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Invited Mini Review

Current status and clinical application of patient-derived tumor organoid model in kidney and prostate cancers

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Urological cancers such as kidney, bladder, prostate, and testicular cancers are the most common types of cancers worldwide with high mortality and morbidity. To date, traditional cell lines and animal models have been broadly used to study pre-clinical applications and underlying molecular mechanisms of urological cancers. However, they cannot reflect biological phenotypes of real tissues and clinical diversities of urological cancers *in vitro* system. *In vitro* models cannot be utilized to reflect the tumor microenvironment or heterogeneity. Cancer organoids in three-dimensional culture have emerged as a promising platform for simulating tumor microenvironment and revealing heterogeneity. In this review, we summarize recent advances in prostate and kidney cancer organoids regarding culture conditions, advantages, and applications of these cancer organoids. [BMB Reports 2023; 56(1): 24-31]

INTRODUCTION

Incidence of urological malignancies such as kidney, bladder, prostate and testicular cancers are gradually increasing worldwide (1). In recent decades, the number of studies on underlying mechanisms of development and progression of urological cancers, particularly kidney and prostate cancers, has increased tremendously. Knowledge of targeted therapies based on genetic alterations of kidney and prostate cancers has substantially progressed, paving the way for a paradigm shift of anti-cancer therapy. Despite accumulating evidences, major hurdle to develop a novel anti-cancer therapy is the translation of findings from bench works to bedside application. Apparently, a myriad of results obtained using pre-clinical cancer models could not translate into real-world clinical practice. In this regard, developing novel *in vitro* and *in vivo* model systems recapitulating the heterogeneity and lineage specificity of human cancers with clinical utility is an unmet need (2, 3).

Immortalized traditional two-dimensional (2D) cancer cell lines are tools widely used for human cancer research due to their low handling costs and ease of use (4, 5). However, cancer cell lines mostly do not reflect the parental tumor or their microenvironments because only a subset of tumors can grow in 2D on a culture dish. Additionally, cancer cell lines have unexpected genetic changes caused by passages during long-term culture. Therefore, they poorly recapitulate the heterogeneity of tumors from which they originated (6, 7). *In vivo* models including genetically engineered mouse models and patient-derived tumor xenograft (PDX) models offer similar histology and genomic alteration patterns of their parental tumors (8). In particular, PDX models can recapitulate the therapeutic behavior of patient's tumors. Nevertheless, mouse models have several drawback such as high maintenance costs and long time for model establishment (9). The most important drawbacks of using *in vivo* mouse models for human cancer are differences in genetic background among interspecies and contamination of mouse cells in a tumor microenvironment.

As mentioned above, 2D culture systems cannot accurately recapitulate *in vivo* real tissues like microenvironment. Cancer cellular biology, including cancer cell proliferation, metastasis, differentiation, and dedifferentiation is strongly influenced by the tumor microenvironment-interacting stroma cells or extracellular matrix (10). Therefore, patient-derived tumor organoid (PDTO) model can overcome limitations of 2D cell line cultures. PDTO can efficiently and closely reconstruct the *in vivo* microenvironment by influencing cell-to-cell and cell-extracellular matrix associations (11). Furthermore, tumor organoid cultures are 3D *in vitro* constructs with self-organizing stem cells and various differentiated cells (12). Here, we review the advantages of PDTO systems, particularly in kidney and prostate cancers. Table 1 summarize advantages and disadvantages of 2D culture, PDX, and PDTO systems (13).

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Table 1. Comparison of advantages and disadvantages of 2D cell lines, patient-derived xenografts (PDXs) and patient-derived tumor organoid (PDTO) models

Models	Advantages	Disadvantages
2D cell lines		
	• Infinite growth	• Lack of heterogeneity
	• Easy handling	• Lack of tumor microenvironment and immune system
	• Highly available and cheap	• Rare source
	• Easy access to genome editing	
PDXs		
	• Retains tumor heterogeneity	• Expensive and time-consuming
	• Correlate with treatment responses in patients	• Differences in genetic interspecies
	• Low contamination of normal cells	• Gaps between mouse and human system
	• Making a metastasis model	• Low-throughput screening
		• Contamination of mouse cells in tumor microenvironment
PDTO		
	• Retains tumor heterogeneity	• Low success rate of culture
	• Preserve genomic characteristic	• Hard to long-term expansion
	• High-throughput screening	• No immune systems
	• Applied to PDX model	• Contamination of normal cells
	• Linked to treatment response in patients	
	• Recapitulation of *in vivo* tissues	

