Human Cancer Modeling: Recapitulating Tumor Heterogeneity Towards Personalized Medicine

Mossa Gardaneh [1,*]

[1] *Department of Stem Cells and Regenerative Medicine, Faculty of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran*

* *Corresponding author: Mossa Gardaneh, Department of Stem Cells and Regenerative Medicine, Faculty of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran. E-mail: Mossabenis65@gmail.com*

Abstract

Despite diagnostic, preventive and therapeutic advances, the growing incidence of cancer and the high rate of mortality among patients affected by specific cancer types indicate that current clinical measures are not ideally useful in eradicating cancer. Chemoresistance and subsequent disease relapse are believed to be mainly driven by the cell-molecular heterogeneity of human tumors, which necessitate personalized approaches to deal with uniquely complex genetic profile of each patient's tumor. Such personalized medicinal therapies require dissection of cancer molecular profiles in order to profoundly understand the mechanisms underlying drug resistance and disease recurrence. Technological advances in comparative genome sequencing have begun to result in identification of common somatic mutations in specific cancer subtypes that potentially constitute bases for prognostic and diagnostic biomarkers and present novel therapeutic targets. These targets have to be tested in reliable platforms, so that data of drug responses obtained can be correlated with those responses elicited in origin by the parental tumor itself. Here, I reviewed different models of cancer in vitro and in vivo and outlined the utilization of these models in drug discovery and novel therapies of cancer with prospect for developing personalized anti-cancer strategies.

INTRODUCTION

Tumor heterogeneity implies that different tumor cells can carry distinct profiles of cellular morphology, gene expression, metabolism, tumor cell motility, proliferation and metastatic potentials [1]. Both inter-tumor and intra-tumor heterogeneity exist. Most human cancers carry intrinsic heterogeneity which is manifested in cancer histology, genomic aberrations and gene expression profile. As a result, each tumor responds to therapies in a unique manner that ultimately determines its clinical outcome. Hence, despite tremendous improvements in patient survival rates achieved in recent years, resistance to treatments drives disease recurrence in many patients and conceivably requires novel treatments to be explored [2]. Heterogeneity is a major problem in applying the concept of personalized medicine to design of effective diagnostic tests, identification of drug resistance mechanisms, discovery of targeted drugs and exploration of novel therapeutic strategies. In many types of cancer, heterogeneity cannot be defined by relying solely on classical histological characteristics of tumors or altered profiles of cancer cell receptors. This means that new platforms have to be created to genuinely recapitulate each human tumor and its microenvironment.

THE ORIGINS OF CANCER HETEROGENEITY

Many cancer types are classified into various subtypes. Differences between these subtypes may be due to their different parental cells of origin, exclusive differentiation blockades, and unique stockpile of mutations [3]. Successful implementation of personalized therapeutic approaches requires detailed identification of these intrinsic differences at cell-molecular levels; however, the traditional histopathological markers used in the clinic are not always useful, given that genetic mutations leading to heterogeneity occur in both inter- and intra-subtype levels [4]. Furthermore, different subtypes may have different rates and forms of mutations, suggesting that detection of new differences in mutation profiles of tumors may introduce new sub-classifications and widen the heterogeneity

[5, 6]. For the reasons outlined above, cell signaling pathways as well as cell–cell and cell–matrix communications within the tumor microenvironment have to be examined following data integration from genomic, transcriptomic and proteomic analyses [7]. Using microarray-based cancer classification that comprises multiple discriminatory molecular markers, Yang and Naiman introduced a statistical approach for classifying multiple disease states in leukemia and obtained improved results compared to benchmark classifiers [8]. Such an approach can also integrate pathway analysis of gene expression to provide accurate and comprehensive classification of cancer. The heterogeneous nature of various tumors indicates that reliable platforms are needed to allow comprehensive analyses for thorough understanding of human tumors before stepping into targeted drug modeling and personalized therapy. Therefore, development of animal models that can recapitulate many aspects of human tumors is required and below, I will discuss various types of these models that are applied to different cancer types to meet the increasing demand for personalized modeling of cancer.

