Modeling the Tumor Microenvironment In Vitro in Prostate Cancer: Current And Future Perspectives

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Abstract

Prostate cancer is the most common cancer in over 50% of the countries and the third most common malignancy worldwide. The tumor microenvironment denotes the non-cancerous cells and components present in the tumor, including the molecules they produce and release. Prostate cancer proliferation, angiogenesis, metastasis and drug resistance are closely associated with the tumor microenvironment. With the continuous development of in vitro tumor models, they have gradually become an important tool for recapitulating parental tumors in vivo and studying the reciprocal interactions between tumors and their microenvironment. In this review, we describe significant in vitro models of prostate cancer, analyze the research results on incorporating the tumor microenvironment into these models, and compare their advantages and disadvantages. Furthermore, we highlight the future developmental direction of prostate cancer in vitro model research according to the hotspots of in vitro model research in other cancer types to facilitate precision medicine in prostate cancer.

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Abstract

Prostate cancer is the most common cancer in over 50% of the countries and the third most common malignancy worldwide. The tumor microenvironment denotes the non-cancerous cells and components present in the tumor, including the molecules they produce and release. Prostate cancer proliferation, angiogenesis, metastasis and drug resistance are closely associated with the tumor microenvironment. With the continuous development of in vitro tumor models, they have gradually become an important tool for recapitulating parental tumors in vivo and studying the reciprocal interactions between tumors and their microenvironment. In this review, we describe significant in vitro models of prostate cancer, analyze the research results on incorporating the tumor microenvironment into these models, and compare their advantages and disadvantages. Furthermore, we highlight the future developmental direction of prostate cancer in vitro model research according to the hotspots of in vitro model research in other cancer types to facilitate precision medicine in prostate cancer.

Keywords: Prostate cancer; Tumor microenvironment; In-vitro model; organoid

1 Introduction

Prostate cancer (PCa) is the most frequently diagnosed cancer among men in more than half of the countries worldwide. It is the leading cause of cancer-related deaths among men in a quarter of the world's countries [1]. Tumor microenvironment (TME) denotes the non-cancerous cells and components present in the tumor, including the molecules they produce and release [2]. Interactions between the TME and tumor cells play a central role in the process of tumor initiation, progression, metastasis, and response to therapies. In vivo models for PCa research, including knockout and transgenic mouse models, patient-derived xenografts, and circulating tumor cell explants, have several limitations, such as long tumor latencies and high expense [3]. Furthermore, a small number of patient PCa specimens obtained from clinics cannot support the establishment of large numbers of in vivo models. By contrast, in vitro model has the advantages of infinite growth, cost-effectiveness, and low demand for clinical specimens, making it promising for PCa research. However, in vitro tumor models primarily consist of a single cell type, the tumor cell, lacking non-cancerous cell types and the extracellular matrix. These models fail to recapitulate 3D organization and reciprocal interactions between the tumor and its microenvironment, contributing to the gap between the research results and real in vivo conditions. Therefore, modeling the TME in vitro to accurately recapitulate patient tumors is crucial for understanding PCa mechanisms and testing new therapeutic agents. In this review, we provide an overview of tumor-mimicking in vitro models, analyze their implications for PCa, and highlight directions for future development.

2 Simple PCa models consisting of cancer cells

2.1 Two-dimensional (2D) cell lines

PCa research initially relied on 2D monoculture models, almost exclusively dependent on immortalized cell lines [3]. These cell lines constitute a spectrum of diseases with various types and stages, partially reflecting the heterogeneity of malignancy [4]. At first, LNCaP, DU-145, and PC-3 cell lines established from metastatic brain, vertebral and lymph node foci were used for PCa investigation [5]. After that, nearly 200 cell lines and sublines derived from primary tumors, metastases and xenografts have been used in PCa research [6].

Moreover, immortalized cell lines can help predict drug responses [7]. Tran et al tested several AR blockers and anti-androgens in LNCAP-AR cells, where enzalutamide was found to be the most effective drug [8].

