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REVIEW

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Harnessing organoid technology in urological cancer: advances and applications in urinary system tumors

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Abstract

The organoid approach preserves the intricate molecular and genetic characteristics of tumor tissues, playing a pivotal role in advancing precision oncology. This preservation enables the exploration of cancer therapies and in vitro validation of drug efficacy. Organoids have emerged as indispensable tools in the study of urological cancers, facilitating research on tumorigenesis, drug testing, and the development of therapeutic combinations. Their superiority over traditional 2D cell cultures and patient-derived xenograft (PDX) models lies in their enhanced ability to more accurately replicate the in vivo environment. Modern organoid platforms integrate 3D bioprinting, co-culture systems, microfluidics, and artificial intelligence to significantly improve the precision, scalability, and efficiency of cancer research. These integrated systems serve as powerful analytical tools, propelling the development of personalized therapies for urological malignancies. This article provides a comprehensive review of the establishment and potential of organoid technologies in treating the three major urogenital system cancers—prostate, bladder, and renal—highlighting their trajectory from basic research to clinical applications and their expanding synergy with bioengineering innovations.

Keywords Organoids, Urinary system tumors, Drug screening, Precision medicine, 3D culture

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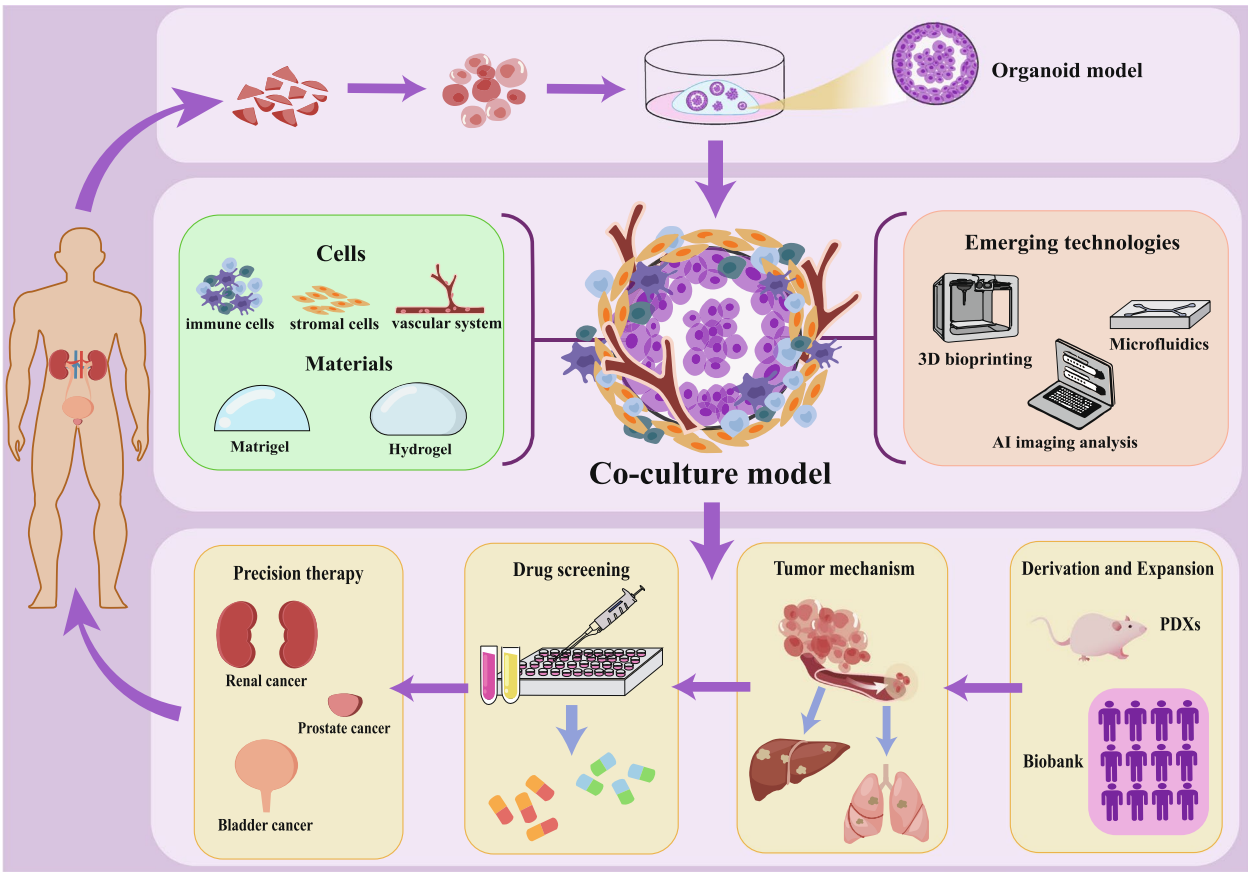
Graphical Abstract

Organoids serve as a bridge between preclinical models and personalized therapies, providing a key preclinical model for addressing urological tumors.



Introduction

Urinary cancers, including prostate, bladder, and renal cancers, are major global health concerns. Prostate cancer ranks as the second most common malignancy in men and is the fifth leading cause of cancer-related death worldwide [1]. Bladder cancer, with 82,290 new cases and 16,710 deaths reported globally in 2023, is the tenth most prevalent cancer, exerting a significant strain on healthcare systems [2]. Kidney cancer accounting for 5% of all cancer cases, is also among the top ten most frequent malignancies [3]. In total, recent reports estimate 168,560 new cases of urological cancers, with approximately 70% occurring in men, underscoring the male population's heightened vulnerability to these diseases [3]. Given the high incidence and associated mortality, developing innovative and effective research models is crucial.

Traditional models for tumor research, such as two-dimensional (2D) cell cultures and patient-derived xenografts (PDX), have been instrumental but have significant limitations. 2D cultures fail to replicate the complex three-dimensional (3D) architecture of tissues, leading to altered cellular behavior that limits their reliability. Hidalgo et al. highlighted the limitations of PDX models, including their slow establishment, high cost, and the confounding influence of the murine microenvironment on tumor behavior. While PDX models retain the in vivo architecture of tumors, they are not ideal for high-throughput drug screening due to these challenges [4]. These shortcomings highlight the urgent need for more accurate, scalable, and cost-effective alternatives for cancer research.

Organoid technology has emerged as a groundbreaking solution, addressing many of the limitations associated with conventional methods. Organoids exhibit biomimetic structural features, enabling them to retain and express essential molecular signatures and genetic characteristics of their tissue of origin over extended periods. Additionally, organoids are relatively simple

to cultivate and can be rapidly expanded, making them suitable for large-scale drug screening and gene-editing applications (Fig. 1A).

Organoids are self-organizing, 3D cell aggregates that replicate the architecture and function of in vivo organs. Derived from embryonic, induced pluripotent, or adult stem cells, organoids preserve the genetic stability and heterogeneity of the originating tissue, offering an in vitro model that mimics the complexities of human organs [5]. Their rapid and cost-effective development makes them an attractive alternative to traditional models, while maintaining the cellular diversity observed in tumor tissues.