IN VITRO MODELS OF HUMAN CANCER

Cancer cell lines have been derived from either high-grade, high-stage cancers or normal lines immortalized by genetic modifications [9, 10]. They have become an indispensable tool in studying cancer biology and screening for cancer drugs. This is due to their attractiveness, as they are inexpensive, immortalized, easily perpetuated, mostly homogeneous, and genetically manipulatable. Cancer cell lines carry many intrinsic characteristics of cancer and share many genetic profiles and genomic modifications with primary human tumors [11, 12]. Cancer cell lines are attractive tools in drug discovery and screening because of their homogenous nature, high rate of proliferation and easy adaptation to cell culture. Notably, the anticancer drug screening program of the National Cancer Institute (NCI) in 1980s was aimed at identifying and prioritizing compounds with selective anticancer activities by screening a panel of 60 cancer cell lines [13]. So far, over 100,000 compounds have been screened by this method, which has led to development of many important anti-cancer drugs including anti-HER2 trastuzumab, anti- tubulin Taxol, anti-angiogenic bevacizumab and anti-proteasome bortezomib [14, 15]. Despite their useful applications in drug screening, cancer cell lines are too simplified to model the heterogeneous nature of human cancers, let alone their ability to reconstitute genuine tumor microenvironment in culture dishes. Once cultured, cancer cell lines undergo genetic transformations that are not restored when they are returned to grow in vivo [16].

Differences in genomic profiles and gene expression patterns can also be envisaged in different isolates of a same single cell line [17]. Culturing also generates homogenous batch of cancer cells by selecting the adapted cells, but eliminating tumor-resident non-cancer cells and cell-interacting proteins [18]. Furthermore in culture, cell lines lack components of cancer stroma that include blood and lymphatic vessels, associated immune cells and fibroblasts, and do not grow in the presence of a complex extracellular matrix [19]. Therefore, cell line-based data often do not match with those obtained from clinical studies [20], a discrepancy that is reflected in different outcomes of transcriptome studies in clinical samples compared to that of established cancer cell lines [21] and is blamed for failure in developing new drugs [22]. It is inferred from these reports that more complex in vivo systems are required to accurately model cancer for its critical steps from tumor formation to progression and metastasis.