The steady accessibility, simplicity, reproducibility has led to the widespread use of PCa cell lines [3]. However, the immortalized cell line is absent of ECM deposition and TME with high serum concentrations, which make them distinctly different from in vivo tumor biology [3]. Furthermore, these PCa cell lines gradually lose the characteristics of patient tumors because of their extended culture and repeated passages. Cells at increasing passages exhibit progressive changes in genotypes and phenotypes including alterations in growth rates, morphology, protein expression, response to drugs and migration [9-11]. The great limitations

of the widely used 2D model gave rise to the establishment of 3D models, making models closer to in vivo conditions.

2.2 3D tumor spheroids

Tumor spheroids are defined as spherical clusters of tumor cells, with or without other cell types, in a 3D system [12]. Cell lines and patient-specific cells are used to establish PCa spheroids in scaffold-free or scaffold-based methods[13].

In comparison with homogeneity of the cell line, PCa spheroids show intratumoral heterogeneity and are used for cell-origin detection. A CD49b high, CD29 high and CD44 high cell population was found to be self-renewing tumor-initiating cells with a high propensity for invasion, migration and tumorigenicity by measuring the prostasphere-forming capacity [14, 15].

Moreover, spheroids allow cell cultivation and cell-cell and cell-matrix interactions in a spatially 3D manner [16]. These interactions lead to changes in morphological and cellular characteristics compared to 2D cell lines, reducing the difference between in vitro and in vivo conditions [12]

PCa spheroids also establish oxygen and nutrient gradient inside the model [17] and shows necrosis if the spheroid grows to a size > 100 μm on average, which mimics in vivo conditions [18].

As a 3D patient derived model, PCa spheroid is more reliable than cell line in drug sensitivity test. Johannes et al. showed that PCa spheroids established from radical prostatectomy specimens responded sensitively to bicalutamide and enzalutamide but are resistant to abiraterone and docetaxel [18].

However, because PCa spheroids are derived from clusters of tumor cells and not stem cells, they lack the ability to self-organize and cannot fully recapitulate intratumoral and intertumoral heterogeneity. As a result, drug sensitivity results based on them may lack uniformity and cannot precisely predict the therapeutic response in clinical settings. Furthermore, simple PCa spheroids consisting of tumor cells fail to mimic heterotypic solid tumors, excluding reciprocal interactions between cancerous and non-cancerous cells. The limitation of the lack of self-organization gives rise to the patient-derived organoid model, and the limitation of the absence of non-cancerous cells gives rise to a complicated co-culture model, which will be discussed in detail later.

2.3 Patient-derived organoids

Patient-derived organoids (PDOs) are self-organizing 3D models established from diseased cells of organs with stem cell properties [19]. The difference between tumor spheroids and organoids is that spheroids are derived from a cluster of tumor cells that may include cells with stem properties [20, 21]. In contrast, organoids process multiple genetic sub-clones and tissue structures derived from single epithelial or mesenchymal stem cell [22]. Tumor PDOs can be established by single malignant cells, minced micro tissues, or inducing normal tissue organoids.

Dong Gao et al. were the first to establish advanced PCa organoids in long-term cultures derived from tissue biopsies or circulating tumor cells. These PCa organoids harbor the genetic and epigenetic characteristics of primary PCa and recapitulate the phenotypic diversity of CRPC, making them amenable to drug testing [22].

Since then, PCa organoids have been widely used to study the cells of origin, screen for genomic mutations, and conduct drug sensitivity assays in PCa (Table 1). Long-term expansion of primary mouse and human organoids revealed a luminal multi-lineage progenitor cell that gave rise to the formation of prostate glands [23]. Furthermore, Park et al. found that both luminal and basal progenitor cells initiate tumorigenesis but contribute to different phenotypes in PCa organoids [24]. Based on the inheritance of various genetic mutations previously reported in distinct subtypes of prostate cancer, such as PTEN, SPOP, TMPRSS2-ERG, LRP5, and CTNNB1 [22, 25], PCa organoid lines further revealed the functions of genes, including ERG, c-Myc, SPOP, and CHD-1, in PCa development[26]. Simultaneously, PCa organoid cell lines are used to evaluate the efficacy of novel drugs found in basic research or clinical trials. Dong Gao et al. found that

only one in seven CRPC organoid lines responded sensitively to enzalutamide [22], consistent with cancer heterogeneity. Beshiri et al. found that the response of mCRPC PCa organoids derived from patient-derived xenografts (PDXs) to olaparib correlated with clinical outcomes in patients [25], demonstrating the potential of PCa organoids in precision medicine.

