID interleukin-2 receptor gamma chain null (NSG) mice have become a common strain used for xenografting. NSG mice are highly immunocompromised because they lack functional T, B and NK cells [81, 82]. It is often assumed that using highly immunocompromised host mice can improve the take rate of PDXs; however, it seems to depend on the patient specimens. Small numbers of melanoma cells have dramatically higher take rates in NSG versus NOD-SCID mice, whereas there is no difference in the engraftment rate of breast cancer tissue in SCID versus NSG mice or prostate cancer tissue in nude versus NOD-SCID mice [11, 42, 65]. Nevertheless, many laboratories now routinely use NOD-SCID or NSG mice for xenografting given that the take rate is greater, or at least equal, to that obtained with other strains. The trade-off for high engraftment rates with immunocompromised mice is the inability to study the interactions between tumour and immune cells, leading to increasing interest in humanised PDX models [83].

In addition to the immune system, steroid hormone levels are another systemic feature of host mice that affects the engraftment and growth of hormone-dependent cancers. Breast cancer PDXs are often established in female mice implanted with oestrogen pellets. Oestrogen supplementation increases the engraftment of both oestrogen receptor-positive and receptor-negative subtypes, presumably due to paracrine signalling from the stroma [17, 42, 84, 85]. Unfortunately, higher doses of exogenous oestrogen can cause side effects in host mice, such as urine retention and hydronephrosis, leading to the development of alternative protocols with lower oestrogen levels [26, 86, 87]. Similar to breast samples, prostate tissue is grafted into male mice with testosterone implants. Higher testosterone levels are required to maintain the differentiation of benign and malignant prostate glands in PDXs, because androgen levels in adult male mice are only equivalent to hypogonadal adult men [10, 24, 88, 89]. Therefore, supplementing steroid hormone levels in host mice is a simple way to maximise the success of PDXs of hormone-dependent cancers.

Local Features of the Mouse Host: Graft Site

The graft site is an important consideration when establishing PDXs, because local features of the mouse host affect the take rate, fidelity and practicality of PDXs. Specimens are often engrafted heterotopically, that is, at a different site compared to their tissue of origin. Subcutaneous grafting into the shoulder or flank of host mice is particularly common. The advantages of subcutaneous grafting include the speed and technical simplicity of the method, the ability to graft large specimens and the ease of monitoring tumour growth [90]. Thus, it is ideal for tumours with high take rates or for serially transplantable PDXs that have already been established at other graft sites. Tumours with low take rates as subcutaneous grafts, such as oesophageal, prostate and low-grade ovarian cancer, often grow more successfully as subrenal capsule or intramuscular grafts [10, 11, 91, 92]. This is thought to be due to the greater blood supply at these sites. Subrenal grafting requires more expertise than subcutaneous grafting; however, once the grafts are implanted, they are held in place by the renal capsule, a thin membrane surrounding the kidney [5, 10, 89, 93]. Another limitation of subrenal capsule grafting is that it is more difficult

to estimate tumour growth by palpating the grafts. Therefore, for heterotopic PDXs, the choice of graft site depends on the balance between optimal take rate and ease of grafting and monitoring of tumour growth.

PDXs can also be established orthotopically [10, 17, 28, 42, 90, 94–97]. It can be more difficult and time-consuming to establish orthotopic grafts, and they may need to be monitored using specialist imaging equipment [98]. Nevertheless, orthotopic grafting ensures that the host microenvironment mimics the patient tissue of origin as closely as possible. This is reflected in the high take rates of orthotopic grafts for many tumour types, compared to subcutaneous grafts [10, 17]. The graft site can also affect the phenotype of PDXs. For example, orthotopic PDXs may be more likely to metastasise [95, 99, 100] and more closely reflect patients' responses to therapy than heterotopic PDXs [101]. Yet, most PDXs closely recapitulate the original patient tumour [2], so the degree to which orthotopic grafting improves fidelity is unclear and might depend on the tumour type. The precise anatomical site used for orthotopic grafting can also be important. For example, breast cancer PDXs exhibit better engraftment and growth in abdominal compared to thoracic fat pads [39]. Furthermore, injecting oestrogen receptor α-positive tumours into milk ducts instead of fad pads produces PDXs that more closely resemble the histopathological features of patient tumours [26]. Collectively, these studies emphasise that the local microenvironment can affect the take rate and phenotype of PDXs.

Authentication of Patient-Derived Xenografts

Once PDXs are established, it is important to verify that they recapitulate the original patient specimens. There is a risk that PDXs can become contaminated, especially with lymphomas, which can rapidly overtake grafts. Lymphomas can originate from the host mouse, particularly in ageing NOD-SCID mice [18, 102, 103]. They can also arise from human B cells within the patient specimen that are transformed by Epstein-Barr virus. PDXs from a diverse range of tumour types have been contaminated with human lymphomas [18, 92, 103–106]. Simple tests can be used to rapidly identify contaminated PDXs, allowing them to be rescued. Contaminating mouse cells can be identified using species-specific analyses of telomeres, Alu repeats and human mitochondrial antigens [26, 92, 107]. Human lymphomas can be detected, and if necessary depleted, based on CD45 expression [105].

PDXs can also be cross-contaminated with one another, so there is a growing need to authenticate that they match the correct patient. It is becoming a routine practice to authenticate immortalised cell lines to ensure that they are derived from the intended source, but this is not yet the case for PDXs. This led the International Cell Line Authentication Committee to recommend that guidelines and protocols should be developed for rigorously characterising PDXs [108]. The identity of PDXs is sometimes confirmed in the process of genomic studies, but other targeted approaches can be used for routine authentication. For example, short tandem repeat (STR) analysis is commonly used for authenticating cell lines, and it has also been used in some PDX studies [42, 109]. An alternative approach is to analyse the

pattern of single nucleotide polymorphisms (SNPs), which provides similar accuracy to STRs in identifying different patient samples. An automated SNP-based PDX Authentication System (PAS) with 32 SNPs was recently validated with PDXs from paediatric acute lymphoblastic leukaemia, Ewing's sarcoma and prostate cancer [110]. This system identifies as little as 3% cross-contamination between PDXs, providing an effective method for authenticating PDXs. Therefore, given the time and expense of PDX experiments, rapid and inexpensive approaches for authenticating the identity of PDXs will become important tools for confirming the validity of the results.

Summary and Conclusions

Over the last few decades, the availability of PDXs in laboratories around the world has increased dramatically. This has been driven by numerous methodological improvements and the realisation that PDXs are invaluable tools in preclinical cancer research. Yet, it is important to note that there is no "best practice" in establishing PDXs. Methods vary depending on the tumour type, and, in many instances, tumour take rates have not been formally compared between various protocols. Instead, a bit like a cottage industry, many individual laboratories have gradually optimised the way they establish PDXs for particular tumour types. This is changing, however, as laboratories publish detailed protocols, and national and international consortia develop large repositories of PDXs. The scale and scope of these PDX platforms is important, because multiple PDXs of each tumour type and each stage of disease progression are required to replicate the diversity of tumours in the clinic. Nevertheless, these PDX platforms depend on the expertise of cancer researchers to grow patient specimens as PDXs. Through further innovation and collaboration, this process is likely to become increasingly successful in the years ahead.

Acknowledgements We thank Laura Porter for her help preparing the manuscript. The Monash University Prostate Cancer Research Program is supported by funding from the Peter and Lyndy White Foundation, the EJ Whitten Foundation and TissuPath Pathology. GPR is supported by a fellowship from the National Health and Medical Research Council (1102752).

References

1. Bruna A, Rueda OM, Greenwood W, Batra AS, Callari M, Batra RN, et al. A biobank of breast cancer explants with preserved intra-tumor heterogeneity to screen anticancer compounds. Cell. 2016;167(1):260–274. e22. doi:10.1016/j.cell.2016.08.041.
2. Gao H, Korn JM, Ferretti S, Monahan JE, Wang Y, Singh M, et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nat Med. 2015;21(11):1318–25. doi:10.1038/nm.3954.
3. Hidalgo M, Amant F, Biankin AV, Budinska E, Byrne AT, Caldas C, et al. Patient-derived xenograft models: an emerging platform for translational cancer research. Cancer Discov. 2014;4(9):998–1013. doi:10.1158/2159-8290.CD-14-0001.

4. Dong X, Guan J, English JC, Flint J, Yee J, Evans K, et al. Patient-derived first generation xenografts of non-small cell lung cancers: promising tools for predicting drug responses for personalized chemotherapy. Clin Cancer Res. 2010;16(5):1442–51. doi:10.1158/1078-0432.CCR-09-2878.
5. Lawrence MG, Taylor RA, Toivanen R, Pedersen J, Norden S, Pook DW, et al. A preclinical xenograft model of prostate cancer using human tumors. Nat Protoc. 2013;8(5):836–48. doi:10.1038/nprot.2013.043.
6. Presnell SC, Werdin ES, Maygarden S, Mohler JL, Smith GJ. Establishment of short-term primary human prostate xenografts for the study of prostate biology and cancer. Am J Pathol. 2001;159(3):855–60. doi:10.1016/S0002-9440(10)61761-0.
7. Toivanen R, Berman DM, Wang H, Pedersen J, Frydenberg M, Meeker AK, et al. Brief report: a bioassay to identify primary human prostate cancer repopulating cells. Stem Cells. 2011;29(8):1310–4. doi:10.1002/stem.668.
8. Lawrence MG, Pook DW, Wang H, Porter LH, Frydenberg M, Kourambas J, et al. Establishment of primary patient-derived xenografts of palliative TURP specimens to study castrate-resistant prostate cancer. Prostate. 2015;75(13):1475–83. doi:10.1002/pros.23039.
9. Risbridger GP, Taylor RA, Clouston D, Sliwinski A, Thorne H, Hunter S, et al. Patient-derived xenografts reveal that intraductal carcinoma of the prostate is a prominent pathology in BRCA2 mutation carriers with prostate cancer and correlates with poor prognosis. Eur Urol. 2015;67(3):496–503. doi:10.1016/j.eururo.2014.08.007.
10. Wang Y, Revelo M, Sudilovsky D, Cao M, Chen W, Goetz L, et al. Development and characterization of efficient xenograft models for benign and malignant human prostate tissue. Prostate. 2005;64(2):149–59.
11. Priolo C, Agostini M, Vena N, Ligon AH, Fiorentino M, Shin E, et al. Establishment and genomic characterization of mouse xenografts of human primary prostate tumors. Am J Pathol. 2010;176(4):1901–13. doi:10.2353/ajpath.2010.090873.
12. Alkema NG, Tomar T, Duiker EW, Jan Meersma G, Klip H, van der Zee AG, et al. Biobanking of patient and patient-derived xenograft ovarian tumour tissue: efficient preservation with low and high fetal calf serum based methods. Sci Rep. 2015;5:14495. doi:10.1038/srep14495.
13. Lin D, Wyatt AW, Xue H, Wang Y, Dong X, Haegert A, et al. High fidelity patient-derived xenografts for accelerating prostate cancer discovery and drug development. Cancer Res. 2014;74(4):1272–83. doi:10.1158/0008-5472.CAN-13-2921-T.
14. Alsop K, Thorne H, Sandhu S, Hamilton A, Mintoff C, Christie E, et al. A community-based model of rapid autopsy in end-stage cancer patients. Nat Biotechnol. 2016;34(10):1010–4. doi:10.1038/nbt.3674.
15. Van Weerden WM, Romijn JC. Use of nude mouse xenograft models in prostate cancer research. Prostate. 2000;43(4):263–71. doi:10.1002/1097-0045(20000601)43:4<263::AID-PROS5>3.0.CO;2-I.
16. Whittle JR, Lewis MT, Lindeman GJ, Visvader JE. Patient-derived xenograft models of breast cancer and their predictive power. Breast Cancer Res. 2015;17:17. doi:10.1186/s13058-015-0523-1.
17. DeRose YS, Wang G, Lin YC, Bernard PS, Buys SS, Ebbert MT, et al. Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. Nat Med. 2011;17(11):1514–20. doi:10.1038/nm.2454.
18. Ilie M, Nunes M, Blot L, Hofman V, Long-Mira E, Butori C, et al. Setting up a wide panel of patient-derived tumor xenografts of non-small cell lung cancer by improving the preanalytical steps. Cancer Med. 2015;4(2):201–11. doi:10.1002/cam4.357.
19. Sivanand S, Pena-Llopis S, Zhao H, Kucejova B, Spence P, Pavia-Jimenez A, et al. A validated tumorgraft model reveals activity of dovitinib against renal cell carcinoma. Sci Transl Med. 2012;4(137):137ra75. doi:10.1126/scitranslmed.3003643.
20. Blance RN, Sims AH, Anderson E, Howell A, Clarke RB. Normal breast tissue implanted into athymic nude mice identifies biomarkers of the effects of human pregnancy levels of estrogen. Cancer Prev Res (Phila). 2009;2(3):257–64. doi:10.1158/1940-6207.CAPR-08-0161.

21. Goldstein AS, Huang J, Guo C, Garraway IP, Witte ON. Identification of a cell of origin for human prostate cancer. Science. 2010;329(5991):568–71. doi:10.1126/science.1189992.
22. Kuperwasser C, Chavarria T, Wu M, Magrane G, Gray JW, Carey L, et al. Reconstruction of functionally normal and malignant human breast tissues in mice. Proc Natl Acad Sci U S A. 2004;101(14):4966–71. doi:10.1073/pnas.0401064101.
23. Montecinos VP, Godoy A, Hinklin J, Vethanayagam RR, Smith GJ. Primary xenografts of human prostate tissue as a model to study angiogenesis induced by reactive stroma. PLoS One. 2012;7(1):e29623. doi:10.1371/journal.pone.0029623.
24. Staack A, Kassis AP, Olshen A, Wang Y, Wu D, Carroll PR, et al. Quantitation of apoptotic activity following castration in human prostatic tissue in vivo. Prostate. 2003;54(3):212–9.
25. Gray DR, Huss WJ, Yau JM, Durham LE, Werdin ES, Funkhouser Jr WK, et al. Short-term human prostate primary xenografts: an in vivo model of human prostate cancer vasculature and angiogenesis. Cancer Res. 2004;64(5):1712–21.
26. Sflomos G, Dormoy V, Metsalu T, Jeitziner R, Battista L, Scabia V, et al. A preclinical model for eralpha-positive breast cancer points to the epithelial microenvironment as determinant of luminal phenotype and hormone response. Cancer Cell. 2016;29(3):407–22. doi:10.1016/j.ccell.2016.02.002.
27. Cooper CS, Eeles R, Wedge DC, Van Loo P, Gundem G, Alexandrov LB, et al. Analysis of the genetic phylogeny of multifocal prostate cancer identifies multiple independent clonal expansions in neoplastic and morphologically normal prostate tissue. Nat Genet. 2015;47(4):367–72. doi:10.1038/ng.3221.
28. Dotto GP. Multifocal epithelial tumors and field cancerization: stroma as a primary determinant. J Clin Invest. 2014;124(4):1446–53. doi:10.1172/JCI72589.
29. DeRose YS, Gligorich KM, Wang G, Georgelas A, Bowman P, Courdy SJ, et al. Patient-derived models of human breast cancer: protocols for in vitro and in vivo applications in tumor biology and translational medicine. Curr Protoc Pharmacol. 2013;60(14.23):14.23.1–14.23.43. doi:10.1002/0471141755.ph1423s60.
30. Kim MP, Truty MJ, Choi W, Kang Y, Chopin-Lally X, Gallick GE, et al. Molecular profiling of direct xenograft tumors established from human pancreatic adenocarcinoma after neoadjuvant therapy. Ann Surg Oncol. 2012;19 Suppl 3:S395–403. doi:10.1245/s10434-011-1839-4.
31. McAuliffe PF, Evans KW, Akcakanat A, Chen K, Zheng X, Zhao H, et al. Ability to generate patient-derived breast cancer xenografts is enhanced in chemoresistant disease and predicts poor patient outcomes. PLoS One. 2015;10(9):e0136851. doi:10.1371/journal.pone.0136851.
32. Allaway RJ, Fischer DA, de Abreu FB, Gardner TB, Gordon SR, Barth RJ, et al. Genomic characterization of patient-derived xenograft models established from fine needle aspirate biopsies of a primary pancreatic ductal adenocarcinoma and from patient-matched metastatic sites. Oncotarget. 2016;7(13):17087–102. doi:10.18632/oncotarget.7718.
33. Zhu Y, Tian T, Li Z, Tang Z, Wang L, Wu J, et al. Establishment and characterization of patient-derived tumor xenograft using gastroscopic biopsies in gastric cancer. Sci Rep. 2015;5:8542. doi:10.1038/srep08542.
34. Julien S, Merino-Trigo A, Lacroix L, Pocard M, Goere D, Mariani P, et al. Characterization of a large panel of patient-derived tumor xenografts representing the clinical heterogeneity of human colorectal cancer. Clin Cancer Res. 2012;18(19):5314–28. doi:10.1158/1078-0432.CCR-12-0372.
35. Juric D, Castel P, Griffith M, Griffith OL, Won HH, Ellis H, et al. Convergent loss of PTEN leads to clinical resistance to a PI(3)Kalpha inhibitor. Nature. 2015;518(7538):240–4. doi:10.1038/nature13948.
36. Bertotti A, Migliardi G, Galimi F, Sassi F, Torti D, Isella C, et al. A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. Cancer Discov. 2011;1(6):508–23. doi:10.1158/2159-8290.CD-11-0109.

37. O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature. 2007;445(7123):106–10. doi:10.1038/nature05372.
38. Rashidi B, Sun FX, Jiang P, An Z, Gamagami R, Moossa AR, et al. A nude mouse model of massive liver and lymph node metastasis of human colon cancer. Anticancer Res. 2000;20(2A):715–22.
39. Fleming JM, Miller TC, Meyer MJ, Ginsburg E, Vonderhaar BK. Local regulation of human breast xenograft models. J Cell Physiol. 2010;224(3):795–806. doi:10.1002/jcp.22190.
40. Liu JF, Palakurthi S, Zeng Q, Zhou S, Ivanova E, Huang W, et al. Establishment of patient-derived tumor xenograft models of epithelial ovarian cancer for pre-clinical evaluation of novel therapeutics. Clin Cancer Res. 2016. doi:10.1158/1078-0432.CCR-16-1237.
41. Verschraegen CF, Hu W, Du Y, Mendoza J, Early J, Deavers M, et al. Establishment and characterization of cancer cell cultures and xenografts derived from primary or metastatic Mullerian cancers. Clin Cancer Res. 2003;9(2):845–52.
42. Zhang X, Claerhout S, Prat A, Dobrolecki LE, Petrovic I, Lai Q, et al. A renewable tissue resource of phenotypically stable, biologically and ethnically diverse, patient-derived human breast cancer xenograft models. Cancer Res. 2013;73(15):4885–97. doi:10.1158/0008-5472.CAN-12-4081.
43. Craft N, Chhor C, Tran C, Belldegrun A, DeKernion J, Witte ON, et al. Evidence for clonal outgrowth of androgen-independent prostate cancer cells from androgen-dependent tumors through a two-step process. Cancer Res. 1999;59(19):5030–6.
44. Li ZG, Mathew P, Yang J, Starbuck MW, Zurita AJ, Liu J, et al. Androgen receptor-negative human prostate cancer cells induce osteogenesis in mice through FGF9-mediated mechanisms. J Clin Invest. 2008;118(8):2697–710. doi:10.1172/JCI33093.
45. McCulloch DR, Opeskin K, Thompson EW, Williams ED. BM18: a novel androgen-dependent human prostate cancer xenograft model derived from a bone metastasis. Prostate. 2005;65(1):35–43.
46. Goncalves A, Bertucci F, Guille A, Garnier S, Adelaide J, Carbuccia N, et al. Targeted NGS, array-CGH, and patient-derived tumor xenografts for precision medicine in advanced breast cancer: a single-center prospective study. Oncotarget. 2016. doi:10.18632/oncotarget.12714.
47. Klein KA, Reiter RE, Redula J, Moradi H, Zhu XL, Brothman AR, et al. Progression of metastatic human prostate cancer to androgen independence in immunodeficient SCID mice. Nat Med. 1997;3(4):402–8.
48. Hong MK, Sapre N, Phal PM, Macintyre G, Chin X, Pedersen JS, et al. Percutaneous image-guided biopsy of prostate cancer metastases yields samples suitable for genomics and personalised oncology. Clin Exp Metastasis. 2014;31(2):159–67. doi:10.1007/s10585-013-9617-2.
49. Kohli M, Wang L, Xie F, Sicotte H, Yin P, Dehm SM, et al. Mutational landscapes of sequential prostate metastases and matched patient derived xenografts during enzalutamide therapy. PLoS One. 2015;10(12):e0145176. doi:10.1371/journal.pone.0145176.
50. Rimondi E, Rossi G, Bartalena T, Ciminari R, Alberghini M, Ruggieri P, et al. Percutaneous CT-guided biopsy of the musculoskeletal system: results of 2027 cases. Eur J Radiol. 2011;77(1):34–42. doi:10.1016/j.ejrad.2010.06.055.
51. Hooper JE, Cantor EL, Ehlen MS, Banerjee A, Malempati S, Stenzel P, et al. A patient-derived xenograft model of parameningeal embryonal rhabdomyosarcoma for preclinical studies. Sarcoma. 2015;2015:826124. doi:10.1155/2015/826124.
52. Rubin MA, Putzi M, Mucci N, Smith DC, Wojno K, Korenchuk S, et al. Rapid ("warm") autopsy study for procurement of metastatic prostate cancer. Clin Cancer Res. 2000;6(3):1038–45.
53. Xie T, Musteanu M, Lopez-Casas PP, Shields DJ, Olson P, Rejto PA, et al. Whole exome sequencing of rapid autopsy tumors and xenograft models reveals possible driver mutations underlying tumor progression. PLoS One. 2015;10(11):e0142631. doi:10.1371/journal.pone.0142631.

54. Gundem G, Van Loo P, Kremeyer B, Alexandrov LB, Tubio JM, Papaemmanuil E, et al. The evolutionary history of lethal metastatic prostate cancer. Nature. 2015;520(7547):353–7. doi:10.1038/nature14347.
55. Kumar A, Coleman I, Morrissey C, Zhang X, True LD, Gulati R, et al. Substantial interindividual and limited intraindividual genomic diversity among tumors from men with metastatic prostate cancer. Nat Med. 2016;22(4):369–78. doi:10.1038/nm.4053.
56. Patch AM, Christie EL, Etemadmoghadam D, Garsed DW, George J, Fereday S, et al. Whole-genome characterization of chemoresistant ovarian cancer. Nature. 2015;521(7553):489–94. doi:10.1038/nature14410.
57. Baccelli I, Schneeweiss A, Riethdorf S, Stenzinger A, Schillert A, Vogel V, et al. Identification of a population of blood circulating tumor cells from breast cancer patients that initiates metastasis in a xenograft assay. Nat Biotechnol. 2013;31(6):539–44. doi:10.1038/nbt.2576.
58. Hodgkinson CL, Morrow CJ, Li Y, Metcalf RL, Rothwell DG, Trapani F, et al. Tumorigenicity and genetic profiling of circulating tumor cells in small-cell lung cancer. Nat Med. 2014;20(8):897–903. doi:10.1038/nm.3600.
59. Williams ES, Rodriguez-Bravo V, Chippada-Venkata U, De Ia Iglesia-Vicente J, Gong Y, Galsky M, et al. Generation of prostate cancer patient derived xenograft models from circulating tumor cells. J Vis Exp. 2015;(105):53182. doi:10.3791/53182.
60. Gao D, Vela I, Sboner A, Iaquinta PJ, Karthaus WR, Gopalan A, et al. Organoid cultures derived from patients with advanced prostate cancer. Cell. 2014;159(1):176–87. doi:10.1016/j.cell.2014.08.016.
61. Grillet F, Bayet E, Villeronce O, Zappia L, Lagerqvist EL, Lunke S, et al. Circulating tumour cells from patients with colorectal cancer have cancer stem cell hallmarks in ex vivo culture. Gut. 2016. doi:10.1136/gutjnl-2016-311447.
62. Yu M, Bardia A, Aceto N, Bersani F, Madden MW, Donaldson MC, et al. Cancer therapy. Ex vivo culture of circulating breast tumor cells for individualized testing of drug susceptibility. Science. 2014;345(6193):216–20. doi:10.1126/science.1253533.
63. Rossi E, Rugge M, Facchinetti A, Pizzi M, Nardo G, Barbieri V, et al. Retaining the long-survive capacity of circulating tumor cells (CTCs) followed by xeno-transplantation: not only from metastatic cancer of the breast but also of prostate cancer patients. Oncoscience. 2014;1(1):49–56. doi:10.18632/oncoscience.8.
64. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A. 2003;100(7):3983–8.
65. Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ. Efficient tumour formation by single human melanoma cells. Nature. 2008;456(7222):593–8. doi:10.1038/nature07567.
66. Hu Y, Smyth GK. ELDA: extreme limiting dilution analysis for comparing depleted and enriched populations in stem cell and other assays. J Immunol Methods. 2009;347(1–2):70–8. doi:10.1016/j.jim.2009.06.008.
67. Cayrefourcq L, Mazard T, Joosse S, Solassol J, Ramos J, Assenat E, et al. Establishment and characterization of a cell line from human circulating colon cancer cells. Cancer Res. 2015;75(5):892–901. doi:10.1158/0008-5472.CAN-14-2613.
68. Morton CL, Houghton PJ. Establishment of human tumor xenografts in immunodeficient mice. Nat Protoc. 2007;2(2):247–50. doi:10.1038/nprot.2007.25.
69. Zhao H, Nolley R, Chen Z, Peehl DM. Tissue slice grafts: an in vivo model of human prostate androgen signaling. Am J Pathol. 2010;177(1):229–39. doi:10.2353/ajpath.2010.090821.
70. Zhao H, Thong A, Nolley R, Reese SW, Santos J, Ingels A, et al. Patient-derived tissue slice grafts accurately depict response of high-risk primary prostate cancer to androgen deprivation therapy. J Transl Med. 2013;11:199. doi:10.1186/1479-5876-11-199.
71. Bernardo C, Costa C, Sousa N, Amado F, Santos L. Patient-derived bladder cancer xenografts: a systematic review. Transl Res. 2015;166(4):324–31. doi:10.1016/j.trsl.2015.02.001.
72. Hutmacher DW, Holzapfel BM, De-Juan-Pardo EM, Pereira BA, Ellem SJ, Loessner D, et al. Convergence of regenerative medicine and synthetic biology to develop standardized and

validated models of human diseases with clinical relevance. Curr Opin Biotechnol. 2015;35:127–32. doi:10.1016/j.copbio.2015.06.001.

73. Toivanen R, Frydenberg M, Murphy D, Pedersen J, Ryan A, Pook D, et al. A preclinical xenograft model identifies castration-tolerant cancer-repopulating cells in localized prostate tumors. Sci Transl Med. 2013;5(187):187ra71. doi:10.1126/scitranslmed.3005688.
74. Clemmesen J. On transplantation of tumor cells to normal and pre-irradiated heterologous organisms. Cancer Res. 1937;29(2):313–32.
75. Grogan JB, Hardy JD. Increased survival of xenogeneic tumor in thymectomized hosts. J Surg Res. 1968;8(1):7–9.
76. Rygaard J, Povlsen CO. Heterotransplantation of a human malignant tumour to "Nude" mice. Acta Pathol Microbiol Scand. 1969;77(4):758–60.
77. Bosma GC, Custer RP, Bosma MJ. A severe combined immunodeficiency mutation in the mouse. Nature. 1983;301(5900):527–30.
78. Bosma MJ. B and T cell leakiness in the scid mouse mutant. Immunodefic Rev. 1992;3(4):261–76.
79. Shultz LD, Schweitzer PA, Christianson SW, Gott B, Schweitzer IB, Tennent B, et al. Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. J Immunol. 1995;154(1):180–91.
80. Liu H, Patel MR, Prescher JA, Patsialou A, Qian D, Lin J, et al. Cancer stem cells from human breast tumors are involved in spontaneous metastases in orthotopic mouse models. Proc Natl Acad Sci U S A. 2010;107(42):18115–20. doi:10.1073/pnas.1006732107.
81. Ito M, Hiramatsu H, Kobayashi K, Suzue K, Kawahata M, Hioki K, et al. NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. Blood. 2002;100(9):3175–82.
82. Shultz LD, Lyons BL, Burzenski LM, Gott B, Chen X, Chaleff S, et al. Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. J Immunol. 2005;174(10):6477–89.
83. Shultz LD, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. Nat Rev Immunol. 2007;7(2):118–30. doi:10.1038/nri2017.
84. Gupta PB, Proia D, Cingoz O, Weremowicz J, Naber SP, Weinberg RA, et al. Systemic stromal effects of estrogen promote the growth of estrogen receptor-negative cancers. Cancer Res. 2007;67(5):2062–71. doi:10.1158/0008-5472.CAN-06-3895.
85. Kabos P, Finlay-Schultz J, Li C, Kline E, Finlayson C, Wisell J, et al. Patient-derived luminal breast cancer xenografts retain hormone receptor heterogeneity and help define unique estrogen-dependent gene signatures. Breast Cancer Res Treat. 2012;135(2):415–32. doi:10.1007/s10549-012-2164-8.
86. Dall G, Vieusseux J, Unsworth A, Anderson R, Britt K. Low dose, low cost estradiol pellets can support MCF-7 tumour growth in nude mice without bladder symptoms. J Cancer. 2015;6(12):1331–6. doi:10.7150/jca.10890.
87. Gakhar G, Wight-Carter M, Andrews G, Olson S, Nguyen TA. Hydronephrosis and urine retention in estrogen-implanted athymic nude mice. Vet Pathol. 2009;46(3):505–8. doi:10.1354/vp.08-VP-0180-N-BC.
88. Michiel Sedelaar JP, Dalrymple SS, Isaacs JT. Of mice and men-warning: intact versus castrated adult male mice as xenograft hosts are equivalent to hypogonadal versus abiraterone treated aging human males, respectively. Prostate. 2013;73(12):1316–25. doi:10.1002/pros.22677.
89. Nicholson TM, Uchtmann KS, Valdez CD, Theberge AB, Miralem T, Ricke WA. Renal capsule xenografting and subcutaneous pellet implantation for the evaluation of prostate carcinogenesis and benign prostatic hyperplasia. J Vis Exp. 2013;(78):e50574. doi:10.3791/50574.
90. Kim MP, Evans DB, Wang H, Abbruzzese JL, Fleming JB, Gallick GE. Generation of orthotopic and heterotopic human pancreatic cancer xenografts in immunodeficient mice. Nat Protoc. 2009;4(11):1670–80. doi:10.1038/nprot.2009.171.

91. Lee CH, Xue H, Sutcliffe M, Gout PW, Huntsman DG, Miller DM, et al. Establishment of subrenal capsule xenografts of primary human ovarian tumors in SCID mice: potential models. Gynecol Oncol. 2005;96(1):48–55. doi:10.1016/j.ygyno.2004.09.025.
92. Read M, Liu D, Duong CP, Cullinane C, Murray WK, Fennell CM, et al. Intramuscular transplantation improves engraftment rates for esophageal patient-derived tumor xenografts. Ann Surg Oncol. 2016;23(1):305–11. doi:10.1245/s10434-015-4425-3.
93. Shultz LD, Goodwin N, Ishikawa F, Hosur V, Lyons BL, Greiner DL. Subcapsular transplantation of tissue in the kidney. Cold Spring Harb Protoc. 2014;2014(7):737–40. doi:10.1101/pdb.prot078089.
94. Fu E, Nelson KE, Ramsey SA, Foley JO, Helton K, Yager P. Modeling of a competitive microfluidic heterogeneous immunoassay: sensitivity of the assay response to varying system parameters. Anal Chem. 2009;81(9):3407–13. doi:10.1021/ac802672v.
95. Fu XY, Theodorescu D, Kerbel RS, Hoffman RM. Extensive multi-organ metastasis following orthotopic onplantation of histologically-intact human bladder carcinoma tissue in nude mice. Int J Cancer. 1991;49(6):938–9.
96. Saar M, Korbel C, Linxweiler J, Jung V, Kamradt J, Hasenfus A, et al. Orthotopic tumorgrafts in nude mice: a new method to study human prostate cancer. Prostate. 2015;75(14):1526–37. doi:10.1002/pros.23027.
97. Topp MD, Hartley L, Cook M, Heong V, Boehm E, McShane L, et al. Molecular correlates of platinum response in human high-grade serous ovarian cancer patient-derived xenografts. Mol Oncol. 2014;8(3):656–68. doi:10.1016/j.molonc.2014.01.008.
98. Kraaij R, van Weerden WM, de Ridder CM, Gussenhoven EJ, Honkoop J, Nasu Y, et al. Validation of transrectal ultrasonographic volumetry for orthotopic prostate tumours in mice. Lab Anim. 2002;36(2):165–72.
99. Fu X, Guadagni F, Hoffman RM. A metastatic nude-mouse model of human pancreatic cancer constructed orthotopically with histologically intact patient specimens. Proc Natl Acad Sci U S A. 1992;89(12):5645–9.
100. Lin D, Watahiki A, Bayani J, Zhang F, Liu L, Ling V, et al. ASAP1, a gene at 8q24, is associated with prostate cancer metastasis. Cancer Res. 2008;68(11):4352–9. doi:10.1158/0008-5472.CAN-07-5237.
101. Garrido-Laguna I, Uson M, Rajeshkumar NV, Tan AC, de Oliveira E, Karikari C, et al. Tumor engraftment in nude mice and enrichment in stroma-related gene pathways predict poor survival and resistance to gemcitabine in patients with pancreatic cancer. Clin Cancer Res. 2011;17(17):5793–800. doi:10.1158/1078-0432.CCR-11-0341.
102. Kato C, Fujii E, Chen YJ, Endaya BB, Matsubara K, Suzuki M, et al. Spontaneous thymic lymphomas in the non-obese diabetic/Shi-scid, IL-2R gamma (null) mouse. Lab Anim. 2009;43(4):402–4. doi:10.1258/la.2009.009012.
103. Wetterauer C, Vlajnic T, Schuler J, Gsponer JR, Thalmann GN, Cecchini M, et al. Early development of human lymphomas in a prostate cancer xenograft program using triple knock-out immunocompromised mice. Prostate. 2015;75(6):585–92. doi:10.1002/pros.22939.
104. Bondarenko G, Ugolkov A, Rohan S, Kulesza P, Dubrovskyi O, Gursel D, et al. Patient-derived tumor xenografts are susceptible to formation of human lymphocytic tumors. Neoplasia. 2015;17(9):735–41. doi:10.1016/j.neo.2015.09.004.
105. Chen K, Ahmed S, Adeyi O, Dick JE, Ghanekar A. Human solid tumor xenografts in immunodeficient mice are vulnerable to lymphomagenesis associated with Epstein–Barr virus. PLoS One. 2012;7(6):e39294. doi:10.1371/journal.pone.0039294.
106. John T, Yanagawa N, Kohler D, Craddock KJ, Bandarchi-Chamkhaleh B, Pintilie M, et al. Characterization of lymphomas developing in immunodeficient mice implanted with primary human non-small cell lung cancer. J Thorac Oncol. 2012;7(7):1101–8. doi:10.1097/JTO.0b013e3182519d4d.
107. Vander Griend DJ, Konishi Y, De Marzo AM, Isaacs JT, Meeker AK. Dual-label centromere and telomere FISH identifies human, rat, and mouse cell contribution to Multispecies recombinant urogenital sinus xenografts. Prostate. 2009;69(14):1557–64. doi:10.1002/pros.21001.

108. Nardone RM, MacLeod RA, Capes-Davis A. Cancer: authenticate new xenograft models. Nature. 2016;532(7599):313.
109. Mattie M, Christensen A, Chang MS, Yeh W, Said S, Shostak Y, et al. Molecular characterization of patient-derived human pancreatic tumor xenograft models for preclinical and translational development of cancer therapeutics. Neoplasia. 2013;15(10):1138–50.
110. El-Hoss J, Jing D, Evans K, Toscan C, Xie J, Lee H, et al. A single nucleotide polymorphism genotyping platform for the authentication of patient derived xenografts. Oncotarget. 2016. doi:10.18632/oncotarget.11125.

Fidelity and Stability of PDX Models

3

Gang Li

Abbreviations

AML	Acute myeloid leukemia
CGH	Comparative genomic hybridization
ER	Estrogen receptor
IFP	Interstitial fluid pressure
mCRC	Metastatic colorectal cancer
NGS	Next generation sequencing
PDAC	Pancreatic ductal adenocarcinoma
PDX	Patient-derived xenograft
SCID	Severe combined immunodeficiency

Introduction

For a cancer drug candidate to enter clinical development, it has to pass many steps within the drug discovery and exploratory development process, and preclinical evaluation in animal models is a key step in transitioning and preparing the drug candidate to enter the clinic. Although much progress has been made from biological understanding of the disease to structure-based drug design, only a fraction of the drug candidates generated meaningful clinical benefit when tested in clinic trials. Although there are many factors that affect the translatability and predictability of preclinical pharmacology studies, the lack of clinical relevance of the animal models has frequently been cited as one of the main reasons for the high attrition rate in oncology. Traditionally, xenograft models derived from monolayers of in vitro cultured human cancer cell lines have been the mainstay of the cancer pharmacology

G. Li, Ph.D.
Ignyta, Inc., 11111 Flintkote Avenue, San Diego, CA 92121, USA
e-mail: gli@ignyta.com

© Springer International Publishing AG 2017
Y. Wang et al. (eds.), *Patient-Derived Xenograft Models of Human Cancer*,
Molecular and Translational Medicine, DOI 10.1007/978-3-319-55825-7_3

toolkit. However, studies have shown that such cell lines often diverge substantially, at molecular and behavior levels, from the original tumors from which they were derived [1–4]. For example, when tumor cells are initially harvested from the patient and subsequently put into tissue culture, the selective pressure of the artificial condition will likely exert a different impact on the heterogeneous population. If cells with a certain physiological property survive and thrive better than others, overtime, the cell population will become more homogeneous, resulting in irreversible loss of important biological characteristics [1]. Even when cells thrive in culture, they are deprived of their native microenvironment and physiological context, which inevitably leads to epigenetic changes and properties that are distinctive from their origin [3]. In some cases, cells gain genetic changes that provide them growth advantage over the cells that don't have such changes. These cells will become dominant or sole populations over generations of culture, which can lead to misrepresentation of the tumor type of origin. Consequently, mouse xenograft models derived from human cancer cell lines, especially the ones that deviate from the origin of tumor due to conditional selection and/or extended culture, are likely to lead to poor predictive power in the translation of preclinical study into clinical practice [5, 6].

In search of clinically relevant and more predictive cancer models, researchers proposed PDX tumors as an advantageous alternative to the cancer cell line-derived xenograft models. Solid tumor PDX models are generally derived from *tumor tissue* freshly harvested from cancer patients undergoing surgical biopsy or needle aspiration. The tumor tissue is implanted immediately into immunocompromised mice with no extended culture [7]. Sometimes, solid tumor PDX models can also be derived from cell suspensions generated by physical or enzymatic dissociation of tumor tissue [8].

For hematological malignancies, such as acute myeloid leukemia (AML) [9, 10], clinically relevant and predictive model systems are also important for preclinical studies to improve understanding of the biology of the disease and to develop effective therapeutic approaches. The initial report on successful engraftment of patient-derived hematological cancer cells in immunocompromised severe combined immunodeficiency (SCID) mice was published more than 20 years ago [11, 12]. However, these models usually suffer from low levels of malignant cell engraftment (as low as 0.1–5% of the mouse bone marrow). In addition, prolonged engraftment of leukemic cells in SCID mice often leads to development of lymphomas and a shortened life-span. To improve the engraftment of human hematopoietic cells, NSG and NOG mice harboring targeted deletion of IL-2 receptor common γ-chain in NOD/SCID background (NOD/LtSz-scid IL2Rγc null) were developed. These mice have severely impaired B-, T-, and NK-cell functions allowing more durable engraftment and development of human normal and malignant hematopoietic cells and less likely develop thymomas compared to SCID mice [13, 14].

The Fidelity of PDX Models

PDX tumor tissue models are increasingly being utilized for cancer drug discovery and development mainly because of the premise that they generally closely recapitulate the histology and architecture, genetic and genomic makeup, and gene- and tissue-level heterogeneity of the original tumors and their immediate

microenvironment from which they are derived [15]. In other words, they more faithfully maintain the fidelity to the human tumor than conventional, cell line-derived xenograft models [16].

Fidelity in Histopathology

One of the obvious issues with cell line-derived xenograft models is the lack of tissue architecture present in the original tumor. The histology of these models tends to show a grossly homogeneous collection of seemingly identical cells without any particular organization pattern, with the exception of mouse blood vessels and infiltrating mouse immune cells. This is usually in great contrast to actual human tumors where distinctive phenotypes are seen for different tumor types or subtypes. It is hard to imagine that the lack of cellular complexity and tissue architecture in cell line-derived models would accurately represent the complex cross talk and interactions between the cancer cells and various components of their microenvironment usually found in the original malignancies. On the other hand, it has been well documented that PDX models generally retain the histopathological features and immunohistological markers [17–21], regardless of the transplantation site.

Freshly harvested tumor fragments or cell suspension can be implanted either heterotopically or orthotopically into the immunocompromised mice. In the heterotopic procedure, subcutaneous implantation by trocar or small surgery is the most commonly used approach followed by subrenal capsular implantation of tumor material [22]. These procedures are generally simple and safe to perform. Usually, it takes about 2–6 months for the tumor to engraft, but the actual time varies tremendously by tumor type, tumor genetics and behavior characteristics, and strain of immunodeficient mice. Although heterotopic xenografts are the predominant type of PDX models available, and they generally retain the architecture of the original specimen within the tumor, the ectopic location of implantation can affect the behavior of the tumor. For example, subcutaneous tumors rarely metastasize and can have an abnormal level of angiogenesis that is significantly deviated from their origin. Subrenal capsular implantation can potentially more faithfully mimic the original tumor stroma but still represents a different anatomical location.

Recently there have been significant efforts in developing orthotopic PDX models in which tumor materials from patients are directly inserted into the corresponding anatomical locations in the host animals. Although the procedures are more technically challenging and time-consuming, it is believed that these models may better replicate the tumor microenvironment than heterotopic models and thus may be more physiopathologically relevant [23]. For example, using mammary fat pad as the receiving site for breast cancer implantation, DeRose et al. [17] reported an initial engraftment rate of 37% and a stable take rate of 24% for four primary tumors, seven pleural effusates, and one ascites. The majority of these PDXs developed metastatic disease at locations similar to those found in human patients. In another report, Zhang and colleagues [24] established a cohort of 35 stable models of breast cancer representing 27 independent patients. These models appeared to be biologically consistent with the tumor of origin, were phenotypically stable across multiple generations at the histologic and molecular levels, and showed treatment responses comparable to those

observed clinically. Of these 35 models, 12 (48%), including two ER+ tumors, developed metastatic lesions in the lungs, a major destination for breast cancer spread in humans [25]. Compared to mammary fat pad, other orthotopic locations can be more technically challenging, but the benefit of these models appeared to outweigh the risk. For instance, Walters et al. [26] reported successful generation of 15 orthotopic pancreatic ductal adenocarcinoma (PDAC) models directly from patient specimens. Interestingly but not surprisingly, they found that the PDX growth and metastatic (peritoneal and liver) rates correlated with the survival rate of the patients from which the original tumor material was obtained. These models faithfully preserved the tumor architecture, nuclear grade, and stromal content.

Fidelity in Molecular Features

In addition to preserving the histopathological features of the original tumors, it is critical for any preclinical model to retain the molecular features to be clinically relevant [5, 6]. One of the key drawbacks of cell line-derived models is their molecular divergence from the original tumors, as the result of extended in vitro culturing and selection under nonphysiological conditions [1–4]. To demonstrate that PDX models are indeed more faithful at the genetic and genomic levels, researchers have employed a number of approaches, such as cytogenetic analysis, next-generation sequencing (NGS), gene expression profiling, and comparative genomic hybridization (CGH), to extensively profile these models. These studies generally show high levels of agreement between original and xenografted tumors, occasionally with more pronounced mutational status in PDXs [17, 20, 27–31].

For example, Ding and colleagues [28] compared deep sequencing results of the primary tumor, patient blood, patient metastasis, and PDX established from the primary tumor specimens of the same triple negative breast cancer patient and found that the PDX retained the mutations of the primary tumors and gained additional mutations also found in the metastasis. Reyal et al. [20] expanded the comparison to include multiple breast cancer PDX models. By CGH comparison of xenografts with their corresponding primary tumors, it was found that the PDX tumors largely reflected the genomic profile of the patients' tumors, with additional DNA gains and losses. Gene expression profiling identified variations between PDX tumors and their corresponding primary tumor specimens, particularly in the expression of a panel of stroma-related genes, suggesting a gradual loss of human stroma and an adaptation to host animal post-tumor implantation.

In the report discussed earlier, Walters et al. [26] profiled all 15 orthotopic PDAC PDX models and found them to closely resemble the molecular features of pancreatic cancer (e.g., high rate of KRAS, P53, SMAD4 mutations, and EGFR activation). The correlation coefficient of gene expression between primary patient specimens and xenografted tumors, propagated through multiple transplantations, was between 93% and 99%. Further profiling of global gene expression showed distinct patterns between PDX and PDAC cell lines, confirming that PDX models more faithfully preserve the molecular characteristics of human cancer than cell lines. Similar

observations were made in lung cancer [32, 33], gastric cancer [34], colon cancer [35], medulloblastoma [31], retinoblastoma [36], prostate cancer [37], bladder cancer [38], ovarian cancer [39], head and neck cancer [40], and other tumor types.

Although the fidelity in molecular features in general has been well preserved in PDX models, as mentioned above, clonal selection and clonal evolution are inevitable during the initial establishment and subsequent passaging of the models, leading to qualitative and quantitative differences between PDXs and the original tumors. An example was provided recently by Eirew et al. [41], who reported that although PDXs are generally faithful molecularly, the initial engraftment and subsequent propagation could impact on the genomic clonal architecture. By deep genome sequencing and single-cell sequencing, they showed that, in all cases examined, both primary and metastatic tumors undergo various levels of initial clonal selection and that the changes continued over time during subsequent propagation. Compared to histopathological characteristics, the changes at molecular levels appear to be relatively more pronounced and dynamic [17, 24, 41].

Fidelity in Stroma/Microenvironment

One of the key differentiating features of PDX models is their ability to maintain the human stroma component during early passages [33]. Tumor stroma was defined as a heterogeneous component that includes both malignant (from tumor) and nonmalignant (from tumor and host) cell components, as well as oxygen and nutrient supply, angiogenesis, and elevated interstitial fluid pressure (IFP) and infiltrating lymphocytes. However, it is still controversial how long/how many passages the human stroma component can last and whether or not the stroma is still functionally relevant [42]. Many studies suggest that as soon as the human tumor fragment is implanted into an immunocompromised mouse, the human stroma is progressively replaced by stroma of murine origin [20, 28, 31, 35, 43, 44]. Therefore, there is a reduction, gradual or steep, in human stroma even in the early passages. Eventually the human stroma is replaced by mouse stroma [45], although the timing of this transition varies and remains to be further clarified [33, 40]. Therefore, when possible, it is wise to use PDX models with a low passage number to preserve the molecular and stromal fidelity of the original tumor [20, 27, 46]. Nonetheless, the presence of human stroma in early passages of PDX models can still provide a platform for studying interactions between tumor cells and their microenvironment [47].

Fidelity in Tumor Heterogeneity

Cancer is a heterogeneous disease as manifested in many forms within the same tumor, in different tumors within the same patient, or in tumors of different patients. However, such heterogeneity is often lost once a tumor is removed from a patient and cultured in vitro, even only for a brief period of time [1]. The lack of heterogeneity or diversity could potentially lead to experimental results that are generated

from a very small number of models with narrow or biased representation of patients [48]. In contrast, PDX models maintain the original tumor heterogeneity, which allows for modeling of a wide spectrum of cancer types or patient heterogeneity when screening larger panels of PDX tumors [35, 49, 50]. Additionally, intra-tumor or intra-patient variation is increasingly recognized as a source of inherent or acquired resistance to targeted anticancer agents, through selection of drug-resistant mutations that potentially preexist in subsets of cells [51, 52].

One of the challenges encountered when building comprehensive panels of PDX tumors is that their engraftment frequencies or "take rates" are highly variable, depending on tumor types and indications. For example, breast cancer PDX models have been more difficult to establish relative to lung, melanoma, and colorectal cancer models [53, 54]. In the case of breast cancer, basal-like cancer models were successfully developed whereas luminal tumors, including estrogen receptor (ER) positive tumors, which inherently have lower pathological grades and slower growth rates in patients, are notoriously challenging to obtain due to their poor engraftment success and slow growth in mice [29, 55–58]. A similar bias was observed in PDX models of prostate cancer [59] and other tumor types [60]. To fully realize the predictive and translational values of PDX models, an unbiased collection of PDX models that approximates the diversity of phase 2/3 clinical trial patient populations should be assembled, mimicking the tumor heterogeneity found within cancer patients. Recently, the introduction and broader use of NGS mice as hosts may be able to allow engraftment of patient samples that is otherwise challenging in traditional SCID or nude mice. In addition, the number of academic institutions and commercial entities that offer PDX models for preclinical or co-clinical testing has been growing dramatically in the past few years, which has resulted in significant improvement in model availability, to cover even the most challenging tumor types and subtypes. It is therefore anticipated that by combining a broader panel of immunocompromised strains such as NSG with optimization of tumor implantation procedures, the bias toward more aggressive and late-stage tumors will become less prominent overtime.

Another consideration during initial establishment and subsequent passaging of PDX models is to take into account the heterogeneity within the donor tumor material. Since each tumor fragment for implantation only represents a small fraction of the tumor, a single recipient animal usually cannot capture the inherent variability of each cancer, and multiple engraftments are thus needed to preserve tumor heterogeneity, even for a single donor tumor [61].

Fidelity in Response and Resistance to Drugs

The ultimate goal of using preclinical tumor models is to understand the mechanism of action of anticancer drugs and to predict patient response and resistance before embarking on expensive clinical trials that impact on human lives. Generally speaking, for dominant oncogenic drivers such as BRAFV600E mutation in melanoma and EGFR L858R mutation in NSCLC, cell lines harboring these drivers in the absence of other concurrent oncogenic mutations can predict clinical responses

fairly accurately when a clinically relevant dose or exposure is applied [62]. Still, in some cases, oncogenic gene amplification can be found in cell lines at levels that are several multitudes higher than in patient tumors, a cell culture-derived artifact that may lead to overprediction of drug responses in the clinic. On the other hand, due to the overall higher fidelity of PDX models to their original human tumors, it has been postulated that PDX models may more accurately reflect clinical responses especially in situations more complicated than single oncogene-driven tumorigenesis. In addition, the diversity and heterogeneity of PDX models allow a mouse clinical trial in which every model represents a distinct cancer patient. This is especially valuable for a population study aimed at discovering patterns of response or predictive features of a patient population. One example is a study by Bertotti et al. using a cohort of 85 PDX models of metastatic colorectal cancer (mCRC) to show HER2 amplification not only as a biomarker for resistance to EGFR inhibition but also as a positive predictor of response to HER2-targeting agents in wild-type mCRC tumors for KRAS, NRAS, BRAF, and PIK3CA. This type of study can only be performed with a large panel of heterogeneous PDX models [63].

There have been a number of studies comparing the response rates observed in PDX model panels in the preclinical setting with those in the clinic. For instance, several reports have demonstrated that, in the case of breast cancer, PDXs recapitulate the heterogeneity of treatment responses seen in the clinic for the same treatments and, more importantly, the response patterns were concordant with the original corresponding patients' responses [56, 58, 64]. Similar observations were made for chemotherapeutic agents [65–67], underscoring the potential predictive value of these models in estimating treatment outcome. Although most of the studies comparing PDX responses with clinical responses have been retrospective, some prospective studies such as the one by Hidalgo et al. [68] have demonstrated early success and real-world potential to use antitumor efficacy of various drugs in PDX models to guide treatment decisions for patients from whom the corresponding models are established. While there are key practical and regulatory hurdles, such as time required for model establishment, variability and unpredictability of engraftment efficacy, and growth rate, the clinical utility of personalized PDXs is an important area for further investigation.

As discussed earlier, PDX models retain the molecular features and the heterogeneity existing in the original human tumor and therefore are useful for studies characterizing mechanisms of drug resistance [69]. For example, a study by Dong et al. [70] identified foci of resistant cells after cisplatin-based treatments in overall responsive NSCLC PDXs. Similar drug-resistant cells also occur in patients after partial or even complete response and are therefore potentially responsible for tumor recurrence. Despite the expanding repertoire of new anticancer drugs, treatment failure due to primary or acquired resistance remains an almost inevitable outcome in most solid tumors. Intrinsic (inherent) or acquired drug resistance remains a fundamental cause of therapeutic failure in cancer therapy. Taking EGFR, for example, various mechanisms of resistance to EGFR-TKIs have been reported in the clinic and reproduced in PDX models. These potential mechanisms include T790M mutation [71]; c-Met amplification [72]; activation of alternative pathways

such as IGF-1, HGF, PI3CA, and AXL [73, 74]; transformation to mesenchymal cells [75]; or small cell features [76]. Recent data further suggested that some of the mechanisms, such as T790M mutation, could be present in a small subset of cancer cells before the treatment [52, 73, 77]. Under treatment, these cells would outlive and outgrow the treatment-sensitive cells and eventually emerge as the dominant population of the resistant tumor.

It is important for the academic and industrial investigators to have access to PDX models recapitulating refractory cancer. Nowadays, most anticancer drug candidates entering clinical development are tested in late-stage cancer patients who have previously failed several lines of chemo- and/or targeted therapies [78]. However, most PDX models currently available are derived from tumor specimens from treatment naïve patients, and the number of treatment refractory tumor PDX models is very limited. Another challenge is that for the models that are established from clinically refractory tumors, whether or not they remain refractory to the same treatment in the mouse, has not been well documented, with the exception that mutation-driven resistance to targeted therapies are usually faithfully preserved. Therefore, a concerted effort in establishing and characterizing refractory PDX models is needed to better serve the need of translating preclinical efficacy into clinical benefit.

Lack of Fidelity in Immune Microenvironment

A key limitation of PDX models based on engraftment in severely immunocompromised mice is the lack of a fully functional immune system and the imperfect cross talk between murine and human cells [17, 79, 80]. As discussed earlier, human immune components are generally present in the initial grafts but will disappear after several passages in vivo. Therefore, currently available PDX models have very limited utility for therapeutic agents whose pharmacological activities require the presence of an intact host immune system. Humanization of the host immune system [81, 82] can, in theory, partially address the issue. However, to prevent rejection of engrafted tumor, the humanized immune cells have to precisely match the tumor, a critical limiting factor, in addition to the prohibitory cost and poor success rate, limiting the practical application of the humanized PDX models.

The Stability of PDX Models

Generally speaking, as discussed earlier in this chapter, established PDX models are considered relatively stable with respect to key genomic features, gene expression patterns, clinically relevant biomarkers, and treatment responses [20, 24].

On one hand, stability is a desirable feature for in vivo models in that it can potentially provide reproducibility and reduce intra- and inter-experimental noise. On the other hand, stability is often achieved by sacrificing the heterogeneity and diversity of original tumors and through clonal selection and enrichment. As a matter of fact, cell lines and cell line-derived xenograft models are very stable models,

and the data are highly reproducible across different geographic locations and times. Therefore stability is not a distinguishing factor for PDX models, especially low passage ones. As discussed earlier, due to their inherent nature of heterogeneity at both molecular and histological levels, clonal selection and clonal evolution are inevitable during the initial establishment and subsequent passaging of the models, leading to qualitative and quantitative differences between passages and tumor-bearing mice [17, 24, 41].

Conclusions

PDX models have gained much attention and momentum in the past few years as they are becoming increasingly available and affordable and are believed to offer a superior predictive value over conventional cell line xenograft models. Ample data indicated that PDX models maintain molecular, histological, and functional heterogeneity, as well as molecular and genetic characteristics reflective of original human tumors. Emerging data start to indicate better a predictive value of the PDX models in translating preclinical efficacy into clinical benefit to patients. At the same time, one cannot overlook the limitations of PDX models and should take their shortcomings into consideration when designing and interpreting studies. Collectively, these new developments emphasize the importance of employing PDX models in key areas of oncology drug discovery and development (Fig. 3.1) [83].

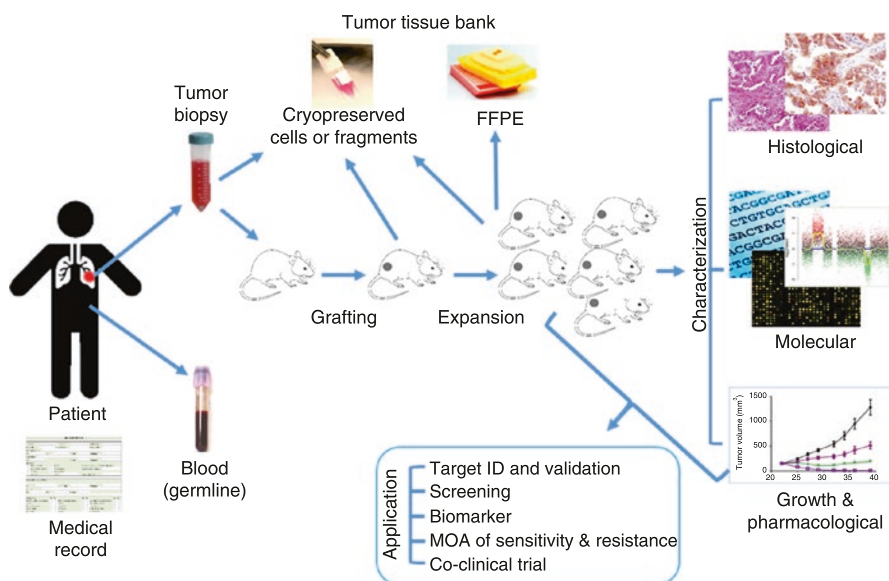


Fig. 3.1 Establishment, characterization, and application of PDX models in cancer drug research and development

References

1. Daniel VC, Marchionni L, Hierman JS, Rhodes JT, Devereux WL, Rudin CM, et al. A primary xenograft model of small-cell lung cancer reveals irreversible changes in gene expression imposed by culture in vitro. Cancer Res. 2009;69(8):3364–73. doi:10.1158/0008-5472.CAN-08-4210.
2. Johnson JI, Decker S, Zaharevitz D, Rubinstein LV, Venditti JM, Schepartz S, et al. Relationships between drug activity in NCI preclinical in vitro and in vivo models and early clinical trials. Br J Cancer. 2001;84(10):1424–31. doi:10.1054/bjoc.2001.1796.
3. Smiraglia DJ, Rush LJ, Fruhwald MC, Dai Z, Held WA, Costello JF, et al. Excessive CpG island hypermethylation in cancer cell lines versus primary human malignancies. Hum Mol Genet. 2001;10(13):1413–9.
4. Szafranska AE, Davison TS, John J, Cannon T, Sipos B, Maghnouj A, et al. MicroRNA expression alterations are linked to tumorigenesis and non-neoplastic processes in pancreatic ductal adenocarcinoma. Oncogene. 2007;26(30):4442–52. doi:10.1038/sj.onc.1210228.
5. Sausville EA, Burger AM. Contributions of human tumor xenografts to anticancer drug development. Cancer Res. 2006;66(7):3351–3354. discussion 4. doi:10.1158/0008-5472.CAN-05-3627.
6. Siolas D, Hannon GJ. Patient-derived tumor xenografts: transforming clinical samples into mouse models. Cancer Res. 2013;73(17):5315–9. doi:10.1158/0008-5472.CAN-13-1069.
7. Malaney P, Nicosia SV, Dave V. One mouse, one patient paradigm: new avatars of personalized cancer therapy. Cancer Lett. 2014;344(1):1–12. doi:10.1016/j.canlet.2013.10.010.
8. Broudy T, Ricono J, Scott C, Nair P, Thatte J, Mirsaidi C. Molecular Response LLC tumor bank and patient-derived tumor xenograft models: a powerful translational engine for discovery and development of novel oncology therapeutics. Proceedings: AACR 106th Annual Meeting 2015. 2015;Abstract 3224.
9. Vick B, Rothenberg M, Sandhofer N, Carlet M, Finkenzeller C, Krupka C, et al. An advanced preclinical mouse model for acute myeloid leukemia using patients' cells of various genetic subgroups and in vivo bioluminescence imaging. PLoS One. 2015;10(3):e0120925. doi:10.1371/journal.pone.0120925.
10. Wang K, Sanchez-Martin M, Wang X, Knapp KM, Koche R, Vu L, et al. Patient-derived xenotransplants can recapitulate the genetic driver landscape of acute leukemias. Leukemia. 2016; doi:10.1038/leu.2016.166.
11. Cesano A, Hoxie JA, Lange B, Nowell PC, Bishop J, Santoli D. The severe combined immunodeficient (SCID) mouse as a model for human myeloid leukemias. Oncogene. 1992;7(5):827–36.
12. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature. 1994;367(6464):645–8. doi:10.1038/367645a0.
13. Ishikawa F, Yoshida S, Saito Y, Hijikata A, Kitamura H, Tanaka S, et al. Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. Nat Biotechnol. 2007;25(11):1315–21. doi:10.1038/nbt1350.
14. Sanchez PV, Perry RL, Sarry JE, Perl AE, Murphy K, Swider CR, et al. A robust xenotransplantation model for acute myeloid leukemia. Leukemia. 2009;23(11):2109–17. doi:10.1038/leu.2009.143.
15. Tentler JJ, Tan AC, Weekes CD, Jimeno A, Leong S, Pitts TM, et al. Patient-derived tumour xenografts as models for oncology drug development. Nat Rev Clin Oncol. 2012;9(6):338–50. doi:10.1038/nrclinonc.2012.61.
16. Garber K. From human to mouse and back: 'tumorgraft' models surge in popularity. J Natl Cancer Inst. 2009;101(1):6–8. doi:10.1093/jnci/djn481.
17. DeRose YS, Wang G, Lin YC, Bernard PS, Buys SS, Ebbert MT, et al. Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. Nat Med. 2011;17(11):1514–20. doi:10.1038/nm.2454.

18. Fu S, Zhao J, Bai H, Duan J, Wang Z, An T, et al. High-fidelity of non-small cell lung cancer xenograft models derived from bronchoscopy-guided biopsies. Thoracic Cancer. 2016;7(1):100–10. doi:10.1111/1759-7714.12291.
19. Loukopoulos P, Kanetaka K, Takamura M, Shibata T, Sakamoto M, Hirohashi S. Orthotopic transplantation models of pancreatic adenocarcinoma derived from cell lines and primary tumors and displaying varying metastatic activity. Pancreas. 2004;29(3):193–203.
20. Reyal F, Guyader C, Decraene C, Lucchesi C, Auger N, Assayag F, et al. Molecular profiling of patient-derived breast cancer xenografts. Breast Cancer Res. 2012;14(1):R11. doi:10.1186/bcr3095.
21. Visonneau S, Cesano A, Torosian MH, Miller EJ, Santoli D. Growth characteristics and metastatic properties of human breast cancer xenografts in immunodeficient mice. Am J Pathol. 1998;152(5):1299–311.
22. Lee CH, Xue H, Sutcliffe M, Gout PW, Huntsman DG, Miller DM, et al. Establishment of subrenal capsule xenografts of primary human ovarian tumors in SCID mice: potential models. Gynecol Oncol. 2005;96(1):48–55. doi:10.1016/j.ygyno.2004.09.025.
23. Rubio-Viqueira B, Hidalgo M. Direct in vivo xenograft tumor model for predicting chemotherapeutic drug response in cancer patients. Clin Pharmacol Ther. 2009;85(2):217–21. doi:10.1038/clpt.2008.200.
24. Zhang X, Claerhout S, Prat A, Dobrolecki LE, Petrovic I, Lai Q, et al. A renewable tissue resource of phenotypically stable, biologically and ethnically diverse, patient-derived human breast cancer xenograft models. Cancer Res. 2013;73(15):4885–97. doi:10.1158/0008-5472.CAN-12-4081.
25. Scully OJ, Bay BH, Yip G, Yu Y. Breast cancer metastasis. Cancer Genomics Proteomics. 2012;9(5):311–20.
26. Walters DM, Stokes JB, Adair SJ, Stelow EB, Borgman CA, Lowrey BT, et al. Clinical, molecular and genetic validation of a murine orthotopic xenograft model of pancreatic adenocarcinoma using fresh human specimens. PLoS One. 2013;8(10):e77065. doi:10.1371/journal.pone.0077065.
27. Bergamaschi A, Hjortland GO, Triulzi T, Sorlie T, Johnsen H, Ree AH, et al. Molecular profiling and characterization of luminal-like and basal-like in vivo breast cancer xenograft models. Mol Oncol. 2009;3(5–6):469–82. doi:10.1016/j.molonc.2009.07.003.
28. Ding L, Ellis MJ, Li S, Larson DE, Chen K, Wallis JW, et al. Genome remodelling in a basal-like breast cancer metastasis and xenograft. Nature. 2010;464(7291):999–1005. doi:10.1038/nature08989.
29. Petrillo LA, Wolf DM, Kapoun AM, Wang NJ, Barczak A, Xiao Y, et al. Xenografts faithfully recapitulate breast cancer-specific gene expression patterns of parent primary breast tumors. Breast Cancer Res Treat. 2012;135(3):913–22. doi:10.1007/s10549-012-2226-y.
30. Povlsen CO, Visfeldt J, Rygaard J, Jensen G. Growth patterns and chromosome constitutions of human malignant tumours after long-term serial transplantation in nude mice. Acta pathologica et microbiologica Scandinavica Section A, Pathology. 1975;83(6):709–16.
31. Zhao X, Liu Z, Yu L, Zhang Y, Baxter P, Voicu H, et al. Global gene expression profiling confirms the molecular fidelity of primary tumor-based orthotopic xenograft mouse models of medulloblastoma. Neuro Oncol. 2012;14(5):574–83. doi:10.1093/neuonc/nos061.
32. Li J, Ye C, Mansmann UR. Comparing patient-derived xenograft and computational response prediction for targeted therapy in patients of early-stage large cell lung cancer. Clin Cancer Res. 2016;22(9):2167–76. doi:10.1158/1078-0432.CCR-15-2401.
33. Moro M, Bertolini G, Tortoreto M, Pastorino U, Sozzi G, Roz L. Patient-derived xenografts of non small cell lung cancer: resurgence of an old model for investigation of modern concepts of tailored therapy and cancer stem cells. J Biomed Biotechnol. 2012;2012:568567. doi:10.1155/2012/568567.
34. Choi YY, Lee JE, Kim H, Sim MH, Kim KK, Lee G, et al. Establishment and characterisation of patient-derived xenografts as paraclinical models for gastric cancer. Sci Rep. 2016;6:22172. doi:10.1038/srep22172.