The formation of organoids begins with isolating stem cells from the target organ or tissue, which are embedded in matrix gels or scaffolds [6]. Under appropriate conditions, these scaffolds undergo structural changes to create a 3D environment that supports cell proliferation and attachment. Growth factors and cell-specific nutrients drive the differentiation of these cells into mature organoid structures, closely mimicking their in vivo behavior [7]. Organoids typically begin forming within days and mature within a week, providing a highly efficient platform for experimental applications (Fig. 1B).

Compared to traditional 2D in vitro models, organoids offer significantly higher accuracy for clinical modeling, with success rates reaching up to 80% in high-throughput drug screening [8]. Furthermore, a recent shift in FDA policy, which eliminated the mandatory use of animal testing in drug development, underscores the growing preference for alternative research methods such as organoid technology [9]. Although challenges remain, such as the inability to fully replicate vascular and immune components, organoids have substantially advanced our understanding of cancer biology and therapeutic responses. The integration of technologies like CRISPR/Cas9 and microfluidics has further enhanced their capacity to model complex tumor microenvironments, providing valuable insights into disease mechanisms and drug efficacy testing [10, 11].

While both traditional 3D cultures and organoid systems aim to mimic aspects of in vivo tissue, there are fundamental differences. Traditional 3D cultures, such as spheroids, often consist of homogeneous cell populations aggregated in a scaffold or matrix. In contrast, organoid cultures are stem cell-derived, self-organizing systems that develop into miniature tissue-like structures, maintaining the cellular diversity, spatial architecture, and functional characteristics of the original organ or tumor.

The design of organoid models is governed by both biochemical and biophysical cues. Biochemically, growth factors and extracellular matrix (ECM) components direct cell fate and lineage specification. Biophysically, mechanical forces and spatial constraints influence morphogenesis, tissue organization, and cellular function. Together, these cues enable organoids to recapitulate complex tissue dynamics, offering a physiologically relevant platform for disease modeling and drug testing.

Organoids are now central to precision medicine, enabling the development of patient-specific disease models. These models facilitate testing various drug combinations and therapeutic strategies, predicting treatment efficacy while minimizing the risks of toxicity and adverse effects. By leveraging patient-derived genetic data, organoid platforms have also become essential for gene-editing experiments, offering new opportunities for genetic correction in disease management (Fig. 1C). In recent years, integrating organoids with advanced bioengineering tools, such as 3D bioprinting, microfluidics and artificial intelligence (AI), has emerged as a novel and promising strategy. 3D bioprinting is a technology that uses advanced manufacturing techniques to precisely arrange cells and biomaterials in three dimensions, creating tissue-like structures that more accurately replicate the tumor microenvironment (TME). This allows for better modeling of cellular interactions within tumors and enhances drug testing (see 3D Bioprinting and tumor organoids for detailed discussion).

Microfluidics involves the use of miniature channels to precisely control the environment in which cells are cultured, mimicking fluid flow and nutrient distribution found in human organs. This technique is especially useful in creating Organoids-on-a-chip, which simulates the behavior of tumors under different conditions (see Microfluidic organoids-on-a-chip for drug screening and personalized therapy for detailed discussion). AI plays a key role in analyzing large datasets generated by organoid experiments, such as imaging data. AI-driven platforms are used for automated analysis of organoid growth and drug response, improving

(See figure on next page.)

Fig. 1 Advantages of Organoid Technology and Its Applications in Precision Medicine: **A**. Organoid technology presents several key advantages over conventional two-dimensional (2D) cell culture systems and patient-derived xenografts (PDX), surpassing them in multiple aspects. **B**. Organoids can be derived from a variety of sources, including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), adult stem cells (ASCs), and tumor cells. These organoids demonstrate rapid expansion potential across multiple generations. **C**. Disease-specific organoid models can be generated from patient tumor cells, providing a platform for in-depth investigation of gene therapies'anti-tumor efficacy. Additionally, these models facilitate the evaluation of both individual and combination drug treatments, supporting clinical decision-making to personalize therapeutic strategies and improve patient outcomes

