Application of patient-derived tumor models in anticancer drug development and individualized medicine

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Kai-Ling Chen, Yu-Fei Deng write and edit the manuscript; Xiao-Ying Hou, Yu-Chen Liu design and revise the manuscript. All four authors have thoroughly read, reviewed, and approved the final version of the manuscript.

Competing interests
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Abbreviations
PDO, patient-derived organoid; PDX, patient-derived xenograft; PDO, patient-derived organoid; PDC, patient-derived cell; HLA, human leukocyte antigen; PBMC, peripheral blood mononuclear cells; HSCs, hematopoietic stem cells; SCLC, small cell lung cancer; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; NSCLC, non-small cell lung cancer; PD-1, programmed death 1; CRC, colorectal cancer; OPCs, oligomeric proanthocyanidins; PLC, primary liver cancer; HCC, hepatocellular carcinoma; HCS, combined hepatocellular; ICC, intrahepatic cholangiocarcinoma; NMIBC, non-muscle invasive bladder cancer; RCC, renal cell cancer; ESCC, esophageal squamous cell carcinoma; NPC, nasopharyngeal carcinoma.

Abstract
Malignant tumor is the second leading cause of death due to its high incidence, lack of effective treatment and poor prognosis. The evaluation of anticancer drugs used to be based on NCI-60 cell line models, but the limited heterogeneity and the divorce from clinical practice of models lead to extremely low success rate of novel anticancer drugs during clinical trials (less than 10%). In recent years, because of the high heterogeneity and human derived tumor matrix, patient-derived tumor models have been gradually applied to the preclinical evaluation of various antitumor drugs, which shows certain advantages in predicting the clinical efficacy of antitumor drugs. Optimize the drug combination through patient-derived tumor models to achieve individualized medicine has gradually become an indispensable strategy in clinical cancer therapy. The current review summarized the development of patient-derived tumor models, characterized the application, advantages and challenges of them in preclinical antitumor drug evaluation and clinical precise medicine, which will provide a scientific basis and novel insights for further basic research, drug development and clinical application.

Keywords: patient-derived xenograft (PDX), patient-derived organoid (PDO), patient-derived cell (PDC), individualized medicine

Citation

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Introduction

With about 19.3 million new diagnosis and 10 million deaths worldwide, cancer will become the main cause of death in the 21st century [1]. For decades, pharmaceutical companies have been committed to develop novel effective anticancer drugs, which requires huge investment in terms of money and time [2]. However, the clinical trial success rates for new anticancer drugs is less than 10%, which is far from meeting the clinical needs [3]. According to ClinicalTrials.gov [https://clinicaltrials.gov/], the limited efficacy in human patients contributes to more than 60% of failures in novel antitumor drugs evaluation [4]. The huge "gap" between the preclinical testing models and the highly heterogeneous tumor tissues in patients restrains the "reproduction" of efficacy in clinical trials for most newly developed drugs. Therefore, it is essential to use a precise and efficient preclinical drug evaluation model that simulate clinical condition.

The traditional cell lines and xenograft models are convenient to operate. However, they are known to be significantly different from real cancer samples, thus have little predictive value [5, 6]. Therefore, patient-derived tumor models, which is more analogous to real cancerous tissue, is gradually applied to the evaluation of antitumor drugs [6]. Patient-derived tumor models mainly include patient-derived xenograft (PDX), patient-derived organoid (PDO) and patient-derived cell (PDC). The application of these models in preclinical research is of great significance for the development of novel anticancer therapies.

Patient-derived tumor models

Preclinical evaluation is an important part of antitumor drug development. In the 1970s, the US National Cancer Institute began to use human derived tumor cells for drug screen. The NCI-60 are sixty immortalized human cancer cell lines from patients. It is simple to screen anticancer drugs in cell lines, and the testing results are highly repeatable. Thus, NCI-60 was widely applied for the screening and evaluation of drugs in vivo and in vitro. During the long-term cell culture, however, irreversible and significant changes of cell genotype, biological behavior as well as the loss of specific cell populations were observed [5, 7]. In addition, it is difficult to simulate the heterogeneity of real tumor tissues when cancer cell line is used alone [1]. These might be the main explanations that most antitumor drug clinical trial are terminated in the phase II because of limited efficacy, although extremely large effort has been invested on preclinical verification and drug optimization [8].