PDOs can accurately recapitulate the intra- and intertumoral biological heterogeneity of tumors. Specifically, PDOs can preserve various cell sub-clones with distinct genetic and phenotypic characteristics intratumorally and recapitulate patient-specific characteristics intertumorally [27]. Therefore, in basic and translational research, PCa organoids have a unique advantage over cell lines and spheroids. However, several limitations preclude their clinical applications. The average success rate of PCa organoid establishment ($< 20\%$) still requires improvement [26]. Moreover, simple PCa organoids do not include the TME, which differs from in vivo tumors. Co-culture models and air-liquid-interface PDO have emerged to address the absence of a TME in PCa organoids, which will be discussed in detail later.

3 Complex PCa models integrating tumor microenvironment components into cancer cells

Cancers are intricate ecosystems composed of tumor and non-cancerous cells embedded in a modified extracellular matrix [28]. TME cell types include immune cells, endothelial cells, pericytes, cancer-associated fibroblasts, and other tissue-resident cell types. These host cells play central roles in cancer pathogenesis and are considered novel targets for cancer therapy [28]. The in vitro prostate cancer model mainly consists of cancer cells without TME components differing from in vivo tumors. Consequently, several studies have attempted to co-culture cancer cells, either cell lines or patient-derived cells, with non-cancerous cells in the TME to discover phenotypic and genomic alterations in cancer cells and examine their potential for drug sensitivity assays. Based on different culturing methods, we classified them into 2D co-culture, spheroid-based, tissue-slice-culture-based, organoid-based, microfluidic organ-on-a-chip-based models.

3.1 2D co-culture system

The 2D co-culture model refers to cancer cells without certain spatial structures interacting with their surrounding microenvironments on the same surface [29].

2D co-culture model is ideal for researchers to study direct and indirect interactions between various types of cells. CAFs gave BPH a more elongated and invasive phenotype than non-malignant prostate tissue fibroblasts (NPFs) through direct contact [30, 31]. PCa cells co-cultured with bone marrow stromal cells attenuated endoglin expression and TGF-B signaling in the stromal and promoted proliferation of cancer cells [32]. Teng et al. showed that HMC-1-SAMD14+ secretions inhibited pro-tumorigenic prostate epithelial morphology and decreased the deposition and arrangement of the matrix generated by CAFs in a co-culture model of primary prostatic CAFs and prostate epithelium [33]. Additionally, the extracellular matrices surrounding tumors have been modelled in a 2D plane. A monolayer ECM deposited from patient-derived CAFs was established, to which BPH-1 cells were added. This showed that the ECM architecture derived from CAFs was stiffer where BPH-1 cells exhibited a more elongated and invasive phenotype compared with NPF [30].

Due to the convenience and ease of 2D cell culture [34], many studies have attempted to recapitulate TME interactions under 2D conditions. However, a significant limitation of the 2D co-culture model is the absence of a spatial structure that plays a central role in cell behavior in vivo [35]. Consequently, the results obtained from the 2D co-culture models may differ from those obtained in vivo. The phenotypic changes in cancer cells co-cultured with non-cancerous cells in the 2D model are sometimes similar to those in the 3D model [30, 36]. Still, gene expression alterations in cancer cells in 2D models are frequently not verified in clinical specimens. Drug screening for cancer treatment utilizing cell lines has seldom led to the discovery of a therapeutically effective agent [34].

3.2 3D co-culture system

3.2.1 Spheroids-based

Spheroids-based co-culture model is a cluster of different types of cells with a spatial structure, either spherical or tissue-shaped.

The model has been further used to study the interaction between cancer cells and non-cancerous in addition to the past use of 2D co-culture models. Compared with 2D models, it is a valuable tool for drug sensitivity study. Eder et al. co-cultured PCa cell lines (LNCAP, DuCaP, and LAPC4) with CAFs in 3D scaffold-free hanging drops [37]. They found that the addition of CAFs affected cancer cells' sensitivity to anti-androgens. and spheroids' anti-androgen resistance could be reversed by PI3K inhibitors [37].