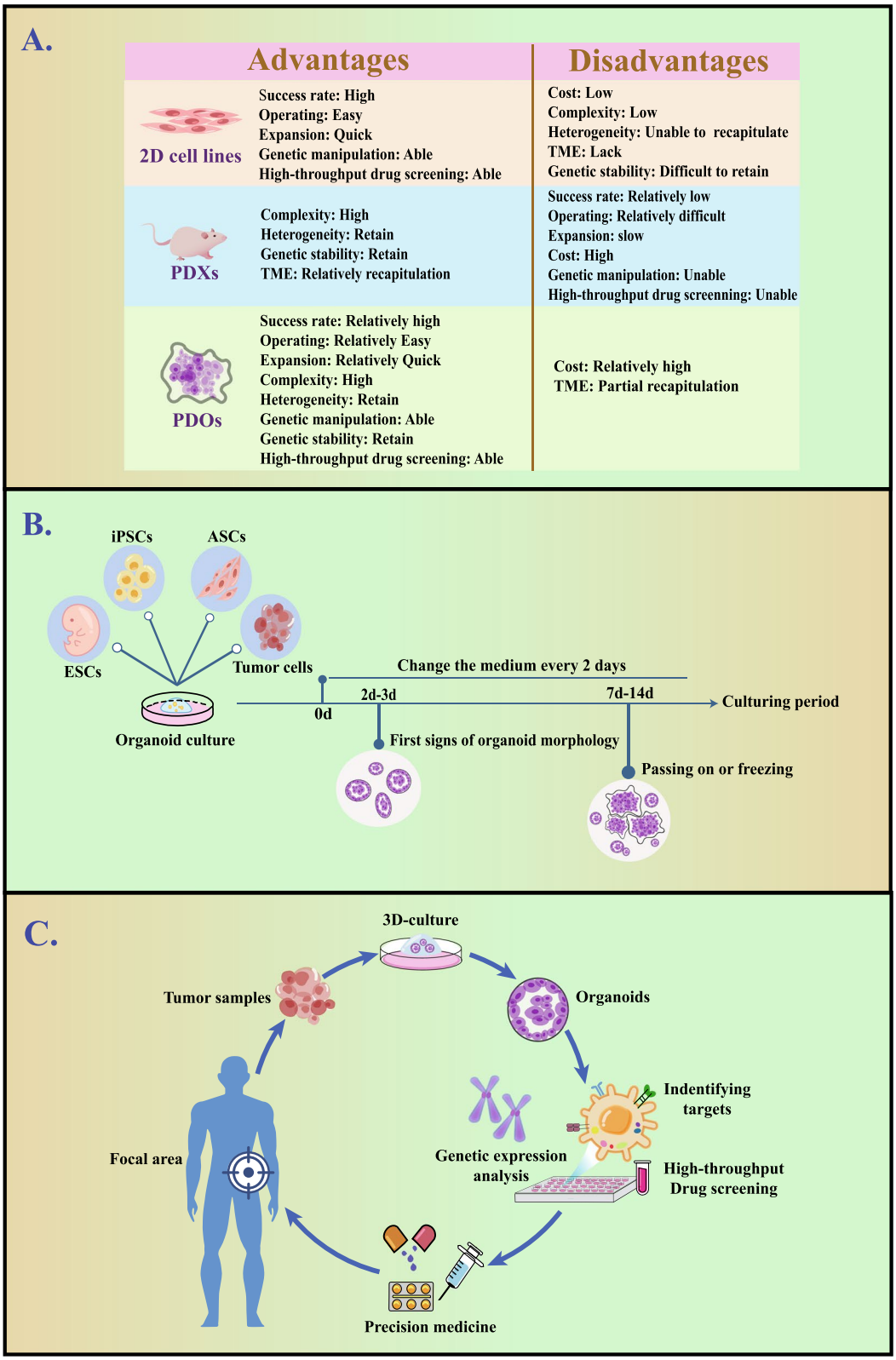


Fig. 1 (See legend on previous page.)

the speed and accuracy of research (see Genetic engineering of tumor organoids for detailed discussion).

These interdisciplinary approaches aim to address key limitations in current urological cancer models, such as lack of a dynamic tumor microenvironment, scalability, and limited predictive power for clinical translation. By employing microfluidic devices, organoids can be cultured under more physiologically relevant conditions that better mimic tissue architecture, while AI-powered image analysis enables more precise quantification of cellular behavior and drug response. This review explores the potential of combining organoid technologies with such bioengineering tools to pave the way for more accurate, high-throughput, and clinically relevant models for urological cancers.

This review not only summarizes the progress of organoid technologies in urological cancers, but also proposes a translational framework that emphasizes their potential to bridge preclinical research and personalized medicine. By introducing a novel classification of organoid systems based on their clinical readiness, and by evaluating the integration of modern tools such as microfluidics, immune co-cultures, and AI, we aim to provide a forward-looking perspective on how organoid platforms can be optimized for real-world clinical application.

Establishment and application of urinary system tumor organoids

The development of the urological tumor organoid platform is divided into four aspects: isolation of tumor cells from patients; 3D culture by scaffolding materials such as Matrigel; validation and characterization by various techniques such as hematoxylin and eosin staining, immunohistochemistry, immunofluorescence, gene profiling, and flow cytometry; and finally, incorporation of validated organoid into organoid biobanks for molecular research, drug discovery, and therapeutic evaluation of precision medicine, tumorigenesis and development. Finally, the validated organoids will be incorporated into organoid biobanks for use in precision medicine, molecular research on tumorigenesis and development, drug discovery and efficacy assessment (Fig. 2).

Prostate cancer organoids

The development of organoid technology has made significant strides since the pioneering work of Sato et al. in 2009, who first established intestinal organoids from Lgr5(+) stem cells [6]. This success laid the groundwork for later advances in cultivating organoids from prostate cancer tissue. Gao et al. were among the first to develop prostate cancer organoids from tumor biopsies and circulating tumor cells, successfully capturing the genomic diversity of metastatic prostate cancer, including PTEN

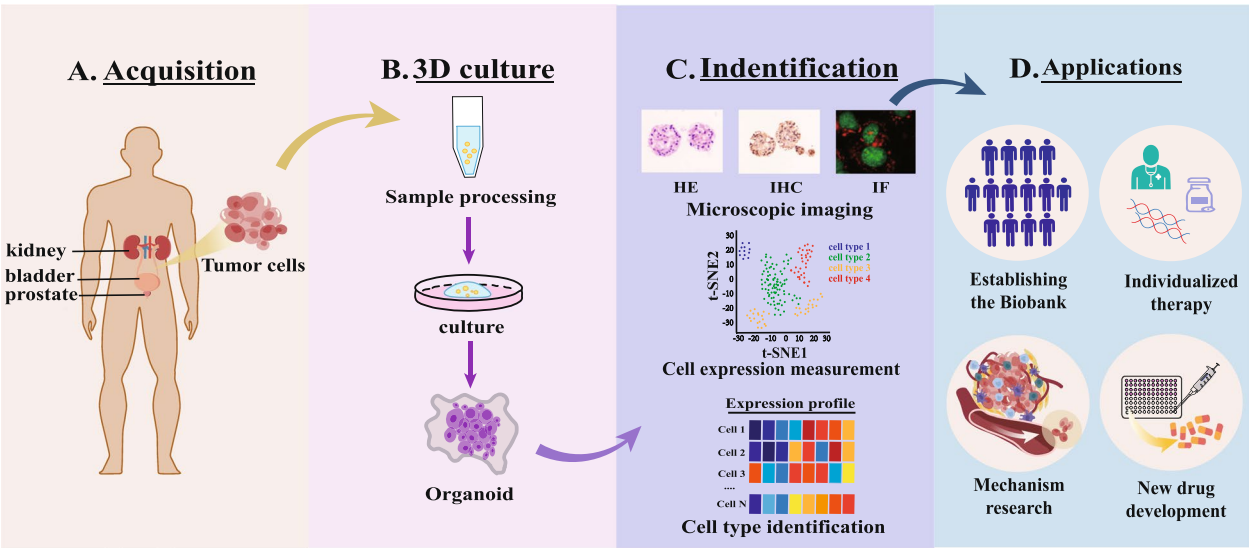


Fig. 2 Development of a Urological Tumor Organoid Platform: **A**. Tumor Cell Acquisition: The process begins with the isolation of tumor cells from the patient, which is the foundational step in establishing the organoid platform. **B**. Three-dimensional (3D) Culture: Using scaffolding materials such as Matrigel, a 3D structure is cultivated to support organoid formation. **C**. Validation and Characterization: The authenticity of the cultured organoids is confirmed through various techniques, including hematoxylin and eosin (H&E) staining, immunohistochemistry (IHC), immunofluorescence (IF), genetic profiling, and flow cytometry analysis. **D**. Applications: These validated organoid models have wide-ranging applications, including their integration into organoid biobanks, use in precision medicine, molecular studies of tumorigenesis and progression, drug discovery, and the evaluation of therapeutic efficacy

deletions, TMPRSS2-ERG fusions, and SPOP mutations [12]. Drost et al. demonstrated that prostate cancer organoids preserve the key features of the primary tumor, including functional androgen receptor signaling, which is critical for prostate cancer growth. These organoids not only maintain the genomic fidelity of the original tumor but also offer a reproducible and scalable platform for functional assays. Importantly, organoids can be genetically manipulated to study specific tumor-driving mutations, something that PDX models or standard 2D cultures cannot replicate as efficiently [13]. These organoids effectively replicate the heterogeneity of prostate cancer, providing a powerful tool for studying disease mechanisms and therapeutic responses.