35. Julien S, Merino-Trigo A, Lacroix L, Pocard M, Goere D, Mariani P, et al. Characterization of a large panel of patient-derived tumor xenografts representing the clinical heterogeneity of human colorectal cancer. Clin Cancer Res. 2012;18(19):5314–28. doi:10.1158/1078-0432.CCR-12-0372.
36. McEvoy J, Ulyanov A, Brennan R, Wu G, Pounds S, Zhang J, et al. Analysis of MDM2 and MDM4 single nucleotide polymorphisms, mRNA splicing and protein expression in retinoblastoma. PLoS One. 2012;7(8):e42739. doi:10.1371/journal.pone.0042739.
37. Rea D, Del Vecchio V, Palma G, Barbieri A, Falco M, Luciano A, et al. Mouse models in prostate cancer translational research: from xenograft to PDX. Biomed Res Int. 2016;2016:9750795. doi:10.1155/2016/9750795.
38. Pan CX, Zhang H, Tepper CG, Lin TY, Davis RR, Keck J, et al. Development and characterization of bladder cancer patient-derived xenografts for molecularly guided targeted therapy. PLoS One. 2015;10(8):e0134346. doi:10.1371/journal.pone.0134346.
39. Liu JF, Palakurthi S, Zeng Q, Zhou S, Ivanova E, Huang W, et al. Establishment of patient-derived tumor xenograft models of epithelial ovarian cancer for pre-clinical evaluation of novel therapeutics. Clin Cancer Res. 2016; doi:10.1158/1078-0432.CCR-16-1237.
40. Peng S, Creighton CJ, Zhang Y, Sen B, Mazumdar T, Myers JN, et al. Tumor grafts derived from patients with head and neck squamous carcinoma authentically maintain the molecular and histologic characteristics of human cancers. J Transl Med. 2013;11:198. doi:10.1186/1479-5876-11-198.
41. Eirew P, Steif A, Khattra J, Ha G, Yap D, Farahani H, et al. Dynamics of genomic clones in breast cancer patient xenografts at single-cell resolution. Nature. 2015;518(7539):422–6. doi:10.1038/nature13952.
42. Lunt SJ, Chaudary N, Hill RP. The tumor microenvironment and metastatic disease. Clin Exp Metastasis. 2009;26(1):19–34. doi:10.1007/s10585-008-9182-2.
43. Chaudary N, Pintilie M, Schwock J, Dhani N, Clarke B, Milosevic M, et al. Characterization of the tumor-microenvironment in patient-derived cervix xenografts (OCICx). Cancer. 2012;4(3):821–45. doi:10.3390/cancers4030821.
44. de Plater L, Lauge A, Guyader C, Poupon MF, Assayag F, de Cremoux P, et al. Establishment and characterisation of a new breast cancer xenograft obtained from a woman carrying a germline BRCA2 mutation. Br J Cancer. 2010;103(8):1192–200. doi:10.1038/sj.bjc.6605900.
45. Braekeveldt N, Wigerup C, Tadeo I, Beckman S, Sanden C, Jonsson J, et al. Neuroblastoma patient-derived orthotopic xenografts reflect the microenvironmental hallmarks of aggressive patient tumours. Cancer Lett. 2016;375(2):384–9. doi:10.1016/j.canlet.2016.02.046.
46. Rubio-Viqueira B, Jimeno A, Cusatis G, Zhang X, Iacobuzio-Donahue C, Karikari C, et al. An in vivo platform for translational drug development in pancreatic cancer. Clin Cancer Res. 2006;12(15):4652–61. doi:10.1158/1078-0432.CCR-06-0113.
47. Simpson-Abelson MR, Sonnenberg GF, Takita H, Yokota SJ, Conway Jr TF, Kelleher Jr RJ, et al. Long-term engraftment and expansion of tumor-derived memory T cells following the implantation of non-disrupted pieces of human lung tumor into NOD-scid IL2Rgamma(null) mice. J Immunol. 2008;180(10):7009–18.
48. Sobel RE, Sadar MD. Cell lines used in prostate cancer research: a compendium of old and new lines—part 1. J Urol. 2005;173(2):342–59. doi:10.1097/01.ju.0000141580.30910.57.
49. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A. 2003;100(7):3983–8.
50. Dolgova EV, Alyamkina EA, Efremov YR, Nikolin VP, Popova NA, Tyrinova TV, et al. Identification of cancer stem cells and a strategy for their elimination. Cancer Biol Ther. 2014;15(10):1378–94. doi:10.4161/cbt.29854.
51. Diaz Jr LA, Williams RT, Wu J, Kinde I, Hecht JR, Berlin J, et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature. 2012;486(7404):537–40. doi:10.1038/nature11219.
52. Shah SP, Roth A, Goya R, Oloumi A, Ha G, Zhao Y, et al. The clonal and mutational evolution spectrum of primary triple-negative breast cancers. Nature. 2012;486(7403):395–9. doi:10.1038/nature10933.

53. Mattern J, Bak M, Hahn EW, Volm M. Human tumor xenografts as model for drug testing. Cancer Metastasis Rev. 1988;7(3):263–84.
54. Vestergaard BF, Bjerrum OJ, Norrild B, Grauballe PC. Crossed immunoelectrophoretic studies of the solubility immunogenicity of herpes simplex virus antigens. J Virol. 1977;24(1):82–90.
55. Beckhove P, Schutz F, Diel IJ, Solomayer EF, Bastert G, Foerster J, et al. Efficient engraftment of human primary breast cancer transplants in nonconditioned NOD/Scid mice. Int J Cancer. 2003;105(4):444–53. doi:10.1002/ijc.11125.
56. Cottu P, Marangoni E, Assayag F, de Cremoux P, Vincent-Salomon A, Guyader C, et al. Modeling of response to endocrine therapy in a panel of human luminal breast cancer xenografts. Breast Cancer Res Treat. 2012;133(2):595–606. doi:10.1007/s10549-011-1815-5.
57. Dangles-Marie V, Pocard M, Richon S, Weiswald LB, Assayag F, Saulnier P, et al. Establishment of human colon cancer cell lines from fresh tumors versus xenografts: comparison of success rate and cell line features. Cancer Res. 2007;67(1):398–407. doi:10.1158/0008-5472.CAN-06-0594.
58. Marangoni E, Vincent-Salomon A, Auger N, Degeorges A, Assayag F, de Cremoux P, et al. A new model of patient tumor-derived breast cancer xenografts for preclinical assays. Clini Cancer Res. 2007;13(13):3989–98. doi:10.1158/1078-0432.CCR-07-0078.
59. Van Weerden WM, de Ridder CM, Verdaasdonk CL, Romijn JC, van der Kwast TH, Schroder FH, et al. Development of seven new human prostate tumor xenograft models and their histopathological characterization. Am J Pathol. 1996;149(3):1055–62.
60. Wang Y, Sudilovsky D, Zhang B, Haughney PC, Rosen MA, Wu DS, et al. A human prostatic epithelial model of hormonal carcinogenesis. Cancer Res. 2001;61(16):6064–72.
61. Whiteford CC, Bilke S, Greer BT, Chen Q, Braunschweig TA, Cenacchi N, et al. Credentialing preclinical pediatric xenograft models using gene expression and tissue microarray analysis. Cancer Res. 2007;67(1):32–40. doi:10.1158/0008-5472.CAN-06-0610.
62. Spilker ME, Chen X, Visswanathan R, Vage C, Yamazaki S, Li GG, et al. Found in translation: maximizing the clinical relevance of nonclinical oncology studies. Clin Cancer Res. 2016; doi:10.1158/1078-0432.CCR-16-1164.
63. Bertotti A, Migliardi G, Galimi F, Sassi F, Torti D, Isella C, et al. A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. Cancer Discov. 2011;1(6):508–23. doi:10.1158/2159-8290.CD-11-0109.
64. Kabos P, Finlay-Schultz J, Li C, Kline E, Finlayson C, Wisell J, et al. Patient-derived luminal breast cancer xenografts retain hormone receptor heterogeneity and help define unique estrogen-dependent gene signatures. Breast Cancer Res Treat. 2012;135(2):415–32. doi:10.1007/s10549-012-2164-8.
65. Dylla SJ, Beviglia L, Park IK, Chartier C, Raval J, Ngan L, et al. Colorectal cancer stem cells are enriched in xenogeneic tumors following chemotherapy. PLoS One. 2008;3(6):e2428. doi:10.1371/journal.pone.0002428.
66. Fichtner I, Rolff J, Soong R, Hoffmann J, Hammer S, Sommer A, et al. Establishment of patient-derived non-small cell lung cancer xenografts as models for the identification of predictive biomarkers. Clin Cancer Res. 2008;14(20):6456–68. doi:10.1158/1078-0432.CCR-08-0138.
67. Fichtner I, Slisow W, Gill J, Becker M, Elbe B, Hillebrand T, et al. Anticancer drug response and expression of molecular markers in early-passage xenotransplanted colon carcinomas. Eur J Cancer. 2004;40(2):298–307.
68. Hidalgo M, Bruckheimer E, Rajeshkumar NV, Garrido-Laguna I, De Oliveira E, Rubio-Viqueira B, et al. A pilot clinical study of treatment guided by personalized tumorgrafts in patients with advanced cancer. Mol Cancer Ther. 2011;10(8):1311–6. doi:10.1158/1535-7163.MCT-11-0233.
69. Krumbach R, Schuler J, Hofmann M, Giesemann T, Fiebig HH, Beckers T. Primary resistance to cetuximab in a panel of patient-derived tumour xenograft models: activation of MET as one mechanism for drug resistance. Eur J Cancer. 2011;47(8):1231–43. doi:10.1016/j.ejca.2010.12.019.

70. Dong X, Guan J, English JC, Flint J, Yee J, Evans K, et al. Patient-derived first generation xenografts of non-small cell lung cancers: promising tools for predicting drug responses for personalized chemotherapy. Clin Cancer Res. 2010;16(5):1442–51. doi:10.1158/1078-0432.CCR-09-2878.
71. Lim SM, Jeong Y, Hong S. Strategies to overcome acquired resistances conferred by mutations in the kinase domain of EGFR. Future Med Chem. 2016;8(8):853–78. doi:10.4155/fmc-2016-0019.
72. Ahsan A. Mechanisms of resistance to EGFR tyrosine kinase inhibitors and therapeutic approaches: an update. Adv Exp Med Biol. 2016;893:137–53. doi:10.1007/978-3-319-24223-1_7.
73. Lee JC, Jang SH, Lee KY, Kim YC. Treatment of non-small cell lung carcinoma after failure of epidermal growth factor receptor tyrosine kinase inhibitor. Cancer Res Treat. 2013;45(2):79–85. doi:10.4143/crt.2013.45.2.79.
74. Zhang Z, Lee JC, Lin L, Olivas V, Au V, LaFramboise T, et al. Activation of the AXL kinase causes resistance to EGFR-targeted therapy in lung cancer. Nat Genet. 2012;44(8):852–60. doi:10.1038/ng.2330.
75. Nurwidya F, Takahashi F, Murakami A, Takahashi K. Epithelial mesenchymal transition in drug resistance and metastasis of lung cancer. Cancer Res Treat. 2012;44(3):151–6. doi:10.4143/crt.2012.44.3.151.
76. Sequist LV, Waltman BA, Dias-Santagata D, Digumarthy S, Turke AB, Fidias P, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. Sci Transl Med. 2011;3(75):75ra26. doi:10.1126/scitranslmed.3002003.
77. Hammerman PS, Janne PA, Johnson BE. Resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. Clin Cancer Res. 2009;15(24):7502–9. doi:10.1158/1078-0432.CCR-09-0189.
78. Kim MP, Truty MJ, Choi W, Kang Y, Chopin-Lally X, Gallick GE, et al. Molecular profiling of direct xenograft tumors established from human pancreatic adenocarcinoma after neoadjuvant therapy. Ann Surg Oncol. 2012;19 Suppl 3:S395–403. doi:10.1245/s10434-011-1839-4.
79. Dumont N, Liu B, Defilippis RA, Chang H, Rabban JT, Karnezis AN, et al. Breast fibroblasts modulate early dissemination, tumorigenesis, and metastasis through alteration of extracellular matrix characteristics. Neoplasia. 2013;15(3):249–62.
80. Fleming JM, Miller TC, Meyer MJ, Ginsburg E, Vonderhaar BK. Local regulation of human breast xenograft models. J Cell Physiol. 2010;224(3):795–806. doi:10.1002/jcp.22190.
81. Garcia S, Freitas AA. Humanized mice: current states and perspectives. Immunol Lett. 2012;146(1–2):1–7. doi:10.1016/j.imlet.2012.03.009.
82. Lux A, Nimmerjahn F. Of mice and men: the need for humanized mouse models to study human IgG activity in vivo. J Clin Immunol. 2013;33 Suppl 1:S4–8. doi:
.
83. Li G. Patient-derived xenograft models for oncology drug discovery. J Cancer Metastasis Treat. 2015;1:8–15.

Humanized Mouse and Rat PDX Cancer Models

4

Fallon K. Noto and Tseten Yeshi

Abbreviations

FSG	F344-scid gamma
GFP	Green fluorescent protein
NK	Natural killer
NOD	Nonobese diabetic
NSG	NOD *scid* gamma
PDTX	Patient-derived tumor xenograft
PDX	Patient-derived xenograft
SCID	Severe combined immunodeficiency

Introduction

One of the main goals of oncology research is to study the molecular, genetic, and pathological characteristics of cancer to be able to predict growth, metastasis rates, and response to therapeutics. With over 100 different types of cancer [1], heterogeneity within a single tumor, within multiple tumors in the same patient, and differences between patient to patient with the same type of cancer, one of the biggest reasons for high failure rates in cancer therapeutics is the lack of models that faithfully recapitulate this heterogeneity. The first models of human cancer were derived via cell culturing techniques. In vitro culturing of cells involves adaptations that allow for the cells to survive outside of their normal three-dimensional environment. The in vivo environment of cancer cells often involves cancer growth-supporting cell types, cytokines, and growth factors from the patient, which are difficult to recapitulate in modeling. In vitro, the cancer cells must learn to survive without that environment, which generally leads to genetic aberrations, altering the

F.K. Noto (✉) • T. Yeshi
Hera BioLabs, Inc., 2277 Thunderstick Dr. #500, Lexington, KY 40505, USA
e-mail: fnoto@herabiolabs.com

© Springer International Publishing AG 2017

Y. Wang et al. (eds.), *Patient-Derived Xenograft Models of Human Cancer*,
Molecular and Translational Medicine, DOI 10.1007/978-3-319-55825-7_4

phenotype and ultimately, the response to therapeutics. These genetic aberrations are not reversed when the cancer cell lines are transplanted in vivo, often resulting in a tumor with different growth kinetics, rates of metastasis, and response to therapeutics in comparison to the originating tumor. Advances in three-dimensional tissue culture, which often involves supplying the cancer cells with extracellular matrices and co-culturing with support cell types, have provided improved in vitro modeling of the tumor microenvironment [2–4]. Three-dimensional or spheroid cultures more accurately mimic cell–cell and cell–matrix interactions, hypoxia observed in tumors, and drug penetration of the tumor. This method is faster to develop and more cost-effective than using animals, but in vivo modeling of cancer remains the most widely used method in preclinical trials of chemotherapeutic agents.

In vivo human tumor growth has been possible due to the ability to genetically modify research animals. In particular, the laboratory mouse has been a vital component of cancer biology research due to the plethora of immunodeficient mouse strains which allow growth of human cells without rejection. There are still drawbacks to the mouse model of human xenografts, including low cell engraftment rate, variability in growth kinetics and histopathology from mouse to mouse transplanted with the same cancer cell line, and lack of survival for some cancer cell lines, even when transplanted into several different mouse strains. Although minimally invasive routes of cancer cell line administration exist such as subcutaneous implantation, this method has the lowest cell survival rate. Other more invasive methods are hindered by technical difficulties with surgical manipulation of the mouse. In addition, the mouse's relatively small size equates to small tumor size due to humane endpoints and small volumes of blood sampling, resulting in an incomplete analysis or splitting the analysis over multiple animals.

More recently, patient-derived xenografts (PDXs) have become popular in the field of cancer research. This involves taking a fragment from a tumor that has been surgically removed or biopsied and transplanting it directly into an animal. Serial transplantation results in a bank of animals with theoretically the same tumor as the patient, allowing for therapeutic testing with multiple drugs. While PDX models more accurately reflect the biology of the original tumor, the field is still hampered by low engraftment rates. If 100 different PDX tissues are subcutaneously transplanted into the same rodent strain, in general, only 20–30 of those will grow and form tumors. In addition, most PDX transplantations are done with mice, which results in small sample sizes, meaning at least 2–3 serial transplantations must occur to have sufficient animals for downstream studies. Nevertheless, PDX models are the most promising choice for personalized medicine to determine which treatment regimen will be most successful with the lowest toxicity for each individual patient [5].

Recent advances in technology for altering the genome have led to the advent of targeted genetic modifications in the rat. These include rats with knocked-out genes implicated in the development and function of the immune system. Such immunodeficient rats, like their mouse counterparts, can potentially be engrafted with multiple different types of cancer with the same efficiency or higher. An

advantage of using the rat as a model system is the means to grow larger tumors and therefore obtain larger sample sizes and volumes of blood for analysis. In addition, the rat's larger size equates to easier surgical manipulation. Furthermore, the rat is the species of choice for toxicology research and is physiologically more similar to humans than the mouse. One could hypothesize that this could result in better host–tumor interactions and, therefore, more accurate modeling of tumor growth and pathology.

This chapter will briefly review advances in mouse strains used for human cancer growth and PDX modeling in mice and discuss the possibility of using the rat for PDX modeling.

Mouse Models of Human Cancer

Immunodeficient Mouse Strains

One of the biggest pieces of the rodent–human xenograft puzzle is the immune status of the recipient animal. To avoid rejection of the human cells, the animal must be immunodeficient. While it is imperative that the animal lacks T cells, lacking mature B and NK cells also enhances human cell engraftment. Likewise, defective macrophages greatly enhance human cell survival in the rodent.

Perhaps the most widely used immunodeficient mouse strain is the NSG or NOD *scid* gamma mouse. This mouse, developed by the laboratory of Dr. Leonard Shultz at The Jackson Laboratory, harbors a functionally null allele for the IL2 receptor gamma chain ($Il2rg^{tm1Wjl}$) and the severe combined immune deficiency mutation ($Prkdc^{scid}$) on the nonobese diabetic NOD/ShiLtJ background [6]. Phenotypically, this mouse lacks mature B, T, and natural killer (NK) cells. In addition, it has defective dendritic cells and absent complement factors, and the NOD background confers higher human cell engraftment rate, especially with regard to immune cells, compared to any other immunodeficient mouse strain [7–10]. Together, this makes that the NSG mouse strain allows the widest range of cancer cell lines and human cell types to be engrafted. The less well-known NOG mouse from Taconic (NOD. Cg-$Prkdc^{scid}$ $Il2rg^{tm1Sug}$/JicTac) has a similar phenotype and, therefore, likely a similar portfolio of human cell types that are able to be engrafted [11].

Before the NSG mouse was established, variations of immunodeficiencies were achieved with different mutations. The Nude ($Foxn1^{nu}$) mouse is athymic and lacks T cells [12] but has a normal complement of B and NK cells, limiting its potential for human cell grafting. However, it is one of the oldest and most published immunodeficient mouse strains, the least expensive commercially available one, and is hairless, making it more accessible to researchers and appealing due to the ease of imaging tumors in the animal. The Rag1 or Rag2 single knockout mice lack B and T cells but have a normal repertoire of other immune cells [13–15], resulting in successful engraftment of some, but not all human cell types and human cancers. Similarly, the $Prkdc^{scid}$ mouse lacks B and T cells but has functional macrophages and NK cells [16]. The *scid* mouse is also hampered by strain-dependent leakiness.

The IL2 receptor gamma chain knockout mouse lacks NK cells [15], such that when combined with the Rag2 knockout, the double knockout mouse lacks B, T, and NK cells, expanding the number of cancer cells types that can potentially be successfully engrafted into the animal.

Cell Lines for Cancer Modeling In Vitro and In Vivo

Although this chapter is aimed at introducing the possibility of human PDX modeling in the rat, it is important to review what is known about human cancer modeling in the mouse. We will briefly review cell-based mouse models of human cancer; however, gene-based models do exist in which an oncogene, or gene implicated in human cancers, is mutated in the mouse to mimic tumor growth [17]. In those cases, the tumors are of mouse origin. Cell-based models of human cancer involve transplanting human cancer cells into the animal. Historically, cells isolated from primary human tumors are cultured and then implanted into the mouse. Establishing cancer cell lines offers the advantage of being able to genetically manipulate the cells, especially with regard to inserting a reporter gene that can be used to track tumor growth, metastasis, and response to therapeutics. Most commonly, these reporter cell lines express GFP (green fluorescent protein) or luciferase for real-time imaging of tumors in live animals through the course of the study. Cell lines have been established for nearly every type of cancer in existence and are generally commercially available to all researchers. Combined with relative ease of propagation, cancer cell lines provide an attractive means of modeling molecular and genetic aspects of their respective primary cancers. In addition, many biotech companies offer profiles for specific cancer lines, even selling animals pre-engrafted with cell lines. For example, Charles River has an extensive database of cancer cell lines with growth profiles in the Nude mouse but also lists popular cancer cell lines for which they do not have a profile, possibly because those cell lines do not survive in mouse models that have been tested. The Jackson Laboratory also offers similar xenografted mouse models.

As previously mentioned, in vitro culturing often leads to accumulation of genetic aberrations, altering the phenotype of the cells, and ultimately to a tumor in the mouse that differs in growth and pathology compared to the original patient's tumor. Notably, these genetic aberrations often result in a phenotypically homogeneous population, which leads to an in vivo tumor that does not represent the heterogeneity of the original tumor. Therefore, oncology drug testing results obtained with cultured cell lines and cell-based tumors do not accurately predict therapeutic efficacy in the patient, leading to high attrition rates of chemotherapeutic clinical trials [18].

The inadequacies of preclinical drug efficacy trials have pushed oncology researchers to devise cancer models that more accurately mimic tumor pathobiology. This led to an increased interest in transplanting tumor tissue directly from the patient into the animal, in hopes that eliminating enzymatic processing and cell culturing would produce a tumor in the animal that more closely resembled that of the patient. The cancer model based on this concept has become widely known as the patient-derived xenograft (PDX).

Patient-Derived Xenografts

The concept of patient-derived xenografts (PDX) or patient-derived tumor xenografts (PDTX) is not a new one. Transplanting primary tumors from patients, in conjunction with cell line-based xenografts, has been used for preclinical drug screening of novel chemotherapeutics for a couple of decades [19]. Historically, cancer cell lines have been used more widely than primary tumor biopsies, but interest in PDX modeling has increased in recent years. The complexity of cancer has shed light on the idea that developing PDX animal models with the same type of cancer from multiple patients will enhance our knowledge of the genetic diversity of the disease and allow us to develop better and more widely successful therapeutics.

PDX models arise when a small piece of tissue obtained from a tumor resection is implanted into an immunodeficient mouse, usually subcutaneously (Fig. 4.1). Not all PDX transplants survive and grow, but some grow and form a large tumor in the animal, which can then be removed, manually cut into smaller pieces, and serially transplanted into multiple mice. This process is repeated until a sufficient number of animals with tumors of appropriate size are obtained for drug efficacy testing. In

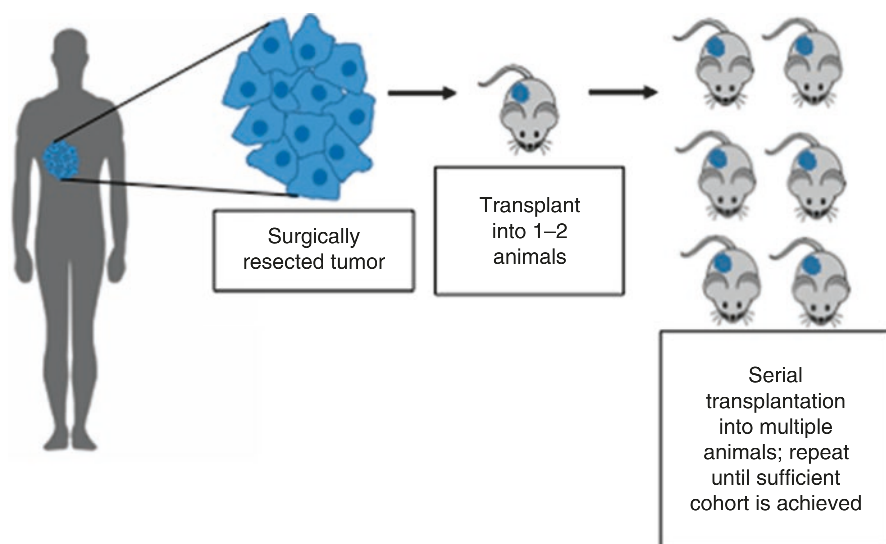


Fig. 4.1 Schematic representation of establishing PDX models. A tumor is surgically removed or a tumor biopsy is taken from the patient. Any tissue remaining after clinical analysis is provided to researchers who then directly transplant the intact tissue into a rodent model. If the tumor survives and grows, it can then be removed from the primary rodent recipient, chopped into smaller pieces, and serially transplanted into secondary, tertiary, etc. recipients until a cohort of rodents with tumors derived from the original PDX tissue is obtained. These cohorts of animals are then subjected to downstream assays such as drug efficacy testing and molecular and genetic analysis to determine how closely the rodent tumors resemble the patient's tumor. A portion of the tumor is cryopreserved at each serial transplantation and can be used for future or follow-up studies

some cases, the primary tumor obtained from the patient is enzymatically digested to obtain single cells or small clusters of cells for in vitro culturing or in vivo subcutaneous, orthotopic, subrenal capsular, or intravenous transplantation, but in most cases, PDX models are established without the tumor tissue ever coming into contact with tissue culture plastic or enzymes.

Locations of PDX engraftment vary, but the subcutaneous graft site is the most widely used. Subcutaneous transplantation is advantageous because tumor monitoring and measurement is easiest with this method. In addition, tumor implantation and surgical removal of the tumor are less invasive than when other methods are used. However, subcutaneous transplants have the lowest engraftment rate, at 40–60% [20]. Subrenal capsular transplantation results in the highest engraftment, i.e., ~95% [21]. However, this method requires a surgical procedure under anesthesia, is the most difficult transplantation technique, and increases stress and pain inflicted on the animal. Another disadvantage of subrenal capsular transplantation is that monitoring of the tumor in an intact animal is impossible without an imaging technique such as fluorescence or luminescence; in which case, the tumor must be labeled with a tracking protein. While there exist many GFP/RFP/other fluorophore-labeled or luciferase-expressing cancer cell lines, creating a reporter PDX tissue would likely require dissociation of the tumor into single cells or small cell clusters. As mentioned previously, most PDX researchers prefer to transplant patients' cancer tissue directly into the animal.

PDX models offer several advantages over cancer cell cultures and traditional cancer modeling by growing cultured cancer cells in animals. One of the most important advantages of PDX models is that they retain major genetic, molecular, and histopathological characteristics of the original tumor, including, to a great extent, its heterogeneity [22–25]. PDX tissues that grow in the mouse can be removed and serially transplanted multiple times, and data suggest that the tumors derived from serial transplantation retain genetic, molecular, and pathological characteristics of the original tumor through successive passages [24, 26]. Therefore, in vivo PDX models offer the possibility to create banks of animals with the same tumor found in a patient which can then be used to test therapeutic efficacy of multiple chemotherapeutics and small molecules to predict the most effective treatment regimen.

Many institutions now offer panels of PDX tissues and animals pre-engrafted with PDX tissues. Such PDX tissues are generally cryopreserved and engraftment-verified and may be characterized in terms of genetics and molecular makeup. Medical centers and hospitals associated with cancer research centers offer a unique advantage to researchers whereby they can obtain freshly isolated tumor biopsies from patients for direct transplantation into the animal.

Predicting Clinical Outcomes with PDX Models

With the increased interest in PDX models in the mouse, researchers have been building a portfolio of how accurately the PDX model mimics the patient's tumor, especially with regard to treatment outcomes. For example, one group created PDX lines by transplanting 49 fresh primary tumors from breast cancer patients into NOD-SCID mice

[27]. Although only 37% of the samples formed tumors, 12 PDX lines from ten different patients were maintained for several rounds of serial transplantation. These tumors represented a variety of breast cancer genetic backgrounds. Their data suggested that the xenografts accurately modeled tumor pathology and molecular characteristics of the original tumors. In addition, xenograft survival was found to correlate with patient prognosis. For example, tumors that were not successfully engrafted, or grew poorly, were derived from patients whose prognosis was positive. On the contrary, well-growing xenografts correlated with aggressive disease outcomes in patients. Many similar studies have been published for other types of cancer indicating that the PDXs reflected disease progression, therapy response, and disease outcomes of the patients. These include non-small cell lung cancer [23, 28], ovarian cancer [29], colorectal cancer [30], renal cell carcinoma [31], pancreatic cancer [32], and others as reviewed by Malaney et al. [5]. Tentler et al. have also systematically reviewed PDX models in the mouse and how treatment outcomes reflect what is seen in the patient [25].

Caveats to PDX Modeling

While PDX modeling in animals holds great promise, the one inadequacy of PDXs is that, on serial transplantation, the human stroma, vasculature, and the entire tumor microenvironment is replaced by host tissues [33]. The tumor microenvironment and tumor–stroma interactions and cross talk then do not completely recapitulate what is seen in the patient. Orthotopic transplants can more accurately reflect tumor–stroma interactions due to similarities of the host tissue to the site of origin of the patient tumor. However, rodent–human cell–cell interactions still do not fully recapitulate tumor–stroma interactions seen in the human patient.

Recent advancements in rodent humanization, where various tissues are reconstituted by human cells, could provide an even more advanced model to study cross talk between the tumor, the microenvironment, and immune system.

Mouse Humanization for Better Host–Patient Tumor Interactions

As mentioned previously, the stroma and vasculature supporting human tumor xenografts in well-established rodent models are derived from host cells. This is also true for host immune cells that invade the tumor. Thus, the tumor microenvironment in a xenograft rodent model does not fully recapitulate what is observed in the patient. Humanizing the immune system of the host has faced many challenges despite the variety of available immunodeficient mouse strains, especially with respect to proper lymphocyte development and function and cytokine production [34]. The development of the NSG mouse and other similarly immunodeficient mice has provided a platform for humanizing the immune system with relatively high efficiency compared to less immunodeficient models that still harbor NK cells and functional macrophages. Growing human cancer xenografts in an immune-humanized rodent is particularly appealing in view of the complex cross talk between the tumor and the immune components of the

stroma [33, 35]. In addition, the immune system plays a role in the body's fight against cancer and, thus, may affect chemotherapeutic efficacy. It is hypothesized that one of the reasons human cancer xenografts in the rodent do not faithfully predict therapeutic efficacy is because the immune component of the stroma is derived from the host, and not of human origin [36, 37]. Therefore, a humanized rodent could better predict therapeutic outcomes. Furthermore, a humanized immune system would allow for better understanding of the mechanism behind immune-mediated antitumor activity. One could also imagine that with a humanized immune system, the rodent would provide a platform for studying cancer immunotherapy, such as that being developed with chimeric antigen receptor T cells (CAR-T) [38, 39].

Rat Models of Human Cancer

The Nude Rat

Until recently, one of the only rat strains that could accept human xenografts was the RNU/nude rat (NIH-*Foxn1*^{rnu}). This athymic rat is devoid of mature T cells but still contains B and NK cells and macrophages [40, 41], limiting its capacity for engraftment and growth of human cell types. While the nude rat has been useful for studying several commercially available cell lines, the number of different cell types that have been successfully grown in the nude rat is far less than that achieved with the NSG mouse. In addition, there are no reports of the nude rat successfully being used to grow and expand PDX tissues.

Genetically Modifying the Rat

One key feature that allows the mouse to be amenable to human xenograft transplantation is the plethora of immunodeficient strains. Several different models exist with different immunophenotypes that lead to varying efficiencies of growing human cells. The mouse has had a clear advantage over the rat with regard to genetic modifications. The first genetically modified mouse was created by inserting DNA into the embryo in 1974 [42]. Since then, hundreds of different genes have been deleted, mutated, and inserted using embryo transgenesis or mouse pluripotent stem cells. Until recently, genetically modifying the rat has faced many challenges that have previously been overcome in the mouse. Despite the ability to isolate bona fide rat embryonic stem cells (ESCs) [43, 44], few groups have demonstrated gene modification or germline transmission using such cells, aside from Transposagen Biopharmaceuticals, Inc., who has successfully created transgenic rats using rat ESCs modified with the piggyBac transposon system. However, prior to the isolation of ESCs, the only methods that could be used for gene modification in the rat were through random mutagenesis, using the chemical ENU or through somatic cell nuclear transfer, also known as cloning, which is highly inefficient.

In 2009, a new technology using zinc finger nucleases was developed for targeted gene disruption. Since then, similar and more efficient technologies, such as TALENs and the CRISPR/Cas9 system, have emerged, paving the way for creating knockout and knock-in rats to complement the mouse models. Furthermore, the use of transposable elements, such as the sleeping beauty transposon and piggyBac transposon [45, 46], has enhanced the efficiency of modifying the rat genome.

Using TALENs and the CRISPR/Cas9 system, Transposagen Biopharmaceuticals, Inc., has pioneered the creation of genetically modified rats. In their quest to transform rat genetics, they created two immunodeficient rat models. Using TALENs (XTN™), Transposagen targeted the Rag2 locus in spermatogonial stem cells (SSCs). The SSCs offer a distinct advantage of generating founder animals in the first generation, expediting the process of creating a knockout animal [47–49]. Transduced SSCs are transplanted into sterile DAZL-deficient male rats, who can then pass on gene mutations directly to offspring when mated. The Rag2-targeted SSCs gave rise to a rat with an in-frame deletion resulting in an immunodeficient phenotype.

In addition to the single Rag2 knockout, Transposagen created a Rag2/Il2rg double knockout rat using the CRISPR/Cas9 system to target the genes in the rat embryo. This system is efficient in that the plasmids to target both genes were injected into the embryos simultaneously, resulting in a founder with out-of-frame deletions in both genes.

Hera BioLabs, Inc., a spin-off contract research organization from Transposagen, has licensed the Rag2 and Rag2/Il2rg knockout rats for commercial services. Hera has characterized the immunophenotype of both knockout rats and is qualifying them for their ability to accept human cancers, PDX tissues, as well as other non-cancer human cell types. The Rag2 knockout rats lack T and B cells but have an increased population of NK cells compared to wild type. The Il2rg single knockout results in significantly reduced numbers of NK cells, such that the combined knockout of Rag2 and Il2rg results in a loss of B, T, and NK cells, similar to the immunophenotype of the Rag2/Il2rg double knockout mouse.

Transposagen is only one of several groups that have created immunodeficient rats. Horizon Discovery markets SAGE® rodent research models which include several immunodeficient rats such as the Rag1 knockout, the Rag2 knockout, and the Prkdc knockout rat, which is similar to the *Prkdc^{scid}* knockout mouse. There are also academic groups that have created their own Rag1 knockout rats [50], *Prkdc^{scid}* rats [51], the Il2rg single knockout rat [52, 53], and a rat that lacks both the *Prkdc* and *Il2rg* genes, referred to as the FSG (F344-*scid* gamma) rat [51]. These immunodeficient rats provide the possibility of humanizing organs and tissues in the rat as well as modeling human cancers.

Human Cancer Xenografts in the Rat

The first reports of human cancer cell line engraftment in the Nude rat suggested that cancer lines that could be engrafted and serially transplanted in the Nude mouse could also be engrafted and grown in the Nude rat [54, 55].

However, the efficiency of engraftment into the Nude rat was found to decrease with age, and there was evidence of tumor regression over time. Although the Nude rat offers several advantages over the Nude mouse, including larger tumor size, severely immunodeficient mouse strains are more widely used due to the higher efficiency of tumor engraftment.

The advances in genetic engineering of the rat and the generation of rats with a higher degree of immunodeficiency than the Nude rat offer the possibility of engrafting a wider range of human cancers and possibly human PDX tissues. Hera BioLabs has performed several studies to determine engraftment potential of different human cancer cell lines in the Rag2 single knockout rat. In the first pilot study, a small cohort of Rag2 null rats was injected intravenously with the human acute lymphocytic leukemia (ALL) cell line, REH. Although the leukemia cells were only detected at very low levels in the peripheral blood (<1%), the cells formed tumors in the lungs and lymph nodes of the recipients. A spinal cord tumor consisting of human cells was also found in one animal.

A human glioblastoma cell line, U87MG-FLuc, was transplanted subcutaneously in the Rag2 knockout rat. All six animals transplanted with the cells, but no heterozygous animals, formed tumors. The tumors grew aggressively, and all tumors reached humane endpoint limits within 45 days of transplantation. Images of tumors found in recipients are shown in Fig. 4.2a. Immunohistochemical analysis of the tumors using an antibody that specifically recognizes a protein found in the mitochondria of all human cells, but not in the cells of other species, demonstrated that these tumors were comprised of human cells (Fig. 4.2b, c).

Three more human leukemia cell lines (KOPN-8, MOLM-13, and MV-4-11) in a third study were transplanted intravenously into the Rag2 knockout rat. Preliminary results suggest that these cells can be found at low levels (<1%) in the peripheral blood of recipients as early as 4 weeks posttransplantation. Similar to the first pilot study with the REH acute lymphocytic leukemia cell line, these leukemia cell lines also formed tumors in the lymph nodes and spinal cord of recipients. Although the mechanism underlying solid tumor formation from human leukemia cell lines is unknown, it is speculated that these cells cannot successfully be engrafted into the bone marrow because the animal is not sufficiently immunodeficient. Supporting this are data suggesting that even the NOD/SCID and NSG mouse strains must undergo irradiation in order for human

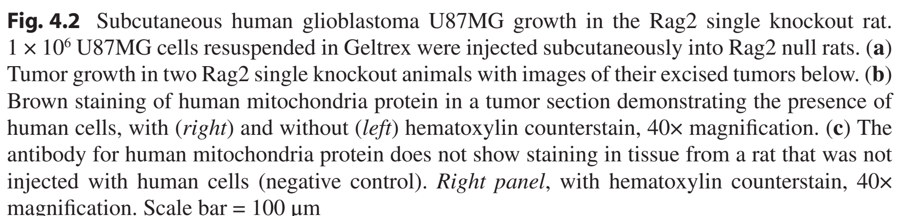
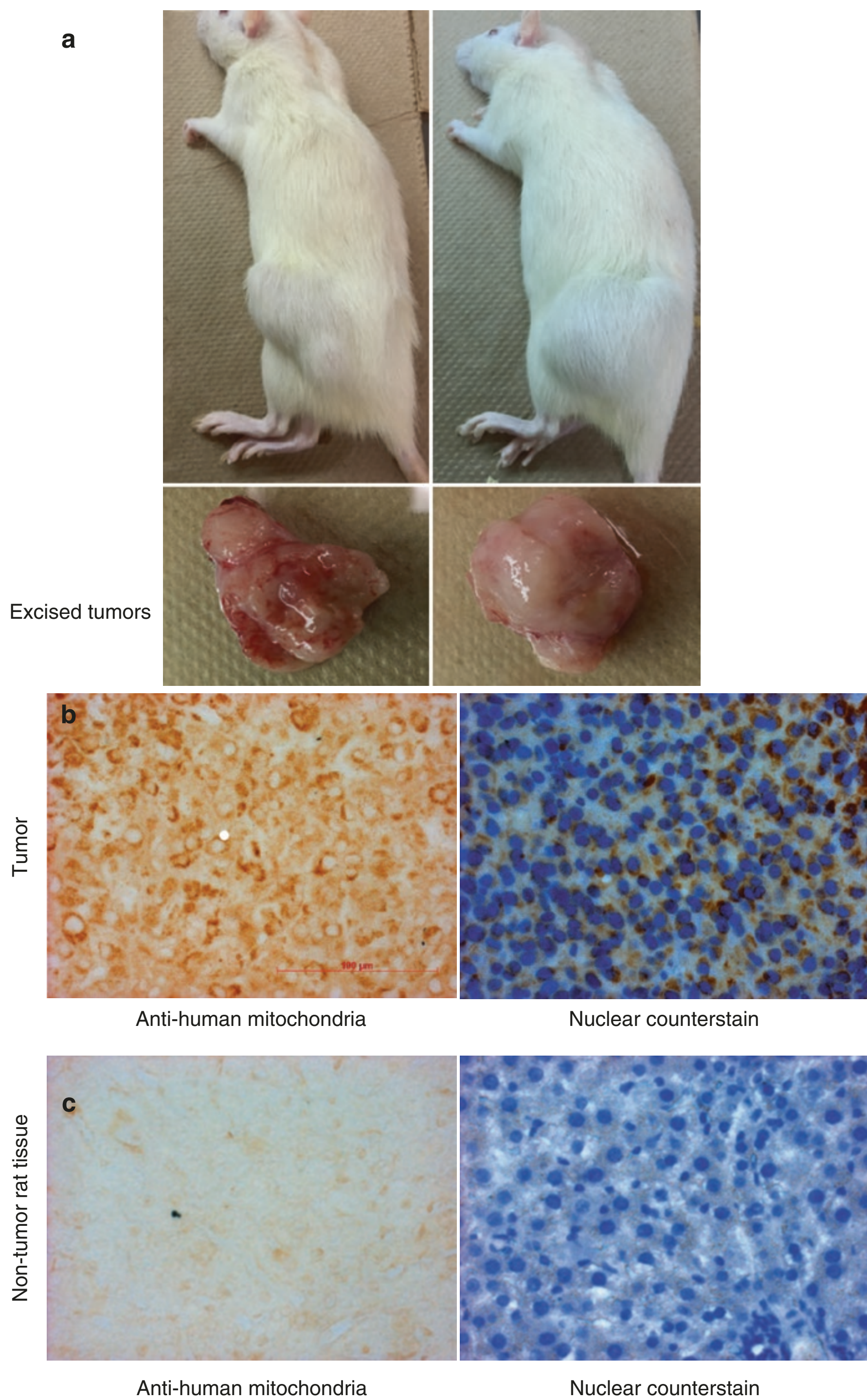


Fig. 4.2 Subcutaneous human glioblastoma U87MG growth in the Rag2 single knockout rat. 1×10^6 U87MG cells resuspended in Geltrex were injected subcutaneously into Rag2 null rats. (**a**) Tumor growth in two Rag2 single knockout animals with images of their excised tumors below. (**b**) Brown staining of human mitochondria protein in a tumor section demonstrating the presence of human cells, with (*right*) and without (*left*) hematoxylin counterstain, 40× magnification. (**c**) The antibody for human mitochondria protein does not show staining in tissue from a rat that was not injected with human cells (negative control). *Right panel*, with hematoxylin counterstain, 40× magnification. Scale bar = 100 μm


a
Excised tumors
b
Tumor
Anti-human mitochondria
Nuclear counterstain
c
Non-tumor rat tissue
Anti-human mitochondria
Nuclear counterstain

leukemia to be engrafted into the bone marrow [56, 57]. Studies are underway to determine the engraftment potential of these cell lines in the Rag2/Il2rg double knockout rat to determine if the absence of NK cells enhances engraftment of these cell lines and others. In addition, experiments are being performed to implant PDX tissues obtained from cryopreserved banks as well as freshly isolated tumor tissue from cancer patients, into the Rag2 single knockout and the Rag2/Il2rg double knockout rats.

The Il2rg single knockout rat, the *Prkdc^{scid}* rat, and the FSG (F344-*scid* gamma) rat are all capable of forming tumors following implantation of human ovarian cancer cell lines [25, 50]; human cancer xenograft efficiencies have not been determined in the Rag1 knockout rat [8]. These models have yet to be characterized for their ability to support the growth of other human cancer cell lines, including those that grow well and those that grow poorly in the mouse, as well as PDX tissues.

Prospects for Human PDX Modeling in the Rat

Preliminary studies suggest that the single Rag2 knockout rat shows promise as a recipient for human xenografts. It has been predicted that the Rag2/Il2rg double knockout rat, because of its lack of NK cells, will more efficiently support the growth of human xenografts and potentially accept a wider range of human cancers. These two models can provide researchers with novel tools with which to study tumor biology and drug efficacy with several advantages over the current mouse models, including relative ease of surgical manipulation, larger tumor size, and larger blood volumes for analysis. Since the rat is the preferred model for toxicology research, rat–human xenograft models could be appealing for downstream drug testing. Furthermore, if we can humanize other host tissues, such as the rat immune system, these humanized rats engrafted with human cancers will provide a novel model for studying host–tumor interactions and the role of the immune system in cancer treatments. Further genetic modification may be necessary to create a rat that phenotypically more closely mimics the NSG mouse to be able to humanize the immune system and engraft the widest range of human cancer cell lines and PDX tissues. Studies are underway to create such an “NSG” rat.

References

1. Cancer Types. National Cancer Institute. https://www.cancer.gov/types. Accessed 21 Nov 2016.
2. Katt ME, Placone AL, Wong AD, Xu ZS, Searson PC. In vitro tumor models: advantages, disadvantages, variables, and selecting the right platform. Front Bioeng Biotechnol. 2016;4:12. doi:10.3389/fbioe.2016.00012.
3. Xu X, Farach-Carson MC, Jia X. Three-dimensional in vitro tumor models for cancer research and drug evaluation. Biotechnol Adv. 2014;32(7):1256–68. doi:10.1016/j.biotechadv.2014.07.009.

4. Zanoni M, Piccinini F, Arienti C, Zamagni A, Santi S, Polico R, et al. 3D tumor spheroid models for in vitro therapeutic screening: a systematic approach to enhance the biological relevance of data obtained. Sci Rep. 2016;6:19103. doi:10.1038/srep19103.
5. Malaney P, Nicosia SV, Dave V. One mouse, one patient paradigm: new avatars of personalized cancer therapy. Cancer Lett. 2014;344(1):1–12. doi:10.1016/j.canlet.2013.10.010.
6. Shultz LD, Lyons BL, Burzenski LM, Gott B, Chen X, Chaleff S, et al. Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. J Immunol. 2005;174(10):6477–89.
7. Strowig T, Rongvaux A, Rathinam C, Takizawa H, Borsotti C, Philbrick W, et al. Transgenic expression of human signal regulatory protein alpha in Rag2-/-gamma(c)-/- mice improves engraftment of human hematopoietic cells in humanized mice. Proc Natl Acad Sci U S A. 2011;108(32):13218–23. doi:10.1073/pnas.1109769108.
8. Takenaka K, Prasolava TK, Wang JC, Mortin-Toth SM, Khalouei S, Gan OI, et al. Polymorphism in Sirpa modulates engraftment of human hematopoietic stem cells. Nat Immunol. 2007;8(12):1313–23. doi:10.1038/ni1527.
9. Theocharides AP, Rongvaux A, Fritsch K, Flavell RA, Manz MG. Humanized hemato-lymphoid system mice. Haematologica. 2016;101(1):5–19. doi:10.3324/haematol.2014.115212.
10. Waern JM, Yuan Q, Rudrich U, Becker PD, Schulze K, Strick-Marchand H, et al. Ectopic expression of murine CD47 minimizes macrophage rejection of human hepatocyte xenografts in immunodeficient mice. Hepatology. 2012;56(4):1479–88. doi:10.1002/hep.25816.
11. Ito M, Hiramatsu H, Kobayashi K, Suzue K, Kawahata M, Hioki K, et al. NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. Blood. 2002;100(9):3175–82.
12. Flanagan SP. 'Nude', a new hairless gene with pleiotropic effects in the mouse. Genet Res. 1966;8(3):295–309.
13. Falk I, Potocnik AJ, Barthlott T, Levelt CN, Eichmann K. Immature T cells in peripheral lymphoid organs of recombinase-activating gene-1/-2-deficient mice. Thymus dependence and responsiveness to anti-CD3 epsilon antibody. J Immunol. 1996;156(4):1362–8.
14. Mombaerts P, Iacomini J, Johnson RS, Herrup K, Tonegawa S, Papaioannou VE. RAG-1-deficient mice have no mature B and T lymphocytes. Cell. 1992;68(5):869–77.
15. Shinkai Y, Rathbun G, Lam KP, Oltz EM, Stewart V, Mendelsohn M, et al. RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. Cell. 1992;68(5):855–67.
16. Nonoyama S, Smith FO, Bernstein ID, Ochs HD. Strain-dependent leakiness of mice with severe combined immune deficiency. J Immunol. 1993;150(9):3817–24.
17. Richmond A, Su Y. Mouse xenograft models vs GEM models for human cancer therapeutics. Dis Model Mech. 2008;1(2–3):78–82. doi:10.1242/dmm.000976.
18. Hutchinson L, Kirk R. High drug attrition rates—where are we going wrong? Nat Rev Clin Oncol. 2011;8(4):189–90. doi:10.1038/nrclinonc.2011.34.
19. Morton CL, Houghton PJ. Establishment of human tumor xenografts in immunodeficient mice. Nat Protoc. 2007;2(2):247–50. doi:10.1038/nprot.2007.25.
20. Jin K, Teng L, Shen Y, He K, Xu Z, Li G. Patient-derived human tumour tissue xenografts in immunodeficient mice: a systematic review. Clin Transl Oncol. 2010;12(7):473–80. doi:10.1007/s12094-010-0540-6.
21. Cutz JC, Guan J, Bayani J, Yoshimoto M, Xue H, Sutcliffe M, et al. Establishment in severe combined immunodeficiency mice of subrenal capsule xenografts and transplantable tumor lines from a variety of primary human lung cancers: potential models for studying tumor progression-related changes. Clin Cancer Res. 2006;12(13):4043–54. doi:10.1158/1078-0432.CCR-06-0252.
22. Aparicio S, Hidalgo M, Kung AL. Examining the utility of patient-derived xenograft mouse models. Nat Rev Cancer. 2015;15(5):311–6. doi:10.1038/nrc3944.
23. Dong X, Guan J, English JC, Flint J, Yee J, Evans K, et al. Patient-derived first generation xenografts of non-small cell lung cancers: promising tools for predicting drug responses for personalized chemotherapy. Clin Cancer Res. 2010;16(5):1442–51. doi:10.1158/1078-0432.CCR-09-2878.

24. Hidalgo M, Amant F, Biankin AV, Budinska E, Byrne AT, Caldas C, et al. Patient-derived xenograft models: an emerging platform for translational cancer research. Cancer Discov. 2014;4(9):998–1013. doi:10.1158/2159-8290.CD-14-0001.
25. Tentler JJ, Tan AC, Weekes CD, Jimeno A, Leong S, Pitts TM, et al. Patient-derived tumour xenografts as models for oncology drug development. Nat Rev Clin Oncol. 2012;9(6):338–50. doi:10.1038/nrclinonc.2012.61.
26. Lee JY, Kim SY, Park C, Kim NK, Jang J, Park K, et al. Patient-derived cell models as preclinical tools for genome-directed targeted therapy. Oncotarget. 2015;6(28):25619–30. doi:10.18632/oncotarget.4627.
27. DeRose YS, Wang G, Lin YC, Bernard PS, Buys SS, Ebbert MT, et al. Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. Nat Med. 2011;17(11):1514–20. doi:10.1038/nm.2454.
28. Fichtner I, Rolff J, Soong R, Hoffmann J, Hammer S, Sommer A, et al. Establishment of patient-derived non-small cell lung cancer xenografts as models for the identification of predictive biomarkers. Clin Cancer Res. 2008;14(20):6456–68. doi:10.1158/1078-0432.CCR-08-0138.
29. Bankert RB, Balu-Iyer SV, Odunsi K, Shultz LD, Kelleher Jr RJ, Barnas JL, et al. Humanized mouse model of ovarian cancer recapitulates patient solid tumor progression, ascites formation, and metastasis. PLoS One. 2011;6(9):e24420. doi:10.1371/journal.pone.0024420.
30. Krumbach R, Schuler J, Hofmann M, Giesemann T, Fiebig HH, Beckers T. Primary resistance to cetuximab in a panel of patient-derived tumour xenograft models: activation of MET as one mechanism for drug resistance. Eur J Cancer. 2011;47(8):1231–43. doi:10.1016/j.ejca.2010.12.019.
31. Karam JA, Zhang XY, Tamboli P, Margulis V, Wang H, Abel EJ, et al. Development and characterization of clinically relevant tumor models from patients with renal cell carcinoma. Eur Urol. 2011;59(4):619–28. doi:10.1016/j.eururo.2010.11.043.
32. Laheru D, Shah P, Rajeshkumar NV, McAllister F, Taylor G, Goldsweig H, et al. Integrated preclinical and clinical development of S-trans, trans-Farnesylthiosalicylic Acid (FTS, Salirasib) in pancreatic cancer. Investig New Drugs. 2012;30(6):2391–9. doi:10.1007/s10637-012-9818-6.
33. Morton JJ, Bird G, Refaeli Y, Jimeno A. Humanized mouse xenograft models: narrowing the tumor-microenvironment gap. Cancer Res. 2016;76(21):6153–8. doi:10.1158/0008-5472.CAN-16-1260.
34. Shultz LD, Brehm MA, Garcia-Martinez JV, Greiner DL. Humanized mice for immune system investigation: progress, promise and challenges. Nat Rev Immunol. 2012;12(11):786–98. doi:10.1038/nri3311.
35. Muenst S, Laubli H, Soysal SD, Zippelius A, Tzankov A, Hoeller S. The immune system and cancer evasion strategies: therapeutic concepts. J Intern Med. 2016;279(6):541–62. doi:10.1111/joim.12470.
36. McMillin DW, Negri JM, Mitsiades CS. The role of tumour-stromal interactions in modifying drug response: challenges and opportunities. Nat Rev Drug Discov. 2013;12(3):217–28. doi:10.1038/nrd3870.
37. Sun Y, Campisi J, Higano C, Beer TM, Porter P, Coleman I, et al. Treatment-induced damage to the tumor microenvironment promotes prostate cancer therapy resistance through WNT16B. Nat Med. 2012;18(9):1359–68. doi:10.1038/nm.2890.
38. Khalil DN, Smith EL, Brentjens RJ, Wolchok JD. The future of cancer treatment: immunomodulation, CARs and combination immunotherapy. Nat Rev Clin Oncol. 2016;13(6):394. doi:10.1038/nrclinonc.2016.65.
39. Vudattu NK, Waldron-Lynch F, Truman LA, Deng S, Preston-Hurlburt P, Torres R, et al. Humanized mice as a model for aberrant responses in human T cell immunotherapy. J Immunol. 2014;193(2):587–96. doi:10.4049/jimmunol.1302455.
40. Hougen HP. The athymic nude rat. Immunobiological characteristics with special reference to establishment of non-antigen-specific T-cell reactivity and induction of antigen-specific immunity. APMIS Suppl. 1991;21:1–39.

41. Hougen HP, Klausen B. Effects of homozygosity of the nude (rnu) gene in an inbred strain of rats: studies of lymphoid and non-lymphoid organs in different age groups of nude rats of LEW background at a stage in the gene transfer. Lab Anim. 1984;18(1):7–14.
42. Jaenisch R, Mintz B. Simian virus 40 DNA sequences in DNA of healthy adult mice derived from preimplantation blastocysts injected with viral DNA. Proc Natl Acad Sci U S A. 1974;71(4):1250–4.
43. Buehr M, Meek S, Blair K, Yang J, Ure J, Silva J, et al. Capture of authentic embryonic stem cells from rat blastocysts. Cell. 2008;135(7):1287–98. doi:10.1016/j.cell.2008.12.007.
44. Li P, Tong C, Mehrian-Shai R, Jia L, Wu N, Yan Y, et al. Germline competent embryonic stem cells derived from rat blastocysts. Cell. 2008;135(7):1299–310. doi:10.1016/j.cell.2008.12.006.
45. Ivics Z, Izsvak Z, Medrano G, Chapman KM, Hamra FK. Sleeping beauty transposon mutagenesis in rat spermatogonial stem cells. Nat Protoc. 2011;6(10):1521–35. doi:10.1038/nprot.2011.378.
46. Izsvak Z, Frohlich J, Grabundzija I, Shirley JR, Powell HM, Chapman KM, et al. Generating knockout rats by transposon mutagenesis in spermatogonial stem cells. Nat Methods. 2010;7(6):443–5. doi:10.1038/nmeth.1461.
47. Chapman KM, Medrano GA, Jaichander P, Chaudhary J, Waits AE, Nobrega MA, et al. Targeted germline modifications in rats using CRISPR/Cas9 and spermatogonial stem cells. Cell Rep. 2015;10(11):1828–35. doi:10.1016/j.celrep.2015.02.040.
48. Hamra FK, Chapman KM, Nguyen DM, Williams-Stephens AA, Hammer RE, Garbers DL. Self-renewal, expansion, and transfection of rat spermatogonial stem cells in culture. Proc Natl Acad Sci U S A. 2005;102(48):17430–5. doi:10.1073/pnas.0508780102.
49. Hamra FK, Chapman KM, Wu Z, Garbers DL. Isolating highly pure rat spermatogonial stem cells in culture. Methods Mol Biol. 2008;450:163–79. doi:10.1007/978-1-60327-214-8_12.
50. Tsuchida T, Zheng YW, Zhang RR, Takebe T, Ueno Y, Sekine K, et al. The development of humanized liver with Rag1 knockout rats. Transplant Proc. 2014;46(4):1191–3. doi:10.1016/j.transproceed.2013.12.026.
51. Mashimo T, Takizawa A, Kobayashi J, Kunihiro Y, Yoshimi K, Ishida S, et al. Generation and characterization of severe combined immunodeficiency rats. Cell Rep. 2012;2(3):685–94. doi:10.1016/j.celrep.2012.08.009.
52. Kuijk EW, Rasmussen S, Blokzijl F, Huch M, Gehart H, Toonen P, et al. Generation and characterization of rat liver stem cell lines and their engraftment in a rat model of liver failure. Sci Rep. 2016;6:22154. doi:10.1038/srep22154.
53. Mashimo T, Takizawa A, Voigt B, Yoshimi K, Hiai H, Kuramoto T, et al. Generation of knockout rats with X-linked severe combined immunodeficiency (X-SCID) using zinc-finger nucleases. PLoS One. 2010;5(1):e8870. doi:10.1371/journal.pone.0008870.
54. Colston MJ, Fieldsteel AH, Dawson PJ. Growth and regression of human tumor cell lines in congenitally athymic (rnu/rnu) rats. J Natl Cancer Inst. 1981;66(5):843–8.
55. Maruo K, Ueyama Y, Kuwahara Y, Hioki K, Saito M, Nomura T, et al. Human tumour xenografts in athymic rats and their age dependence. Br J Cancer. 1982;45(5):786–9.
56. Lock RB, Liem N, Farnsworth ML, Milross CG, Xue C, Tajbakhsh M, et al. The nonobese diabetic/severe combined immunodeficient (NOD/SCID) mouse model of childhood acute lymphoblastic leukemia reveals intrinsic differences in biologic characteristics at diagnosis and relapse. Blood. 2002;99(11):4100–8.
57. Patel B, Dey A, Castleton AZ, Schwab C, Samuel E, Sivakumaran J, et al. Mouse xenograft modeling of human adult acute lymphoblastic leukemia provides mechanistic insights into adult LIC biology. Blood. 2014;124(1):96–105. doi:10.1182/blood-2014-01-549352.

Studies of Cancer Heterogeneity Using PDX Models

5

Danyi Wen, Feifei Zhang, and Yuan Long

Abbreviations

ABC	Activated B-cell
AI	Allelic imbalance
BCR	B-cell receptor
CAF	Cancer-associated fibroblasts
CCC	Consensus clustering classification
CNA	Copy number alterations
DLBCL	Diffuse large B-cell lymphoma
ECM	Extracellular matrix
EGFR	Epidermal growth factor receptor
HNSCC	Head and neck squamous cell carcinomas
JUND	Jun D proto-oncogene
LBCL	Large B-cell lymphoma
PDXs	Patient-derived xenografts
TAM	Tumor-associated macrophages
TGFBR3	Transforming growth factor b receptor 3
TNBCs	Triple negative breast cancers
T_{reg}	Regulatory T-cell

Introduction

Preclinical cancer models are of paramount importance in the development of anti-cancer drugs. Xenograft models based on cultured cancer cell lines have played a key role in this process. Thus, the NCI-60 cancer cell line panel has been used for over 25 years for anticancer drug screening. As valuable a resource as the cell lines

D. Wen, M.D., M.B.A. (✉) • F. Zhang • Y. Long
Shanghai LIDE Biotech, Co. Ltd., Pudong, Shanghai 201203, People's Republic of China
e-mail: Danyi.wen@lidebiotech.com

© Springer International Publishing AG 2017
Y. Wang et al. (eds.), *Patient-Derived Xenograft Models of Human Cancer*,
Molecular and Translational Medicine, DOI 10.1007/978-3-319-55825-7_5

have been, their grafts have significant limitations, such as lack of predictive power in drug efficacy tests as they fail to mimic the enormous complexity of human cancers, including their tumor heterogeneity and tissue architecture. It is also not precisely known how these cell lines differ genomically and functionally from the primary cancer cells from which they were derived. Moreover, they lack clinical information, such as treatments administered, patient outcome, response to therapy and stage of disease at diagnosis, etc. To overcome such limitations, transplantable patient-derived xenografts (PDXs) have been generated in the last decade by engrafting fresh human tumor *tissue* into immunodeficient mice. These PDXs more closely mimic the patients' tumors with regard to tumor microenvironment, retaining interactions between cancer cells and stromal cells. They exhibit a high degree of genomic stability in comparison to their parental tumors, even after serial transplantation of tumor sections. As well, a PDX model significantly retains the heterogeneity of the tumor from which it is derived.

In this chapter, we review genomic, transcriptomic, and proteomic heterogeneities of PDX models, compared to parental tissues, in the light of current advances in research and understanding.

Genomic and Proteomic Heterogeneity of PDX Models

Intra-tumor heterogeneity is thought to stem from two factors: (1) cell autonomous, including genomic, transcriptomic, and proteomic heterogeneity and (2) non-cell autonomous, for example, stromal heterogeneity. Tumor heterogeneity has clinical implications for patient-specific responses to therapy and resistance to targeted therapy [1]. PDX models are capable of retaining tumor heterogeneity, so these models have clear advantages over traditional cell line-based models and are becoming the preferred tools in drug discovery and preclinical studies [2, 3].

Gaub et al. established seven PDX models in nude mice for human colonic tumors (from stages B1 to D) in order to study correlations between initial tumors and PDX models. They used allelotyping analysis to test 45 loci (on 18 chromosomes) on the seven original tumors and their sequential PDXs and scored retention of the genetic alterations present in the original tumors after xenografting. The original tumors showed chromosome profile instability between fragments of the same tumor in an allelic imbalance (AI) assay. After the xenografting, all the AIs were maintained in PDX models compared to the original tumors, and the maintenance of the genetic profiles of the tumors could be observed even after serial transplantation for up to 14 passages. These results proved that intra-tumor clonal heterogeneity was conserved in the PDX models of the seven colonic tumors [4].

In Landen et al.'s research, it was demonstrated that PDX models of ovarian cancer can also recapitulate the original tumor's heterogeneity. They examined oncogenic expression, proliferation, and response to chemotherapy and found that xenografts recapitulated the heterogeneity of tumor-initiating cells in the original patient tumor, although the stromal component was murine. The PDX models had similar oncogene expressions as the original tumor and responded to chemotherapy

in a similar manner as the patients from which the original tumors had been harvested [5].

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous B-cell cancer defined by signaling and survival pathways, multiple genetic alterations, and transcriptional classifications. Rodig et al. generated nine large B-cell lymphoma (LBCL) PDX models, including eight DLBCL and one plasmablastic lymphoma. They used whole-exome sequencing to identify mutations and chromosomal alterations and whole-transcriptome sequencing to classify cells of origin and consensus clustering classification (CCC) subtypes. Six of the eight DLBCL models were activated B-cell (ABC)-type tumors and exhibited ABC-associated mutations such as CARD11, CD79B, MYD88, and PIM1. The other two DLBCL models were germinal B-cell type and showed alterations of CREBBP, EZH2, and GNA13 and chromosomal translocations involving IgH and either BCL2 or MYC. Six of the eight DLBCL PDX models were B-cell receptor (BCR)-type tumors identified by CCC criteria, and they exhibited BCR selective surface immunoglobulin expression. The reflection of the transcriptional, genetic, and immune-phenotypic heterogeneity of primary DLBCL in PDX models indicates that PDX models for DLBCL are effective and faithful as reported for solid tumors [6].

PDX models have been developed for a few malignancies, including colonic [4, 7], ovarian [5], pancreatic [8], and breast cancers [9, 10], non-small cell lung cancers [11], as well as large B-cell lymphoma [6] and medulloblastoma [12]. Although these PDX models were shown to closely recapitulate the histology and gene expression patterns of the primary tumors, some genomic, transcriptomic, or proteomic differences were also observed between the PDXs and the patients' tumors.

In Fang et al.'s study, a collection of PDX models for hepatocellular carcinoma (HCC) was established. These models recapitulated the complexity of the original tumors, including gene expression profiles, mutational status, and DNA copy number alterations (CNA), with few differences found. For example, of the gene expression profiles, genes related to DNA replication and cell cycling were upregulated in the PDXs. They compared 286 HCC patient samples with 42 HCC PDX models and found copy number gains in the following genes: *PBX1* (76.2%), *PRCC* (76.2%), *ARNT* (61.9%), *BCL9* (59.5%), *MTDH* (52.4%), *COX6C* (52.4%), *ABL2* (50%), *MET* (42.9%), *CCND1* (16.7%), *FGF19* (14.3%), and losses of *AFF1* (76%), *RAP1GDS1* (71%), *WRN* (71.4%), *PCM1* (71.4%), *WHSC1L1* (66.7%), *RB1* (59.5%), *BRCA2* (57.1%), *CDKN2A* (57.1%), *CDH1* (50%), *CDKN2B* (45.2%), *TSC2* (38.1%), *SMAD4* (33.3%), *APC* (28.6%), *STK11* (26.2%), *WT1* (23.8%), *MLH1* (21.4%), *TNFAIP3* (21.4%), *PTEN* (19.1%), *CDKN2C* (16.7%), *ARID1A* (14.3%), and *TNFRSF14* (11.9%). The results suggest that oncogenes were enriched during the xenografting [13].

Mardis et al. established a panel of PDX models for human basal-like breast cancer and analyzed four DNA samples for one patient to get genomic information on peripheral blood, primary tumor, brain metastasis, and the xenograft derived from the primary tumor. Compared with the primary tumor, the metastasis exhibited enrichment for 20 shared mutations, a large deletion not present in the primary tumor and two de novo mutations. The PDX models retained all primary tumor mutations as

expected, while the mutation enrichment pattern of the PDX highly resembled that of the metastasis. They identified 50 novel somatic point mutations and small indels (insertion/deletion). The wide range of mutant allele frequencies displayed genetic heterogeneity in the cell population of the primary tumor. The mutation frequency range narrowed in the brain metastasis and PDX, which may indicate that the metastatic and xenografting processes selected for cells carrying a distinct subset of the primary tumor mutation repertoire. The overlap between the mutation frequency both changed in the metastatic and xenograft samples suggested that cellular selection during xenografting was similar to that during metastasis [14].

Differences between original tumors and PDX models have also been observed in head and neck squamous cell carcinomas (HNSCC). Grandis JR et al. compared the protein expressions of PDXs with those of HNSCCs and found that, whereas the majority of proteins were similarly expressed, 64 proteins were differentially expressed in the PDXs: 30 proteins showing increased expression, whereas 34 showed reduced expression. There were only six proteins, i.e., AKT, c-Myc, PR, BCL2, c-Kit, and HSP70, with more than half of the PDX models outside the expression range of primary HNSCCs. AKT, c-Myc, and PR showed increased expression in PDXs, whereas the expressions of BCL2, c-Kit, and HSP70 were decreased. This protein expression panel indicates that proteins associated with cell proliferation may be preferentially selected during the development of the xenografts [15].

Differences between original tumors and PDX models have been reported for several types of cancer, such as breast [14, 16, 17], colonic [18], and liver cancer [13] and head and neck squamous cell carcinoma [15]. The differences may be explained by the following theories: (1) In response to stress-inducing events, specific cells which had preference expression patterns could survive more easily than others, (2) the xenografts evolve dynamically in order to adapt to growth in different hosts [17], (3) the replacement of the human stroma with mouse stromal cells after engraftment, and (4) loss of non-transformed epithelial cells.

PDX Models Retain Cell-Autonomous Heterogeneity

Human tumor heterogeneity creates a complex microenvironment that enables cell growth, development of therapy resistance, and metastasis [19, 20]. Cell lines cultured from cancer samples which were collected decades ago are still used in laboratories, yet pronounced differences in molecular profiles have been found between commonly used ovarian cancer cell lines and high-grade serous ovarian cancer samples [21]. In vitro cell cultures lack the stroma and mesenchymal elements present in human tumors to generate the paracrine production of growth factors and signaling pathways necessary to support tumor proliferation and metastasis formation [22–24]. Continuous subculturing of cells and passaging with enzyme treatment used for in vitro cell maintenance may be selecting a genetically and phenotypically uniform cancer cell subclone that flourishes in the plastic dish of the laboratory setting which, however, lacks the heterogeneous microenvironment seen in human tumors [25].