Moreover, resistance mechanism can be explored. Neuwirt et al. found that CAFs contributed to upregulating the HMGCS2 gene, AKR1C3 gene, and the biosynthesis pathway of steroids and cholesterol, which enabled cancer cells to escape androgen deprivation. Simvastatin, which targets cholesterol and steroid biosynthesis with an AKR1C3 inhibitor, could potentially reverse it by conducting gene expression analysis of spheroids[38]. Kato, M et al. found that ADT led to an increase in the CD105+ fibroblastic subpopulation and downstream SFRP1 in a spheroid-based co-culture model, which induced neuroendocrine differentiation of prostate cancer cells in a paracrine manner [39].

Spheroid models have also been used to mimic the metastatic environment in PCa combined with bioengineered materials. Paindelli et al. further modified a tissue-engineered bone model by seeding human mesenchymal stem cells (hMECs) on a calcium phosphate polycaprolactone scaffold (mPCL-CaP). They found that tumors became resistant to the chemotherapeutic drug docetaxel in a bone stroma-dependent way, and Radium-223 targeting the bone stroma could induce cytotoxicity [40]. Similarly, PCa cells in bone-mimetic environment models derived from culturing osteoprogenitor cells on polymer scaffolds displayed molecular and functional features consistent with in vivo bone metastatic condition [41, 42].Anti-androgen drug sensitivity assays were further conducted in this type of model, where enzalutamide contributed to stronger adaptive responses of cancer cells, osteomimicry, and a better treatment response than bicalutamide, correlating with enzalutamide delaying the onset of bone-related events and prolonging survival in mCRPC[42]. Bioengineering models integrating prostate cancer spheroids into bone-mimetic environments have demonstrated their potential in precision medicine for late-stage prostate cancer where bone metastasis occurs.

Owing to their accessibility and cost-effectiveness, spheroid-based co-culture models have been widely used to study the interactions between tumor and non-cancerous cells and model the pre-metastatic niche in bone. Although it has a 3D structure, the spheroid lacks self-organization and intertumoral heterogeneity. Thus, it is not the most ideal model for mimicking patient tumors in vitro.

3.2.2 Organoids-based

PDOs are a revolutionary model for heterogeneous recapitulation and personalized medicine which preserve the features of the parent tumor at the morphological, genetic, proteomic, and pharmaceutical levels [27]. Recent studies have emphasized the development of organoid culture methods that can accurately recapitulate TME cell heterogeneity and model heterotypic cell interaction. Modeling ECM in organoids widely used naturally derived hydrogels, such as the EHS matrix, which are poorly tunable and ill-defined [43]. A recent study integrated microscopy, spatial omics, proteomics, and transcriptomics on patient biopsies to define the ECM tumor environment in castration-resistant prostate cancer adenocarcinoma (CRPC-Adeno) as neuroendocrine prostate cancer (CRPC-NEPC) [44]. Based on these findings, synthetic hydrogels were developed to grow CRPC-NEPC organoids, where the tumor-specific ECM differentially regulated the mobilized genes, epigenetic methylation, and therapeutic response to drugs of the organoids. Using synthetic hydrogel-grown organoids, they discovered a putative therapeutic drug, the DRD2 inhibitor ONC-201, to treat CRPC-NEPC. They clarified how ECM-integrin interactions could make these tumors vulnerable to DRD2 and EZH2 antagonist activity [44].