Research using prostate cancer organoids has revealed critical insights into tumorigenesis. Studies, such as those by Karthaus et al., have identified luminal progenitor cells as key contributors to prostate cancer development [14]. By closely mimicking the native prostate architecture, luminal cell-derived organoids have advanced our understanding of cancer initiation and progression. Additionally, prostate cancer organoid models have been pivotal in evaluating androgen receptor (AR) signaling, which is central to prostate cancer pathogenesis [15]. Androgen deprivation therapy (ADT) is widely used to treat prostate cancer, but resistance mechanisms, particularly in castration-resistant prostate cancer (CRPC), remain a major clinical challenge [16].

Organoid models have become indispensable for high-throughput drug screening. Jansson et al., for example, screened 110 compounds on 15 prostate cancer organoid lines and identified HSP90 inhibitors as potent agents against prostate cancer [17]. Quantitative high-throughput imaging has also been integrated with organoid models to monitor drug responses in real-time, providing detailed insights into structural and compositional changes post-treatment [18]. Several therapeutic candidates, such as RGFP966, an HDAC3 inhibitor, and NEO2734, a BET-CBP/p300 dual inhibitor, have shown promise in prostate cancer organoid studies [19, 20] (Table 1).

Targeted therapies have also been explored using organoid models. The dual-mTOR inhibitor RapaLink-1 and the fatty acid synthase inhibitor IPI-9119 have demonstrated efficacy in inhibiting tumor growth in organoids [22, 62]. Additionally, O-GlcNAc transferase (OGT)

Table 1 Progress of urinary tumor-derived organoid in development of new therapeutic strategies in the last decade

Urinary tumor	Drug	Target	Model validation effect		Clinical translational phase	Reference
Prostate cancer			PDO growth inhibition	PDX growthinhibition		
	Skp2/Cks1 pocket inhibitor C1	Skp2/Cks1	√	√	—	[21]
	Ganetespib	HSP90	√	—	Phase II trial NCT01270880	[17]
	RGFP966	HDAC3	√	—	—	[19]
	IPI-9119	FASN	√	√	—	[22]
	RapaLink-1	mTORC1/2	√	—	—	[62]
	Pro-A	UPR	√	√	—	[24]
	OSMI-2 and AT7519	O-GlcNAc Transferase and CDK9	√	—	—	[62]
	Patitumab and U3-1402	HER3	HER3 high expression √HER3 low expression ×	HER3 high expression √ HER3 low expression ×	—	[25]
	NEO2734	BET- CBP/p300	√	√	Phase I trial in progress NCT05488548	[20]
	DS7300a	B7-H3	√	√	—	[26]
	PTUPB	AKR1C3	√	√	—	[27]
	TQB3720	Androgen receptor	√	√	Phase I NCT04853498	[28]
Renal cancer	ICG-001 and DAPT	WNT and NOTCH signaling	√	√	—	[29]
	Crizotinib	C-Met, ALK, ROS1	√	√	Phase II trial NCT01524926	[30]
	5-BDBD	P2X4R	√	—	—	[31]
Bladder cancer	NCT-502	PHGDH	√	—	—	[32]

inhibitors, when combined with CDK9 inhibitors, have shown enhanced anticancer activity in prostate cancer models [23]. Despite their success, scaling organoid models and fully replicating the complex biology of in vivo tissues remain key challenges. Future research will focus on refining culture methods and integrating advanced imaging technologies to improve clinical relevance.

Renal cancer organoids

Renal cancer organoid models have been developed from both adult and pluripotent stem cells, offering new avenues for studying kidney cancer. Early work by Lam et al. and Morizane et al. showed that renal progenitor cells could be differentiated into structures resembling nephron units [54, 63]. These kidney organoids represent key functional units of the kidney, such as podocytes and tubules. However, the development of functional vascular systems within kidney organoids remains a significant hurdle. Studies utilizing decellularized extracellular matrix (dECM) hydrogels and endothelial cell co-culture systems have advanced vascularization efforts, although challenges persist [55]. In a related study by Schutgens et al., kidney cancer organoids (tubuloids) were developed from patient-derived urine and kidney tissues. These organoids more accurately reflected the histological structure and functional behavior of renal cell carcinoma compared to 2D cell cultures. Moreover, compared to PDX models, organoids offer a scalable and efficient platform for testing drug responses in a manner that aligns more closely with the complex biology of renal cancers [56].

Renal cell carcinoma (RCC) accounts for the majority of kidney cancers, and recent advancements in organoid technology have allowed researchers to model specific RCC subtypes, including clear cell RCC (ccRCC). Na et al. successfully cultivated ccRCC organoids that retained key histopathological features of the original tumor, including lipid-rich cytoplasm and clear cell morphology 57. These organoids have been instrumental in drug screening efforts and in understanding the molecular drivers of RCC.

Given RCC's resistance to conventional therapies, organoids have been valuable in testing novel treatment approaches. Anti-VEGF therapies, multi-kinase inhibitors, and immunotherapies have all been evaluated in RCC organoids. For example, Grassi et al. used organoids to assess foretinib, a multi-kinase inhibitor, demonstrating its potential to induce apoptosis in RCC cells [58]. Organoid models have also been applied to immunotherapy research, with studies on CAR-T cells targeting CD70 and c-MET showing promising results in both in vitro and in vivo models [59]. However, the lack of immune components in current organoid models limits their utility for immunotherapy research, highlighting the need for more complex co-culture systems (experimental models that integrate multiple cell types—such as immune cells, fibroblasts, and tumor cells—into a shared environment to more accurately replicate the tumor microenvironment).

Bladder cancer organoids

Bladder cancer research has traditionally been limited by a lack of effective in vitro models. While early studies focused on culturing human urothelial cells, these models failed to fully capture the complexity of bladder cancer. Recent efforts have shifted toward organoid systems, which better replicate tumor architecture and cellular diversity. Kang et al. and Shin et al. made key advances in differentiating pluripotent stem cells into bladder urothelial cells, providing new insights into bladder cancer development and progression [60, 61]. As highlighted in Lee et al., bladder cancer organoids derived from patient samples exhibited superior genomic stability and heterogeneity compared to traditional 2D cultures. These organoids not only preserved the molecular characteristics of the primary tumors but also provided more accurate predictions of drug responses, mirroring clinical outcomes better than the monolayer cultures typically used in preclinical drug testing [33].

Bladder cancer organoid biobanks have been established to provide a diverse array of models for studying drug responses and tumor evolution. Lee et al. created a biobank of organoids derived from 22 bladder cancer patients, which has since expanded to 53 samples [33]. These organoids retain the histopathological and genetic characteristics of the original tumors, offering a valuable resource for personalized medicine and drug testing.

