Current research developments of patient-derived tumour xenograft models (Review)

ZHENYU YIN[1*], EWETSE PAUL MASWIKITI[1*], QIAN LIU[1], YUPING BAI[1], XIAOMEI LI[1], WENBO QI[1], LE LIU[1], YANLING MA[1] and HAO CHEN[2]

[1]The Second Clinical Medical College of Lanzhou University; [2]Department of Oncology, Second Hospital of Lanzhou University, Lanzhou, Gansu 730030, P.R. China

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Abstract. Patient-derived tumor xenograft (PDTX) models are established by transferring patient tumors into immunodeficient mice. In these murine models, the characteristics of the primary tumor are retained, including the microenvironment of tumor cell growth and histopathology. Due to this, it has become the most reliable *in vivo* human cancer model. However, the success rates differ by type of tumor, site of transplantation and tumor aggressiveness. Subcutaneous transplantation is a standard method for PDTX, and subrenal capsule transplantation improves the engraftment rate. Recently, PDTX models are frequently used in the fields of precision medicine, predictive biomarkers, evaluation of drug efficacy and preclinical research on tumor immunotherapeutic drugs. The aim of the present article was to review the establishment, clinical applications and limitations of the PDTX model in tumor research.

Contents

Correspondence to: Professor Hao Chen, Department of Oncology, Second Hospital of Lanzhou University, 82 Cuiying Door, Lanzhou, Gansu 730030, P.R. China
E-mail: ery_chenh@lzu.edu.cn

*Contributed equally

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1. Introduction

Cancer poses a major threat to human health. In 2020 there were an estimated 19.3 million new cancer cases and 10 million cancer-related fatalities worldwide (1). The continuously growing population is associated with aging and various lifestyle changes, and the number of cancer cases and deaths is also expected to increase rapidly (2). In-depth cancer research requires models that accurately represent the characteristics of human cancer. Due to ethical considerations, human oncology research is mainly limited to analytical and observational research; however, clinical trials for treatment are the most crucial in research, and the involvement of humans in these trials is subject to ethical restrictions. Therefore, preclinical mouse tumor models are an indispensable intermediate experimental model system, which effectively combines *in vitro* studies with human studies (3).

The classical xenograft tumor model is a cell line-derived tumor xenograft model; this is an animal model formed by injecting a tumor cell line cultured *in vitro* into an immunodeficient mouse. The single tumor cell line is repeatedly passaged *in vitro*, adapted to and freely exposed to the external culture environment, and loses most of the patient characteristics (4). In addition, the lack of tumor-related matrix and, specifically, the addition of fetal calf serum to the culture medium, may lead to cellular differentiation and significant genetic aberrations. Furthermore, gene expression profiling has further demonstrated that cell lines obtained from diverse tumors resemble each other more than the corresponding clinical samples from which they were derived, and serum-cultured cell lines can lose drug resistance mechanisms (5). The microenvironment of the cell line *in vitro* and the original primary tumor are completely different. It should further be noted that the genetic variations and tumor heterogeneity produced by cell lines cannot be predicted, and the role of drugs in clinical trials cannot be accurately predicted. The average success rate of the transformation from animal tumor-bearing models to the clinical tumor patient trials is <8% (6). To overcome these limitations, medical researchers urgently need more accurate and effective methods for predicting and evaluating the efficacy of drugs, and the patient-derived tumor xenograft (PDTX) model has, to a certain extent, solved some of these problems.

Therefore, the aim of the present article was to review the clinical applications and limitations of the PDTX model in tumor research in order to develop improved and more efficient treatment strategies through implanting xenograft tumors from oncology patients to immunodeficient mice.

2. Characteristics of the PDTX model

Comparison with primary tumors. PDTX models possess certain characteristics that are relatively similar to those observed in primary tumors, and researchers have incorporated and utilised them in experiments on hepatocellular carcinoma, pancreatic cancer, small cell lung cancer, non-small cell lung cancer (NSCLC), breast cancer, ovarian cancer, uterine sarcoma, prostate cancer, renal cell carcinoma, head and neck cancer, melanoma and several other tumors (7). From the perspective of histopathology and genetics, it was confirmed that the transplanted tumor maintained the pathological and histological characteristics of the primary tumor, retained the characteristics of the tumor cell matrix, and reflected the genetic diversity of the patient's tumor (8). Genome-wide gene expression analysis studies have also demonstrated that PDTX models maintain the activities of most key genes and complete pathways in primary tumors. Fichtne *et al* (9) reported that, in a PDTX model of NSCLC, unsupervised hierarchical clustering of the whole genome gene expression profile revealed that 9 of the 17 primary tumors are almost identical with the transplanted PDTX model and the coefficient was 0.78-0.95. The key factor is that the correlation coefficient in 10 of the 17 pairs of primary and PDTX tumors was >0.90, indicating a high degree of similarity between the primary tumor and the corresponding PDTX model.