Because in vitro cell cultures lack heterogeneity, researchers have investigated alternative models that more closely resemble human tumors. Xenograft models, generated by engrafting established cell lines in mice, are widely used by researchers, but the functional utility [26] and the accuracy of such conventional xenografts lacking the donor tumor heterogeneity and tumor microenvironment [27] have been questioned. For decades, preclinical research in malignancies has largely relied upon cloned cancer-derived cell lines and tumor xenografts derived from these cell lines. However, the cell lines used for translational research have disadvantages, as genetic and phenotypic alterations from serial passaging have resulted in expression profiles that are different from those of the original patient tumors. Preclinical models, such as cell line-based xenograft models, often fail to retain the diverse heterogeneity of human tumors and hence lack clinical predictive power.

Intra-tumoral heterogeneity plays an important role in driving the extent of drug response and the development of therapy resistance. The existence of multiple subclones in human tumors explains variable response rates to therapy, even within a single tumor mass, and the rapid emergence of drug resistance. For example, the presence of a minor KRAS-mutant clone can predict which colorectal cancer patients will develop resistance to epidermal growth factor receptor (EGFR)-targeted therapy [28]. Curtis et al. showed that breast cancer had at least ten distinct molecular subtypes with significant differences in disease outcome and responses to therapy [29]. There is an association between clonal diversity and drug resistance for at least some tumor types—notably ovarian [30] and esophageal [31]. Basal-like triple negative breast cancers (TNBCs) have previously been linked to shorter disease-free survival when compared to non-basal-like triple negative breast cancers and tend to be associated with higher clonal diversity [32]. Clearly, although more work has to be done, it seems likely that the clonal composition of tumors will have potential use for predicting disease outcome and informing treatment choice.

The above implications suggest that we need to advance toward using highly characterized tumor models, representative of the large variability of tumors in humans. Next-generation sequencing and single-cell sequencing studies have identified multiple genetically distinct clonal variants within a single human tumor, demonstrating the level of heterogeneity that exists in human tumors [33, 34]. Therefore, the models we choose to study the development of therapeutic drug resistance need to reflect (1) genetic variation and (2) the tumor microenvironment. Both factors will affect the sensitivity or response and eventual resistance of a tumor to therapy. There are several heterogeneity factors in a developing tumor, such as the presence of distinct clonal variants in the original tumor population, tumor-initiating subpopulations, and cells carrying "mutator" phenotypes that allow a tumor to develop therapy resistance. The better we model all these aspects of intra-tumoral heterogeneity, the more likely we are to capture the dynamic nature of resistance. In order to create better models of human cancers, PDX models have been developed. The PDX models, derived from patient tumor tissues as distinct from cultured cell lines, have, by virtue of recapitulating as much of the human variation as possible, emerged as a powerful technology showing better representation of the heterogeneity of tumors, and part of the human tumor microenvironment, with preservation of

cellular complexity, genetics, vascular, and stromal tumor architecture. PDX cancer models are likely best suited for (1) studying the emergence of multiple resistance mechanisms, (2) guiding therapeutic strategies to overcome relapsed tumors, and (3) using drug efficacy tests in the discovery and preclinical development of superior anticancer agents.

To examine whether PDXs are able to show varying responses of patients' tumors to chemotherapy, the Shanghai LIDE Biotech Company designed a reverse validation trial with S-1, a drug combination used for therapy of gastric cancers. The trial was performed on four gastric cancer samples (GAPF155, GAPF157, GAPF161, and GAPF187) that had successfully been engrafted into mice. Xenografts were propagated and treatment cohorts of 16 mice were generated for each implanted cancer specimen. When tumors reached an average volume of 200 mm^3, mice were randomized to receive either a placebo or S-1. Consistent with the treatment response of patients to S-1, as shown in Fig. 5.1, GAPF155 and GAPF157 were sensitive to S-1, whereas GAPF161 and GAPF187 were not sensitive to S-1. These results indicate that PDXs can reflect variable responses of patients' tumors to therapy.

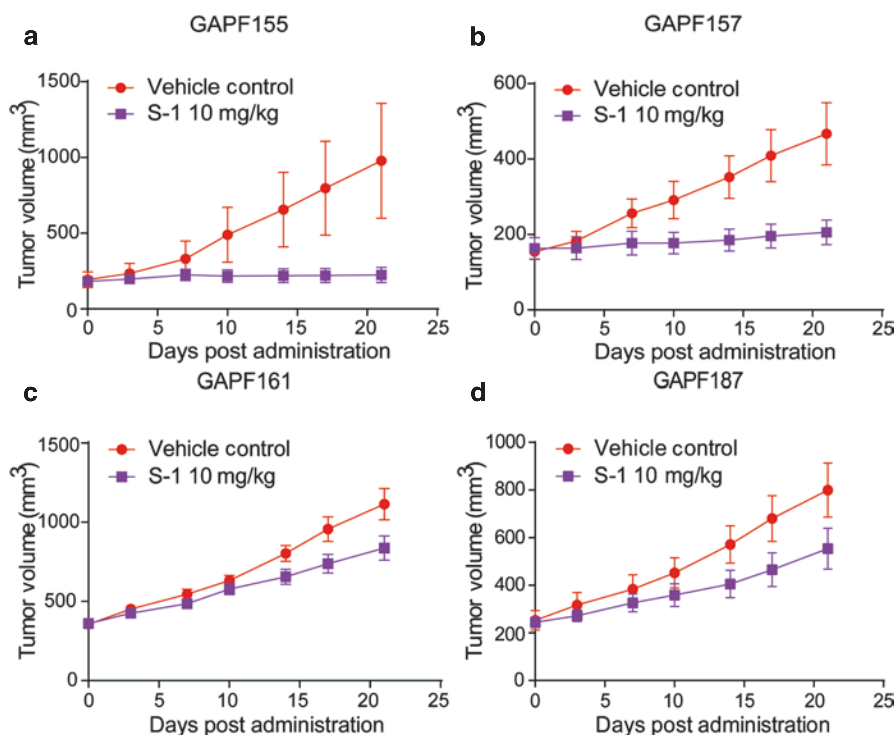


Fig. 5.1 Efficacy study of S-1 in four gastric PDX models. **a** and **b** show that GAPF155 and GAPF157 were sensitive to S-1; **c** and **d** show that GAPF161 and GAPF187 were not sensitive to S-1

PDX Models May Fail to Fully Account for Many Non-cell-Autonomous Drivers of Heterogeneity

PDXs are arguably the best models of tumor heterogeneity and therefore perhaps the most powerful tools for investigating tumor biology. Although PDX models maintain the genomic architecture, histology, and drug responsiveness of the original patients' tumors, the clonal profiles and tumor microenvironment of PDX tumors can change during their propagation in immunodeficient mice. Analysis of genome-wide variant allele frequencies in serial passages of PDX tumors showed that clonal selection occurs more frequently in initial engraftment steps than in propagation steps, but the detailed clonal dynamics differ depending on the various tumor samples of the same tumor type [35]. The clonal dynamics in PDX tumors is probably generated by selection acting on preexisting clones rather than by generation of new clones [36]. As a result, it is probable that the more aggressive clones become dominant in PDX tumors, and, in some cases, PDX models indeed showed the genomic and transcriptomic signature of metastatic and relapsed cancers [37]. These aggressive clones could be particularly important targets in cancer therapy.

As well, stromal and immune interactions in PDXs may be altered by cellular component deficiencies and interspecies compatibility in host models. However, the tumor microenvironment has long been known to play an essential role in tumor progression, and its role in drug response is becoming apparent [38, 39]. Aside from clonal dynamics driven by intrinsic differences in a cell's genetic or epigenetic background, intra-tumor heterogeneity can be influenced by tumor-extrinsic factors in the non-cell-autonomous compartment [40]. Cellular interactions with the extracellular matrix (ECM) can alter gene expression programs, drive differentiation, and profoundly alter cell behavior. As cancers develop, tight regulation of the ECM is lost and tissue architecture begins to degrade [38]. A study by Wang and colleagues [41] provides direct evidence that ECM-dependent signaling confers dynamic switching between transforming growth factor b receptor 3 (TGFBR3) and jun D proto-oncogene (JUND)-related expression signatures. ECM-driven oscillations between signaling pathways such as those described could have profound effects on propensity to malignancy. Furthermore, solid-state ECM interactions are necessary for cells to maintain stem cell properties, and regulated ECM helps maintain the stem cell niche [42]. In PDX models, Matrigel is often used to increase the engraftment efficiency; however, it is worth noting that this is a murine basement membrane extract, and suitable synthetic human alternatives are available. The presence of growth factors in Matrigel may favor the engraftment of one cell type over another. Finally, as ECM structure is tissue specific [42], researchers should consider the use of orthotopic transplantations where possible.

The tumor microenvironment is further characterized by an influx of stromal cells. Infiltrating cancer-associated fibroblasts (CAF) can often confer resistance to cytotoxic and targeted therapies [39]. Because of the high levels of CAF infiltrates seen in some tumor types, heterogeneity within their population would undoubtedly

confer differential properties to the tumor bulk. In PDX models, human stromal cells are gradually replaced by murine equivalents upon engraftment in the mouse, suggesting that implanted human cancer cells retain the ability to recruit murine accessory cells to their niche. However, it should be noted that some differences exist between ligand repertoires of human and murine fibroblasts. Clearly, stromal architecture and activity are mimicked in the murine host; however, it is currently unclear how this reflects human stroma with regard to supporting tumor growth and development.

The immune system also plays a crucial role in tumor progression, and perhaps it is the most obvious disadvantage in PDX models, because of engraftment into severely immune-deficient host animals. Tumor cells are broadly thought to be antigenic which emerge point mutations in coding exons in a developed tumor and result in a large repertoire of neoantigens. Targeting of these neoantigens can lead to significant $CD8^+$cytotoxic T-cell infiltration and tumor cell death. However, most tumors eventually progress and evade the immune system often through the dominant inhibitory effects of suppressive pathways (the so-called immune checkpoints such as CTLA-4/B7 and PD-1/PD-L1). This is supported by the prognostic value of the $CD8^+$ to $FOXP3^+$ (cytotoxic to regulatory T-cell, T_{reg}) ratio in many solid tumors and the recently reported clinical efficacy of a variety of checkpoint inhibitors [43, 44]. The proinflammatory microenvironment established by M1-polarized tumor-associated macrophages (TAM), $CD8^+$T-cells, NK cells, and others can lead to the recruitment of numerous immune-suppressive components. In addition, $CD4^+$T-cell and macrophage recruitment following intensive chemotherapy in breast cancer patients is associated with significantly reduced recurrence-free survival [44].

All in all, heterogeneity within a tumor is governed by both cell-autonomous (e.g., genetic and epigenetic heterogeneity) and non-cell-autonomous (e.g., stromal heterogeneity) drivers. Although PDXs can largely recapitulate the genomic architecture, histology, and drug responsiveness of human tumors, they may not fully account for heterogeneity in the tumor microenvironment. However, these models have substantial utility in basic and translational research in cancer biology, but study of stromal or immune drivers of tumor progression may be limited. Similarly, PDX models offer the ability to conduct in vivo and ex vivo patient-specific drug screens, but stromal contributions to treatment responses may be underrepresented.

References

1. Gao H, Korn JM, Ferretti S, Monahan JE, Wang Y, Singh M, et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nat Med. 2015;21(11):1318–25. doi:10.1038/nm.3954.
2. Sausville EA, Burger AM. Contributions of human tumor xenografts to anticancer drug development. Cancer Res. 2006;66(7):3351–3354. discussion 4. doi:10.1158/0008-5472.CAN-05-3627.
3. Siolas D, Hannon GJ. Patient-derived tumor xenografts: transforming clinical samples into mouse models. Cancer Res. 2013;73(17):5315–9. doi:10.1158/0008-5472.CAN-13-1069.

4. Guenot D, Guerin E, Aguillon-Romain S, Pencreach E, Schneider A, Neuville A, et al. Primary tumour genetic alterations and intra-tumoral heterogeneity are maintained in xenografts of human colon cancers showing chromosome instability. J Pathol. 2006;208(5):643–52. doi:10.1002/path.1936.
5. Dobbin ZC, Katre AA, Steg AD, Erickson BK, Shah MM, Alvarez RD, et al. Using heterogeneity of the patient-derived xenograft model to identify the chemoresistant population in ovarian cancer. Oncotarget. 2014;5(18):8750–64. doi:10.18632/oncotarget.2373.
6. Chapuy B, Cheng H, Watahiki A, Ducar MD, Tan Y, Chen L, et al. Diffuse large B-cell lymphoma patient-derived xenograft models capture the molecular and biological heterogeneity of the disease. Blood. 2016;127(18):2203–13. doi:10.1182/blood-2015-09-672352.
7. Seol HS, Kang HJ, Lee SI, Kim NE, Kim TI, Chun SM, et al. Development and characterization of a colon PDX model that reproduces drug responsiveness and the mutation profiles of its original tumor. Cancer Lett. 2014;345(1):56–64. doi:10.1016/j.canlet.2013.11.010.
8. Kim MP, Evans DB, Wang H, Abbruzzese JL, Fleming JB, Gallick GE. Generation of orthotopic and heterotopic human pancreatic cancer xenografts in immunodeficient mice. Nat Protoc. 2009;4(11):1670–80. doi:10.1038/nprot.2009.171.
9. Fleming JM, Miller TC, Meyer MJ, Ginsburg E, Vonderhaar BK. Local regulation of human breast xenograft models. J Cell Physiol. 2010;224(3):795–806. doi:10.1002/jcp.22190.
10. Petrillo LA, Wolf DM, Kapoun AM, Wang NJ, Barczak A, Xiao Y, et al. Xenografts faithfully recapitulate breast cancer-specific gene expression patterns of parent primary breast tumors. Breast Cancer Res Treat. 2012;135(3):913–22. doi:10.1007/s10549-012-2226-y.
11. Fichtner I, Rolff J, Soong R, Hoffmann J, Hammer S, Sommer A, et al. Establishment of patient-derived non-small cell lung cancer xenografts as models for the identification of predictive biomarkers. Clin Cancer Res. 2008;14(20):6456–68. doi:10.1158/1078-0432.CCR-08-0138.
12. Zhao X, Liu Z, Yu L, Zhang Y, Baxter P, Voicu H, et al. Global gene expression profiling confirms the molecular fidelity of primary tumor-based orthotopic xenograft mouse models of medulloblastoma. Neuro-Oncology. 2012;14(5):574–83. doi:10.1093/neuonc/nos061.
13. Gu Q, Zhang B, Sun H, Xu Q, Tan Y, Wang G, et al. Genomic characterization of a large panel of patient-derived hepatocellular carcinoma xenograft tumor models for preclinical development. Oncotarget. 2015;6(24):20160–76. doi:10.18632/oncotarget.3969.
14. Ding L, Ellis MJ, Li S, Larson DE, Chen K, Wallis JW, et al. Genome remodelling in a basal-like breast cancer metastasis and xenograft. Nature. 2010;464(7291):999–1005. doi:10.1038/nature08989.
15. Li H, Wheeler S, Park Y, Ju Z, Thomas SM, Fichera M, et al. Proteomic characterization of head and neck cancer patient-derived xenografts. Mol Cancer Res. 2016;14(3):278–86. doi:10.1158/1541-7786.MCR-15-0354.
16. Kabos P, Finlay-Schultz J, Li C, Kline E, Finlayson C, Wisell J, et al. Patient-derived luminal breast cancer xenografts retain hormone receptor heterogeneity and help define unique estrogen-dependent gene signatures. Breast Cancer Res Treat. 2012;135(2):415–32. doi:10.1007/s10549-012-2164-8.
17. Kanaya N, Somlo G, Wu J, Frankel P, Kai M, Liu X et al. Characterization of patient-derived tumor xenografts (PDXs) as models for estrogen receptor positive (ER+HER2− and ER+HER2+) breast cancers. J Steroid Biochem Mol Biol. 2016. doi:10.1016/j.jsbmb.2016.05.001.
18. Burgenske DM, Monsma DJ, Dylewski D, Scott SB, Sayfie AD, Kim DG, et al. Establishment of genetically diverse patient-derived xenografts of colorectal cancer. Am J Cancer Res. 2014;4(6):824–37.
19. Lengyel E. Ovarian cancer development and metastasis. Am J Pathol. 2010;177(3):1053–64. doi:10.2353/ajpath.2010.100105.
20. Lengyel E, Burdette JE, Kenny HA, Matei D, Pilrose J, Haluska P, et al. Epithelial ovarian cancer experimental models. Oncogene. 2014;33(28):3619–33. doi:10.1038/onc.2013.321.
21. Domcke S, Sinha R, Levine DA, Sander C, Schultz N. Evaluating cell lines as tumour models by comparison of genomic profiles. Nat Commun. 2013;4:2126. doi:10.1038/ncomms3126.

22. Rasmussen AA, Cullen KJ. Paracrine/autocrine regulation of breast cancer by the insulin-like growth factors. Breast Cancer Res Treat. 1998;47(3):219–33.
23. van Roozendaal CE, Gillis AJ, Klijn JG, van Ooijen B, Claassen CJ, Eggermont AM, et al. Loss of imprinting of IGF2 and not H19 in breast cancer, adjacent normal tissue and derived fibroblast cultures. FEBS Lett. 1998;437(1–2):107–11.
24. Vermeulen L, De Sousa EMF, van der Heijden M, Cameron K, de Jong JH, Borovski T, et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. Nat Cell Biol. 2010;12(5):468–76. doi:10.1038/ncb2048.
25. Williams SA, Anderson WC, Santaguida MT, Dylla SJ. Patient-derived xenografts, the cancer stem cell paradigm, and cancer pathobiology in the 21st century. Lab Invest. 2013;93(9):970–82. doi:10.1038/labinvest.2013.92.
26. Scott CL, Becker MA, Haluska P, Samimi G. Patient-derived xenograft models to improve targeted therapy in epithelial ovarian cancer treatment. Front Oncol. 2013;3:295. doi:10.3389/fonc.2013.00295.
27. Stordal B, Timms K, Farrelly A, Gallagher D, Busschots S, Renaud M, et al. BRCA1/2 mutation analysis in 41 ovarian cell lines reveals only one functionally deleterious BRCA1 mutation. Mol Oncol. 2013;7(3):567–79. doi:10.1016/j.molonc.2012.12.007.
28. Diaz Jr LA, Williams RT, Wu J, Kinde I, Hecht JR, Berlin J, et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature. 2012;486(7404):537–40. doi:10.1038/nature11219.
29. Curtis C, Shah SP, Chin SF, Turashvili G, Rueda OM, Dunning MJ, et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature. 2012;486(7403):346–52. doi:10.1038/nature10983.
30. Bashashati A, Ha G, Tone A, Ding J, Prentice LM, Roth A, et al. Distinct evolutionary trajectories of primary high-grade serous ovarian cancers revealed through spatial mutational profiling. J Pathol. 2013;231(1):21–34. doi:10.1002/path.4230.
31. Maley CC, Galipeau PC, Finley JC, Wongsurawat VJ, Li X, Sanchez CA, et al. Genetic clonal diversity predicts progression to esophageal adenocarcinoma. Nat Genet. 2006;38(4):468–73. doi:10.1038/ng1768.
32. Shah SP, Roth A, Goya R, Oloumi A, Ha G, Zhao Y, et al. The clonal and mutational evolution spectrum of primary triple-negative breast cancers. Nature. 2012;486(7403):395–9. doi:10.1038/nature10933.
33. Anderson K, Lutz C, van Delft FW, Bateman CM, Guo Y, Colman SM, et al. Genetic variegation of clonal architecture and propagating cells in leukaemia. Nature. 2011;469(7330):356–61. doi:10.1038/nature09650.
34. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012;366(10):883–92. doi:10.1056/NEJMoa1113205.
35. Eirew P, Steif A, Khattra J, Ha G, Yap D, Farahani H, et al. Dynamics of genomic clones in breast cancer patient xenografts at single-cell resolution. Nature. 2015;518(7539):422–6. doi:10.1038/nature13952.
36. Aparicio S, Hidalgo M, Kung AL. Examining the utility of patient-derived xenograft mouse models. Nat Rev Cancer. 2015;15(5):311–6. doi:10.1038/nrc3944.
37. Rosfjord E, Lucas J, Li G, Gerber HP. Advances in patient-derived tumor xenografts: from target identification to predicting clinical response rates in oncology. Biochem Pharmacol. 2014;91(2):135–43. doi:10.1016/j.bcp.2014.06.008.
38. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med. 2013;19(11):1423–37. doi:10.1038/nm.3394.
39. Straussman R, Morikawa T, Shee K, Barzily-Rokni M, Qian ZR, Du J, et al. Tumour micro-environment elicits innate resistance to RAF inhibitors through HGF secretion. Nature. 2012;487(7408):500–4. doi:10.1038/nature11183.
40. Michor F, Weaver VM. Understanding tissue context influences on intratumour heterogeneity. Nat Cell Biol. 2014;16(4):301–2. doi:10.1038/ncb2942.

41. Wang CC, Bajikar SS, Jamal L, Atkins KA, Janes KA. A time- and matrix-dependent TGFBR3-JUND-KRT5 regulatory circuit in single breast epithelial cells and basal-like premalignancies. Nat Cell Biol. 2014;16(4):345–56. doi:10.1038/ncb2930.
42. Cassidy JW. Nanotechnology in the regeneration of complex tissues. Bone Tissue Reg Insights. 2014;5:25–35. doi:10.4137/BTRI.S12331.
43. Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T, et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. Nature. 2014;515(7528):577–81. doi:10.1038/nature13988.
44. Schmidt M, Bohm D, von Torne C, Steiner E, Puhl A, Pilch H, et al. The humoral immune system has a key prognostic impact in node-negative breast cancer. Cancer Res. 2008;68(13):5405–13. doi:10.1158/0008-5472.CAN-07-5206.

6 The Plasticity of Stem-Like States in Patient-Derived Tumor Xenografts

Alastair H. Davies, Fraser Johnson, Kirsi Ketola, and Amina Zoubeidi

Abbreviations

CAF	Cancer-associated fibroblast
CDMs	Cell-derived matrices
CSC	Cancer stem-like cell
ECM	Extracellular matrix
EGF	Epidermal growth factor
FAK	Focal adhesion kinase
HDACis	Histone deacetylase inhibitors
HIFs	Hypoxia inducible factors
NK	Natural killer
PDX	Patient-derived xenograft
PI3K	Phosphoinositide 3-kinase
TME	Tumor microenvironment

Introduction

Despite remarkable advances in our understanding of the molecular events that underpin tumor survival and progression, the harsh reality is that about 85% of preclinical anticancer therapies fail in early clinical trials [1, 2]. One of the most often cited reasons for the disconnection between drug efficacy in conventional cancer

A.H. Davies • F. Johnson • K. Ketola • A. Zoubeidi, Ph.D. (✉)
Vancouver Prostate Centre, 2660 Oak Street, Vancouver, BC, V6H 3Z6, Canada

Department of Urologic Sciences, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada
e-mail: azoubeidi@prostatecentre.com

© Springer International Publishing AG 2017

Y. Wang et al. (eds.), *Patient-Derived Xenograft Models of Human Cancer*, Molecular and Translational Medicine, DOI 10.1007/978-3-319-55825-7_6

cell line models and clinical response is that preclinical models fail to mimic the complexity of human cancers [3–5]. Unlike in vitro cell cultures, which are largely homogeneous, cancer cells within individual tumors often exist in any of several distinct phenotypic states that differ in functional attributes. For example, subpopulations of cancer stem-like cells (CSCs) with increased tumor-initiating ability and drug resistance have been identified in many tumor types, including malignant germ cell cancers [6, 7], leukemias [8, 9], as well as solid tumors in the brain [10], breast [11], pancreas [12], and prostate [13]. This heterogeneity has important therapeutic implications because anticancer therapies preferentially target specific cancer cell states and, thus, can yield selective changes in phenotypic proportions within tumors [14–17].

The CSC state is governed by both cell-autonomous (e.g., genetic and epigenetic) and cell-extrinsic (e.g., stromal and immune) factors (Table 6.1). This complexity is further compounded by evidence of epigenetic shifts allowing tumor cell populations to reversibly transition between functional states, including between stem-like and more committed cells [18, 19]. In contrast to conventional cell line xenografts, patient-derived xenograft (PDX) cancer tissue models retain the molecular signatures and epigenetic architecture of the donor tumor, as well as recapitulate interactions of the host microenvironment [20]. Thus, PDX models are better suited to capture the interplay between genetics/epigenetics and microenvironment that dictates how cancer cell states coexist and evolve within tumors.

Table 6.1 Cell-autonomous and cell-extrinsic factors that regulate cell plasticity

	Factor	Implications on cell plasticity	Representation in PDX models
Cell-autonomous	Genetics	Genetic aberrations, such as *K-RAS* mutations, activate signal transduction cascades that can endow cells with tumor-initiating and self-renewal properties	Copy number alterations and exome sequencing data show concordance between PDX models and donor tumors
	Epigenetics	Epigenetic marks (e.g., DNA methylation and histone modifications) change dynamically as cells move between pluripotent and differentiated states	DNA methylation patterns are conserved between PDX and donor tumor

Table 6.1 (continued)

	Factor	Implications on cell plasticity	Representation in PDX models
Cell-extrinsic	ECM	ECM ligands and nanotopology modulate intracellular signaling pathways (e.g., Notch) that maintain the stem cell compartment	PDXs are implanted with murine basement membrane (Matrigel), but this may not fully mimic the architecture and content of human ECM
	Stromal Cells	CAFs secrete growth factors and cytokines (e.g., CCL5), which act in a paracrine fashion to activate stem cell-associated signaling pathways and induce dedifferentiation	PDXs in early passage retain stromal elements from the donor tumor. However, these are eventually replaced with mouse stroma. Orthotopic models should be prioritized as they better replicate the ECM of the donor TME
	Immune cells	Interplay between the immune system and tumor regulates cell state dynamics as stem-like cells exhibit immunosuppressive properties. Non-stem cells may dedifferentiate to escape immune surveillance	PDXs are often established in highly immunocompromised mice; however, immune cell aggregates and NK cell infiltration have been reported in less immunocompromised models (e.g., nude mice). Humanized PDX models are under development that harbor an intact immune system
	Hypoxic TME	HIF2α activates stem cell factors and signaling pathways that promote expansion of the stem cell pool under hypoxic conditions	PDX models maintain a hypoxic microenvironment
	Acidic TME	Low extracellular pH promotes a stem cell-like gene expression signature	An acidic microenvironment is commonly observed in PDX models

CAF cancer-associated fibroblast, *ECM* extracellular matrix, *NK* natural killer, *PDX* patient-derived xenograft, *TME* tumor microenvironment

The Cancer Stem Cell Paradigm

While the initial observation that cancer cells share similarities with embryonic cells was made as early as 1875 [21], it was not until the last decade that the cancer research community began to embrace the notion that tumors are hierarchically organized and maintained by a population of "cancer stem cells." However one frames the concept, most cancer biologists accept that there are subpopulations of cells within a tumor that exhibit self-renewal and tumor-initiating capability [22–24]. This framework is largely based on a series of elegant genetic tracing studies showing that cancers are comprised of a heterogeneous population of cells that differ in their capacity of self-renewal, metastatic ability, and resistance to radio- and chemotherapy [25–27].

It is important to acknowledge that the CSC hypothesis does not address whether cancer arises from normal stem cells. Rather it suggests that irrespective of the cell-of-origin, cancers are hierarchically organized in much the same manner as normal tissues. Just as normal stem cells differentiate into more committed progeny, CSCs can undergo genetic and/or epigenetic changes analogous to the differentiation of normal cells to form phenotypically diverse nontumorigenic cells that contribute to tumor heterogeneity.

In contrast to the rigid hierarchical model of tumors, evidence is emerging that tumor cell populations can reversibly shift between functional states. In other words, CSCs can arise de novo from non-stem cells. In a paradigm-shifting study, Gupta and colleagues identified that breast cancer cell populations can interconvert between phenotypic states [18]. This dynamic transition between non-CSC and CSC states was found to be regulated by contextual signals from the microenvironment, in particular TGFβ [28, 29], as well as certain stressors, such as nutrient deprivation and therapeutics [30].

Cell-Autonomous Regulation of Cancer Stem Cell Plasticity: Genomic and Epigenomic Features in PDX Models

PDX Models Preserve Genetic and Epigenetic Heterogeneity

From a histological and cell population standpoint, heterogeneity has long been observed in both normal and neoplastic tissues. Through the course of tumor evolution, cancer cells undergo repeated mutational events coupled with chaotic shifts in the epigenome that together may result in increased fitness relative to neighboring cells. In this concept, subclones arise in bursts of expansion in response to pressures from Darwinian selection—including those imposed by therapeutic intervention—and may be stable or transient [31–33]. Certainly, driver mutations such as those for *K-RAS* in a variety of tumor types [34], *APC* in colorectal cancer [35], and *VHL* in renal cancers [36] likely are the central forces that induce the appearance of cells with tumor-initiating and self-renewal properties. Similarly, epigenetic changes, e.g., promotor focal gains of DNA methylation, are also key factors that may be especially important for regulating cell plasticity and formation of stem-like cell populations in cancers [37].

Cell lines, and by extension cell line xenografts, undergo extensive evolutionary adaptation to grow indefinitely in artificial culture conditions and thus rarely recapitulate the biology of parental tumors when reimplanted [3, 5]. The rationale for developing PDX models for cancer research is based on the assumption that these models will faithfully recapitulate the heterogeneity of the donor tumor and that this characteristic will be maintained through successive mouse-to-mouse passages in vivo [38–42]. In general terms, this appears to be correct. Studies using basic morphological assessments do indeed show that PDX models share the same histological structure as the donor tumor, including fine tissue structure and subtle microscopic details, such as gland architecture, grade of differentiation, and relative abundance of tumor and stroma.

PDX Models Retain Genetic Characteristics of the Donor Tumor

Analysis of copy number alterations and exome sequencing data show extraordinary concordance between patient tumors and the PDX models derived from them. In a recent study, whole genome sequencing of several primary tumors and matched PDXs in breast cancer showed that PDXs have relatively stable genomes without a significant accumulation of DNA structural rearrangements [43]. Likewise, PDX models developed from *TMPRSS2-ERG*-positive prostate tumors expressed the *TMPRSS2-ERG* fusion gene and exhibited high levels of ERG protein [40]. Finally, analysis of gene expression profiles in 25 non-small cell lung cancer tumors showed that there are no substantive differences between the donor tumor and corresponding PDX, with only genes involved in the stromal compartment and immune function being less represented in the models [44]. This high degree of similarity extends to tumor-PDX pairs from many cancers as unsupervised clustering analysis shows that in most studies PDX models cluster with their counterpart primary tumor [40].

Epigenetics Impacts Cancer Cell Heterogeneity

Our appreciation of epigenetic complexity and plasticity has dramatically increased over the last decade largely due to the development of several global proteomic and genomic technologies. Briefly, the concept of epigenetic control refers to a stable change in gene expression without any changes in DNA sequence [45, 46]. Covalent modifications of DNA (such as methylation) and histones (such as methylation and acetylation) are dynamically laid down and removed by chromatin-modifying enzymes to facilitate active/permissive or condensed/closed chromatin states that, in turn, modulate transcription. In a holistic sense, epigenetic modifications serve to maintain stable patterns of transcription for the maintenance of cell phenotypes. Thus, histone modifications, in addition to DNA methylation, are vital to the control of cellular states.

The earliest indications of a link between epigenetics and cancer were derived from correlation studies between gene expression and DNA methylation patterns.

These studies are too numerous to review but have been comprehensively described elsewhere [47]. These early observations have been strengthened by recent research indicating that cancer cells exhibit focal gains in promoter DNA hypermethylation and associated transcriptional silencing. Interestingly, a large proportion of genes with this cancer-specific change are those with a history of chromatin regulation in adult stem cells [37], which has been further traced back to embryonic stem cells [48, 49]. Thus, epigenetic changes likely contribute functionally to the heterogeneity of tumor cell populations, maintaining the balance between self-renewal and commitment to differentiation.

PDX Models Retain Epigenomic Patterns of the Donor Tumor

To specifically assess the fidelity of the epigenome between PDXs and the original patient tumors, Guilhamon and colleagues performed methylome sequencing on matched PDX and primary tumor from osteosarcoma and colon cancer patients [50]. Notably, less than 3% of the methylome underwent major changes as a result of the xenografting procedure. In a series of separate studies, *IDH1*-mutant glioblastomas as well as the corresponding PDXs were found to exhibit a marked increase in the oncometabolite D-2-hydroxyglutarate, which inhibits various histone demethylases as well as the TET family of enzymes that catalyze DNA demethylation. Indeed, these tumors had an abnormal buildup of DNA methylation that, in turn, was linked to suppression of cellular differentiation. Treatment with DNA demethylating agents, such as 5-azacytidine, yielded a reduction in DNA methylation of promoter loci and induction of glial differentiation [51, 52]. Together, these studies suggest a prominent role for the epigenome in controlling phenotypic heterogeneity in patient tumors that is retained by PDX models.

It is important to note that PDX models exhibit a relatively stable methylome, with no significant methylation changes during mouse-to-mouse propagation [50]. This is in stark contrast to cell line models where differences in DNA methylation patterns have been reported between primary cultures and tissues [53]. Changes in DNA methylation will affect gene expression and, in turn, influence cellular processes and even responses to therapy. Therefore, PDXs may provide a more realistic model than cell line panels for deciphering the basic facets of the tumor epigenome as well as testing epigenetic therapies.

Cell-Extrinsic Regulation of Cancer Stem Cell Plasticity: The PDX Microenvironment

The Cancer Stem Cell Niche

The interplay between tumor cells and their microenvironment is strikingly complex and is accepted to be a key determinant of clonal evolution and therapeutic response. As is the case with normal stem cells, CSCs are believed to reside in

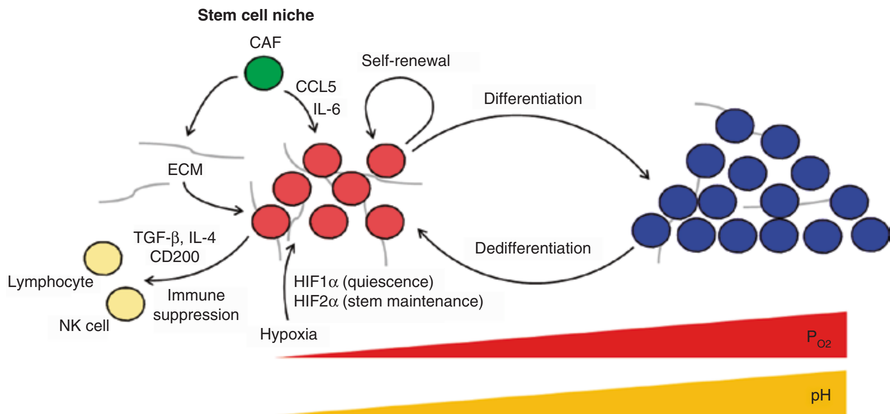


Fig. 6.1 Interplay between the tumor microenvironment and stem cell niche. A complex network of reciprocal signaling between tumor cells, stromal cells, and immune infiltrate maintain the stem cell niche by controlling the balance between cell self-renewal and commitment to differentiation. Stromal and tissue architecture, such as ECM stiffness, also has a profound effect on transcriptional regulation to mediate the interconversion between tumor cell states. In addition to cellular contributions, several extracellular properties contribute to maintaining the stem cell niche, including low oxygen tension and acidity. *CAF* cancer-associated fibroblast, *ECM* extracellular matrix, *NK* natural killer

niches, which provide cues via cell-cell contacts and secreted factors to regulate stem cell survival and identity [54, 55]. This specialized microenvironment is comprised of fibroblastic cells, immune cells, endothelial cells or their progenitors, extracellular matrix (ECM) components, and networks of cytokines and growth factors. The CSC niche itself is part of the larger tumor microenvironment, which encapsulates the adjacent stroma and non-CSC tumor cells. Thus, the CSC state depends critically on the tumor microenvironment and potentially on the CSC niches within it (Fig. 6.1).

To fully appreciate the effect of CSCs on tumor biology and response to therapy, an appropriate model system that preserves the tumor microenvironment and CSC niches is paramount. Notably, PDXs recapitulate many facets of the donor tumor microenvironment, including the architecture of the ECM, composition of stromal cells, and physiological conditions such as hypoxia and acidity.

Tumor Architecture and the Extracellular Matrix

Cellular interactions with the ECM can profoundly change stem cell behavior by altering gene expression programs that control the balance between self-renewal and differentiation. In particular, interactions between ECM and stem cells can be directly mediated by a number of cell receptors, namely, integrins, which signal via focal adhesion kinase (FAK) and phosphoinositide 3-kinase (PI3K) to regulate self-renewal and proliferation of stem cells [56–58]. For

example, ECM-dependent signaling mediated by β1 integrins is essential to preserving the neural and mammary stem cell pool by controlling the activity of Notch and epidermal growth factor (EGF) receptors [59, 60]. In addition, the mechanical properties of the ECM, including stiffness and nanotopology, act as potent regulators of growth and differentiation [61]. Thus, the interaction between cells and their microenvironment plays an important role in controlling their fate.

In contrast to in vitro cell lines, which are largely propagated in two dimensions on a plastic substratum, PDX models more closely mimic the topology and cell-ECM interactions found in patient tumors. However, a number of caveats need to be taken into account when generating PDXs to ensure the interplay between cells and ECM is conserved. First, the ECM structure is tissue specific [62]; therefore, PDXs should be established orthotopically whenever possible. Second, Matrigel, a murine basement membrane extract, is often used to increase engraftment efficiency but may not fully mimic the properties of human ECM in terms of growth factors, spatial heterogeneity, and stiffness. As an alternative, cell-derived matrices (CDMs), which are the product of matrix secretion and assembly by cells cultured at high density in vitro, have been shown to better recapitulate the architecture and content observed in human tissue stroma [63, 64].

The Tumor Stroma

In addition to ECM components of a patient's tumor, the PDX tumor architecture also contains, at least initially, stromal cells from the donor tumor. These cells, in particular the cancer-associated fibroblasts (CAFs), produce tumor-supportive ECM and secrete growth factors and cytokines that mediate signaling pathways to affect tumor cell states [65–67]. They therefore play a key role in maintaining the stem cell niche by regulating the balance between self-renewal and differentiation. For example, interleukin-6 (IL-6) secretion by CAFs in head and neck squamous cell carcinoma coincides with an increased CSC population and treatment with an IL-6 receptor blocking antibody inhibits CSC-mediated tumor initiation [68]. Similarly, in colon cancer, myofibroblast-secreted factors, such as hepatocyte growth factor, activate Wnt signaling to induce the dedifferentiation of tumor cells to a CSC-like state in vivo [69]. Finally, it has recently emerged that bone marrow mesenchymal stromal cells (BM-MSCs) are recruited to tumor sites where they secrete the chemokine CCL5/RANTES, which in a paracrine fashion signals cancer cells to undertake a stem cell-like phenotype [70, 71].

One important consideration when assessing CSCs in PDX models is the substitution of human stromal components with murine stroma. This new murine stroma may result in changes in paracrine regulation of the tumor as well as its physical properties [72]. However, in general, PDX models in early passage (<5 passages) retain stromal components and microenvironment features of the donor patient tumor [20] and can be used reliably to address the effect of the microenvironment, in particular, the stem cell niche, on tumor biology and response to therapy.

Immune Cells

While relatively little is known about the specific immunological properties of the CSC population, recent work suggests that these cells may be able to modulate immune responses. CSCs isolated from breast cancer cell lines have been shown to escape natural killer (NK) cell-mediated killing as well as antibody-dependent cell-mediated cytotoxicity in response to trastuzumab [73]. This is likely due, at least in part, to the fact that CSCs secrete more of the immunosuppressive cytokine TGF-β compared to non-CSC tumor cells [74, 75]. Likewise, colon CSCs are known to secrete interleukin-4, which inhibits anti-tumor immune responses [76]. CSCs also express CD200 [77], a molecule that inhibits myeloid cells and could thus play a major role in mediating the immunosubversive nature of CSCs.

A major limitation of current generation PDX models is their reliance on immunodeficient mouse strains in order to avoid allograft rejection. NSG mice (which lack functional T, B, and NK cells) are routinely employed to establish PDXs, thus precluding the interplay between the immune system and CSCs that could affect cell plasticity and tumor heterogeneity. Interestingly, a recent study suggests that, at least initially, immune-tumor interactions are preserved in PDX models established in less immunocompromised mice, such as nude mice (which only lack functional T cells; [78]). However, to fully appreciate the role of CSCs in tumor initiation and therapy resistance, tumors should ideally be propagated in models possessing a functional immune system. Toward this end, efforts are underway to engineer "humanized" PDX models, in which the tumor is co-engrafted with hematopoietic stem cells to reconstitute the full repertoire of immune cells of the individual donor [79, 80].

The Hypoxic Microenvironment

It has been robustly shown that stem cells localize within the hypoxic niche, and CSCs are similarly enriched and maintained in hypoxic tumors [55, 81]. Disorganized blood vessel architecture restricts tumor perfusion and causes inadequate oxygen delivery in vivo; the resulting oxygen gradient is prone to transient fluctuations and dynamically impacts CSC behavior [82]. The hypoxia-inducible factors (HIFs) are master regulators of the hypoxic response that elicit numerous mechanisms affecting stem cell maintenance [83]. Notably, HIF-mediated suppression of growth signals is important for sustaining the long-term survival of stem cells within the hypoxic niche [84]. Hypoxia stimulates HIF1α to inhibit the transcription of c-myc, thereby alleviating transcriptional repression of cyclin-dependent kinase inhibitors p21 and p27 [85]. This inhibitory effect on cell cycle progression maintains a quiescent CSC population that confers protection from therapies that target mitotically dividing cells [86].

Not only is stem cell differentiation inhibited under low oxygen conditions, but transcriptional and epigenetic reprograming in response to hypoxic conditions brings about a phenotypic shift in tumor cells to a less differentiated state [87, 88].

HIF2α is activated as a cellular response to hypoxia and subsequent activation of Oct4, Notch, TGF-β, and WNT signaling pathways together promotes a stem-like phenotype and expansion of the CSC pool [55, 89–91]. The epigenetic shift following HIF activation is being actively investigated, and chromatin remodeling in the hypoxic niche may explain how CSCs arise from more committed cancer cell populations [92]. The histone demethylase genes *JARID1B, KDM3A, KDM4B*, and *KDM4C* have independently been described to command a phenotypic switch to the CSC state and are upregulated in response to hypoxia, although the extent to which these genes cooperate within the hypoxic niche is not well understood [93–99]. The numerous phenotypic consequences that arise from hypoxia raise concern that long-term passaging of cancer cell lines in vitro at ambient partial oxygen pressure (~760 mmHg) selects against CSC populations that are adapted to thrive in the hypoxic tumor microenvironment (<10 mmHg; [100]). PDX models have not been compromised in this regard and thus more accurately embody the physiological conditions observed in patients.

Acidification of the Microenvironment

It has long been recognized that tumors reprogram their metabolic pathways, upregulating glycolytic enzymes leading to the accumulation of lactate that, in turn, acidifies the tumor microenvironment [101, 102]. However, the biological significance of tumor acidification remains poorly understood. Low pH tends to coincide with low partial oxygen pressure, as hypoxia-induced carbonic anhydrase IX activity couples with transmembrane ion exchange to acidify the extracellular compartment [103]. Although acidification is frequently observed alongside hypoxia, evidence suggests that low extracellular pH promotes a CSC gene expression pattern independently of partial oxygen pressure. Human glioma cell cultures depleted of CSCs showed a HIF2α-dependent activation of stem cell-associated genes when acidified. These changes were found to be hypoxia-independent and were reversed when normal pH was restored, suggesting that the CSC niche is responsive to pH fluctuations [104]. To further investigate the dynamic influence pH exerts on CSC plasticity, the PDX model will surely play an important role as it conserves the donor tumor physiology and, accordingly, preserves the CSC niche.

Cancer Stem Cell Dynamics in PDX Models

Isolation and Characterization of CSCs in PDX Models

Genetic aberrations and chromatin regulation of key transcription factors are critical for locking cells into a particular differentiation state [105–107]. As PDX models retain the genetic and epigenetic properties of the donor tumor, it follows that these models could be used as a tool for studying CSC populations. Analysis of PDX models developed from renal cell carcinomas revealed these tumors to harbor both

CSC and non-CSC populations, based on expression of the CSC-associated cell surface markers CD133 and CD105 [108]. Isolating and sorting for CSCs confirmed these cells to exhibit stem cell-associated properties, including expression of pluripotency genes (such as *Oct4* and *Nanog*), ALDH activity, capacity to form spheroids, and ability to form tumors at limiting dilution in SCID mice. Likewise, stem-like cells have also been identified and isolated from glioblastoma PDXs. These cells were characterized as having a mesenchymal gene signature and expressing high levels of the stem cell markers CD133, Sox2, and Nestin [109]. Finally, both ovarian PDX tumors and the original patient tumors were found to harbor a similar proportion of cells expressing the CSC markers ALDH1A1 and CD133 [110]. Together, these studies convey that the polyclonality and, in particular, the stem cell compartment of human tumors is maintained in PDX models.

CSCs in the Establishment of PDX Models

Eirew and colleagues recently examined clonal dynamics in a panel of breast cancer PDX models at single-cell resolution [111]. Notably, the dynamics were highly skewed toward minor prevalence clones that represented less than 5% of the starting population, expanding to dominate the xenograft. The most parsimonious explanation for this phenomenon of clonal dominance is that preexisting clones exhibit variations in clonal fitness, with a stem cell/tumor-initiation compartment establishing the tumor and, in turn, differentiating to reestablish tumor polyclonality. In support, similar clonal dynamics were observed in parallel xenografts established from the same sample [111], implying that the basis of selection is nonrandom and likely linked to a particular genotype or epigenotype.

Indeed, a number of studies have suggested that the CSC population plays a principal role in repopulating a tumor following xenotransplantation. Ding and colleagues reported that PDX models derived from basal-like breast cancer more closely resemble the mutational enrichment pattern of the patient's metastatic lesion than the primary tumor [112]. CSCs have been implicated as the "seeds" for metastasis [113], suggesting that the CSC subpopulation may facilitate PDX engraftment and expand to repopulate the xenograft. In a separate study, the engraftment frequency of xenografts from human acute lymphoblastic leukemia was found to be directly proportional to the number of CD34+ leukemia-initiating cells [114]. Finally, sorting disaggregated tumor cells from hepatocellular carcinoma patients for the putative stem cell marker GEP was found to facilitate PDX establishment [115]. From these studies it is clear, and perhaps obvious, that the stem cell/tumor-initiating subpopulation within tumors is more adept at tumor engraftment.

CSCs in PDX Models After Chemotherapy

It has been proposed that CSCs contribute to treatment resistance [116]; indeed, these cells may be a key determinant of the therapy failures that plague many

cancers. For example, chronic myeloid leukemia appears to be driven by leukemia stem cells that exhibit heightened resistance to imatinib compared to their more differentiated progeny [117, 118]. Likewise, there is also evidence that CSCs in gliomas [119] and breast cancers [120] are intrinsically more resistant to therapy than other cells in the tumor. As PDX models closely mimic patient tumors, including the CSC compartment, they represent a versatile tool for simulating CSC-mediated resistance generated in response to treatment strategies used in the clinic.

In an elegant study by Kreso and colleagues, lentiviral lineage tracing was used to trace the chemotherapy tolerance of different clones in colorectal cancer PDXs [121]. It was found that previously minor "type IV" subclones repopulated the tumor bulk after treatment with chemotherapy. Notably, this cell population was linked to the BMI1-positive stem cells important for intestinal and colonic crypt maintenance [122]. Likewise, in a PDX model of acute myeloid leukemia, lymphoid-primed multipotent progenitors were enriched following treatment with the BET bromodomain inhibitor iBET [123]. Finally, combined carboplatin/paclitaxel treatment enriched CSC populations in ovarian cancer PDXs [110]. Global RNA-seq transcriptome profiling of the relapsed tumors revealed upregulation of the drug efflux pump ABCG1 as well as Sphingosine-1-phosphate signaling, which has been linked to protection of oocytes from apoptosis.

For over a decade, targeting CSCs has been proposed as a therapeutic goal. However, clinical trials, targeting the stem cell state in solid tumors with agents such as histone deacetylase inhibitors (HDACis), have been disappointing with few durable responses observed [124, 125]. As PDXs closely mimic the heterogeneity of patient tumors, with respect to both the CSC and non-CSC subpopulations, they represent a robust platform for deciphering the contribution of CSCs to therapy resistance as well as guiding empirical treatments targeting the CSC population.

Alternative PDX Models for Assessing Cancer Stem Cells

In addition to the murine PDX models described above, zebrafish PDX models have emerged in the last decade as an alternative and powerful tool to study tumor initiation and drug responses of various cancers [126, 127]. Transplants of human cells into zebrafish were first reported in 2005 by Lee et al. in a study where human metastatic melanoma cells were engrafted in zebrafish [128]. Later, successful transplantation of traditional cancer cell lines and primary human tumor cells was established for a variety of human tumors. These include leukemic blood cell lines and primary chronic myeloid leukemia cells in zebrafish embryos as well as engraftment of small pieces or dissociated cells from human pancreas, colon, and stomach carcinomas [129–132]. Since the zebrafish model provides a shorter time interval to complete in vivo studies compared to conventional murine PDXs, the model has recently gained attention as a system to rapidly assess anticancer agent responses in a live animal model. The advantage of the zebrafish system is also that the adaptive immune response has not been developed before 48 h postfertilization, and thus the procedure does not require immune suppression [129, 131, 133].

Zebrafish models may also reflect the biology of cancer in a similar way as mouse models and enable studies on tumorigenicity and CSCs. Notably, zebrafish are less costly and easily assessable and as such the system may be ideal for limiting dilution studies to define self-renewal cell frequency by tracking single tumor-initiating cells in vivo. Bansal et al. assessed tumor-initiation properties of six primary prostate cancer patients in the zebrafish xenograft model [134]. In the assay, $\alpha 2\beta 1^{hi}$/CD44^{hi} tumor-initiating cells were isolated and transplanted to zebrafish embryos, and the results indicated that the tumor-initiating cells displayed significantly shorter survival rates and rapid death from tumor burden and that they invaded both local and remote tissues. Conversely, benign prostate epithelial cells survived but failed to initiate tumors in the model. These studies indicate that zebrafish PDX models can be utilized for tumor-initiating studies. As the successful engraftment of cells in zebrafish can be achieved from fewer cells compared to mouse models and host numbers can be scaled up easily to provide more robust statistical analyses, the zebrafish model is especially suitable for CSC studies where there is limited material available [135, 136].

However, limitations in utilizing zebrafish PDX models have been reported as the engraftment of human cells in zebrafish has been more difficult than in mice. For example, Pruvot et al. were unsuccessful in transplanting and maintaining healthy human CD34+ hematopoietic stem and progenitor cells in zebrafish embryos [137]. This could be the result of differences in the microenvironment in zebrafish compared to mouse models. For example, there are different temperature requirements for maintaining zebrafish as these are traditionally maintained at a lower temperature (28 °C) than human cells which are cultured at human body temperature (37 °C). Thus, various groups have suggested that zebrafish embryos should be kept at 35 °C where they still develop normally and in which xenotransplanted human cells are able to proliferate [129, 137, 138].

Although zebrafish PDXs cannot displace the gold standard murine PDX model and use of mammalian model systems, they serve as a well-established alternative to rapidly model clinically relevant human-derived cells, self-renewal, and tumor initiation. Thus, the zebrafish PDXs can be thought of as providing a complementary and cost-effective alternative to mouse PDX models.

Summary

PDX models are capable of recapitulating the complexity of human cancers remarkably well. Tumors are not simply a homogeneous mass of cells but rather a hierarchy of genetically and epigenetically distinct populations of cells that exist dynamically in space and time, competing, and perhaps cooperating, to further increase the fitness of the tumor as a whole. As PDX models preserve both cell-autonomous and cell-extrinsic factors that control cell plasticity, they will lead to a better understanding of how cancer cell states coexist within a tumor, as well as how the equilibrium between self-renewal and differentiation is

disrupted by therapy. However, it is critical that such models be viewed in light of their inherent limitations. Optimizations to better mimic the stem cell niche of the donor tumor, possibly via co-engraftment of stromal and immune components, will further improve the precision of PDXs in modeling cell plasticity and stem-like states.

References

1. Arrowsmith J. Trial watch: phase III and submission failures: 2007–2010. Nat Rev Drug Discov. 2011;10(2):87. doi:10.1038/nrd3375.
2. Ledford H. Translational research: 4 ways to fix the clinical trial. Nature. 2011;477(7366):526–8. doi:10.1038/477526a.
3. Esquenet M, Swinnen JV, Heyns W, Verhoeven G. LNCaP prostatic adenocarcinoma cells derived from low and high passage numbers display divergent responses not only to androgens but also to retinoids. J Steroid Biochem Mol Biol. 1997;62(5–6):391–9.
4. Johnson JI, Decker S, Zaharevitz D, Rubinstein LV, Venditti JM, Schepartz S, et al. Relationships between drug activity in NCI preclinical in vitro and in vivo models and early clinical trials. Br J Cancer. 2001;84(10):1424–31. doi:10.1054/bjoc.2001.1796. S0007092001917963 [pii]
5. Wenger SL, Senft JR, Sargent LM, Bamezai R, Bairwa N, Grant SG. Comparison of established cell lines at different passages by karyotype and comparative genomic hybridization. Biosci Rep. 2004;24(6):631–9. doi:10.1007/s10540-005-2797-5.
6. Illmensee K, Mintz B. Totipotency and normal differentiation of single teratocarcinoma cells cloned by injection into blastocysts. Proc Natl Acad Sci USA. 1976;73(2):549–53.
7. Kleinsmith LJ, Pierce Jr GB. Multipotentiality of single embryonal carcinoma cells. Cancer Res. 1964;24:1544–51.
8. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med. 1997;3(7):730–7.
9. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, et al. A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature. 1994;367(6464):645–8. doi:10.1038/367645a0.
10. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, et al. Identification of human brain tumour initiating cells. Nature. 2004;432(7015):396–401.
11. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA. 2003;100(7):3983–8.
12. Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, et al. Identification of pancreatic cancer stem cells. Cancer Res. 2007;67(3):1030–7. doi:10.1158/0008-5472.CAN-06-2030.
13. Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res. 2005;65(23):10946–51. doi:10.1158/0008-5472.CAN-05-2018.
14. Creighton CJ, Li X, Landis M, Dixon JM, Neumeister VM, Sjolund A, et al. Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. Proc Natl Acad Sci U S A. 2009;106(33):13820–5. doi:10.1073/pnas.0905718106.
15. Gupta PB, Onder TT, Jiang G, Tao K, Kuperwasser C, Weinberg RA, et al. Identification of selective inhibitors of cancer stem cells by high-throughput screening. Cell. 2009;138(4):645–59. doi:10.1016/j.cell.2009.06.034.
16. Li X, Lewis MT, Huang J, Gutierrez C, Osborne CK, Wu MF, et al. Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. J Natl Cancer Inst. 2008;100(9):672–9. doi:10.1093/jnci/djn123.
17. Woodward WA, Chen MS, Behbod F, Alfaro MP, Buchholz TA, Rosen JM. WNT/beta-catenin mediates radiation resistance of mouse mammary progenitor cells. Proc Natl Acad Sci U S A. 2007;104(2):618–23. doi:10.1073/pnas.0606599104.

18. Gupta PB, Fillmore CM, Jiang G, Shapira SD, Tao K, Kuperwasser C, et al. Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells. Cell. 2011;146(4):633–44. doi:10.1016/j.cell.2011.07.026.
19. Meacham CE, Morrison SJ. Tumour heterogeneity and cancer cell plasticity. Nature. 2013;501(7467):328–37. doi:10.1038/nature12624.
20. Choi SY, Lin D, Gout PW, Collins CC, Xu Y, Wang Y. Lessons from patient-derived xenografts for better in vitro modeling of human cancer. Adv Drug Deliv Rev. 2014;79–80:222–37. doi:10.1016/j.addr.2014.09.009.
21. Cohnheim J. Congenitales, quergestreiftes Muskelsarkom der Nieren. Archiv für pathologische Anatomie und Physiologie und für klinische Medicin. 1875;65(1):64–9. doi:10.1007/bf01978936.
22. Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, et al. Cancer stem cells—perspectives on current status and future directions: AACR workshop on cancer stem cells. Cancer Res. 2006;66(19):9339–44. doi:10.1158/0008-5472.CAN-06-3126.
23. Nguyen LV, Vanner R, Dirks P, Eaves CJ. Cancer stem cells: an evolving concept. Nat Rev Cancer. 2012;12(2):133–43. doi:10.1038/nrc3184.
24. Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer. 2008;8(10):755–68. doi:10.1038/nrc2499.
25. Chen J, Li Y, Yu TS, McKay RM, Burns DK, Kernie SG, et al. A restricted cell population propagates glioblastoma growth after chemotherapy. Nature. 2012;488(7412):522–6. doi:10.1038/nature11287.
26. Driessens G, Beck B, Caauwe A, Simons BD, Blanpain C. Defining the mode of tumour growth by clonal analysis. Nature. 2012;488(7412):527–30. doi:10.1038/nature11344.
27. Schepers AG, Snippert HJ, Stange DE, van den Born M, van Es JH, van de Wetering M, et al. Lineage tracing reveals Lgr5+ stem cell activity in mouse intestinal adenomas. Science. 2012;337(6095):730–5. doi:10.1126/science.1224676.
28. Chaffer CL, Brueckmann I, Scheel C, Kaestli AJ, Wiggins PA, Rodrigues LO, et al. Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. Proc Natl Acad Sci U S A. 2011;108(19):7950–5. doi:10.1073/pnas.1102454108.
29. Chaffer CL, Marjanovic ND, Lee T, Bell G, Kleer CG, Reinhardt F, et al. Poised chromatin at the ZEB1 promoter enables breast cancer cell plasticity and enhances tumorigenicity. Cell. 2013;154(1):61–74. doi:10.1016/j.cell.2013.06.005.
30. Flavahan WA, Wu Q, Hitomi M, Rahim N, Kim Y, Sloan AE, et al. Brain tumor initiating cells adapt to restricted nutrition through preferential glucose uptake. Nat Neurosci. 2013;16(10):1373–82. doi:10.1038/nn.3510.
31. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012;366(10):883–92. doi:10.1056/NEJMoa1113205.
32. Hansen KD, Timp W, Bravo HC, Sabunciyan S, Langmead B, McDonald OG, et al. Increased methylation variation in epigenetic domains across cancer types. Nat Genet. 2011;43(8):768–75. doi:10.1038/ng.865.
33. Navin N, Kendall J, Troge J, Andrews P, Rodgers L, McIndoo J, et al. Tumour evolution inferred by single-cell sequencing. Nature. 2011;472(7341):90–4. doi:10.1038/nature09807.
34. Moon BS, Jeong WJ, Park J, Kim TI, Min do S, Choi KY. Role of oncogenic K-Ras in cancer stem cell activation by aberrant Wnt/beta-catenin signaling. J Natl Cancer Inst. 2014;106(2):djt373. doi:10.1093/jnci/djt373.
35. Suzuki H, Watkins DN, Jair KW, Schuebel KE, Markowitz SD, Chen WD, et al. Epigenetic inactivation of SFRP genes allows constitutive WNT signaling in colorectal cancer. Nat Genet. 2004;36(4):417–22. doi:10.1038/ng1330.
36. Myszczyszyn A, Czarnecka AM, Matak D, Szymanski L, Lian F, Kornakiewicz A, et al. The role of hypoxia and cancer stem cells in renal cell carcinoma pathogenesis. Stem Cell Rev. 2015;11(6):919–43. doi:10.1007/s12015-015-9611-y.
37. Easwaran H, Johnstone SE, Van Neste L, Ohm J, Mosbruger T, Wang Q, et al. A DNA hypermethylation module for the stem/progenitor cell signature of cancer. Genome Res. 2012;22(5):837–49. doi:10.1101/gr.131169.111.

38. Beltran H, Eng K, Mosquera JM, Sigaras A, Romanel A, Rennert H, et al. Whole-exome sequencing of metastatic cancer and biomarkers of treatment response. JAMA Oncol. 2015;1(4):466–74. doi:10.1001/jamaoncol.2015.1313.
39. Kohli M, Wang L, Xie F, Sicotte H, Yin P, Dehm SM, et al. Mutational landscapes of sequential prostate metastases and matched patient derived xenografts during enzalutamide therapy. PLoS One. 2015;10(12):e0145176. doi:10.1371/journal.pone.0145176.
40. Lin D, Wyatt AW, Xue H, Wang Y, Dong X, Haegert A, et al. High fidelity patient-derived xenografts for accelerating prostate cancer discovery and drug development. Cancer Res. 2014;74(4):1272–83. doi:10.1158/0008-5472.CAN-13-2921-T.
41. Priolo C, Agostini M, Vena N, Ligon AH, Fiorentino M, Shin E, et al. Establishment and genomic characterization of mouse xenografts of human primary prostate tumors. Am J Pathol. 2010;176(4):1901–13. doi:10.2353/ajpath.2010.090873.
42. Wang Y, Xue H, Cutz JC, Bayani J, Mawji NR, Chen WG, et al. An orthotopic metastatic prostate cancer model in SCID mice via grafting of a transplantable human prostate tumor line. Lab Invest. 2005;85(11):1392–404.
43. Li S, Shen D, Shao J, Crowder R, Liu W, Prat A, et al. Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts. Cell Rep. 2013;4(6):1116–30. doi:10.1016/j.celrep.2013.08.022.
44. Fichtner I, Rolff J, Soong R, Hoffmann J, Hammer S, Sommer A, et al. Establishment of patient-derived non-small cell lung cancer xenografts as models for the identification of predictive biomarkers. Clin Cancer Res. 2008;14(20):6456–68. doi:10.1158/1078-0432.CCR-08-0138.
45. Bird A. Perceptions of epigenetics. Nature. 2007;447(7143):396–8. doi:10.1038/nature05913.
46. Waddington CH. The epigenotype. 1942. Int J Epidemiol. 2012;41(1):10–3. doi:10.1093/ije/dyr184.
47. Feinberg AP, Tycko B. The history of cancer epigenetics. Nat Rev Cancer. 2004;4(2):143–53. doi:10.1038/nrc1279.
48. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, et al. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell. 2006;125(2):315–26. doi:10.1016/j.cell.2006.02.041.
49. Harikumar A, Meshorer E. Chromatin remodeling and bivalent histone modifications in embryonic stem cells. EMBO Rep. 2015;16(12):1609–19. doi:10.15252/embr.201541011.
50. Guilhamon P, Butcher LM, Presneau N, Wilson GA, Feber A, Paul DS, et al. Assessment of patient-derived tumour xenografts (PDXs) as a discovery tool for cancer epigenomics. Genome Med. 2014;6(12):116. doi:10.1186/s13073-014-0116-0.
51. Borodovsky A, Salmasi V, Turcan S, Fabius AW, Baia GS, Eberhart CG, et al. 5-azacytidine reduces methylation, promotes differentiation and induces tumor regression in a patient-derived IDH1 mutant glioma xenograft. Oncotarget. 2013;4(10):1737–47. doi:10.18632/oncotarget.1408.
52. Turcan S, Fabius AW, Borodovsky A, Pedraza A, Brennan C, Huse J, et al. Efficient induction of differentiation and growth inhibition in IDH1 mutant glioma cells by the DNMT Inhibitor Decitabine. Oncotarget. 2013;4(10):1729–36. doi:10.18632/oncotarget.1412.
53. Varley KE, Gertz J, Bowling KM, Parker SL, Reddy TE, Pauli-Behn F, et al. Dynamic DNA methylation across diverse human cell lines and tissues. Genome Res. 2013;23(3):555–67. doi:10.1101/gr.147942.112.
54. Morrison SJ, Spradling AC. Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. Cell. 2008;132(4):598–611. doi:10.1016/j.cell.2008.01.038.
55. Plaks V, Kong N, Werb Z. The cancer stem cell niche: how essential is the niche in regulating stemness of tumor cells? Cell Stem Cell. 2015;16(3):225–38. doi:10.1016/j.stem.2015.02.015.
56. Buitenhuis M. The role of PI3K/protein kinase B (PKB/c-akt) in migration and homing of hematopoietic stem and progenitor cells. Curr Opin Hematol. 2011;18(4):226–30. doi:10.1097/MOH.0b013e32834760e5.
57. Legate KR, Wickstrom SA, Fassler R. Genetic and cell biological analysis of integrin outside-in signaling. Genes Dev. 2009;23(4):397–418. doi:10.1101/gad.1758709.

58. Lu P, Weaver VM, Werb Z. The extracellular matrix: a dynamic niche in cancer progression. J Cell Biol. 2012;196(4):395–406. doi:10.1083/jcb.201102147.
59. Brisken C, Duss S. Stem cells and the stem cell niche in the breast: an integrated hormonal and developmental perspective. Stem Cell Rev. 2007;3(2):147–56.
60. Campos LS, Decker L, Taylor V, Skarnes W. Notch, epidermal growth factor receptor, and beta1-integrin pathways are coordinated in neural stem cells. J Biol Chem. 2006;281(8):5300–9. doi:10.1074/jbc.M511886200.
61. Guilak F, Cohen DM, Estes BT, Gimble JM, Liedtke W, Chen CS. Control of stem cell fate by physical interactions with the extracellular matrix. Cell Stem Cell. 2009;5(1):17–26. doi:10.1016/j.stem.2009.06.016.
62. Cassidy JW. Nanotechnology in the regeneration of complex tissues. Bone Tissue Regen Insights. 2014;5:25–35. doi:10.4137/BTRI.S12331.
63. Amatangelo MD, Bassi DE, Klein-Szanto AJ, Cukierman E. Stroma-derived three-dimensional matrices are necessary and sufficient to promote desmoplastic differentiation of normal fibroblasts. Am J Pathol. 2005;167(2):475–88. doi:10.1016/S0002-9440(10)62991-4.
64. Quiros RM, Valianou M, Kwon Y, Brown KM, Godwin AK, Cukierman E. Ovarian normal and tumor-associated fibroblasts retain in vivo stromal characteristics in a 3-D matrix-dependent manner. Gynecol Oncol. 2008;110(1):99–109. doi:10.1016/j.ygyno.2008.03.006.
65. Hu Y, Yan C, Mu L, Huang K, Li X, Tao D, et al. Fibroblast-derived exosomes contribute to chemoresistance through priming cancer stem cells in colorectal cancer. PLoS One. 2015;10(5):e0125625. doi:10.1371/journal.pone.0125625.
66. Kinugasa Y, Matsui T, Takakura N. CD44 expressed on cancer-associated fibroblasts is a functional molecule supporting the stemness and drug resistance of malignant cancer cells in the tumor microenvironment. Stem Cells. 2014;32(1):145–56. doi:10.1002/stem.1556.
67. Lotti F, Jarrar AM, Pai RK, Hitomi M, Lathia J, Mace A, et al. Chemotherapy activates cancer-associated fibroblasts to maintain colorectal cancer-initiating cells by IL-17A. J Exp Med. 2013;210(13):2851–72. doi:10.1084/jem.20131195.
68. Krishnamurthy S, Warner KA, Dong Z, Imai A, Nor C, Ward BB, et al. Endothelial interleukin-6 defines the tumorigenic potential of primary human cancer stem cells. Stem Cells. 2014;32(11):2845–57. doi:10.1002/stem.1793.
69. Vermeulen L, De Sousa EMF, van der Heijden M, Cameron K, de Jong JH, Borovski T, et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. Nat Cell Biol. 2010;12(5):468–76. doi:10.1038/ncb2048.
70. Luo J, Ok Lee S, Liang L, Huang CK, Li L, Wen S, et al. Infiltrating bone marrow mesenchymal stem cells increase prostate cancer stem cell population and metastatic ability via secreting cytokines to suppress androgen receptor signaling. Oncogene. 2014;33(21):2768–78. doi:10.1038/onc.2013.233.
71. Zhang Y, Yao F, Yao X, Yi C, Tan C, Wei L, et al. Role of CCL5 in invasion, proliferation and proportion of CD44+/CD24− phenotype of MCF-7 cells and correlation of CCL5 and CCR5 expression with breast cancer progression. Oncol Rep. 2009;21(4):1113–21.
72. Ostman A. The tumor microenvironment controls drug sensitivity. Nat Med. 2012;18(9):1332–4. doi:10.1038/nm.2938.
73. Reim F, Dombrowski Y, Ritter C, Buttmann M, Hausler S, Ossadnik M, et al. Immunoselection of breast and ovarian cancer cells with trastuzumab and natural killer cells: selective escape of CD44high/CD24low/HER2low breast cancer stem cells. Cancer Res. 2009;69(20):8058–66. doi:10.1158/0008-5472.CAN-09-0834.
74. Lottaz C, Beier D, Meyer K, Kumar P, Hermann A, Schwarz J, et al. Transcriptional profiles of CD133+ and CD133− glioblastoma-derived cancer stem cell lines suggest different cells of origin. Cancer Res. 2010;70(5):2030–40. doi:10.1158/0008-5472.CAN-09-1707.
75. Shipitsin M, Campbell LL, Argani P, Weremowicz S, Bloushtain-Qimron N, Yao J, et al. Molecular definition of breast tumor heterogeneity. Cancer Cell. 2007;11(3):259–73. doi:10.1016/j.ccr.2007.01.013.