Bladder cancer treatment strategies are highly dependent on tumor pathology, with non-muscle-invasive bladder cancer (NMIBC) typically treated with transurethral resection and muscle-invasive bladder cancer (MIBC) requiring more aggressive approaches, such as radical cystectomy. Organoids have been used to explore new therapeutic approaches, including Sirtuin 1 activators and selective MEK inhibitors. For example, SRT1720 has shown promise in inhibiting bladder cancer organoid growth, while trametinib, a MEK1/2 inhibitor, has been effective in targeting ERK pathway activation in organoid models [34, 64].

Recent technological advances, such as microfluidic biochips and 3D bioprinting, have further expanded the utility of bladder cancer organoids. These innovations enable the creation of more physiologically relevant models that incorporate immune cells and endothelial cells, enhancing the accuracy of drug screening efforts. However, further refinement is needed to improve scalability and clinical translation.

Limitations and challenges

In the past decade, significant progress has been made in developing organoid models for urological cancers, including prostate, kidney, and bladder tumors. Organoid biobanks have been established, providing invaluable tools for studying tumor heterogeneity and drug responses. These models offer a promising platform for precision medicine, allowing for the evaluation of personalized therapeutic strategies while minimizing patient risk. Continued research will focus on overcoming current limitations, such as scaling production and incorporating immune and vascular systems, to further enhance the clinical applicability of organoid technology.

While organoid models for urological cancers have significantly advanced in recent years, there are still some limitations, for example: incomplete microenvironment, heterogeneity loss over passages, lack of standardization, throughput constraints and limited clinical validation. Recent advancements in organ-on-a-chip platforms, co-culture models, and bioengineering tools are playing a key role in overcoming these limitations, offering new pathways to enhance the relevance, scalability, and predictive accuracy of organoid models for urological cancers (Table 2, Fig. 3).

Tumor organoids and bioengineering technologies

3D bioprinting and tumor organoids

3D bioprinting has revolutionized biomedical research by allowing precise replication of complex biological tissues. This technology uses advanced manufacturing techniques, such as magnetic bioprinting and bio-inks (cell suspensions within hydrogels), coupled with computer-aided design to construct physiologically accurate structures [35]. By customizing scaffold parameters like morphology, pore size, and elasticity, 3D bioprinting replicates the tumor microenvironment (TME) with remarkable precision. These models enhance cell proliferation, migration, and nutrient delivery, offering more reliable conditions for studying tumor behavior.

One key advantage of 3D bioprinting is its ability to incorporate various biomaterials, including collagen, gelatin, and polylactic acid, which mimic the ECM. By integrating different cell types—immune cells, fibroblasts, and tumor cells—bioprinting creates complex co-culture systems that provide deeper insights into cellular interactions within the TME. This goes beyond traditional organoid cultures, offering enhanced physical and biochemical complexity for cancer research.

Recent applications of 3D bioprinting in urological cancer research include developing ex vivo models of the TME. For example, magnetic bioprinting has been used to co-culture renal cancer cells with fibroblasts, allowing a sophisticated representation of intercellular signaling [36]. Similarly, prostate cancer research has leveraged 3D bioprinting to explore interactions between cancer-associated fibroblasts (CAFs) and hyaluronic acid (HA), key drivers of tumor proliferation and metastasis. In this model, optimized bio-inks allowed the study of how CAFs and HA promote cancer cell growth, providing valuable insights into potential therapeutic targets [37].

Bladder cancer research has also benefited from bioprinting. Acoustic droplet bioprinting, for example, has enabled the rapid creation of bladder cancer organoids co-cultured with immune cells. These models have shown the ability to generate tumor-reactive T cells, pointing to the potential of 3D bioprinting in developing personalized immunotherapy models. This technology thus holds promise for translating organoid research into clinical applications, particularly in immuno-oncology.

Overall, 3D bioprinting offers a powerful tool for enhancing traditional organoid models, allowing the recreation of complex TMEs. While challenges such as scalability and reproducibility remain, the precision and biomimetic capabilities of 3D bioprinting position it as a transformative technology in preclinical cancer research.

Table 2 Limitations and solutions on organoid models of urologic cancer

Limitations	Consequences	Solutions
Incomplete Microenvironment	Inability to reproduce the tumor microenvironment	Co-culture systems, Organ-on-a-chip platforms
Heterogeneity Loss over Passages	Limiting the ability of organoids to reproduce clinical tumor complexity	Single-cell sequencing, CRISPR-Cas9 gene editing
Lack of Standardization	Differences between studies leading to urologic tumor-like organs	Develop optimized protocols
Throughput Constraints	Restricting the utility in high-content drug screening	Integration with AI-driven automated platforms and microfluidics
Limited Clinical Validation	Limiting clinical use	Development of more robust in vivo models, Integration of organoid models with the PDX systems