Provision of sufficient tumor samples by the PDTX model. The PDTX model can make up for insufficient sample sizes in tumor-related research. Therefore, for PDTX models to produce more convincing and reliable results, as mentioned earlier, it is more convenient to utilize fresh tumor tissues harvested from patients and xenografted in animal models. Therefore, PDTX models require a small sample size. Post-establishment, the first-generation model can be used in the subsequent model, which directly achieves an increment in the sample size. This makes it markedly easier to obtain tumor tissues. Tumors harvested from patients with pancreatic acinar cell carcinoma, small cell lung cancer and liver metastases from intestinal cancer are very aggressive and more difficult to obtain and, therefore, PDTX models are useful in these cases.

Difference of a small number of PDTX models from primary tumors. Due to the high degree of consistency and accuracy between the PDTX model and the primary tumor, this type of model has been a major breakthrough in translational medicine. Although humans and mice are different, tumor formation is a gradual development process from a single tumor cell, involving a variety of etiologies and resulting in various pathologies. Changes in this process, involving transformations and translations, are complicated, and a small number of PDTX models exhibit histopathological differences from the primary tumor. In a PDTX model of 63 cases of gastric cancer using non-obese diabetic (NOD)/severe combined immunodeficiency (SCID) mice, Zhu *et al* (8) reported that the concordance rate of differentiation between primary tumors of patients and xenografts was 90.5% (57/63), which became 98.4% (62/63) after three passages of the xenograft cells. In three patients with moderately differentiated primary tumors the differentiation of the xenografts changed to poor, and in one patient with a poorly differentiated primary tumor the differentiation of the xenograft changed to moderate. Chijiw *et al* (10) observed that, in 8 cases (7% of all engraftments), the transplantable xenograft tumors were composed of large unifrom non-epithelial cells, the morphology of which differed from that of the original tumor. The reasons underlying these differences require further investigation to elucidate.

3. Preparation of the PDTX model

Materials and methods involving surgical specimens and biopsies. To create a PDTX model, fresh tumor tissue must be obtained from patients and implanted into immunodeficient mice in a timely manner without delay. The methods include surgical specimen and biopsy collection. However, in several cases, obtaining surgical specimens may pose a challenge for researchers and investigators, particularly in patients with a risk of delayed surgery due to complications, such as patients with advanced gastric cancer. In such cases, the main technique utilized for tumor sample collection for construction of a PDTX model is gastroscopic biopsy (8).

The methods for collection of surgical specimens are as follows: Addition of penicillin/streptomycin to medium 199 or RPMI-1640 at 1:100 to prepare a solution (6). Tumor cells and tissues are then transferred to a sterile Petri dish containing medium 199 (or RPMI-1640) and penicillin/streptomycin. Finally, tumors are cut into 5x5x5-mm^3 pieces. Care and proper precautions must be taken to ensure that the tissue blocks are as uniform as possible and that no necrotic tissues are used in the transplantation and implantation procedures. Necrotic tissues are distinct and differ across various types of tumors, but they usually appear as dark or blackish, resembling a scar located centrally in large tumors (11). The methods for collecting biopsy specimens are as follows: Four fresh biopsy tissue blocks are taken from the patient's tumor tissue sample, all of which measure 2x2x2 mm^3 in size (8). It must be ensured that the tumor tissue obtained from the biopsy is non-necrotic. Thereafter, it is stored in a culture medium 199 or RPMI-1640 at 4˚C until use.

The obtained surgical and biopsy specimens are placed in a 4˚C environment for the next transplantation step. Specimens should be transplanted within no more than 2 h after *ex vivo* experiments (12). Portions of the tissue samples are used for transplantation, and additional tissue samples are quickly frozen and stored at -80˚C for genetic, genomic and proteomic analyses.