76. Todaro M, Alea MP, Di Stefano AB, Cammareri P, Vermeulen L, Iovino F, et al. Colon cancer stem cells dictate tumor growth and resist cell death by production of interleukin-4. Cell Stem Cell. 2007;1(4):389–402. doi:10.1016/j.stem.2007.08.001.
77. Kawasaki BT, Mistree T, Hurt EM, Kalathur M, Farrar WL. Co-expression of the toleragenic glycoprotein, CD200, with markers for cancer stem cells. Biochem Biophys Res Commun. 2007;364(4):778–82. doi:10.1016/j.bbrc.2007.10.067.
78. Choi SY, Xue H, Wu R, Fazli L, Lin D, Collins CC, et al. The MCT4 gene: a novel, potential target for therapy of advanced prostate cancer. Clin Cancer Res. 2016;22(11):2721–33. doi:10.1158/1078-0432.CCR-15-1624.
79. Morton JJ, Bird G, Keysar SB, Astling DP, Lyons TR, Anderson RT, et al. XactMice: humanizing mouse bone marrow enables microenvironment reconstitution in a patient-derived xenograft model of head and neck cancer. Oncogene. 2016;35(3):290–300. doi:10.1038/onc.2015.94.
80. Rongvaux A, Willinger T, Martinek J, Strowig T, Gearty SV, Teichmann LL, et al. Development and function of human innate immune cells in a humanized mouse model. Nat Biotechnol. 2014;32(4):364–72. doi:10.1038/nbt.2858.
81. Ezashi T, Das P, Roberts RM. Low O2 tensions and the prevention of differentiation of hES cells. Proc Natl Acad Sci U S A. 2005;102(13):4783–8. doi:10.1073/pnas.0501283102.
82. Bennewith KL, Durand RE. Quantifying transient hypoxia in human tumor xenografts by flow cytometry. Cancer Res. 2004;64(17):6183–9. doi:10.1158/0008-5472.CAN-04-0289.
83. Semenza GL. Hypoxia-inducible factors in physiology and medicine. Cell. 2012;148(3):399–408. doi:10.1016/j.cell.2012.01.021.
84. Eliasson P, Rehn M, Hammar P, Larsson P, Sirenko O, Flippin LA, et al. Hypoxia mediates low cell-cycle activity and increases the proportion of long-term-reconstituting hematopoietic stem cells during in vitro culture. Exp Hematol. 2010;38(4):301–10.e2. doi:10.1016/j.exphem.2010.01.005.
85. Gordan JD, Bertout JA, Hu CJ, Diehl JA, Simon MC. HIF-2alpha promotes hypoxic cell proliferation by enhancing c-myc transcriptional activity. Cancer Cell. 2007;11(4):335–47. doi:10.1016/j.ccr.2007.02.006.
86. Ye J, Wu D, Wu P, Chen Z, Huang J. The cancer stem cell niche: cross talk between cancer stem cells and their microenvironment. Tumour Biol. 2014;35(5):3945–51. doi:10.1007/s13277-013-1561-x.
87. Deheeger M, Lesniak MS, Ahmed AU. Cellular plasticity regulated cancer stem cell niche: a possible new mechanism of chemoresistance. Cancer Cell Microenviron. 2014;1(5):e295. doi:10.14800/ccm.295.
88. Heddleston JM, Li Z, McLendon RE, Hjelmeland AB, Rich JN. The hypoxic microenvironment maintains glioblastoma stem cells and promotes reprogramming towards a cancer stem cell phenotype. Cell Cycle. 2009;8(20):3274–84. doi:10.4161/cc.8.20.9701.
89. Guo W. Concise review: breast cancer stem cells: regulatory networks, stem cell niches, and disease relevance. Stem Cells Transl Med. 2014;3(8):942–8. doi:10.5966/sctm.2014-0020.
90. Mohyeldin A, Garzon-Muvdi T, Quinones-Hinojosa A. Oxygen in stem cell biology: a critical component of the stem cell niche. Cell Stem Cell. 2010;7(2):150–61. doi:10.1016/j.stem.2010.07.007.
91. Visvader JE, Lindeman GJ. Cancer stem cells: current status and evolving complexities. Cell Stem Cell. 2012;10(6):717–28. doi:10.1016/j.stem.2012.05.007.
92. Yun Z, Lin Q. Hypoxia and regulation of cancer cell stemness. Adv Exp Med Biol. 2014;772:41–53. doi:10.1007/978-1-4614-5915-6_2.
93. Beyer S, Kristensen MM, Jensen KS, Johansen JV, Staller P. The histone demethylases JMJD1A and JMJD2B are transcriptional targets of hypoxia-inducible factor HIF. J Biol Chem. 2008;283(52):36542–52. doi:10.1074/jbc.M804578200.
94. Krieg AJ, Rankin EB, Chan D, Razorenova O, Fernandez S, Giaccia AJ. Regulation of the histone demethylase JMJD1A by hypoxia-inducible factor 1 alpha enhances hypoxic gene expression and tumor growth. Mol Cell Biol. 2010;30(1):344–53. doi:10.1128/MCB.00444-09.

95. Loh YH, Zhang W, Chen X, George J, Ng HH. Jmjd1a and Jmjd2c histone H3 Lys 9 demethylases regulate self-renewal in embryonic stem cells. Genes Dev. 2007;21(20):2545–57. doi:10.1101/gad.1588207.
96. Mimura I, Nangaku M, Kanki Y, Tsutsumi S, Inoue T, Kohro T, et al. Dynamic change of chromatin conformation in response to hypoxia enhances the expression of GLUT3 (SLC2A3) by cooperative interaction of hypoxia-inducible factor 1 and KDM3A. Mol Cell Biol. 2012;32(15):3018–32. doi:10.1128/MCB.06643-11.
97. Ramadoss S, Guo G, Wang CY. Lysine demethylase KDM3A regulates breast cancer cell invasion and apoptosis by targeting histone and the non-histone protein p53. Oncogene. 2016; doi:10.1038/onc.2016.174.
98. Yamamoto S, Wu Z, Russnes HG, Takagi S, Peluffo G, Vaske C, et al. JARID1B is a luminal lineage-driving oncogene in breast cancer. Cancer Cell. 2014;25(6):762–77. doi:10.1016/j.ccr.2014.04.024.
99. Yang J, Ledaki I, Turley H, Gatter KC, Montero JC, Li JL, et al. Role of hypoxia-inducible factors in epigenetic regulation via histone demethylases. Ann N Y Acad Sci. 2009;1177:185–97. doi:10.1111/j.1749-6632.2009.05027.x.
100. Vaupel P, Hockel M, Mayer A. Detection and characterization of tumor hypoxia using pO2 histography. Antioxid Redox Signal. 2007;9(8):1221–35. doi:10.1089/ars.2007.1628.
101. Kato Y, Ozawa S, Miyamoto C, Maehata Y, Suzuki A, Maeda T, et al. Acidic extracellular microenvironment and cancer. Cancer Cell Int. 2013;13(1):89. doi:10.1186/1475-2867-13-89.
102. Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. J Gen Physiol. 1927;8(6):519–30.
103. Svastova E, Hulikova A, Rafajova M, Zat'ovicova M, Gibadulinova A, Casini A, et al. Hypoxia activates the capacity of tumor-associated carbonic anhydrase IX to acidify extracellular pH. FEBS Lett. 2004;577(3):439–45. doi:10.1016/j.febslet.2004.10.043.
104. Hjelmeland AB, Wu Q, Heddleston JM, Choudhary GS, MacSwords J, Lathia JD, et al. Acidic stress promotes a glioma stem cell phenotype. Cell Death Differ. 2011;18(5):829–40. doi:10.1038/cdd.2010.150.
105. Khavari DA, Sen GL, Rinn JL. DNA methylation and epigenetic control of cellular differentiation. Cell Cycle. 2010;9(19):3880–3. doi:10.4161/cc.9.19.13385.
106. Lunyak VV, Rosenfeld MG. Epigenetic regulation of stem cell fate. Hum Mol Genet. 2008;17(R1):R28–36. doi:10.1093/hmg/ddn149.
107. Ohbo K, Tomizawa S. Epigenetic regulation in stem cell development, cell fate conversion, and reprogramming. Biomol Concepts. 2015;6(1):1–9. doi:10.1515/bmc-2014-0036.
108. Hasmim M, Bruno S, Azzi S, Gallerne C, Michel JG, Chiabotto G, et al. Isolation and characterization of renal cancer stem cells from patient-derived xenografts. Oncotarget. 2016;7(13):15507–24. doi:10.18632/oncotarget.6266.
109. Garner JM, Ellison DW, Finkelstein D, Ganguly D, Du Z, Sims M, et al. Molecular heterogeneity in a patient-derived glioblastoma xenoline is regulated by different cancer stem cell populations. PLoS One. 2015;10(5):e0125838. doi:10.1371/journal.pone.0125838.
110. Dobbin ZC, Katre AA, Steg AD, Erickson BK, Shah MM, Alvarez RD, et al. Using heterogeneity of the patient-derived xenograft model to identify the chemoresistant population in ovarian cancer. Oncotarget. 2014;5(18):8750–64. doi:10.18632/oncotarget.2373.
111. Eirew P, Steif A, Khattra J, Ha G, Yap D, Farahani H, et al. Dynamics of genomic clones in breast cancer patient xenografts at single-cell resolution. Nature. 2015;518(7539):422–6. doi:10.1038/nature13952.
112. Ding L, Ellis MJ, Li S, Larson DE, Chen K, Wallis JW, et al. Genome remodelling in a basal-like breast cancer metastasis and xenograft. Nature. 2010;464(7291):999–1005. doi:10.1038/nature08989.
113. Oskarsson T, Batlle E, Massague J. Metastatic stem cells: sources, niches, and vital pathways. Cell Stem Cell. 2014;14(3):306–21. doi:10.1016/j.stem.2014.02.002.
114. Notta F, Mullighan CG, Wang JC, Poeppl A, Doulatov S, Phillips LA, et al. Evolution of human BCR-ABL1 lymphoblastic leukaemia-initiating cells. Nature. 2011;469(7330):362–7. doi:10.1038/nature09733.

115. Cheung PF, Yip CW, Ng LW, Lo KW, Chow C, Chan KF, et al. Comprehensive characterization of the patient-derived xenograft and the paralleled primary hepatocellular carcinoma cell line. Cancer Cell Int. 2016;16:41. doi:10.1186/s12935-016-0322-5.
116. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer. 2005;5(4):275–84.
117. O'Hare T, Corbin AS, Druker BJ. Targeted CML therapy: controlling drug resistance, seeking cure. Curr Opin Genet Dev. 2006;16(1):92–9. doi:10.1016/j.gde.2005.11.002.
118. Oravecz-Wilson KI, Philips ST, Yilmaz OH, Ames HM, Li L, Crawford BD, et al. Persistence of leukemia-initiating cells in a conditional knockin model of an imatinib-responsive myeloproliferative disorder. Cancer Cell. 2009;16(2):137–48. doi:10.1016/j.ccr.2009.06.007.
119. Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature. 2006;444(7120):756–60. doi:10.1038/nature05236.
120. Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, et al. Association of reactive oxygen species levels and radioresistance in cancer stem cells. Nature. 2009;458(7239):780–3. doi:10.1038/nature07733.
121. Kreso A, O'Brien CA, van Galen P, Gan OI, Notta F, Brown AM, et al. Variable clonal repopulation dynamics influence chemotherapy response in colorectal cancer. Science. 2013;339(6119):543–8. doi:10.1126/science.1227670.
122. Kreso A, van Galen P, Pedley NM, Lima-Fernandes E, Frelin C, Davis T, et al. Self-renewal as a therapeutic target in human colorectal cancer. Nat Med. 2014;20(1):29–36. doi:10.1038/nm.3418.
123. Fong CY, Gilan O, Lam EY, Rubin AF, Ftouni S, Tyler D, et al. BET inhibitor resistance emerges from leukaemia stem cells. Nature. 2015;525(7570):538–42. doi:10.1038/nature14888.
124. Nebbioso A, Carafa V, Benedetti R, Altucci L. Trials with 'epigenetic' drugs: an update. Mol Oncol. 2012;6(6):657–82. doi:10.1016/j.molonc.2012.09.004.
125. Qiu T, Zhou L, Zhu W, Wang T, Wang J, Shu Y, et al. Effects of treatment with histone deacetylase inhibitors in solid tumors: a review based on 30 clinical trials. Future Oncol. 2013;9(2):255–69. doi:10.2217/fon.12.173.
126. Konantz M, Balci TB, Hartwig UF, Dellaire G, Andre MC, Berman JN, et al. Zebrafish xenografts as a tool for in vivo studies on human cancer. Ann N Y Acad Sci. 2012;1266:124–37. doi:10.1111/j.1749-6632.2012.06575.x.
127. MacRae CA, Peterson RT. Zebrafish as tools for drug discovery. Nat Rev Drug Discov. 2015;14(10):721–31. doi:10.1038/nrd4627.
128. Lee LM, Seftor EA, Bonde G, Cornell RA, Hendrix MJ. The fate of human malignant melanoma cells transplanted into zebrafish embryos: assessment of migration and cell division in the absence of tumor formation. Dev Dyn. 2005;233(4):1560–70. doi:10.1002/dvdy.20471.
129. Haldi M, Ton C, Seng WL, McGrath P. Human melanoma cells transplanted into zebrafish proliferate, migrate, produce melanin, form masses and stimulate angiogenesis in zebrafish. Angiogenesis. 2006;9(3):139–51. doi:10.1007/s10456-006-9040-2.
130. Marques IJ, Weiss FU, Vlecken DH, Nitsche C, Bakkers J, Lagendijk AK, et al. Metastatic behaviour of primary human tumours in a zebrafish xenotransplantation model. BMC Cancer. 2009;9:128. doi:10.1186/1471-2407-9-128.
131. Nicoli S, Ribatti D, Cotelli F, Presta M. Mammalian tumor xenografts induce neovascularization in zebrafish embryos. Cancer Res. 2007;67(7):2927–31. doi:10.1158/0008-5472.CAN-06-4268.
132. Weiss FU, Marques IJ, Woltering JM, Vlecken DH, Aghdassi A, Partecke LI, et al. Retinoic acid receptor antagonists inhibit miR-10a expression and block metastatic behavior of pancreatic cancer. Gastroenterology. 2009;137(6):2136–45 e1–7. doi:10.1053/j.gastro.2009.08.065.
133. Lam SH, Chua HL, Gong Z, Lam TJ, Sin YM. Development and maturation of the immune system in zebrafish, *Danio rerio*: a gene expression profiling, in situ hybridization and immunological study. Dev Comp Immunol. 2004;28(1):9–28.

134. Bansal N, Davis S, Tereshchenko I, Budak-Alpdogan T, Zhong H, Stein MN, et al. Enrichment of human prostate cancer cells with tumor initiating properties in mouse and zebrafish xenografts by differential adhesion. Prostate. 2014;74(2):187–200. doi:10.1002/pros.22740.
135. Eguiara A, Holgado O, Beloqui I, Abalde L, Sanchez Y, Callol C, et al. Xenografts in zebrafish embryos as a rapid functional assay for breast cancer stem-like cell identification. Cell Cycle. 2011;10(21):3751–7. doi:10.4161/cc.10.21.17921.
136. Smith AC, Raimondi AR, Salthouse CD, Ignatius MS, Blackburn JS, Mizgirev IV, et al. High-throughput cell transplantation establishes that tumor-initiating cells are abundant in zebrafish T-cell acute lymphoblastic leukemia. Blood. 2010;115(16):3296–303. doi:10.1182/blood-2009-10-246488.
137. Pruvot B, Jacquel A, Droin N, Auberger P, Bouscary D, Tamburini J, et al. Leukemic cell xenograft in zebrafish embryo for investigating drug efficacy. Haematologica. 2011;96(4):612–6. doi:10.3324/haematol.2010.031401.
138. Corkery DP, Dellaire G, Berman JN. Leukaemia xenotransplantation in zebrafish—chemotherapy response assay in vivo. Br J Haematol. 2011;153(6):786–9. doi:10.1111/j.1365-2141.2011.08661.x.

Modeling Cancer Metastasis

7

Nora M. Navone and Estefania Labanca

Abbreviations

3D	Three dimensional
cPDXs	Cancer cells derived from PDXs
ECM	Extracellular matrix
PBS	Phosphate-buffered saline
PCa	Prostate cancer
PDXs	Patient-derived xenografts
UICC	Union for International Cancer Control

Introduction and Background

When tumor cells spread and grow in sites noncontiguous to the organ of origin (primary site) and become metastatic, cancer enters a treatment-resilient stage. At this stage, the prospects for a cure using conventional therapy (e.g., surgery, radiotherapy) are greatly reduced, and although chemotherapy and targeted therapies may prolong the lives of patients, these treatment modalities are, in general, not curative, and the disease eventually becomes treatment resistant.

Metastases result from a multistage process that cancer cells at the primary site must undergo to establish tumors at distant sites. Put simply, these steps include local invasion of the surrounding extracellular environment, degradation, and passage through a basement membrane followed by intravasation into the blood or lymphatic system, survival in circulation (hematogenous and/or lymphatic), arrest at a distant organ (involving lodging in a distant capillary bed), extravasation out of the blood vessel, survival in the new environment, and invasion and proliferation in what is

N.M. Navone (✉) • E. Labanca
Department of Genitourinary Medical Oncology, The University of Texas-MD Anderson Cancer Center, Houston, TX, USA
e-mail: nnavone@mdanderson.org; ELabanca@mdanderson.org

© Springer International Publishing AG 2017

Y. Wang et al. (eds.), *Patient-Derived Xenograft Models of Human Cancer*, Molecular and Translational Medicine, DOI 10.1007/978-3-319-55825-7_7

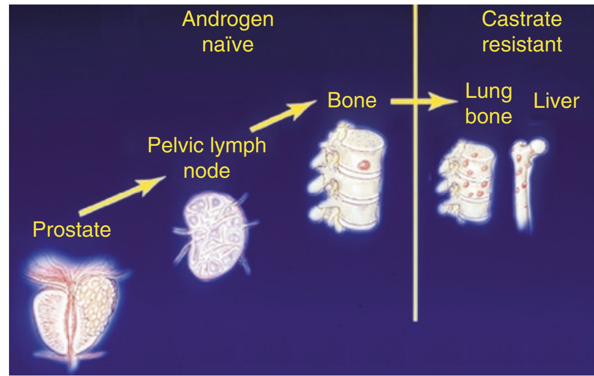


Fig. 7.1 A model of PCa progression. PCa starts as a localized tumor. In its natural progression, it typically metastasizes to the lymph nodes and then the bone, producing bone-forming metastases. Most PCas respond to androgen ablation therapy, but relapse usually occurs, and bone is the primary site of progression

now a foreign cellular microenvironment (reviewed in [1]). During the development of metastases, tumor cells must have or acquire specific traits to successfully go through this multistep process, survive, and interact with the microenvironment, including the immune system [1–5]. In Stephen Paget's 1889 work, he proposed that metastasis depends on cross talk among cancer cells (the "seeds") and organ microenvironments (the "soil"). This seed and soil hypothesis still holds true today. In the 1970s, authors documented the selective nature of metastasis. A detailed analysis of experimental metastases in syngeneic mice indicated that mechanical arrest of tumor cells in the capillary bed of distant organs did indeed occur but that subsequent cell proliferation and growth into secondary lesions were influenced by specific organ cells [6]. A clear example of the selectivity of cancer cells for specific sites of metastasis is the pattern of progression to the bone of prostate cancer (PCa). Indeed, 80% of patients who die of PCa have bone metastasis, and their metastases are consistently bone forming, although an osteolytic component in PCa bone metastases is always present (Fig. 7.1) [7–9]. The bone houses the hematopoietic stem cell niche, which comprises hematopoietic and mesenchymal cell populations that regulate hematopoietic stem cell self-renewal [10]. Authors suggested that disseminated PCa cells can precondition the metastatic niche and that PCa cells competes with and occupy the hematopoietic stem cell niche to facilitate metastasis [11, 12].

A more comprehensive understanding of PCa resulted in clinical division of the disease into subgroups having various patterns of progression to metastasis [13]. The pathologic classification of PCa is defined using the Gleason sum score (UICC), which is based on morphologic criteria [14]. Researchers categorized the clinical course of PCa from diagnosis to death as a series of clinical stages or treatment statuses (e.g., extent of the local disease, hormonal status, the absence or presence of detectable metastases on an imaging study). Prostate-specific antigen level is widely used to monitor disease progression and response to treatment. Although these clinical stages or treatment statuses are currently used to establish therapeutic objectives and outcomes, investigators have proposed a new molecular classification of PCa that incorporates androgen receptor level, oncogenes/tumor suppressors, and the tumor-bone microenvironment in the disease model. This proposed classification may facilitate the implementation of current and emerging therapies [15].

The cascade of events that lead to metastatic dissemination starts long before a tumor is clinically detected at a distant site [4, 16]. Metastatic dissemination possibly continues throughout the progression of the disease. Therefore, precise understanding of the molecular mechanism underlying each step in the metastatic process requires longitudinal analyses of human cancer progression in addition to laboratory-based studies. Some such studies have demonstrated the anarchic evolution of metastases, which in some cases caused local recurrence, whereas others had cross-metastatic site seeding [4, 16, 17]. In fact, one study demonstrated the presence of metastatic and primary tumor clones in blood even years after removal of the primary tumor, supporting the concept the anarchic evolution of metastases [16].

Solid tumors are typically heterogeneous, and studies have suggested the existence of clonal evolution of metastases as well as genetic and epigenetic diversity of primary and metastatic clones [4, 18]. This clonal diversity may be reduced or enhanced at both primary and metastatic sites by systemic treatment.

In this chapter, we primarily discuss epithelial-derived cancers with special emphasis on modeling the metastasis of PCa. PCa is the second leading cause of cancer deaths among American men. PCa cells are particularly difficult to grow on two-dimensional platforms, which are evidenced by the paucity of PCa cell lines available for study. Thus, PDXs are not only clinically relevant but, in the case of PCa, essential for PCa research given the paucity of cancer cell lines.

PCa PDX Models

In 1996, with the support of the Prostate Cancer Foundation, we established the PCa PDX program in the Department of Genitourinary Medical Oncology at MD Anderson. This program has the goal of developing PDXs from annotated tissue samples obtained from men undergoing radical prostatectomy, cystoprostatectomy/pelvic exenteration, or resection/biopsy analysis of metastatic lesions. PDXs are derived from advanced therapy-naïve prostate tumors or prostate tumors resistant to various therapies. By generating PDXs from different areas of the same tumor, we have developed models of PCa heterogeneity. PDXs developed in our facility include adenocarcinomas derived from therapy-naïve and therapy-resistant primary tumors and metastases [19–21]. Atypical clinical and histopathological variants PCa are also available [21, 22]. To date, the program has processed tumor samples derived from more than 270 patients with PCa for PDX development and established two PCa cell lines (MDA PCa 2a and 2b) and PCa PDXs from more than 76 donors. PDXs developed in our program are derived from patients with advanced PCa. We derived PCa PDXs from tumors in the prostate; areas of direct tumor extension to adjacent organs, the bone, lymph nodes, the liver, the thyroid, a testis, the adrenal gland, the brain, and unusual sites (e.g., skin, chest wall, soft tissue); ascites; and pleural effusions. PDXs are identified by the prefix MDA PCa followed by a number that is unique to the donor tumor, tumor site, and procedure date (e.g., MDA PCa 144). Because this is an ongoing program, the clinical and molecular evolution of PCa as a result of the development of resistance to new and upcoming

therapies will be reflected in our dynamic repository. Because this PDX repository is dynamic, the number of developed PDXs provided above will change over time. The use of these PCa PDXs by investigators has been described in various reports of their research [19–44].

Also, to be able to study and understand the heterogeneity of PCa, when the tumor of origin is large, we submit human PCa tissue samples from different areas of the same tumor for PDX development, which are established as independent PDXs and independently identified according to a unique suffix, such as MDA PCa 144-2 and MDA PCa 144-4.

The PCa PDX program operates within a highly integrated network of physicians, scientists, staff, and resources in the Tissue Biospecimen and Pathology Resource at MD Anderson. These individuals include urologists, oncologists, pathologists, staff of the Department of Diagnostic Radiology (when tissue samples are obtained using image-guided biopsies), and staff who provide regulatory and compliance support for archived tissue and blood sample requests, consent validation, protocol submissions to the MD Anderson Institutional Review Board, material transfer agreements, data management, and logistic issues concerning sample shipments. This team ensures that complete patient information is captured for comparison with the derived PDXs.

Patient-Derived Xenografts as Models for Studying Metastasis

In recent years, the scientific community has recognized that patient-derived xenografts (PDXs) are far superior to cell lines for studying mechanisms of response of cancer to treatment with US Food and Drug Administration-approved and investigational agents and in discerning mechanisms of treatment resistance of human cancer. Furthermore, specific pathways involved in the metastatic process may be better reflected by early-passage PDXs, although cell lines are still useful in mechanistic studies.

Selection of approaches to modeling metastases using PDXs should be based on the specific pattern of progression of the disease of interest while taking into consideration the stages of the metastatic process that are to be modeled as well as heterogeneity and clonal evolution. For example, when studying the growth of PCa cells in the bone, in addition to using bone metastasis-derived PDXs, considering whether the process to be modeled would be influenced by the treatment status of the patient may be beneficial.

Modeling Specific Steps Involved in Metastasis

Cell Migration and Invasion

As described previously [1], migration and invasion are the initial events required for a solid tumor to spread and disseminate throughout the body. Cell migration is a

multistep process; the cell motility cycle is the basic component of cell migration and consists of a series of well-organized events. These events begin with asymmetric morphology and definition of leading and trailing edges in the cancer cell. Intracellular signals then orient protrusions in the leading edge of the cell, which is followed by a sequence of contraction and detachment movements via integrin-mediated adhesion [45].

Multiple factors can influence cancer cell migration, including the topography of the extracellular matrix (ECM), cell polarity, and cell adhesion [45]. In vitro experiments have demonstrated that tumor cells invading three-dimensional (3D) matrices remodel the ECM microenvironment to migrate. These experiments also have shown that collagen fibers aligned in parallel can promote or at least enable cancer cell invasion, whereas a disorganized, nonlinear matrix reduces invasive behavior. These data suggest that oriented ECMs play a part in directional migration and invasion of cancer cells in vivo [46]. In addition to single-cell migration, collective migration is a principal mode of cell movement in which cells remain connected as they move. Collective migration of cohesive cell groups in vivo is a feature of many invasive tumor types [47].

The invasive phenotype, which distinguishes benign from malignant neoplasms (cancer), is defined by the ability to actively breach or cross tissue barriers, including the bone marrow [48]. This phenotype is manifested at different steps of the metastatic cascade, including escape of cancer cells from the primary tumor, intravasation into and extravasation from the bloodstream, and establishment of a secondary tumor at a distant site. Invasion requires adhesion to and degradation of ECM components and restructuring of the cytoskeleton along with transcriptional and epigenetic changes. Furthermore, invasive cells can adopt different morphogenetic programs, and this transition to a different program is influenced by the tumor microenvironment. When invasive cancer cells utilize a mesenchymal invasion program, the switch from epithelial to mesenchymal cell phenotype is often referred to as epithelial to mesenchymal transition [48]. Recently reported evidence indicated that cells are in cell-cycle arrest when they enter an invasive state. This cell-cycle arrest state is frequently associated with the invasive phenotype acquired via epithelial-to-mesenchymal transition [48–51].

Processing Fresh PCa PDXs for In Vitro Studies

The use of cancer cells derived from PDXs (cPDXs) in laboratory-based research in vitro requires the separation of cancer cells from the mouse stroma. This process constitutes a challenge of differing magnitude depending on the cancer of origin. In the case of PCa, cPDXs do not grow in vitro for long periods, so studies using them must be performed with short-term cultures. cPDXs derived from other malignancies are more amenable to growth through several passages in vitro and may even develop into cell lines. However, during the development of these cell lines, selection of cells for in vitro growth is likely. Thus, short-term cultures are thought to better reflect the phenotype typical of 3D growth and the heterogeneity typical of

PDXs than are cell lines. The method we use to isolate cPDXs enriched in PCa cells is described below.

1. Anesthetize PDX-bearing mice by administering isoflurane according to training in the Department of Veterinary Medicine and using an approved inhalation-induction-vapor recovery apparatus. Administer isoflurane at concentrations of 4–5% for induction and 2–3% for maintenance of anesthesia.
2. Kill tumor-bearing host mice via cervical dislocation.
3. Aseptically remove subcutaneous tumors from euthanized host mice as follows: clean the skin over the tumor with 70% ethanol spray, open the skin with a scalpel, and remove the tumor by using scissors to dissect it free from surrounding loose connective tissue (PCa PDXs growing subcutaneously usually do not invade surrounding tissues).
4. Place tumor tissue in a sterile 50-ml conical tube. Rinse the tumor tissue three times using fresh phosphate-buffered saline (PBS) each time.
5. Place the rinsed tumor tissue in a sterile tissue culture dish. Using scissors, remove any remaining connective or other nontumor tissues surrounding the tumor as well as any visible necrotic sections of the tumor.
6. Place the cleaned tumor tissue in a sterile tissue culture dish, and cut it into small pieces with a sterile scalpel. Use a sharp blade to avoid compression damage to the tissue. Place tissue pieces and any spilled cells into a new sterile 50-ml conical tube, wash them with 1× PBS, spin the tissue down in a centrifuge at 300 × *g* for 5 min, and discard supernatant. This is done under a sterile laminar flow hood.
7. Add Accumax enzyme solution (Innovative Cell Technologies) to pelleted PCa tissue in a conical tube (add enough enzyme to cover the pellet). Incubate the tube for 20 min at 37 °C in a rotation shaker set at 150 rounds/min.
8. Filter out cell clamps from the resulting solution by using a sterile 70-μm-diameter pore cell strainer into a new 50-ml conical tube. This is done under a sterile laminar flow hood.
9. Spin the resulting filtrate in a centrifuge at 300 × *g* for 5 min at room temperature. Remove and discard the supernatant. Resuspend the pellet in 20 ml of alpha-MEM medium containing 10% fetal bovine serum to neutralize any remaining Accumax enzymes.
10. Rinse tumor solution three to four times, using fresh PBS each time
11. Count the viable cells in the suspension prepared in **J** using a hemocytometer under a light microscope with a trypan blue assay.
12. Spin the solution prepared in **J** again in a centrifuge at 300 × *g* for 5 min at room temperature. Discard the supernatant as described above, and then adjust the tumor cell concentration with growth medium to the desired cell density, and plate it in tissue culture dishes according to the study to be performed.

As previously mentioned, the genetic manipulation (e.g., transfection, silencing of gene expression) of PCa cPDXs is challenging because most of these cPDXs can be propagated in vitro only in short-term cultures. One approach to genetically

manipulate cPDXs that is promising is the use of a green fluorescent protein (or green fluorescent protein-like)-tagged virus or bicistronic expression vectors for simultaneous expression of green fluorescent-like proteins and an insert DNA whose expression would lead to modulation of protein expression by overexpression, expression of mutant variants, or gene silencing. GFP tagged CRISPR-Cas9 viral systems can also be utilized for targeted genome editing of cPDXs. Via sequential grafting of infected cPDXs in mice and ex vivo selection of labeled PCa cells using cell sorting, a PDX containing genetically manipulated cancer cells can be established in vivo. This PDX retains the benefits of heterogeneity and in vivo growth, although a certain level of selection of cells probably occurs. This procedure is difficult, and the difficulty is primarily related to the feasibility of growing cPDXs in vitro in monolayers, with PCa cPDXs being the most challenging.

Recent reports described the establishment of organoids from PCa tissue or PDXs. These 3D in vitro procedures are more enabling of genetic manipulation of cPDXs than short-term cultures of cPDXs in monolayers, although these procedures are laborious. Full, detailed protocols for the development of organoids were recently published [52, 53].

Modeling Cell Migration and Invasion In Vitro

Single or collective cell migration in two-dimensional in vitro models can be measured using video microscopy (random motility) or by scraping a monolayer of confluent cells in culture and monitoring their ability to migrate back into the "wounded" area (wound healing assay; directed motility). This method allows for the study of polarization, force generation, and mechanisms of cell-cell cohesion during the movement of confluent monolayers [47, 54, 55].

A Boyden chamber-type system (i.e., a barrier in culture through which cellular invasion can be monitored and quantitated) has been used to model cellular invasion. Several filters through which the cells crawl have been used. More frequently, an 8-μm pore filter is covered with a reconstituted ECM such as Matrigel (Corning Life Sciences and BD Biosciences), which mimics a collagen IV-rich basement membrane, fibronectin, laminin, or a fibrillar collagen I-like meshwork [54]. Similarly, the ability of cancer cells to enter and traverse thick (~2-mm) collagen gels or a monolayer of ECM-producing stromal cells can be used to recapitulate cellular invasion. In each case, chemotactic invasion can be measured by filling the lower chamber of the Boyden chamber-type system with a source of growth factors.

In collective cell migration, several mechanisms polarize the cells as "leader" cells that guide "followers" behind them. The differences between leaders and followers are their clear differences in cell morphology and gene expression. Whereas cells at the leading edge of migration are often less ordered and mesenchyme-like, cells at the rear form more tightly packed assemblies, such as rosettes and tubular networks [47]. Other approaches to studying the invasion of cancer cells include the use of 3D scaffolds overlying ECM alone or with mesenchymal cells. These

platforms demonstrate how collective cancer cell invasion is facilitated by both leading cancer cells and leading stromal fibroblasts [56, 57]. For example, authors reported that squamous cell carcinomas that retain epithelial markers cannot remodel the surrounding matrix but instead follow stromal fibroblasts that remodel the ECM [56].

Researchers have developed new technology to monitor cell migration/invasion. For example, the xCELLigence RTCA DP instrument (ACEA Biosciences Inc.) can kinetically measure cell invasion and migration using an electronically integrated Boyden chamber (CIM-Plate 16). Another new development is the 3D spatially organized cancer invasion platform, a microfluidic cancer invasion platform capable of spatially organizing 3D cell-embedded hydrogel matrices while enabling real-time 3D capture of cancer invasion within heterogeneous ECMs [47, 58, 59].

Cancer Cell-Host Cell Interactions

Metastasis requires interaction between cancer cells and their microenvironment. This is particularly striking in the case of PCa, which is the only major malignancy that consistently produces bone-forming metastases, suggesting that factors secreted by PCa cells induce bone formation. In this context, a vicious cycle mediated by soluble factors released by cancer cells and the bone is implicated to support cancer growth. Authors originally described this cycle in the interaction of breast tumor cells with bone cells [60]. Bone homeostasis is maintained by equilibrium of bone formation (mediated by osteoblasts) and bone resorption (mediated by osteoclasts). This balance is disrupted when cancer cells arrive and grow in the bone. Researchers proposed that tumor cells, osteoblasts, osteoclasts, and bone matrices are the four components of the vicious cycle necessary for the initiation and development of bone metastases [60]. Gene expression in tumor cells is modified by factors released from the bone matrix by osteoclast-mediated resorption. In PCa patients, bone lesions are frequently osteoblastic; thus, osteoblast activation is suggested to produce factors that favor the growth of PCa cells [19, 31, 32, 61, 62].

Boyden chamber-type systems have been used to study the effect of soluble factors released by cancer cells and host cell in the cancer cell-host cell interaction. The benefit of these systems is that the effect of soluble factors in the two cell types can be analyzed separately at the end of the study. With the use of this system, we reported that soluble factor release by PCa cells induced the expression of osteoblast-specific factors in osteoblasts and that the Wnt canonical pathway mediated (at least in part) PCa-induced new bone formation (Fig. 7.2) [19, 62, 63].

Another approach to study cancer cell-host cell interactions involves the use of encapsulated cPDX tumor cells in a 3D hyaluronan-based hydrogel. Given the ubiquity of hyaluronic acid in the bone marrow ECM, this approach may be useful in studying PCa cell-bone cell interaction. Use of this approach demonstrated that the hydrogel maintained PDX cell viability with continuous native androgen receptor expression (Fig. 7.3) [26].

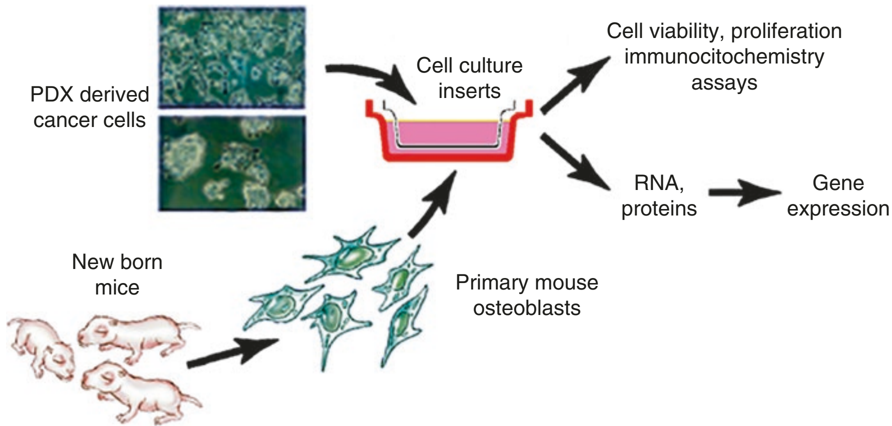


Fig. 7.2 Illustration of a Boyden chamber-type system used to study the interaction between PDX-derived cells (short-term cultures) and primary osteoblasts derived from the calvariae of 4-day-old mice

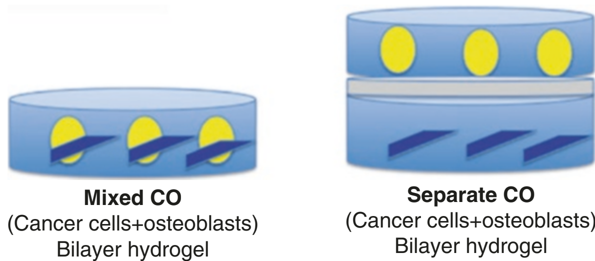


Fig. 7.3 Schematic representation of a 3D hydrogel system for PCa cPDXs and the mouse osteoblast cell line MC 3T3-E1. The hydrogel system comprises the thiolated hyaluronic acid and acrylate-functionalized peptides GRGDS (integrin binding) and PQ (matrix metalloproteinase sensitive). *Left*, cPDXs are co-encapsulated with osteoblastic (MC 3T3-E1) cells. Thus, cPDXs and MC 3T3-E1 cells are in direct contact. *Right*, cPDXs and osteoblastic (MC 3T3-E1) cells are encapsulated separately. Thus, their effects are mediated by soluble factors

Tumor Cell Intravasation

The chorioallantoic membrane in chicks is an accessible, blood vessel-rich membrane onto which cPDXs can be placed to test their ability to intravasate into nearby blood vessels and disseminate. This can be studied in vivo in live chick embryos [64, 65] (reviewed in [66]).

Modeling Metastases in Mice In Vivo. Historically, the mouse has been widely used as a model organism to study and molecularly dissect the process of metastasis in vivo. Studies may involve the entire metastatic process when cells are injected orthotopically (in the site of origin of the cancer), and metastatic spread is monitored macroscopically or by labeling the input cells with a

transgene-expressing luciferase, which enables them to be tracked in vivo. In general, subcutaneous grafting of PDXs does not result in distant metastasis. Orthotopic engraftment of colorectal carcinoma PDXs has resulted in the development of liver and lung metastases in a proportion of cases [67]. Reports on metastases of breast cancer PDXs implanted orthotopically in the mammary fat pad in mice included different metastatic outcomes. In one report, metastases occurred at a very low rate (3 of 144 mice) after long periods of observation following resection of primary tumors [68]. Furthermore, these metastases did not result in a more aggressive metastatic phenotype upon retransplantation into new host tumors [68]. In two other reported studies, lymph node and lung metastases were frequently detected [69], with lung metastases found in 48% of cases [70]. Recently, authors reported that thin tissue slice grafts of renal cell carcinomas had the potential to metastasize to clinically relevant sites, including the liver, lung, and bone. Also, lymph node metastases were reported to develop after intramuscular implantation (in the quadriceps of mice) of PDXs derived from highly aggressive squamous cell carcinomas of the uterine cervix [71].

Frequently, researchers design their studies to focus on specific aspects of the metastatic process in vivo. For example, cell motility and invasion in vivo can be studied using intravital microscopy. The ability of cells to reach specific organs and grow can be studied with intracardiac injection of cancer cells into the left ventricle (termed "experimental" metastasis). In this scenario, the initial steps of metastasis are bypassed. Finally, the interaction of cancer cells with the host cells at the metastatic site can be studied using direct injection of the cancer cells into the organ subject of study. This strategy does not provide information about the metastatic process but does provide important information on the tumor/stroma interactions that lead to tumor growth at the metastatic site.

These experimental procedures have strengths and shortcomings, and determining the value of each of these methods comes down to the scientific question that is being addressed and how well the methods for answering the question were selected. For example, intracardiac injection of cancer cells into the left ventricle will provide important evidence regarding the preferred site of metastasis of a given cPDX and how this tropism can be altered by genetically manipulating the injected cells. However, the effects of genetic manipulation of the injected cells in the initial steps of metastasis cannot be studied using intracardiac injection. Also, the effects of genetic manipulation of the injected cPDX on cancer cell-host cell interaction at the metastatic site cannot be accurately assessed because the pattern of metastasis is unpredictable. A more informative method for the latter would be direct injection of a cPDX into the metastatic site because the subject of the study is controlled. For preclinical/co-clinical studies, direct injection of a cPDX into the organ of interest is preferred if the purpose of the study is to identify means of controlling the growth of established metastases. If the goal is to prevent the development of metastases, either orthotopic or intracardiac injection would be adequate. These methods are described below.

Intravital Microscopy

An approach to studying cell motility and invasion in vivo is labeling injected cells with a vital dye or a green fluorescent protein-tagged transgene, which allows for evaluation of the initial steps of metastasis using intravital microscopy. However, this approach can only be used when the tissue of interest can be accessed directly through an imaging window or surgically [72–74].

Briefly, multiphoton microscopy is based on the use of two or more low energy photons rather than a single higher-energy photon in examination of a sample. As a result, the focus is only on a dot, and no excitation or bleaching occurs above or below it. A significant advantage of using lower-energy excitation is penetration. Both deep-tissue imaging [75, 76] and imaging resolution at the single-cell level for monitoring of tumor cell behavior during metastasis [77–79] are possible thanks to multiphoton excitation. This technology allows for imaging even at the subcellular level, resulting in definition of parameters such as cell kinetics, morphology, the presence and nature of protrusions, and the proliferative state to further understand tumor heterogeneity and better outline its role in the different steps of metastasis.

A great benefit of assessing tissues using this technology is that they generate intrinsic signals on their own. For example, collagen fibers making up the ECM in bone tissue become optically accessible by second harmonic generation. Additionally, third harmonic generation permits visualization of cell and tissue interfaces, such as water-lipid interfaces, including adipocytes, microvesicles, exosomes, and nerves, owing to the high density of myelin in them. Technologies that do not require external probes include optical frequency domain imaging for deep, continuous imaging over time [80] and Coherent anti-Stokes Raman spectroscopy for studying lipid distribution [81]. Even moving nonfluorescent host cells can be imaged as a "side effect" of fluorescent tumor cells caused by light scattering by the resident cells, particularly immune cells, that form shadows outlining their shapes [82]. Many of the components of the tumor microenvironment are readily capable of being imaged.

The role of the microenvironment in metastasis can be studied via simultaneous imaging (using intravital microscopy) of stroma and tumor cell components stained with dyes in multiple colors [77, 83]. A wide variety of vital dyes can be injected directly into mice, enabling staining of specific cell types and/or subcellular structures endogenously [84]. For example, AngioSense (PerkinElmer), fluorescent dextrans, quantum dots (nanocrystals), and fluorescently labeled lectins that selectively bind to endothelial cells [85] can be used to visualize blood vessels and lymph nodes. Also, distinct cell populations, such as macrophages and monocytes, can be labeled with iron oxide nanoparticles. Furthermore, the activity of some enzymes can be examined, such as metalloproteinases using MMPSense (PerkinElmer) and cathepsins using ProSense®. Other agents, such as blue Hoechst 33342, are used for ex vivo cell staining. Stable expression of the fluorescence ubiquitination cell cycle sensor reporter system can be used to monitor the proliferative status of tumors implanted subcutaneously in mice [86]. OsteoSense 680 EX (PerkinElmer) is an in vivo imaging agent used to study bone growth and resorption by defining areas of

microcalcification and bone remodeling using fluorescent bisphosphonate. Photoswitchable flourophores such as Dendra2, PSmOrange, and Kaede, which act by switching the color of a subset of cells, are applied to tracking the position and motility of tumor cells in subsequent imaging over multiple days. With the use of this feature, studies of primary cancer cell colonization at distant organs in a mouse orthotopic mammary cancer model revealed that some tumor microenvironments could stimulate metastatic behavior, whereas others exhibited lower rates of tumor cell invasion and intravasation [87].

Single-cell resolution studies identified different modes of migration of tumor cells—mesenchymal, ameboid and blebby, streaming, and collective—which can be found together in strands, clusters, or single cells in the same lesion. Multiphoton excitation allows for optical examination of morphologic cell changes. Reduction in compactness of ECM network during carcinogenic progression frees up cell movement and morphology. In general, the shapes of the tumor cells are not elongated, rigid, or fibroblastic anymore after reduction of ECM network but tend to be ameboidal with extending lamellipodia at their edges [76, 79]. At the subcellular level, the arrangement of the cytoskeleton components in migrating cells, assayed via fluorescent labeling of the myosin light chain in the actomyosin, demonstrated how the myosin light chain localizes to extending protrusions in motile cells and that these protrusions, or invadopodia, cause ECM deformation [88]. Signaling pathways involved in migration in situ also can be visualized.

As described previously, intravital microscopy can be used only when the tissue of interest can be accessed directly through an imaging window [72, 74]. Researchers have developed different approaches to accessing tumor tissue optically. The dorsal skinfold chamber is one widely used technique, although a disadvantage of this method is that the tumor grows ectopically (except for skin-derived cancers) and the size of the tumor is limited to that of the slide inside the chamber. Also, investigators developed a cranial window implant to image the brain cortex, which is facilitated by the shallow thickness and thus the relative transparency of the brain surface [89]. Abdominal imaging windows are used to study tumors in the intestine, pancreas, and liver. Mammary imaging windows are used to recapitulate breast tumors orthotopically. Other organs can be made more accessible using surgical procedures with variable degrees of invasiveness. A small skin incision enables imaging of popliteal lymph nodes [90], whereas the liver can be visualized via previous exteriorization and superfusion with physiological saline solution [91]. In developing models of other organs, such as the kidney, pancreas, spleen, and heart, researchers must consider inflammatory responses as well as muscle contractions, respiration, and blood vessel pulses, which can cause unwanted movements during imaging that must be restrained under deep anesthesia and with some form of physical immobilization.

Further methods to accessing tumor tissue optically and model the specific microenvironmental site in an ectopical imaging chamber context have been introduced. The application of engineered scaffolds to mimicking the bone as a preferred site of metastasis of breast and PCa and as a key site of stem cell development has

been useful. These are called implantable microenvironments and can be visualized in a dual skinfold chamber [92]. Tissue engineering is another field that will help further model metastases for the study of invasion, migration, and cancer cell-host cell interaction.

Intracardiac Injection of Tumor Cells into the Left Ventricle

Intracardiac injection of tumor cells measures experimental metastases and is aimed at the direct release of cPDXs into the left ventricle so that they can reach all organs via the blood vessels. The cPDXs can be labeled with luciferase so that they can be visualized when they grow in distant organs. The methods we use to implant tumor cells into the left ventricle are described below:

1. Using the solution prepared in **J** (processing fresh PCa PDXs for in vitro studies), spin it again in a centrifuge at 300 × *g* for 5 min at room temperature. Discard the supernatant, and then adjust the tumor cell concentration by adding 1× PBS as needed to produce a final suspension containing 0.5×10^6 to 1.5×10^6 tumor cells per 50–100 µl. Place the tube containing the final suspension on ice in preparation for implantation of the cells into mice as described below.
2. Anesthetize 5- to 6-week-old male severe combined immunodeficiency mice that can receive tumor cells using isoflurane as described above.
3. With the mouse placed in supine position (dorsal side down), fix the legs with tape. The mouse should be symmetrically positioned. Sanitize the surgical field (chest) by spraying it with 70% alcohol, and dry it with sterile gauze.
4. Identify and mark the sternal notch and xyphoid process. Mark the middle point between them slightly to the left of the sternum (in the intercostal space).
5. Gently finger flip a few times a tube containing a prepared and iced tumor cell-PBS suspension to remix and aspirate 200 µl of the suspension into a syringe with a 26.5-gauge needle, leaving 200 µl of air space. Air must be near the plunger to allow for blood pumping when the needle enters the left ventricle. Note: the tumor cells must be free of aggregates to prevent embolic obstruction.
6. Inject cells into the marked point, maintaining the needle and syringe in a vertical position at 90°. Successful insertion into the left cardiac ventricle should result in a distinct bright red pulse of blood in the syringe.
7. While holding the syringe steady (with the wrist fixed to the bench), slowly inject 50–100 µl of the cell suspension over 20–30 s. Once the injection is complete, the needle must be taken straight out quickly.
8. Remove the needle quickly and dry the wound with gauze. Quickly withdrawing the needle from the chest prevents seeding of tumor cells in the heart and/or lung. Place pressure on the chest with alcohol wipes for about 30 s to prevent the bleeding.
9. Return the mouse to a clean cage and observe it for bleeding or other unusual behavior. Wait for the mouse to recover completely from the anesthesia.

Metastases to the bone can be visualized in x-ray analyses. Metastatic lesions in the bone are believed to grow in the medullary cavity first. The tumor cells then interact with the surrounding bone cells, activating osteoblasts (bone-forming cells) and osteoclasts (bone-degrading cells). This results in various degrees of bone formation (mediated by osteoblasts) and resorption (mediated by osteoclasts). Bone metastases that predominantly induce bone formation, bone resorption, or mixed bone formation/resorption display predominant osteoblastic, osteolytic, or mixed patterns on radiographs. PCas typically produce bone-forming metastases, whereas breast cancers typically produce osteolytic bone metastases. Furthermore, macroscopic metastases can be identified at necropsy and should be confirmed via histopathological analysis of involved organs. Typically, osteolytic metastases are easily recognized on x-rays, appearing as C-like notches. Figure 7.4 shows two examples of PC3 PCa cells (which are osteolytic) after intracardiac injection in male severe combined immunodeficiency mice.

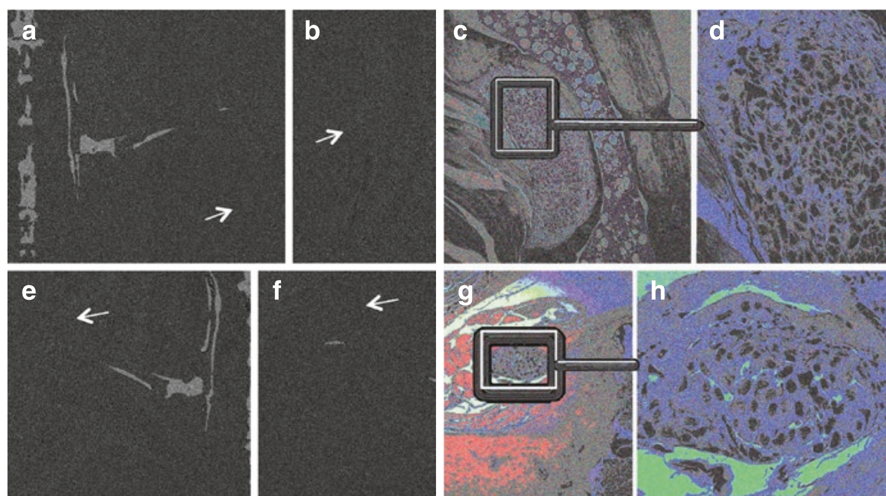


Fig. 7.4 (**a**) Radiographs of a mouse hemipelvis and rear limb illustrating a radiolucent area suspicious of an osteolytic bone metastasis in the right tibia (*arrow*). (**b**) Magnification of the radiolucent area in the tibia. (**c**) Formalin-fixed, paraffin-embedded section of the suspicious area in A demonstrating the presence of a tumor (100× magnification). (**d**) A higher magnification (400×) image of the indicated area in **c**. (**e**) Radiographs of a mouse hemipelvis and rear limb illustrating a radiolucent area suspicious of an osteolytic bone metastasis in the left femur (*arrow*). (**f**) Magnification of the radiolucent area in the femur. (**g**) Formalin-fixed, paraffin-embedded section of the suspicious area in E demonstrating the presence of a tumor (100× magnification). (**h**) A higher magnification (400×) image of the indicated area in **c**

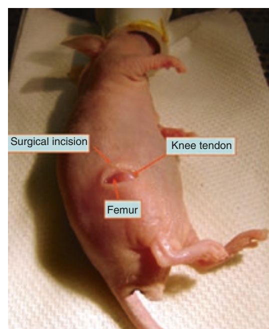
This method has proven very popular, as cells tend to home to the bone, and key events in the metastatic process after dissemination of tumor cells into the circulation can be monitored.

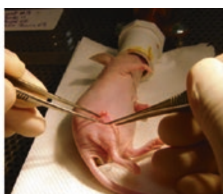
Direct Injection of Tumor Cells into the Metastatic Site

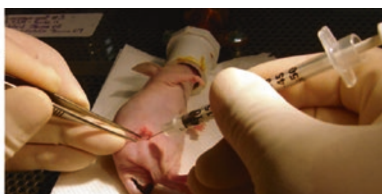
Direct injection of tumor cells is useful for studying cancer cell microenvironment interactions. In particular, bone metastases of PCa are sites of treatment resistance; thus, this approach is useful in co-clinical (i.e., treatment with the same drug or drugs in humans and mice performed in parallel with PDXs with genotypes representing those of the patients) and preclinical studies for assessment of the impact of treatment on not only tumor volume but also PCa cell-bone interaction.

Some investigators perform these studies using intratibial implantation of cancer cells, but we prefer to perform intrafemoral injection, because the bone marrow cavity in the femur is bigger and better defined than that in the tibia. Thus, we can better emulate the way in which PCa cells reach the bone. In addition, we perform our studies with PCa cells confined to the bone. This variable can be better controlled in the femur, which is bigger than the tibia. The methods we use for implantation of tumor cells into mouse bone (femur) are described below.

1. Using the solution prepared in **J** (processing fresh PCa PDXs for in vitro studies), spin it again in a centrifuge at 300 × *g* for 5 min at room temperature. Discard the supernatant, and then adjust the tumor cell concentration by adding 1× PBS as needed to produce a final suspension containing 0.5×10^6–1.5×10^6 tumor cells per 5–10 μl. Place the tube containing the final suspension on ice in preparation for implantation of the cells into mice as described below.
2. Anesthetize 5- to 6-week-old male severe combined immunodeficiency mice that can receive tumor cells using isoflurane as described above.
3. With the mouse placed in the left lateral decubitus position, shave the skin over the distal femur (usually the right femur in our laboratory), and sanitize the surgical field (lateral side of the distal end of the femur) by spraying it with 70% alcohol.
4. Gently finger flip a few times a tube containing a prepared and iced tumor cell-PBS suspension to remix and aspirate 15 μl of it into a sterile, glass-tight 25-μl Hamilton syringe with a 28.5-gauge needle. Place the filled syringe and tube with the cell suspension back on ice. Note: the Hamilton needle must be cut to a length of 2.5–3.0 cm. Next, use a Dremel 10.8-V cordless rotary tool and 541 aluminum oxide grinding wheel to sharpen, polish, and make a smooth blunt end of the Hamilton needle.







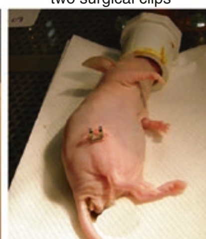


Fig. 7.5 The method used for intrafemoral injection of cPDXs in the bone in male severe combined immunodeficiency mice

5. With sharp 9-cm straight scissors, make a 0.5-cm skin incision on the lateral side of the distal end of the femur (Fig. 7.5). Expose the bone, and manually create a track through the cortical bone into the bone marrow cavity by pushing a sterile 28.5-gauge needle (attached to an insulin syringe) into the femur until the marrow cavity is reached. Note: feeling for the reduction of resistance that occurs when the needle passes from the cortical bone into the bone marrow cavity is important.
6. When the track is made, gently remove the needle, and insert the 28.5-gauge needle attached to the Hamilton syringe containing the tumor cell suspension through the established needle track into the bone marrow cavity. Slowly inject 3–5 μl of the cell suspension, depending on the number of cells desired, into the cavity.
7. Carefully remove the needle and spray the injection area with 70% ethanol. Close the skin wound with one or two surgical clips.
8. Observe the mouse for 24 h after the implantation procedure for any apparent physiologic disturbances, such as infection or inability to walk. Kill any mice with such signs of physiologic distress.
9. Remove the wound clips in the usual manner 10–14 days after the implantation procedure. Note: only manual restraint without anesthesia is necessary during clip removal.

Figure 7.6 shows radiographic images of two PCa PDXs and one PCa cell line with osteoblastic and osteolytic phenotypes. Tumor volume after direct injection of tumor cells in the bone can be monitored using magnetic resonance imaging, and bone reaction to cancer cell growth can be monitored using micro-computed tomography [19, 29, 43, 93]. Histomorphometry of undecalcified bone provides an accurate measure of tumor-induced bone reaction and treatment effects on normal bone and tumor-induced bone reaction [43]. However, interpretation of bone histomorphometric results should consider that these measurements are usually performed in the mid-cancellous region of the distal metaphysis of the femur (Fig. 7.7). Thus, when measuring tumor-bearing bones, the heterogeneity of bone lesions induced by cancer-induced bone remodeling requires a high number of biologic replicates to provide meaningful results.

Although preclinical/co-clinical studies also can be performed after intracardiac injection of cancer cells, the random cancer cell distribution limits quantification of the assay results. Also, these studies cannot distinguish tropism and growth.

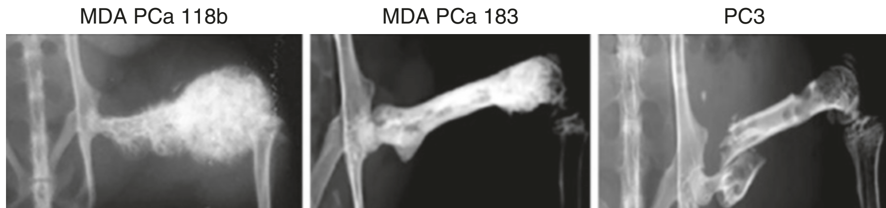


Fig. 7.6 X-ray images of PCa cells growing in bone in 6- to 8-week-old mice (MDA PCa 118b and MDA PCa 183) or 3-week-old mice (PC3) after injection of 106 cells into the distal end of a femur

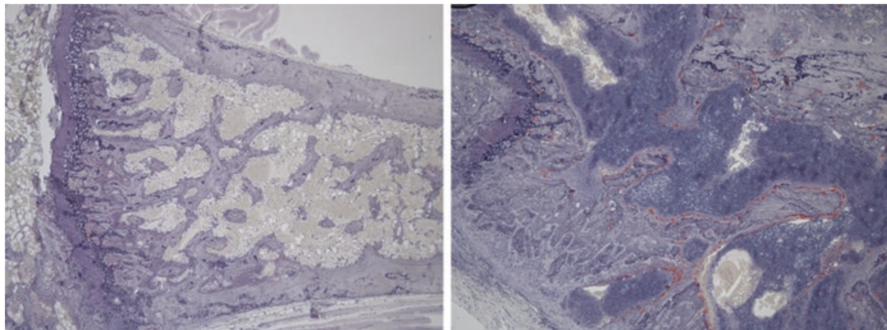


Fig. 7.7 Representative longitudinal section images of undecalcified femurs stained with tartrate-resistant acid phosphatase and counterstained with hematoxylin. *Left*, normal bone. *Right*, tumor-bearing bone. *Growth plate. Magnification, 50×

References

1. Talmadge JE, Fidler IJ. AACR centennial series: the biology of cancer metastasis: historical perspective. Cancer Res. 2010;70(14):5649–69. doi:10.1158/0008-5472.CAN-10-1040.
2. Massague J, Obenauf AC. Metastatic colonization by circulating tumour cells. Nature. 2016;529(7586):298–306. doi:10.1038/nature17038.
3. Sethi N, Kang Y. Unravelling the complexity of metastasis—molecular understanding and targeted therapies. Nat Rev Cancer. 2011;11(10):735–48. doi:10.1038/nrc3125.
4. Turajlic S, Swanton C. Metastasis as an evolutionary process. Science. 2016;352(6282):169–75. doi:10.1126/science.aaf2784.
5. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. Cell. 2011;147(2):275–92. doi:10.1016/j.cell.2011.09.024.
6. Hart IR, Fidler IJ. Role of organ selectivity in the determination of metastatic patterns of B16 melanoma. Cancer Res. 1980;40(7):2281–7.
7. Catalona WJ. Management of cancer of the prostate. N Engl J Med. 1994;331(15):996–1004.
8. Cook GB, Watson FR. Events in the natural history of prostate cancer: using salvage curves, mean age distributions and contingency coefficients. J Urol. 1968;96:87–96.
9. Loberg RD, Logothetis CJ, Keller ET, Pienta KJ. Pathogenesis and treatment of prostate cancer bone metastases: targeting the lethal phenotype. J Clin Oncol. 2005;23(32):8232–41.
10. Taichman RS, Wang Z, Shiozawa Y, Jung Y, Song J, Balduino A, et al. Prospective identification and skeletal localization of cells capable of multilineage differentiation in vivo. Stem Cells Dev. 2010;19(10):1557–70. doi:10.1089/scd.2009.0445.
11. Decker AM, Jung Y, Cackowski F, Taichman RS. The role of hematopoietic stem cell niche in prostate cancer bone metastasis. J Bone Oncol. 2016;5(3):117–20. doi:10.1016/j.jbo.2016.02.005.
12. Shiozawa Y, Pedersen EA, Havens AM, Jung Y, Mishra A, Joseph J, et al. Human prostate cancer metastases target the hematopoietic stem cell niche to establish footholds in mouse bone marrow. J Clin Invest. 2011;121(4):1298–312. doi:10.1172/JCI43414.
13. Beltran H, Tomlins S, Aparicio A, Arora V, Rickman D, Ayala G, et al. Aggressive variants of castration-resistant prostate cancer. Clin Cancer Res. 2014;20(11):2846–50. doi:10.1158/1078-0432.CCR-13-3309.
14. Gleason DF. Classification of prostatic carcinomas. Cancer Chemother Rep. 1966;50(3):125–8.
15. Logothetis CJ, Gallick GE, Maity SN, Kim J, Aparicio A, Efstathiou E, et al. Molecular classification of prostate cancer progression: foundation for marker-driven treatment of prostate cancer. Cancer Discov. 2013;3(8):849–61. doi:10.1158/2159-8290.CD-12-0460.
16. Hong MK, Macintyre G, Wedge DC, Van Loo P, Patel K, Lunke S, et al. Tracking the origins and drivers of subclonal metastatic expansion in prostate cancer. Nat Commun. 2015;6:6605. doi:10.1038/ncomms7605.
17. Crockford A, Jamal-Hanjani M, Hicks J, Swanton C. Implications of intratumour heterogeneity for treatment stratification. J Pathol. 2014;232(2):264–73. doi:10.1002/path.4270.
18. de Bruin EC, McGranahan N, Mitter R, Salm M, Wedge DC, Yates L, et al. Spatial and temporal diversity in genomic instability processes defines lung cancer evolution. Science. 2014;346(6206):251–6. doi:10.1126/science.1253462.
19. Li ZG, Mathew P, Yang J, Starbuck MW, Zurita AJ, Liu J, et al. Androgen receptor-negative human prostate cancer cells induce osteogenesis in mice through FGF9-mediated mechanisms. J Clin Invest. 2008a;118(8):2697–710. doi:10.1172/JCI33093.
20. Roychowdhury S, Iyer MK, Robinson DR, Lonigro RJ, Wu YM, Cao X, et al. Personalized oncology through integrative high-throughput sequencing: a pilot study. Sci Transl Med. 2011;3(111):111ra21. doi:10.1126/scitranslmed.3003161.
21. Tzelepi V, Zhang J, Lu JF, Kleb B, Wu G, Wan X, et al. Modeling a lethal prostate cancer variant with small-cell carcinoma features. Clin Cancer Res. 2012;18(3):666–77. doi:10.1158/1078-0432.CCR-11-1867.
22. Aparicio A, Tzelepi V, Araujo JC, Guo CC, Liang S, Troncoso P, et al. Neuroendocrine prostate cancer xenografts with large-cell and small-cell features derived from a single patient's

tumor: morphological, immunohistochemical, and gene expression profiles. Prostate. 2011;71(8):846–56. doi:10.1002/pros.21301.