IN VIVO MODELS OF HUMAN TUMORS

Animal models of cancer provide a more reliable platform to investigate basic, translational as well as clinical aspects of cancer biology [23]. Faithful reproduction of cellular and molecular pathologies of cancer is a prerequisite to accurately recapitulate the disease, and animal models are supposed to preserve genotypic and phenotypic characteristics of cancer. However, there will be some compromise when using mice as model animals since they cannot perfectly reflect certain features of human cancer [24]. One reason for this is that within the microenvironment, there also exist multiple non-malignant cell types as well as the extracellular matrix to maintain the tumor [23]. The interactions between malignant and non-malignant cell types determine the tumor fate and identity and so are determinants of novel anti-cancer therapies [23]. Substitution of each component of this complex structure with animal counterpart or changes in sites of tumor growth may alter the tumor microenvironment and influence the stromal and vascular interactions. In fact, upon implantation of human cancer cells into an immunodeficient mouse host, the xenograft will inevitably grow beside murine stromal and endothelial cells [24]. In addition, suppression of animal's immune system to disallow rejection of human implants compromises normal immune responses and function of immune cells in the tumor microenvironment. Various approaches have been applied to develop animal models of cancer and as a result, several models exist (Table 1) [25]. Figure 1 shows both the resources of human tumor modeling and various forms of produced models. To generate each model, a choice of immune-compromised mice exists, as outlined in Table 2 [26].
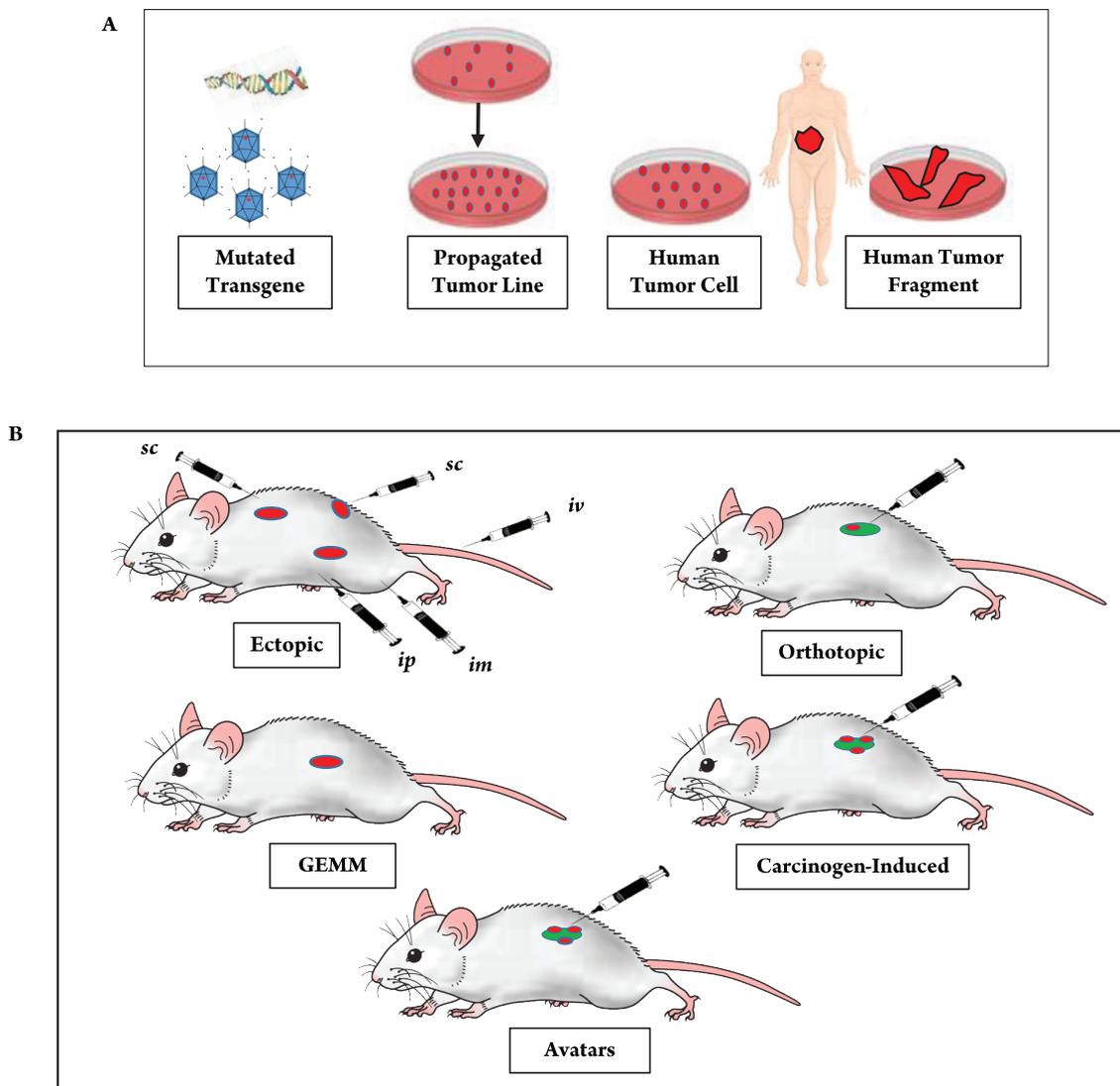

Figure 1: Human Cancer Modeling. (A) Sources of modeling that include mutated transgene as in non-viral or viral vectors, established tumor cell line propagated in culture, tumor fragments or tumor cells directly isolated from human tumor. (B) Various tumor models: ectopic xenografts formed by different injection routes, genetically engineered mouse model (GEMM), orthotopic models, carcinogen-induced models and Avatars. Green pads indicate the right tissue and red ones represent tumor models. im, intramuscular; ip, intraperitoneal; iv, intravenous, sc, subcutaneous. See text and Table 1 for more information.

GENETICALLY ENGINEERED MOUSE MODELS

Gene-targeting technologies combined with transgenesis have provided unique opportunities for closely recapitulating human tumors by producing genetically engineered mouse models (GEMMs) [33]. Mice can be genetically modified by microinjection of DNA in the pronuclei of fertilized zygotes whereby the transgene is integrated into the genome [34]. If the aim of this DNA manipulation is to overexpress an oncogene or delete a tumor-suppressor gene; the host animals will provide suitable models of cancer. Several GEMMs have been developed for sporadic cancers with a high impact on oncology, drug discovery and preclinical translational biology [35]. By introducing specific mutations known to be linked to human tumors and induction of cancer-initiating mutations in a spatiotemporal manner, generation of GEMMs can be designed specifically for studying tumorigenesis and cancer behavior [33]. Another advantage of GEMMs to transplantation models is that the tumor grows as the host animal grows without need for immune compromising. Therefore, the host remains immune-competent and preserves the complex interactions that occur between tumor cells and the immune system [19]. Moreover, developmental stages of GEMMs occur spontaneously without further induction or manipulation and so mimic those of human tumors more closely than other models do [35]. Furthermore, GEMMs continue to grow in number and so allow for studying the effect of combinations

of mutations on tumorigenesis. This makes it possible to dissect complex molecular events that happen in the course of tumor formation and progression.