One very important recent finding is patient-derived tumor models. As a predictable and effective preclinical drug evaluation system, patient-derived tumor models are now widely accepted. And the three patient-derived tumor models (PDX, PDO and PDC) have been greatly developed in the past five years.

Patient-derived xenograft (PDX):

Although cancer cell lines and derived xenograft tumors have been widely used in the past decades, scientific reports indicate that they differ significantly from the actual tumors. After being isolated from human body and cultured into immortalized cell lines, the biological characteristics such as growth, metastasis and natural heterogeneity of tumors gradually disappear. Whereas PDX uses a piece of cancerous tissue removed from surgery, and then directly inoculated into mice with severe immunodeficiency or humanized immune system. PDX model efficiently preserves the genotype and microenvironment of the parental tumors, and its function as a valid oncology research platform is significantly superior [9]. PDX model can be created either by subcutaneous inoculation which facilitates subsequent accurate monitoring, or by renal capsule transplantation which increases the success rate of transplantation.

Since the creation of PDX model usually takes 4-8 months, researchers established alternative models such as mini PDX model. In the development of mini PDX model, tumor tissues are digested into cell suspension, and wrapped in tiny capsules before inoculated subcutaneously into experimental animals, which significantly accelerate the procedure of drug screen [10]. In addition, immune system humanized models is established to expand the drug evaluation spectrum of PDX [11]. Specifically, mice are first injected with human peripheral blood mononuclear cells (PBMC) or hematopoietic stem cells (HSCs) into immunodeficient mice to simulate human immune system. Then tumor tissue is inoculated as described before. PDX model created this way is excellent evaluating immunotherapy drugs.

PDX model reacts to chemotherapy, immunotherapy and targeted therapy highly similar as parental tumor. Thus, PDX based evaluation of novel antitumor drugs show higher pass rate in clinical trial. And it is suitable for evaluating and predicting clinical outcome of anticancer therapy on actual patients [12]. Studies showed that PDX could guarantee 80% of effective rate even for patients who developed drug resistance after the first round of treatment.

PDX model has several disadvantages such as high cost since mice needs maintenance and only the first three generations of PDX model can well maintain the oncological characteristics of parent tumor. And the development period of PDX can be so long, that patients might not have the luxury of time to wait. Collectively, the extensive application of PDX model needs further investigation and improvement.

Patient-derived organoid (PDO):

Two-dimensional (2D) cultured cell lines is still the main tool for oncology research. However, limited percentage of cells isolated from fresh tumor tissues can be successfully grown in petri dish, and 2D cultured cells exhibit different morphological characteristics. Analysis suggested that the levels of cell proliferation, metastasis, metabolism and drug resistance proteins in 2D cultured cells were significantly different from actual tumor cells [13].

The first long-term organoid culture (3D culture model) of intestinal epithelium was reported in 2009. In the substrate with specific growth factors, mouse intestinal Lgr5+ stem cells can construct microspheres with crypts and finger-like processes, that is, small intestinal epithelial organoids with crypt villi structure [14]. As for the construction of PDOs, fresh tumor tissues from patients were digested into individual cells or cell clusters (diameter < 100 microns) by trypsin or collagenase, which were then transplanted into the basement membrane extract with specific medium, finally the cells will grow successfully in clusters within a few weeks [15]. It is confirmed that PDO preserves the key genetic information and phenotypic characteristics of parental tissues, and maintains tumor heterogeneity. When compared with PDX models, PDO model has the advantages of quick development, high-throughput screening ability, relatively low cost, etc. Therefore, PDOs, as a prediction platform for precision medicine, are gradually applied to the evaluation and screening of antitumor drugs in preclinical and clinical studies [16]. Collectively, application of PDO and PDC accelerates the development of precision medicine against cancer.