23. Brenner JC, Ateeq B, Li Y, Yocum AK, Cao Q, Asangani IA, et al. Mechanistic rationale for inhibition of poly(ADP-ribose) polymerase in ETS gene fusion-positive prostate cancer. Cancer Cell. 2011;19(5):664–78. doi:10.1016/j.ccr.2011.04.010.
24. Dafni H, Burghardt AJ, Majumdar S, Navone NM, Ronen SM. Vascular patterning and permeability in prostate cancer models with differing osteogenic properties. NMR Biomed. 2012;25(6):843–51. doi:10.1002/nbm.1800.
25. Dayyani F, Parikh NU, Varkaris AS, Song JH, Moorthy S, Chatterji T, et al. Combined inhibition of IGF-1R/IR and Src family kinases enhances antitumor effects in prostate cancer by decreasing activated survival pathways. PLoS One. 2012;7(12):e51189. doi:10.1371/journal.pone.0051189.
26. Fong EL, Martinez M, Yang J, Mikos AG, Navone NM, Harrington DA, et al. Hydrogel-based 3D model of patient-derived prostate xenograft tumors suitable for drug screening. Mol Pharm. 2014;11(7):2040–50. doi:10.1021/mp500085p.
27. Fong EL, Wan X, Yang J, Morgado M, Mikos AG, Harrington DA, et al. A 3D in vitro model of patient-derived prostate cancer xenograft for controlled interrogation of in vivo tumor-stromal interactions. Biomaterials. 2016;77:164–72. doi:10.1016/j.biomaterials.2015.10.059.
28. Karlou M, Lu JF, Wu G, Maity S, Tzelepi V, Navone NM, et al. Hedgehog signaling inhibition by the small molecule smoothened inhibitor GDC-0449 in the bone forming prostate cancer xenograft MDA PCa 118b. Prostate. 2012;72(15):1638–47. doi:10.1002/pros.22517.
29. Kundra V, Ng CS, Ma J, Bankson JA, Price RE, Cody DD, et al. In vivo imaging of prostate cancer involving bone in a mouse model. Prostate. 2007a;67(1):50–60. doi:10.1002/pros.20494.
30. Lee YC, Cheng CJ, Bilen MA, Lu JF, Satcher RL, Yu-Lee LY, et al. BMP4 promotes prostate tumor growth in bone through osteogenesis. Cancer Res. 2011;71(15):5194–203. doi:10.1158/0008-5472.CAN-10-4374.
31. Lee YC, Gajdosik MS, Josic D, Clifton JG, Logothetis C, Yu-Lee LY, et al. Secretome analysis of an osteogenic prostate tumor identifies complex signaling networks mediating cross-talk of cancer and stromal cells within the tumor microenvironment. Mol Cell Proteomics. 2015a;14(3):471–83. doi:10.1074/mcp.M114.039909.
32. Lee YC, Lin SC, Yu G, Cheng CJ, Liu B, Liu HC, et al. Identification of bone-derived factors conferring de novo therapeutic resistance in metastatic prostate cancer. Cancer Res. 2015b;75(22):4949–59. doi:10.1158/0008-5472.CAN-15-1215.
33. Li L, Chang W, Yang G, Ren C, Park S, Karantanos T, et al. Targeting poly(ADP-ribose) polymerase and the c-Myb-regulated DNA damage response pathway in castration-resistant prostate cancer. Sci Signal. 2014;7(326):ra47. doi:10.1126/scisignal.2005070.
34. Liu J, You P, Chen G, Fu X, Zeng X, Wang C, et al. Hyperactivated FRS2alpha-mediated signaling in prostate cancer cells promotes tumor angiogenesis and predicts poor clinical outcome of patients. Oncogene. 2015; doi:10.1038/onc.2015.239.
35. Mandelin J, Cardo-Vila M, Driessen WH, Mathew P, Navone NM, Lin SH, et al. Selection and identification of ligand peptides targeting a model of castrate-resistant osteogenic prostate cancer and their receptors. Proc Natl Acad Sci U S A. 2015;112(12):3776–81. doi:10.1073/pnas.1500128112.
36. Mohamedali KA, Li ZG, Starbuck MW, Wan X, Yang J, Kim S, et al. Inhibition of prostate cancer osteoblastic progression with VEGF121/rGel, a single agent targeting osteoblasts, osteoclasts, and tumor neovasculature. Clin Cancer Res. 2011;17(8):2328–38. doi:10.1158/1078-0432.CCR-10-2943.
37. Pasqualini R, Millikan RE, Christianson DR, Cardo-Vila M, Driessen WH, Giordano RJ, et al. Targeting the interleukin-11 receptor alpha in metastatic prostate cancer: a first-in-man study. Cancer. 2015; doi:10.1002/cncr.29344.
38. Prantl L, Muehlberg F, Navone NM, Song YH, Vykoukal J, Logothetis CJ, et al. Adipose tissue-derived stem cells promote prostate tumor growth. Prostate. 2010;70(15):1709–15. doi:10.1002/pros.21206.

39. Prensner JR, Chen W, Iyer MK, Cao Q, Ma T, Han S, et al. PCAT-1, a long noncoding RNA, regulates BRCA2 and controls homologous recombination in cancer. Cancer Res. 2014;74(6):1651–60. doi:10.1158/0008-5472.CAN-13-3159.
40. Salameh A, Lee AK, Cardo-Vila M, Nunes DN, Efstathiou E, Staquicini FI, et al. PRUNE2 is a human prostate cancer suppressor regulated by the intronic long noncoding RNA PCA3. Proc Natl Acad Sci U S A. 2015; doi:10.1073/pnas.1507882112.
41. Sircar K, Huang H, Hu L, Cogdell D, Dhillon J, Tzelepi V, et al. Integrative molecular profiling reveals asparagine synthetase is a target in castration-resistant prostate cancer. Am J Pathol. 2012a;180(3):895–903. doi:10.1016/j.ajpath.2011.11.030.
42. Sircar K, Huang H, Hu L, Liu Y, Dhillon J, Cogdell D, et al. Mitosis phase enrichment with identification of mitotic centromere-associated kinesin as a therapeutic target in castration-resistant prostate cancer. PLoS One. 2012b;7(2):e31259. doi:10.1371/journal.pone.0031259.
43. Wan X, Corn PG, Yang J, Palanisamy N, Starbuck MW, Efstathiou E, et al. Prostate cancer cell-stromal cell crosstalk via FGFR1 mediates antitumor activity of dovitinib in bone metastases. Sci Transl Med. 2014;6(252):252ra122. doi:10.1126/scitranslmed.3009332.
44. Wan X, Liu J, Lu JF, Tzelepi V, Yang J, Starbuck MW, et al. Activation of beta-catenin signaling in androgen receptor-negative prostate cancer cells. Clin Cancer Res. 2012;18(3):726–36. doi:10.1158/1078-0432.CCR-11-2521.
45. Petrie RJ, Doyle AD, Yamada KM. Random versus directionally persistent cell migration. Nat Rev Mol Cell Biol. 2009;10(8):538–49. doi:10.1038/nrm2729.
46. Provenzano PP, Inman DR, Eliceiri KW, Trier SM, Keely PJ. Contact guidance mediated three-dimensional cell migration is regulated by rho/ROCK-dependent matrix reorganization. Biophys J. 2008;95(11):5374–84. doi:10.1529/biophysj.108.133116.
47. Friedl P, Gilmour D. Collective cell migration in morphogenesis, regeneration and cancer. Nat Rev Mol Cell Biol. 2009;10(7):445–57. doi:10.1038/nrm2720.
48. Kohrman AQ, Matus DQ. Divide or conquer: cell cycle regulation of invasive behavior. Trends Cell Biol. 2016; doi:10.1016/j.tcb.2016.08.003.
49. Hagedorn EJ, Ziel JW, Morrissey MA, Linden LM, Wang Z, Chi Q, et al. The netrin receptor DCC focuses invadopodia-driven basement membrane transmigration in vivo. J Cell Biol. 2013;201(6):903–13. doi:10.1083/jcb.201301091.
50. Matus DQ, Li XY, Durbin S, Agarwal D, Chi Q, Weiss SJ, et al. In vivo identification of regulators of cell invasion across basement membranes. Sci Signal. 2010;3(120):ra35. doi:10.1126/scisignal.2000654.
51. Matus DQ, Lohmer LL, Kelley LC, Schindler AJ, Kohrman AQ, Barkoulas M, et al. Invasive cell fate requires G1 cell-cycle arrest and histone deacetylase-mediated changes in gene expression. Dev Cell. 2015;35(2):162–74. doi:10.1016/j.devcel.2015.10.002.
52. Drost J, Karthaus WR, Gao D, Driehuis E, Sawyers CL, Chen Y, et al. Organoid culture systems for prostate epithelial and cancer tissue. Nat Protoc. 2016;11(2):347–58. doi:10.1038/nprot.2016.006.
53. Gao D, Vela I, Sboner A, Iaquinta PJ, Karthaus WR, Gopalan A, et al. Organoid cultures derived from patients with advanced prostate cancer. Cell. 2014;159(1):176–87. doi:10.1016/j.cell.2014.08.016.
54. Justus CR, Leffler N, Ruiz-Echevarria M, Yang LV. In vitro cell migration and invasion assays. J Vis Exp. 2014;88 doi:10.3791/51046.
55. Nobes CD, Hall A. Rho GTPases control polarity, protrusion, and adhesion during cell movement. J Cell Biol. 1999;144(6):1235–44.
56. Gaggioli C, Hooper S, Hidalgo-Carcedo C, Grosse R, Marshall JF, Harrington K, et al. Fibroblast-led collective invasion of carcinoma cells with differing roles for RhoGTPases in leading and following cells. Nat Cell Biol. 2007;9(12):1392–400. doi:10.1038/ncb1658.
57. Wolf K, Wu YI, Liu Y, Geiger J, Tam E, Overall C, et al. Multi-step pericellular proteolysis controls the transition from individual to collective cancer cell invasion. Nat Cell Biol. 2007;9(8):893–904. doi:10.1038/ncb1616.

58. Haycock JW. 3D cell culture: a review of current approaches and techniques. Methods Mol Biol. 2011;695:1–15. doi:10.1007/978-1-60761-984-0_1.
59. Truong D, Puleo J, Llave A, Mouneimne G, Kamm RD, Nikkhah M. Breast cancer cell invasion into a three dimensional tumor-stroma microenvironment. Sci Rep. 2016;6:34094. doi:10.1038/srep34094.
60. Kozlow W, Guise TA. Breast cancer metastasis to bone: mechanisms of osteolysis and implications for therapy. J Mammary Gland Biol Neoplasia. 2005;10(2):169–80. doi:10.1007/s10911-005-5399-8.
61. Logothetis CJ, Navone NM, Lin SH. Understanding the biology of bone metastases: key to the effective treatment of prostate cancer. Clin Cancer Res. 2008;14(6):1599–602. doi:10.1158/1078-0432.CCR-07-4603.
62. Yang J, Fizazi K, Peleg S, Sikes CR, Raymond AK, Jamal N, et al. Prostate cancer cells induce osteoblast differentiation through a Cbfa1-dependent pathway. Cancer Res. 2001;61(14):5652–9.
63. Li ZG, Yang J, Vazquez ES, Rose D, Vakar-Lopez F, Mathew P, et al. Low-density lipoprotein receptor-related protein 5 (LRP5) mediates the prostate cancer-induced formation of new bone. Oncogene. 2008b;27(5):596–603. doi:10.1038/sj.onc.1210694.
64. Kim J, Yu W, Kovalski K, Ossowski L. Requirement for specific proteases in cancer cell intravasation as revealed by a novel semiquantitative PCR-based assay. Cell. 1998;94(3):353–62.
65. McClatchey AI. Modeling metastasis in the mouse. Oncogene. 1999;18(38):5334–9. doi:10.1038/sj.onc.1203086.
66. Quigley JP, Armstrong PB. Tumor cell intravasation alu-cidated: the chick embryo opens the window. Cell. 1998;94(3):281–4.
67. Brown KM, Xue A, Mittal A, Samra JS, Smith R, Hugh TJ. Patient-derived xenograft models of colorectal cancer in pre-clinical research: a systematic review. Oncotarget. 2016; doi:10.18632/oncotarget.11184.
68. Paez-Ribes M, Man S, Xu P, Kerbel RS. Development of patient derived xenograft models of overt spontaneous breast cancer metastasis: a cautionary note. PLoS One. 2016;11(6):e0158034. doi:10.1371/journal.pone.0158034.
69. DeRose YS, Wang G, Lin YC, Bernard PS, Buys SS, Ebbert MT, et al. Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. Nat Med. 2011;17(11):1514–20. doi:10.1038/nm.2454.
70. Zhang X, Claerhout S, Prat A, Dobrolecki LE, Petrovic I, Lai Q, et al. A renewable tissue resource of phenotypically stable, biologically and ethnically diverse, patient-derived human breast cancer xenograft models. Cancer Res. 2013;73(15):4885–97. doi:10.1158/0008-5472.CAN-12-4081.
71. Rofstad EK, Huang R, Galappathi K, Andersen LM, Wegner CS, Hauge A, et al. Functional intratumoral lymphatics in patient-derived xenograft models of squamous cell carcinoma of the uterine cervix: implications for lymph node metastasis. Oncotarget. 2016; doi:10.18632/oncotarget.10931.
72. Chishima T, Miyagi Y, Wang X, Yamaoka H, Shimada H, Moossa AR, et al. Cancer invasion and micrometastasis visualized in live tissue by green fluorescent protein expression. Cancer Res. 1997;57(10):2042–7.
73. Ellenbroek SI, van Rheenen J. Imaging hallmarks of cancer in living mice. Nat Rev Cancer. 2014;14(6):406–18. doi:10.1038/nrc3742.
74. Koop S, Schmidt EE, MacDonald IC, Morris VL, Khokha R, Grattan M, et al. Independence of metastatic ability and extravasation: metastatic ras-transformed and control fibroblasts extravasate equally well. Proc Natl Acad Sci U S A. 1996;93(20):11080–4.
75. Andresen V, Alexander S, Heupel WM, Hirschberg M, Hoffman RM, Friedl P. Infrared multiphoton microscopy: subcellular-resolved deep tissue imaging. Curr Opin Biotechnol. 2009;20(1):54–62. doi:10.1016/j.copbio.2009.02.008.
76. Condeelis J, Segall JE. Intravital imaging of cell movement in tumours. Nat Rev Cancer. 2003;3(12):921–30. doi:10.1038/nrc1231.

77. Sahai E, Wyckoff J, Philippar U, Segall JE, Gertler F, Condeelis J. Simultaneous imaging of GFP, CFP and collagen in tumors in vivo using multiphoton microscopy. BMC Biotechnol. 2005;5:14. doi:10.1186/1472-6750-5-14.
78. Wang BG, Riemann I, Schubert H, Schweitzer D, Konig K, Halbhuber KJ. Multiphoton microscopy for monitoring intratissue femtosecond laser surgery effects. Lasers Surg Med. 2007a;39(6):527–33. doi:10.1002/lsm.20523.
79. Wang W, Wyckoff JB, Frohlich VC, Oleynikov Y, Huttelmaier S, Zavadil J, et al. Single cell behavior in metastatic primary mammary tumors correlated with gene expression patterns revealed by molecular profiling. Cancer Res. 2002;62(21):6278–88.
80. Vakoc BJ, Lanning RM, Tyrrell JA, Padera TP, Bartlett LA, Stylianopoulos T, et al. Three-dimensional microscopy of the tumor microenvironment in vivo using optical frequency domain imaging. Nat Med. 2009;15(10):1219–23. doi:10.1038/nm.1971.
81. Evans CL, Potma EO, Puoris'haag M, Cote D, Lin CP, Xie XS. Chemical imaging of tissue in vivo with video-rate coherent anti-stokes Raman scattering microscopy. Proc Natl Acad Sci U S A. 2005;102(46):16807–12. doi:10.1073/pnas.0508282102.
82. Wyckoff JB, Jones JG, Condeelis JS, Segall JE. A critical step in metastasis: in vivo analysis of intravasation at the primary tumor. Cancer Res. 2000;60(9):2504–11.
83. Wang W, Wyckoff JB, Goswami S, Wang Y, Sidani M, Segall JE, et al. Coordinated regulation of pathways for enhanced cell motility and chemotaxis is conserved in rat and mouse mammary tumors. Cancer Res. 2007b;67(8):3505–11. doi:10.1158/0008-5472.CAN-06-3714.
84. Romanelli E, Sorbara CD, Nikic I, Dagkalis A, Misgeld T, Kerschensteiner M. Cellular, subcellular and functional in vivo labeling of the spinal cord using vital dyes. Nat Protoc. 2013;8(3):481–90. doi:10.1038/nprot.2013.022.
85. Veiseh M, Kwon DH, Borowsky AD, Tolg C, Leong HS, Lewis JD, et al. Cellular heterogeneity profiling by hyaluronan probes reveals an invasive but slow-growing breast tumor subset. Proc Natl Acad Sci U S A. 2014;111(17):E1731–9. doi:10.1073/pnas.1402383111.
86. Dan S, Okamura M, Mukai Y, Yoshimi H, Inoue Y, Hanyu A, et al. ZSTK474, a specific phosphatidylinositol 3-kinase inhibitor, induces G1 arrest of the cell cycle in vivo. Eur J Cancer. 2012;48(6):936–43. doi:10.1016/j.ejca.2011.10.006.
87. Kedrin D, Gligorijevic B, Wyckoff J, Verkhusha VV, Condeelis J, Segall JE, et al. Intravital imaging of metastatic behavior through a mammary imaging window. Nat Methods. 2008;5(12):1019–21. doi:10.1038/nmeth.1269.
88. Wyckoff JB, Pinner SE, Gschmeissner S, Condeelis JS, Sahai E. ROCK- and myosin-dependent matrix deformation enables protease-independent tumor-cell invasion in vivo. Curr Biol. 2006;16(15):1515–23. doi:10.1016/j.cub.2006.05.065.
89. Lo Celso C, Lin CP, Scadden DT. In vivo imaging of transplanted hematopoietic stem and progenitor cells in mouse calvarium bone marrow. Nat Protoc. 2011;6(1):1–14. doi:10.1038/nprot.2010.168.
90. Mempel TR, Scimone ML, Mora JR, von Andrian UH. In vivo imaging of leukocyte trafficking in blood vessels and tissues. Curr Opin Immunol. 2004;16(4):406–17. doi:10.1016/j.coi.2004.05.018.
91. Egen JG, Rothfuchs AG, Feng CG, Horwitz MA, Sher A, Germain RN. Intravital imaging reveals limited antigen presentation and T cell effector function in mycobacterial granulomas. Immunity. 2011;34(5):807–19. doi:10.1016/j.immuni.2011.03.022.
92. Lee J, Li M, Milwid J, Dunham J, Vinegoni C, Gorbatov R, et al. Implantable microenvironments to attract hematopoietic stem/cancer cells. Proc Natl Acad Sci U S A. 2012;109(48):19638–43. doi:10.1073/pnas.1208384109.
93. Ravoori M, Czaplinska AJ, Sikes C, Han L, Johnson EM, Qiao W, et al. Quantification of mineralized bone response to prostate cancer by noninvasive in vivo microCT and non-destructive ex vivo microCT and DXA in a mouse model. PLoS One. 2010;5(3):e9854. doi:10.1371/journal.pone.0009854.

Modeling Drug Resistance in PDX Models

8

Erin L. Stewart and Ming Sound Tsao

Abbreviation

PDX Patient-derived xenograft

Introduction

Cancer remains a major cause of death worldwide. Along with advancing cancer prevention, more effective treatments are desperately required. Despite significant strides over the past 30 years, resistance to systemic therapies remains a massive obstacle [1]. Diverse mechanisms of acquired resistance to cancer chemotherapies have been discovered [2]. Moreover, multiple mechanisms of resistance may exist against a single therapy [3–9]. Despite being able to identify some of the mechanisms underlying drug resistance, many of them are still unknown. Furthermore, biomarkers to predict how resistance will occur and optimal subsequent treatment methods remain to be determined in the majority of cancer cases. Resistance to a therapy may be innate or acquired [10]. To combat innate resistance, improved patient stratification strategies for therapies are required. To combat acquired resistance, a better understanding of the numerous mechanisms of resistance that can arise, and how to avoid or overcome them, is imperative.

To study a phenomenon as complex as acquired therapy resistance, optimal tumor models are required. Models based on cultured cancer cell lines have been shown to poorly predict patients' responses to drugs, in part due to a lack

E.L. Stewart • M.S. Tsao (✉)
Princess Margaret Cancer Research Tower,
Rm 14-401, 101 College Street, Toronto, ON M5G 1L7, Canada
e-mail: erin.stewart@mail.utoronto.ca; Ming.Tsao@uhn.ca

© Springer International Publishing AG 2017
Y. Wang et al. (eds.), *Patient-Derived Xenograft Models of Human Cancer*,
Molecular and Translational Medicine, DOI 10.1007/978-3-319-55825-7_8

of tumor heterogeneity, lack of three-dimensional tissue architecture and stromal support, and lack of dynamic and naturally occurring physiological phenomena such as hypoxia [11]. In contrast, patient-derived xenograft (PDX) tumor models have been reported to recapitulate major complexities of patients' malignancies, including their responses to therapies [8, 9, 12–14]. Furthermore, such models can be leveraged to study the heterogeneity within a patient's tumor; a single tumor sample may be split and implanted into multiple biological replicates, each potentially representing an array of subclones. The PDX models may also lend themselves to molecular characterizations of tumors during the course of treatment. Such procedures are not yet routinely performed in patients due to clinical challenges in deciding which tumor site to re-biopsy, as well as ethical issues such as the invasiveness of the biopsy procedure [15]. With PDXs, however, multiple biological replicates can be implanted allowing for subsets to be studied at various time points of the treatment regimen. As such, PDXs lend themselves as excellent models for studying the development of resistance to therapies.

We recently carried out a PubMed search based on "patient-derived xenograft models" and "resistance" which led to numerous studies, the majority of which describe experimental models of innate resistance. Often these studies involve treatment of multiple PDX models with a therapy aimed at determining what is molecularly different between the responders and the nonresponders. They then generally use these differences to predict the responses of another cohort to the same therapeutic [16–19]. As these studies fringe on "biomarker studies," they will not be the major focus of this chapter. The same PubMed search yielded a handful of studies of which at least one section investigated acquired resistance using PDXs. We selected 15 of these investigations for comparative analysis as they cover the scope of methods and motivations (summarized in Table 8.1) for studying acquired therapy resistance by PDX models.

Therapeutic Doses for Development of Drug Resistance

One of the first steps in studying acquired resistance is to identify a model that is sensitive to the therapy of interest. Often more than one drug dosage will be explored during this initial PDX screening phase, in an effort to determine the optimal dose that produces tumor sensitivity while avoiding mouse toxicity; a theoretical example of such a study is presented in Fig. 8.1. From these initial screening studies, a dose can be determined for future investigations, such as modeling acquired resistance. Such initial drug screening studies are not always published together with the acquired resistance studies. In the case of vemurafenib, Das Thakur et al. [20] reported their initial screening study in the same paper as their resistance study. They investigated the growth-inhibitory activity of vemurafenib at three separate doses, 5, 15, and 45 mg/kg and found that the

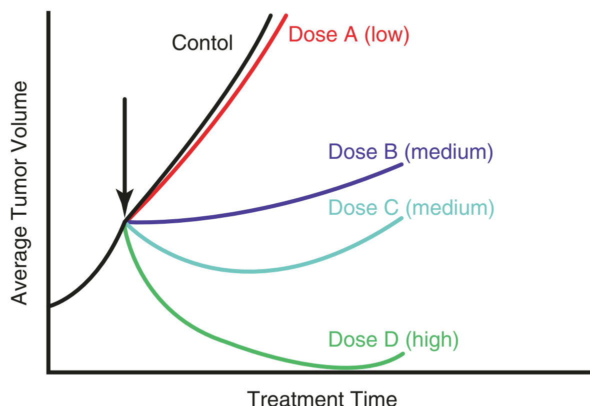


Fig. 8.1 Example of an initial drug screening study to determine optimal dose for subsequent experiments. The average tumor volume (y-axis) of five mouse cohorts was plotted over time (x-axis). The *gray arrow* represents the start of treatment for the four dosed cohorts. In this example Dose A has the lowest drug concentration, Dose D has the highest, and Dose B and C are in between

lowest dose was ineffective (similar to Dose A in Fig. 8.1), whereas the two higher doses were able to shrink the tumors. Surprisingly, 15 mg/kg was almost as effective as the higher dose. However, it became apparent that 45 mg/kg was the more effective dose as it maintained minimal disease, whereas tumors treated at 15 mg/kg began to escape inhibition after approximately 1 month (similar to Dose C in Fig. 8.1). As such, the authors decided to use the highest dose for their resistance modeling (similar to Dose D in Fig. 8.1).

Shen et al. [21] also reported their preliminary drug sensitivity screening in the same paper as their acquired resistance study. Two doses, 12.5 or 25 mg/kg of crizotinib once a day for 21 days, were investigated. However, data from the lower dose cohort were shown for only one of the PDX models studied. Regardless, using the model that was most sensitive to crizotinib, the authors chose to continue to treat at the lower dose of 12.5 mg/kg for another month to develop resistance. Using our example shown in Fig. 8.1, the authors essentially explored Doses C and D and opted to use Dose C for developing resistance.

Other investigators used different ways of screening drug dosages to generate therapy resistance. Gaponova et al. performed a drug screen for STA-8666 at 75 mg/kg for over a month and achieved stable disease or tumor regression (similar to Dose D in Fig. 8.1); they then increased the dosage to 150 mg/kg which led to increased regression. However, two of the three tumors escaped therapeutic control and grew uncontrollably [22]. Micel et al. similarly did a drug screen of the MEK inhibitor TAK-733, examining 25 or 10 mg/kg, and then used 100 mg/kg to develop resistance [23].

Tumor Volume at Treatment Initiation

Tumor volume is often the main characteristic used to determine when to start PDX dosing. Some studies use rolling enrollment, i.e., treatment of individual mice is started whenever their tumors reach a specific volume (Fig. 8.2a). Treatment of mice can also be started when their tumors reach a prespecified average volume (Fig. 8.2b). Given the technical difficulties of regularly measuring orthotopically implanted tumors (aside from mammary fat pad implantations), studies using these models tend to rely more on timing rather than specific tumor volumes [24, 25]. Among the studies using subcutaneously implanted PDXs, however, the tumor volume at dosing initiation varies widely, regardless of which enrollment method is used.

A number of studies did not explicitly state at what tumor volume treatment was started. Some of these studies reported the growth curves, with actual tumor volumes on the y-axis, allowing readers to extrapolate the starting volumes [20, 21, 26]. Unfortunately, other studies either did not report a starting tumor volume and published graphs with transformed data [22, 27, 28] or stated parameters such as "during log phase," which could cover a wide range of volumes [29]. Monsma et al. [30] reported that treatment was started when tumors were in exponential growth; however, they also gave an approximate tumor volume to indicate what this might mean [30]. Ter Brugge et al. [31], Micel et al. [23], Gao et al. [32], and Tentler et al. [33] specifically stated either the mean or the individual tumor volume criteria that they used for treatment initiation, all of which seem to have been chosen around

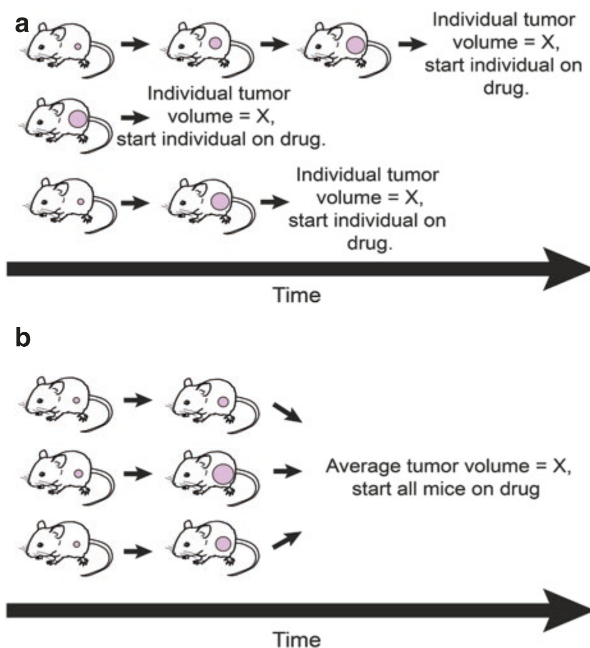


Fig. 8.2 Schema depicting two methods for determining when to start treatment. (**a**) Rolling enrollment: starting to treat individual mice when their respective tumors reach a predetermined volume. (**b**) Mass enrollment: starting to treat all mice when the average tumor volumes reach a predetermined volume

100–300 mm^3 [23, 31–33]. Ciamporcero et al. [34] also reported the approximate tumor volumes for treatment initiation; however, this was reported in mm^2 instead of mm^3, making comparisons with other studies less direct [34].

Methods for Developing Drug Resistance

The overall method for developing a resistant model also varies from study to study. Parameters, such as constant versus escalating doses, constant versus cycled doses, and dosing across one or more passages, were varied across experiments. The majority of studies utilized continuous dosing at one concentration until resistance developed (Fig. 8.3a). The resistant tumors were either molecularly characterized or serially passaged to maintain a resistant model for further studies [20, 21, 23, 25, 26, 28, 29, 32, 33]. Ciamporcero et al. [34] also used continuous dosing; however, once tumors regrew under therapeutic pressure to double their initial volume, PDXs were considered "resistant" and randomized into experimental arms to determine the effectiveness of alternate therapies (Fig. 8.3b). Gaponova et al. [22] also employed continuous dosing to develop resistance, yet increased their dosage from 75 to 150 mg/kg STA-8666 after approximately 1 month (Fig. 8.3c). Cottu et al. [27] dosed their PDXs continuously, yet performed three further serial passages, with continued dosing, before they considered the model to be truly drug-resistant and used it for further studies (Fig. 8.3d).

Aside from variations on continuous dosing, some studies also employ cycling methods to develop drug resistance. Again, specifics such as number of passages

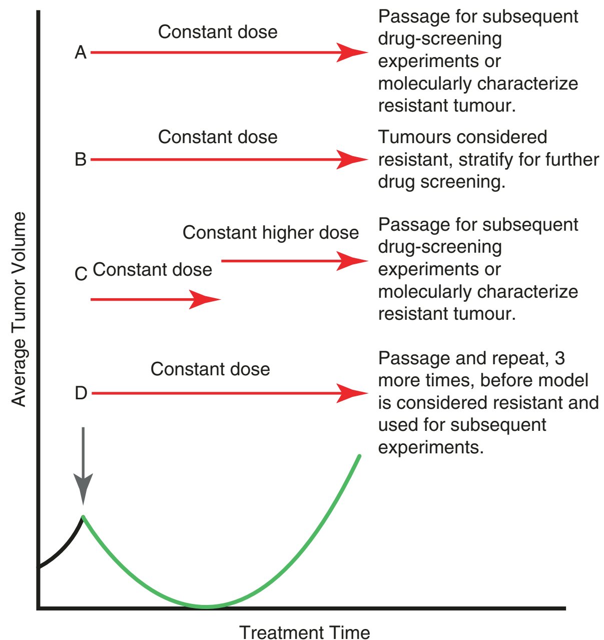


Fig. 8.3 Schema depicting the four major continuous dosing regimens to develop resistance. *Gray vertical arrow* represents when treatment was started. *Red horizontal arrows* represent length of dosing

and drug dosages vary across studies. Monsma et al. [30] treated PDX tumors of ~160 mm^3 for 28 days, during which average tumor volumes regressed. After this treatment period, the researchers allowed three tumors to relapse (off treatment) and passaged them into a new cohort of mice and again allowed them to reach ~160 mm^3 before treatment was continued. Upon the therapeutic rechallenge, the tumors exhibited decreased drug sensitivity and did not regress but continued to grow while under therapeutic pressure (albeit at a slower rate than untreated control tumors) (Fig. 8.4a). Vidal et al. [24] also cycled therapies across multiple passages to generate a resistant model; however, they increased the dose at each passage (Fig. 8.4b). The mice received one cycle of cisplatin at 2 mg/kg once a week for 3 weeks, and the tumors were then allowed to relapse. Tumors were then passaged into the next cohort of mice and allowed to again grow until intra-abdominal masses were

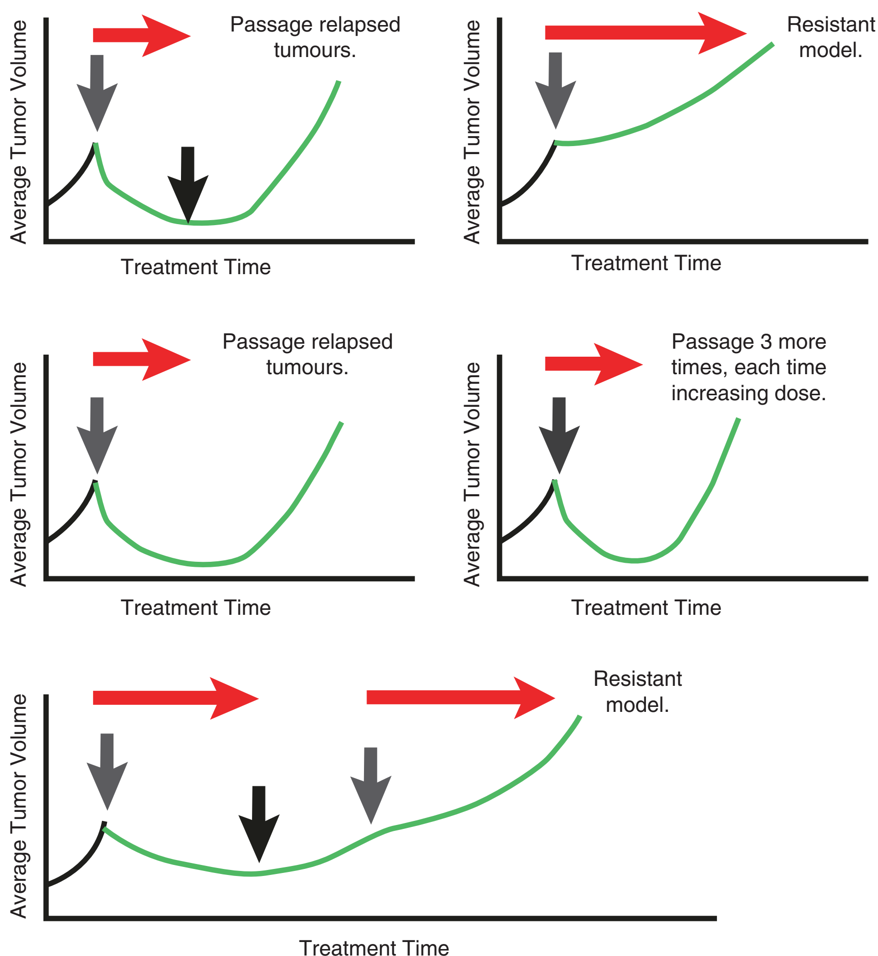


Fig. 8.4 Schema depicting the three major cycled dosing regimens to develop resistance. *Gray vertical arrows* represent when treatment was started. *Dark gray vertical arrows* indicate higher dose. *Black vertical arrows* indicate stopping treatment. *Red horizontal arrows* represent length of dosing

palpable. These PDXs received one cycle of cisplatin at 3 mg/kg once a week for 3 weeks and were then allowed to relapse. This process was repeated for up to five passages and cycles with increasing doses (cycle 3, 3.5 mg/kg; cycle 4, 4 mg/kg; cycle 5, 5 mg/kg). After the fifth cycle, a stably shortened time to relapse was observed for the PDXs, and they were considered "cisplatin-resistant" and used for further studies. Lastly, Ter Brugge et al. [31] cycled tumors within a single passage (Fig. 8.4c). Mice were randomized when their tumors reached a volume of 100 mm^3, and treatment was initiated at 200 mm^3. Treatment was stopped if tumors regressed to <50% of the initial tumor volume and was resumed once the starting volume was regained.

Motivation for Developing Drug-Resistant PDX Models

Resistance was developed in PDXs across these studies for a number of reasons. While many of the studies using PDXs with acquired resistance had more than one focus (as depicted in Table 8.1), there seem to be four major themes. Studying the mechanism of acquired resistance was, as expected, the most common theme found in the studies reviewed. For some studies such as those by Nathanson et al. [28],

Table 8.1 Summary of major focus of studies using resistant PDXs[a]

References	Novel MoR to drug	Optimal subsequent therapies	Clinical utility of model	Possibility of AR to a new drug
Nathanson et al. [28]	✓			
Kopetz et al. [26]	✓			
Das Thakur et al. [20]	✓	✓		
Shen et al. [21]	✓			
Ter Brugge et al. [31]	✓			
Monsma et al. [29]	✓	✓		
Ciamporcero et al. [34]		✓		
Cottu et al. [27]	✓	✓		
Monsma et al. [30]	✓	✓		
Vidal et al. [24]		✓		
Gao et al. [32]	✓		✓	
Zhao et al. [25]	✓		✓	
Tentler et al. [33]	✓			✓
Gaponova et al. [22]				✓
Micel et al. [23]	✓			✓

[a]Only major conclusions from the PDX portions of the studies are reported; papers may have gone on to explore other avenues and drawn more conclusions using alternative models. While all reports mentioned at least some sort of molecular anomaly that was observed in the resistant PDX but not in the sensitive parental model, only those that specifically sought the exact mechanisms of resistance, and not just potential passenger anomalies, are noted under the MoR studies. *MoR* mechanism of resistance, *AR* acquired resistance

Kopetz et al. [26], Shen et al. [21], and Ter Brugge et al. [31], resistant PDXs were generated to study novel mechanisms of resistance. Das Thakur et al. [20], Monsma et al. [29, 30], Ciamporcero et al. [34], Cottu et al. [27], and Vidal et al. [24] focused on determining optimal subsequent therapies to combat the acquired resistance that had developed. Gao et al. [32] and Zhao et al. [25] developed resistance in large part to demonstrate the benefit of using their models to study clinically relevant issues. Finally, Tentler et al. [33], Gaponova et al. [22], and Micel et al. [23] investigated the efficacy of novel therapies and wanted to determine whether acquired resistance might occur and through what mechanisms.

Perspectives and Future Directions

Determining how best to model acquired resistance by choosing a treatment regimen including drug concentration, PDX enrollment, and treatment timing can be a subjective matter. As such, some perspectives on each matter deserve a discussion.

The use of a lower dose that decreases tumor growth rate, but does not cause actual tumor shrinkage, may seem ineffective [21]. By the end of Shen et al.'s initial screen of 21 days, the tumor had actually continued to progress, albeit at a slower rate than the control tumors, suggesting that the optimal dose had not been achieved. The higher dose of crizotinib seems more optimal as it shrank tumors, and no toxicities were reported, thus making it the appropriate dose for modeling acquired resistance. However, achieving higher, optimal doses is not always possible in humans, and treatments that merely slow the growth of tumors may be clinically relevant.

Clearly reporting parameters such as tumor volume at treatment initiation is imperative as this criterion can have a large effect on a study's outcome. Some treatments may be affected by the volume of the tumors. For example, large tumors may have more hypoxic and/or less vascularized regions, potentially decreasing effectiveness of the therapy employed. In contrast, small tumors may not be fully established in their implantation site, lacking supportive stroma that is observed in clinically detectable tumors, making them more vulnerable to treatments. As such, clearly stating important experimental parameters such as tumor volume at treatment initiation allows readers to draw their own conclusions regarding the reported results of the study.

Lastly, reporting individual growth curves for all replicates in a study provides potentially useful information to readers. For example, if all replicates develop resistance, a highly distributed subclone (Fig. 8.5a) or therapy-induced event, such as epigenetic reprogramming or increased transcription of pro-survival genes (Fig. 8.5b), may be responsible for the resistance. If only a few replicates develop resistance, perhaps only those replicates contained rare subclones with innate mechanisms of resistance, such as mutations within the target pathway (Fig. 8.5c). At the very least, authors should state how many replicates actually developed resistance. Furthermore, the growth kinetics of tumors developing resistance may be

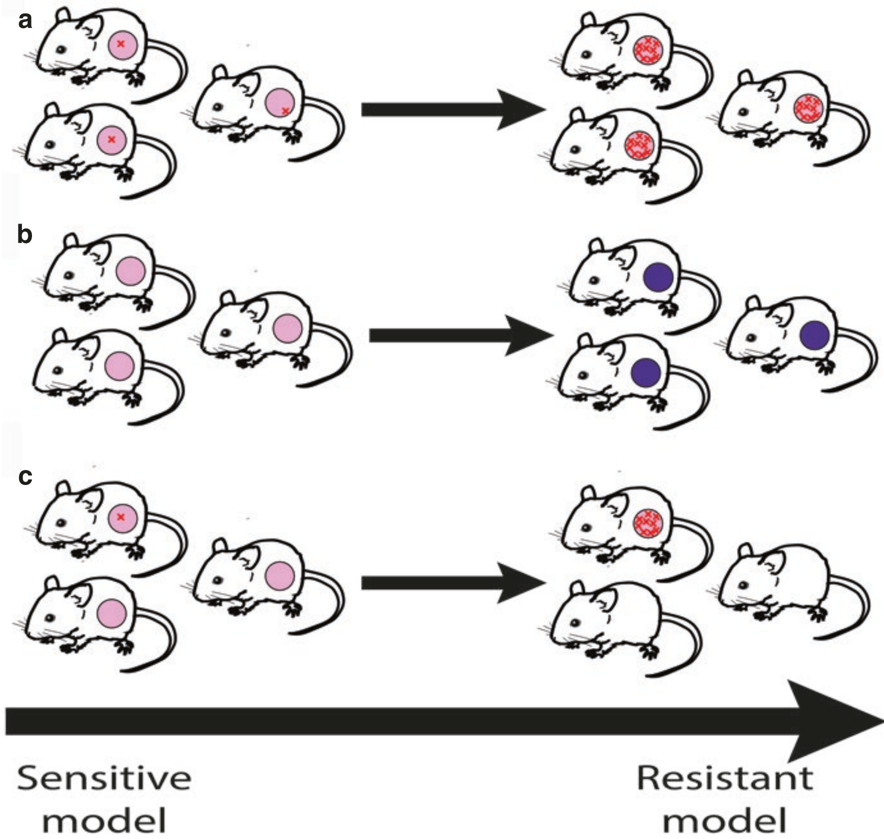


Fig. 8.5 Schema demonstrating how various mechanisms of resistance may impact the number of biological replicates that actually develop resistance in a study. (**a**) Acquired resistance is due to a highly distributed subclone with innate resistance. (**b**) Acquired resistance is due to a therapy-induced cellular event such as epigenetic and/or transcriptional reprogramming. (**c**) Acquired resistance is due to only a few rare subclones with innate resistance

informative too. Slow-growing "resistant" tumors may simply be demonstrating a decrease in sensitivity to drug and may actually be inhibited by higher doses, whereas quickly growing "resistant" tumors may have acquired novel mechanisms of resistance that completely overcome any therapy-specific effect regardless of dose. Among the manuscripts reviewed, approximately half showed either all replicates [20, 22, 27, 31] or at least showed examples of individual growth curves that did develop resistance [26, 29, 33]. Some of the subcutaneous PDX studies did not show any growth curves for the development of acquired resistance and only reported on downstream applications of the resistant model(s) [23, 32].

Conclusions

PDXs provide versatile cancer models for studying multiple facets of acquired drug resistance. It has been shown that, following exposure to pharmacological pressure, they develop resistance via similar mechanisms and with similar timelines as patients' tumors [25, 32]. PDXs have been used to study novel mechanisms of resistance to current therapies [21, 26, 28, 31] and to investigate what to do once resistance develops [24, 27, 34]; they also have been used to try to stay ahead of the game and determine the propensity of tumors to acquire resistance to novel therapies [22, 23, 33]. PDXs are even becoming incorporated into clinical trial studies: PDXs with acquired resistance have been used in lieu of post-progression biopsies to determine potential mechanisms of resistance observed in a clinical trial [26]. Given the versatility of these models to explore clinically relevant issues in a timely manner, we believe that PDX incorporation into the clinic will become more common in the future.

References

1. Stewart BW, Wild CP. World Cancer Report 2014.
2. Hu X, Zhang Z. Understanding the genetic mechanisms of cancer drug resistance using genomic approaches. Trends Genet. 2016;32(2):127–37. doi:10.1016/j.tig.2015.11.003.
3. Braun DA, Burke KP, Van Allen EM. Genomic approaches to understanding response and resistance to immunotherapy. Clin Cancer Res. 2016;22(23):5642–50. doi:10.1158/1078-0432.CCR-16-0066.
4. Gillis NK, McLeod HL. The pharmacogenomics of drug resistance to protein kinase inhibitors. Drug Resist Updat. 2016;28:28–42. doi:10.1016/j.drup.2016.06.008.
5. Hotte SJ. Addressing taxane resistance in metastatic castration-resistant prostate cancer: a focus on chaperone proteins. Future Oncol. 2017;13(4):369–79. doi:10.2217/fon-2016-0279.
6. Paoletti C, Larios JM, Muniz MC, Aung K, Cannell EM, Darga EP, et al. Heterogeneous estrogen receptor expression in circulating tumor cells suggests diverse mechanisms of fulvestrant resistance. Mol Oncol. 2016;10(7):1078–85. doi:10.1016/j.molonc.2016.04.006.
7. Shi H, Zhang W, Zhi Q, Jiang M. Lapatinib resistance in HER2+ cancers: latest findings and new concepts on molecular mechanisms. Tumour Biol. 2016; doi:10.1007/s13277-016-5467-2.
8. Stewart EL, Mascaux C, Pham NA, Sakashita S, Sykes J, Kim L, et al. Clinical utility of patient-derived xenografts to determine biomarkers of prognosis and map resistance pathways in EGFR-mutant lung adenocarcinoma. J Clin Oncol. 2015a;33(22):2472–80. doi:10.1200/JCO.2014.60.1492.
9. Stewart EL, Tan SZ, Liu G, Tsao MS. Known and putative mechanisms of resistance to EGFR targeted therapies in NSCLC patients with EGFR mutations-a review. Transl Lung Cancer Res. 2015b;4(1):67–81. doi:10.3978/j.issn.2218-6751.2014.11.06.
10. Gottesman MM. Mechanisms of cancer drug resistance. Annu Rev Med. 2002;53:615–27. doi:10.1146/annurev.med.53.082901.103929.
11. Choi SY, Lin D, Gout PW, Collins CC, Xu Y, Wang Y. Lessons from patient-derived xenografts for better in vitro modeling of human cancer. Adv Drug Deliv Rev. 2014;79–80:222–37. doi:10.1016/j.addr.2014.09.009.
12. Dodbiba L, Teichman J, Fleet A, Thai H, Starmans MH, Navab R, et al. Appropriateness of using patient-derived xenograft models for pharmacologic evaluation of novel therapies for esophageal/gastro-esophageal junction cancers. PLoS One. 2015;10(3):e0121872. doi:10.1371/journal.pone.0121872.

13. Nunes M, Vrignaud P, Vacher S, Richon S, Lievre A, Cacheux W, et al. Evaluating patient-derived colorectal cancer xenografts as preclinical models by comparison with patient clinical data. Cancer Res. 2015;75(8):1560–6. doi:10.1158/0008-5472.CAN-14-1590.
14. Wong NC, Bhadri VA, Maksimovic J, Parkinson-Bates M, Ng J, Craig JM, et al. Stability of gene expression and epigenetic profiles highlights the utility of patient-derived paediatric acute lymphoblastic leukaemia xenografts for investigating molecular mechanisms of drug resistance. BMC Genomics. 2014;15:416. doi:10.1186/1471-2164-15-416.
15. Meghan Campo ZP, Muzikansky A, Rizzo C, Gainor JF, Shaw AT, Engelman JA, Sequist LV, editors. The challenges of repeat biopsies at acquired resistance to EGFR TKIs. ASCO Annual Meeting. J Clin Oncol. 2016:e20571.
16. Chen H, Ye Q, Lv J, Ye P, Sun Y, Fan S, et al. Evaluation of trastuzumab anti-tumor efficacy and its correlation with HER-2 status in patient-derived gastric adenocarcinoma xenograft models. Pathol Oncol Res. 2015;21(4):947–55. doi:10.1007/s12253-015-9909-8.
17. Forest A, Amatulli M, Ludwig DL, Damoci CB, Wang Y, Burns CA, et al. Intrinsic resistance to cixutumumab is conferred by distinct isoforms of the insulin receptor. Mol Cancer Res. 2015;13(12):1615–26. doi:10.1158/1541-7786.MCR-15-0279.
18. Krumbach R, Schuler J, Hofmann M, Giesemann T, Fiebig HH, Beckers T. Primary resistance to cetuximab in a panel of patient-derived tumour xenograft models: activation of MET as one mechanism for drug resistance. Eur J Cancer. 2011;47(8):1231–43. doi:10.1016/j.ejca.2010.12.019.
19. Wu X, Zhang J, Zhen R, Lv J, Zheng L, Su X, et al. Trastuzumab anti-tumor efficacy in patient-derived esophageal squamous cell carcinoma xenograft (PDECX) mouse models. J Transl Med. 2012;10:180. doi:10.1186/1479-5876-10-180.
20. Das Thakur M, Salangsang F, Landman AS, Sellers WR, Pryer NK, Levesque MP, et al. Modelling vemurafenib resistance in melanoma reveals a strategy to forestall drug resistance. Nature. 2013;494(7436):251–5. doi:10.1038/nature11814.
21. Shen A, Wang L, Huang M, Sun J, Chen Y, Shen YY, et al. c-Myc alterations confer therapeutic response and acquired resistance to c-Met inhibitors in MET-addicted cancers. Cancer Res. 2015;75(21):4548–59. doi:10.1158/0008-5472.CAN-14-2743.
22. Gaponova AV, Nikonova AS, Deneka AY, Kopp MC, Kudinov AE, Skobeleva N, et al. A novel HSP90 inhibitor-drug conjugate to SN38 is highly effective in small cell lung cancer. Clin Cancer Res. 2016;22(20):5120–9. doi:10.1158/1078-0432.CCR-15-3068.
23. Micel LN, Tentler JJ, Tan AC, Selby HM, Brunkow KL, Robertson KM, et al. Antitumor activity of the MEK inhibitor TAK-733 against melanoma cell lines and patient-derived tumor explants. Mol Cancer Ther. 2015;14(2):317–25. doi:10.1158/1535-7163.MCT-13-1012.
24. Vidal A, Munoz C, Guillen MJ, Moreto J, Puertas S, Martinez-Iniesta M, et al. Lurbinectedin (PM01183), a new DNA minor groove binder, inhibits growth of orthotopic primary graft of cisplatin-resistant epithelial ovarian cancer. Clin Cancer Res. 2012;18(19):5399–411. doi:10.1158/1078-0432.CCR-12-1513.
25. Zhao H, Thong A, Nolley R, Reese SW, Santos J, Ingels A, et al. Patient-derived tissue slice grafts accurately depict response of high-risk primary prostate cancer to androgen deprivation therapy. J Transl Med. 2013;11:199. doi:10.1186/1479-5876-11-199.
26. Kopetz S, Desai J, Chan E, Hecht JR, O'Dwyer PJ, Maru D, et al. Phase II pilot study of vemurafenib in patients with metastatic BRAF-mutated colorectal cancer. J Clin Oncol. 2015;33(34):4032–8. doi:10.1200/JCO.2015.63.2497.
27. Cottu P, Bieche I, Assayag F, El Botty R, Chateau-Joubert S, Thuleau A, et al. Acquired resistance to endocrine treatments is associated with tumor-specific molecular changes in patient-derived luminal breast cancer xenografts. Clin Cancer Res. 2014;20(16):4314–25. doi:10.1158/1078-0432.CCR-13-3230.
28. Nathanson DA, Gini B, Mottahedeh J, Visnyei K, Koga T, Gomez G, et al. Targeted therapy resistance mediated by dynamic regulation of extrachromosomal mutant EGFR DNA. Science. 2014;343(6166):72–6. doi:10.1126/science.1241328.
29. Monsma DJ, Cherba DM, Eugster EE, Dylewski DL, Davidson PT, Peterson CA, et al. Melanoma patient derived xenografts acquire distinct vemurafenib resistance mechanisms. Am J Cancer Res. 2015;5(4):1507–18.

30. Monsma DJ, Cherba DM, Richardson PJ, Vance S, Rangarajan S, Dylewski D, et al. Using a rhabdomyosarcoma patient-derived xenograft to examine precision medicine approaches and model acquired resistance. Pediatr Blood Cancer. 2014;61(9):1570–7. doi:10.1002/pbc.25039.
31. Ter Brugge P, Kristel P, van der Burg E, Boon U, de Maaker M, Lips E et al. Mechanisms of therapy resistance in patient-derived xenograft models of BRCA1-deficient breast cancer. J Natl Cancer Inst. 2016;108(11). doi:10.1093/jnci/djw148.
32. Gao H, Korn JM, Ferretti S, Monahan JE, Wang Y, Singh M, et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nat Med. 2015;21(11):1318–25. doi:10.1038/nm.3954.
33. Tentler JJ, Ionkina AA, Tan AC, Newton TP, Pitts TM, Glogowska MJ, et al. p53 family members regulate phenotypic response to aurora kinase a inhibition in triple-negative breast cancer. Mol Cancer Ther. 2015;14(5):1117–29. doi:10.1158/1535-7163.MCT-14-0538-T.
34. Ciamporcero E, Miles KM, Adelaiye R, Ramakrishnan S, Shen L, Ku S, et al. Combination strategy targeting VEGF and HGF/c-met in human renal cell carcinoma models. Mol Cancer Ther. 2015;14(1):101–10. doi:10.1158/1535-7163.MCT-14-0094.

Using PDX for Biomarker Development

9

Werner J. Struss and Peter C. Black

Abbreviations

CTC	Circulating tumor cells
ctDNA	Cell-free tumor DNA
ncRNA	Non(protein)-coding RNA
NOD-SCID	Nonobese diabetic, severe combined immune deficient
NSCLC	Non-small cell lung cancer
PDAC	Pancreatic ductal adenocarcinoma
PDX	Patient-derived xenograft
SPIDER	Serial Patient-Derived Xenograft Models to Eliminate Cancer Therapy Resistance Trial

Introduction

We aim to highlight how patient-derived xenografts (PDX) have been utilized in cancer research in the development of biomarkers. Biomarkers are important in oncology for early diagnosis, risk stratification, selection of optimal treatment, and monitoring of response to therapy. Biomarkers are the cornerstone of individualized therapy according to specific parameters of the patient and the patient's malignancy. Better biomarkers are considered essential in our pursuit of reduced treatment toxicity, improved quality of life, and better overall survival.

Biomarker discovery is dependent on the comprehensive understanding of tumor biology. Patient-derived primary xenografts (PDX) represent an advanced preclinical model that facilitates investigation of patient tumor biology. By implanting

W.J. Struss, M.D. • P.C. Black, M.D. (✉)
Vancouver Prostate Centre, Vancouver, BC, Canada

Department of Urologic Sciences, University of British Columbia,
Level 6, 2775 Laurel St., Vancouver, BC V5Z 1M9, Canada
e-mail: pblack@mail.ubc.ca

© Springer International Publishing AG 2017

Y. Wang et al. (eds.), *Patient-Derived Xenograft Models of Human Cancer*,
Molecular and Translational Medicine, DOI 10.1007/978-3-319-55825-7_9

patient tumor tissue directly into immunodeficient mice, the architectural integrity of the tumor and especially the associated stroma is preserved. As a result of this preservation of the tumor microenvironment, these models more closely represent the patient tumor than any other preclinical models, especially xenograft models utilizing tumor cell lines from tissue culture. Cell lines are altered by prolonged growth in culture and a complete lack of stroma, so that xenograft models from these only poorly reflect the clinical disease. These variations also affect the pharmacodynamics and pharmacokinetics of novel drugs being tested. Proteomic and genomic alterations that are critical for understanding drug efficacy and targeted therapy may be aberrant in cell lines compared to the true patient disease.

The ability of PDX to closely mimic the human cancer from which they were derived makes them a valuable tool in biomarker discovery and validation. Typically the parental tumor and the PDX undergo careful molecular analysis to demonstrate preservation of the patient tumor biology in the model system and to identify priority pathways and targets. Molecular findings can be linked to observed phenotypic behavior, for example, the propensity to metastasize. PDX are particularly well suited for treatment studies and subsequent studies on markers of response to treatment. Here we will discuss how these features of PDX make them particularly suitable for biomarker development.

Definition of a Biomarker

The term biological marker or biomarker was coined in the early 1980s and has been defined as a particular biological feature that serves as an indicator of normal processes, pathological mechanisms, or pharmacological reactions to therapeutic intervention. Crucial to a biomarker is that it must be objectively measurable and appraisable [1, 2]. Hence, a cancer biomarker refers to a measurable biological parameter, often a particular protein, mRNA, or genomic alteration, that reflects the presence of cancer in the body, the aggressiveness of a cancer, or the likelihood that the cancer will respond to a therapy. Many markers are tissue based, but ideally biomarkers should be harvested in a noninvasive manner, and biofluids (especially blood, urine, and saliva) are often the ideal medium for marker development [3]. This allows repeated collection of samples for biological analysis without burdening patients with intrusive procedures.

Patient-Derived Xenografts (PDX)

Xenotransplantation is the transplantation of living cells, tissues, or organs from one species to another (WHO Definition). Patient-derived xenografting is defined as the transplantation of human living cells or tissues into another species, commonly into rodents such as mice and rats. It was thought that the response to treatment of the human tissues in the xenograft-bearing mice would more accurately reflect the clinical response in patients than would the treated cell lines in a petri dish. The intratumoral heterogeneity and histologic characteristics of the original tumor were maintained, allowing for improved proof of principle studies [4].

A key feature that has driven the development of PDX is the ability of PDX to predict drug resistance in tumors. In one study, the accuracy of PDX for predicting

drug response was 90%, compared to 60–70% accuracy in 3D cell cultures (My Mighty Mouse, The Scientist, Scudellari M, April 1, 2015). Several international companies have now specialized in the breeding of PDX mice for research purposes, and several different subtypes of immunodeficient strains are available. The development of the NOD/SCID/IL2R mouse strains, for example, by additionally blocking the maturation of natural killer cells, offers a particularly immunodeficient mouse useful for engrafting difficult tumors more efficiently. These mice allow for excellent engraftment rates (approaching 95–100%) [5].

Unfortunately xenografting has its limitations. Although the preservation of tumor stroma is a strength of this model, the integrity of the stroma is gradually lost over the course of early passage, and it is quickly replaced with host stroma [6]. This affects drug distribution and metabolism, and tumor growth does not necessarily reflect the exact biology of the original tumor. Testing therapies that target the stromal component of tumors is of limited value in PDX models. In addition, due to the risk of rejection of the tumor implant, only immunocompromised mice are used for xenografting. The study of immunomodulating therapies in PDX is therefore not possible [4].

Classification of Cancer Biomarkers

There are several ways to classify biomarkers including the origin of the specimen (e.g., blood, urine, cerebrospinal fluid, tissue) or according to the structural component of the biomarker (e.g., DNA, RNA, protein) [3]. Figure 9.1 groups biomarkers according to proposed clinical functionality. Any given biomarker can be represented

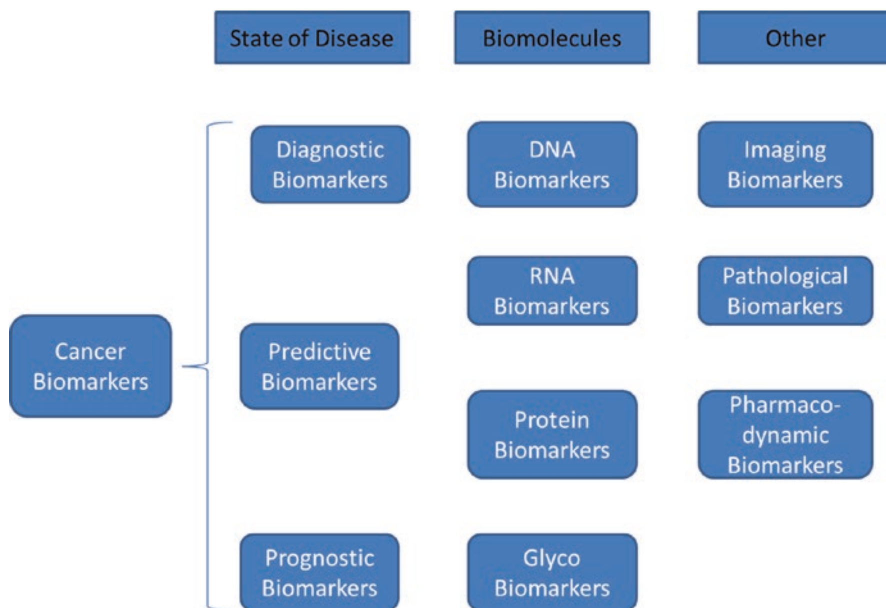


Fig. 9.1 Biomarker Classification. Adapted from Mishra A, Verma M. Cancer biomarkers: are we ready for the prime time? Cancers. 2010;2(1):190–208. doi:10.3390/cancers2010190

in more than one subcategory in this classification. Diagnostic biomarkers indicate the presence of disease, while prognostic biomarkers indicate the likely course the disease will take independent of the treatment administered. Predictive biomarkers indicate the likely response to a specific treatment. Predictive biomarkers are the basis for individualized therapy. The following discussion of biomarker development in the context of PDX is based on the structural component of the biomarker.

DNA Biomarkers

The most fundamental event in the development of cancer is the alteration of genetic information in the cancer cells that leads to autonomous growth. Changes may include especially mutations, copy number changes, and gene rearrangements. Gene expression may also be altered in similar fashion by epigenetic changes. Specific DNA alterations may or may not be relevant to the biology of the tumor, and this will be reflected in the utility of these alterations as DNA biomarkers. Driver alterations can be used to select treatment. The overall mutation rate may also be a relevant marker, as reflected in the use of mutational burden as a marker of response to checkpoint immunotherapies [7].

In a typical example of identifying an important DNA biomarker in a PDX model, Kortmann et al. established a PDX derived from a patient with ovarian serous carcinoma and a germline BRCA2 mutation. The PARP inhibitor olaparib alone and in combination with carboplatin markedly inhibited growth in this model but not in a second serous carcinoma PDX with normal BRCA status [8]. A subsequent randomized clinical trial showed that BRCA status can be used to enrich ovarian cancer patients responsive to olaparib [9].

The new frontier in DNA biomarkers is the measurement of DNA alterations in the blood of patients with cancer. Tumor DNA spills into the bloodstream with the natural turnover of tumor cells, and this circulating cell-free tumor (ct)DNA can be identified within the background of plasma DNA derived from normal cells and hematopoietic cells [10–13]. In its simplest form, a recent study showed that the amount of detectable ctDNA correlates with the patient tumor burden [14, 15]. ctDNA can, however, be exploited for much more with next-generation sequencing, which allows precise determination of tumor-specific DNA alterations. Since the ctDNA should reflect the entire tumor burden, it may overcome some of the limitations of tumor heterogeneity that arise with tissue sampling. Because it only requires a blood draw, ctDNA is an assay that can be repeated longitudinally to monitor disease progression and response to therapy. Treatment-induced alterations in the genomic landscape can be identified, and appropriate, rational changes in therapy can be instituted. This is discussed below in the context of precision oncology.

RNA Biomarkers

Synonymous with the decoding of genes has been the translational function of messenger RNA (mRNA), converting the genetic information into functional proteins [16]. However in the last two decades, the discovery of many different regulatory

nonprotein-coding RNA (ncRNA) has revolutionized the understanding of fundamental biological mechanisms and consequences of dysregulation resulting in disease [17]. ncRNA has also become the focus of diagnostic and therapeutic biomarker development. The FDA has approved several RNA sequencing tests (Table 9.1) [18].

Proof of principle studies to validate in vitro biomarker discoveries are being performed using PDX models. These models closely reflect the patient tumor microenvironment but enable biomarker investigation without burdening the patients directly. Crea et al. demonstrated this principle in PDX models of prostate cancer [19]. In RNA sequencing of paired metastatic and nonmetastatic PDX, they identified the long non-coding RNA PCAT18 as the most highly upregulated transcript. Cancer-specific upregulation of PCAT18 was confirmed in an independent

Table 9.1 Selected examples of current RNA-based clinical tests

RNA biomolecule	Method	Examples	Use
Viral RNA	qRT-PCR	• Influenza virus • Dengue virus • HIV • Ebola virus	Viral detection and typing
mRNA	qRT-PCR	• AlloMap (CareDx; heart transplant) • Cancer type ID (Biotheranostics)	Diagnosis
	Microarray	Afirma thyroid nodule assessment (Veracyte)	Diagnosis
	qRT-PCR	• Oncotype DX (Genomic Health; breast, prostate, and colon cancer) • Breast cancer index (Biotheranostics) • Prolaris (Myriad; prostate cancer)	Prognosis
	Digital bar-coded mRNA analysis	Prosigna breast cancer prognostic gene signature (NanoString)	Prognosis
	Microarray	• MammaPrint (Agendia; breast cancer) • ColoPrint (Agendia; colon cancer) • Decipher (GenomeDX; prostate cancer)	Prognosis
miRNA	Microarray	Cancer origin (Rosetta Genomics)	Diagnosis
Fusion transcript	qRT-PCR	AML (*RUNX1-RUNX1T1*)	Diagnosis
	qRT-PCR	*BCR-ABL1* (REF. 21)	Monitoring molecular response during therapy
	qRT-PCR (exosomal RNA)	ExoDx Lung (ALK) (Exosome Dx)	Fusion detection
	RNA-seq	FoundationOne Heme	Fusion detection

Adapted from Table 1 of Byron SA, Van Keuren-Jensen KR, Engelthaler DM, Carpten JD, Craig DW. Translating RNA sequencing into clinical diagnostics: opportunities and challenges. Nat Rev. Genet. 2016;17(5):257–71

AML acute myeloid leukemia, *BCR* breakpoint cluster region, *miRNA* microRNA, *qRT-PCR* quantitative reverse transcription PCR, *RNA-seq* RNA sequencing, *RUNX1* runt-related transcription factor 1, *RUNX1T1* runt-related transcription factor 1 translocated to 1 (cyclin D related)

prostate cancer patient cohort. PCAT18 was also detectable in plasma samples, and levels increased with more advanced disease.

The same group in another study compared differential RNA expression between a metastatic and nonmetastatic prostate cancer PDX and discovered a circulating microRNA signature that differentiated localized from metastatic prostate cancer [20]. A subsequent analysis of patient specimens showed some overlap with the PDX-derived signature [21].

A more recent study has taken a more comprehensive approach to the identification of RNA biomarkers in PDX [22]. A group linked to AstraZeneca performed RNA sequencing on 79 PDX models from various solid tumors. Since the human stroma of PDX is replaced by mouse stroma within a few passages, species-specific RNA sequencing allowed this group to perform comprehensive analysis of interactions between the human tumor and the murine microenvironment. They were able to establish independent tumor and stromal biomarkers. The clinical relevance of this approach remains to be determined.

Protein Biomarkers

As DNA and RNA biomarkers have strongly influenced our understanding of cancer dynamics in terms of disease identification, progression, treatment modulation, and development of resistance to treatment, so has the development of proteomics. The proteome represents the entire set of proteins modified or produced by an organism [23]. To some degree, the proteome represents the final product of the innumerable events that happen at the DNA and RNA level. Analysis of the human genome has identified over 20,000 protein-coding genes (an integrated encyclopedia of DNA elements in the human genome [24]). Posttranslational modifications add to the complexity of the proteome. A wide variety of protein cancer biomarkers has been described, but only a small number has been approved for clinical use by the FDA (Table 9.2).

The PDX model system lends itself also to protein biomarker discovery. Chiang et al. established two different PDX from a single patient with prostate cancer. One model was metastatic and the other was nonmetastatic [25]. Analysis of differential gene expression between the two models revealed six candidate master regulatory genes as drivers of metastasis in prostate cancer. One of these markers was GATA2, a transcription factor that facilitates the recruitment of additional transcription factors. Subsequent analysis revealed that elevated GATA2 expression in metastatic prostate cancer tissues correlated with poor patient prognosis. Additional in vitro and in vivo investigations showed that GATA2 may also be a relevant target of novel therapies.

Another example of protein biomarker discovery in PDX can be found in models of pancreatic ductal adenocarcinoma (PDAC). In PDX treated with gemcitabine, expression of the gemcitabine-activating enzyme deoxycytidine kinase was found to be a predictor of response to gemcitabine [26]. A subsequent investigation of patient tissue samples confirmed these results [27].

Table 9.2 Current protein cancer biomarkers approved by the FDA

Nr	Type of tumor marker	Biomarker	Type of tumor	Application	Type of specimen	Methods of detection
1	Oncofetal antigens	Alpha-fetoprotein (AFP)	Testicular hepatocellular	Risk assessment, diagnostics, and disease monitoring	Serum	Immunoassay
2		Carcinoembryonic antigen (CEA)	Colorectal	Disease monitoring, treatment response, progression	Plasma	Immunoassay
3	Cancer antigens	CA125	Ovarian	Monitoring disease, treatment response	Serum, plasma	Immunoassay
4		CA19-9	Pancreatic	Monitoring disease, treatment response	Serum, plasma	Immunoassay
5		CA 27, 29	Breast	Monitoring disease, treatment response	Serum, plasma	Immunoassay
6		CA 15-3	Breast	Monitoring disease, treatment response	Serum, plasma	Immunoassay
7		Human epididymis protein 4 (HE4)	Ovarian	Monitoring disease, treatment response, recurrence	Serum	Immunoassay
8		OVA1	Ovarian	Risk assessment, diagnostics	Serum	Immunoassay
9		ROMA (CA125,HE4)	Ovarian	Risk assessment, diagnostics	Serum	Immunoassay
10		Fibrin, fibrinogen degradation product DR-70	Colorectal	Disease monitoring, diagnostics	Serum	Immunoassay
11		Thyroglobulin	Thyroid	Monitoring disease	Serum, plasma	Immunoassay
12	Enzymes	Prostate-specific antigen (PSA)	Prostate	Disease monitoring, diagnostics	Serum	Immunoassay

(continued)

Table 9.2 (continued)

Nr	Type of tumor marker	Biomarker	Type of tumor	Application	Type of specimen	Methods of detection
13	Receptors	Estrogen receptor (ER)	Breast	Prognosis, treatment selection and response	FFPE	Immunohistochemistry
14		Progesterone receptor (PgR)	Breast	Prognosis, treatment response	FFPE	Immunohistochemistry
15		Human epidermal growth factor receptor 2 (HER/Neu)	Breast	Prognosis, treatment selection	FFPE	Immunohistochemistry
16		Mast/stem cell growth factor receptor (SCFR)/c-Kit	Gastrointestinal	Diagnosis, treatment selection	FFPE	Immunohistochemistry
17	Cell nuclear proteins	p63	Prostate	Differential diagnosis	FFPE	Immunohistochemistry
18		Nuclear mitotic apparatus protein NMP22/NuMa	Bladder	Early detection, cancer monitoring	Urine	Immunoassay

Adapted from Mäbert K(1), Cojoc M, Peitzsch C, Kurth I, Souchelnytskyi S, Dubrovska, A. Cancer biomarker discovery: current status and future perspectives, Int J Radiat Biol. 2014;90(8):659–77. doi:10.3109/09553002.2014.892229

Precision Oncology

The underlying principal of precision oncology is to anticipate the driving genomic alterations of an individual tumor and to select targeted drug treatment accordingly. Durable responses even to these molecularly guided therapeutic modulations, however, are rare and drug resistance invariably arises. Identifying markers of resistance and developing novel agents to overcome this resistance are key unmet needs in cancer research and treatment. Precision oncology is an iterative process driven by biomarker discovery. It involves all the components of biomarkers described above.