PATIENT-DERIVED TUMOR ORGANOIDS (PDTO)

PDTO culture can be accomplished in a short time (several weeks) with a high efficiency compared to iPSC-induced organoids (2, 14). With advantages of representing microenvironments and heterogeneities of parental tumors, PDTO systems might be employed in preclinical tumor models of human cancers. For example, PDTO systems can be used to study mechanisms of oncogenesis and anti-cancer drug resistance and to determine the origin of cancer cells. Furthermore, considering that cancer stem cells can contribute to resistance to chemotherapy and initiation of the process of cancer, PDTO can help us identify cancer stem cells initiating tumor that leads to resistance to anti-cancer drugs (10, 13). In this context, PDTO platform opens up the opportunity to apply personalized medicine. It can be used as a convenient model for high-throughput drug screening and for studying anti-cancer drug resistance.

Studies on urological cancers including kidney, prostate, and bladder tumors have elucidated the distinct genetic alterations (15). These genetic alterations are responsible for intra-tumor or inter-tumor heterogeneity. Studies on the underlying molecular mechanisms of urologic cancers have been accumulated with advanced sequencing technologies and experimental methodologies. Particularly, 3D organoid models such as PDTO systems highlight advanced cancer research. They can maintain the heterogeneity of parental cancer and simulate the real environment *in vivo* (16). Among various type of urological malignancies, prostate and kidney cancer organoids are focused in this review (Fig. 1) (17-19).

PROSTATE CANCER ORGANOIDS

Prostate cancer is one of the most common adenocarcinomas among men worldwide. Since most prostate cancers are androgen-driven adenocarcinomas, androgen deprivation therapy (ADT) is the treatment of choice for patients with advanced prostate cancer (20). Tumor volume of prostate cancer is dramatically reduced at an early period of ADT in patients with advanced prostate cancer. However, inhibiting the activation of androgen receptor is no longer sufficient to suppress tumor cell growth after several years of therapy due to the development of castration-resistant prostate cancer (CRPC) or neuroendocrine prostate cancer (NEPC) lacking AR-activity (21, 22). Resistance to AR inhibitors and NEPC can occur due to genomic rearrangements and copy number alterations known to contribute to inter-tumor or intra-tumor heterogeneity (23).

So far, *in vitro* and *in vivo* model systems have been used to study the oncologic mechanisms underlying prostate cancer pathology. However, as mentioned above, conventional *in vitro* models might be clinically unreliable due to several critical limitations. Most *in vitro* studies have been performed using cell lines such as LNCaP, 22Rv1, PC3, or DU145. However, most cells cannot represent real prostate cancer tissues because they do not have an intact AR signaling pathway. In addition, 2D culture systems could not encompass the complex tumor microenvironment of prostate cancer (5, 6). Moreover, prostate cancer cell line model does not reflect the heterogeneity of prostate cancer. For these reasons, prostate cancer organoid has been developed as a suitable model system to overcome these limitations (13, 24). Prostate cancer organoids can recapitulate