Mouse selection. Nude mice (congenitally athymic mice), have been used in research since 1962. These are primarily T lymphocyte-immunodeficient mice. In 1980, Shultz *et al* (13) observed that human cells could be transplanted into NOD mice lacking macrophages and NK cells. A series of subsequent mouse models were developed based on NOD

mice. The appearance of SCID mice in 1983 provided the conditions for human cell implantation. This type of mouse harbored mutations in the protein kinase, DNA-activated, catalytic subunit (Prkdc) gene, which caused inactivation of T- and B-cell surface receptors *in vivo* (14). NOD/SCID mice have been used in research since 1995. Due to the lack of dendritic cells, T cells, B cells, NK cells, macrophages and complement functions, the success rate of the transplantation of human cells and tissues improved significantly compared with SCID mice, which was a landmark in the history of mouse models (15). Subsequently, the first IL-2Rγ mutant mouse model emerged in 1996, in which the IL-2Rγ mutation characteristically inhibited the activity of NK, T and B cells, while reducing the lymphoma conversion rate (16). NOG mice were developed by Taconic Japan in 2002 and NSG mice by Jackson Laboratory in 2005 (17-19). Their characteristics included low conversion rate of thymic lymphoma, no residual lymphocytes, dual immune deficiency with innate immunity and adaptive immunity, long lifespan and high success rate in transplantation in *in vivo* experiments. In 2015, the Nanjing Institute of Biomedicine independently developed NCG mice. CRISPR/Cas9 technology was used to directly knock out the Prkdc and IL-2Rγ genes in NOD/ShiLtJNju mice, which had characteristics similar to those of NOG and NSG mice. Rats and mice have the advantages of higher fertility rate and better outcomes in immune and tumor-related studies. NOG, NSG and NCG mice presently have the highest tumor formation rate. However, research on the success rate of gastric tumor xenografts in different immunodeficient mice has yet to be performed and put into practice.

Transplantation steps and experimental procedures. Gastric cancer tissue specimens obtained from an isolated surgical or endoscopic biopsy specimen must be implanted in immunodeficient mice within 2 h. The specific steps are as follows:

Subcutaneous transplantation. Tumor specimens are collected from onco-surgical patients in the operating theater and cut into 2x2x3-mm^3 pieces; biopsy tissue specimens are also cut into pieces of the same size (2x2x2 mm^3), and washed 3 times with the aforementioned culture solution. Under isoflurane anesthesia, a small incision and subcutaneous pouches are created on the back or on the sides of the groin of immunodeficient mice (aged 5-6 weeks). A tumor is then implanted into each pouch (11) and penicillin/streptomycin solution is dripped into each surgical incision. Post-transplantation, the muscle and skin are sutured. Tumor length (a) and width (b) are measured by Vernier calipers, and the growth of the tumor xenograft is monitored at least twice per week. Tumor volume is calculated using the formula (a x b^2)/2.

Subrenal capsule transplantation. A portion of surgical specimen/biopsy tissue measuring 1x3x3 mm^3 is isolated. Under sterile conditions, an incision of ~2.0 cm is made along the midline of the back skin of anesthetized NOD/SCID mice. Pressure is applied to one side of the kidney with the investigator's index finger and thumb to slide the kidney out of the body cavity. The subrenal capsule is gently clamped with a #5 fine forceps, and a 2-4-mm incision is made in the subrenal capsule with a pair of fine spring scissors. A pocket is then created between the kidney and the subrenal capsule by blunt dissection, taking extra caution not to damage the kidney parenchyma or cause bleeding. The graft is then placed on the surface of the kidney with blunt-end forceps. The incision of the subrenal capsule is lifted with a pair of fine forceps and the graft is inserted in the pocket under the capsule with a polished glass pipette. Two to three grafts may be placed under the kidney capsule without significant adverse effects on the mice. Post-transplantation, the kidney is gently returned into the body cavity, and the layers are carefully sutured (12).