The main limitation of current concepts of precision oncology lies in our inability to predict which genomic alterations are driving growth and metastasis of an individual tumor. PDX can serve as the foundation of a program of evidence-based precision oncology by facilitating the determination of the driving alterations

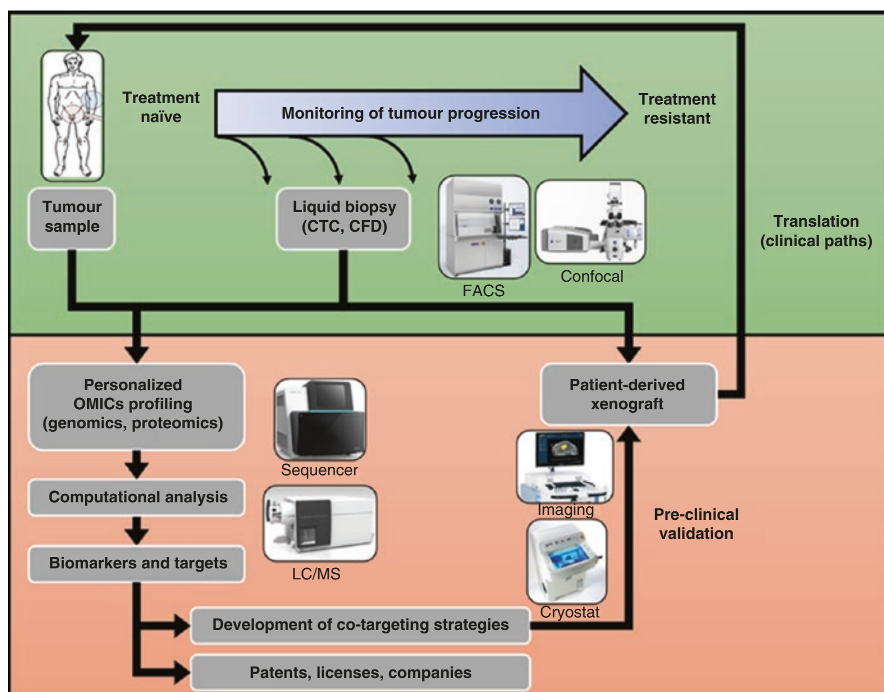


Fig. 9.2 The Vancouver Prostate Centre model of evidence-based precision oncology, employing comprehensive molecular profiling of patient tumor tissue, establishment of PDX for target validation, and longitudinal monitoring of molecular landscape of tumor with liquid biopsy

before administering therapy to patients (Fig. 9.2). Tumor tissue taken from patients is implanted into mice as PDX, but in parallel also undergoes comprehensive molecular characterization. The established PDX are characterized in a similar fashion to ensure that it remains comparable to the original patient tumor. Computational algorithms are applied in order to predict key driving alterations and identify putative drug targets. The PDX-bearing mice are then treated with the corresponding drugs, and antineoplastic effects are measured. Only if a clear response is observed, is the agent administered also to the patient from whom the PDX was derived. This may involve rational co-targeting of more than one gene or gene network to overcome potential mechanisms of resistance. If treatment resistance is observed, new PDX can be grown and the process can be repeated, or the already established PDX can be investigated for molecular alterations that may explain the resistance.

A similar model of precision oncology has been developed at the University of California, Davis, with the Serial Patient-Derived Xenograft Models to Eliminate Cancer Therapy Resistance (SPIDER) trial. In this study tumor biopsies are being taken from patients prior to treatment initiation and then again once therapy

resistance has emerged. The PDX models are treated with the same targeted agents that are administered to the patients. This allows the research team to study the developing genomic resistance mechanisms seen within the patients and their representative PDX. Targeted therapy can be modulated to the evolving molecular changes, and even future mutational changes can perhaps be anticipated. This was demonstrated by Hidalgo et al. where targeted treatment was introduced to patients once modulated drug therapy successes were achieved in their corresponding PDX models [28].

Stewart et al. have demonstrated biomarker-driven precision oncology in PDX models of EGFR-mutated non-small cell lung cancer (NSCLC) [29]. Only 6 of 33 early-stage lung adenocarcinoma samples were successfully engrafted, and these were shown to be more proliferative than those that failed engraftment. The corresponding patients had reduced overall and disease-free survival compared to the patients from whom the tissue failed to engraft. The response to EGFR-targeted therapy in these PDX closely recapitulated the course in the corresponding patients. Although the sample size was very small, there was some indication that c-MET amplification with high protein expression was associated with primary resistance to EGFR-targeted therapy. The PDX therefore revealed not only a potential marker of resistance, but also a potential target for therapy. Targeting c-MET with crizotinib induced tumor regression alone in one PDX and in combination with erlotinib in a second PDX with lower c-MET protein expression. The evidence for c-MET inhibition from clinical trials has been inconsistent, and more investigation is required to determine the true value of this marker. The MARQUEE trial showed a benefit for the c-MET inhibitor tivantinib in combination with erlotinib in a subset of patients with c-MET amplification, but the METLung phase III trial showed no additional benefit of onartuzumab in advanced NSCLC with c-MET overexpression.

In a similar fashion, Bertotti et al. put together a cohort of 85 PDX from patients with colorectal cancer [30]. The PDX responded to EGFR-targeted therapy (cetuximab) at a rate and to a degree that would be expected in a similar patient population. Based on a panel of predictive biomarkers the investigators were able to prospectively stratify the likelihood of response. As expected, KRAS mutation was an important marker of resistance, but so too was HER2 amplification in KRAS wild-type tumors. Combined HER2 and EGFR inhibition in these HER2-amplified tumors resulted in durable responses in the PDX model, and this was later validated in clinical trials.

Both of these examples demonstrate that PDX are often used to identify mechanisms of primary drug resistance. Another strategy is to treat PDX that are primarily responsive to a specific drug until these PDX develop acquired resistance. Analysis of the resistant tumors can then elucidate mechanisms of resistance. This recapitulates the usual scenario in patients. This has been done, for example, with cisplatin in platinum-sensitive ovarian cancer [31]. It has also been tested with acquired resistance to the BRAF inhibitor vemurafenib in melanoma PDX models [32].

The ultimate model for using mouse models of cancer to inform precision oncology is the “mouse hospital”. Although based primarily on genetically engineered

mouse models, PDX also fit this paradigm. Clohessy et al. [33] reported on this concept recently in conjunction with the concept of the co-clinical trial [34], whereby patients and the corresponding PDX are treated with the same drugs in a clinical trial setting, and the PDX models are used for detailed molecular evaluation of response and resistance. Co-clinical trials allow for rapid real-time transfer of information from mouse models to human trials.

Gao et al. at Novartis established 1075 PDX models from some of the most common adult solid tumors [35]. Each tumor type was represented by between 6 and 215 individual PDX. Each PDX was profiled at the RNA and DNA level, and a panel of 38 therapies was administered depending on the tumor type and the genomic changes identified. Each mouse received one treatment, but each patient tumor was represented in different mice, so that any single tumor was treated with multiple agents. Response rates based on tumor type and genomic alterations closely resembled what would be expected in a similar patient population. While exploratory analyses were conducted to identify biomarkers of resistance in these PDX, the models were also used to validate markers of resistance that had been hypothesized in vitro. These potentially predictive biomarkers were, however, not validated in patients.

Real-time patient monitoring to identify emerging resistance is another crucial component of precision oncology. Minimally invasive liquid biopsies are a particularly attractive means to achieve this goal. Liquid biopsy refers to the molecular characterization of a tumor by analysis of ctDNA and circulating tumor cells (CTC). CTC can be analyzed also for RNA and protein changes, so that these parameters together provide detailed information on the evolving molecular landscape of the tumor under the selective pressures of ongoing treatment. Liquid biopsies are able to overcome the common barrier of not being able to profile patient tissue routinely in the metastatic setting.

While the analysis of ctDNA and CTC in patient samples continues to evolve, PDX offer the opportunity to refine methodology prior to clinical implementation. Giuliano et al. demonstrated in PDX models of breast cancer that CTC can be detected in the majority of cases. Hayashi et al. developed a digital drop PCR assay to detect tumor-specific EWS-ETS breakpoints in Ewing sarcoma cell lines, PDX, and patients. The EWS-ETS translocation is known to drive the growth of this tumor type, but the intronic breakpoint is specific to each individual tumor. Detection of EWS-ETS in ctDNA of PDX-bearing mice correlated closely with disease burden, and, for example, was able to detect metastatic disease recurrence after resection of the primary tumor.

It is important to acknowledge limitations of PDX models with respect to precision oncology. As soon as the PDX models are transitioned to in vitro cultured cell lines, they are likely to drift rapidly from the parental tumor [33]. They are also likely to drift at a molecular level with each successive generation of propagation in mice. A key limitation of PDX is also the engraftment rate, which can vary by tumor type, organ site of inoculation, and research group. Only a selection of tumors can be studied in the PDX model, which may mean that PDX do not represent the broader patient population [36]. Furthermore, PDX may underrepresent

the heterogeneity of the original tumor, as more aggressive clones are likely to dominate. Finally, in the current era of rapidly evolving immunotherapy, PDX are severely limited by the lack of an intact immune system in the host mouse, which is most frequently the nonobese diabetic, severe combined immune-deficient (NOD-SCID) mouse. However, mouse models with a humanized immune system have been developed [37, 38]. Unfortunately these add expense to an already costly model system.

Conclusion

As cancer research and clinical oncology move increasingly towards molecularly directed therapy, PDX are an important tool not only in drug testing but also in biomarker development. Whether studying biomarkers at the RNA, DNA, or protein level, and whether in tissue, blood, or other bodily fluid, the PDX provide a model system that recapitulates patient disease with the highest fidelity. The PDX are most important for deciphering the driver from the passenger alterations, thereby facilitating target validation and enabling evidence-based precision oncology.

References

1. Aronson JK. Research priorities in biomarkers and surrogate end-points. Br J Clin Pharmacol. 2012;73(6):900–7.
2. Strimbu K, Tavel JA. What are biomarkers? Curr Opin HIV AIDS. 2010;5(6):463–6. doi:10.1097/COH.0b013e32833ed177.
3. Mishra A, Verma M. Cancer biomarkers: are we ready for the prime time? Cancers. 2010;2(1):190–208.
4. Kopetz S, Lemos R, Powis G. The promise of patient-derived xenografts: the best laid plans of mice and men. Clin Cancer Res. 2012;18(19):5160–2.
5. Shultz LD, Goodwin N, Ishikawa F, Hosur V, Lyons BL, Greiner DL. Human cancer growth and therapy in NOD/SCID/IL2Rγ(null) (NSG) mice. Cold Spring Harb Protoc. 2014;2014(7):694–708. doi:10.1101/pdb.top073585.
6. Bagby S, Messersmith WA, Pitts TM, Capasso A, Varella-Garcia M, Klauck PJ, et al. Development and maintenance of a preclinical patient derived tumor xenograft model for the investigation of novel anti-cancer therapies. J Vis Exp. 2016;30(115):54393.
7. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science. 2015;348(6230):124–8.
8. Kortmann U, McAlpine JN, Xue H, Guan J, Ha G, Tully S, et al. Tumor growth inhibition by olaparib in BRCA2 germline-mutated patient-derived ovarian cancer tissue xenografts. Clin Cancer Res. 2011;17(4):783–91. doi:10.1158/1078-0432.CCR-10-1382. 1078-0432.CCR-10-1382 [pii]
9. Oza AM, Cibula D, Benzaquen AO, Poole C, Mathijssen RH, Sonke GS, et al. Olaparib combined with chemotherapy for recurrent platinum-sensitive ovarian cancer: a randomised phase 2 trial. Lancet Oncol. 2015;16(1):87–97.
10. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012;366(10):883–92.

11. Leon SA, Shapiro B, Sklaroff DM, Yaros MJ. Free DNA in the serum of cancer patients and the effect of therapy. Cancer Res. 1977;37(3):646–50.
12. Sorenson GD, Pribish DM, Valone FH, Memoli VA, Bzik DJ, Yao SL. Soluble normal and mutated DNA sequences from single-copy genes in human blood. Cancer Epidemiol Biomarkers Prev. 1994;3(1):67–71.
13. Vasioukhin V, Anker P, Maurice P, Lyautey J, Lederrey C, Stroun M. Point mutations of the N-ras gene in the blood plasma DNA of patients with myelodysplastic syndrome or acute myelogenous leukaemia. Br J Haematol. 1994;86(4):774–9.
14. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med. 2014;6(224):224ra24. doi:10.1126/scitranslmed.3007094.
15. Thierry AR, Mouliere F, Gongora C, Ollier J, Robert B, Ychou M, et al. Origin and quantification of circulating DNA in mice with human colorectal cancer xenografts. Nucleic Acids Res. 2010;38(18):6159–75.
16. Taft RJ, Pang KC, Mercer TR, Dinger M, Mattick JS. Non-coding RNAs: regulators of disease. J Pathol. 2010;220(2):126–39.
17. Cooper TA, Wan L, Dreyfuss G. RNA and disease. Cell. 2009;136(4):777–93. doi:10.1016/j.cell.2009.02.011.
18. Byron SA, Van Keuren-Jensen KR, Engelthaler DM, Carpten JD, Craig DW. Translating RNA sequencing into clinical diagnostics: opportunities and challenges. Nat Rev Genet. 2016;17(5):257–71. doi:10.1038/nrg.2016.10.
19. Crea F, Watahiki A, Quagliata L, Xue H, Pikor L, Parolia A, et al. Identification of a long noncoding RNA as a novel biomarker and potential therapeutic target for metastatic prostate cancer. Oncotarget. 2014;5(3):764–74.
20. Watahiki A, Wang Y, Morris J, Dennis K, O'Dwyer HM, Gleave M, et al. MicroRNAs associated with metastatic prostate cancer. PLoS One. 2011;6(9):30.
21. Watahiki A, Macfarlane RJ, Gleave ME, Crea F, Wang Y, Helgason CD, et al. Plasma miRNAs as biomarkers to identify patients with castration-resistant metastatic prostate cancer. Int J Mol Sci. 2013;14(4):7757–70.
22. Bradford JR, Wappett M, Beran G, Logie A, Delpuech O, Brown H, et al. Whole transcriptome profiling of patient-derived xenograft models as a tool to identify both tumor and stromal specific biomarkers. Oncotarget. 2016;7(15):20773–87.
23. Wilkins MR, Pasquali C, Appel RD, Ou K, Golaz O, Sanchez JC, et al. From proteins to proteomes: large scale protein identification by two-dimensional electrophoresis and amino acid analysis. Biotechnology. 1996;14(1):61–5.
24. ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. Nature. 2012;489(7414):57–74. http://www.nature.com/nature/journal/v489/n7414/abs/nature11247.html#supplementary-information
25. Chiang YT, Wang K, Fazli L, Qi RZ, Gleave ME, Collins CC, et al. GATA2 as a potential metastasis-driving gene in prostate cancer. Oncotarget. 2014;5(2):451–61.
26. Rubio-Viqueira B, Jimeno A, Cusatis G, Zhang X, Iacobuzio-Donahue C, Karikari C, et al. An in vivo platform for translational drug development in pancreatic cancer. Clin Cancer Res. 2006;12(15):4652–61.
27. Sebastiani V, Ricci F, Rubio-Viqueira B, Kulesza P, Yeo CJ, Hidalgo M, et al. Immunohistochemical and genetic evaluation of deoxycytidine kinase in pancreatic cancer: relationship to molecular mechanisms of gemcitabine resistance and survival. Clin Cancer Res. 2006;12(8):2492–7.
28. Hidalgo M, Bruckheimer E, Rajeshkumar NV, Garrido-Laguna I, De Oliveira E, Rubio-Viqueira B, et al. A pilot clinical study of treatment guided by personalized tumor grafts in patients with advanced cancer. Mol Cancer Ther. 2011;10(8):1311–6.
29. Stewart EL, Mascaux C, Pham NA, Sakashita S, Sykes J, Kim L, et al. Clinical utility of patient-derived xenografts to determine biomarkers of prognosis and map resistance pathways in EGFR-mutant lung adenocarcinoma. J Clin Oncol. 2015;33(22):2472–80.

30. Bertotti A, Migliardi G, Galimi F, Sassi F, Torti D, Isella C, et al. A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. Cancer Discov. 2011;1(6):508–23.
31. Vidal A, Munoz C, Guillen MJ, Moreto J, Puertas S, Martinez-Iniesta M, et al. Lurbinectedin (PM01183), a new DNA minor groove binder, inhibits growth of orthotopic primary graft of cisplatin-resistant epithelial ovarian cancer. Clin Cancer Res. 2012;18(19):5399–411.
32. Das Thakur M, Salangsang F, Landman AS, Sellers WR, Pryer NK, Levesque MP, et al. Modelling vemurafenib resistance in melanoma reveals a strategy to forestall drug resistance. Nature. 2013;494(7436):251–5.
33. Clohessy JG, Pandolfi PP. Mouse hospital and co-clinical trial project—from bench to bedside. Nat Rev Clin Oncol. 2015;12(8):491–8. doi:10.1038/nrclinonc.2015.62.
34. Lunardi A, Ala U, Epping MT, Salmena L, Clohessy JG, Webster KA, et al. A co-clinical approach identifies mechanisms and potential therapies for androgen deprivation resistance in prostate cancer. Nat Genet. 2013;45(7):747–55. doi:10.1038/ng.2650. http://www.nature.com/ng/journal/v45/n7/abs/ng.2650.html#supplementary-information
35. Gao H, Korn JM, Ferretti S, Monahan JE, Wang Y, Singh M, et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nat Med. 2015;21(11):1318–25. doi:10.1038/nm.3954. http://www.nature.com/nm/journal/vaop/ncurrent/abs/nm.3954.html#supplementary-information
36. Gandara DR, Lara Jr PN, Mack PC. Patient-derived xenografts for investigation of acquired resistance in oncogene-driven cancers: building a better mousetrap. J Clin Oncol. 2015;33(26):2839–40. doi:10.1200/JCO.2015.61.9692.
37. Aryee KE, Shultz LD, Brehm MA. Immunodeficient mouse model for human hematopoietic stem cell engraftment and immune system development. Methods Mol Biol. 2014;1185:267–78.
38. Simpson-Abelson MR, Sonnenberg GF, Takita H, Yokota SJ, Conway Jr TF, Kelleher Jr RJ, et al. Long-term engraftment and expansion of tumor-derived memory T cells following the implantation of non-disrupted pieces of human lung tumor into NOD-scid IL2Rgamma(null) mice. J Immunol. 2008;180(10):7009–18.

10 Patient-Derived Xenografts as Cancer Models for Preclinical Drug Screening

Juliet A. Williams

Abbreviations

CAFs	Cancer-associated fibroblasts
CR	Complete regression
CRC	Colorectal cancer
ER	Estrogen receptor
HSCs	Hematopoietic stem cells
MCTs	Mouse clinical trials
NSCLC	Non-small cell lung carcinoma
PBMCs	Peripheral blood mononuclear cells
PD	Pharmacodynamics
PDAC	Pancreatic ductal adenocarcinoma
PK	Pharmacokinetics
PR	Partial regression
PRL	Prolactin
RTK	Receptor tyrosine kinase
SD	Stable disease
TCGA	The Cancer Genome Atlas
TGF	Transforming growth factor

History of the Utility of Mouse Models in Oncology Drug Discovery and Development

New drugs discovered for oncology clinical trials require profiling in preclinical species before entering humans. One aim of preclinical experiments is to screen compounds to identify those that engage the target in vivo and have the best drug-like

J.A. Williams
Oncology Disease Area, Novartis Institutes for Biomedical Research, Cambridge, MA, USA
e-mail: juliet.williams@novartis.com

© Springer International Publishing AG 2017
Y. Wang et al. (eds.), *Patient-Derived Xenograft Models of Human Cancer*,
Molecular and Translational Medicine, DOI 10.1007/978-3-319-55825-7_10

properties. Another aim is to assess the translatability of the identified drugs to the clinic, i.e., to understand potential efficacy of the compound in different types of cancer and subpopulations of those cancers. Mouse models have long been used for both of these aims of drug identification and translatability, because of their ease of use.

The original mouse models for oncology drug discovery were mice carrying mouse tumors which had arisen spontaneously or been created by exposure to carcinogenic agents. The ability to grow and expand these mouse cancer cell lines in vitro enabled the same models to be widely used and disseminated between researchers, resulting in approximately 20 well-known and utilized syngeneic mouse models for drug discovery research. The first human tumor xenografts were published in the late 1960s and early 1970s after the advent of immunocompromised mice [1, 2]. These mice, because they are immunocompromised, do not reject human cells, allowing human tumors to grow in them. The original xenografts were constructed using cancer cell lines developed by culturing tumor tissue from patients in vitro. Unfortunately, continuous passaging of the cells in vitro tend to select for cellular subsets that thrive in plastic dishes, leading to loss of the original tumor heterogeneity. In addition, tumor growth-supporting factors such as those arising from tumor stroma and extracellular matrix are eliminated—a major departure from the natural tumor state [3, 4]. Nevertheless, cell line in vivo xenografts have been, and still are, very useful in screening compounds for drug-like properties. In drug discovery, these kinds of models are often used to assess the drug exposures needed to modulate the biological effect and what biological effect is needed to observe efficacy, i.e., data crucial for identifying the best compounds to pursue. However, when it comes to the translatability question, predicting which populations of patients in the clinic may respond, patient-derived xenografts, obtained by directly engrafting cancer tissue from patients in mice without the in vitro culturing step, are thought to have more utility. This is because PDXs retain more key characteristics of the original tumors than cell line-based xenografts and also because of the impressive diversity of tumors available. Indeed, for some programs, cell line-based models are not relevant. For example, cell lines grown in 2D lose their dependency on the Hedgehog (Hh) pathway as shown with cultured medulloblastoma cells [5], making it impossible to use such cell line models to assess the efficacy of Hh pathway inhibitors.

Consequently, the need for drug discovery programs, such as those targeting developmental pathways, has contributed to the rapid expansion in the industry to obtain and characterize PDXs in the belief they are more translatable to the clinic. Supporting this argument, genomic analysis of PDXs compared to cell lines and The Cancer Genome Atlas (TCGA) has shown that various signaling pathways are under- or overrepresented in cell lines across lineages to a greater degree than in PDXs [6]. It should be noted, however, that all models are imperfect in their own way and that the best models which answer the specific question or objective in hand should be chosen.

Status of PDX Models Available for Drug Discovery

Many PDX models have been successfully established for pancreatic ductal adenocarcinoma (PDAC), breast carcinoma, gastric carcinoma, colorectal carcinoma (CRC), non-small cell lung carcinoma (NSCLC), esophageal carcinoma, ovarian carcinoma, AML, and many other cancers [7]. There are some gaps in PDX collections: for example, prostate cancer models have been notoriously hard to establish, although exceptions are found, and head and neck cancers (HPV causative) have also been difficult to grow as PDXs, although some research groups have valuable collections. Estrogen receptor (ER)-positive and ER-dependent PDX models are limited; even if ER positivity is retained, lack of response to estrogen-targeted agents is often noted [8]. A potential method to overcome this is to graft the cells into the milk ducts instead of implanting them in the subcutaneous compartment. The ER^+ tumor cells develop, like their clinical counterparts, in the presence of physiological hormone levels. Intraductal ER^+ PDXs are retransplantable, predictive, and appear genomically stable, providing opportunities for translational research and the study of physiologically relevant hormone action in breast carcinogenesis [9].

Histological and genetic characterization has shown that once a PDX is established, the tumor architecture is largely preserved and, at most, undergoes limited genetic drift [7, 10, 11]. Furthermore, engrafted tumor tissue retains the genetic and epigenetic abnormalities found in patients. Although xenograft tissue can be excised from the patient to include the surrounding human stroma [12], it should be noted that after a couple of passages in mice, the human stroma component is completely replaced by mouse stroma, making it impossible to use xenografts for studying all cancer cell-stroma interactions. For example, the human Met receptor does not recognize the mouse Met ligand, so paracrine Met signaling is not recapitulated. Also, mouse prolactin (PRL) antagonizes the human PRL receptor, thereby impairing the ability of PRL receptor-positive human tumors or cell lines to grow in mice [13]. Additionally, as with cell line-based xenografts, the vasculature in subcutaneously injected tumors is not mimicked well, being exceptionally leaky, so caution should be taken if anti-angiogenic drugs are assessed with these models [12]; orthotopic models may be more appropriate to evaluate this category of agents. Finally, as PDXs are grown in immunocompromised mice, many immune components are lacking in most models [14], leading to an inability to evaluate immunomodulatory agents in these mice. However, it should be noted that more complex models can be created to "humanize" the immune system in such mice by adoptive transfer protocols and other methodologies [15, 16].

Site-specific microenvironmental differences and the lack of an immune system are likely to have substantial roles in the selection of cells of the original donor tumor that preferentially get established as a PDX in the immunodeficient animal. The clonal dynamics of breast xeno-engraftment have been examined at single cell resolution [17]. While the initial clonal selection, i.e., the first passage in mice,

seems quite variable, PDXs are, once established, in most cases relatively genetically stable. This could have a profound impact when using personalized PDXs to guide patient therapy, as in the avatar trial setting. However, if PDXs are used to represent any patient with a similar genetic profile, the profile of the original sample becomes moot.

Recently, considerable genomic analysis of over 400 PDXs has revealed that, for the most part, panels of PDXs can represent the genetic heterogeneity found in the patient population [6]. When comparing PDXs and cell lines with the TCGA, it was found that PDXs more faithfully represent the patient population than cell lines where various signaling pathways are under- or overrepresented across lineages. For example, in cell lines, there is under-representation of alterations in the PI3K pathway in NSCLC and overrepresentation of the transforming growth factor (TGF) beta pathway in PDAC and receptor tyrosine kinase (RTK) alterations in BRCA. In contrast, in the PDX collection, these pathways are accurately represented at a similar mutation frequency as reported for patient tumors. The discrepancies between in vitro and in vivo data may well reflect the clonal bias inherent in immortalized cells propagated on plastic. Given that there are considerably many more PDX models available than cell lines also leads to the use of PDXs to model the diverse inter-patient heterogeneity seen in man.

The ability to quickly profile protein phosphorylation of many samples, now catching up with the ability to readily carry out genetic profiling, means that the PDX tumor models will soon be even better characterized than even 5 years ago, with information at the protein cell signaling pathway level [18].

Utilizing PDX Tumor Models for Drug Discovery and Translatability

In drug discovery, various steps are applied to identify and profile lead compounds and ultimately new drug candidates. One stage, often referred to as lead optimization, is the process in which numerous compounds are profiled through assays using “work horse” models to determine how a molecule can be optimized so the final version is a compound with excellent drug-like properties. Some of these assays are in vivo assays which, for oncology programs, most often utilize cancer xenograft models. Here, target engagement is assessed in an in vivo context, and three data sets are combined to ultimately understand the relationship of three parameters: drug exposure or pharmacokinetics (PK), target engagement or pharmacodynamics (PD), and drug efficacy. Once the optimal chemical matter has been identified to pursue, xenograft models are somewhat expanded to look at PK-PD- efficacy in a handful more models. It is important to assess the compound in a variety of in vivo models, as the

predicted exposure of the drug, needed to see efficacy in humans, is calculated from the ability of a drug to halt tumor growth (tumor stasis) or regress the tumor in these models—with the understanding that a range of models may likely aid a better prediction. Although cell line-based xenografts are often used at these stages of drug discovery due to their ease of use, PDX models may, dependent on the availability of specific models, be more useful. For example, recently identified mutations in the estrogen receptor, ESR1, in endocrine therapy-resistant ER-positive breast cancer patients are only found in established PDX models and not in cell lines [19]. Regardless, even if many cell lines can be used for a given target, it may be prudent to use at least one PDX model for determining a human dose prediction, as sometimes the PDX models may be more resistant to treatment. Additionally, a range of models with different sensitivities is helpful to more accurately predict the human exposure needed to have a significant effect.

After the lead optimization stage of the drug discovery process is complete, and a lead compound has been identified to take into humans, then the candidate drug enters the translational phase of drug discovery. One aspect of this phase is to determine where the drug may have further applications, i.e., expand upon the initial hypothesis of where the drug may be effective, to other indications and subsets of populations in those indications. Traditionally, to examine "indication expansion," only a few representative models for each indication were chosen. Additionally, experiments were done with these models by using $N = 8–10$ mice for each dose of agent being tested, numbers potentially necessary for statistical evaluation of the agent to overcome growth variations of the tumors. However, oncology drugs have had low rates of success in clinical trials [20, 21], and although this may be attributed to a range of reasons, one is that cancer is a very heterogeneous disease. The implications of this are that testing drugs with only a few models for each cancer indication may not capture the heterogeneity of the disease and subsequently overpredict the patient population that will benefit from the drug or, contrary, not be powered to identify the individuals who will benefit most. Profiling drugs across a large panel of oncology models may seem a large undertaking, highly costly, and time-consuming. However, with the increasing standards for what robust efficacy means, from reducing the rate of growth (growth control) to wanting tumor stasis or stable disease (SD), and preferably regression [partial regression (PR) or complete regression (CR)], the need for large Ns for each model diminishes.

More recently, groups have been performing large-scale screening using the "mouse clinical trial" or MCT approach first described by Migliardi et al. [22]. Here, a limited number of mice carry one PDX and are treated with one drug, but the number of PDXs from different patients is large. When looking at responses in this paradigm, it is not the response of an individual mouse/tumor per se that is important when evaluating a drug but the population response, just like in a human

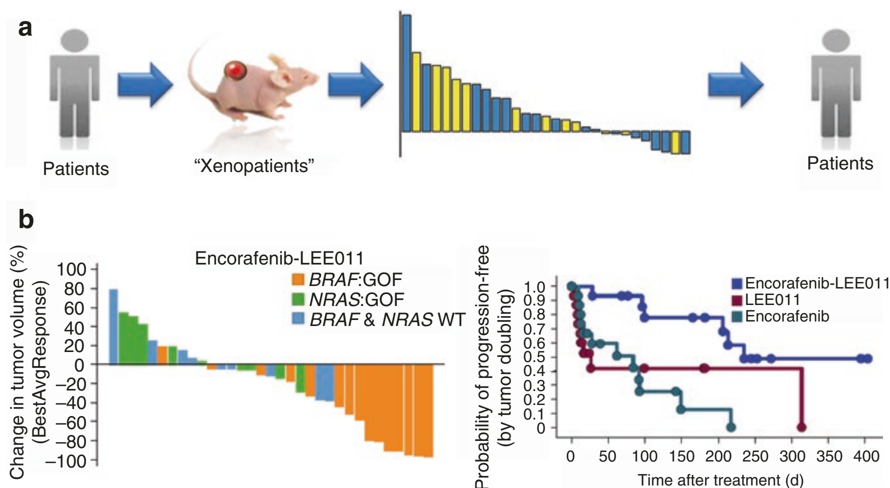


Fig. 10.1 Modeling inter-patient response heterogeneity with mouse clinical trials (MCTs). (**a**) Schematic depicting the mouse clinical trial paradigm where mice bear one representative PDX tumor, originally created from the tumor of one patient and treated with one therapy or combination of therapies (mirroring a human clinical trial setting). Data are collected in much like the fashion it would be in the clinic, and those results influence which therapies are given to current patients, i.e., guided clinical trials. (**b**) Representative data from a mouse clinical trial showing "best average response" data in a waterfall plot and also pseudo-survival data approximated by the probability of time to endpoint by tumor doubling. This example shows the efficacy of encorafenib, a Raf inhibitor, combined with LEE011, a CDK4/CDK6 inhibitor in a melanoma mouse clinical trial. The data show that this combination has a profound effect on *BRAF* gain-of-function xenopatients and also some *BRAF* and *NRAS* wild-type patients. They also show that the effects of the drugs are more pronounced when administered in combination than as single agents

clinical trial. MCT data are assessed, close to how one would in a human clinical trial, for example, measuring "best response" and "time to progression" (Fig. 10.1). Migliardi et al. [22] chose to evaluate the effect of four different treatment arms (ERK, MEK, and PI3K inhibitors) across 40 different colon cancer PDXs each with an $n = 4$. Another study [23] took a similar screening approach, in this case using over 100 different colorectal cancer models ($n = 5$ or 6) to profile the efficacy of epidermal growth factor receptor inhibitor, cetuximab, and found concordance in the response to this drug of EGFR-amplified models and CRC patients in the clinic. In the latter case, all tumors were well annotated with complete exome sequence and copy number analyses, to enable determination of the population response relative to genetic profile.

Also more recently, large-scale mouse clinical trials using the 1 × 1 × 1 approach have been published. In this paradigm, only one mouse is used, bearing one type of PDX, and given one treatment [6, 24]. In both cases investigators determined that using the one animal per cohort study design has outstanding reproducibility for data collected with cohorts using an $n = 4$ or greater. A very comprehensive study of a panel of pediatric tumors [25] substantially analyzed the individual tumor response by taking a randomly chosen mouse and comparing the response to the

group median. In a total of 2134 comparisons, the single tumor response accurately predicted the group median response in 1604 comparisons (75.16%) (mean tumor response correct prediction rate 78%). Allowing for the misprediction of +/− one response category (SD, PR, CR), the overall mean correct single mouse prediction rate was 95.28% and predicted overall object response rates for group data in 66 of 67 drug studies. The ability to use the 1 × 1 × 1 approach of course enables many more types of PDX and treatment groups to be assessed operationally and the heterogeneity of patients to be captured experimentally. This has been most comprehensively demonstrated in a study where 62 treatments were assessed across six indications comprised of 29–45 models per indication [6]. Here modified RESIST criteria were used to assess responses comparable to the clinic (Fig. 10.2, Table 10.1) as well as “best average response” (a calculation taking into account best response, time to response, and duration of response) and pseudo-survival calculations (probability of being progression-free by tumor doubling time) (Fig. 10.1). The 1 × 1 × 1

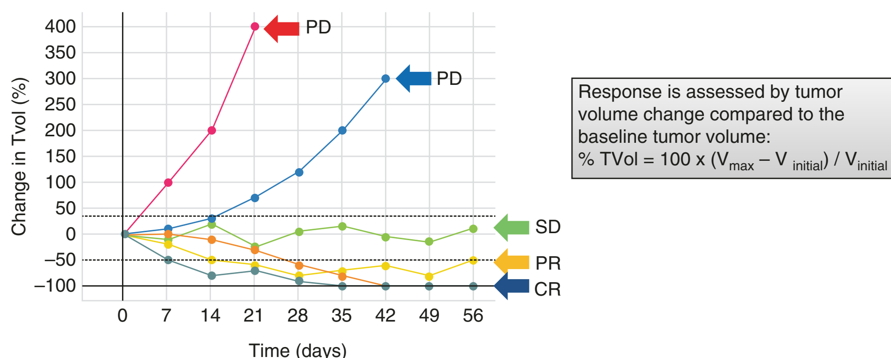


Fig. 10.2 Determination of progressive disease, stable disease, partial response, and complete response in mouse clinical trials. The schematic depicts data from a mouse clinical trial showing change in tumor volume relative to baseline resulting in the assessment of progressive disease, stable disease, partial response, and complete response

Table 10.1 Mouse clinical trial assessment of tumor response to treatment with sensitivity parameters based on RECIST

Clinical term	Clinical	Preclinical
Complete response (CR)	Disappearance	Complete regression
Partial response (PR)	>30% decrease in the sum of longest diameters	>50% decrease in tumor volume
Stable disease (SD)	<30% decrease and <20% increase in the sum of longest diameters	<50% decrease and <35% increase in tumor volume
Progressive disease (PD)	>20% increase in sum of longest diameters	>35% increase in tumor volume

Table depicting the similarities and differences of the terms complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) used for clinical data and preclinical data in the mouse clinical trial setting, i.e., how RECIST criteria have been modified

MCT approach has also proven to be successful in a “phase II”-like study using B-ALL PDXs, again in well-annotated tumors [24], as well as the pediatric MCT where 67 agents were profiled across 84 xenograft models [25]. This mouse clinical trial approach has now been adapted by many pharmaceutical companies following suit, many of whom use CROs with extensive PDX collections to perform such studies.

The PDX mouse clinical trials really allow insight into inter-patient response heterogeneity in an efficient manner and help to identify responsive subpopulations and thus enable the discovery of predictive biomarkers [26, 27]. In addition, the approach can be used to identify clinically relevant mechanisms of resistance and potentially enhance predictability for clinical trials having shown the beginnings of retrospective translatability [6]. The field still needs to assess whether translatability of MCTs to the clinic is influenced by using naive PDXs versus PDXs derived from pretreated tumors. Most large panels of PDX models that are available to the community have been derived from patient tumors that have not undergone any therapy, but there are exceptions. It is probable that when agents directly target a driver mutation, the initial response would be demonstrated by both naive and pretreated tumors. The translatability with targeted therapy seen in an extensive MCT study [6] suggests that could be so. However, the translatability for standard of care chemotherapeutics was less robust. For chemotherapeutics, inability to match exposure and dosing regimens, such as infusion in mice, may play a part in the disconnect between clinical trial response and the mouse clinical trial approach. Additional evidence that naive tumors may be used to assess targeted therapies is demonstrated in a B-ALL MCT, where both pretreated and naive tumor PDX models responded to targeted therapy (MDM2 inhibitor) in a similar fashion [24]. However, it could be reasonably hypothesized that the range and temporal response of resistance mechanisms could differ, depending whether naive or pretreated models are used, and this could also be influenced by the kind of pretreatment. Indeed, it is known, for example, that some agents such as TMZ can change even the driver mutations, so care should be taken if researchers are trying to model TMZ-treated patient populations to understand which drugs may be effective for this group of people [28]. How important it is to use naive vs. pretreated PDXs when assessing standard epigenetic targeting agents has yet to be determined, although it could be potentially more problematic.

There is evidence that the liabilities with cell line-based models, previously discussed above, are borne out in discrepancies with respect to drug response [6]. An in vitro combination screen in melanoma failed to identify the combinatorial effect of the CDK4/CDK6 inhibitor with other targeted therapies, whereas the MCT subsequently did reveal this effect (as exemplified by the combination of LEE011 and encorafenib). The studies additionally show differential combination effects with IGF1R inhibitors in vitro and in vivo. IGF1R inhibitors have long been shown to have great results in vitro as single agents [29] and in combinations with MEK1/MEK2 [29–31] or with PI3K/mTOR [32]. Also importantly, these combinations with IGF1R inhibitors have been efficacious in select cell-derived xenografts and PDXs in vivo. These positive results have led to a number of clinical trials in CRC

and NSCLC as well as in several other indications, yet data from these clinical trials are fairly disappointing [33–35]. Likewise, in the mouse clinical trial clinic where a large portfolio of PDXs is examined for efficacy, performance of IGF1R inhibitors across the board was unimpressive [6], concordant with results in the human clinical setting. Given the high rate of failure of oncology drugs, where under 10% of compounds which go into clinical trials eventually get approved [20, 21, 36], it is hoped that, by assessing the heterogeneity of response across models representing the diversity of the population, the number of successful clinical trials can substantially improve.

Avatars

Another methodology to attempt to improve success rates in the clinic is a rapidly developing application for PDXs commonly referred to as the avatar approach. This is the practice of using PDXs to preclinically guide treatment decisions for patients from whom the tumors were derived [37, 38]. As patient surrogates, these avatar PDX models hope to represent a power tool for addressing individualized therapy for the patient from whom the PDX model was created.

For this paradigm to work, the patient's PDX must establish in mice within the life span of the patient. Given that PDX models often do not grow in mice and, if they do, can take months to establish, only certain types of cancer can benefit from this approach. Additional issues include that patients and mice often tolerate different levels of drug toxicity, which may in part be due to differing dosing schedules and that drug exposures may not be fully translatable. There is also the added complexity that patients are often on additional medications and diets, which are not factored into PDX treatments [15]. Another potential caveat, particular to avatars, is the consequence of cancer being a heterogeneous disease; the biopsy of the tumor captured and grown in the mouse may or may not represent the bulk of the tumor growing in the patient. However, the proclaimed success of avatars [39, 40] may suggest that this may only be an issue at a later stage of disease progression, when the disease is more heterogeneous and potentially does not reflect the PDX derived from the primary tumor.

The successful avatar approach has been shown for a variety of tumor types. Weroha and colleagues used nine high-grade serous ovarian avatar models, treated with four rounds of carboplatin/paclitaxel. When compared to patient response, nine out of nine PDXs demonstrated in vivo platinum response reflective of the patient's clinical response [39]. In a breast cancer model, Zhang and colleagues established PDX models representing a variety of breast cancer subtypes. In this report a significant association between the PDX and patient treatment response was observed, with 12 of the 13 PDX responses matching the patient's clinical response [40]. Garralda and colleagues used avatar models along with whole-exome sequencing analysis in order to inform the treatment of patients with advanced stage solid tumors, including CRC, glioblastoma, NSCL, melanoma,

and PDAC. Here the response of 11 of the 13 models mimicked the patient response [41].

However, it is true that not all PDXs represent the patient's tumor they were derived from. As only a part of the originally patient tumor is used to create the avatar PDX, the sample taken may not truly represent the whole disease. In addition, it is known that the site-specific microenvironmental differences in the subcutaneous layer of skin in a mouse cause selection pressure. These determine which cells from the original host tumor preferentially are established in the animal. The clonal dynamics of breast xeno-engraftment have been examined at single cell resolution [17] and shown that the initial clonal selection, i.e., the first passage in mice, seems quite variable which could have a profound implication on some avatar trials.

To address the potential concern of intra-tumor heterogeneity further, PDXs are being generated from tumor samples obtained from warm autopsy. The aim of this approach is to obtain multiple biopsies from different metastatic sites from the one patient at the time of treatment failure and then to directly compare similarities and differences between samples. This would address the issue of heterogeneity at the end of the patient's journey and enable a direct comparison with PDXs generated from chemo-naive patients at the time of diagnostic surgery, prior to any cancer treatment, when tumor heterogeneity may be less of an issue. Indeed, analysis of melanoma PDXs from vemurafenib therapy-refractory metastases in a patient revealed that multiple resistance mechanisms were present within one metastasis and between metastases. This heterogeneity, both inter- and intra-tumorally, caused an incomplete capture in the PDXs of the resistance mechanisms observed in the patient, indicating that PDXs may not harbor the full genetic heterogeneity seen in the patient's melanoma [42]. This obviously could have profound implications on the avatar/co-clinical trial approach. Further studies like these may enable a greater prediction to which avatars could be beneficial/predictive for patients, i.e., which types of tumor and at which stage. Again, the fact that many studies report comparable outcomes from a PDX derived from a single tumor site, when compared with the patient response to treatment, suggests that tumor heterogeneity may not be such an issue at first diagnosis, at least for some cancers.

"Humanized" PDX Models

One obvious short coming of PDX tumor models is that they have to be created in immunodeficient mice to prevent immune attacks against the xenotransplanted tumor. Therefore, a critical component of the known tumor microenvironment interaction is missing in the tumor. The consequence is that cancer agents that target the immune system components cannot be studied using xenograft models. This is important as it has been well documented that the immune system is critically involved in cancer initiation and expansion which has led to the relatively recent success and ballooned interest in targeting the immune system [43, 44]. Consequently, researchers are beginning to explore the use of what is labeled as

"humanized-xenograft" models. Humanized-xenograft models are created by co-engrafting a patient tumor fragment (or cell line) and human peripheral blood mononuclear cells (PBMCs) or hematopoietic stem cells (HSCs) into immunodeficient mice [15, 16]. Humanized-xenograft models for acute lymphoblastic leukemia and acute myeloid leukemia have been created [45]. However, these strategies have yet to be validated for most tumor types, and there remain questions over whether the reconstituted immune system will behave in the same way as it does in the patient. For example, the immune system could be "hyper-activated" due to exposure to mouse tissues in a similar fashion to graft-versus-host disease [46]. How close HLA matching needs to be with the human tumor and human immune cells to mitigate potential rejection of the tumor also remains to be understood.

The strain of mice used influences human PBMC and HSC engraftment success; the development of three different murine strains with IL-2 receptor mutations has increased rates of engraftment: NOD.Cg-PrkdcscidIl2rgtm1Wjl (NSG mice), NODShi.CgPrkdcscidIl2rgtm1Sug (NOG mice), and C;129S4-Rag2tm1FlvIl2rgtm1Flv (BRG mice) [47, 48]. Of these models, NSG mice lack the IL-2 receptor, while NOG and BRG mice express a truncated IL-2 receptor. However the result is all these models lack important resulting in all these models lacking cytokine responses and express defective NK cells [48].

When creating "humanized" mice with HSC engraftment, the myeloid subsection of blood cells is severely underrepresented. To increase this population, various GEMM mice expressing certain human cytokines have been created to more faithfully mimic the usual human situation, thereby creating a more "realistic" model. Human versions of genes encoding human MCSF (csf1), human interleukin 3 (IL-3) and GM-CSF, and human thrombopoietin have been generated as a transgenic model in respective mouse loci in Rag2−/− Il2rg−/− mice (MITRG mice). The resulting human cytokines support the development and function of monocytes, macrophages, and NK cells derived from human fetal liver or adult CD34(+) progenitor cells co-injected into the mice [49]. Mice such as these are important as they may model the interplay the various immune components have on each other which could be crucial when trying to understand the translatability of a drug on complex systems. Regardless, "humanized mice" can be useful if the question being asked is specific to the model. Although the myeloid fraction is usually underrepresented, the T cell population is well modeled and can be used in drug discovery to identify drug candidates for various immunotherapies, including the clinically successful checkpoint inhibitors, and bispecific molecules carrying the CD3 arm.

The "humanized" PDX/avatar approach can circumvent allogeneic effects seen with non-compatible PDXs and engrafted PBMCs or HSCs. Here the PBMCs are taken from the same patient the PDX is derived from, ensuring HLA compatibility. Therefore, these co-engrafted systems can aid the recapitulation of many aspects of the tumor microenvironment [48]. Another aspect missing from the PDXs are the cancer-associated fibroblasts (CAFs), which are also a key determinant in the malignant progression of cancer and represent an important target for cancer therapies [10, 50]. Co-injecting CAFs from the same patient can also increase the faithfulness of the tumor model; mouse fibroblasts, however, ultimately take over the tumor. There is a real need to develop models

that allow us to characterize the interactions between immune cells, CAFs, and cancer cells in the tumor microenvironment, to aid our ability to identify potential new drugs and determine how some agents will translate in the clinic.

Summary

PDXs can be used to aid drug discovery in two aspects, the first to help identify compounds with drug-like properties and the second to identify patient populations who will respond to the drug. For some drug discovery programs, PDX models are critical to understand efficacious response, as cell lines either do not possess the required lesion or have lost dependency on certain pathways. PDXs are most useful in aiding the translatability to the clinic, as the range and breadth of available PDXs are diverse, enabling these models to capture much of the inter-patient heterogeneity seen across patients. This is critical, as less than 10% of drugs which we put into oncology clinical trials gets approved, potentially because of our lack of understanding of the diversity of the disease. Large mouse clinical trials are now commonly used to identify which cancers and subsets of these cancers may respond to the drug and identify potential biomarkers to select potential responders.

PDX models are now also being used in what is commonly referred to as the avatar approach. Here, a PDX model is created from a patient's tumor, and, if established in mice in the survival time frame of the patient, the model is treated with various agents to determine which therapies to use to treat person from whom the PDX was created. This approach has had success in some cases, but caution should be taken with heterogeneous cancers, since the PDX may well not represent the bulk of the tumor from which it originated and therefore not be reflective of that patient's disease.

All models have their limitations, and for PDXs, as all xenografts, the lack of an immune system is most problematic. In this era of heightened awareness of the immune system's role in cancer and the relatively recent success of immunomodulatory agents, it is critical that we can create PDX models with humanized immune systems. Efforts to do this are progressing, with the hope that in future years large-scale mouse clinical trials can be performed with humanized mice to enable the assessment of many immunomodulatory agents (as single agents and in combinations with drugs with other mechanisms of action) across a diverse range of models.

References

1. Giovanella BC, Stehlin JS. Heterotransplantation of human malignant tumors in "nude" thymusless mice. I. Breeding and maintenance of "nude" mice. J Natl Cancer Inst. 1973;51:615–9.
2. Rygaard J, Povlsen CO. Heterotransplantation of a human malignant tumour to "nude" mice. Acta Pathol Microbiol Scand. 1969;77:758–60.
3. Daniel VC, Marchionni L, Hierman JS, et al. A primary xenograft model of small-cell lung cancer reveals irreversible changes in gene expression imposed by culture in vitro. Cancer Res. 2009;69:3364–73.

4. Williams SA, Anderson WC, Santaguida MT, et al. Patient-derived xenografts, the cancer stem cell paradigm, and cancer pathobiology in the 21st century. Lab Invest. 2013;93:970–82.
5. Sasai K, Romer JT, Lee Y, et al. Shh pathway activity is down-regulated in cultured medulloblastoma cells: implications for preclinical studies. Cancer Res. 2006;66:4215–22.
6. Gao H, Korn JM, Ferretti S, et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nat Med. 2015;21:1318–25.
7. Tentler JJ, Tan AC, Weekes CD, et al. Patient-derived tumour xenografts as models for oncology drug development. Nat Rev Clin Oncol. 2012;9:338–50.
8. Whittle JR, Lewis MT, Lindeman GJ, et al. Patient-derived xenograft models of breast cancer and their predictive power. Breast Cancer Res. 2015;17:17–20.
9. Sflomos G, Dormoy V, Metsalu T, et al. A preclinical model for ERα-positive breast cancer points to the epithelial microenvironment as determinant of luminal phenotype and hormone response. Cancer Cell. 2016;29:407–22.
10. Cassidy JW, Caldas C, Bruna A. Maintaining tumour heterogeneity in patient derived tumour xenografts. Cancer Res. 2015;75:2963–8.
11. Malaney P, Nicosia SV, Davé V, et al. One mouse, one patient paradigm: new avatars of personalized cancer therapy. Cancer Lett. 2014;344:1–12.
12. Richmond A, Su Y. Mouse xenograft models vs GEM models for human cancer therapeutics. Dis Model Mech. 2008;1:78–82.
13. Utama FE, Tran TH, Ryder A, et al. Insensitivity of human prolactin receptors to nonhuman prolactins: relevance for experimental modeling of prolactin receptor-expressing human cells. Endocrinology. 2009;150:1782–90.
14. DeRose YS, Wang G, Lin YC, et al. Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. Nat Med. 2011;17:1514–20.
15. Siolas D, Hannon GJ. Patient-derived tumor xenografts: transforming clinical samples into mouse models. Cancer Res. 2013;73:5315–9.
16. Talmadge JE, Singh RK, Fidler IJ, et al. Murine models to evaluate novel and conventional therapeutic strategies for cancer. Am J Pathol. 2007;170:793–804.
17. Eirew P, Steif A, Khattra J, et al. Dynamics of genomic clones in breast cancer patients xenografts at single cell resolution. Nature. 2015;518:422–6.
18. Lim Y. Mining the tumor phosphoproteome for cancer markers. Clin Cancer Res. 2005;11:3163–9.
19. Li S, Shen D, Shao J, et al. Endocrine-therapy resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts. Cell Rep. 2013;4:1116–30.
20. Arrowsmith J. Trial watch: phase II failures: 2008–2010. Nat Rev Drug Discov. 2011;10:328–9.
21. Arrowsmith J, Miller P. Trial watch: phase II and phase III attrition rates 2011–2012. Nat Rev Drug Discov. 2013;12:569.
22. Migliardi G, Sassi F, Torti D, et al. Inhibition of MEK and PI3K/mTOR suppresses tumor growth but does not cause tumor regression in patient-derived xenografts of RAS-mutant colorectal carcinomas. Clin Cancer Res. 2012;18:2515–25.
23. Bertotti A, Migliardi G, Galimi F, et al. A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. Cancer Discov. 2011;1:508–23.
24. Townsend EC, Murakami MA, Christodoulou A, et al. The public repository of xenografts enables discovery and randomized phase II-like trials in mice. Cancer Cell. 2016;29(4):574–86.
25. Murphy B, Yin H, Maris JM, et al. Evaluation of alternative in vivo drug screen methodology: a single mouse analysis. Cancer Res. 2016;76(19):5798–809.
26. Julien S, Merino-Trigo A, Lacroix L, et al. Characterization of a large panel of patient derived tumor xenografts representing the clinical heterogeneity of human colorectal cancer. Clin Cancer Res. 2012;18:5314–28.
27. Lièvre A, Bachet JB, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res. 2006;66:3992–5.

28. Johnson BE, Mazor T, Hong C, et al. Mutational analysis reveals the origin and therapy driven evolution of recurrent glioma. Science. 2014;343:189–93.
29. Ebi H, Corcoran RB, Singh A, et al. Receptor tyrosine kinases exert dominant control over PI3K signaling in human KRAS mutant colorectal cancers. J Clin Invest. 2011;121:4311–21.
30. Flanigan SA, Pitts TM, Newton TP, et al. Overcoming IGF1R/IR resistance through inhibition of MEK signaling in colorectal cancer models. Clin Cancer Res. 2013;19:6219–29.
31. Molina-Arcas M, Hancock DC, Sheridan C, et al. Coordinate direct input of both KRAS and IGF1 receptor to activation of PI3 kinase in KRAS-mutant lung cancer. Cancer Discov. 2013;3:548–63.
32. Friedbichler K, Hofmann MH, Kroez M, et al. Pharmacodynamic and antineoplastic activity of BI 836845, a fully human IGF ligand-neutralizing antibody, and mechanistic rationale for combination with rapamycin. Mol Cancer Ther. 2014;13:399–409.
33. Brana I, Berger R, Golan T, et al. A parallel-arm phase I trial of the humanised anti-IGF-1R antibody dalotuzumab in combination with the AKT inhibitor MK-2206, the mTOR inhibitor ridaforolimus, or the NOTCH inhibitor MK-0752, in patients with advanced solid tumours. Br J Cancer. 2014;111:1932–44.
34. Moran T, Felip E, Keedy V, et al. Activity of dalotuzumab, a selective anti-IGF1R antibody, in combination with erlotinib in unselected patients with non-small-cell lung cancer: a phase I/II randomized trial. Exp Hematol Oncol. 2014;3:26–33.
35. Scagliotti GV, Bondarenko I, Blackhall F, et al. Randomized, phase III trial of figitumumab in combination with erlotinib versus erlotinib alone in patients with nonadenocarcinoma nonsmall-cell lung cancer. Ann Oncol. 2015;26:497–504.
36. Hutchinson L, Kirk R. High drug attrition rates—where are we going wrong? Nat Rev Clin Oncol. 2011;8:189–90.
37. Hidalgo M, Amant F, Biankin AV, et al. Patient-derived xenograft models: an emerging platform for translational cancer research. Cancer Discov. 2014;4:998–1013.
38. Lodhia KA, Hadley AM, Haluska P, et al. Prioritizing therapeutic targets using patient-derived xenograft models. Biochim Biophys Acta. 2015;1855:223–34.
39. Weroha SJ, Becker MA, Enderica-Gonzalez S, et al. Tumorgrafts as in vivo surrogates for women with ovarian cancer. Clin Cancer Res. 2014;20:1288–97.
40. Zhang X, Claerhout S, Prat A, et al. A renewable tissue resource of phenotypically stable, biologically and ethnically diverse, patient-derived human breast cancer xenograft models. Cancer Res. 2013;73:4885–97.
41. Garralda E, Paz K, Lopez-Casas PP, et al. Integrated next-generation sequencing and avatar mouse models for personalized cancer treatment. Clin Cancer Res. 2014;20:2476–84.
42. Kemper K, Krijgsman O, Cornelissen-Steijger P, et al. Intra- and inter-tumor heterogeneity in a vemurafenib-resistant melanoma patient and derived xenografts. EMBO Mol Med. 2015;7:1104–18.
43. Farkona S, Eleftherios P, Diamandis P, et al. Cancer immunotherapy: the beginning of the end of cancer? BMC Med. 2016;14:73–9.
44. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. Nature. 2011;480:480–9.
45. Meyer LH, Debatin KM. Diversity of human leukemia xenograft mouse models: implications for disease biology. Cancer Res. 2011;71:7141–4.
46. Schroeder MA, DiPersio JF. Mouse models of graft-versus-host disease: advances and limitations. Dis Model Mech. 2011;4:318–33.
47. Quintana E, Shackleton M, Sabel MS, et al. Efficient tumour formation by single human melanoma cells. Nature. 2008;456:593–8.
48. Shultz LD, Brehm MA, Garcia-Martinez JV, et al. Humanized mice for immune system investigation: progress, promise and challenges. Nat Rev Immunol. 2012;12:786–98.
49. Rongvaux A, Willinger T, Martinek J, et al. Development and function of human innate immune cells in a humanized mouse model. Nat Biotechnol. 2014;32:364–72.
50. Kalluri R, Zeisberg M. Fibroblasts in cancer. Nat Rev Cancer. 2006;6:392–401.

First-Generation Tumor Xenografts: A Link Between Patient-Derived Xenograft Models and Clinical Disease

11

Xin Dong, Peter W. Gout, Lu Yi, Yinhuai Wang, Yong Xu, and Kuo Yang

Abbreviations

ECM	Extracellular matrix
NSCLC	Non-small cell lung cancer
PCa	Prostate cancer
PDX	Patient-derived xenograft
S.C.	Subcutaneous
SRC	Subrenal capsule

Introduction

Despite new insights into the cancer biology and continuous identification of targetable genetic alterations, more than 85% of novel, potential therapeutic agents fail the late phases of clinical trials due to lack of efficacy [1–3]. This high failure rate has been largely attributed to a lack of clinically relevant cancer models for

X. Dong (✉)
Living Tumor Laboratory, BC Cancer Agency/Vancouver Prostate Centre, Vancouver, BC, Canada
e-mail: xdong@bccrc.ca

P.W. Gout
Living Tumor Laboratory, BC Cancer Agency/Vancouver Prostate Centre, Vancouver, BC, Canada

Department of Experimental Therapeutics, BC Cancer Research Centre, Vancouver, BC, Canada

L. Yi • Y. Wang
Department of Urology, The Second Xiangya Hospital, Zhongnan University, Changsha, China

Y. Xu • K. Yang
Tianjin Institute of Urology, Tianjin Medical University, Tianjin, China

© Springer International Publishing AG 2017
Y. Wang et al. (eds.), *Patient-Derived Xenograft Models of Human Cancer*, Molecular and Translational Medicine, DOI 10.1007/978-3-319-55825-7_11

preclinical drug screening and the inability to individualize cancer treatment toward target-specific therapies [4–6]. The conventional cell culture systems often lack the tumor heterogeneity and microenvironment components of cancer tissues, resulting in substantial differences with the original patients' tumors and poor clinical predictive power of drug efficacies [7]. There is, therefore, a critical need for more sophisticated and reliable preclinical models that better represent the complexity of cancers and more closely mimic the tumor microenvironment.

In recent years, patient-derived xenograft (PDX) tumor models, based on grafting of fresh tumor fragments from patients into immunodeficient mice, have become preferred platforms in translational cancer research and drug development [5, 6]. An increasing amount of evidence suggests that the PDXs recapitulate tumor pathogenesis and progression with high fidelity and predict tumor behavior with increased accuracy, including disease progression and drug sensitivity in the clinic [8, 9]. It is expected that integration in PDXs of genomic profiling with preclinical drug screening will lead to guided treatment for individual patients, which will greatly shorten the process from preclinical drug discovery to clinical application and improve patient outcomes in cancer medicine.

First-generation xenografts, also called primary xenografts, are PDXs in the initial human-to-mouse generation harboring the tumor tissues directly derived from patients. Although they have not been widely used in translational cancer research or thoroughly characterized, first-generation xenografts are advantageous in several aspects compared to established PDXs of subsequent generations, such as retention of intra-tumoral heterogeneity, tumor microenvironment, and drug sensitivity of the original patients' cancer tissues [10–12]. In this review, we discuss the current comprehension of first-generation xenografts and then focus on the potential application of first-generation xenograft models in contemporary preclinical research and oncology fields.

Methodology

Methods for generating first-generation xenografts are similar to those used in developing transplantable PDX models [12–15]. Regardless of specific differences between various approaches, one fundamental concept is to transplant fresh tumor fragments from the patient into immunodeficient mice. To this end, fresh tumor tissues obtained by surgery or biopsy are sectioned into 2–3 mm^3 pieces which are directly implanted into host mice. A variety of mouse strains harboring different immunosuppression levels have been used as recipient mice. The most common graft site is the subcutaneous (s.c.) flank of a mouse. Orthotopic grafting involves direct implantation of patients' fresh tumor tissue into the homologous mouse organ, e.g., ovary, prostate, pancreas, or mammary fat pad. Tumor tissues can also be implanted under the renal capsules of a host mouse. The first-generation xenograft is usually termed G0 or F0, with subsequent generations numbered consecutively.

One of the biggest challenges in producing high-quality first-generation xenografts for immediate usage is to achieve a high tumor engraftment rate. Obtaining a high engraftment rate is affected by multiple factors, including the mouse strain used, types and quality of the patients' original tumor tissues, and the transplantation site employed.

Severely immunosuppressed mouse strains, such as NOD/SCID or NOD/SCID/IL2RG null mice, are commonly preferred rodent strains for generating high engraftment rates, although very few studies have been properly designed to compare the engraftment rates of the various types of recipient mice. Clinically aggressive, metastatic tumors generally have higher engraftment rates than less aggressive, nonmetastatic primary tumors [4, 16, 17], which may indicate utility of first-generation xenografts for predicting or monitoring tumor aggressiveness in donor patients. Although subcutaneous grafting allows straightforward surgery and is convenient for accurately monitoring of xenograft growth, it is generally associated with low engraftment rates, ranging from 25% to 60% [18–21]. Some hormone-sensitive tumors, such as prostate cancer and hormone-dependent breast cancers, are particularly difficult to engraft at s.c. graft sites. Alternatively, the subrenal capsule graft site is employed and has consistently yielded high engraftment rates for various cancer types [14, 22–24]. The high engraftment rate obtained with the use of the subrenal capsule graft site is likely a result of superior nutrient supply and enhancement of graft microvascularity provided by the host kidney [25]. This is of great importance to difficult-to-graft cancer types such as prostate cancer [24]. For example, by subrenal capsule grafting, a panel of prostate cancer PDX models has been successfully established from tiny amounts of needle biopsy tissues obtained at diagnosis [26]. A high engraftment rate can be further ensured if viable tumor tissue can be identified prior to implantation, either by macro methods (e.g., macro pathology, fluorescence illumination) or microscopic methods (e.g., cryostat sectioning of randomly selected pre-grafting fragments) [10].

Major Characteristics of PDX Tumor Models

It has become increasingly clear that widely used conventional human cancer cell cultures, as well as in vivo xenografts generated by implanting the cultured cells into immunodeficient mice, lack predictive power for clinical outcomes and are the major reason for the discrepancy between results of preclinical studies and clinical trials. The cell cultures, established in an artificially normoxic and growth factor-rich environment, undergo irreversible alterations such as loss of specific cell populations, loss of stromal components, gain and loss of genetic information, and alterations in growth and tissue invasive properties [27–29]. Consequently, the cell cultures cannot reflect the complexity of patient tumors such as tissue architecture, cancer-stromal interaction, and inter- or intra-tumor heterogeneity and hence are not suitable for preclinical drug screening or personalized medicine.

To overcome the above limitations of conventional cancer cell cultures, major efforts have been made to establish PDX tumor models in which key genetic and biologic characteristics of the patients' original tumors are reliably retained [5, 9, 30]. Many studies, comparing PDX models developed from various tumor types with their corresponding patients' (donors') original tumors, have indicated that the PDX models can serve as superior platforms for applications in oncology research and drug development. At the histopathological level, it has been shown that PDX models closely resemble their original patients' tumors, retaining major histopathological characteristics such as microscopic tissue architecture, glandular structure,

cyst development, and mucin production. Expressions of tumor markers in the original patients' tumors were not only retained in PDX models but have shown alterations similar to those observed in patients' original tumors in response to treatments such as hormonal therapy [26]. At the genetic level, comprehensive genome-wide gene expression analysis studies of various cancers have shown high degrees of similarities between PDX models and the original patients' tumors [14, 16, 26, 27, 31, 32]. For example, in non-small cell lung cancer (NSCLC) PDX models, unsupervised hierarchical clustering of genome-wide gene expression profiles revealed that PDX models clustered directly with the paired patients' original tumors [32]. Similarly, in prostate cancer (PCa) PDX models, unsupervised hierarchical clustering of copy-number segmentation profiles confirmed that all patient and xenograft pairs clustered together. As well, combined genome copy-number analysis of each independent transplantable tumor line demonstrated that they largely recapitulated both the heterogeneity of prostate cancer and key chromosomal alterations frequently observed such as loss of TP53, NKX3-1 and RB1 tumor suppressors, as well as gains of 8q and oncogenes, such as ETV1, EZH2, and BRAF [26]. At the biological level, the PDX models closely mimic the biological behavior of patients' original tumors, including disease progression, tumor heterogeneity, and responses to treatment. Correlations between the success of engraftment and poor patient outcomes have been reported in studies of cancers of the breast, kidney, pancreas, ovary, and skin [16, 17, 33–35]. The PDX models also recapitulate tumor heterogeneity to a certain extent. For example, in a study published in 2014, Lin et al. reported five transplantable tumor lines derived from needle biopsy specimens from five different foci of a patient's primary prostate cancer showing different metastatic abilities and growth rates in vivo. Further copy-number profiling analysis showed not only major chromosomal alterations shared among the five lines, suggesting all lines shared a common ancestor, but also several unique chromosomal alterations that were only observed in particular tumor lines, indicating the existence of functionally heterogeneous subpopulations in the patient's primary tumors [26]. Another important biological advantage of PDX models is that they have shown reasonable concordance with patients' tumors in responses to treatments with drugs [17, 36, 37]. As such, PDX cancer models are thought to quite accurately represent the complex biochemical and physical interactions between cancer cells and their microenvironment and hence can serve as preclinical tools with enhanced predictive value for patients' cancer biology and responses to treatments [9, 38].

Advantages of Using First-Generation Xenograft Models

Although the method of transplanting patient-derived tumor cells or tissues into immunocompromised mice has been used for more than 40 years to test chemotherapeutic and other anticancer agents in vivo, application of first-generation xenografts as a reliable tool for cancer research and preclinical drug development has been greatly hampered, presumably due to the generally low engraftment rate at the s.c. graft site and insufficient numbers of first-generation xenografts generated.

