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ABSTRACT

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.

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 patient-derived tumor organoid (PDTO) systems, particularly in kidney and prostate cancers. Table 1 summarize advantages and disadvantages of 2D culture, patient-derived xenografts (PDX), and patient-derived tumor organoid (PDTO) systems, particularly in kidney and prostate cancers.

Table 1. Comparison of advantages and disadvantages of 2D cell lines, patient-derived xenografts (PDXs) and patient-derived tumor organoid (PDTO) models (13).

Models	Advantages	Disadvantages
2D cell lines	<ul style="list-style-type: none"> • Infinite growth • Easy handling • Highly available and cheap • Easy access to genome editing 	<ul style="list-style-type: none"> • Lack of heterogeneity • Lack of tumor microenvironment and immune system • Rare source
PDXs	<ul style="list-style-type: none"> • Retains tumor heterogeneity • Correlate with treatment responses in patients • Low contamination of normal cells • Making a metastasis model 	<ul style="list-style-type: none"> • Expensive and time-consuming • Differences in genetic interspecies • Gaps between mouse and human system • Low-throughput screening • Contamination of mouse cells in tumor microenvironment
PDTO	<ul style="list-style-type: none"> • Retains tumor heterogeneity • Preserve genomic characteristic • High-throughput screening • Applied to PDX model • Linked to treatment response in patients • Recapitulation of <i>in vivo</i> tissues 	<ul style="list-style-type: none"> • Low success rate of culture • Hard to long-term expansion • No immune systems • Contamination of normal cells

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 (Figure 1).