Despite their unique potential to be applied for translational research and drug development [35], GEMMs suffer from few pitfalls in modeling human tumors. One major disadvantage is the different genetics and physiology between tumors developed in human and those produced in mouse [15]. Secondly, only few tumor-associated genes can be manipulated by reverse genetics as the base of GEMMs production and the level and pattern of gene expression cannot be controlled precisely with random integration, leading to unexpected phenotypes [35]. Thirdly, the rapid evolution of GEMMs solely by genetic manipulation of tumor-associated oncogenes or tumor-suppressor genes does not necessarily recapitulate the slow pace of tumor formation as it naturally takes decades to complete in humans, even though the same genes are altered in both [36].

PATIENT-DERIVED XENOGRAFT MODELS

Patient-derived xenograft models (PDXs) have gained credit as translation tools for bridging cell-molecular biology studies of human tumors with clinical studies, by mimicking their key characteristics important for drug discovery and novel therapies [37]. Tumor xenografts are advantageous for using advanced or metastatic cancer as the starting material. In these stages, tumor cells presumably represent real human tumors replete with genetic complexity and intra-tumor heterogeneity. Syngeneic models of cancer made of murine cancer cells implanted in mice can be especially useful because their host's immune system remains intact to interact with the molecules under investigation and allow the activity of candidate drugs to be more accurately evaluated [38]. Xenografts are also advantageous to cancer cell lines in propagating through successive generations in animal host in the absence of high selection pressure, recapitulating gene expression patterns specific to primary tumors, exhibiting stable patterns of protein expression, and bearing relatively stable genomes over time [39].

GENERATION OF XENOGRAFT MODELS

To produce xenograft models, tumors derived from primary surgical resection are cut into smaller pieces and injected into mice either directly or upon enzymatic digestion to produce cell suspensions. Expansion and early passage preparation of frozen stocks from tumor cell suspension is a critical step that would allow inoculation of mice in any given cohort with the same number of tumor cells [40]. Recent technical progresses in xenograft formation improved the rate of success. Such progresses in breast cancer (BC), for instance, include not only injection into the orthotopic site, but also supplementation of estrogen, the use of more highly immune-suppressed mice, and alteration of the microen-

vironment through addition of mesenchymal stem cells and/or Matrigel [41]. The orthotopic transplantation into the inguinal mammary fat pad is the best procedural option of all, as it more faithfully recapitulates the breast tumor stromal microenvironment [42], whereas a combination of approaches described in the literature are needed to model colorectal cancer [43].

The content of the stroma includes the vasculature, adipocytes, tumor-associated macrophages and other immune cells, as well as cancer-associated fibroblasts that affect tumor cell behavior by supplying growth factors/cytokines [23]. These interactions are important determinants of the local microenvironment in promoting tumorigenesis. In this case, some murine growth factors and cytokines do not interact with their human counterpart receptors, orthotopic tumors vascularize more significantly than do subcutaneous tumors, and implantation to inguinal rather than thoracic fat pads show improved engraftment rates [44]. Humanization of tumor microenvironment to support tumor engraftment and growth [45] and its manipulation to narrow tumor-microenvironment gap [46] that recently produced Xactmice [47] are two interesting areas that add to the improved modeling of human tumors in mice.

APPLICATIONS OF ANIMAL MODELS FOR HUMAN CANCER STUDIES

Duplication of Molecular Heterogeneity

The increasing use of genome-wide analyses over the recent years has unraveled the molecular heterogeneity of cancer and its impact on patient prognosis [48]. These developments provide compelling evidence for poor translation of drug responses from cell line-based in vitro and in vivo models. As a result, models that more faithfully reflect the clinical diversity of human cancer are at high demand.

PDX for Tissue-cell-molecular Recapitulation

There are no comprehensive reports available to compare patient tumor with xenograft. Despite this, PDX models presumably reflect the genuine entity of the parental tumor by maintaining its histology, gene expression patterns and genome profile [49]. The models preserve histological characteristics of the parental patient tumors, mutation profiles, as well as the response patterns to targeted therapies [50]. Moreover, in some PDX models, critical post-therapeutic tumor characteristics such as residual disease and tumor relapse can be observed.