In recent years, with the improvement of grafting techniques and development of severely immunosuppressed mouse strains such as NOD/SCID or NOD/SCID/IL2RG null mice, enhanced engraftment efficiency has become possible, even for tumors that are particularly difficult to engraft. In our laboratory, more than 1500 first-generation xenografts have been derived from surgically removed NSCLC tissues of 32 patients by subrenal capsule grafting in NOD/SCID, with a total engraftment rate of 90% [11]. Such improvement in engraftment efficiency makes broad usage of first-generation xenografts possible for translational cancer research, preclinical drug testing, as well as individualized drug screening. First-generation xenografts have a number of advantages, in comparison with later generations of PDX models, which are described in detail in the following paragraphs.

The time required to establish a first-generation xenograft is much shorter than for establishing a transplantable tumor line. This is of crucial importance when one tries to implement real-time PDXs for personalized cancer drug screening, so-called tumor avatars. Ideally, a tumor avatar should closely mimic the biology of the original tumor in the patient such as retention of major genotypes and phenotypes and biological behavior such as tumor progression and drug responses. More importantly, a tumor avatar needs to be established within a short time frame to conform to timely initiation of patients' therapy. It normally takes 2–4 months to develop a transplantable PDX model that is suitable for preclinical studies, a time frame many patients with aggressive disease do not have. In trying to overcome this limitation, we have found, using severe immunocompromised mouse strains in combination with subrenal capsule grafting, that first-generation NSCLC xenografts can be established within 2 weeks after implantation, ready for use in personalized drug testing [11]. This relatively short time frame makes first-generation xenografts, as distinct from transplantable tumor lines [39–41], suitable for personalized drug testing during the time between patients' diagnosis or surgery and initiation of treatment.

First-generation xenografts retain human stromal components and extracellular matrix (ECM) interaction better than transplantable, later-generation PDX models. In patients, the cancer cells are in a microenvironment consisting of surrounding lymphatic and blood capillaries, fibroblasts, the extracellular matrix, immune cells, and other normal cells. Bidirectional communications between cancer cells and their immediate environment play a significant role in oncogenesis, tumor progression, and patient prognosis. Alterations in the cellular microenvironment may lead to cell proliferation and facilitate tumor initiation and direct metastasis [42–44]. Cancer-associated fibroblasts have been found to produce tumor-supportive ECM and secrete growth factors and chemokines; they often confer resistance to cytotoxic and targeted therapies [45]. A shift in tissue 3D architecture can lead to altered paracrine signaling and significantly contribute to disease progression [44, 46, 47]. The aberrant vascularity found in solid tumors, a complex status regulated by pro- and anti-angiogenesis factors produced by both tumor cells and the stromal compartment [48, 49], may lead to hypoxia, extracellular acidosis, nutrient deprivation, and energy depletion and often plays a role in treatment resistance, suppression of apoptosis and immune surveillance, and metastasis. It is therefore important that experimental

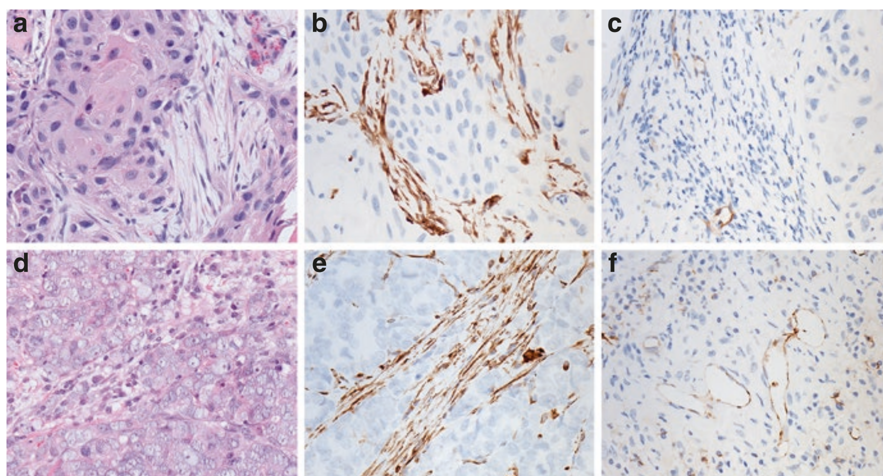


Fig. 11.1 H&E and immunohistochemistry staining of first-generation xenografts of a lung squamous cell carcinoma (**a**–**c**) and an ovarian serous carcinoma (**d**–**f**) shows that they retain human fibroblasts (**b**, **e**, stained by a human-specific anti-vimentin antibody) and human endothelial cells (**c**, **f**, stained by a human-specific anti-CD31 antibody) of the patients' original cancer tissues

translational models retain human extracellular matrix and derived vasculature in order to accurately recapitulate the tumor microenvironment. Although no substantial changes were found in transplantable PDX models, in comparison to their donor tumors, expression of genes involved in the stromal compartment, immune function and angiogenesis were found to be less represented in transplantable PDX models, due to replacement of the human stroma by murine elements [50]. It has been shown that, after three to five serial passages, the human stroma in PDXs is completely replaced by murine stroma [5]. This loss of human stroma in later generations of transplantable PDX models greatly limits their use for accurately predicting treatment responses in individualized drug screening or for studying species-specific therapeutic reagents targeting the human tumor microenvironment. Alternatively, first-generation patient-derived xenografts retain the human tumor microenvironment to the greatest extent. In our laboratory, a panel of first-generation patient-derived xenografts of NSCLCs, ovary cancers and prostate cancers has been screened for expression of human-specific markers for stromal compartment cells. The results revealed retention by the first-generation xenografts of tumor-associated human stroma components, including human fibroblast and vascular epithelial cells (Fig. 11.1), in contrast to loss of human stroma in serially transplanted later generations of PDX models [15, 26]. Thus, the first-generation xenograft models represent improved models in recapitulating cancer-stroma interactions.

First-generation xenograft models may also provide valuable tools for studying limited aspects of cancer immunology. The ability of cancer cells to evade the immune system is considered an emerging hallmark of cancer [51]. As a main battleground where the immune system succeeds or fails to eliminate tumor cells, the tumor microenvironment harbors two major components, immune-stimulatory

elements (e.g., cytotoxic CD8+ T cells, natural killer (NK) cells, IL-2) and immunosuppressive elements (e.g., regulatory T cells (T_{reg}), type 2 macrophages). Cross talks between cancer cells and tumor-infiltrating immune cells via specific chemokines, adhesion molecules, and exosomes lead either to elimination of cancer cells or induction of an immunosuppressive network that protects the malignant cells from attacks by anticancer immune cells and promotes their survival and migration. There is increasing clinical evidence that tumor-infiltrating immune cells are significantly associated with patient prognosis. Decreased ratios of CD8+ T cells to Foxp3+ T_{reg} cells have been shown to correlate with poor patient prognosis for many cancer types, including ovarian [52, 53], breast [54], and gastric cancer [55]. High regulatory T-cell densities in tumor tissues have been significantly correlated with poor patient outcomes for the majority of solid tumors such as kidney and breast cancers and melanomas [56]. In the past decade, immunotherapy has become an important part of cancer treatments, and clinically relevant, preclinical screening models for immunotherapy, especially in the personalized medicine setting, are urgently needed. Despite the close resemblance of transplantable PDXs to their human donor tumors, critical shortcomings of these models are the required usage of hosts that lack functional immune cells and also the loss of patient immune cells in later PDX generations [8]. Transplantable PDX models are thus not adequate models for testing immunotherapies and anticancer agents that target the immune system. With regard to the hosts used, one solution is to reconstitute the human immune system into recipient mice to produce partially humanized mouse models [57, 58]. The cost of such mice is excessively high and thus prevents their wide-scale use in preclinical drug screening. Moreover, these humanized mice are not matched to individual patients' tumors and may generate an immune attack that may not be relevant to the immunotherapy tested [29]. On the other hand, it has been reported that first-generation xenografts established in NOD/SCID/IL2RG mice were able to maintain human tumor-associated leukocytes such as effector memory T cells for up to 9 weeks after implantation [58]. In our laboratory, CD45+ leukocytes, including cytotoxic CD8+ T cells as well as FOXP3+ regulatory T cells, were also observed in first-generation xenografts of a prostate cancer (Fig. 11.2), suggesting that it may be possible to use first-generation xenografts to test the effect of immunomodulatory agents on the levels of patient immune cells. However, the evidence in this field is very limited and further studies are required.

One important reason for using first-generation xenografts in cancer research and drug testing is that they have clear advantages over cell lines and transplantable PDX models as they better capture the intra-tumor heterogeneity of the patient's original tumor. It is well known that intra-tumor heterogeneity exists in almost all types of cancers. Cancers consist of highly diverse cell populations that can be recognized or detected at the phenotypic, genetic, and epigenetic levels. Mechanisms contributing to the intra-tumor heterogeneity include autonomous mutations of the cancer cells, persistence of cancer stem cells, and the dynamic interaction between tumor cells and their microenvironments and between subclones of tumor cells. Pre-existing intra-tumor heterogeneity is considered a major cause of treatment resistance or failure to chemo- and targeted therapy. Indeed, in patients with colorectal cancer,

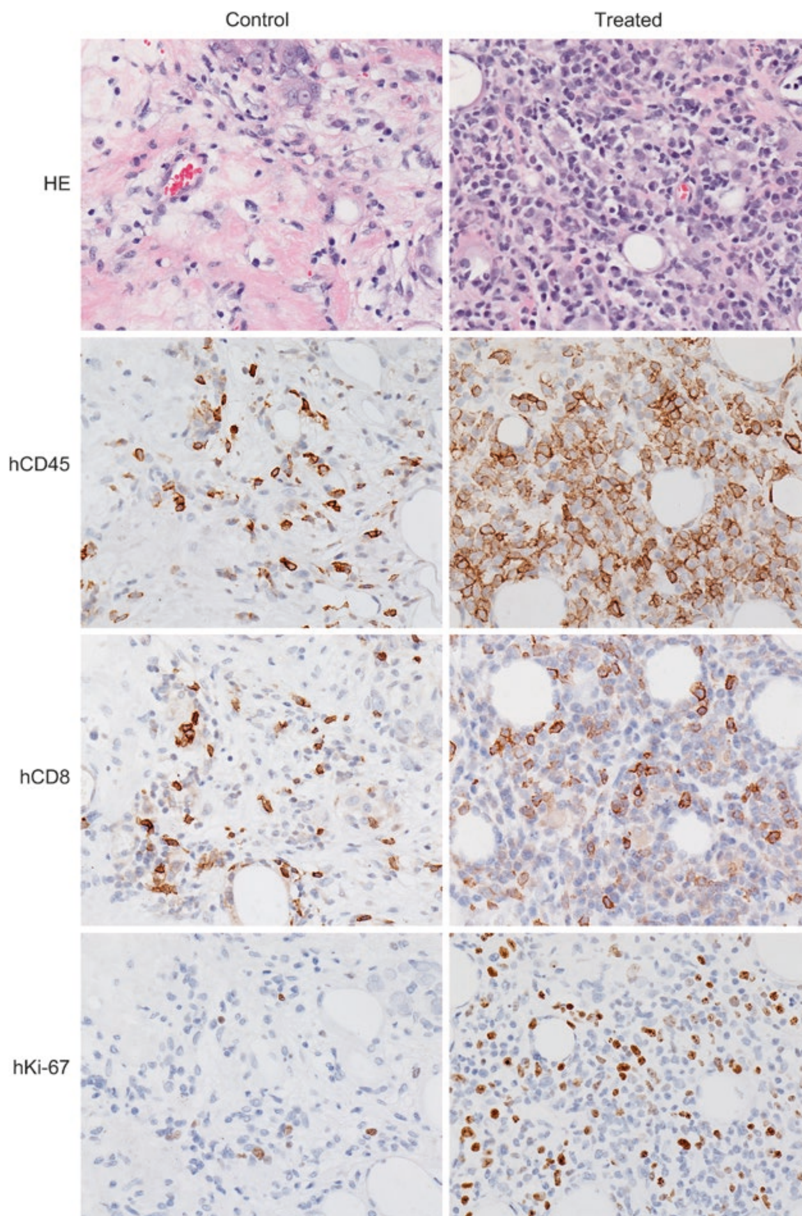


Fig. 11.2 An example of using first-generation xenografts to test an immunotherapeutic agent. Metastatic prostate cancer tissues from a patient's lymph nodes were obtained and implanted into immunodeficient mice to develop first-generation xenografts. *Left panel*, xenografts in untreated control group contain infiltrating human immune cells, labeled by immunohistochemistry staining of human-specific anti-CD45 (hCD45) for the whole population of leukocytes and human-specific anti-CD8 (hCD8) for cytotoxic T-cell subpopulations. A human-specific anti-Ki-67 (hKi-67) was used to evaluate the proliferative status of the immune cells. *Right panel* showing significantly increased CD8+ cytotoxic T-cell infiltration in xenografts treated with a drug candidate that can indirectly promote the proliferation of cell-killing immune cells. The increased numbers of cytotoxic T cells are highly proliferative, as indicated by the anti-hKi67 antibody

pre-existing KRAS-mutant clones may expand exponentially following administration of the anti-EGFR antibody cetuximab. Similar dynamic changes have been observed in a variety of other cancer types, including lung cancer [59, 60] and leukemia [61, 62]. This highlights the necessity to generate a comprehensive genomic and molecular profile of individual patients and, more importantly, to functionally validate the genomic and molecular aberrations that truly reflect the intra-tumor heterogeneity and treatment responses of the cancer cell subpopulations to various chemo- and targeted therapeutics. Many approaches have been proposed or studied in order to achieve such validations, including the use of relevant cell lines, genetically engineered mouse models, organoid cultures, and PDX models [63, 64]. The transplantable PDXs are considered to be the best models for maintaining intra-tumor heterogeneity as well as other molecular traits such as mutations, chromosomal aberrations, fusion events, and gene expression profiles [65–68]. However, long-term in vitro and in vivo manipulations may lead to clonal evolution and loss of intra-tumoral heterogeneity. It has been suggested that serial tumor engraftment may result in vast differences between the patient's original tumor and the derived transplantable PDX models in terms of molecular landscape [68, 69]. Notably, the first passage (from the first to the second generation) is when most of the changes occur, and the genes affected are mostly associated with immune pathways and cancer-stromal interaction [68, 70, 71]. First-generation xenografts, which are derived by directly transplanting a patient's tumor tissue into the host, are the closest generation to the patient's original tumor and are thought to have minimal alterations at genetic, epigenetic, or stromal levels [10]. Thus, first-generation xenografts may serve as improved cancer models reflecting the complex intra-tumor heterogeneity of the patient's original tumor to the greatest extent and, as such, provide better tools for evaluating patient-specific responses to therapy in personalized medicine.

Application of the First-Generation Xenografts

Prediction of Tumor Progression

The ability to accurately predict progression of tumors is of major importance to the clinical management of the disease. The plasticity of tumors highlights the critical need for dynamic platforms capturing variations among patients' tumors or cell subpopulations within a tumor. In recent years, a number of methods or systems have been developed to meet such a demand, including monitoring of predictive molecular biomarkers, circulating tumor cells, cell-free tumor DNA, and analysis of large omics, as well as functional assays using PDX models or other ex vivo assays [72]. It has been reported that the tumor engraftment rate during the process of establishing first-generation xenografts from a patient's tumor tissue might reflect the aggressiveness of the original tumor. The rate of engraftment has been associated with the risk of metastasis, disease recurrence, or patient survival in various types of cancer. For patients with pancreatic ductal adenocarcinoma, successful engraftment of the patients' tumor tissues transplanted into immunodeficient mice was significantly

predictive of reduced patient survival in a multivariate analysis, with a median survival time of 299 days for patients whose tumors were successfully engrafted, in contrast to the median survival time of more than 800 days for patients whose cancers failed engraftment [34]. In PDX models of NSCLC, the engraftment success was significantly associated with a shorter disease-free survival in patients [18, 73]. A correlation of engraftment success and poor patient outcome has also been reported for patients with other types of malignancies, including kidney [17], breast [18], ovary [47], and skin [53] cancers. The results of these studies suggest that the rate of engraftment of a patient's tumor into immunodeficient mice could be useful as a prognostic indicator of tumor aggressiveness and risk of disease progression.

First-Generation Xenografts for Precision Medicine

It has become evident that cancer is a heterogeneous disease, as revealed by differences in histology, drastically different genomic and molecular profiles, and greatly varied biological behavior among cancers of the same type. These inter-tumoral differences have led to the development of widely used histology-based definition systems and, more recently, to refined classification systems with integration of molecular data for improved prediction of cancer prognosis and responses to specific treatments. In addition to inter-tumoral heterogeneity, there is intra-tumor heterogeneity within the same tumor, a result of the continuous evolution of cancer cells through sequential alterations of the genome during carcinogenesis and disease progression. Understanding the inter- and intra-tumoral heterogeneities has led to significant progress in the identification of hundreds of cancer-driving abnormalities and new therapeutic targets. In the past few decades, the field of oncology has seen a rapid shift from the cytotoxic, nonspecific approach to cancer therapy to molecularly tailored, rationally designed precision medicine [74]. Some cancer types, such as colorectal cancer, NSCLC, and human breast cancers, have been routinely profiled to aid in the treatment decision-making process [75]. Tumor heterogeneity has also caused great challenges for cancer treatment and is considered one of the major reasons for the development of resistance to conventional cytotoxic chemotherapeutics or targeted therapy in the clinic. So far little is known about what type and what level of heterogeneity is responsible for the failure of a treatment. Real-time genetic and molecular profiling of the patient may identify multiple targets that could serve as potential candidates for targeted therapy, adding another layer of complexity to therapy decision-making. Furthermore, for patients whose tumors do not express any therapeutic targets or biomarkers of drug efficacy, or patients that will receive conventional cytotoxic chemotherapy, it will be particularly difficult to have their treatment personalized. To this end, predictive information acquired by functional validation of cytotoxic chemotherapeutic regimens, or a specific targeted therapy for a patient's tumor, will be of great value to therapy decision-making in the clinic and to avoid potential drug toxicities and unnecessary financial burden.

Transplantable PDX tumor models, or so-called avatars, have been used to personalize cancer treatment. In a pilot clinical study published in 2011, advanced cancer patients received guided treatments that were prospectively selected, based on

drug responses of PDX models developed from their own cancers. The study demonstrated a remarkable correlation between drug efficacy in the model and clinical outcome [76]. In another study of patients with lung cancer, it was shown that the use of PDX testing led to the identification of appropriate drug combinations that could not have been identified by genetic testing alone [77]. The positive correlation between treatment responses of PDX models and patients has also been seen in patients with breast cancer [78, 79]. However, a few limitations of this PDX-based approach may challenge a broad clinical application of personalized drug evaluation. It usually takes 6–8 months from the implantation of a patient's tumor tissue into immunodeficient mice to the first passage of a PDX. This period may be suitable for patients who have relatively slow-growing tumors but will not be appropriate for patients with aggressive tumors who may die before they could benefit from the PDX testing results. Another major challenge to precision medicine is the high failure rate of engraftment of implanted tumor tissues, especially at the commonly used s.c. graft site and for slow-growing cancer types such as prostate cancer. Consequently, large amounts of fresh tumor tissues and resources are required to generate enough xenografts for randomly grouped drug testing studies. In order to acquire enough tumor xenografts for drug assessment, PDXs often are further passaged. However, changes in the peri-tumoral microenvironment and host immune system induced by the longer time of the PDX in vivo may alter the subpopulations and molecular landscapes of the tumor cells within a single tumor specimen, resulting in intra-tumoral heterogeneity that is different from that of the patient's original tumor [80–83].

As a relatively low engraftment rate of tumors in immunodeficient mice is a main limiting factor in the application of first-generation PDXs as avatars in precision medicine, it is advantageous to use a high engraftment rate-producing grafting technique such as subrenal capsule grafting. A study by Dong et al. describes the utilization of first-generation NSCLC xenografts, established by subrenal capsule grafting, for testing the efficacies of three of the most commonly used first-line chemotherapy regimens for NSCLC [10]. In this study, 1573 first-generation xenografts from 32 surgically removed NSCLCs were established with a high engraftment rate of 90%. The consistently achieved high engraftment rates by subrenal capsule implantations [14, 23, 24, 27, 84–86], in contrast to the much lower and unpredictable rates at other graft sites [31, 40, 41, 87], were assumed to be a result of superior nutrient supply, enhancement of graft microvascularity provided by the subrenal capsule site, and rigorous selection of viable tumor tissue by fluorescence illumination and pathological evaluation before grafting. The response rate of the xenografts to chemotherapeutic regimens and chemo-induced histological changes were similar to those observed in the clinic. The study revealed the NSCLCs were not equally sensitive to all the regimens and that there were nonresponsive subpopulations even though the tumor was sensitive to chemotherapy, suggesting that the first-generation xenografts reflected both inter- and intra-tumoral heterogeneities of the tumors. Although the response rate to individual chemotherapy regimens was ranging from 28% to 44%, approximately two thirds of the NSCLCs were sensitive to at least one or more first-line chemotherapies,

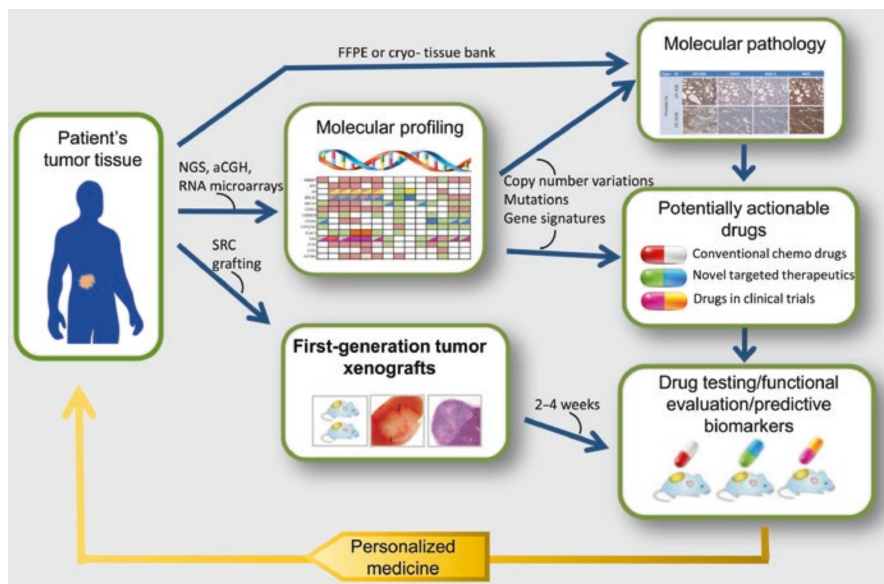


Fig. 11.3 Utilization of first-generation xenografts in real-time personalized medicine. A patient's tumor tissues are obtained and processed for molecular profiling analysis and implantation into immunodeficient mice to generate first-generation tumor xenografts. The tumor xenografts are allowed to stabilize in the hosts for 2–4 weeks. Potentially actionable drugs, as suggested by parallel genetic and molecular analyses, are then tested and ranked in the xenografts before they are administrated to the patient. *FFPE* formalin-fixed-paraffin-embedded, *NGS* next-generation sequencing, *aCGH* microarray-based comparative genomic hybridization, *SRC* subrenal capsule

indicating a potential benefit to improve the drug response rate by selecting optimal regimens for personalized chemotherapy. The use of the subrenal capsule grafting technique significantly shortened the engraftment time, one of the most important variables in avatar drug testing for cancer treatment. The entire process from implantation of a patient's tumor into mice to completion of drug testing in the xenografts for chemotherapeutic drugs took only 6–8 weeks, conforming to the clinical schedules of the patients. The study suggests use of a longer adaptation time before treatment (2–4 weeks) for xenografts to stabilize in the hosts and develop microvasculature, in contrast to 6–11 days used by other groups [84, 88]. Notably, the application of first-generation xenografts in personalized cancer therapy can be hampered by difficulties in monitoring tumor growth partially due to the small volume of the first-generation xenografts. Imaging techniques such as ultrahigh-frequency ultrasound could be introduced to accurately measure the volumes of first-generation xenografts. Taken together, the high engraftment rate and short establishment time obtained for first-generation xenografts with subrenal capsule grafting makes them more suitable than transplantable PDX lines for real-time personalized drug testing. Using patients' own tumor avatars, potentially actionable targeted therapies can be quickly screened before they are administrated to patients (Fig. 11.3).

For Preclinical Drug Screening and Development of Predictive Biomarkers

One of the major issues in oncology drug development is the high failure rate of new investigational cancer drugs in late-phase clinical trials, which has frequently been attributed to lack of preclinical screening models that can recapitulate the heterogeneity of patients' tumors [6, 9, 89, 90]. The traditional preclinical models are mainly based on human cancer cell lines cultured in vitro or xenografts derived from such cell lines. Although the use of the cell lines has yielded valuable insights into cancer biology and drug discovery, the artificial growth conditions, selection stress from in vitro cultivation, and serial passages promote outgrowth of rapidly growing subpopulations and cause major and irreversible changes in biologic and genetic properties of the cells. The established cell lines are hence no longer representative of the heterogeneity of patients' tumors and cannot reflect the complex cancer-stromal interactions and angiogenesis of the tumors. As a result, the use of such homogeneous cell lines can lead to overestimation of the effectiveness of a drug candidate and to discrepancies between drug efficacy results in preclinical studies and clinical trials.

Increasing amounts of evidence have suggested that PDX models are much more patient-relevant models compared to the conventional cancer cell lines. A direct comparison of the PDXs and cell lines was made by comparing PDX models and cell lines that were derived from the same specimen of a patient's primary small cell lung cancer [27]. The data show that the gene expression profile of a PDX model was similar to that of the original patient's tumor, while an in vitro cultured cell line derived from the same tumor specimen displayed a different expression profile that could not be restored when the derivative cell line was returned to growth in vivo as a xenograft. The results indicate that in vitro cell cultures do not provide correct models of a disease. The resemblance of PDXs to their donor tumors was investigated at the histopathological, epigenetic, genomic, and biological levels in a large body of studies across various types of cancer [26, 31, 32, 65, 67, 68, 91, 92]. These studies demonstrate that the PDXs offer high genetic stability as well as histopathological and molecular fidelity and tend to maintain the complex tumor heterogeneity and the cancer-stromal interactions of the patients' tumors. The PDX cancer models have been reported to be predictive of tumor responses to therapeutic agents and patient outcomes [4, 32, 38, 39, 93–95]. All the evidence suggests that PDXs may serve as a valuable platform for preclinical drug testing for many types of cancer and for the discovery of novel predictive biomarkers for drug sensitivity or patient prognosis.

Although PDX models are now becoming a preferential tool for studying the efficacy of anticancer agents and biomarker development, there are a few challenges in their utilization. It has been suggested that the process of establishing a transplantable PDX results in the selection of more aggressive and increasingly metastatic cancers [16, 17, 33–35, 96]. A genome-wide analysis of variant allele frequencies in serial passages of human breast cancer PDXs showed that clonal selection occurs as early as the time when the transplantable line is established [80], indicating that established PDXs likely have some reduced intra-tumoral heterogeneity compared to the original patient's tumor. Another critical drawback of the established PDX models is the gradual replacement of human stromal cells,

including tumor-infiltrating immune cells, by murine stromal cells. Gene expression analysis has shown that genetic changes in PDXs compared to their original patients' tumors are mainly associated with microenvironment and immune responses [33]. Since the cancer-stromal interactions play a critical role in numerous aspects of cancer biology, the mismatch between human tumor cells and murine stromal cells may affect the sensitivity of PDXs to therapeutic agents and cause a discrepancy between the preclinical testing results and clinical findings. The depletion of human stromal and immune cells in established PDXs also limits the application of PDX models in studying immunotherapeutic drugs or anticancer drugs targeting patient-matched stroma.

These limitations of established PDXs outlined above may be overcome, at least in part, by utilizing first-generation xenografts as improved models for tumor heterogeneity and cancer-stromal interaction. Unlike established PDX lines that have been kept in immunodeficient hosts for a relatively longer period and possibly have undergone genetic, stromal, and immune drifts from the original patients' tumors, first-generation xenografts are implanted for only 2–4 weeks before the initiation of drug testing [31]. First-generation xenografts are thus thought to mimic more closely the intra-tumoral heterogeneity and better preserve the polyclonal subpopulations of the original patient's tumor than established PDXs. As previously mentioned, our collective observations revealed that the human stromal components, including tumor-associated fibroblasts, human vascular cells, and human immune cells, are faithfully retained in first-generation xenografts up to 8–10 weeks after the initial implantation (Figs. 11.1 and 11.2). In one of our preclinical studies (unpublished), the feasibility of using first-generation xenografts for testing immunotherapeutic agents has been investigated. Biopsy-derived metastatic prostate cancer tissues from a patient's lymph nodes were implanted into immunodeficient mice to develop first-generation xenografts. The xenografts were then treated with a drug candidate that can indirectly promote the proliferation of cell-killing immune cells and thus enhance the anticancer immunity of the tumor microenvironment. The status of immune cells in the treated xenografts was compared to that in untreated control xenografts. The results showed significantly increased CD8+ cytotoxic T-cell infiltration and tumor death in xenografts treated with the drug compared to the untreated xenografts (Fig. 11.2). Although the result is preliminary and further validation is still needed, it indicates that with proper study design the use of first-generation xenografts could help evaluate the efficacy of therapies designed to target tumor stroma or tumor-associated immune cells.

First-Generation Xenografts for Translational Cancer Research

An important, potential use of first-generation xenografts in translational cancer research consists of studying the tumor microenvironment and cancer-stromal cross talks. In the traditionally used cell line models, either cultured in vitro or grown in vivo, the complete absence of a tissue-specific tumor microenvironment has been a major problem for studying bidirectional communications between tumor cells

and the ECM or tumor-associated stromal cells. Given the important roles of the tumor microenvironment and its components in regulating multiple crucial aspects of cancer biology [97, 98], the cell lines likely lack some important biological properties of cancer tissue, thus compromising the translational value of the models [17, 99]. Significant efforts have been made in designing in vitro or in vivo models that can mimic some aspects of native tissue microenvironments in humans, such as coculturing of cancer cells with cancer-associated fibroblasts [100], and use of cell-derived matrices containing ECM components naturally produced by selected types of tumor-associated cells [101, 102]. However, these models still cannot recapitulate the in vivo behavior of human cancers in situ, due to inter-tumor heterogeneity of microenvironmental components [8, 99]. In addition, the cell-based xenografts often contain mouse stromal and endothelial cells, which may result in changes in the paracrine regulation of the cancer cells [103, 104].

Since first-generation xenograft models rather faithfully retain the tumor microenvironment as well as the genotype and phenotype of the original patient's tumor, such models could be particularly advantageous for studying cancer-stromal biology. A good example is the utilization of a first-generation xenograft model for studying tumor angiogenesis. Using first-generation xenografts of prostate cancer, it was found that there was a marked angiogenic response induced by endogenous human blood vessels from the original patient's tumor [11, 105]. The angiogenic response, which was possibly induced by androgen-driven expression of VEGF by the prostate stroma, occurred between days 6–14 after the initial implantation and ceased by day 15. In another study [106], the relationships of vascular architecture, hypoxia, and proliferation were examined using first-generation xenografts derived from patients with head-and-neck squamous cell carcinoma. The study showed that the xenografts retained their original distribution pattern of hypoxia found in the original patient's tumors, with coexistence of both hypoxic and proliferating tumor cells. These studies suggest that first-generation xenograft models could be well suited for investigations of cancer-stromal interactions, allowing in vivo modeling of the biological process in tumor stroma (e.g., angiogenesis/neovascularization) in the context of an intact human cancer tissue microenvironment.

Conclusions

In the era of massive parallel sequencing technologies, novel agents targeting specific genetic alterations in individual patients have been continuously developed. Preclinical cancer models are among the most critical elements in translating experimental discoveries into clinical applications. PDXs are superior to the conventional cell lines in many aspects and have become preferred platforms in translational cancer research and therapeutic development. First-generation xenograft models are the initial human-to-mouse generations of PDXs that harbor the tumor tissues directly derived from patients. First-generation xenograft models closely retain the histopathological and molecular characteristics of their parental patients' tumors. More importantly, they keep the human stromal component and immune cells, which are absent in cell line models or lost in transplantable PDX models following extended serial passaging in vitro or in vivo.

First-generation PDX models therefore provide highly valuable tools for cancer-stromal interaction studies, precision medicine/personalized cancer therapy, preclinical drug development, and limited cancer immunology investigations. The combination of transplantable PDX models and multi-omics analyses will significantly improve anticancer drug development, precision medicine, and our understanding of the mechanisms underlying cancer progression.

Acknowledgments We thank Drs. Dong Lin and Hui Xue at the Living Tumor Laboratory (www.livingtumorlab.com) for their original inputs and suggestions. This study was supported by Dr. Yuzhuo Wang's grants from the Canadian Institutes of Health Research, Terry Fox Research Institute, BC Cancer Foundation, Prostate Cancer Canada, and Princess Margaret Hold'em for Life.

References

1. Arrowsmith J. Trial watch: phase II failures: 2008–2010. Nat Rev Drug Discov. 2011;10(5):328–9. doi:10.1038/nrd3439.
2. Arrowsmith J, Miller P. Trial watch: phase II and phase III attrition rates 2011–2012. Nat Rev Drug Discov. 2013;12(8):569. doi:10.1038/nrd4090.
3. DiMasi JA, Reichert JM, Feldman L, Malins A. Clinical approval success rates for investigational cancer drugs. Clin Pharmacol Ther. 2013;94(3):329–35. doi:10.1038/clpt.2013.117.
4. Cho SY, Kang W, Han JY, Min S, Kang J, Lee A, et al. An integrative approach to precision cancer medicine using patient-derived xenografts. Mol Cell. 2016;39(2):77–86. doi:10.14348/molcells.2016.2350.
5. Hidalgo M, Amant F, Biankin AV, Budinska E, Byrne AT, Caldas C, et al. Patient-derived xenograft models: an emerging platform for translational cancer research. Cancer Discov. 2014;4(9):998–1013. doi:10.1158/2159-8290.CD-14-0001.
6. Izumchenko E, Meir J, Bedi A, Wysocki PT, Hoque MO, Sidransky D. Patient-derived xenografts as tools in pharmaceutical development. Clin Pharmacol Ther. 2016;99(6):612–21. doi:10.1002/cpt.354.
7. Wilding JL, Bodmer WF. Cancer cell lines for drug discovery and development. Cancer Res. 2014;74(9):2377–84. doi:10.1158/0008-5472.CAN-13-2971.
8. Choi SY, Lin D, Gout PW, Collins CC, Xu Y, Wang Y. Lessons from patient-derived xenografts for better in vitro modeling of human cancer. Adv Drug Deliv Rev. 2014;79–80:222–37. doi:10.1016/j.addr.2014.09.009.
9. Tentler JJ, Tan AC, Weekes CD, Jimeno A, Leong S, Pitts TM, et al. Patient-derived tumour xenografts as models for oncology drug development. Nat Rev Clin Oncol. 2012;9(6):338–50. doi:10.1038/nrclinonc.2012.61.
10. Dong X, Guan J, English JC, Flint J, Yee J, Evans K, et al. Patient-derived first generation xenografts of non-small cell lung cancers: promising tools for predicting drug responses for personalized chemotherapy. Clin Cancer Res. 2010;16(5):1442–51. doi:10.1158/1078-0432.CCR-09-2878.
11. Montecinos VP, Godoy A, Hinklin J, Vethanayagam RR, Smith GJ. Primary xenografts of human prostate tissue as a model to study angiogenesis induced by reactive stroma. PLoS One. 2012;7(1):e29623. doi:10.1371/journal.pone.0029623.
12. Morton CL, Houghton PJ. Establishment of human tumor xenografts in immunodeficient mice. Nat Protoc. 2007;2(2):247–50. doi:10.1038/nprot.2007.25.
13. Calles A, Rubio-Viqueira B, Hidalgo M. Primary human non-small cell lung and pancreatic tumorgraft models—utility and applications in drug discovery and tumor biology. Curr Protoc Pharmacol. 2013;Chapter 14:Unit 14.26. doi:10.1002/0471141755.ph1426s61.

14. Cutz JC, Guan J, Bayani J, Yoshimoto M, Xue H, Sutcliffe M, et al. Establishment in severe combined immunodeficiency mice of subrenal capsule xenografts and transplantable tumor lines from a variety of primary human lung cancers: potential models for studying tumor progression-related changes. Clin Cancer Res. 2006;12(13):4043–54. doi:10.1158/1078-0432.CCR-06-0252.
15. Rubio-Viqueira B, Hidalgo M. Direct in vivo xenograft tumor model for predicting chemotherapeutic drug response in cancer patients. Clin Pharmacol Ther. 2009;85(2):217–21. doi:10.1038/clpt.2008.200.
16. Nemati F, Sastre-Garau X, Laurent C, Couturier J, Mariani P, Desjardins L, et al. Establishment and characterization of a panel of human uveal melanoma xenografts derived from primary and/or metastatic tumors. Clin Cancer Res. 2010;16(8):2352–62. doi:10.1158/1078-0432.CCR-09-3066.
17. Sivanand S, Pena-Llopis S, Zhao H, Kucejova B, Spence P, Pavia-Jimenez A, et al. A validated tumorgraft model reveals activity of dovitinib against renal cell carcinoma. Sci Transl Med. 2012;4(137):137ra75. doi:10.1126/scitranslmed.3003643.
18. Ilie M, Nunes M, Blot L, Hofman V, Long-Mira E, Butori C, et al. Setting up a wide panel of patient-derived tumor xenografts of non-small cell lung cancer by improving the preanalytical steps. Cancer Med. 2015;4(2):201–11. doi:10.1002/cam4.357.
19. Jin K, Teng L, Shen Y, He K, Xu Z, Li G. Patient-derived human tumour tissue xenografts in immunodeficient mice: a systematic review. Clin Transl Oncol. 2010;12(7):473–80. doi:10.1007/s12094-010-0540-6.
20. Oh BY, Lee WY, Jung S, Hong HK, Nam DH, Park YA, et al. Correlation between tumor engraftment in patient-derived xenograft models and clinical outcomes in colorectal cancer patients. Oncotarget. 2015;6(18):16059–68. doi:10.18632/oncotarget.3863.
21. Siolas D, Hannon GJ. Patient-derived tumor xenografts: transforming clinical samples into mouse models. Cancer Res. 2013;73(17):5315–9. doi:10.1158/0008-5472.CAN-13-1069.
22. Houghton JA, Houghton PJ, Green AA. Chemotherapy of childhood rhabdomyosarcomas growing as xenografts in immune-deprived mice. Cancer Res. 1982;42(2):535–9.
23. Lee CH, Xue H, Sutcliffe M, Gout PW, Huntsman DG, Miller DM, et al. Establishment of subrenal capsule xenografts of primary human ovarian tumors in SCID mice: potential models. Gynecol Oncol. 2005;96(1):48–55. doi:10.1016/j.ygyno.2004.09.025.
24. Wang Y, Revelo MP, Sudilovsky D, Cao M, Chen WG, Goetz L, et al. Development and characterization of efficient xenograft models for benign and malignant human prostate tissue. Prostate. 2005;64(2):149–59. doi:10.1002/pros.20225.
25. Bogden AE, Cobb WR, Lepage DJ, Haskell PM, Gulkin TA, Ward A, et al. Chemotherapy responsiveness of human tumors as first transplant generation xenografts in the normal mouse: six-day subrenal capsule assay. Cancer. 1981;48(1):10–20.
26. Lin D, Wyatt AW, Xue H, Wang Y, Dong X, Haegert A, et al. High fidelity patient-derived xenografts for accelerating prostate cancer discovery and drug development. Cancer Res. 2014;74(4):1272–83. doi:10.1158/0008-5472.CAN-13-2921-T.
27. Daniel VC, Marchionni L, Hierman JS, Rhodes JT, Devereux WL, Rudin CM, et al. A primary xenograft model of small-cell lung cancer reveals irreversible changes in gene expression imposed by culture in vitro. Cancer Res. 2009;69(8):3364–73. doi:10.1158/0008-5472.CAN-08-4210.
28. Ruggeri BA, Camp F, Miknyoczki S. Animal models of disease: pre-clinical animal models of cancer and their applications and utility in drug discovery. Biochem Pharmacol. 2014;87(1):150–61. doi:10.1016/j.bcp.2013.06.020.
29. Williams SA, Anderson WC, Santaguida MT, Dylla SJ. Patient-derived xenografts, the cancer stem cell paradigm, and cancer pathobiology in the 21st century. Lab Invest. 2013;93(9):970–82. doi:10.1038/labinvest.2013.92.
30. Whittle JR, Lewis MT, Lindeman GJ, Visvader JE. Patient-derived xenograft models of breast cancer and their predictive power. Breast Cancer Res. 2015;17:17. doi:10.1186/s13058-015-0523-1.

31. de Plater L, Lauge A, Guyader C, Poupon MF, Assayag F, de Cremoux P, et al. Establishment and characterisation of a new breast cancer xenograft obtained from a woman carrying a germline BRCA2 mutation. Br J Cancer. 2010;103(8):1192–200. doi:10.1038/sj.bjc.6605900.
32. Fichtner I, Rolff J, Soong R, Hoffmann J, Hammer S, Sommer A, et al. Establishment of patient-derived non-small cell lung cancer xenografts as models for the identification of predictive biomarkers. Clin Cancer Res. 2008;14(20):6456–68. doi:10.1158/1078-0432.CCR-08-0138.
33. DeRose YS, Wang G, Lin YC, Bernard PS, Buys SS, Ebbert MT, et al. Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. Nat Med. 2011;17(11):1514–20. doi:10.1038/nm.2454.
34. Garrido-Laguna I, Uson M, Rajeshkumar NV, Tan AC, de Oliveira E, Karikari C, et al. Tumor engraftment in nude mice and enrichment in stroma- related gene pathways predict poor survival and resistance to gemcitabine in patients with pancreatic cancer. Clin Cancer Res. 2011;17(17):5793–800. doi:10.1158/1078-0432.CCR-11-0341.
35. Kleine W. Prognostic significance of growth characteristics of xenotransplanted ovarian carcinomas into nude mice. Gynecol Oncol. 1986;25(1):65–72.
36. Fichtner I, Slisow W, Gill J, Becker M, Elbe B, Hillebrand T, et al. Anticancer drug response and expression of molecular markers in early-passage xenotransplanted colon carcinomas. Eur J Cancer. 2004;40(2):298–307.
37. Migliardi G, Sassi F, Torti D, Galimi F, Zanella ER, Buscarino M, et al. Inhibition of MEK and PI3K/mTOR suppresses tumor growth but does not cause tumor regression in patient-derived xenografts of RAS-mutant colorectal carcinomas. Clin Cancer Res. 2012;18(9):2515–25. doi:10.1158/1078-0432.CCR-11-2683.
38. Garber K. From human to mouse and back: 'tumorgraft' models surge in popularity. J Natl Cancer Inst. 2009;101(1):6–8. doi:10.1093/jnci/djn481.
39. Fiebig HH, Schuchhardt C, Henss H, Fiedler L, Lohr GW. Comparison of tumor response in nude mice and in the patients. Behring Inst Mitt. 1984;74:343–52.
40. Merk J, Rolff J, Becker M, Leschber G, Fichtner I. Patient-derived xenografts of non-small-cell lung cancer: a pre-clinical model to evaluate adjuvant chemotherapy? Eur J Cardiothorac Surg. 2009;36(3):454–9. doi:10.1016/j.ejcts.2009.03.054.
41. Perez-Soler R, Kemp B, Wu QP, Mao L, Gomez J, Zeleniuch-Jacquotte A, et al. Response and determinants of sensitivity to paclitaxel in human non-small cell lung cancer tumors heterotransplanted in nude mice. Clin Cancer Res. 2000;6(12):4932–8.
42. Bhowmick NA, Chytil A, Plieth D, Gorska AE, Dumont N, Shappell S, et al. TGF-beta signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. Science. 2004;303(5659):848–51. doi:10.1126/science.1090922.
43. Bissell MJ, Hines WC. Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression. Nat Med. 2011;17(3):320–9. doi:10.1038/nm.2328.
44. Kaplan RN, Rafii S, Lyden D. Preparing the "soil": the premetastatic niche. Cancer Res. 2006;66(23):11089–93. doi:10.1158/0008-5472.CAN-06-2407.
45. Curtis C, Shah SP, Chin SF, Turashvili G, Rueda OM, Dunning MJ, et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature. 2012;486(7403):346–52. doi:10.1038/nature10983.
46. Schedin P, Keely PJ. Mammary gland ECM remodeling, stiffness, and mechanosignaling in normal development and tumor progression. Cold Spring Harb Perspect Biol. 2011;3(1):a003228. doi:10.1101/cshperspect.a003228.
47. Sundararaghavan HG, Saunders RL, Hammer DA, Burdick JA. Fiber alignment directs cell motility over chemotactic gradients. Biotechnol Bioeng. 2013;110(4):1249–54. doi:10.1002/bit.24788.
48. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell. 1996;86(3):353–64.
49. Jung YD, Ahmad SA, Liu W, Reinmuth N, Parikh A, Stoeltzing O, et al. The role of the microenvironment and intercellular cross-talk in tumor angiogenesis. Semin Cancer Biol. 2002;12(2):105–12. doi:10.1006/scbi.2001.0418.

50. Julien S, Merino-Trigo A, Lacroix L, Pocard M, Goere D, Mariani P, et al. Characterization of a large panel of patient-derived tumor xenografts representing the clinical heterogeneity of human colorectal cancer. Clin Cancer Res. 2012;18(19):5314–28. doi:10.1158/1078-0432.CCR-12-0372.
51. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74. doi:10.1016/j.cell.2011.02.013.
52. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med. 2004;10(9):942–9. doi:10.1038/nm1093.
53. Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F, et al. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proc Natl Acad Sci U S A. 2005;102(51):18538–43. doi:10.1073/pnas.0509182102.
54. Bates GJ, Fox SB, Han C, Leek RD, Garcia JF, Harris AL, et al. Quantification of regulatory T cells enables the identification of high-risk breast cancer patients and those at risk of late relapse. J Clin Oncol Off J Am Soc Clin Oncol. 2006;24(34):5373–80. doi:10.1200/JCO.2006.05.9584.
55. Sasada T, Kimura M, Yoshida Y, Kanai M, Takabayashi A. CD4+CD25+ regulatory T cells in patients with gastrointestinal malignancies: possible involvement of regulatory T cells in disease progression. Cancer. 2003;98(5):1089–99. doi:10.1002/cncr.11618.
56. Shang B, Liu Y, Jiang SJ. Prognostic value of tumor-infiltrating FoxP3+ regulatory T cells in cancers: a systematic review and meta-analysis. Sci Rep. 2015;5:15179. doi:10.1038/srep15179.
57. Ishikawa F, Yasukawa M, Lyons B, Yoshida S, Miyamoto T, Yoshimoto G, et al. Development of functional human blood and immune systems in NOD/SCID/IL2 receptor {gamma} chain(null) mice. Blood. 2005;106(5):1565–73. doi:10.1182/blood-2005-02-0516.
58. Zeng J, See AP, Phallen J, Jackson CM, Belcaid Z, Ruzevick J, et al. Anti-PD-1 blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas. Int J Radiat Oncol Biol Phys. 2013;86(2):343–9. doi:10.1016/j.ijrobp.2012.12.025.
59. Ambrogio C, Carmona FJ, Vidal A, Falcone M, Nieto P, Romero OA, et al. Modeling lung cancer evolution and preclinical response by orthotopic mouse allografts. Cancer Res. 2014;74(21):5978–88. doi:10.1158/0008-5472.CAN-14-1606.
60. Dawson SJ, Tsui DW, Murtaza M, Biggs H, Rueda OM, Chin SF, et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med. 2013;368(13):1199–209. doi:10.1056/NEJMoa1213261.
61. Ding L, Ley TJ, Larson DE, Miller CA, Koboldt DC, Welch JS, et al. Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. Nature. 2012;481(7382):506–10. doi:10.1038/nature10738.
62. Wong TN, Ramsingh G, Young AL, Miller CA, Touma W, Welch JS, et al. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. Nature. 2015;518(7540):552–5. doi:10.1038/nature13968.
63. Bui JD, Schreiber RD. Cancer immunosurveillance, immunoediting and inflammation: independent or interdependent processes? Curr Opin Immunol. 2007;19(2):203–8. doi:10.1016/j.coi.2007.02.001.
64. Pufnock JS, Rothstein JL. Oncoprotein signaling mediates tumor-specific inflammation and enhances tumor progression. J Immunol. 2009;182(9):5498–506. doi:10.4049/jimmunol.0801284.
65. Bogachek MV, Park JM, De Andrade JP, Kulak MV, White JR, Wu T, et al. A novel animal model for locally advanced breast cancer. Ann Surg Oncol. 2015;22(3):866–73. doi:10.1245/s10434-014-4174-8.
66. Mohseni MJ, Amanpour S, Muhammadnejad S, Sabetkish S, Muhammadnejad A, Heidari R, et al. Establishment of a patient-derived Wilms' tumor xenograft model: a promising tool for individualized cancer therapy. J Pediatr Urol. 2014;10(1):123–9. doi:10.1016/j.jpurol.2013.07.009.

67. Zhang H, Cohen AL, Krishnakumar S, Wapnir IL, Veeriah S, Deng G, et al. Patient-derived xenografts of triple-negative breast cancer reproduce molecular features of patient tumors and respond to mTOR inhibition. Breast Cancer Res. 2014;16(2):R36. doi:10.1186/bcr3640.
68. Zhao X, Liu Z, Yu L, Zhang Y, Baxter P, Voicu H, et al. Global gene expression profiling confirms the molecular fidelity of primary tumor-based orthotopic xenograft mouse models of medulloblastoma. Neuro-Oncology. 2012;14(5):574–83. doi:10.1093/neuonc/nos061.
69. Bogner PN, Patnaik SK, Pitoniak R, Kannisto E, Repasky E, Hylander B, et al. Lung cancer xenografting alters microRNA profile but not immunophenotype. Biochem Biophys Res Commun. 2009;386(2):305–10. doi:10.1016/j.bbrc.2009.06.021.
70. Ding L, Ellis MJ, Li S, Larson DE, Chen K, Wallis JW, et al. Genome remodelling in a basal-like breast cancer metastasis and xenograft. Nature. 2010;464(7291):999–1005. doi:10.1038/nature08989.
71. Reyal F, Guyader C, Decraene C, Lucchesi C, Auger N, Assayag F, et al. Molecular profiling of patient-derived breast cancer xenografts. Breast Cancer Res. 2012;14(1):R11. doi:10.1186/bcr3095.
72. Herter-Sprie GS, Kung AL, Wong KK. New cast for a new era: preclinical cancer drug development revisited. J Clin Invest. 2013;123(9):3639–45. doi:10.1172/JCI68340.
73. John T, Kohler D, Pintilie M, Yanagawa N, Pham NA, Li M, et al. The ability to form primary tumor xenografts is predictive of increased risk of disease recurrence in early-stage non-small cell lung cancer. Clin Cancer Res. 2011;17(1):134–41. doi:10.1158/1078-0432.CCR-10-2224.
74. Dancey JE, Bedard PL, Onetto N, Hudson TJ. The genetic basis for cancer treatment decisions. Cell. 2012;148(3):409–20. doi:10.1016/j.cell.2012.01.014.
75. Garraway LA. Genomics-driven oncology: framework for an emerging paradigm. J Clin Oncol Off J Am Soc Clin Oncol. 2013;31(15):1806–14. doi:10.1200/JCO.2012.46.8934.
76. Hidalgo M, Bruckheimer E, Rajeshkumar NV, Garrido-Laguna I, De Oliveira E, Rubio-Viqueira B, et al. A pilot clinical study of treatment guided by personalized tumorgrafts in patients with advanced cancer. Mol Cancer Ther. 2011;10(8):1311–6. doi:10.1158/1535-7163.MCT-11-0233.
77. Crystal AS, Shaw AT, Sequist LV, Friboulet L, Niederst MJ, Lockerman EL, et al. Patient-derived models of acquired resistance can identify effective drug combinations for cancer. Science. 2014;346(6216):1480–6. doi:10.1126/science.1254721.
78. Marangoni E, Vincent-Salomon A, Auger N, Degeorges A, Assayag F, de Cremoux P, et al. A new model of patient tumor-derived breast cancer xenografts for preclinical assays. Clin Cancer Res. 2007;13(13):3989–98. doi:10.1158/1078-0432.CCR-07-0078.
79. Zhang X, Claerhout S, Prat A, Dobrolecki LE, Petrovic I, Lai Q, et al. A renewable tissue resource of phenotypically stable, biologically and ethnically diverse, patient-derived human breast cancer xenograft models. Cancer Res. 2013;73(15):4885–97. doi:10.1158/0008-5472.CAN-12-4081.
80. Eirew P, Steif A, Khattra J, Ha G, Yap D, Farahani H, et al. Dynamics of genomic clones in breast cancer patient xenografts at single-cell resolution. Nature. 2015;518(7539):422–6. doi:10.1038/nature13952.
81. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. Nat Immunol. 2013;14(10):1014–22. doi:10.1038/ni.2703.
82. Mlecnik B, Tosolini M, Kirilovsky A, Berger A, Bindea G, Meatchi T, et al. Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. J Clin Oncol Off J Am Soc Clin Oncol. 2011;29(6):610–8. doi:10.1200/JCO.2010.30.5425.
83. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med. 2003;348(3):203–13. doi:10.1056/NEJMoa020177.
84. Bogden AE, Griffin W, Reich SD, Costanza ME, Cobb WR. Predictive testing with the subrenal capsule assay. Cancer Treat Rev. 1984;11(Suppl A):113–24.

85. Cunha GR. Epithelial-stromal interactions in development of the urogenital tract. Int Rev Cytol. 1976;47:137–94.
86. Cunha GR, Lung B, Kato K. Role of the epithelial-stromal interaction during the development and expression of ovary-independent vaginal hyperplasia. Dev Biol. 1977;56(1):52–67. doi:10.1016/0012-1606(77)90154-3.
87. Johnson JR, Hammond WG, Benfield JR, Tesluk H. Successful xenotransplantation of human lung cancer correlates with the metastatic phenotype. Ann Thorac Surg. 1995;60(1):32–6. discussion 6–7
88. Griffin TW, Bogden AE, Reich SD, Antonelli D, Hunter RE, Ward A, et al. Initial clinical trials of the subrenal capsule assay as a predictor of tumor response to chemotherapy. Cancer. 1983;52(12):2185–92.
89. Gao H, Korn JM, Ferretti S, Monahan JE, Wang Y, Singh M, et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nat Med. 2015;21(11):1318–25. doi:10.1038/nm.3954.
90. Johnson JI, Decker S, Zaharevitz D, Rubinstein LV, Venditti JM, Schepartz S, et al. Relationships between drug activity in NCI preclinical in vitro and in vivo models and early clinical trials. Br J Cancer. 2001;84(10):1424–31. doi:10.1054/bjoc.2001.1796.
91. Bertotti A, Migliardi G, Galimi F, Sassi F, Torti D, Isella C, et al. A molecularly annotated platform of patient-derived xenografts ("xenopatients") identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. Cancer Discov. 2011;1(6):508–23. doi:10.1158/2159-8290.CD-11-0109.
92. Kortmann U, McAlpine JN, Xue H, Guan J, Ha G, Tully S, et al. Tumor growth inhibition by olaparib in BRCA2 germline-mutated patient-derived ovarian cancer tissue xenografts. Clin Cancer Res. 2011;17(4):783–91. doi:10.1158/1078-0432.CCR-10-1382.
93. Jimeno A, Tan AC, Coffa J, Rajeshkumar NV, Kulesza P, Rubio-Viqueira B, et al. Coordinated epidermal growth factor receptor pathway gene overexpression predicts epidermal growth factor receptor inhibitor sensitivity in pancreatic cancer. Cancer Res. 2008;68(8):2841–9. doi:10.1158/0008-5472.CAN-07-5200.
94. Rajeshkumar NV, Tan AC, De Oliveira E, Womack C, Wombwell H, Morgan S, et al. Antitumor effects and biomarkers of activity of AZD0530, a Src inhibitor, in pancreatic cancer. Clin Cancer Res. 2009;15(12):4138–46. doi:10.1158/1078-0432.CCR-08-3021.
95. Seguin L, Kato S, Franovic A, Camargo MF, Lesperance J, Elliott KC, et al. An integrin beta(3)-KRAS-RalB complex drives tumour stemness and resistance to EGFR inhibition. Nat Cell Biol. 2014;16(5):457–68. doi:10.1038/ncb2953.
96. Li S, Shen D, Shao J, Crowder R, Liu W, Prat A, et al. Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts. Cell Rep. 2013;4(6):1116–30. doi:10.1016/j.celrep.2013.08.022.
97. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med. 2013;19(11):1423–37. doi:10.1038/nm.3394.
98. Zou W. Immunosuppressive networks in the tumour environment and their therapeutic relevance. Nat Rev Cancer. 2005;5(4):263–74. doi:10.1038/nrc1586.
99. Cassidy JW, Caldas C, Bruna A. Maintaining tumor heterogeneity in patient-derived tumor xenografts. Cancer Res. 2015;75(15):2963–8. doi:10.1158/0008-5472.CAN-15-0727.
100. Gaggioli C, Hooper S, Hidalgo-Carcedo C, Grosse R, Marshall JF, Harrington K, et al. Fibroblast-led collective invasion of carcinoma cells with differing roles for RhoGTPases in leading and following cells. Nat Cell Biol. 2007;9(12):1392–400. doi:10.1038/ncb1658.
101. Kutys ML, Doyle AD, Yamada KM. Regulation of cell adhesion and migration by cell-derived matrices. Exp Cell Res. 2013;319(16):2434–9. doi:10.1016/j.yexcr.2013.05.030.
102. Quiros RM, Valianou M, Kwon Y, Brown KM, Godwin AK, Cukierman E. Ovarian normal and tumor-associated fibroblasts retain in vivo stromal characteristics in a 3-D matrix-dependent manner. Gynecol Oncol. 2008;110(1):99–109. doi:10.1016/j.ygyno.2008.03.006.
103. De Wever O, Mareel M. Role of tissue stroma in cancer cell invasion. J Pathol. 2003;200(4):429–47. doi:10.1002/path.1398.

104. Junttila MR, de Sauvage FJ. Influence of tumour micro-environment heterogeneity on therapeutic response. Nature. 2013;501(7467):346–54. doi:10.1038/nature12626.
105. Gray DR, Huss WJ, Yau JM, Durham LE, Werdin ES, Funkhouser Jr WK, et al. Short-term human prostate primary xenografts: an in vivo model of human prostate cancer vasculature and angiogenesis. Cancer Res. 2004;64(5):1712–21.
106. Ljungkvist AS, Bussink J, Rijken PF, Kaanders JH, van der Kogel AJ, Denekamp J. Vascular architecture, hypoxia, and proliferation in first-generation xenografts of human head-and-neck squamous cell carcinomas. Int J Radiat Oncol Biol Phys. 2002;54(1):215–28.

Application of PDX Cancer Models in Co-clinical Trials and Personalized/ Precision Medicine

12

Hongwei Cheng, Zongwen Liu, Hui Xue, Peter W. Gout, and Hong Shan

Abbreviations

APL	Acute promyelocytic leukemia
ATRA	All trans-retinoic acid
CML	Chronic myeloid leukemia
EOC	Epithelial ovarian cancer
ESMO	European Society for Medical Oncology
HGS	High-grade serous
HPSC	Hematopoietic stem cells
PDX	Patient-derived xenograft

H. Cheng (✉) • H. Xue
Living Tumor Laboratory, BC Cancer Agency/Vancouver Prostate Centre, Vancouver, BC, Canada
e-mail: hcheng@bccrc.ca

Z. Liu
Department of Radiotherapy, The Second Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China

P.W. Gout
Living Tumor Laboratory, BC Cancer Agency/Vancouver Prostate Centre, Vancouver, BC, Canada

Department of Experimental Therapeutics, BC Cancer Research Centre, Vancouver, BC, Canada

H. Shan
The 5th Affiliated Hospital, Sun Yat-Sen University, No. 52, Meihua Dong Road, Zhuhai, China

© Springer International Publishing AG 2017
Y. Wang et al. (eds.), *Patient-Derived Xenograft Models of Human Cancer*, Molecular and Translational Medicine, DOI 10.1007/978-3-319-55825-7_12

Background: An Era of Personalized/Precision Cancer Medicine

The medical care of cancer patients is entering an era of personalized cancer medicine, promoted by revolutionary advances in genomic technologies such as high-throughput sequencing methods of the genome, transcriptome, and epigenome [1, 2]. As proposed by the European Society for Medical Oncology (ESMO), "personalized medicine" refers to "the tailoring of medical treatments to the characteristics of an individual patient and moves beyond the current approach of stratifying patients into treatment groups based on phenotypic biomarkers" [1]. The term "personalized medicine" is often used interchangeably with terms such as "genomic medicine" and "precision medicine," with the latter more emphasizing the use of personalized molecular information to aid diagnosis, prognosis, treatment, and cancer prevention for individual patients [1].

It could be argued that precision medicine in oncology started with the clinical approval of imatinib (Glivec), a small molecule inhibitor rationally designed to target the protein product of the common t(9;22) chromosome translocation in chronic myeloid leukemia (CML) [3]. This achievement has revolutionized the treatment of CML and, ever since, made CML a manageable disease. A similar situation also prevails with the treatment of acute promyelocytic leukemia (APL) with all trans-retinoic acid (ATRA), in which the proliferation of APL tumor cells is primarily driven by the *PML-RAR* alpha fusion gene product, resulting from a t(15;17) translocation [4]. Other successful precision medicine examples also include the use of the monoclonal antibody trastuzumab to target elevated expression of *HER2* in breast cancer patients [5], of vemurafenib to specifically target the *BRAF* (V600E) mutation in metastatic melanoma patients [6], of PARP inhibitors to treat breast cancer patients with *BRCA1* mutations [7], and recently, of the immunotherapeutic checkpoint antibodies, ipilimumab and nivolumab, for targeting CTLA4 [8] and PD1 [9] in many types of cancer.

Preliminary successes of precision medicine have also been reported in large-scale clinical settings. For example, a recent meta-analysis study of phase II clinical trials revealed that a personalized strategy was an independent predictor for better patient outcomes and fewer toxic deaths, while non-personalized targeted therapies were associated with significantly poorer patient outcomes [10]. Another very recent study also reported at the 2016 ASCO annual meeting that meta-analysis of 346 phase I clinical trials (involving more than 13,000 patients) showed that the tumor shrinkage rate in biomarker-directed precision medicine arms was 30.6%, compared to 4.9% for patients not treated with biomarker-directed precision medicine (https://www.asco.org). In the same study, it was also found that patients receiving precision medicine treatment had longer progression-free survival times compared to patients who did not get precision therapy (median 5.7 months vs. 2.95 months).

Despite all these encouraging achievements, precision medicine is still in its infant stage and faces immense challenges. Firstly, tumors exhibit extensive inter- and intratumor heterogeneity, which causes widespread phenotypic diversity and hence poses a significant challenge to personalized cancer medicine [11]. For example, it was shown in clear cell renal cell carcinomas that ~75% of driver gene aberrations were subclonal and therefore greatly confounded accurate estimation of driver mutation prevalence in the disease [12]. Secondly, tumor evolution, a

continuous process that is subject to dynamic changes during disease progression, from tumorigenesis to development of metastasis and to post-therapy relapse [13], further complicates our understanding of the dynamic nature of cancer biology. The third significant challenge is from the practice of using prognostic and predictive biomarkers. This includes defining the positive/negative cutoffs for biomarkers without dichotomous interpretation, the choice of validated genetic assays and laboratory tests to detect biomarkers, and the time to complete assay results within a time frame compliant with ongoing clinical care [2]. Other major challenges come from bioinformatics and economy perspectives, because of unmet needs for more efficient computational analytic methods for accurate translation of large-scale genomic data into patient clinical guidance and of economic solutions to support the significantly increased costs incurred in personalized medicine practice [2].

PDX System: Brief Overview and Advantages

Patient-derived xenograft (PDX) tumor models are established by direct implantation of patients' tumor tissues into immune-deficient mice. As PDXs have been reported to faithfully recapitulate the tumors of the patients and provide excellent clinical predictive power [14], they have gradually become preferred tools in many cancer research fields such as anticancer drug development, biomarker discovery, and tumor biology investigation [15]. The recent integration with high-throughput technologies further enabled the development of genetically characterized PDX models that provide significant molecular information along with their application [16]. Since detailed descriptions of the unique features of PDX tumor tissue models are provided elsewhere in this book, we will here mainly focus on the advantages of the PDX system that are related to its application in co-clinical trials and personalized/precision medicine.

In the normal clinical setting, large-scale molecular profiling of a patient's tumor can usually be carried out at only a few time points, i.e., when the tumor is surgically removed or a biopsy is taken. This limited access to patient tumor samples severely curtails our understanding of the mechanisms underlying the malignant progression of a patient's cancer [17]. In contrast, the use of PDX tumor models allows frequent examination of PDX tissue specimens as a function of time, thus providing exceptional opportunities to investigate the molecular and cellular mechanisms that underlie tumor progression and therapy resistance [18, 19].

Another major advantage of PDX tumor models is their capability to capture therapy-induced cancer progression [16]. For example, in our prostate cancer PDX panels, developed at the Living Tumor Laboratory (www.livingtumorlab.com), the LTL-331 PDX line is initially a typical prostatic adenocarcinoma line which, following castration of the host, eventually develops into a neuroendocrine prostate cancer subline (LTL-331R) [14]. As such, this model system provides a useful platform to study important molecular/cellular pathways that are responsible for the development of currently incurable neuroendocrine prostate cancer from prostatic adenocarcinoma, a phenomenon well recognized in the clinic [20].

The relatively stable feature of PDXs in retaining genetic alterations of patient tumor cells represents another advantage of these models [16]. For instance, *KRAS*

and *PIK3CA* mutational statuses in PDXs of pancreatic ductal adenocarcinoma and colorectal cancer were found to be 100% preserved in as many as eight passages [15]. A similar observation has been reported for other PDX cancer models [14, 21], where genetic mutations were faithfully preserved from passage to passage. As such, the stable retention of genetic alterations by the PDXs, in combination with their accurate cancer progression modeling power, makes them an excellent system for studying molecular mechanisms underlying the development and progression of cancer.

The resource-sustainable feature of transplantable PDX models also makes them an unlimited and relatively economic source for carrying out, and repeating, all kinds of investigations, such as sequential, combinational, and long-term pharmacological studies important for the development of new therapeutic modalities and for a better understanding of therapy-induced drug resistance mechanisms [22]. Additionally, this resource-sustainable feature also gives PDX tumor models a massive potential to explore tailored personalized medicine strategies that exhibit the best therapeutic effects for their potential translation into clinical patient care.

PDX Co-clinical Trials: From Concept to Paradigm Development

Recent progress in high-throughput sequencing technologies has enabled systematic cataloguing of cancer genomes, which in turn stimulated the development of biomarker-directed clinical trials with targeted therapy against certain altered genes or pathways in individual cancer patients [23]. As a consequence, new clinical trial designs are developed to evaluate targeted agents based on genetic alterations in enrolled patients; such trials are also called “basket trials.” Despite significant benefits produced in recently published basket trials, fundamental challenges, such as high patient drop-off rates in clinical trials, complex tumor heterogeneity, and differences between patients’ tumors, often confound the interpretation of the efficacy of an investigational drug [18]. From such conditions, co-clinical trial approaches have emerged in attempts to accelerate the translation of genomic and therapeutic discoveries into clinical benefits for cancer patients.

A “co-clinical trial” project was first proposed and initiated by Pandolfi and coworkers in 2011 in which they used high-fidelity genetically engineered mouse models (GEMM) of human cancers to conduct preclinical trials in parallel with human phase I/II clinical trials [4]. The trials typically involve collection, comparison, and integration of data obtained from the two systems. Although the “co-clinical trial” idea was originally inspired by the successful translation of preclinical “arsenic trioxide and retinoid” combination therapy into clinical treatment of APL [24, 25], co-clinical trial practice has recently, and also increasingly, been applied to genetically well-defined PDX models [26].