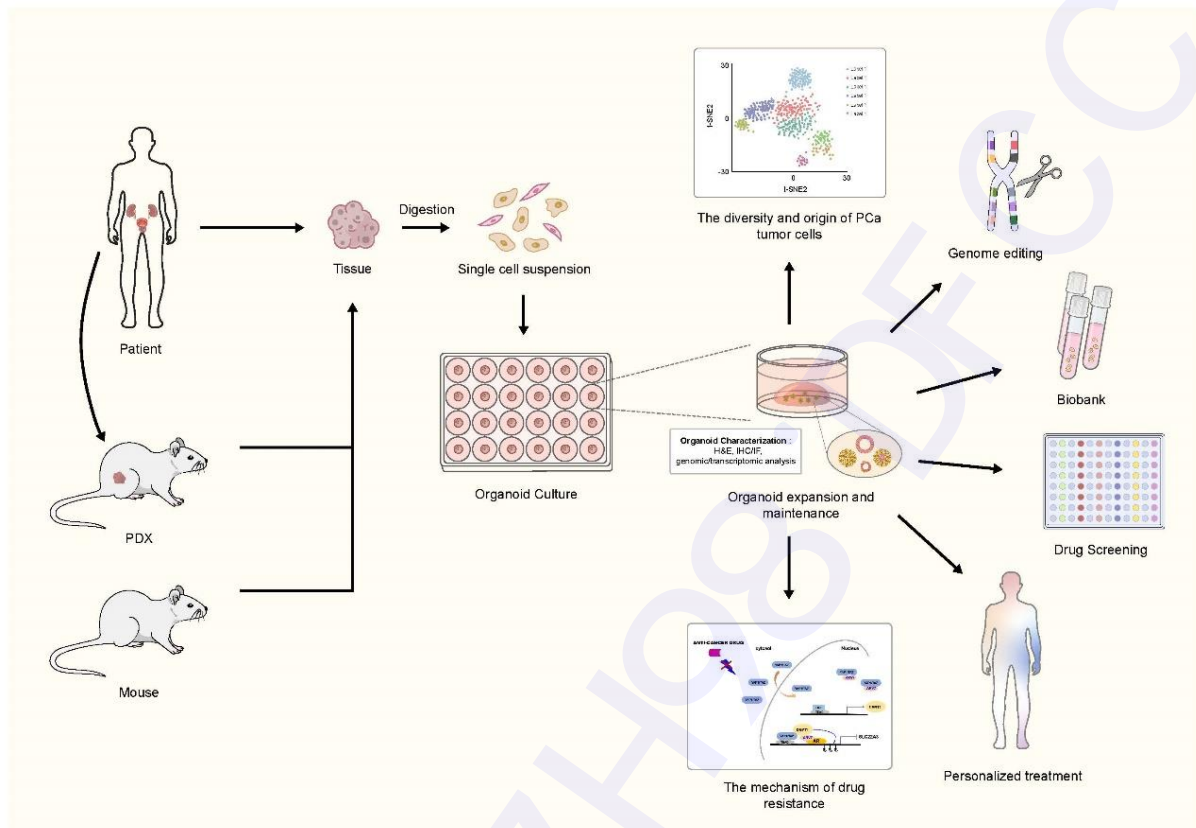


Figure 1. Generation and applications of patient-derived tumor organoid (PDTO) from kidney or prostate cancer tissues. Kidney or prostate cancer tissues of patients or mouse are isolated and mechanically minced and enzymatically digested. The dissociated cell suspension is strained to remove clumpy tissues, 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 intra/-inter tumor at the genomic level, genomic/or transcriptomic analysis assessed a degree of variance or similarity of organoids corresponding prostate cancer patients. Genome editing by using CRISPR Cas9 is employed in organoids (17). Prostate cancer or kidney cancer organoids are used for drug response, preclinical models, anti-cancer drug screening of patient's biopsy, or biobanks (18, 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 as mentioned earlier. 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 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 SB20190 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, n-acetylcysteine, 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- β) 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 PDO 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 PDOs (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 prostate 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). 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 (44). 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 (45). Grassi et al. have also cultured normal kidney organoids and RCC PDTO from surgically resected tissues (46). 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. (50) have suggested effects of a single drug or the combination of targeted

drugs (such as SU11274, foretinib, cabozantinib, and the combination of levavantinib 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 (51). 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-glutamyltransferases) in proximal tubules is essential to the γ -glutamyl cycle in the detoxification of xenobiotics (51, 52). 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 (52) 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 (53). 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 (54) and found that treating these organoids with NEO2734 could result in BET inhibitor (JQ1)-resistant SPOP mutant prostate cancer organoids with drug sensitivity (54). Therefore, PDO 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. (55) 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 (55). McCray et al.(56) 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 PDO, 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 (57). 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 (58). 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 (59). Furthermore, transcriptomic analyses of metastatic castration-resistant prostate cancer (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 (60).

In case of RCC organoids, single-cell RNA sequencing provides genetic landscape of patient-derived RCC organoids to reveal intra-tumor heterogeneity (61). Bockl et al. have established RCC organoids that could recapitulate intra-heterogeneity confirmed by extensive DNA sequencing (62). 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 inter- and intra-heterogeneity in RCC (61). Kazama et al. (63) have developed an *ex-vivo* drug testing assay for screening drugs using PDO of patients with RCC. They demonstrated a concordance between primary tumors and their corresponding PDOs based on histopathological and molecular characteristics by whole-exosome sequencing. Taken together, genomic/or transcriptomic profiles of PDO 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 (64). Organoids have since been applied to model various type of cancers. In this review, we concisely summarized recent advances in the establishment of PDO 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. PDO 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 PDO 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 declare no conflict of interest.

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. *CA Cancer J Clin.* 2021;71(1):7-33.
2. Gao D, Chen Y. Organoid development in cancer genome discovery. *Current opinion in genetics & development.* 2015;30:42-8.
3. Karantanos T, Corn PG, Thompson TC. Prostate cancer progression after androgen

deprivation therapy: mechanisms of castrate resistance and novel therapeutic approaches. *Oncogene*. 2013;32(49):5501-11.