Patient-derived cell (PDC):

As an alternative preclinical model, PDC is widely accepted. With similar principles as PDX and PDO, PDC is accurate in antitumor drug screening as well as in individualized medicine. The malignant ascites, pleural effusion or pericardial effusion of cancer patients were collected, the cells were re-suspended and cultured in the culture medium. PDCs were passaged to detach cells when the cells reached 80-90% confluence. PDC maintains better the genotype of parental tumor when compared with traditional NCI-60 cell lines. And PDC is easier to operate when compared to PDX and PDO. Moreover, PDC avoids the disadvantage of time and budget consumption of PDX and PDO. Therefore, PDC is proposed as a predictive high-throughput evaluation platform for antitumor drugs and clinical individualized medicine [17].
**Application of patient-derived tumor models in antitumor drugs evaluation**

With the increased awareness of the limitations of traditional cell line-based evaluation model, pharmaceutical companies take patient-derived tumor models (PDO, PDO, and PDX) as an integral platform of preclinical antitumor drug evaluation. And it is now common to “re-evaluate” drugs prior to anticancer therapy with the patient-derived tumor models to find out the optimal treatment.

**Lung cancer**

Lung cancer has the highest morbidity and mortality among all cancers. The overall five-year survival rate of lung cancer is only about 15%, which is even lower in the small cell lung cancer (SCLC). Lung adenocarcinoma accounts for 70–80% of lung cancer cases, and EGFR-mutant (48%) is the most common among its molecular types. Thus, several EGFR targeted drugs, known as Epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) are developed, which mainly include AXL, ALK, ROS1, NTRK, RET, BRAF and MET inhibitors [18]. However, the extensively existing primary resistance (approximately 60%) and the rapidly developed acquired resistance (in about 6–12 months) are the major obstacles of EGFR-TKIs therapy against non-small cell lung cancer (NSCLC).

PDO models have been applied in developing strategies to increase EGFR-TKI efficacy against NSCLC. Researchers found that delayed degradation of AXL, a receptor tyrosine kinase from TAM family, induced resistance to gefitinib and osimertinib in PDO models. And a natural product-derived antitumor agent, yuanhuadine (YD) was confirmed to be able to modulate AXL degradation and effectively prevent the development of EGFR-TKIs resistance using PDX models. Thus, the combination of EGFR-TKI and AXL degraders may be a potential therapeutic strategy to overcome the acquired resistance in NSCLC [19]. Furthermore, PDO combined with immune system humanized mouse model provides an improved platform for evaluation of immunotherapy. For example, fresh cord blood CD34+ stem cells derived PDO model simulates better the human immune system and tumor microenvironment [20]. Anti-PD-1 (programmed death 1) checkpoint inhibitors were tested in this optimized PDO model, and showed significantly better results controlling lung cancer.

PDO is also used as a promising preclinical lung cancer model. Motoki Takagi et al. constructed a high throughput PDO platform for anti-lung cancer targeted drug evaluation and tested small molecule inhibitors, monoclonal antibodies and antibody-drug conjugates. Results confirmed that PDOs were more suitable to reflect pathological conditions and evaluate the molecularly targeted drugs [21].

In addition, a PDC pharmacogenomics platform was established by Namhee Yu et al. to explore the potential drug resistance mechanisms and individualized treatments for advanced lung cancer [22]. They constructed PDCs from 102 lung cancer cases and analyzed the genetic variation and clinical features to explore the correlation of genomic characteristics between PDCs and solid lung cancer tissues. Subsequently, they selected XAV939, a WNT-TNKS-β-catenin inhibitor, to treat the osimertinib-resistant PDCs. Result suggested that the osimertinib resistance cells exhibit increased invasive characteristics, the expression YAP/TAZ-AXL axis, and is more sensitive to XAV939. Their PDC models recapitulated the molecular characteristics of lung cancer, and pharmacogenomics analysis provided plausible individualized treatment candidates.