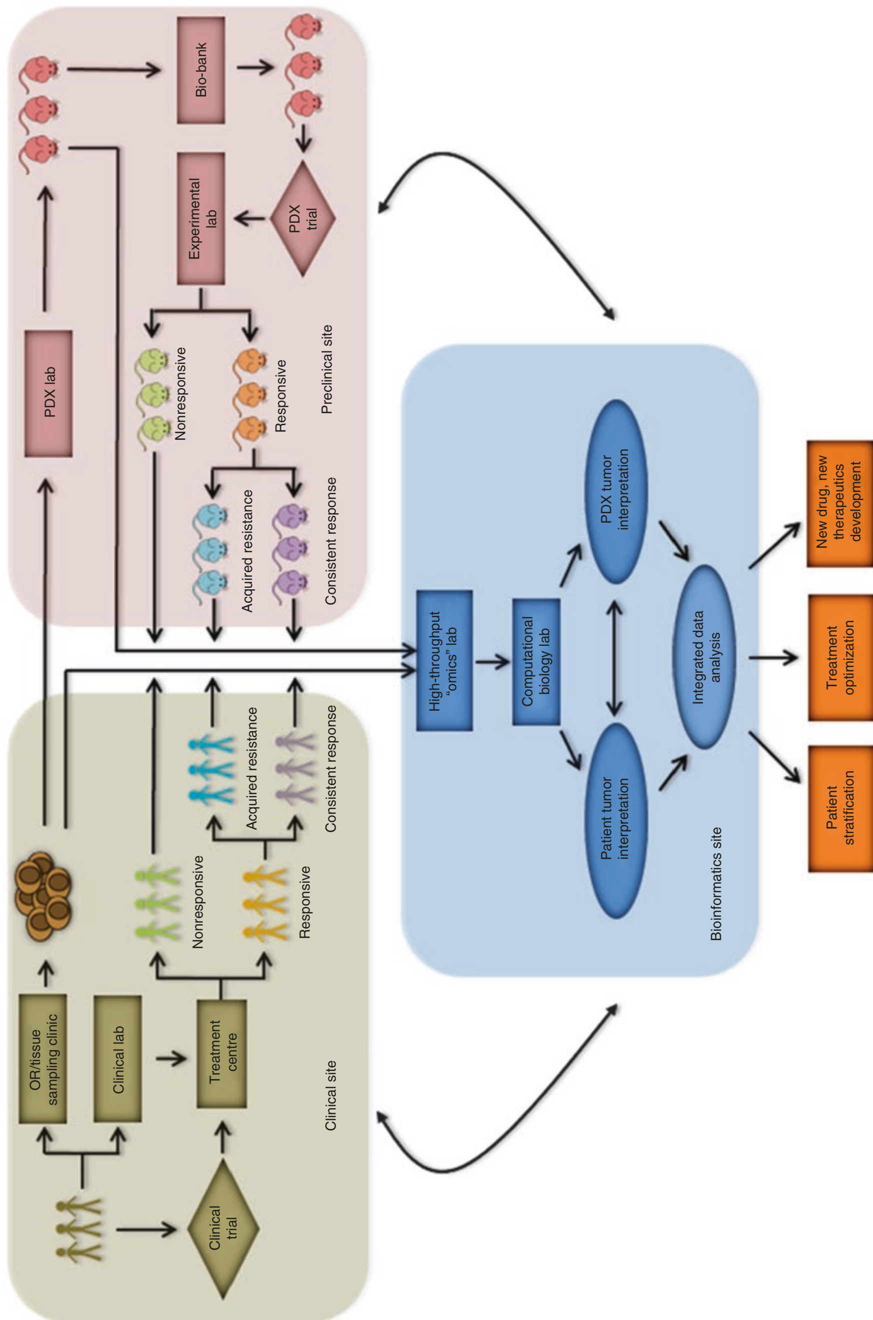
Based on relationships between the PDX models used in preclinical trials and the patients enrolled in the trials, co-clinical trials can be divided into three approaches – PDXs derived from patients who are enrolled in the clinical trial; PDXs not derived from enrolled patients, but which are histologically and genetically similar; and the use of a very large number of PDXs (e.g., ~1000) mimicking a clinical trial setting.

In the first approach, PDXs are developed directly from donor patients who are enrolled in a clinical trial [27–29]. Since it usually takes 6–9 months to generate the mouse "avatars" and only a portion of patient tumor models can be established, the efficacy of treatment with the same drugs in the models can only be compared retrospectively to clinical responses—therefore only providing relative retrospective information for drug and new therapy discovery. However, this approach still significantly complements clinical trials and can be valuable for patient stratification and prioritizing second-line treatment options for patient care. This approach is also common practice in personalized patient medicine, which will be discussed below.

The second approach is to use preestablished PDX tumor models that match the tumors of the patients enrolled in an ongoing clinical trial with regard to tumor histology and/or genetic variants. This approach requires early molecular characterization of the patients' tumors [30, 31]. Since this type of co-clinical trial uses existing models, the timeline for preclinical results is significantly shorter than the first approach, where PDX avatars need to be firstly developed. However, depending on the complexity of the signatures in the patient population and the depth to which they need to be matched in the PDXs, finding the highly relevant population to match models may be a challenging task.

The third approach, which was recently pioneered at Novartis, makes use of a large number (*n*~1000) of PDX tumor models, which are histologically homogeneous but molecularly heterogeneous, to identify pharmacological and efficacy characteristics of targeted drugs [32]. With this approach, investigators performed in vivo drug efficacy screening, using a so-called "one animal per model per treatment" approach (1 × 1 × 1), to assess the population responses to 62 treatments across six indications [32]. Although this strategy is totally new, when compared to that used in conventional preclinical studies where many animals of one model are used, it was demonstrated both to be reproducible and clinically translatable with regard to identifying relationships between a genotype and drug response. Furthermore, this approach also achieved markedly similar results when comparing the therapeutic responses of the PDXs with the clinical data, further proving the validity of this new approach.

In spite of the different methodologies used, the above approaches share the common basic concept and workflow. All co-clinical studies are composed of two trials, the PDX preclinical trial and the patient clinical trial. All patients' tumors and PDX tumors are molecularly profiled in the first place. The analyzed bioinformatics data are then applied for therapy decision-making. The therapeutic results derived from both trials are compared and examined for correlations aimed at (1) stratifying patients in terms of drug responsiveness on the basis of genetic and molecular criteria, (2) identifying mechanisms responsible for therapy resistance, and (3) evaluating the effectiveness of drug combinations to overcome such resistance based on a mechanistic understanding [33]. Figure 12.1 illustrates the overall procedure and basic infrastructure framework for carrying out a co-clinical trial study; a detailed description can be found in its legend.



PDX lab
Bio-bank
Experimental lab
PDX trial
Nonresponsive
Responsive
Preclinical site
Acquired resistance
Consistent response
OR/tissue sampling clinic
Clinical lab
Treatment centre
Clinical trial
Nonresponsive
Responsive
Acquired resistance
Consistent response
Clinical site
High-throughput "omics" lab
Computational biology lab
PDX tumor interpretation
Patient tumor interpretation
Integrated data analysis
Bioinformatics site
New drug, new therapeutics development
Treatment optimization
Patient stratification

Fig. 12.1 Basic infrastructure and simplified workflow of a PDX-based co-clinical trial. The infrastructure of co-clinical trials is composed of three basic sites—the clinical, preclinical, and bioinformatics sites. At the clinical site, centralized key facilities include an operating room and a biopsy clinic, where patients' tumor specimens are collected, and a clinical laboratory where clinical conditions of patients and pathological features of tumors are assessed. At the preclinical site, a PDX lab for PDX tumor model development, an experimental lab for therapeutics testing, and a biobank facility for storing PDX seed tissues are fundamental prerequisites. At the bioinformatics site, a high-throughput molecular "omics" lab for assessing patients' and PDX tumors' molecular profiles and a computational biological lab for data mining and interpretation are the core facilities. A simplified workflow for co-clinical trial implementation goes as follows. Starting from the clinical site, patient tumor samples are first collected by an oncologist at the operating room or biopsy clinic and then sent to (1) the high-throughput "omics" lab (at the bioinformatics site) for genetic, transcriptomic, proteinomic, or metabolomic information extraction and (2) the PDX lab at the preclinical site for PDX model development. After mining and interpretation of data at the computational biology lab (bioinformatics site), molecular characterization and interpretation of the information will be evaluated together with clinical information of the patient for his/her enrollment into a clinical trial program. When at the preclinical site a patient's PDX tumor model has successfully been established, the xenograft will undergo bioinformatics analysis as previously carried out with a patient tumor sample. Also, consistent with the molecular profiling information derived from PDX tumors, a cohort of PDX models will be enrolled into a preclinical trial in which they will be subjected, in the experimental therapeutic lab of the preclinical site, to the same therapeutic regimens applied in the corresponding clinical trial. In both clinical and preclinical trials, patient tumors and PDXs will be first stratified into responsive and nonresponsive categories. After prolonged treatment with therapeutics, some initially responsive patient tumors and PDXs will develop acquired treatment resistance, and the initial responsive category of patient tumors and PDXs will be further stratified into acquired resistance and consistently responsive subcategories. Tumor samples collected from each category will undergo further molecular characterization analyses and will mutually be compared at multiple molecular levels to ensure that the de novo and acquired resistance mechanisms identified in the PDX models can be translated to the same subtype of patient tumors. This comparative PDX co-clinical trial approach is aimed at facilitating molecular-defined patient stratification, prioritizing treatment options, and accelerating the development of new therapeutic agents and strategies

PDX-Assisted Personalized/Precision Medicine: A Promising and Fast Growing Field

Personalized medicine is a patient-centered exercise, in which a patient is treated as an individual rather than as a representative of a disease group with similar histological designation, the latter forming the basis of stratified medicine. Two fundamental bases of personalized cancer medicine are (1) increasing knowledge of the cancer genomic alteration repertoire and (2) availability of therapeutic agents that target altered genes or pathways. While the challenges of integrating genomic testing into cancer treatment decision-making are complex and wide-ranging, one challenge in particular is well recognized, namely, the risk of analysis and interpretation of large-scale genomic data without validated supportive information from relevant experiments [21]. This is despite our ever-improving understanding of cancer biology and increasing ability of identifying molecular drivers of tumor growth and survival. Increasing evidence has shown that PDXs faithfully recapitulate human tumor biology and may be used to predict a patient's drug response, as a direct correlation is observed between the drug responses of patients and those of their tumor xenografts [22]. PDX models therefore, especially when integrated with patient tumor genomic information, provide the most clinically relevant experimental platform to monitor patients' responses and to identify and validate hypothesis-driven therapies for patients who do not respond or who develop resistance to treatments [34].

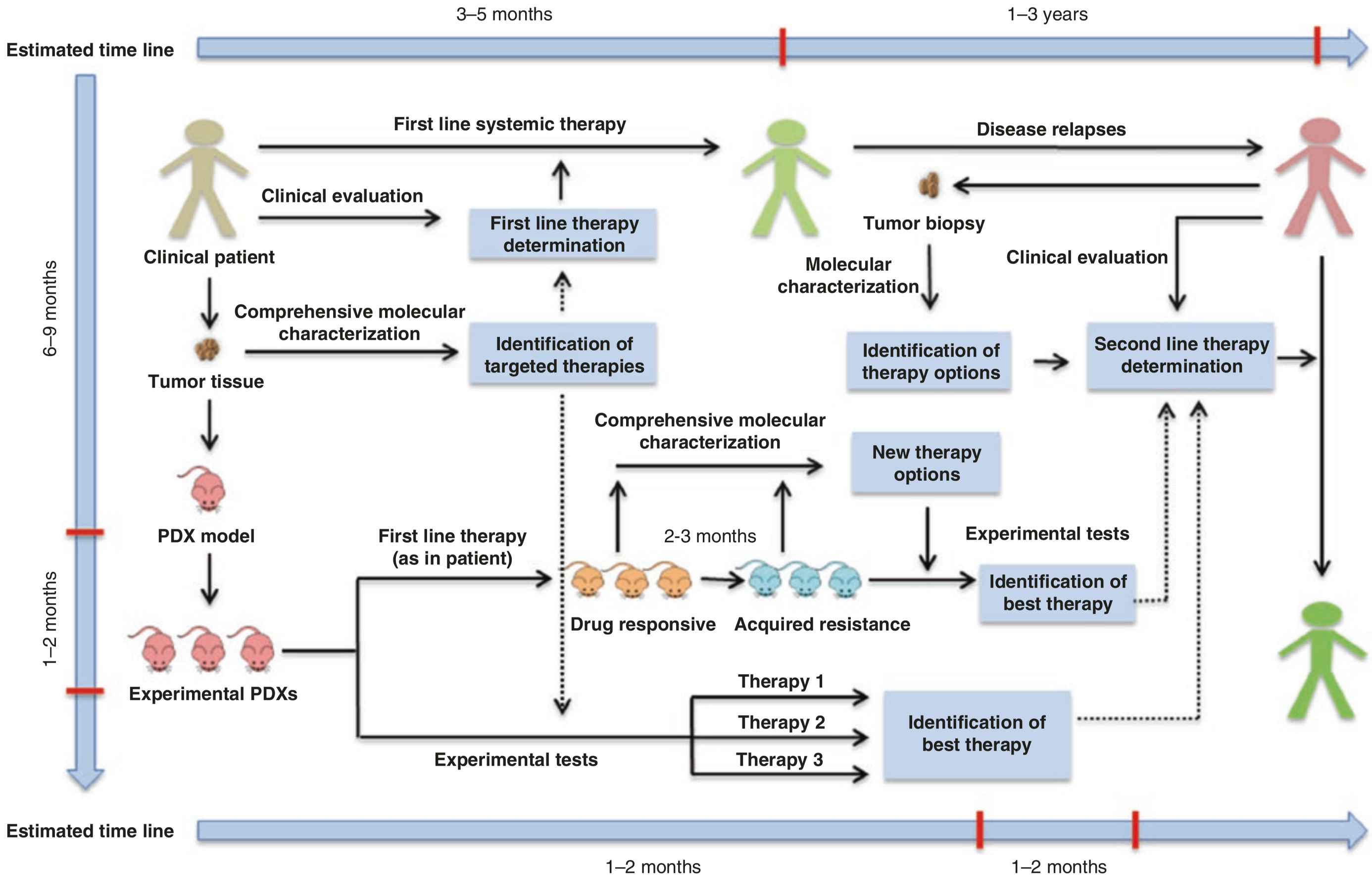
Serving as an example, good evidence of high reliability of PDX tumor models in personalized medicine was presented in a study of high-grade serous (HGS) epithelial ovarian cancer (EOC) [35]. In this study, HGS-EOC samples, obtained from patients before and after neoadjuvant platinum-based chemotherapy, were collected and used to establish PDXs. It was found that PDXs derived from a naïve HGS-EOC showed responsiveness to carboplatin, trabectedin, and gemcitabine. PDXs propagated from a tumor mass of the same patient, grown after carboplatin therapy, did no longer respond to trabectedin and gemcitabine and showed a heterogeneous response to carboplatin. Consistent with this observation, the patient experienced platinum sensitivity first and then discordant responses of different tumor sites to platinum rechallenge. The loss of PDX responsiveness to chemo-drugs was associated with a fourfold increase in NR2F2 gene expression. In another HGS-EOC case, PDXs from a naïve tumor showed a complete response to pegylated liposomal doxorubicin (PLD), which was lost in the PDXs derived from the tumor mass in the same patient after platinum-based chemotherapy. Consistently, this patient showed platinum refractoriness and responded poorly to PLD as a second-line treatment.

Another major application of PDX-empowered personalized medicine is to explore combination therapies for treating malignancies of different types which show similar genetic alterations [36], as single drug targeted therapy has shown limited efficiency against tumors harboring concurrent genetic lesions [37]. For

example, a combination treatment of the MEK inhibitor trametinib with paclitaxel was tested effective in a PDX model of a metastatic melanoma exhibiting a non-*V600 BRAF* mutation (G466E), a concurrent *HRAS* mutation (G13 V), and a loss-of-function *RB1* mutation [34]. Although the patient did not have the opportunity to receive this personalized combination treatment, a recent phase I clinical trial with a trametinib/paclitaxel (PACMEL) combination reported <40% partial response rates in patients with non-*V600* mutant *BRAF* [38], indirectly validating the clinical relevance of using a PDX-empowered approach to explore combination therapies for cancers with concurrent genetic lesions.

It is worthwhile to point out that due to the substantial time it takes to establish a patient's PDX tumor model (usually 6–9 months), real-time PDX-guided clinical care for a cancer patient is usually not practical. Although this is the general situation, exceptions have been reported for extremely aggressive cancers (e.g., metastatic melanoma), from which patients' PDX tumor models can be quickly established and applied in PDX-guided treatment decision-making in a real-time manner [39]. Nonetheless, the PDX-assisted personalized cancer medicine approach appears to be a feasible practice for second-line therapy decision-making in the case of relapsed cancer patients. Figure 12.2 illustrates the estimated timeline and the basic workflow of PDX-empowered personalized medicine, as described below.

In brief, pieces of a patient's tumor tissue are implanted into immune-deficient mice to establish a transplantable PDX line; this usually takes 6–9 months. Comprehensive molecular characterization of the patient's tumor is performed and analyzed for identification of potential targeted therapies. After integration of the molecular findings with the patient's clinical conditions, first-line systemic chemotherapy is administered to the patient. Once the patient's PDX model has successfully been generated, it is subjected to the same first-line therapy as received by the patient and used to test other identified targeted therapeutic options. A portion of treatment-sensitive mice will be subjected to prolonged treatment aimed at developing acquired therapy resistance by the tumors and establishing drug-resistant PDXs. Tissue samples collected from drug-resistant and drug-sensitive PDXs are subjected to genetic testing to explore de novo and acquired drug-resistant mechanisms. According to hypothesized potential mechanisms, new therapeutic options are formulated and tested in drug-resistant PDXs for identification of most effective therapeutic approaches. The entire PDX study is expected to be completed within 15 months after the initial engraftment of the patient's tumor tissue. Following recurrence of the patient's cancer after the first-line treatment (at about 1–3 years), it will be biopsied and molecularly characterized. The molecular information obtained is then compared with that generated from the PDX models. If a similar drug resistance mechanism is identified for the recurrent tumor, the patient will be treated with the therapy that was most effective against the drug-resistant PDXs. If not, the patient will be treated with the best targeted therapy initially identified with the patient's tumor and validated with the PDXs.



Estimated time line
3–5 months
1–3 years
First line systemic therapy
Disease relapses
Clinical evaluation
First line therapy determination
Tumor biopsy
Clinical patient
Molecular characterization
Clinical evaluation
Comprehensive molecular characterization
Identification of targeted therapies
Identification of therapy options
Second line therapy determination
Tumor tissue
Comprehensive molecular characterization
New therapy options
PDX model
First line therapy (as in patient)
2-3 months
Experimental tests
Identification of best therapy
Drug responsive
Acquired resistance
Experimental PDXs
Therapy 1
Therapy 2
Therapy 3
Identification of best therapy
Experimental tests
6–9 months
1–2 months
Estimated time line
1–2 months
1–2 months

Fig. 12.2 Workflow and estimated timelines of PDX-assisted personalized medicine. A portion of obtained patient tumor tissues is subjected to comprehensive molecular analyses; the other portion is used for development of a patient PDX model, usually taking 6–9 months. First-line therapy of patients is determined based on patient clinical information and patient tumor's molecular evaluation. After successful PDX model development and number expansion (taking 1–2 months), experimental PDXs will receive the same first-line therapy as administered to the patients and subjected to efficacy testing of candidate-targeted therapies suggested by molecular information of the patient tumor. PDX mice treated with first-line therapy will be exposed to prolonged treatment to generate drug-resistant PDX models (usually taking 2–3 months). Tumor samples from these drug-resistant mice and the parental drug-responsive mice are processed for comprehensive molecular evaluation to explore the mechanisms underlying development of acquired resistance. Based on hypothesized resistance mechanisms, new candidate therapies will be formulated and tested using therapy-resistant PDXs. At the time when first-line therapy-treated patients develop drug resistance (estimated 1–3 years), patient-relapsed tumors will be biopsied and evaluated by molecular analyses to explore resistance mechanisms and new candidate therapies. By this time, PDX-derived experimental findings can be used to assist patient second-line therapy decision-making in the following two ways: if a similar drug resistance mechanism is also revealed in a patient-relapsed tumor, the patient will be treated with the best therapy as validated with the therapy-resistant PDX model, and if no agreement is observed with the patient-relapsed tumor, the patient will be treated with the best targeted therapy as initially suggested by patient primary tumor analysis and validated in patient PDX tests

Infrastructure and Implementation: Integrated Multidisciplinary Care

Both PDX-assisted co-clinical trials and personalized medicine require sustained and coordinated efforts by both academicians and clinicians. They are extremely resource intensive and demand extensive infrastructure to support clinical, preclinical, and bioinformatics management. Consequently, the development of integrated facilities, composed of, for instance, a clinical oncology center, a PDX mouse laboratory, and a genomic bioinformatics consortium, is highly recommended prior to commencement of such projects [4]. Furthermore, rigorous clinical trial and PDX experimental protocols, SOPs and good laboratory practice, cooperation of pharmaceutical companies, data integration and co-clinical evaluations, as well as clinical and patient education are all warranted for better implementation of PDX-assisted co-clinical trials, personalized medicine, and patient benefits [40].

Figure 12.1 illustrates a simplified infrastructure layout for PDX-assisted co-clinical trial studies. The basic co-clinical infrastructure constitutes three execution sites—the clinical, the preclinical, and the bioinformatics centralized bases, with several key functional facilities involved at each site. At the clinical site, an operating room (or biopsy clinic), clinical laboratory, and treatment center are essential for carrying out clinical trial studies. A PDX lab and experimental therapeutic lab are essential for preclinical studies at the preclinical site, while high-throughput molecular experimental and computational biology laboratories are fundamental for data generation, data mining, and result interpretation.

The PDX tumor models used in all the approaches described above require extensive molecular characterization, including genetic, transcriptomic signature, and standardized PDX response data. These data would ideally be stored in a database that links tumor molecular characteristics to PDX treatment responses. Moreover, patient data would also be needed to determine whether there are correlations between PDX and patients' drug responses for various classes of therapeutics. As well, these data could be used to suggest potential novel therapeutic regimens for patients whose tumors share similar genetic or expression signatures with a particular PDX or, more ideally, a group of PDX models. Thus, PDXs could potentially be used to help stratify molecularly defined tumor subsets, prioritize treatment options, and find new combination regimens to benefit enrolled patients.

As personalized cancer medicine is based on the concept of cancer being a systemic, highly heterogeneous, and complex disease, a multidisciplinary team of highly qualified healthcare professionals is required to provide quality care to individual cancer patients. Since medical oncologists act as the "patient interface" within a multidisciplinary team [40], they should take the leading role in the multidisciplinary team as they not only are aware of current advances in research but also guide their patients in seeking out available clinical trials and in making treatment decisions.

Open Questions and Challenges of PDX-Based Approaches: A Journey for Improvement

It is well documented that PDX tumor models in general faithfully maintain the histology, genomic architecture, and drug responsiveness of the original patients' tumors. However, a recent study of the effects of engraftment and propagation in immune-deficient mice on the genomic clonal architecture of human breast tumors has shown that changes may occur in the clonal dynamics of the xenografts. Thus, clonal selection was observed for both primary and metastatic tumors, occurring more frequently in the initial engraftment stage than in the propagation stage of the xenografts [41]. While the changes in the clonal dynamics of the PDXs may be due to selection of preexisting clones [42], it remains an open question on how the spontaneous clonal evolution affects the predictive power of PDXs with regard to drug responsiveness and how this is going to influence the decision-making with regard to patient treatment [15].

As PDX models are skewed toward more aggressive types of cancer [22], a higher engraftment failure rate is encountered with less aggressive malignancies. As well, the development of PDX models for drug efficacy testing may involve a long establishment time of about 6–9 months plus an expansion time of 1–2 months. Both factors greatly limit immediate application of PDXs in treatment decision-making for personalized patient care. As illustrated in Fig. 12.2, a more feasible approach would be to perform PDX-based drug testing during the period of initial standard therapy and pursue optimization of the second-line treatment.

Recently, the realization that cancer cells develop complex mechanisms to evade host immune responses has led to rapid development of targeted immunotherapies [43]. However, the lack of an intact immune system in immune-deficient mice bearing PDX tumor models does not allow elucidation of the immune-suppressive activities of the grafted tumor in such systems [44], raising the biggest challenge in the PDX field. One potential solution may be to generate a humanized PDX model featuring a restored immune system by co-engrafting human hematopoietic stem cells (HPSC) and a patient's tumor into an NSG mouse [45]. Such an approach could make it possible to study the complex interaction between cancer cells and the human immune system and allow assessment of novel immunotherapies targeting primary and disseminated tumor cells.

A critical economical challenge is the cost associated with PDX approaches, as maintaining "live tumor banks," mouse-housing facilities, and histopathological cores proves to be expensive [1]. Increased popularity of the PDX tumor model systems also warrants the regulation of their use by a federally run centralized body similar to the FDA, to regulate costs and streamline disparate practices with respect to health insurance. Such a regulatory body would oversee fair competition and uniformity of services provided by various commercial enterprises [1].

Conclusions

In conclusion, PDX tumor models provide a proof of principle opportunity to aid clinical trial design and improve patient outcomes following treatment with personalized/precision medicine. While it is not practical to develop PDXs in real time to direct front-line therapy of most patients, the xenografts may be useful for selection of subsequent lines of treatment. Although resource intensive, PDX studies complement approaches of molecularly targeted clinical oncology, allowing prediction of probable mechanisms of drug resistance and informing design of appropriate therapeutic strategies. PDX models also make it possible to test multiple therapeutic options for prioritizing patient treatment, which could not be possibly carried out in the clinical setting. Although still facing many challenges, PDX systems are likely to become increasingly useful tools as they are more and more integrated with molecular biology techniques. As such they have a tremendous potential to develop into an essential modality in the field of clinical oncology.

Acknowledgments We thank all past and current members at the Living Tumor Laboratory (www.livingtumorlab.com) for their original inputs and suggestions. This study was supported by Dr. Yuzhuo Wang's grants from the Canadian Institutes of Health Research, Terry Fox Research Institute, BC Cancer Foundation, Prostate Cancer Canada, and Princess Margaret Hold'em for Life.

References

1. Ciardiello F, Arnold D, Casali PG, Cervantes A, Douillard JY, Eggermont A, et al. Delivering precision medicine in oncology today and in future-the promise and challenges of personalised cancer medicine: a position paper by the European Society for Medical Oncology (ESMO). Ann Oncol. 2014;25(9):1673–8. doi:10.1093/annonc/mdu217.
2. Lander ES. Initial impact of the sequencing of the human genome. Nature. 2011;470(7333):187–97. doi:10.1038/nature09792.
3. Capdeville R, Buchdunger E, Zimmermann J, Matter A. Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. Nat Rev Drug Discov. 2002;1(7):493–502. doi:10.1038/nrd839.
4. Nardella C, Lunardi A, Patnaik A, Cantley LC, Pandolfi PP. The APL paradigm and the "co-clinical trial" project. Cancer Discov. 2011;1(2):108–16. doi:10.1158/2159-8290.CD-11-0061.
5. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med. 2001;344(11):783–92. doi:10.1056/NEJM200103153441101.
6. Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med. 2010;363(9):809–19. doi:10.1056/NEJMoa1002011.
7. Benafif S, Hall M. An update on PARP inhibitors for the treatment of cancer. Onco Targets Ther. 2015;8:519–28. doi:10.2147/OTT.S30793.
8. Day F, Kumar M, Fenton L, Gedye C. Durable response of metastatic squamous cell carcinoma of the skin to ipilimumab immunotherapy. J Immunother. 2017;40(1):36–8. doi:10.1097/CJI.0000000000000146.

9. Rizzo MM, Mando P, De La Puente CP, Puparelli C, Losco F, Chacon M, et al. P2.38: nivolumab in clinical practice: real world experience in an argentina oncologic center: track: immunotherapy. J Thorac Oncol. 2016;11(10S):S240–S1. doi:10.1016/j.jtho.2016.08.109.
10. Schwaederle M, Zhao M, Lee JJ, Eggermont AM, Schilsky RL, Mendelsohn J, et al. Impact of precision medicine in diverse cancers: a meta-analysis of phase II clinical trials. J Clin Oncol. 2015;33(32):3817–25. doi:10.1200/JCO.2015.61.5997.
11. Burrell RA, McGranahan N, Bartek J, Swanton C. The causes and consequences of genetic heterogeneity in cancer evolution. Nature. 2013;501(7467):338–45. doi:10.1038/nature12625.
12. Gerlinger M, Horswell S, Larkin J, Rowan AJ, Salm MP, Varela I, et al. Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing. Nat Genet. 2014;46(3):225–33. doi:10.1038/ng.2891.
13. Yap TA, Gerlinger M, Futreal PA, Pusztai L, Swanton C. Intratumor heterogeneity: seeing the wood for the trees. Sci Transl Med. 2012;4(127):127ps10. doi:10.1126/scitranslmed.3003854.
14. Lin D, Wyatt AW, Xue H, Wang Y, Dong X, Haegert A, et al. High fidelity patient-derived xenografts for accelerating prostate cancer discovery and drug development. Cancer Res. 2014;74(4):1272–83. doi:10.1158/0008-5472.CAN-13-2921-T.
15. Tignanelli CJ, Herrera Loeza SG, Yeh JJ. KRAS and PIK3CA mutation frequencies in patient-derived xenograft models of pancreatic and colorectal cancer are reflective of patient tumors and stable across passages. Am Surg. 2014;80(9):873–7.
16. Tentler JJ, Tan AC, Weekes CD, Jimeno A, Leong S, Pitts TM, et al. Patient-derived tumour xenografts as models for oncology drug development. Nat Rev Clin Oncol. 2012;9(6):338–50. doi:10.1038/nrclinonc.2012.61.
17. Cheon DJ, Orsulic S. Mouse models of cancer. Annu Rev Pathol. 2011;6:95–119. doi:10.1146/annurev.pathol.3.121806.154244.
18. Malaney P, Nicosia SV, Dave V. One mouse, one patient paradigm: new avatars of personalized cancer therapy. Cancer Lett. 2014;344(1):1–12. doi:10.1016/j.canlet.2013.10.010.
19. Richmond A, Su Y. Mouse xenograft models vs GEM models for human cancer therapeutics. Dis Model Mech. 2008;1(2–3):78–82. doi:10.1242/dmm.000976.
20. Beltran H, Tomlins S, Aparicio A, Arora V, Rickman D, Ayala G, et al. Aggressive variants of castration-resistant prostate cancer. Clin Cancer Res. 2014;20(11):2846–50. doi:10.1158/1078-0432.CCR-13-3309.
21. Garraway LA. Genomics-driven oncology: framework for an emerging paradigm. J Clin Oncol. 2013;31(15):1806–14. doi:10.1200/JCO.2012.46.8934.
22. Hidalgo M, Amant F, Biankin AV, Budinska E, Byrne AT, Caldas C, et al. Patient-derived xenograft models: an emerging platform for translational cancer research. Cancer Discov. 2014;4(9):998–1013. doi:10.1158/2159-8290.CD-14-0001.
23. Simon R, Roychowdhury S. Implementing personalized cancer genomics in clinical trials. Nat Rev Drug Discov. 2013;12(5):358–69. doi:10.1038/nrd3979.
24. Rego EM, He LZ, Warrell Jr RP, Wang ZG, Pandolfi PP. Retinoic acid (RA) and As2O3 treatment in transgenic models of acute promyelocytic leukemia (APL) unravel the distinct nature of the leukemogenic process induced by the PML-RARalpha and PLZF-RARalpha oncoproteins. Proc Natl Acad Sci U S A. 2000;97(18):10173–8. doi:10.1073/pnas.180290497.
25. Rego EM, Ruggero D, Tribioli C, Cattoretti G, Kogan S, Redner RL, et al. Leukemia with distinct phenotypes in transgenic mice expressing PML/RAR alpha, PLZF/RAR alpha or NPM/RAR alpha. Oncogene. 2006;25(13):1974–9. doi:10.1038/sj.onc.1209216.
26. Hidalgo M, Bruckheimer E, Rajeshkumar NV, Garrido-Laguna I, De Oliveira E, Rubio-Viqueira B, et al. A pilot clinical study of treatment guided by personalized tumorgrafts in patients with advanced cancer. Mol Cancer Ther. 2011;10(8):1311–6. doi:10.1158/1535-7163.MCT-11-0233.
27. Glaser G, Weroha SJ, Becker MA, Hou X, Enderica-Gonzalez S, Harrington SC, et al. Conventional chemotherapy and oncogenic pathway targeting in ovarian carcinosarcoma using a patient-derived tumorgraft. PLoS One. 2015;10(5):e0126867. doi:10.1371/journal.pone.0126867.

28. Scott CL, Becker MA, Haluska P, Samimi G. Patient-derived xenograft models to improve targeted therapy in epithelial ovarian cancer treatment. Front Oncol. 2013;3:295. doi:10.3389/fonc.2013.00295.
29. Weroha SJ, Becker MA, Enderica-Gonzalez S, Harrington SC, Oberg AL, Maurer MJ, et al. Tumorgrafts as in vivo surrogates for women with ovarian cancer. Clin Cancer Res. 2014;20(5):1288–97. doi:10.1158/1078-0432.CCR-13-2611.
30. Amendt C, Staub E, Friese-Hamim M, Storkel S, Stroh C. Association of EGFR expression level and cetuximab activity in patient-derived xenograft models of human non-small cell lung cancer. Clin Cancer Res. 2014;20(17):4478–87. doi:10.1158/1078-0432.CCR-13-3385.
31. Li S, Shen D, Shao J, Crowder R, Liu W, Prat A, et al. Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts. Cell Rep. 2013;4(6):1116–30. doi:10.1016/j.celrep.2013.08.022.
32. Gao H, Korn JM, Ferretti S, Monahan JE, Wang Y, Singh M, et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nat Med. 2015;21(11):1318–25. doi:10.1038/nm.3954.
33. Lunardi A, Pandolfi PP. A co-clinical platform to accelerate cancer treatment optimization. Trends Mol Med. 2015;21(1):1–5. doi:10.1016/j.molmed.2014.10.008.
34. Girotti MR, Gremel G, Lee R, Galvani E, Rothwell D, Viros A, et al. Application of sequencing, liquid biopsies, and patient-derived xenografts for personalized medicine in melanoma. Cancer Discov. 2016;6(3):286–99. doi:10.1158/2159-8290.CD-15-1336.
35. Erriquez J, Olivero M, Mittica G, Scalzo MS, Vaira M, De Simone M, et al. Xenopatients show the need for precision medicine approach to chemotherapy in ovarian cancer. Oncotarget. 2016;7(18):26181–91. doi:10.18632/oncotarget.8325.
36. Dancey JE, Bedard PL, Onetto N, Hudson TJ. The genetic basis for cancer treatment decisions. Cell. 2012;148(3):409–20. doi:10.1016/j.cell.2012.01.014.
37. Lopez-Chavez A, Thomas A, Rajan A, Raffeld M, Morrow B, Kelly R, et al. Molecular profiling and targeted therapy for advanced thoracic malignancies: a biomarker-derived, multiarm, multihistology phase II basket trial. J Clin Oncol. 2015;33(9):1000–7. doi:10.1200/JCO.2014.58.2007.
38. Coupe N, Corrie P, Hategan M, Larkin J, Gore M, Gupta A, et al. PACMEL: a phase 1 dose escalation trial of trametinib (GSK1120212) in combination with paclitaxel. Eur J Cancer. 2015;51(3):359–66. doi:10.1016/j.ejca.2014.11.018.
39. Einarsdottir BO, Bagge RO, Bhadury J, Jespersen H, Mattsson J, Nilsson LM, et al. Melanoma patient-derived xenografts accurately model the disease and develop fast enough to guide treatment decisions. Oncotarget. 2014;5(20):9609–18. doi:10.18632/oncotarget.2445.
40. Clohessy JG, de Stanchina E. Infrastructure needs for translational integration of mouse and human trials. Cold Spring Harb Protoc. 2013;2013(12):1109–14. doi:10.1101/pdb.top078782.
41. Eirew P, Steif A, Khattra J, Ha G, Yap D, Farahani H, et al. Dynamics of genomic clones in breast cancer patient xenografts at single-cell resolution. Nature. 2015;518(7539):422–6. doi:10.1038/nature13952.
42. Aparicio S, Hidalgo M, Kung AL. Examining the utility of patient-derived xenograft mouse models. Nat Rev Cancer. 2015;15(5):311–6. doi:10.1038/nrc3944.
43. Eggermont AM, Robert C. Melanoma: smart therapeutic strategies in immuno-oncology. Nat Rev Clin Oncol. 2014;11(4):181–2. doi:10.1038/nrclinonc.2014.36.
44. Giovanella BC, Stehlin JS, Wall ME, Wani MC, Nicholas AW, Liu LF, et al. DNA topoisomerase I—targeted chemotherapy of human colon cancer in xenografts. Science. 1989;246(4933):1046–8.
45. Yokota SJ, Facciponte JG, Kelleher Jr RJ, Shultz LD, Loyall JL, Parsons RR, et al. Changes in ovarian tumor cell number, tumor vasculature, and T cell function monitored in vivo using a novel xenograft model. Cancer Immun. 2013;13:11.

Prospectives 13

Dong Lin, Yuzhuo Wang, and Peter W. Gout

Abbreviations

BLI	Bioluminescence imaging
COG	Children's Oncology Group
NCI	National Cancer Institute
NIBR PDXE	Novartis Institutes for Biomedical Research PDX Encyclopedia
PPTC	Pediatric Preclinical Testing Consortium
PRoXe	Public Repository of Xenografts
RECIST	Response Evaluation Criteria in Solid Tumors
SRC	Subrenal capsule

Although the advantages of PDX cancer tissue models over traditional cell line-based models are well appreciated, PDXs do have their limitations and deficiencies. Current PDX cancer models will benefit from optimization in a number of areas, such as improvement of engraftment rate and efficiency, availability of new, more amenable host strains, post-implantation xenograft monitoring, standardization of model development and experimental design, and inter-institutional collaborations. The use of such optimized, next-generation PDX models will accelerate the advancement of preclinical drug development and personalized cancer medicine.

D. Lin • Y. Wang
Vancouver Prostate Centre, Vancouver, BC, Canada

Department of Urologic Sciences, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada

Department of Experimental Therapeutics, BC Cancer Research Centre, Vancouver, BC, Canada

P.W. Gout (✉)
Living Tumor Laboratory, BC Cancer Agency/Vancouver Prostate Centre, Vancouver, BC, Canada

Department of Experimental Therapeutics, BC Cancer Research Centre, Vancouver, BC, Canada
e-mail: pgout@bccrc.ca

© Springer International Publishing AG 2017

Y. Wang et al. (eds.), *Patient-Derived Xenograft Models of Human Cancer*, Molecular and Translational Medicine, DOI 10.1007/978-3-319-55825-7_13

Improvement of Engraftment Rate and Efficiency

Establishment of a panel of PDX cancer models, derived from various stages of a particular type of cancer, is important for better mimicking a patient's disease. Also, to develop PDX models timely and efficiently is crucial, particularly for personalized medicine applications. Although large collections of PDX models have been reported by various research groups, the number of models of difficult-to-engraft cancers, such as prostate cancer, is still limited. The development of transplantable models in particular from primary, untreated prostate cancer samples is much less successful [1–4]. This low success rate might simply reflect low aggressiveness of the cancer type, which is beyond a researcher's control. On the other hand, it suggests that these cancers may be more sensitive to grafting conditions, including the graft site, grafting methods, and the mouse strain used.

As reviewed in Chap. 2, three graft sites, i.e., the subrenal capsule (SRC) graft site, the subcutaneous site, and the orthotopic site, are commonly used for tissue grafting. The SRC site is most efficient for growing human prostate tumors as well as normal prostate cells [5]. Furthermore, it has been reported that successful development of PDXs from needle biopsies of primary prostate cancer tissues can be achieved by grafting specimens into the SRC site of NOD-SCID mice [6]. Such findings suggest important advantages of SRC grafting in developing models of difficult-to-engraft cancer types, which are likely based on the greater vascularity of the renal graft site (www.livingtumorlab.com). Thus there is an exceptionally high fluid circulation within the extracellular space of the kidney [7]. This provides high graft perfusion, and the abundant supply of nutrients, hormones, growth factors, and oxygen to transplanted cells and tissues (before they become vascularized) is likely instrumental to the success of SRC engraftment, in particular of cancer subpopulations which are critically dependent on growth-stimulating factors and oxygen [8–12]. It appears that SRC xenografting may not only maximize the tumor engraftment rate but also retention of the original cellular complexity of the primary tumor. This interpretation is supported by the high similarity observed between SRC xenografts and the parent tumors in histopathology, genetic and transcriptomic profiles, and their response to hormone deprivation [5, 13–15]. Accordingly, cancer tissue lines developed at the SRC site should better reflect the wide spectrum of cancer subpopulations in the primary tumor than tumor lines developed at the relatively anoxic subcutaneous graft site, although the latter is more convenient for grafting as it involves less intricate surgery and easier tumor size monitoring. Furthermore, once SRC tumor tissue lines are well established, they can be regrafted to, for example, the orthotopic site for assessment of metastatic ability.

It has also been reported that co-implanting of Matrigel or embryonic or neonatal mouse mesenchyme with tumor tissues can provide a supportive microenvironment and improve the vasculature of the models [2, 16–21]. Such supportive material provides growth factors and extracellular matrix to enhance the growth of the patient tissue and host vasculature. However, it is still not clear whether the supportive material can lead to artificial changes at the genomic and transcriptomic levels. In addition, the source of grafting samples and sample preservation methods may also influence the success rate of model development. However, a direct comparison is

lacking the effects of fresh tissue, rapid autopsy tissue, circulating tumor cells, and other sample sources on the establishment of the models. The effect of sample preservation media on the modeling is also unknown. We believe that a better understanding of these factors will significantly improve the success rate of PDX model establishment, particularly for difficult-to-engraft cancer types.

Improvement of Recapitulating Tumor Heterogeneity

It is well established that cancer is a heterogeneous disease and that tumor heterogeneity is a prominent contributor to therapeutic failure. The intra-tumoral heterogeneity and subpopulation evolution during tumor progression form a major hurdle in developing effective anticancer therapeutics. In view of this, it is essential that cancer models faithfully recapitulate the tumor heterogeneity of a patient's malignancy (Chap. 5). As mentioned above, xenografting at the SRC graft site, as distinct from the more convenient subcutaneous graft site, likely optimizes retention of the cancer subpopulations of the patients' tumor. Accordingly, SRC PDX cancer models should better reflect the heterogeneity of cancers. Furthermore, to maximize recapitulation of the tumor heterogeneity of a patient's tumor, a panel of models will be needed which are derived from multiple biopsy specimens or circulating tumor cells collected at different stages of the patient's cancer development.

A clear understanding of the molecular foundation of cancer appears to be required for optimal assessment of its potential for disease progression. Recently, multiple molecular alterations have been identified in various types of cancer which demonstrate inter-tumoral heterogeneity and provide a rationale for molecular subclassification of the disease. PDX models have provided valuable tools for studying various molecular alterations of the disease, and it can be expected that a large panel of such models, covering a number of molecular subtypes of the disease, will be useful for elucidating the actions of molecular alterations in cancer progression and for developing novel therapeutic approaches for the disease.

To obtain reproducible and reliable results with cancer tissue lines, it is crucial that their cellular characteristics and composition are maintained. Although following continual in vivo passaging of tumor tissue lines only minimal changes were observed in their key characteristics (i.e., gross chromosome copy number, cell morphology, growth rates, and gene expression profiles) compared to early generation xenografts, it is likely that more aggressive subclones will be enriched during the process of PDX model establishment, and increasing histopathological and molecular differences between patient tumors and xenografts are foreseeable (Chap. 3). It is therefore prudent to establish a permanent stock of a xenograft line at an early generation, to ensure that cellular characteristics and composition are preserved and avoid alterations generated by extended tumor passaging and unnecessary use of mice. A number of groups have routinely preserved PDX models for long-term storage and can successfully resurrect them with a high recovery rate. These xenograft tissue stocks can be used as an unlimited supply of a particular patient's tumor and allow reproducible and reliable results.

Establishment of Humanized PDX Models

In the last decades, the development of PDX models has much benefited from the production and customization of immunodeficient host mice which lack a functional immune system to allow establishment of xenografts. On the other hand, the lack of a functional immune system is a major limitation of PDX models, limiting their application in studying cancer immunology and preclinical screening of immunomodulatory therapeutics (Chap. 4). To circumvent this hurdle, establishing PDX models with a human immune repertoire is of major interest, and it has recently become possible to generate PDXs in NSG mice with limited human immunity by co-grafting patient cancer tissue and human peripheral lymphoid cells or hematopoietic stem cells [22]. The development of such humanized mice is still at a relatively early stage and is time- and cost-consuming. However, although generating immunodeficient rodent hosts with further enhanced human immunity is challenging, we believe that the next generation of humanized mice will soon be available and that PDX models established with such hosts will provide a new, improved platform for investigating the role of the immune system in cancer development and testing the efficacy of immune-based cancer therapies.

Improvement of Cancer-Stromal Interaction

Cancer-associated stroma plays an important role in the development, progression, and treatment response of cancers (Chaps. 6 and 11). As PDX models recapitulate the 3D structure of the original tumor, they should provide a valuable tool for studying cancer-stromal interaction in a clinically relevant setting. However, it has consistently been observed that, upon serial passaging, the human stromal components of established PDXs are replaced by murine stroma [17, 23, 24]. This may lead to a lack of cross talk between cancer cells and stroma and probably cause paracrine changes in such PDXs that may limit their usefulness in studying therapeutic agents directed against tumor stroma [25]. First-generation PDX models, however, not only retain the intra-tumor heterogeneity of patients' original tumors but also their human stroma and its components, including human cancer-associated fibroblasts and vasculature (Chap. 11). Furthermore, it has been observed that first-generation PDXs can retain human immune cells, such as cytotoxic CD8+ and regulatory T cells, for up to 10 weeks after tumor implantation. As such, first-generation PDXs may be used for studying limited aspects of immunotherapy, such as measuring the effect of immunotherapeutics on human stromal components and levels of human immune cells associated with anticancer immunity.

Recently, 3D or spheroid cultures have successfully been established for a variety of cancers; they mimic cell-cell and cell-matrix interactions, hypoxia, and drug penetration through the tumor more accurately than traditional cell line-based models [26]. This in vitro method can be developed faster, and is more cost-effective, than animal models and therefore provides an important complementary modeling option for cancer research and drug development. However, ex vivo manipulation

may lead to modifications of the tumor in terms of biologic, genetic, and epigenetic properties, and, at present, it is not fully understood to what extent these in vitro models can mimic the patients' disease. A comprehensive comparative analysis of 3D/organoid culture and PDX models is needed for a better understanding of these in vitro models and their optimization.

Improvement of Post-implantation Xenograft Monitoring

It is critical to establish an efficient monitoring method that allows quick measurement of the growth of xenografts and their responses to therapies in a noninvasive, longitudinal fashion, particularly considering the increasing utilization of orthotopic and subrenal capsule grafting techniques in the development of PDX models and drug screening. Although small animal imaging techniques, such as computed tomography, magnetic resonance imaging, and positron emission tomography, allow detailed, real-time assessment of tumor 3D structure, angiogenesis, and metabolic activity [27], these approaches are often time-consuming and costly and are therefore not suitable for high-throughput implementation. Alternatively, high-frequency micro-ultrasound imaging may be used as a rapid, comparatively inexpensive tool for visualizing tumor anatomy and vascularization of PDXs. Recently, new technology has enabled the frequency of ultrasound systems, based on linear transducer arrays, to be extended from 15 to above 50 MHz, leading to significant improvements in depth of field, visualization of microvasculature, two- or three-dimensional sampling of anatomic data in high resolution, and ability to image contrast agents [28]. Bioluminescence imaging (BLI), a noninvasive imaging technology that uses a light-emitting enzyme (e.g., luciferase) to monitor biological processes of tumor cells in small animals, may also represent a cost-effective and relatively high-throughput preclinical imaging modality [29]. Studies have shown that exogenous proteins, including luciferase, can be efficiently expressed in patient-derived tumor cells of suspension and spheroid cultures. However, a wide usage of such a technique with PDX tumor models is still a challenge [30]. We can imagine that innovations in small animal imaging will enable a broad range of new applications, including longitudinal, high-throughput studies of the growth and metastasis of orthotopic and subrenal capsule PDXs that otherwise would be extremely difficult to perform.

Inter-institutional Collaboration, Data Sharing, and Quality Control

As mentioned above, individual PDX models may provide only a partial picture of the disease due to intra- and inter-tumoral heterogeneities. Preclinical evaluation of candidate drugs using a limited number of PDX models may therefore not accurately predict their clinical efficacy nor correctly guide selection of therapies for patients. In view of this, establishing an extensive collection of xenograft models of a particular cancer,

covering a range of its subtypes, will provide a valuable platform for exploring mechanisms underlying treatment resistance, predicting potential clinical trial outcomes in population-scale studies, and developing novel personalized therapeutic approaches for the disease (Chaps. 8, 9, 10, and 12). Considering the complexity and enormous workload, the establishment and maintenance of such a large-scale PDX collection are beyond the capability of individual laboratories or institutes. Last but not least, the high cost and amount of human resources associated with their use, compared to traditional cell line-based systems, form another major factor hampering the widespread use of PDX cancer models. Consequently, a number of academic and industrial institutions have joined forces to develop a collaborative network for sharing large-scale PDX collections, including the National Cancer Institute (NCI) repository of patient-derived models, the Public Repository of Xenografts (PRoXe), the Children's Oncology Group (COG) Cell Culture and Xenograft Repository, the Pediatric Preclinical Testing Consortium (PPTC), the European EurOPDX resource, and the Novartis Institutes for Biomedical Research PDX Encyclopedia (NIBR PDXE).

While each of the above academic and industrial groups has successfully developed PDX cancer models, there are significant differences in their working protocols and practices, such as the selection of host animals, grafting technique, nomenclature, annotation, and model characterization. For large-scale inter-institutional collaboration, standardization of methodological procedures is crucial for classification of the models, their distribution, data integration and interpretation, and experimental reproducibility. Meanwhile, thoroughly characterizing established models and sharing their genomic and transcriptomic data are essential. Furthermore, it is crucial to reach a consensus for the selection of drug efficacy endpoints and criteria used in the evaluation of in vivo responses to treatment. There is no doubt that the use of a quantitative assessment system to categorize xenografts' responses to treatment will provide a more accurate evaluation of drug efficacy in preclinical PDX trials. Recently, the modified clinical Response Evaluation Criteria in Solid Tumors (RECIST) has been used to classify treatment response in a population-based PDX trial [31]. Optimization of such modified RESCIST criteria will improve the data interpretation and provide more precise information for better guidance of patients' therapy selection and outcome prediction.

Acknowledgments We thank all past and current members of the Living Tumor Laboratory (www.livingtumorlab.com) for their valuable contributions and thoughtful discussions. This study was supported by Dr. Yuzhuo Wang's grants from the Canadian Institutes of Health Research, Terry Fox Research Institute, BC Cancer Foundation, Prostate Cancer Canada, and Princess Margaret Hold'em for Life.

References

1. Alsop K, Thorne H, Sandhu S, Hamilton A, Mintoff C, Christie E, et al. A community-based model of rapid autopsy in end-stage cancer patients. Nat Biotechnol. 2016;34(10):1010–4. doi:10.1038/nbt.3674.

2. Toivanen R, Berman DM, Wang H, Pedersen J, Frydenberg M, Meeker AK, et al. Brief report: a bioassay to identify primary human prostate cancer repopulating cells. Stem Cells. 2011;29(8):1310–4. doi:10.1002/stem.668.
3. Van Weerden WM, Romijn JC. Use of nude mouse xenograft models in prostate cancer research. Prostate. 2000;43(4):263–71. doi:10.1002/1097-0045(20000601)43:4<263::AID-PROS5>3.0.CO;2-I.
4. Whittle JR, Lewis MT, Lindeman GJ, Visvader JE. Patient-derived xenograft models of breast cancer and their predictive power. Breast Cancer Res. 2015;17:17. doi:10.1186/s13058-015-0523-1.
5. Wang Y, Revelo MP, Sudilovsky D, Cao M, Chen WG, Goetz L, et al. Development and characterization of efficient xenograft models for benign and malignant human prostate tissue. Prostate. 2005;64(2):149–59. doi:10.1002/pros.20225.
6. Lin D, Wyatt AW, Xue H, Wang Y, Dong X, Haegert A, et al. High fidelity patient-derived xenografts for accelerating prostate cancer discovery and drug development. Cancer Res. 2014;74(4):1272–83. doi:10.1158/0008-5472.CAN-13-2921-T.
7. Pinter G. Renal lymph: vital for the kidney and valuable for the physiologist. Physiology. 1988;3(5):189–93.
8. Bogden AE, Griffin W, Reich SD, Costanza ME, Cobb WR. Predictive testing with the subrenal capsule assay. Cancer Treat Rev. 1984;11 Suppl A:113–24.
9. Cunha GR. Epithelial-stromal interactions in development of the urogenital tract. Int Rev Cytol. 1976;47:137–94.
10. Cunha GR, Lung B, Kato K. Role of the epithelial-stromal interaction during the development and expression of ovary-independent vaginal hyperplasia. Dev Biol. 1977;56(1):52–67.
11. Griffin TW, Bogden AE, Reich SD, Antonelli D, Hunter RE, Ward A, et al. Initial clinical trials of the subrenal capsule assay as a predictor of tumor response to chemotherapy. Cancer. 1983;52(12):2185–92.
12. Tunstead JR, Thomas M, Hornsby PJ. Early events in the formation of a tissue structure from dispersed bovine adrenocortical cells following transplantation into scid mice. J Mol Med (Berl). 1999;77(9):666–76.
13. Lee CH, Xue H, Sutcliffe M, Gout PW, Huntsman DG, Miller DM, et al. Establishment of subrenal capsule xenografts of primary human ovarian tumors in SCID mice: potential models. Gynecol Oncol. 2005;96(1):48–55. doi:10.1016/j.ygyno.2004.09.025.
14. Priolo C, Agostini M, Vena N, Ligon AH, Fiorentino M, Shin E, et al. Establishment and genomic characterization of mouse xenografts of human primary prostate tumors. Am J Pathol. 2010;176(4):1901–13. doi:10.2353/ajpath.2010.090873.
15. Zhao H, Nolley R, Chen Z, Peehl DM. Tissue slice grafts: an in vivo model of human prostate androgen signaling. Am J Pathol. 2010;177(1):229–39. doi:10.2353/ajpath.2010.090821.
16. Bernardo C, Costa C, Sousa N, Amado F, Santos L. Patient-derived bladder cancer xenografts: a systematic review. Transl Res. 2015;166(4):324–31. doi:10.1016/j.trsl.2015.02.001.
17. DeRose YS, Wang G, Lin YC, Bernard PS, Buys SS, Ebbert MT, et al. Tumor grafts derived from women with breast cancer authentically reflect tumor pathology, growth, metastasis and disease outcomes. Nat Med. 2011;17(11):1514–20. doi:10.1038/nm.2454.
18. Lawrence MG, Taylor RA, Toivanen R, Pedersen J, Norden S, Pook DW, et al. A preclinical xenograft model of prostate cancer using human tumors. Nat Protoc. 2013;8(5):836–48. doi:10.1038/nprot.2013.043.
19. Montecinos VP, Godoy A, Hinklin J, Vethanayagam RR, Smith GJ. Primary xenografts of human prostate tissue as a model to study angiogenesis induced by reactive stroma. PLoS One. 2012;7(1):e29623. doi:10.1371/journal.pone.0029623.
20. Risbridger GP, Taylor RA, Clouston D, Sliwinski A, Thorne H, Hunter S, et al. Patient-derived xenografts reveal that intraductal carcinoma of the prostate is a prominent pathology in BRCA2 mutation carriers with prostate cancer and correlates with poor prognosis. Eur Urol. 2015;67(3):496–503. doi:10.1016/j.eururo.2014.08.007.

21. Toivanen R, Frydenberg M, Murphy D, Pedersen J, Ryan A, Pook D, et al. A preclinical xenograft model identifies castration-tolerant cancer-repopulating cells in localized prostate tumors. Sci Transl Med. 2013;5(187):187ra71. doi:10.1126/scitranslmed.3005688.
22. Shultz LD, Brehm MA, Garcia-Martinez JV, Greiner DL. Humanized mice for immune system investigation: progress, promise and challenges. Nat Rev Immunol. 2012;12(11):786–98. doi:10.1038/nri3311.
23. Cutz JC, Guan J, Bayani J, Yoshimoto M, Xue H, Sutcliffe M, et al. Establishment in severe combined immunodeficiency mice of subrenal capsule xenografts and transplantable tumor lines from a variety of primary human lung cancers: potential models for studying tumor progression-related changes. Clin Cancer Res. 2006;12(13):4043–54.
24. Delitto D, Pham K, Vlada AC, Sarosi GA, Thomas RM, Behrns KE, et al. Patient-derived xenograft models for pancreatic adenocarcinoma demonstrate retention of tumor morphology through incorporation of murine stromal elements. Am J Pathol. 2015;185(5):1297–303.
25. Junttila MR, de Sauvage FJ. Influence of tumour micro-environment heterogeneity on therapeutic response. Nature. 2013;501(7467):346–54. doi:10.1038/nature12626.
26. Edmondson R, Broglie JJ, Adcock AF, Yang L. Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. Assay Drug Dev Technol. 2014;12(4):207–18. doi:10.1089/adt.2014.573.
27. Van der Meel R, Gallagher WM, Oliveira S, O'Connor AE, Schiffelers RM, Byrne AT. Recent advances in molecular imaging biomarkers in cancer: application of bench to bedside technologies. Drug Discov Today. 2010;15(3–4):102–14. doi:10.1016/j.drudis.2009.12.003.
28. Foster FS, Hossack J, Adamson SL. Micro-ultrasound for preclinical imaging. Interface Focus. 2011;1(4):576–601. doi:10.1098/rsfs.2011.0037.
29. O'Neill K, Lyons SK, Gallagher WM, Curran KM, Byrne AT. Bioluminescent imaging: a critical tool in pre-clinical oncology research. J Pathol. 2010;220(3):317–27. doi:10.1002/path.2656.
30. Welm BE, Dijkgraaf GJ, Bledau AS, Welm AL, Werb Z. Lentiviral transduction of mammary stem cells for analysis of gene function during development and cancer. Cell Stem Cell. 2008;2(1):90–102. doi:10.1016/j.stem.2007.10.002.
31. Gao H, Korn JM, Ferretti S, Monahan JE, Wang Y, Singh M, et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. Nat Med. 2015;21(11):1318–25. doi:10.1038/nm.3954.

Index

A
Acidific microenvironment, 80
Activated B-cell (ABC)-tumors, 61
Acute myeloid leukemia (AML), 30
Acute promyelocytic leukemia (APL), 178
Allelic imbalance (AI) assay, 60
AngioSense, 103
AstraZeneca, 132
Avatar approach, 149–150, 164–165

B
B-cell receptor (BCR), 61
Biomarkers
  classification
    DNA biomarkers, 130
    protein biomarkers, 132–134
    RNA biomarkers, 130–132
  definition, 128
  discovery, 127–128
  precision oncology, 134–138
Blood/liquid biopsies, 17
Boyden chamber-type system, 99, 101
Breast cancer
  engraftment frequencies, 33
  genetic characteristics, 75
  oestrogen supplementation, 18
  orthotopic PDX models, 31

C
Cancer cell lines
  glioblastoma cell line, 52
  leukemia cell lines, 52
  mouse models, 46
  rat models, 51–54
  U87MG-FLuc, 52
Cancer genesis, 2
Cancer heterogeneity. *See* Heterogeneity
Cancer stem cells (CSCs)
  chemotherapy tolerance, 81–82
  isolation and characterization, 80–81
  murine PDX models, 81
  zebrafish PDX model, 82–83
Cancer-associated fibroblasts (CAF), 65, 78
Cancer-stromal interaction, 196–197
Cell sorting, 17
Cell-autonomous heterogeneity, 62–64
Cell-derived matrices (CDMs), 78
Cell-free tumor (ct)DNA, 130
Chronic myeloid leukemia (CML), 178
Circulating tumour cell (CTC), 16–17, 137
Clonal selection and evolution, 33
Co-clinical trials. *See* PDX co-clinical trials
Comparative genomic hybridization (CGH), 32
Consensus clustering classification (CCC), 61
CRISPR/Cas9 system, 51
Cultured cancer cells, 17

D
Diagnostic biomarker, 130
Diffuse large B-cell lymphoma (DLBCL), 61
DNA biomarkers, 130
DNA methylation, 75–76
Drug discovery, 37
  compounds identification, 144, 145
  lead optimization stage, 144–145
  mouse clinical trial, 145–146
  mouse models utility in, 141–142
  patient identification, 148
  PDX models, 143–144
  translatability
  tumor response to treatment, 147

© Springer International Publishing AG 2017

Y. Wang et al. (eds.), *Patient-Derived Xenograft Models of Human Cancer*,
Molecular and Translational Medicine, DOI 10.1007/978-3-319-55825-7

Drug resistance, 34–36
  mass enrollment, 118
  methods, 119–121
  motivation for development, 121–122
  optimal dose determination, 117
  pro-survival genes, 122, 123
  rolling enrollment, 118
  target pathway, 122, 123
  therapeutic doses, 116–117, 122
  therapy-induced event, 122, 123
  tumor volume, 118–119, 122

E
Embryonic stem cells (ESCs), 50
Engraftment rate, 194–195
Epigenetics
  heterogeneity, 74–75
  plasticity, 75–76
Epithelial-derived cancers, 95–96
European Society for Medical Oncology (ESMO), 178–179
Extracellular matrix (ECM), 65
  cell migration, 97
  plasticity, 77–78

F
Fidelity
  drugs response and resistance, 34–36
  histopathology, 31–32
  immune microenvironment, 36
  molecular features, 32–33
  stroma/microenvironment, 33
  tumor heterogeneity, 33–34
First-generation xenografts, 13–14
  advantages, 158–163
  cancer immunology, 160–161
  cell lines and transplantable PDX models, 161
  immunohistochemistry staining, 160
  methodology, 156–157
  precision medicine, 164–166
  preclinical drug screening, 167–168
  predictive biomarkers development, 167–168
  translational cancer research, 168–169
  tumor progression, 163–164
Focal adhesion kinase (FAK), 77

G
Gastric cancer, 64
GATA2, 132
Gemcitabine, 132
Genetics
  heterogeneity, 74–75
  plasticity, 75
Gleason sum score, 94
Glioblastoma cell line, 52
Green fluorescent protein (GFP), 46

H
Head and neck squamous cell carcinomas (HNSCC), 62
Hematopoietic stem cells (HSCs), 151
Hepatocellular carcinoma (HCC), 61
Hera BioLabs, 51
Heterogeneity
  cell-autonomous, 62–64
  genetic and epigenetics, 74–75
  genomics and proteomics, 60–62
  improvement, 195
  non cell-autonomous, 65–66
Heterotopic implantation, 31
Histone demethylase genes, 80
Host immunity, 4
Humanized-xenograft models, 4–5, 150–152
  improvement, 196
  mouse models
    cancer cell lines, 46
    host–patient tumor interactions, 49–50
    immunodeficient mouse strains, 45–46
    PDX, 47–49
  rat models
    cancer cell line, 51–54
    genetic modification, 50–51
    nude rat, 50
Hypoxia-inducible factors (HIFs), 79
Hypoxic microenvironment, 79–80

I
IGF1R inhibitors, 148, 149
Imatinib (Glivec), 178
Immune cells, plasticity, 79
Immune-deficient mice, 5–6
Immunodeficient mouse strains
  allograft rejection, 79
  host–patient tumor interactions, 49
  human cancer, 45–46
Intracardiac injection, 105–107
Intrafemoral injection, 108
Intravital microscopy, 103–105

L
Leukemia cell lines, 52
Localised tumour tissue, 15
Luciferase, 46
Lymphomas, 20

M
Matrigel, 18, 78
Metastases, 15–16
  cell migration and invasion, 96–97, 99–100
  cPDXs, 97–99
  direct injection, 107–109
  host cell interactions, 100–101
  intracardiac injection, 105–107
  intravital microscopy, 103–105
  tumor cell intravasation, 101–102
Microenvironment
  enrichment of, 17–18
  fidelity, 33
MMPSense, 103
Murphy's hypothesis, 3

N
NOD scid gamma mouse, 45
Non cell-autonomous heterogeneity, 65–66
Non-malignant tissues, 14–15
Non-obese diabetic (NOD) strain, 5–6, 18, 19
Nonprotein-coding RNA (ncRNA), 131
NSG mice, 79
NSG mouse strain, 45
Nude mouse, 45
Nude rat, 50

O
Oncology drug discovery, 37
Orthotopic graft, 20
OsteoSense 680 EX, 103

P
Patient-derived xenografts (PDX)
  advantages, 179–180
  cancer-stromal interaction, 196–197
  characteristics, 157–158
  data sharing, 197–198
  definition, 128
  development, 128–129
  engraftment rate and efficiency, 194–195
  graft preparation, 12, 17–18
  graft validation/authentication, 12, 20–21
  grafting site, 12, 19–20
  history, 2–5
  host impacts, 18–20
  humanized PDX models, 196
  inter-institutional collaboration, 197–198
  limitations, 129
  post-implantation, 197
  primary *vs.* serially transplantable PDX, 13–14
  quality control, 197–198
  recapitulating tumor heterogeneity, 195
  sample collection, 12, 14–17
  strains, 12, 18–19
  tissue sources for, 14–17
PDX Authentication System (PAS), 21
PDX co-clinical trials
  challenges, 189
  clinical site, 182, 183
  data interpretation, 182, 183
  derived not from enrolled patients, 181
  from enrolled patients, 181
  infrastructure and workflow, 182–183, 188
  preclinical site, 182, 183
  prolonged treatment, 182, 183
Peripheral blood mononuclear cells (PBMCs), 151
Personalized/precision cancer medicine
  challenges, 189
  early development, 178–179
  largescale clinical settings, 178
  PDX-assisted, 184–187
  tumor evolution, 178–179
Peyrilhe's experiment, 2
Phosphoinositide 3-kinase (PI3K), 77
Plasticity
  cell-autonomous
    epigenetics, 75–76
    genetics, 75
  cell-extrinsic factors
    acidific microenvironment, 80
    cancer stem cell niche, 76–77
    extracellular matrix, 77–78
    hypoxic microenvironment, 79–80
    immune cells, 79
    tumor architecture, 77–78
    tumor stroma, 78
Precision medicine, 164–166. *See also* Personalized/precision cancer medicine
Predictive biomarker, 130
Primary cell culture, 17
Primary PDX, 13–14. *See also* First-generation xenografts
Prognostic biomarkers, 130
Prostate cancer, 95–96
  engraftment frequencies, 33
Protein biomarkers, 132–134

R
Rag1/Rag2 mouse strain, 45
Rapid autopsy, 16
RNA biomarkers, 130–132

S
Serially transplantable PDX, 13–14
Severe combined immunodeficiency (SCID) mice, 5, 30
Short tandem repeat (STR) analysis, 20
Single nucleotide polymorphisms (SNPs), 21
Solid tumor PDX models, 30
Sorted/cultured cancer cells, 17
Spermatogonial stem cells (SSCs), 51
Spontaneous tumor formation, 2, 3
Stability, of PDX models, 36–37
Stem cell niche, 76, 77
Steroids, 19
Stroma
 fidelity, 33
 plasticity, 78
Subcutaneous grafting, 19
Subrenal capsular implantation, 31
Subrenal capsule (SRC) graft site, 194
Subrenal grafting, 19

T
TALENs, 51
The Cancer Genome Atlas (TCGA), 142
Tissue graft, 14–17
TMPRSS2-ERG, 75
Transplant human cancers, 3
Transposagen, 51
Triple negative breast cancers (TNBCs), 63
Tumor cell intravasation, 101–102
Tumor heterogeneity, 33–34. *See also* Heterogeneity
Tumor microenvironment
 acidific, 80
 hypoxic, 79–80
Tumor stroma, 33, 78

U
U87MG-FLuc, 52

V
Vancouver Prostate Centre model, 135

X
Xenotransplantation. *See* Patient-derived xenografts (PDX)