Orthotopic transplantation procedure. Hiroshima *et al* (20) first invented and described an orthotopic transplantation model of a soft tissue sarcoma. The tumor tissue was harvested from the biopsy of a patient with retroperitoneal soft tissue sarcoma, and a piece measuring 3.0 mm^3 was transplanted into the left retroperitoneum of the nude mice. A small (6-10 mm) incision was made in the left lumbar region of the nude mouse through the skin and muscle. The location of retroperitoneal fat and the left kidney in the nude mouse corresponded to the location of the original tumor in the patient, and a gap between the kidney and the retroperitoneal fat was created through this incision. A 3-mm^3 tumor piece was implanted into the cavity and sutured using 8-0 nylon surgical sutures. After completion, the incision was closed with a layer of 6-0 nylon surgical sutures. Tumor pieces of the same size were also implanted subcutaneously in nude mice using standard techniques. Sun *et al* (21) used the embedding method for orthotopic transplantation of gastric cancer. The operation steps were as follows: Using a 1-ml disposable sterile syringe, 0.1 ml 0.9% (g/ml) sodium chloride was gently injected into the gastric serosa of the animal, forming a small subserosal cavity. The edge of the constructed cavity was gently pinched with a pair of tweezers, a small opening (~3 mm) was created with a pair of ophthalmic scissors, and then small forceps were used to transfer the tumor mass into the cavity. In this manner, the tumor tissue did not adhere to other tissues, and the injury to the stomach was minimal. Finally, the abdominal cavity was sutured layer-wise, and the wound was carefully cleaned and disinfected with an iodine solution.

Intramuscular transplantation. Read *et al* (22) used matrix gel-coated tumor tissue sections and implanted them into the lateral subcutaneous or posterior muscles of immunocompromised mice. PDTX models of esophageal and anal cancer were successfully constructed using fresh and cryopreserved biopsy tumor tissues. Compared with subcutaneous implantation, the rate of intramuscular implantation was relatively higher. The tumor formation rate of surgical tissue specimens was 95 and 25% intramuscularly and subcutaneously, respectively, and the tumor formation rate of biopsy tissues was 48 and 7%, respectively. In addition, intramuscular tumors grew faster compared with subcutaneous PDTX tumors. The tumor differentiation, protein expression, mutation profile and response to chemotherapy were consistent with those of the primary tumors.

Application of in vivo animal optical imaging technology in gastric cancer mouse models. *In vivo* imaging technology refers to the qualitative and quantitative research of living organisms at the tissue, cellular and molecular levels by

non-invasive methods. At present, *in vivo* animal imaging technology is mainly divided into optical imaging, nuclear imaging and positron emission tomography (PET)-single photon emission computed tomography. Magnetic resonance imaging (MRI), computed tomography (CT) and ultrasound imaging are also among the *in vivo* imaging technologies utilized. Amongst these imaging techniques, optical imaging in living animals (23,24) mainly includes two technologies: Bio-luminescence and fluorescence imaging. These two optical imaging techniques are mainly used to detect the success rate and size of tumor transplantation in tumor cell line-derived xenograft models. As regards the PDTX model, MRI is also used to determine the success rate and size of the tumor during the transplantation procedure. Comparable to the human anatomical systems, the successful transplantation of tumor tissues is associated with obvious abnormal signal shadows on plain MRI scans.

Passaging of tumor tissue. When the tumor grows to a size of ~750 mm^3 (8), it can be sub-cultured. The tumor-bearing animals are sacrificed by cervical dislocation, placed in an ice water bath for 2 min, then placed in 75% ethanol for 2 min and dissected in a sterile environment. The tumor tissues are minced under sterile conditions to a tissue block size of 3x3x3 mm^3 (7). They are then transplanted into immunodeficient mice, as described above. The tumors may be passaged no more than 10 times after the surgical transplantation procedure. Post-transplantation, the tumor is allowed to grow to 200-500 mm^3 before initializing chemotherapy.

Cryopreservation and resuscitation. A large number of samples from early passages are stored in a tissue bank refrigerator, frozen in liquid nitrogen and used for further *in vivo* experiments. Half of the freshly prepared xenograft tumor tissues are immersed in RPMI-1640 solution, to which 10% fetal bovine serum and 10% dimethyl sulfoxide are added. After storing overnight at -80˚C, the vial is transferred to liquid nitrogen (25).

For re-transplantation, the tumor tissues are quickly thawed at 37˚C, washed with PBS solution, and transplanted subcutaneously into nude mice (26). Zhang *et al* (27) implanted frozen biopsy NSCLC tissue in SCID mice, achieving a similar success rate as with fresh tumor tissue (32%, 10/31 implantations). Anderson *et al* (28) further proved that the PDTX model with cryopreserved small cell lung cancer biopsy tissue exhibited highly consistent histological characteristics compared with the primary tumor.