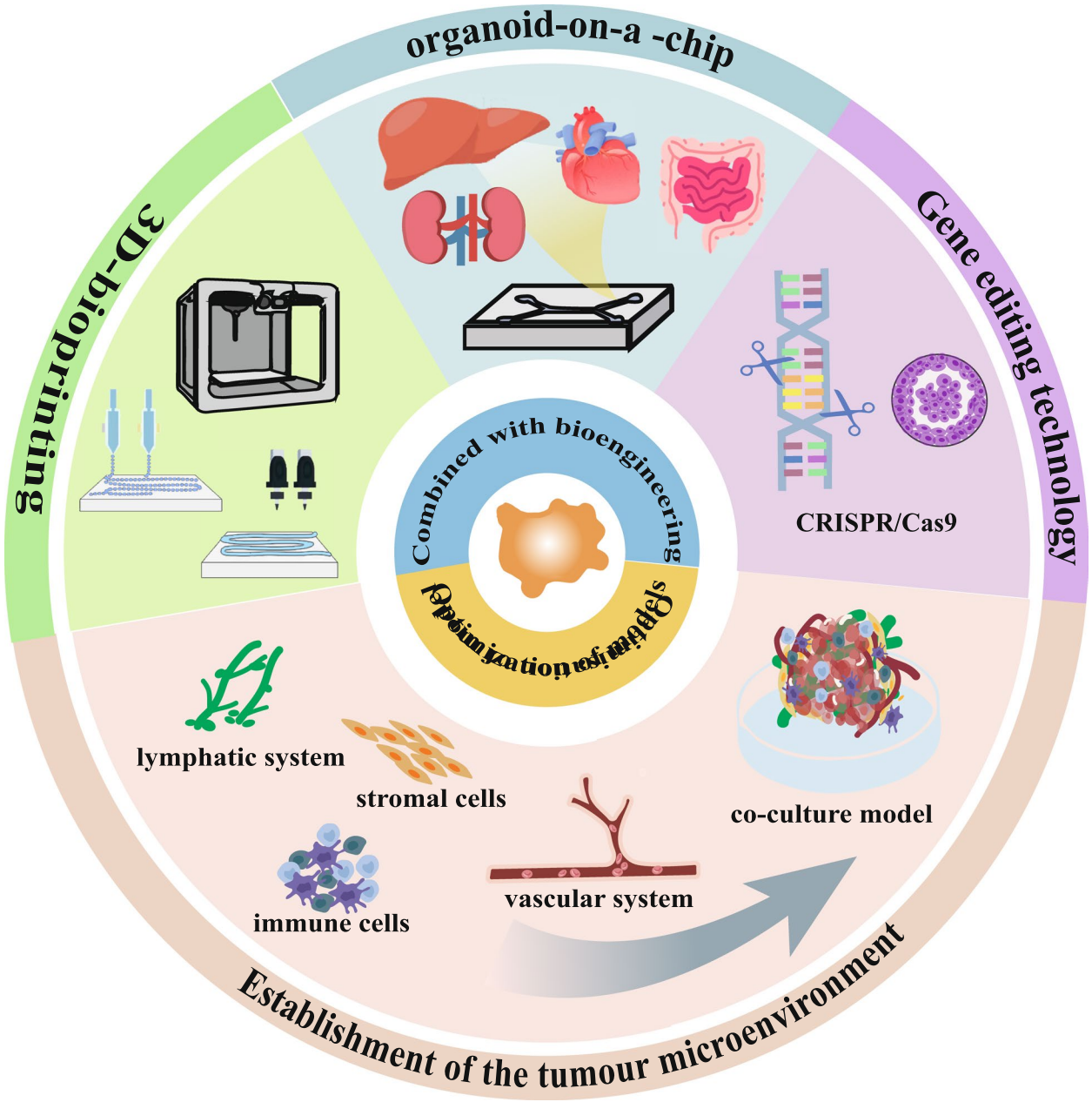


Fig. 3 Recent advancements in tumor organoid technology: bioengineering integration: the convergence of bioengineering with organoid technology has driven significant innovations. Advances in microfluidics and 3D bioprinting have enabled the construction of highly biomimetic tissue structures, both in terms of geometry and function. These innovations have led to the development of the"organoid-on-a-chip"model, which has progressed from single-organ systems to multi-organ and multi-system constructs. This approach enhances inter-system connectivity, improving the simulation of drug actions and metabolic pathways in vitro. Additionally, the incorporation of artificial intelligence (AI) allows for more precise and efficient organoid image analysis. Model Optimization: To better replicate the immune and vascular systems, and to recreate a biomimetic tumor microenvironment in vitro, researchers have introduced additional cell types, such as vascular endothelial and immune cells, into organoid models. This refinement is crucial for enhancing the physiological relevance of these models in cancer research

Microfluidic organoids-on-a-chip for drug screening and personalized therapy

Microfluidic technology, utilizing micron-scale channels, enables the creation of precise in vitro disease models by controlling the physical and chemical conditions that influence cell growth [38]. These platforms simulate in vivo environments, offering key advantages such as the ability to mimic physiological perfusion and create drug concentration gradients for detailed therapeutic testing.

Organoids-on-a-chip (OOC) systems, which integrate microfluidic technology with biological culturing methods, have greatly enhanced the study of organ functions and tumor behavior [39]. These systems allow precise manipulation of cells and fluids, mimicking the TME and enabling real-time monitoring of drug responses. By incorporating genetic and phenotypic characteristics of patient-derived tissues, OOCs hold significant promise for personalized cancer therapies.

In renal cancer research, OOCs have been used to model the TME and study drug resistance mechanisms. A recent study utilizing CXCR4 and CXCL-12 chemokines demonstrated significant changes in gene expression in renal cancer cells treated with cisplatin, offering new insights into therapeutic efficacy [40]. Bladder cancer research has also employed OOCs for drug screening. For instance, microfluidic chips have been used to evaluate the effectiveness of recombinant Bacillus Calmette-Guérin (BCG) treatments, revealing superior efficacy in novel formulations compared to traditional BCG therapy [41].

Despite these advancements, challenges remain in scaling microfluidic platforms for widespread clinical use. Improving the efficiency of microchannel construction and reducing the time required for drug response analysis are critical areas for future research. Nonetheless, as the technology evolves, microfluidic organoid systems are expected to play an increasingly important role in advancing personalized cancer therapies.

Genetic engineering of tumor organoids

Genetic engineering has become a powerful tool for investigating tumor biology, enabling researchers to introduce specific mutations into organoid models. CRISPR/Cas9 technology has revolutionized cancer research by allowing precise gene editing, facilitating the study of gene function and disease mechanisms in a controlled environment.

A landmark study using CRISPR/Cas9 corrected the CFTR gene in intestinal organoids derived from cystic fibrosis patients, demonstrating the potential of gene editing in disease modeling [42]. Since then, CRISPR/Cas9 has been widely applied to cancer research. For example, prostate cancer organoids have been engineered with TMPRSS2-ERG gene fusions, providing an accurate model for studying tumorigenesis and drug resistance [43].

Gene editing combined with organoid technology offers a valuable platform for drug discovery. Studies using CRISPR/Cas9 to manipulate genes such as JMJD6 in renal cancer organoids have identified new therapeutic targets, with inhibitors like SKLB325 showing synergistic effects when combined with traditional treatments [44]. These findings underscore the importance of organoid models in validating the efficacy of novel therapies and advancing clinical gene therapy.

However, challenges remain, particularly in managing the heterogeneity of organoid cultures. Single-organoid sequencing offers a solution by optimizing sgRNA design and improving the consistency of genetic screening outcomes. As CRISPR/Cas9 technology continues to evolve, its applications in cancer research will expand, further enhancing our understanding of disease mechanisms and guiding the development of personalized treatments.

Co-culture systems to mimic the tumor microenvironment

The tumor microenvironment (TME) is a complex network of signaling molecules, immune cells, fibroblasts, and extracellular matrix components that play a crucial role in regulating tumor growth and metastasis [45]. Co-culture systems, which incorporate these elements into organoid models, offer a more accurate representation of the TME, providing valuable insights into tumor biology.

CAR-T cell therapies, for instance, have shown promise in hematological cancers but have faced challenges in solid tumors due to the immunosuppressive nature of the TME. Co-culture models that combine CAR-T cells with chemotherapeutic agents have been shown to enhance immune cell infiltration and improve treatment outcomes, as demonstrated in prostate cancer studies [46]. Similarly, co-culture systems with macrophages have been used to investigate drug resistance in pancreatic cancer, revealing key feedback loops that contribute to treatment failure [47].

The development of vascular and lymphatic systems in organoid models is also critical for replicating the TME. Advances in microfluidic chip technology have enabled the creation of vascularized organoids, improving nutrient delivery and cellular maturation [48]. These models offer new opportunities for studying cancer progression and developing more effective therapeutic strategies.

Despite the promise of co-culture systems, limitations such as incomplete immune cell representation and immature vascular structures remain challenges. Future research will focus on refining these models to more accurately mimic the complex interactions within the TME, particularly in urological cancers.