4. Sobel R, Sadar M. Cell lines used in prostate cancer research: a compendium of old and new lines—part 2. *The Journal of urology*. 2005;173(2):360-72.
5. Namekawa T, Ikeda K, Horie-Inoue K, Inoue S. Application of prostate cancer models for preclinical study: advantages and limitations of cell lines, patient-derived xenografts, and three-dimensional culture of patient-derived cells. *Cells*. 2019;8(1):74.
6. van Bokhoven A, Varella-Garcia M, Korch C, Johannes WU, Smith EE, Miller HL, et al. Molecular characterization of human prostate carcinoma cell lines. *The Prostate*. 2003;57(3):205-25.
7. Chen H, Zhang W, Maskey N, Yang F, Zheng Z, Li C, et al. Urological cancer organoids, patients' avatars for precision medicine: past, present and future. *Cell & Bioscience*. 2022;12(1):1-22.
8. Jacob F, Salinas RD, Zhang DY, Nguyen PT, Schnoll JG, Wong SZH, et al. A patient-derived glioblastoma organoid model and biobank recapitulates inter-and intra-tumoral heterogeneity. *Cell*. 2020;180(1):188-204. e22.
9. Kopper O, De Witte CJ, Löhmussaer K, Valle-Inclan JE, Hami N, Kester L, et al. An organoid platform for ovarian cancer captures intra-and interpatient heterogeneity. *Nature medicine*. 2019;25(5):838-49.
10. Fan H, Demirci U, Chen P. Emerging organoid models: leaping forward in cancer research. *Journal of hematology & oncology*. 2019;12(1):1-10.
11. Vela I, Chen Y. Prostate cancer organoids: a potential new tool for testing drug sensitivity. *Expert review of anticancer therapy*. 2015;15(3):261-3.
12. . !!! INVALID CITATION !!! (12-14).
13. Zhou L, Zhang C, Zhang Y, Shi C. Application of organoid models in prostate cancer research. *Frontiers in Oncology*. 2021;11:736431.
14. Usui T, Sakurai M, Nishikawa S, Umata K, Nemoto Y, Haraguchi T, et al. Establishment of a dog primary prostate cancer organoid using the urine cancer stem cells. *Cancer science*. 2017;108(12):2383-92.
15. Inoue T, Terada N, Kobayashi T, Ogawa O. Patient-derived xenografts as in vivo models for research in urological malignancies. *Nature Reviews Urology*. 2017;14(5):267-83.
16. Xu R, Zhou X, Wang S, Trinkle C. Tumor organoid models in precision medicine and investigating cancer-stromal interactions. *Pharmacology & therapeutics*. 2021;218:107668.
17. Gopal S, Rodrigues AL, Dordick JS. Exploiting CRISPR Cas9 in three-dimensional stem cell cultures to model disease. *Frontiers in Bioengineering and Biotechnology*. 2020;8:692.
18. Li Y, Tang P, Cai S, Peng J, Hua G. Organoid based personalized medicine: from bench to bedside. *Cell Regeneration*. 2020;9(1):1-33.
19. Botti G, Di Bonito M, Cantile M. Organoid biobanks as a new tool for pre-clinical

validation of candidate drug efficacy and safety. *International Journal of Physiology, Pathophysiology and Pharmacology*. 2021;13(1):17.