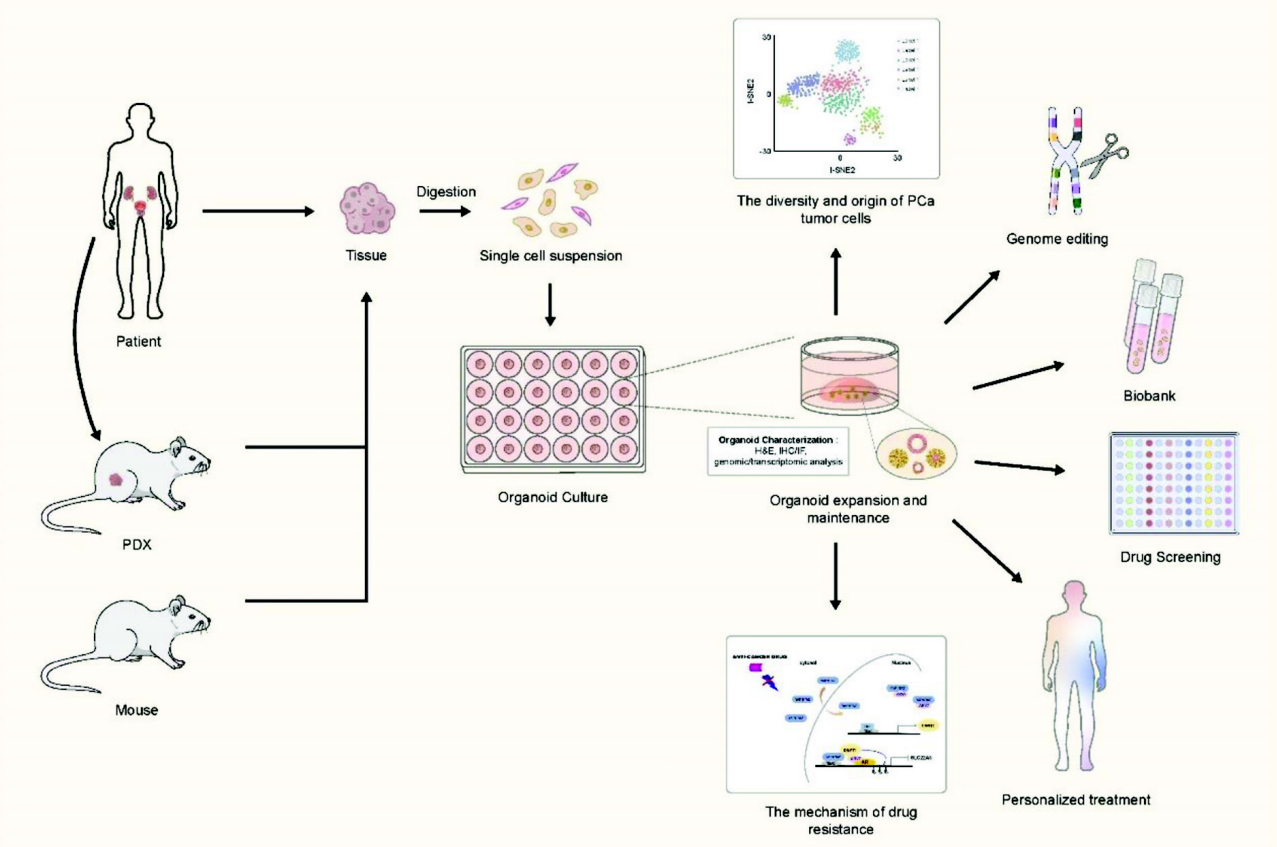

Fig. 1. Generation and applications of patient-derived tumor organoid (PDTO) from kidney or prostate cancer tissues. Kidney or prostate cancer tissues of patients are isolated, mechanically minced and then enzymatically digested. The dissociated cell suspension is strained to remove clumpy tissues, washed and embedded within matrigel to form organoids. To compare the diversity and similarity between *in vivo* tissues and organoids or to analyze a distinct inter-/intratumoral-heterogeneity at the genomic level, genomic/or transcriptomic analysis assessed a degree of variance or similarity of organoids corresponding parental tissues from cancer patients. Genome editing by using CRISPR Cas9 is employed in organoids. Prostate cancer or kidney cancer organoids can be used for preclinical models, such as anti-cancer drug screening, or biobanks.

organ-like structures. They possess the AR signaling pathway with diverse heterogeneity (24). In fact, adult prostate consists of heterogeneous populations of cells including basal, luminal, and rare neuroendocrine cells. The potential of prostate cells to gain stemness characteristic, alteration in androgen receptor, or a certain cell that subsequently undergoes clonal expansions can give rise to heterogeneous prostate cancer like androgen independence.

Gao *et al*. (25) first established seven metastatic prostate cancer organoids and demonstrated that prostate cancer organoids could recapitulate genetic features such as copy number and mutation profiles commonly found in prostate cancer. Additionally, prostate cancer organoids are genetically highly consistent with original cancer. They can be stably maintained for a long-time. Jarno *et al*. (26) first developed organoids derived from a small piece of biopsy tissue from patients with metastatic prostate cancer. They established specific protocols for the growth of both luminal and basal prostatic epithelial lineages.