Integrating other cell-based factors in the TME, such as CAFs and immune cells, into organoids has also been performed. The direct co-culture of organoids with other cell populations enables studying cell interactions and drug targeting within the TME. Human prostate cancer organoids co-cultured with cancer-associated

fibroblasts purified from the patient-derived xenograft model, CWR22Pc, showed increased resistance to androgen deprivation therapy [45]. Furthermore, NRG1 secreted by CAFs improves ADT resistance [45]. On the other hand, androgen induced the migration of CAFs to cancer cells and, finally, an enlarged cancer organoid size in the co-culture mode. While co-culturing TME components with patient-derived organoids has been widely used in various cancer types, such as esophageal, colorectal, and pancreatic cancer [46-48], attempts to develop a co-culture model for prostate cancer are limited. This limitation is partially due to the relatively low success rate of prostate cancer organoid establishment itself, which remains at 16% [49]. Moreover, simultaneously supporting the growth viability of cancer organoids and specific TME components is in high demand for ECMs and culture media. For example, to maintain the viability of CD8+ T-cells in clear renal cell carcinoma organoids, a series of chemokines, including IL-2, must be added to the ECM and culture medium at specific concentrations [50].

Despite organoids from purely patient-derived epithelial cell populations, the air-liquid-interface patient-derived organoid (ALI-PDO) cultures both epithelial and non-epithelial cell populations by embedding millimeter-scale tumor fragments in a 3D matrix [51]. The ALI-PDO model was established for various types of cancers to study the preservation of the TME and its potential for personalized medicine. Li et al. cultured both epithelial and mesenchymal cell populations in the organoids from mouse colonic, gastric, and pancreatic malignancies [52]. The organoids exhibited extremely detailed histologic endpoints for transformation and dysplasia after oncogenic reprogramming, which were not previously observed in epithelial-only organotypic modeling in the past [52]. Neal et al. successfully co-cultured tumor epithelia and stromal components with endogenous syngeneic immune cells in an organoid model from more than 100 human or mouse tumor biopsies, including human pancreatic, colon, lung, and ampullary adenocarcinomas and so on [53]. Furthermore, they demonstrated that organoids accurately recapitulated the T cell repertoire and modeled responses to immune checkpoint inhibitors in patient tumors [53]. The ALI-PDO platform has also been developed to predict the response of tumors to immunotherapy in other studies [47, 50, 54], promoting precision medicine in cancers. However, no organoids utilize air-liquid interface methodology in PCa to allow for the co-culture of epithelial and non-epithelial cells. We look forward to breakthroughs in establishing air-liquid-interface PCa organoids, given the unique advantages of the methodology in TME heterogeneity recapitulation.

3.2.3 Microfluidic organ-on-a-chip system

Microfluidic-based systems enable the introduction of physiological factors, such as mechanical stress, flow, pressure, and tissue-to-tissue interfaces, into cancer models in vitro [55]. These systems employ microchips, often engineered with various lateral channels and chambers with fluidic flow [56]. Microfluidic systems have demonstrated unique advantages for PCa modeling in vitro. Hsiao et al. utilized a microfluidic system to culture tumor spheroids composed of PC-3 cells, endothelial cells, and osteoblasts to mimic the bone metastatic environment, which led to uniform cell distribution and easy cell tracking during the culture course [57]. Padmyastuti et al. found that microRNAs promoting PCa progression were mostly upregulated, whereas PSA secretion remained constant in spheroids derived from LNCAP cells when cultured in a microfluidic system, which showed that the micro-physiological system could induce significant phenotypic changes in PCa models [58]. Furthermore, the microfluidic device aided precision medicine in PCa by simultaneously delivering drugs with reproducible concentration gradients to patient derived PCa spheroids of multiple sizes [59]. PCa spheroids cultivated in a Microwell Flow Device (MFD) demonstrated decreased necrotic cores, downregulated cell stress genes, enhanced proliferation, improved cellular structural integrity, and better recapitulation of chemotherapy responses, which prompted feasible research into hypoxia modulation and cancer metabolism under pathophysiological conditions [60]. Hence, more research into PCa TME remodeling using microfluidic devices considering physiological factors is warranted.

Microfluidic systems give rise to an organ-on-a-chip (OoC) model, designed to model the functional units of single or multiple human organs [61]. The OoC comprises simplified essential elements necessary for the physiological functions of one or multiple organs in a microfabricated device [61]. Owing to the multiple biomechanical stimuli and complex connectivity between the elements inside the device, OoCs are often

regarded as ideal in vitro models for recapitulating self-regulating biochemical and biophysical networks in vivo[62]. Several organs, including the heart, lungs, kidneys, and liver, have been established in OoCs [55]. The notable features, such as gut microbiome enzymatic activity in the intestine, secretion of anionic drugs in the kidney, and biliary duct formation in the liver, have been recapitulated [58]. Although an OoC model composed of human osteogenically differentiated MSCs, PCa spheroids, and collagen matrices for further research and drug testing has been established [63], more attempts to establish OoC models to study invasion, progression, metastasis, and interactions with the TME and other organs in PCa are needed.