Translational implications: from bench to bedside

The translational potential of organoid technologies lies in their ability to recapitulate patient-specific tumor biology while allowing for rapid, iterative testing of therapeutic responses. However, realizing this potential requires addressing several challenges, including standardization, scalability, and regulatory alignment.

To better contextualize the clinical maturity of organoid models, we propose a three-tier classification system:

Tier 1: Preclinical Research Models**, mainly used for studying tumor biology and drug mechanisms.
-Tier 2: Clinical Decision Support Models**, where patient-derived organoids are used to guide therapeutic choices via in vitro drug testing.
Tier 3: Predictive Therapeutic Models**, designed for direct application in clinical trials or treatment stratification, pending regulatory validation.

Recent advances in bioengineering have significantly accelerated progress along this trajectory. Microfluidic organoid-on-a-chip systems simulate dynamic tumor microenvironments; AI-powered phenotypic screening enables robust, quantitative analysis; and immune-stromal co-culture platforms restore crucial components of tumor-immune interaction. These integrated approaches enhance physiological relevance, support high-throughput testing, and enable more confident translation of findings into personalized treatment plans.

Future perspectives

The application of in vitro organoid models for the study of urinary system tumors has led to significant advancements in understanding tumor biology and identifying novel therapeutic targets. These models have provided unprecedented insights into tumor heterogeneity, drug resistance mechanisms, and the role of the tumor microenvironment (TME) in cancer progression. Despite these achievements, several technical and biological limitations still need to be addressed to fully exploit the potential of organoid technology for translational research and precision medicine.

Challenges in organoid cultivation and standardization

Organoid cultivation and standardization present several significant challenges that must be addressed for the broad applicability of organoid systems in research and clinical settings. Although organoids offer advantages over traditional models, their complexity introduces variability that can hinder reproducibility and comparability across studies. Key challenges in standardization include:

Cell Source Variability: The use of different stem cell populations (e.g., pluripotent stem cells, organ-specific adult stem cells) leads to variability in organoid morphology, growth, and functionality. For instance, variations in the genetic background or differentiation protocols can yield organoids with distinct characteristics, even when derived from the same tissue type.
Medium Composition and Culture Conditions: The culture media used for organoid maintenance can vary widely, including differences in growth factors, ECM components, and supplement formulations [49]. The absence of standardized, commercially available media leads to inconsistent results across different labs, affecting reproducibility.
Biophysical Factors: The mechanical properties of the culture environment—such as matrix stiffness, nutrient gradients, and oxygen levels—can significantly influence organoid growth and differentiation. These factors often vary between studies, contributing to differences in tissue architecture and cellular behavior.
Long-Term Cultivation and Passage Effects: Over extended passages, organoids may lose key features such as functional maturity or genetic integrity, leading to reduced reliability in long-term studies. This phenomenon underscores the need for standardized protocols to preserve organoid characteristics over time.
High-Throughput Compatibility: While organoid systems are increasingly used in drug screening and large-scale studies, they remain difficult to integrate into high-throughput workflows due to challenges in consistency and scalability. Standardized protocols for large-scale production of organoids are essential to facilitate reproducible and efficient drug testing.

Addressing these issues requires the development of unified protocols that encompass cell source selection, medium optimization, and culture system design. Additionally, advancements in automation technologies (e.g., microfluidics, 3D bioprinting) hold great promise in enabling standardized, high-throughput organoid production, reducing inter-lab variability, and improving reproducibility.

Incorporating vascular, lymphatic, and immune systems into organoid models

Current organoid models lack critical components, such as functional vasculature, lymphatic systems, and immune elements, which are crucial for replicating the dynamic interactions within the TME. The absence of these components limits the physiological relevance of organoid models and restricts their use in studying immune evasion mechanisms and the effects of immunotherapies.

Recent advances in bioengineering and microfluidic technologies have enabled the creation of vascularized organoids that more closely mimic in vivo blood perfusion and nutrient exchange. For example, vascular networks integrated into organoid cultures via microfluidic chips or 3D bioprinting have shown promise in maintaining tissue homeostasis and improving drug delivery efficiency.Ilan et al. developed a 3D human lymphatic vessel-on-chip that may provide a unique platform to explore mechanisms of lymphatic junction morphogenesis and sprouting under different flow conditions and growth factors [50]. Chen et al. presented a perfusable, hierarchical microvasculature-on-a-chip model, which preserves the ability to measure vessel permeability, and allows for analysis of flow dynamics, arrest, and extravasation of various cell types [51]. In addition, a microfluidics-based, patient-specific 'glioblastoma-on-a-Chip' microphysiological system was constructed by Cui et al. to screen personalized immunotherapy for glioblastoma patients and dissect the heterogeneous tumor immune microenvironments, which facilitate precision immuno-oncology [52].

Additionally, the development of co-culture systems incorporating immune cells, such as tumor-associated macrophages and T cells, has provided platforms for studying immune-tumor interactions and the impact of checkpoint inhibitors. Moreover, incorporating lymphatic systems into organoid models could offer new perspectives on metastatic spread and immune cell trafficking. Although the development of such complex models is still in its infancy, preliminary work using endothelial and lymphatic cell co-cultures within organoids has shown that these systems can be engineered to form functional networks. Further integration of vascular and lymphatic systems with immune components could lead to the establishment of comprehensive in vitro models that recapitulate the entire TME, enhancing the predictive power of these models for evaluating therapeutic efficacy and toxicity.

Integration of advanced technologies: 3D bioprinting, microfluidics, and Artificial Intelligence

Combining organoid technology with advanced bioengineering tools, such as 3D bioprinting and microfluidics, has the potential to revolutionize the field by creating more physiologically relevant models. 3D bioprinting allows for the spatial arrangement of multiple cell types within ECM-like scaffolds, enabling the recreation of complex tissue architectures and stromal heterogeneity. These bioprinted structures can incorporate precise gradients of cytokines, growth factors, and oxygen tension, better mimicking in vivo conditions.

Microfluidic systems, often referred to as "organs-on-chips, "can simulate interstitial flow, shear stress, and dynamic perfusion, providing a controlled environment for real-time analysis of organoid growth, differentiation, and response to therapies. For instance, the integration of microfluidic chips with renal organoids has allowed researchers to study the role of mechanical forces in kidney function and disease progression. By mimicking blood flow and waste removal, these systems can replicate organ-specific microenvironments, providing more accurate platforms for drug screening and toxicity testing.

The incorporation of AI into organoid research is another promising avenue. AI-driven platforms, such as OrganoID, are capable of analyzing complex datasets, identifying subtle morphological changes, and predicting drug responses with high precision [53]. AI can also automate image analysis, reducing observer bias and increasing throughput in large-scale studies. Moreover, the integration of AI with multi-dimensional data from organoid experiments can uncover novel insights into cellular behaviors and disease mechanisms, accelerating the discovery of new therapeutic strategies.