To recapitulate mini organ-like structures and to model organ development or disease for treatment, a suitable medium including growth factors or chemicals can be used to efficiently and successfully culture prostate cancer organoids. Organoid formation also requires the growth factors. Human tissues-derived organoids can be cultured with different factors including FGF10, FGF2, PGE2, nicotinamide, and SB202190 for the efficient organoid formation (26). The general organoid culture method uses a universal organoid medium based on DMEM/F12 medium containing epidermal growth factor (EGF), Noggin, and Wnt agonist R-spondin-1 (27). Additionally, culturing human prostate cancer organoids requires lymphoma kinase (ALK) 3/4/5 inhibitor A83-01, dihydrotestosterone (DHT), fibroblast growth

factor-10 (FGF10), fibroblast growth factor-2 (FGF2), prostaglandin E2 (PGE2), nicotinamide, and p38 inhibitor SB202190, nacetylcysteine, B27 supplement, and Rho kinase inhibitor Y-27632 (26, 28). However, culturing murine-derived prostate cancer organoids does not need FGF10, FGF2, PGE2, nicotinamide, or SB202190. Basically, prostate cancer organoids can be stably formed within two weeks (26). Each growth factor has its own functions when culturing prostate cancer organoids. R-Spondin-1 and Noggin can promote the formation and expansion of organoids by activating Wnt signaling and bone morphogenetic protein (BMP) signaling pathways, respectively (28, 29). Addition of EGF can make prostate cancer organoids lose sensitivity to androgen resistance, meaning that elimination of EGF is needed for pharmacological studies on sensitivities of prostate cancer organoids to anti-androgen receptor drugs (30). FGF2, PGE2, nicotinamide, B27 supplement, and transforming growth factor beta (TGF-b) inhibitor A83-01 can promote the proliferation of prostate cancer organoid cells for a long-term culture (31). SB202190 can improve the stemness characteristic ability of prostate organoids (28). DHT can increase the growth rate of organoid cells with activation of androgen receptor and FGF10 signaling pathway (28). Rho kinase inhibitor Y-27632 can promote the proliferation of epithelial cells and inhibit the apoptosis of single stem cells (32). Y-27632 is added to stem cells when organoids are digested into single cells. N-acetylcysteine is an antioxidant scavenger of ROS (reactive oxygen species). It can help cells proliferate (33). These components are basically required for a long-term culture of prostate cancer organoids.

IDENTIFICATION OF ORIGIN IN PROSTATE CANCER USING PDTO MODEL

Identifying prostate cancer cells' origin gives an important clue to discover the pathogenesis of prostate cancer initiation. The differentiating process of cells from human prostate cancer tissues can be simulated in prostate cancer organoids. It can help us identify cells of origin that triggers tumorigenesis. Normal/or cancer stem cells are differentiated into CK5+ basal cells and CK8 luminal cells in PDTOs (34). CK5+ basal cells and CK8+ luminal cells reside the outer layer and the inside of prostate organoids, respectively (28). Therefore, the origin of prostate cancer can be identified according to molecular subtypes during the formation of organoids. The luminal type prostate cancer featured by atypical gland lumens originates from luminal cells, whereas basal cells are able to transform in prostate cancer, leading to tumorigenesis (35). On the other hand, a study reported that both luminal and basal cells can be cells of origin to initiate prostate cancer by overexpressing Myc proto-oncogene and activated Akt, respectively (36). Supporting this result, luminal cells overexpressing Myc proto-oncogene and activated Akt show well-differentiated tumor, whereas basal cells-derived organoids are more aggressive, meaning that both cells are capable of initiating cancer, although their features are different (37). Additionally, castration-resistant cells expressing NKX3-1 reside in the luminal layer of ADT-treated prostate cancer. These luminal cancer stem cells (CD38-low luminal cells) can initiate prostate cancer and generate both luminal and basal cells in the organoids (38). Thus, treatment responses to anti-cancer drugs might greatly vary among different human prostate cancer organoid lines. As an example, Pappas *et al*. reported that MSKPCA2 organoid lines expressing androgen receptor and luminal marker (CK8) were inhibited by anti-androgen agents, whereas MSKPCA3 organoids showed resistance to anti-androgen agents and expressed CK5, a basal cell marker (39). Like these, identification of cells of origin can help us understand the underlying mechanisms of the initiation, heterogeneity, and progression of human prostate cancer.