**Colorectal cancer**

The global incidence of colorectal cancer (CRC) exceeds 1 million new cases per year, and the disease-specific mortality is about 33% [23]. Notably, over 50% of metastatic colorectal cancers are primarily resistant to EGFR-targeted drugs [24]. Andrea Bertott confirmed that tumor tissues could largely maintain their phenotypic and genomic characteristics during early passages in CRC PDXs. Subsequent validation using cetuximab proved that PDX was a valid preclinical research model. Collectively, drug evaluation and mechanism exploration based on the PDX models effectively promote the discovery of novel and effective treatment strategies against CRC [25]. Meanwhile, PDOs can be applied for cell amplification and drug evaluation in a 3D environment. By establishing PDO model, Toden S et al. demonstrated that oligomeric proanthocyanidins (OPCs) could significantly inhibit the expression of tumor stem cell biomarkers, induce cancer cell apoptosis and cycle arrest to inhibit tumor development, implicating OPCs might be a novel drug for clinical cancer therapy. In conclusion, PDO model is also an effective tool for preclinical drug evaluation of colorectal cancer [26].

**Liver cancer**

Primary liver cancer (PLC) has the highest incidence in China among all cancers. The main pathological types of PLC include hepatocellular carcinoma (HCC, accounting for more than 90% of PLC), intrahepatic cholangiocarcinoma (ICC) and combined hepatocellular (CHC). A lot of molecular targeting therapies have been developed to promote precision medicine for PLC. However, due to the lack of obvious phenotypic and molecular diversity, the prognosis of PLC is still poor, an efficient preclinical evaluation model is urgently needed to promote the effective transformation of fundamental research into clinical practice and realize individualized treatment. Darko Castven et al. established several patient-derived cell lines from fresh liver tumors, confirmed that the cell lines maintained key oncogenic mutations, and gene expression profiles of the primary tumor [27]. These PDCs will facilitate direct exploration of therapeutic targets, drug discovery and individualized treatment of liver cancer.

**Pancreatic cancer**

Pancreatic cancer is one of the most clinically challenging malignant tumors. Its commonly late diagnosis and resistance to chemotherapy calls for urgent development of novel treatment strategies. Patient-derived tumor models have actually become indispensable for anti-pancreatic cancer drugs evaluation. Using PDO model of pancreatic cancer, Laheru et al. found that RAS inhibitor SaliRasib combined with gemcitabine could down-regulate RAS expression and inhibit tumor development, thus proposed a new phase II study of pancreatic cancer treatment [28].

**Bladder cancer**

Bladder cancer is the most malignancy in urinary tract which is mainly divided into non-muscle invasive bladder cancer (NMIBC) and advanced bladder cancer. And NMIBC accounts for 80% of bladder cancer, which can be primarily treated by transurethral resection. However, about 60% of the patients relapsed within two years and 25% progressed to advanced stage. Pan et al. explored the potential mechanisms of bladder cancer malignancy and drug resistance by PDO. And PDO shows good correlation with the patient at the genomic level and known patient response to treatment. It proves the patient-derived bladder cancer xenograft model is useful for bladder cancer screening of effective drugs and individualized therapy [29].

**Ovarian cancer**

About 70% of ovarian cancer are diagnosed with advanced stage, and the five-year survival rate is as low as 28% [30]. Scientists in Cambridge constructed PDX model of ovarian cancer, and confirmed that the expression of cell adhesion molecule CDH6 is closely related to ovarian cancer development. They subsequently designed an antibody-coupled drug HKT288 that targeted CDH6. They examined HKT288 on PDO models and showed that HKT288 significantly inhibited the development of ovarian cancer and even induced tumor regression in 40% of cases. Therefore, PDO based efficacy evaluation of HKT288 provides solid scientific proof for its first-in-human clinical trial [31].

**Other tumors**

Patient-derived tumor models are also used for the screening and re-evaluation of antitumor drugs for other malignant tumors, including renal cell cancer (RCC), esophageal squamous cell
carcinoma (ESCC), nasopharyngeal carcinoma (NPC), melanoma, glioma etc. [32–37].