Recently, a strategy termed organoid-on-a-chip combines organ-on-a-chip with organoids. These two complementary approaches mimic the complexity of human tissues in vitro [61]. Organoid-on-a-chip allows the presence of other biochemical and biophysical elements in the TME, such as cell-secreted soluble factors, mechanical flow, and blood vessels, with precise control, to study organoids changes in genes, phenotypes, and drug sensitivity [61]. In an intestinal organoid-on-a-chip system, opposing gradients of BMP and WNT signaling were used to culture self-renewing epithelial cells, finally leading to the compartmentalization of non-proliferative and proliferative cells [64]. The vascularization and perfusion of tumor organoids were observed under physiological flow conditions in a breast cancer organoid-on-a-chip [65]. Fluid shear stress promotes the maturation and vascularization of kidney organoids, as validated by the high expression of vascular markers and increased vascular density in kidney organoids-on-a-chip [66]. Drug discovery is among the most promising applications of the organoids-on-a-chip technology. The correlation between organoids and actual organs makes them more effective for therapeutic target identification, while organs-on-a-chip are more controllable and reproducible engineered devices, better suitable for efficient screening [61]. Currently, there are no PCa organoid-on-a-chip of great interest for future research.

3.2.4 Tissue slice culture

To maintain the architecture and heterogeneity of the TME, a tissue slice culture (TSC) was developed by precisely cutting the slices to a certain thickness in vitro [67]. Precision-cut slices were first developed for pharmacological metabolism studies in the liver and kidney and were extended to other organs, including the prostate [68]. Initially, several issues, including secretory cell degeneration, basal cell hyperplasia, and poor in vitro survival, were observed in prostate TSC culture [69]. Maund et al. demonstrated a PCa TSC model recapitulating patient tumors' genetic, cellular, and structural characteristics during a 5-day culture period by optimizing the culture condition [70]. The survival of monocytes, macrophages, and endothelial cells was shown in the model for the first time, and ADT drug sensitivity assays were conducted [70]. Centenera et al. evaluated a novel HSP90 inhibitor in organotypic PCa TSC as well as 2D cell lines and found that tissue cultures offered insights into the drug response not previously seen in animal models or cell lines before [71]. Zhang et al. tested enzalutamide and olaparib, a PRAP-1 inhibitor, in a PCa TSC model whose viability was maintained for 6 days [72]. Besides drug testing, TSC culture has a unique advantage in spatial transcriptomic methods because co-detection by indexing (CODEX) imaging enables the examination of more than 60 markers in one TSC model [67].

TSC is patient-specific, maintains the local microenvironment, and holds promise for basic translational research. A major limitation of TSC culture is that its structure and cell viability cannot be maintained for more than 1 week [70, 72], which hampers reproducible and high-throughput drug screening. When treated with samples with mixed pathology, commonly observed in clinics, one specific subtype of cancer cells often outgrows and finally homogenizes the entire tissue in the TSC culture [67]. Additionally, unlike spheroids and organoids, studying the interactions between cancerous and non-cancerous cells in the TME using a TSC culture model is difficult.

The advantages, limitations, and applications of the different in vitro PCa models are summarized in Table 2.

4 Discussion

With the rapid development of in vitro cancer models from cell lines and spheroids to organoids, models that integrate the TME into in vitro tumor cells to recapitulate parental tumors have advanced significantly from

2D, spheroid-based, organoid-based co-culture to air-liquid-interface organoids, and finally to microfluidic organoid-on-a-chip systems (Figure 1). These models are becoming increasingly intricate, incorporating an increasing number of TME components, including the ECM, non-cancerous cellular components, and physiological factors, into cancer cells.