KIDNEY CANCER ORGANOIDS

Kidney cancers have several histologic subtypes, including clear cell, papillary, chromophobe, and collecting duct subtypes. Renal cell carcinoma (RCC) accounts for approximately 90% of all kidney cancers (40). As previously mentioned, there are differences between cell culture or PDX modeling and cancer patients' tissues depending on heterogeneous trait, tumor microenvironment, and genetic landscape (41, 42). Many scientists have contributed to the establishment of kidney cancer organoid culture. Interestingly, normal kidney cells show greater proliferation than tumor cells in 2D cells and organoid formation than other tissues (43, 44). As disadvantages of *in vitro* models of prostate cancer cells, renal cancer cells also have limitations such as decline in proliferation for long-term maintenance and the lack of heterogeneities or tumor microenvironments (45). Na *et al*. have established PDTO culture systems directly from surgical resected clear cell RCC tissues and validated that PDTO could preserve the morphology and biomarker expression of parental tumors (46). Grassi *et al*. have also cultured normal kidney organoids and RCC PDTO from surgically resected tissues (44). Methodologies and basic medium components of RCC organoids are similar to them of prostate cancer organoids. As an example, Annika *et al*. have developed clear cell RCC organoids based on the methods of prostate cancer and bladder cancer organoids (47). They added amphotericin to RCC organoids' culture medium which included heparin, bFGF, B27, and N-acetylcysteine for expansion and long-term cultures. For well-formation of *in vivo*-like structures of RCC organoids, growth factors and chemicals in medium are added to control several signaling pathways for RCC organoids. The addition of R-spondin1 into single cells after dissociation of kidney tissues can trigger the activation of Wnt signaling in order to initiate the proliferation of cancer stem cells without inducing differentiation. Thus, FGF signaling can affect the maintenance of stemness of kidney progenitor cells (47, 48). Batchelder *et al*. have successfully cultured RCC organoids from patients-derived ccRCC samples and maintained these organoids for up to 21 days (49). Like these, many

studies reported an efficient establishment of PDTO from kidney cancer samples for the recapitulation of clinical tumor *ex vivo* for a long culture period.

PRECISION MEDICINE USING KIDNEY CANCER ORGANOID

Grassi *et al*. (44) have suggested effects of a single drug or the combination of targeted drugs (such as SU11274, foretinib, cabozantinib, and the combination of lenvatinib and everolimus) on clear cell RCC organoids. Interestingly, clear cell RCC organoids responsive to SU11274 and foretinib show reduced expression levels of pAKT and pERk, which are important for regulating cell proliferation and arrest. However, only foretinib could sustainably induce cleaved-Caspase 3 activation, resulting in apoptosis of clear cell RCC organoids. Like these, RCC organoids can be applied to study targeted therapeutic drugs and their mechanisms. Sun *et al*. have established human urine stem cells (USC)-derived kidney organoids and demonstrated that they show similar histological features and specific gene expression (50). Specific proximal tubule markers Aquaporin-1, erythropoietin, Podocin, and Synaptopodin are expressed in USC-organoids, suggesting that such organoids can mimic part of kidney's structure and function. Using kidney organoids as a pre-clinical model, it has been demonstrated that expression of GGT (gamma-glutamyltansferases) in proximal tubules is essential to the γ-glutamyl cycle in the detoxification of xenobiotics (50, 51). From these results, kidney organoid formation and GGT assay can be used to assess patients' kidney functions. These organoids can also be used to assess drug sensitivity and tolerance.

APPLICATION OF PATIENT-DERIVED ORGANOIDS IN PROSTATE AND KIDNEY CANCERS: DRUG SCREENING, DRUG RESISTANCE MECHANISM, AND GENOMIC/TRANSCRIPTOMIC PROFILING

Patient-derived prostate cancer organoids can be used as a platform to perceive precision medicine by performing drug screening using individual patient samples (51) and to study mechanisms underlying drug resistance. Importantly, the ability to identify drugs showing high clinical effectiveness prior to clinical trials could enhance the efficiency of translational research into clinical practice. Gao *et al*. have successfully established prostate cancer organoids for *in vitro* and *in vivo* screening of various anti-cancer drugs (25). They evaluated efficacies of three anti-cancer drugs, enzalutamide (the next-generation anti-androgen) and two phosphoinositide 3 kinase pathway inhibitors, BKM-120, and everolimus and found that AR amplification, PIK3R1 mutation, and PTEN loss caused high sensitivity to enzalutamide. Prostate cancer organoids harboring SPOP-W131R mutation show resistance to BET inhibitors-induced cell growth arrest and apoptosis via stabilization of BET protein and activation of AKT-mTORC1 signaling (52). Furthermore, Yan *et al*. have evaluated anti-cancer effects of NEO2734 (novel BET-CBP/p300 dual inhibitor) in organoids expressing SPOP mutation from prostate cancer patients (53) and found that treating these organoids with NEO2734 could result in BET inhibitor (JQ1)-resistant SPOP mutant prostate cancer organoids with drug sensitivity (53). Therefore, PDTO can be used to screen drugs and elucidate mechanisms of drug resistance.