Future directions: building next-generation organoid models

The future of organoid technology lies in the development of next-generation models that integrate cutting-edge technologies, enhance scalability and reproducibility, and broaden their clinical relevance. Several emerging directions hold significant promise in addressing current limitations and maximizing the potential of organoid systems, particularly in the realms of personalized medicine and cancer research.

Integration of AI and machine learning

The integration of AI and machine learning (ML) technologies is poised to revolutionize organoid research. AI-driven platforms are increasingly employed to analyze large and complex datasets derived from organoid

cultures, including gene expression profiles, high-resolution imaging, and drug response assays. Machine learning models are instrumental in identifying hidden patterns within these datasets, facilitating the identification of biomarkers, predicting drug efficacy, and developing personalized treatment strategies. Additionally, AI technologies can assist in automating the monitoring of organoid growth and morphogenesis, reducing human error and enabling high-throughput screening of drug candidates (see Incorporating vascular, lymphatic, and immune systems into organoid models for further details on AI integration).

Scalability and reproducibility**

A key challenge in advancing organoid technology for clinical applications is scalability and reproducibility. To meet the demand for large numbers of organoids, particularly for patient-specific models, the optimization of automated culture systems and bioreactor technologiesis is essential. Advances in microfluidics and 3D bioprinting are already making strides toward scaling organoid production while minimizing variability between batches. Furthermore, establishing robust and standardized protocols for organoid growth and differentiation will be crucial to ensure the reproducibility necessary for large-scale drug screening and clinical applications.

Ethical and regulatory considerations

As patient-derived organoid biobanks continue to grow, addressing the associated ethical and regulatory concerns is imperative. The use of patient tissue, particularly in oncology, raises critical issues related to informed consent, privacy, and genetic data protection. Additionally, establishing comprehensive regulatory frameworks is necessary to govern the development and clinical use of organoids, ensuring their safety, efficacy, and ethical use in patient-specific therapies. Developing clear guidelines for the biobanking of organoids, including standards for storage, use, and sharing, will be essential for advancing organoid-based medicine.

Personalized organoid platforms in cancer modeling and precision medicine

The potential of personalized organoid platforms in transforming cancer modeling and precision medicine is immense. Organoids derived from individual patients can closely replicate the genetic and histological characteristics of tumors, enabling more accurate drug testing and biomarker discovery. As these platforms evolve, they will play a pivotal role in predicting treatment responses, optimizing chemotherapy regimens, and identifying drug-resistant mutations in real-time. Furthermore, personalized organoid systems could facilitate the development of organ-on-a-chip models, enabling more accurate, patient-specific predictions of treatment outcomes.

Towards clinical applications

For organoid models to be effectively translated to clinical settings, further standardization of protocols and clinical validation are required. Ongoing efforts to improve the long-term culture and cryopreservation of organoids will facilitate their storage and transport for clinical use. As more clinical data is gathered, organoid-based models will become central in the development of personalized cancer therapies and regenerative medicine applications.

To fully realize the potential of organoid models, future research should aim at overcoming existing technical limitations and expanding the scope of their applications. One promising approach is the development of the Universal Coupling Culture Array (UCCA), which integrates 3D bioprinting, microfluidics, and co-culturing technologies to simulate complex inter-organ communications. UCCA could provide new opportunities for constructing multi-tissue platforms that replicate the physiological interactions between the liver, kidney, and bladder, offering novel insights into disease progression and evaluating systemic drug effects.

Advancements in gene-editing technologies

The development of more sophisticated organoid models is also facilitated by advancements in gene-editing technologies, such as CRISPR/Cas9. These tools will allow researchers to engineer patient-specific organoids that capture genetic mutations and epigenetic modifications, enabling personalized platforms for testing therapeutic responses and optimizing treatment regimens. Such advancements pave the way for more effective precision oncology, an approach that uses the genetic, molecular, and environmental data of an individual patient's tumor to tailor cancer treatment, ensuring higher effectiveness and fewer side effects.

Integration with single-cell omics and high-resolution imaging

The future of organoid models will also be shaped by the integration of single-cell omics and high-resolution imaging technologies. Single-cell RNA sequencing, paired with spatial transcriptomics, can provide detailed maps of cellular heterogeneity and lineage tracing within organoids, offering deeper insights into tumor evolution and clonal dynamics. Additionally, high-resolution imaging techniques, such as light-sheet microscopy, will enable real-time monitoring of cellular behaviors within organoids, enhancing the study of invasion, metastasis, and drug responses at a single-cell resolution.

AI-powered phenotypic screening

In the context of urological cancer models, the integration of AI technologies will enable phenotypic screening that allows for the precise quantification of cellular behavior in response to various treatments. This will facilitate personalized therapeutic strategies based on the unique tumor profiles of individual patients.

Co-culture systems and organoid-on-a-chip models

Incorporating co-culture systems will help to restore missing components of the tumor microenvironment, such as immune and stromal cells, which are vital for improving the physiological relevance of organoid models. These systems will enhance the prediction of therapeutic responses and tumor progression. Additionally, organoid-on-a-chip platforms will provide more accurate models of organ-organ interactions and mechanical cues, advancing our understanding of tumor growth and metastasis.

Gene editing in organoids for clinical relevance

The CRISPR-Cas9 gene-editing technology will continue to be a crucial tool for modeling genetic mutations and drug resistance mechanisms in organoids, thereby improving the clinical relevance of these models. This will enable more accurate simulations of cancer biology and facilitate the development of targeted therapies.

These innovations hold immense promise for advancing organoid-based research in urological cancers and ensuring the successful translation of preclinical findings into clinical applications. Moving forward, the integration of AI, bioengineering tools, and gene-editing technologies will be instrumental in overcoming the current limitations of organoid models, paving the way for more personalized, effective, and clinically relevant cancer therapies.

Conclusion

The integration of organoid technology with cutting-edge bioengineering and computational tools has the potential to transform our understanding of urinary system tumors. While current limitations, such as inefficient culturing and the absence of key microenvironmental components, present significant challenges, ongoing research is poised to overcome these barriers. By leveraging innovations in 3D bioprinting, microfluidics, co-culture systems, and AI, researchers can build next-generation organoid models that more accurately replicate the complexity of human tumors.

These advanced models will not only facilitate more effective drug screening and preclinical testing but also enable the development of personalized therapeutic strategies. As the field continues to evolve, organoid technology will likely play an increasingly pivotal role in bridging the gap between bench and bedside, ultimately contributing to improved patient care and outcomes in the fight against urinary system tumors. With ongoing technological innovation, organoid-based systems are poised to serve not just as experimental models, but as clinically actionable tools that inform personalized therapy and drug development in urological oncology. The integration of these models into standardized clinical workflows will be a crucial next step in translating laboratory insights into patient benefit.

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Authors' contributions
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None.

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