However, challenges associated with PCa organoid cultures have impeded the development of in vitro models. No ALI-PDO and organoids-on-a-chip model has been reported in PCa research, underscoring the need for more research in this area. Although the use of drug screening in advanced in vitro models has been a heat map in recent years, the correlation between the results of drug sensitivity assays and clinical outcomes is seldom verified in clinical settings. Possible reasons for this include unapproved medication combinations based on drug screening results used in real patients and long follow-up times required to observe clinical outcomes. Consequently, there remains a gap between the design of complicated in vitro models and their implementation in precision medicine.

In conclusion, research on establishing more efficient and complex organoids should be conducted in PCa to produce in vitro models that recapitulate patient tumors regarding TME components for basic and translational research.

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References

1. Bergengren, O., K.R. Pekala, K. Matsoukas, J. Fainberg, S.F. Mungovan, O. Bratt, F. Bray, O. Brawley, A.N. Luckenbaugh, L. Mucci, T.M. Morgan, and S.V. Carlsson, Eur Urol 2023, **84** , 22. Xiao, Y. and D. Yu, Pharmacol Ther 2021, **221** , 3. Ellem, S.J., E.M. De-Juan-Pardo, and G.P. Risbridger, Adv Drug Deliv Rev 2014,**79-80** , 4. van Bokhoven, A., M. Varella-Garcia, C. Korch, W.U. Johannes, E.E. Smith, H.L. Miller, S.K. Nordeen, G.J. Miller, and M.S. Lucia, Prostate 2003, **57** , 35. Sobel, R.E. and M.D. Sadar, J Urol 2005, **173** , 26. Sobel, R.E. and M.D. Sadar, J Urol 2005,**173** , 27. Abate-Shen, C. and F. Nunes de Almeida, Cancer Res 2022, **82** , 98. Tran, C., S. Ouk, N.J. Clegg, Y. Chen, P.A. Watson, V. Arora, J. Wongvipat, P.M. Smith-Jones, D. Yoo, A. Kwon, T. Wasielewska, D. Welsbie, C.D. Chen, C.S. Higano, T.M. Beer, D.T. Hung, H.I. Scher, M.E. Jung, and C.L. Sawyers, Science 2009, **324** , 59289. Esquenet, M., J.V. Swinnen, W. Heyns, and G. Verhoeven, J Steroid Biochem Mol Biol 1997, **62** , 5-610. Chang-Liu, C.M. and G.E. Woloschak, Cancer Lett 1997, **113** , 1-211. Wenger, S.L., J.R. Senft, L.M. Sargent, R. Bamezai, N. Bairwa, and S.G. Grant, Biosci Rep 2004, **24** , 612. Ma, H.L., Q. Jiang, S. Han, Y. Wu, J. Cui Tomshine, D. Wang, Y. Gan, G. Zou, and X.J. Liang, Mol Imaging 2012,**11** , 613. Nunes, A.S., A.S. Barros, E.C. Costa, A.F. Moreira, and I.J. Correia, Biotechnol Bioeng 2019, **116** , 114. Bansal, N., M. Bartucci, S. Yusuff, S. Davis, K. Flaherty, E. Huselid, M. Patrizii, D. Jones, L. Cao, N. Sydorenko, Y.C. Moon, H. Zhong, D.J. Medina, J. Kerrigan, M.N. Stein, I.Y. Kim, T.W. Davis, R.S. DiPaola, J.R. Bertino, and H.E. Sabaawy, Clin Cancer Res 2016, **22** , 2415. Bansal, N., S. Davis, I. Tereshchenko, T. Budak-Alpdogan, H. Zhong, M.N. Stein, I.Y.