Saeed et al. established patient-derived cells from different tumor regions of 4 RCC patients, and conducted drug sensitivity tests of 460 oncological drugs to investigate the impacts of genetic heterogeneity on drug response. Results confirmed the contribution of genomic heterogeneity to the variability in drug responses and suggested PDC models were efficient in the development of novel treatment strategies [32]. Another study established 8 PDCs from 123 patients with ESCC. And 46 targeted drugs were selected for the following screening based on genotypes of PDCs and associated pathways. Results indicate that PDCs can predict the sensitivity of ESCC with different mutations to targeted drugs [34]. Study of nasopharyngeal carcinoma also used PDX models. Combining whole exome sequencing and genome mutation analysis in 5 PDX models confirmed the homologues of xenografts to parent tumors [35]. Then potential drugs were applied in 2 PDX mice models and revealed that a cyclin-dependent kinase inhibitor Palbociclib is a novel candidate drug for NPC. In summary, the integrated information from high-throughput analysis and drug screening in patient-derived tumor models provides powerful guidelines for personalized precision treatments.

**Personalized medicine based on patient-derived tumor models**

Individualized therapy aims to optimize anti-cancer treatment according to differential pathological conditions of cancer patients. We retrieved publications with the keyword “tumor & individualized therapy”, published from 2013 to 2023 from the Web of Science, and found that the related research articles increased significantly in recent years (Figure 1). The popularity of patient-derived tumor models is evident from the increasing number of patent applications in this field since 2021. In addition, according to the statistics of TianYanCha.com, there are more than 50 enterprises had patents granted in China.

**Advantages and challenges of patient-derived tumor models**

The development of PDX, PDO and PDC models have achieved great success over the last 10 years. They closely recapitulate the original features of the primary tumors and allow the acquisition of more reliable screening results. However, each patient-derived model has its limitations (Table 1).

![Figure 1 The number of published papers about “tumor & individualized therapy”](https://www.tmrjournals.com/lr20240002)

**Table 1 The advantages, disadvantages and applications of PDX, PDO, PDC models.**

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<th>PDX</th>
<th>PDO</th>
<th>PDC</th>
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<td><strong>Advantages</strong></td>
<td>Easy to monitor;</td>
<td>Maintains the genetic information as</td>
<td>Also maintains the heterogeneity of the</td>
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<td></td>
<td>2. Preserves the genotype and</td>
<td>well as phenotypic characteristics of</td>
<td>maternal tumor cells;</td>
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<td>microenvironment characteristics of</td>
<td>the parental tumor;</td>
<td>2. Convenient and economic to operate;</td>
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<td></td>
<td>primary tumors;</td>
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<td>3. Higher success rate for model</td>
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<td>3. Highly similar reacts to treatments as</td>
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<td>the parental tumor.</td>
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<td><strong>Disadvantages</strong></td>
<td>1. High cost;</td>
<td>1. High cost;</td>
<td>1. Loss of the origin and quality of the</td>
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<td></td>
<td>2. Long development cycle;</td>
<td>2. Difficult of operation;</td>
<td>parental model after several generations</td>
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<td></td>
<td>3. Only the first three generations of</td>
<td>2. Organoid source is limited to</td>
<td>of cultivation;</td>
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<td></td>
<td>PDX models retained the characteristics</td>
<td>epithelial tissue tumors.</td>
<td>2. The different tumor microenvironments</td>
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<td>of the maternal tumor;</td>
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<td>affect the accuracy of research.</td>
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<td>4. Limited success rate, only suitable</td>
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<td>for tumors with high malignancy;</td>
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<td>5. Built in immunodeficient mice, cannot</td>
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<td>evaluate the drug by their immune system.</td>
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<td><strong>Applications</strong></td>
<td>1. Preclinical drug evaluation;</td>
<td>1. High throughput screening of drugs;</td>
<td>1. Large-scale drug screening;</td>
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<td>2. Re-evaluation of drugs after marketing;</td>
<td>2. Drug mechanism exploration;</td>
<td>2. Drug mechanism exploration</td>
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<td>4. Clinical individualized therapy.</td>
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Patient-derived xenograft