Using cancer patient samples, advances in high-throughput sequencing technologies have enabled us to recapitulate molecular diversity, heterogeneity, and genetic landscape of cancer. However, due to the difficulty of accessing patient tissues multiple times for different experiments, organoid models can be important alternatives for genomic or transcriptomic research. Comparison cancer organoids with corresponding human tissues should be performed to faithfully reflect biological characteristics and genetic diversity in patient tissues. Single-cell sequencing is a more powerful method for assessing the quality of cancer organoids in comparison with patient' tissues than conventional bulk sequencing. For example, Song *et al*. (54) have performed single-cell RNA sequencing analysis using prostate cancer biopsies and patient-derived organoid to characterize prostate cancer tissues and organoids. They found that prostate cancer tissues contained more AR-responsive club cells usually found in normal tissues than prostate cancer organoids which also harbored club cells observed in tumor (54). McCray *et al*. (55) have compared primary prostate tissues with organoids from the same patient's specimen by single-cell RNA sequencing and identified epithelial stem population expressing high levels of Keratin 13 mRNA in both patient tissues and prostate organoids. These results suggest that transcriptomic profiles at the single cell level allow us to characterize prostate cancer organoids as suitable representative *in vitro* models instead of patient tissues.

With accumulating data of transcriptomic and genomic sequencing using prostate cancer tissues and PDTO, androgen-independent prostate cancer has been found to have three general mechanisms involved in drug resistance (39). The first is by activating mutations that result in restoration of androgen receptor signaling (56). The second is by activating bypass signaling pathways to escape from androgen receptor inhibitor such as activating glucocorticoid receptor to compensate for the loss of androgen receptor signaling (57). The third resistance mechanism involves lineage plasticity, in which cancer cells can acquire resistance by switching a fate of cell type from a drug target-dependent cell type to a drug target-independent one (58). Furthermore, transcriptomic analyses of metastatic CRPC (mCRPC) tissues, mCRPC organoids, and patient-derived xenografts have been performed to be classified into a positive androgen receptor pathway, mesenchymal stem like prostate cancer, and neuroendocrine prostate cancer (59).

In case of RCC organoids, single-cell RNA sequencing provides genetic landscape of patient-derived RCC organoids to reveal intra-tumor heterogeneity (60). Boclk *et al*. have esta-

blished RCC organoids that could recapitulate intratumoral-heterogeneity confirmed by extensive DNA sequencing (61). Fendler *et al*. have characterized cancer stem cells in clear cell RCC, performed transcriptional profiling and single cell sequencing, and revealed the activation of Wnt and NOTCH signaling pathways in clear cell RCC organoids (47). Li *et al*. have cultured 33 kidney organoid lines from kidney cancer subtypes, performed single cell RNA sequencing, and provided intertumoral and intratumoral-heterogeneity in RCC (60). Kazama *et al*. (62) have developed an *ex-vivo* drug testing assay for screening drugs using PDTO of patients with RCC. They demonstrated a concordance between primary tumors and their corresponding PDTO based on histopathological and molecular characteristics by whole-exome sequencing. Taken together, genomic/transcriptomic profiles of PDTO in prostate and kidney cancers can be used to elucidate the mechanisms of drug resistance and develop drug screening assays or pathologic and molecular characteristics recapitulating the cancer in a patient.

CONCLUSIONS

In summary, 3D organoid culture systems that can capture phenotypic and molecular heterogeneity found in various organs have been developed (63). Organoids have since been applied to model various type of cancers. In this review, we concisely summarized recent advances in the establishment of PDTO models for patients with prostate cancer and kidney cancer. As a model preserving majority of the characteristics of patients' tumor, prostate and kidney cancer organoids are promising in various applications such as high-throughput drug screening, disease modeling, platform for biobanks, and personalized treatment. PDTO can overcome limitations of 2D cell lines and PDX that cannot recapitulate heterogeneity, microenvironment, interspecific genetic variation, or genetic landscape. Therefore, 3D organoid culture systems open opportunities to overcome limitations regarding heterogeneity and long-term culture of prostate and kidney cancer cells. We believe that PTDO is an essential platform for personalized medicine. It can be used as an alternative pre-clinical model for more accurate recapitulation of *in vivo* patient tumor.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

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