Preservation of Genome/genetic Compositions

Whole-genome analysis of a patient's peripheral blood, primary breast tumor and brain metastasis with PDX model of the tumor demonstrated preservation of ma-

jority of mutations and genomic variations between the tumor, metastasis and the model [51]. More solid evidence for retaining histological and molecular stability between the tumor and its PDX model was shown by DeRose et al. who analyzed 12 primary serially-transplanted tumor grafts derived from different BC patients with different profiles for several clinical markers and demonstrated high similarity of PDX to the original patient tumors even in fine molecular aspects such as gene clustering [45]. The only exception was that model developed both lymphomas and adenocarcinomas upon transplantation, conceivably exhibiting its heterogeneous composition.

Orthotopic Transplantation for Genuine Microenvironment

Cancer physiology is best mimicked by orthotopic injection that engrafts the tumor directly into the relevant animal organ. However, in large cohorts of animals, this might face challenges. Alternative methods such as subcutaneous and renal capsule transplantation as non-native sites of tumor growth or metastasis are applied to provide easy access to tissue, but their relevance to cancer modeling is questionable as they may not provide natural microenvironments [52]. In modeling colorectal cancer, for example, orthotopic transplantation of the human colon tumor graft line, COL-2-JCK, as intact tissue into the caecum resulted in 100% take rate, with the xenograft displaying extensive local tumor growth and a high incidence of metastases to the regional lymph nodes, peritoneum, liver, and lung [53]. No metastasis occurred, however, when a cell suspension of this same tumor graft line was applied. Therefore, models such as PDX produce maximum molecular closeness to the original tumor, provided they are produced by orthotopic transplantation and are, therefore, invaluable platforms to study details of each cancer type, develop specific and targeting drugs, and adopt the most effective personalized therapy to cure it.

PREDICTION OF ANTI-TUMOR RESPONSE

To what extent can xenograft results and clinical trial data be relevant? How much power do xenograft models have to predict activity in clinical setting? A number of studies have been undertaken to address these questions, but ended up with discrepancies in results. In one study carried out on various cancer types, only non-small cell lung cancer (NSCLC) xenografts were predictive of clinical activity in the same histology and correlations in other cancer types were poor [20]. In another study, parallel test of 31 cytotoxic drugs on xenograft models and phase II clinical trial showed that the xenograft was able to predict NSCLC and ovarian cancer but not breast or colon cancer [54]. Another study demonstrated that PDXs were superior to cell line-based models in that they correctly predicted treatment response

in 90% of tumor samples and drug resistance in 97% [55]. The subrenal capsule (SRC) implantation technique has been developed, which optimizes combined personalized chemotherapy for individual patients [27]. SRC implantation maintains the tumor integrity within a fragment for a limited period of time in a well-vascularized location, so tumor responses can be assessed within short windows. Short periods below tissue rejection time allow use of normal immunocompetent mice, which have claimed very good associations with clinical outcome. Longer periods of time using nude mice, on the other hand, allow the tissue microenvironment with the tumor fragments to be maintained at this well vascularized site, as well as a sufficient time frame to assess tumor response. Such an approach applied for non-small cell lung cancer models resulted in the retention of morphological characteristics of the tumor with > 90% successful take rate for implantation [56].

DEFINITION OF TARGETED THERAPEUTIC STRATEGIES

An attractive approach in personalized modeling is to select for xenografts that naturally encompass certain molecular abnormalities. Although such targeted abnormalities can be created by engineering GEMMs, using xenografts for molecular targeting while properly simulating tumor heterogeneity would be more appropriate [57]. Such selected xenografts can be compared with the background of models lacking these specific abnormalities. Work on such platforms is expandable by manipulating expression of specific gene candidates and examining the effects of targeted inhibitors. Such studies have been applied to BC where overexpression of estrogen receptor has occurred in 70% of the cases, used for prediction of response. Therapy with anti-estrogen agents or aromatase inhibitors ultimately ends up with endocrine resistance and disease relapse. Xenograft models have shown their utility in analyzing profiles of gene expression regulated under estrogen influence and the resulting data established molecular correlations between in vitro and in vivo settings [58]. Xenografts remove barriers of analyzing in details the impact of a drug on gene expression time courses, as such barriers are faced when dealing with human patients. Dynamic effect of tamoxifen on gene expression was shown in a xenograft model of ER + BC patient [59]. Application of xenografts has further shown that silencing of estrogen signaling upon endocrine modulation can activate HER2 downstream pathways [54]. Similar studies are growing in number to produce more exciting data, but the overall trend is in favor of utilizing xenografts for targeted molecular analyses and adapting such models to replace GEMMs in many specific applications. Combined inhibitor studies such as using HER2 + BC models have been instrumental in unraveling expression shuffling between various receptor tyro-

sine kinases [60, 61] and successful in xenograft models of non-small cell lung cancer [62] and in phase II clinical trial of BC [63].