20. Litwin MS, Tan H-J. The diagnosis and treatment of prostate cancer: a review. *Jama*. 2017;317(24):2532-42.
21. Conteduca V, Oromendia C, Eng KW, Bareja R, Sigouros M, Molina A, et al. Clinical features of neuroendocrine prostate cancer. *European journal of cancer*. 2019;121:7-18.
22. Chandrasekar T, Yang JC, Gao AC, Evans CP. Mechanisms of resistance in castration-resistant prostate cancer (CRPC). *Translational andrology and urology*. 2015;4(3):365.
23. . !!! INVALID CITATION !!! (21, 23, 24).
24. Wyatt AW, Mo F, Wang K, McConeghy B, Brahmbhatt S, Jong L, et al. Heterogeneity in the inter-tumor transcriptome of high risk prostate cancer. *Genome biology*. 2014;15(8):1-14.
25. Gao D, Vela I, Sboner A, Iaquinta PJ, Karthaus WR, Gopalan A, et al. Organoid cultures derived from patients with advanced prostate cancer. *Cell*. 2014;159(1):176-87.
26. Drost J, Karthaus WR, Gao D, Driehuis E, Sawyers CL, Chen Y, et al. Organoid culture systems for prostate epithelial and cancer tissue. *Nature protocols*. 2016;11(2):347-58.
27. Sato T, Vries RG, Snippert HJ, Van De Wetering M, Barker N, Stange DE, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature*. 2009;459(7244):262-5.
28. Karthaus WR, Iaquinta PJ, Drost J, Gracanin A, Van Boxtel R, Wongvipat J, et al. Identification of multipotent luminal progenitor cells in human prostate organoid cultures. *Cell*. 2014;159(1):163-75.
29. Cook C, Vezina CM, Allgeier SH, Shaw A, Yu M, Peterson RE, et al. Noggin is required for normal lobe patterning and ductal budding in the mouse prostate. *Developmental biology*. 2007;312(1):217-30.
30. Cheaito K, Bahmad HF, Jalloul H, Hadadeh O, Msheik H, El-Hajj A, et al. Epidermal growth factor is essential for the maintenance of novel prostate epithelial cells isolated from patient-derived organoids. *Frontiers in cell and developmental biology*. 2020:1253.
31. Kato M, Ishii K, Iwamoto Y, Sasaki T, Kanda H, Yamada Y, et al. Activation of FGF2-FGFR signaling in the castrated mouse prostate stimulates the proliferation of basal epithelial cells. *Biology of reproduction*. 2013;89(4):81, 1-10.
32. Liu X, Ory V, Chapman S, Yuan H, Albanese C, Kallakury B, et al. ROCK inhibitor and feeder cells induce the conditional reprogramming of epithelial cells. *The American journal of pathology*. 2012;180(2):599-607.
33. Khanna S, Mitra S, Lakhera PC, Khandelwal S. N-acetylcysteine effectively mitigates cadmium-induced oxidative damage and cell death in Leydig cells in vitro. *Drug and Chemical Toxicology*. 2016;39(1):74-80.
34. Maitland NJ, Frame FM, Polson ES, Lewis JL, Collins AT. Prostate cancer stem cells: do they

have a basal or luminal phenotype? *Hormones and Cancer*. 2011;2(1):47-61.

35. . !!! INVALID CITATION !!! (29, 36).

36. . !!! INVALID CITATION !!! (37, 38).

37. Park JW, Lee JK, Phillips JW, Huang P, Cheng D, Huang J, et al. Prostate epithelial cell of origin determines cancer differentiation state in an organoid transformation assay. *Proceedings of the National Academy of Sciences*. 2016;113(16):4482-7.

38. Wang X, Julio MK-d, Economides KD, Walker D, Yu H, Halili MV, et al. A luminal epithelial stem cell that is a cell of origin for prostate cancer. *Nature*. 2009;461(7263):495-500.

39. Pappas KJ, Choi D, Sawyers CL, Karthaus WR. Prostate organoid cultures as tools to translate genotypes and mutational profiles to pharmacological responses. *JoVE (Journal of Visualized Experiments)*. 2019(152):e60346.

40. Hsieh JJ, Purdue MP, Signoretti S, Swanton C, Albiges L, Schmidinger M, et al. Renal cell carcinoma. *Nature reviews Disease primers*. 2017;3(1):1-19.

41. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl j Med*. 2012;366:883-92.

42. Kuroda N, Tanaka A. Recent classification of renal epithelial tumors. *Medical molecular morphology*. 2014;47(2):68-75.

43. . !!! INVALID CITATION !!! (53, 54).

44. Gedye C, Sirskyj D, Lobo NC, Meens J, Hyatt E, Robinette M, et al. Cancer stem cells are underestimated by standard experimental methods in clear cell renal cell carcinoma. *Scientific reports*. 2016;6(1):1-13.

45. Na JC, Kim J-H, Kim SY, Gu Y-R, Jun D-Y, Lee HH, et al. Establishment of patient-derived three-dimensional organoid culture in renal cell carcinoma. *Investigative and clinical urology*. 2020;61(2):216-23.

46. Grassi L, Alfonsi R, Francescangeli F, Signore M, De Angelis M, Addario A, et al. Organoids as a new model for improving regenerative medicine and cancer personalized therapy in renal diseases. *Cell Death Dis* 10: 201. 2019.

47. Fendler A, Bauer D, Busch J, Jung K, Wulf-Goldenberg A, Kunz S, et al. Inhibiting WNT and NOTCH in renal cancer stem cells and the implications for human patients. *Nature communications*. 2020;11(1):1-16.