Key factors for the successful establishment of the PDTX model. Tumor *in vitro* time and implantation time were the most important factors affecting the success rate, and the tumor was transplanted within 2 h after *in vitro* processing. Chijiw *et al* (10) demonstrated that tumors transplanted into NOG mice two or more days after surgical removal exhibited a higher establishment rate (61%) compared with those transplanted on the day of surgery or the next day (51%), but the difference was not statistically significant.

The success rate of tumor transplantation has been found to be higher in patients with tumors exhibiting high malignancy and low differentiation, with different tumors exhibiting different tumor formation rates. Okada *et al* (29) reported that the tumor formation rate following subrenal capsule and orthotopic transplantation was higher compared with that of subcutaneous transplantation. Chijiw *et al* (10) reported that the success rates differ with tumor origin: Respiratory tumors are 67% successful, while gastrointestinal tumors are 58% successful and urinary tumors are 57% successful. Furthermore, the tumor formation rate of metastatic tumors was found to be higher compared with that of primary tumors (65 and 27%, respectively). The success rate of tumor tissue obtained before chemotherapy was also better compared with that post-chemotherapy. Zhu *et al* (8) demonstrated that the transplantation rate of biopsy before chemotherapy (52.1%, 37/71) was higher compared with that post-chemotherapy (21.9%, 25/114). The success rates of PDTX modeling in different transplant sites is summarized in Table I.

4. Clinical applications of the PDTX model

Precision medicine and predictive biomarkers. During the treatment of clinical tumors, the individual differences between patients with the same tumor manifestations represent a major challenge, but the treatment plan applied is often non-personalized (17). The results of pre-clinical experiments using the PDTX model are often highly consistent with clinical facts and expectations, which is helpful for individualizing the treatment plan for each patient. Practically, the concept of precision medicine is to group patients into subpopulations based on sophisticated genomic profiling, enabling certain therapies to specifically target the subgroup. Based on this concept, PDTX is an appropriate model as it retains the genomic characteristics of the individual tumor and represents a subgroup with a similar genetic profile. Moreover, the PDTX model can even recapitulate heterogeneity within the same tumor specimen (intratumoral heterogeneity) (30).

A study from Johns Hopkins University (31) reported that a patient with stage IV gastroesophageal adenocarcinoma with liver and lung metastases was initially treated with an epirubicin-cisplatin-capecitabine regimen achieving a partial response (PR) that lasted for 8 months. Subsequently, disease progression developed with lung and liver metastases and an increase in the levels of carcinoembryonic antigen. At that point, a tumorgraft generated from a resected liver metastasis had been treated with 17 different drugs in 35 combinations. The tumorgraft responded to the combination of irinotecan, bevacizumab and cetuximab, which was the recommended for clinical use. With this treatment, the patient achieved a PR in the liver metastasis that lasted for 14 months. c-MYC controls >15% of genes responsible for cancer cell proliferation, differentiation and metabolism in pancreatic and other cancers, making this transcription factor a major target for patient treatment. The transcriptome of 55 patient-derived xenografts reported by Bian *et al* (32) revealed that 30% shared an exacerbated expression profile of MYC transcriptional targets (MYC-high); it was then demonstrated that cells from MYC-high patients were more sensitive to JQ1 treatment compared to MYC-low cells, as determined by cell monolayer cultures, 3D cultured spheroids and *in vivo* xenografted tumors, due to induced cell cycle arrest followed by apoptosis. Therefore, these results may provide new markers and potentially novel therapeutic

Table I. Patient-derived tumor xenograft modeling comparison among different transplant sites.

Transplant site	Advantages	Disadvantages	Tumor type	Tumor formation rate	Tumor formation time (days)	(Refs.)
Subcutaneous	Simple operation and easy to observe	Lack of blood supply	Colorectal cancer	56%	49	(45)
Subrenal capsule	Supports growth, proliferation and infiltration of transplanted tumor tissues and other biological activities. Good blood supply.	Inevitably leads to phenotypic changes of tumor-related molecules	Lung cancer	96%	20	(46)
Orthotopic	Microenvironment closer to the original tumor	Difficult operation	Pancreatic cancer	43%	107	(47)
Intramuscular	Well-vascularized graft bed at a constant temperature	Xenograft produces lymphoma transformation	Esophageal cancer	65%	-	(48)

modalities for distinct subgroups of pancreatic tumors, and may be applicable for the future management of such patients in the setting of individualized medicine.