The PDX model highly preserves the genotype and microenvironment characteristics of primary tumors, and shows better predictability for antitumor drugs than traditional models. Meanwhile, as grafted in vivo, PDX is more accurate and reliable compared with in vitro models. However, there are still many challenges. First, the construction of PDX models takes 4 to 8 months and is relatively expensive, which can be unavailable for cancer patients. Second, for tumors with specific phenotypes such as breast cancer, the success rate of PDX model is still unsatisfied, it is more applicable for tumors with high malignancy [38]. Third, the current PDX set is biased toward certain cancer subtypes, it cannot broadly represent diseases when used for drug screening. Fourth, the human cancer derived matrix will be replaced by mouse matrix after three to five in vivo passages, only the first 3 passages have valid clinical annotation. In addition, immune system especially T cells is known to play key roles in cancer development. Since PDX model is established in immuno-deficient mice, it is impossible to evaluate drug acts through immune system. And the development of immune system humanized PDX models cannot fully satisfy the simulation of human immune response.

Nevertheless, PDX has already become an efficient integral part of pharmaceutical development including drug screening and biomarker exploitation. In conclusion, PDX models show great perspectives in preclinical trials of novel antitumor drugs and can be efficient strategies in individualized medicine.

Patient-derived organoid:

PDO generated from primary cancer material allows long-term maintenance of near-native 3D tumorous tissues in vitro, thus represents the cutting edge of science and technology [39]. Meanwhile, the predictability of PDO for clinical efficacy has been intensely verified by high-throughput screening. Science published a clinical examination of organoid’s chemotherapeutic sensitivity. Result showed that when compared with the actual clinical effectiveness, the prediction specificity of PDO was 93%, with a positive prediction accuracy of 88%, and a negative prediction accuracy reach 100% [40]. In addition, PDO can also be used to establish tumor model with immunologic function. Peripheral blood lymphoid tissue co-cultured with PDO can enrich tumor-reactive T cells in CRC and NSCLC, which can be used to evaluate the killing efficiency of PDO, which serves to explore more strategies for cancer treatment [41].

The culture medium for PDO is natural extract supplied with a variety of growth factors, which is expensive. Reducing the expense is one of the important directions for further development of organ-like platforms. Moreover, the current organs analogous are limited to tumors originated from epithelial tissues, the exploration of organoids derived from non-epithelial tissues will also be an important research field.

Patient-derived cell

PDC model well represents the heterogeneity of primary tumors [42]. Like PDX and PDO, PDC maintains molecular, genetic as well as the pathological characteristics of tumors. PDC also simulates well the therapeutic efficacy of cancer patients [43] while it is more convenient and economical to operate. With the higher success rate for model establishment, PDC is more suitable for large-scale drug screening. However, similar to the NCI-60 cell lines, the origin and quality of the PDC model were difficult to control after several generations of culture. Thus, it is difficult to reproduce satisfactory results in different PDC models. In addition, the different tumor microenvironment between PDC model and in vivo model also affects the accuracy of antitumor investigation. As the best alternative to NCI-60-derived models, PDC is also expected to be more widely used in basic cancer research as well as in drug evaluation [44].

In summary, PDX, PDO and PDC models are currently ideal preclinical evaluation platforms for anticancer translational research. These patient-derived models simulate the real complex system of cancer well that they gradually become efficient tools in personalized medicine. There are still obstacles and challenges for establishment and application of patient-derived tumor models. While the continuing development and optimization such as miniPDX and immune system humanized PDX models, make sure that patient-derived tumor models have broader prospects to promote precision medicine and improve cancer prognosis in the future.

References


Conclusions

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42. Dijkstra KK, Cattaneo CM, Weeber F, et al. Generation of...