48. Bauer D, Birchmeier W, Fendler A. Establishment of kidney cancer organoid cultures. 2020.

49. Batchelder CA, Martinez ML, Duru N, Meyers FJ, Tarantal AF. Three dimensional culture of human renal cell carcinoma organoids. *PLoS One*. 2015;10(8):e0136758.

50. Grassi L, Alfonsi R, Francescangeli F, Signore M, De Angelis ML, Addario A, et al. Organoids as a new model for improving regenerative medicine and cancer personalized therapy in renal diseases. *Cell death & disease*. 2019;10(3):1-15.

51. Sun G, Ding B, Wan M, Chen L, Jackson J, Atala A. Formation and optimization of three-dimensional organoids generated from urine-derived stem cells for renal function in vitro. *Stem cell research & therapy*. 2020;11(1):1-12.
52. Wang S, Gao D, Chen Y. The potential of organoids in urological cancer research. *Nature Reviews Urology*. 2017;14(7):401-14.
53. Zhang P, Wang D, Zhao Y, Ren S, Gao K, Ye Z, et al. Intrinsic BET inhibitor resistance in SPOP-mutated prostate cancer is mediated by BET protein stabilization and AKT-mTORC1 activation. *Nature medicine*. 2017;23(9):1055-62.
54. Yan Y, Ma J, Wang D, Lin D, Pang X, Wang S, et al. The novel BET-CBP/p300 dual inhibitor NEO2734 is active in SPOP mutant and wild-type prostate cancer. *EMBO molecular medicine*. 2019;11(11):e10659.
55. Song H, Weinstein HN, Allegakoen P, Wadsworth MH, Xie J, Yang H, et al. Single-cell analysis of human primary prostate cancer reveals the heterogeneity of tumor-associated epithelial cell states. *Nature communications*. 2022;13(1):1-20.
56. McCray T, Moline D, Baumann B, Vander Griend DJ, Nonn L. Single-cell RNA-Seq analysis identifies a putative epithelial stem cell population in human primary prostate cells in monolayer and organoid culture conditions. *American journal of clinical and experimental urology*. 2019;7(3):123.
57. Robinson D, Van Allen EM, Wu Y-M, Schultz N, Lonigro RJ, Mosquera J-M, et al. Integrative clinical genomics of advanced prostate cancer. *Cell*. 2015;161(5):1215-28.
58. Arora VK, Schenkein E, Murali R, Subudhi SK, Wongvipat J, Balbas MD, et al. Glucocorticoid receptor confers resistance to antiandrogens by bypassing androgen receptor blockade. *Cell*. 2013;155(6):1309-22.
59. Mu P, Zhang Z, Benelli M, Karthaus WR, Hoover E, Chen C-C, et al. SOX2 promotes lineage plasticity and antiandrogen resistance in TP53-and RB1-deficient prostate cancer. *Science*. 2017;355(6320):84-8.
60. Han H, Wang Y, Curto J, Gurrapu S, Laudato S, Rumandla A, et al. Mesenchymal and stem-like prostate cancer linked to therapy-induced lineage plasticity and metastasis. *Cell Reports*. 2022;39(1):110595.
61. Li Z, Xu H, Yu L, Wang J, Meng Q, Mei H, et al. Patient-derived renal cell carcinoma organoids for personalized cancer therapy. *Clinical and Translational Medicine*. 2022;12(7):e970.
62. Bolck HA, Corró C, Kahraman A, von Teichman A, Toussaint NC, Kuipers J, et al. Tracing clonal dynamics reveals that two-and three-dimensional patient-derived cell models capture tumor heterogeneity of clear cell renal cell carcinoma. *European urology focus*. 2021;7(1):152-62.
63. Kazama A, Anraku T, Kuroki H, Shirono Y, Murata M, Bilim V, et al. Development of patient-derived tumor organoids and a drug testing model for renal cell carcinoma. *Oncology Reports*. 2021;46(4):1-8.

64. Clevers H. Modeling development and disease with organoids. *Cell*. 2016;165(7):1586-97.