Different tumors, including those of the liver, stomach and colon, display distinct responses to therapeutic interventions. The therapeutic options for hepatoblastoma and hepatocellular carcinoma are limited, and PDTX models may broaden the treatment options for managing liver cancer (33). Serine/threonine-protein kinase PLK-1, also known as polo-like kinase 1 (PLK-1) or serine/threonine-protein kinase 13, has a significant impact on the survival of gastric cancer cells *in vivo* as shown by analyzing tumorigenesis in PDTX models, and the inhibition of PLK1 activity by BI6727 was found to significantly decrease the volume and weight of the tumors compared with the control group, indicating that PLK-1 may represent a prognostic factor, a potential therapeutic target and a preventive biomarker in gastric cancer (34). Furthermore, circPTK2 has been found to play key roles in colorectal cancer growth and metastasis, and it may serve as a potential therapeutic target for colorectal cancer metastasis and also as a promising biomarker for the early diagnosis of metastasis (35).

Evaluation of drug efficacy. When evaluating the anticancer activity of a drug, the results of *in vivo* experiments are mainly considered, whereas the results of *in vitro* experiments are referenced to reach correct and accurate conclusions. Traditionally, in *in vivo* experiments, a model is constructed by transplanting the corresponding cell line into mice. However, the cell line cannot maintain a close association with the primary tumor, resulting in greater variations in dose range and drug efficacy. PDTX models do not carry this disadvantage and ensure a more reliable evaluation of drug anticancer activity. Furthermore, PDTX models also play an important role in antitumor drug resistance experiments and antitumor metastasis drug experiments *in vivo*. Bertotti *et al* (36) treated 47 cases of metastatic colon cancer in PDTX models with cetuximab, an anti-EGFR receptor antibody. The results demonstrated that the effectiveness rate was 10.6% (5/47), the disease stability rate was 29.8% (14/47), and the disease progression rate was 59.6% (28/47), which was consistent with the clinical efficacy observed in the patients. Recently, the BRAF V600 inhibitor, vemurafenib, has revolutionized the therapeutic management of metastatic melanoma; however, adverse effects and the onset of resistance are frequently observed, limiting the efficacy of this agent. Guerreschi *et al* (37) established a PDTX model of BRAF V600E melanoma to test the efficacy of vemurafenib. First, they validated the stability of the model, which was similar to the original tumor with respect to histology, immunohistochemistry, mutational status and findings on ^{18}F-fluorodeoxyglucose (FDG)-PET/CT. Next, the sensitivity of the xenografts to vemurafenib was determined by tumor growth inhibition and decreased standardized uptake value on ^{18}F-FDG-PET/CT. The results, using a personalized PDTX, allowed successful rechallenge with vemurafenib in a patient who was administered a lower dose of vemurafenib due to the onset of adverse events. The outcome demonstrated that PDTX may provide 'real-time' results in an animal that phenocopies the biology and expected vemurafenib responses of the tumor in a patient with BRAF V600E melanoma. Thus, 'coclinical' trials using PDTX may help guide vemurafenib treatment for patients with metastatic melanoma.

Preclinical research on tumor immunotherapeutic drugs. In clinical practice, it has been found that different patients respond differently to tumor immunotherapy. Accumulating evidence shows that the heterogeneity of the tumor microenvironment results in different antitumor immune responses in different individuals (38). The tumor microenvironment is crucial for the evaluation of tumor immune drugs. PDTX models can simulate the tumor microenvironment in humans. Currently, by transplanting hematopoietic stem cells derived from human bone marrow, peripheral blood or embryonic tissues in highly immunodeficient mice, these can express the human immune system (HIS) *in vivo*, thereby reconstructing mouse models of the HIS (39). Lee *et al* (40) used scRNA-seq to depict the tumor landscape of a single