DRUG DEVELOPMENT AND TESTING

With the accelerating pace of progress in molecular modeling and computational simulation of drug-target interactions, tumor models can now be extensively analyzed for their molecular profile including gene expression, gene copy numbers, single nucleotide polymorphisms, mutation profiles and chemosensitivity to routinely-applied and novel compounds [64]. In search of effective combination drugs and to explore avenues to overcome drug resistance, a set of desired models can then be selected to test new drugs and identify the underlying molecular characteristics of sensitive and resistant tumor subpopulations. As outlined above, PDXs may provide the most faithful representation of human tumors in vivo. But are they the best in predicting drug efficacy too? Studies have demonstrated the value of PDXs as preclinical models for drug evaluation. In non-small cell lung cancer, PDXs have demonstrated their capacity in replicating clinical response to cisplatin but not to mitomycin C (MMC) when formed in orthotopic sites, whereas created opposite effects when grown in subcutaneous tissues [65]. Similarly in colorectal cancer, application of 5-fluorouracil (5-FU) and MMC induced clinically more relevant responses in orthotopic xenografts versus in subcutaneous models [66]. Xenograft models of childhood cancers can accurately identify active agents and effective drug combinations [58]. A Preclinical Testing Program (PPTP) supported by the National Cancer Institute was set up to identify new anticancer agents that have the potential for significant activity when clinically applied against selected childhood cancers [67]. A panel of over 80 xenograft lines derived from a range of different human tumors that preserved the parental genomic and transcriptomic patterns [68, 69] were treated with two anti-cancer compounds, vincristine and cyclophosphamide [67]. Both drugs displayed their broad-range activity and reproduced their activity against specific childhood cancers.

Co-clinical trials are based on simultaneous examination of xenografts in the laboratory and treatment of patients in the clinic. They provide the opportunity for personalizing therapies for the patient by allowing for real-time integration of murine and human tumor data. For example, treatment of xenografts with anti-cancer agents and xenograft responses have helped to identify effective treatment regimens for patients [37]. A response rate of 88% was produced by the model and tested in the patients, which is significantly greater than the 10% expected with phase I agents [37, 70]. Combination models may be inevitably needed to study various aspects of each individual tumor using a uniquely specific set of models. The combined application of multiple models can be more beneficial in developing new agents, as shown in developing anti-HER2 humanized antibody trastuzumab [71]. Co-clinical trials in which clinical and xenograft analyses are carried out in parallel form is another combined approach to explore correlation of response, resistance mechanism, potential biomarkers and novel combined treatments [37].

CONCLUSIONS

Faithful reproduction of human tumors reflecting their heterogeneous entity in host animals is the prime goal of cancer modeling. Then there comes the potential utility of the model for studying stages of cancer development, drug exploration and resolution of chemoresistance for better cure. The intra-tumoral heterogeneity that even results in expansion of each tumor subtype classification states that the idea of "one mouse, one patient paradigm" [50] may need to be inevitably materialized. PDXs and the avatar models have tremendous potentials in guiding therapy and quick assessment of safety and efficacy of new drugs and drug combinations, especially in those patients with deteriorating situation and so ineligible for clinical trial. When combined with cell-molecular approaches, xenograft models predict the outcome of the tumor more robustly and accurately. PDXs as well as GEMMs can form one arm of co-clinical trials that is sought to analyze human tumors more comprehensively than single trials in evaluating drug response. This is why mouse hospitals are being established in various institutions [72]. The models also are increasingly finding their ways to molecular profiling of each tumor at genomic (or even exomic), transcriptomic and proteomic levels for personalized solutions. PDXomics as a bioinformatics filtering tool is being employed to eliminate misreads caused by contaminated mouse xenografts. Computational, mathematical and predictive models of cancer are being vigorously developed to simulate tumor heterogeneity and meet demands for pharmacokinetic analyses in drug discovery [73-76]. The ultimate integration of all these approaches will be critical for forthcoming design of patient-specific cancer therapy in personalized settings.

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CONFLICTS OF INTEREST

The author declared that there was no conflict of interests.

ETHICS APPROVAL

Not Applicable.

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