case of chemoresistant metastatic, muscle-invasive urothelial bladder cancer (MIUBC) addicted to an activating Harvey rat sarcoma viral oncogene homolog (HRAS) mutation. In order to analyze tumor evolution and microenvironmental changes upon treatment, the authors also applied scRNA-seq to the corresponding PDTX before and after treatment with tipifarnib, a HRAS-targeting agent under clinical evaluation. In the parallel analysis of the human MIUBC and the PDX, diverse stromal and immune cell populations recapitulated the cellular composition in the human and mouse tumor microenvironment, and treatment with tipifarnib achieved notable anticancer effects but was unable to achieve a complete response. Importantly, the comparative scRNA-seq analysis between pre- and post-tipifarnib-treated PDTX revealed the nature of tipifarnib-refractory tumor cells and the tumor-supporting microenvironment. The findings of that study demonstrated the value of scRNA-seq for visualizing the tumor microenvironment and identifying molecular and cellular therapeutic targets in a treatment-refractory cancer patient. Yao *et al* (41) used NOD, SCID, gamma (NSG)™ or NSG™-SGM3 mice as hosts for the establishment of HIS following hematopoietic stem cell injection and for engraftment of human tumors, and demonstrated that a wide range of PDTX tumors grow in humanized mice without obvious indications of rejection. Human $CD45^+$ immune cells infiltrated tumor tissues that were present in PDTX-bearing humanized mice, but not in PDTX-bearing control NSG™ mice; in addition, PDTX-bearing humanized mice responded to standard-of-care chemotherapeutics and to the checkpoint inhibitor pembrolizumab. These observations may be used in preclinical pharmacological and toxicological experiments to provide more information for the evaluation of immunotoxicity. Moreover, tumor tissues of patients with head and neck tumors were surgically removed and transplanted subcutaneously to the lateral surface of the reconstructed HIS mice to study the interaction between human stem cell progenitor cells and transplanted human tumors (42).

Recently developed programmed cell death protein 1 (PD-1) pathway inhibitors have revolutionized cancer treatment for a proportion of the patients, but the majority of patients do not achieve a complete response; therefore, there is a need for methods to test the potential antitumor activity of rational combination therapies (43). Conventional murine xenograft models are restricted by their immunocompromised status; thus, Capasso *et al* (44) humanized BALB/c-$Rag2^{null}Il2r\gamma^{null}SIRP\alpha^{NOD}$ (BRGS) pups through transplantation of cord blood (CB)-derived $CD34^+$ cells. The mice were evaluated for human chimerism in the blood and assigned into experimental untreated or nivolumab groups based on chimerism. triple-negative breast cancer cell lines or tumor tissue from established colorectal cancer PDTX models were implanted into both flanks of humanized mice and treatments were initiated once the tumors reached a volume of ~150 mm^3. The results demonstrated that Hu-CB-BRGS mice may represent a reliable *in vivo* model for studying immune checkpoint blockade in human tumors. The HIS in the mice is inherently suppressed, similar to a tumor microenvironment, and thus allows the growth of human tumors. However, this suppression may be lifted by anti-PD-1 therapies and inhibit the growth of some tumors. This model offers ample access to lymph and tumor cells for in-depth immunological analysis. The tumor growth inhibition is associated with increased numbers of CD8 $IFN\gamma^+$ tumor-infiltrating T cells, and these hu-CB-BRGS mice provide a relevant preclinical animal model to facilitate prioritization of hypothesis-driven combination immunotherapies.

5. Outlook in humanized tumor animal models

Although significant progress has been made in therapeutic approaches based on humanized tumor animal models, researchers are still faced with major challenges that must be addressed. First, the dependability of the models may be questionable. Along with the importance of the tumor microenvironment in several aspects of tumor biology, the transformation of PDTX tumor matrix components from the original human patient to the tumor-bearing mouse matrix components is likely to represent a related defect in these models. This limitation is particularly more pronounced when specific anticancer compounds that target the microenvironment, such as bevacizumab, are administered.

Second, the lack of human immune microenvironment may represent a problem. Since none of the modeled mice have a mature immune system, the PDTX model cannot be used to evaluate the efficacy of immunosuppressants and immunomodulators. The human immune microenvironment can be made up by rebuilding the HIS. However, the degree of reconstruction of the human immune microenvironment must be improved. More efforts should be focused on constructing an animal model with higher similarity and efficacy to the primary tumor.

Third, the mean tumor formation time is relatively long, which is not conducive for the provision of accurate treatment options for patients with advanced-stage disease. Among patients with rapid tumor development and short expected survival, a proportion may succumb to the disease before obtaining the results of drug sensitivity tests.

Finally, it is difficult to determine tumor formation in deeply situated organs, and the methods utilized for assessing tumor formation in deep organs in PDTX models must be improved. MRI, CT, PET-CT and other means may be employed, but these methods are difficult to adapt to a large number of PDTX models.

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Availability of data and materials

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Authors' contributions

WQ, LL and YM contributed to the conception and design of the present study. QL, YB and XL consulted relevant references and performed the literature data collection. ZY and EPM wrote the first draft of the manuscript. HC revised the manuscript. Data authentication is not applicable. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

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