Kim, R.S. Dipaola, J.R. Bertino, and H.E. Sabaawy, Prostate 2014,**74** , 216. Baal, N., R. Widmer-Teske, T. McKinnon, K.T. Preissner, and M.T. Zygmunt, Lab Invest 2009, **89** , 217. Nath, S. and G.R. Devi, Pharmacol Ther 2016, **163** , 18. Linxweiler, J., M. Hammer, S. Muhs, M. Kohn, A. Pryalukhin, C. Veith, R.M. Bohle, M. Stockle, K. Junker, and M. Saar, J Cancer Res Clin Oncol 2019,**145** , 319. Schutgens, F. and H. Clevers, Annu Rev Pathol 2020,**15** , 20. Clevers, H., Nat Med 2011, **17** , 321. S, S.F., K. Szczesna, M.S. Iliou, M. Al-Qahtani, A. Mobasheri, J. Kobolák, and A. Dinnyés, BMC Cancer 2016, **16** , Suppl 222. Gao, D., I. Vela, A. Sboner, P.J. Iaquinta, W.R. Karthaus, A. Gopalan, C. Dowling, J.N. Wanjala, E.A. Undvall, V.K. Arora, J. Wongvipat, M. Kossai, S. Ramazanoglu, L.P. Barboza, W. Di, Z. Cao, Q.F. Zhang, I. Sirota, L. Ran, T.Y. MacDonald, H. Beltran, J.M. Mosquera, K.A. Touijer, P.T. Scardino, V.P. Laudone, K.R. Curtis, D.E. Rathkopf, M.J. Morris, D.C. Danila, S.F. Slovin, S.B. Solomon, J.A. Eastham, P. Chi, B. Carver, M.A. Rubin, H.I. Scher, H. Clevers, C.L. Sawyers, and Y. Chen, Cell 2014, **159** , 123. Karthaus, W.R., P.J. Iaquinta, J. Drost, A. Gracanin, R. van Boxtel, J. Wongvipat, C.M. Dowling, D. Gao, H. Begthel, N. Sachs, R.G.J. Vries, E. Cuppen, Y. Chen, C.L. Sawyers, and H.C. Clevers, Cell 2014,**159** , 124. Park, J.W., J.K. Lee, J.W. Phillips, P. Huang, D. Cheng, J. Huang, and O.N. Witte, Proc Natl Acad Sci U S A 2016,**113** , 1625. Beshiri, M.L., C.M. Tice, C. Tran, H.M. Nguyen, A.G. Sowalsky, S. Agarwal, K.H. Jansson, Q. Yang, K.M. McGowen, J. Yin, A.N. Alilin, F.H. Karzai, W.L. Dahut, E. Corey, and K. Kelly, Clin Cancer Res 2018, **24** , 1726. Buskin, A., E. Scott, R. Nelson, L. Gaughan, C.N. Robson, R. Heer, and A.C. Hepburn, Oncogene 2023, **42** , 3227. LeSavage, B.L., R.A. Suhar, N. Broguiere, M.P. Lutolf, and S.C. Heilshorn, Nat Mater 2022, **21** , 228. de Visser, K.E. and J.A. Joyce, Cancer Cell 2023, **41** , 329. Liu, R., X. Meng, X. Yu, G. Wang, Z. Dong, Z. Zhou, M. Qi, X. Yu, T. Ji, and F. Wang, Int J Mol Sci 2022, **23** , 2130. Jaeschke, A., A. Jacobi, M.G. Lawrence, G.P. Risbridger, M. Frydenberg, E.D. Williams, I. Vela, D.W. Hutmacher, L.J. Bray, and A. Taubenberger, Mater Today Bio 2020, **8** , 31. Clark, A.K., A.V. Taubenberger, R.A. Taylor, B. Niranjan, Z.Y. Chea, E. Zotenko, S. Sieh, J.S. Pedersen, S. Norden, M. Frydenberg, J.P. Grummet, D.W. Pook, B. Australian Prostate Cancer, C. Stirzaker, S.J. Clark, M.G. Lawrence, S.J. Ellem, D.W. Hutmacher, and G.P. Risbridger, Biomaterials 2013, **34** , 2032. O'Connor, J.C., M.C. Farach-Carson, C.J. Schneider, and D.D. Carson, Mol Cancer Res 2007, **5** , 633. Teng, L.K.H., B.A. Pereira, S. Keerthikumar, C. Huang, B. Niranjan, S.N. Lee, M. Richards, R.B. Schittenhelm, L. Furic, D.L. Goode, M.G. Lawrence, R.A. Taylor, S.J. Ellem, G.P. Risbridger, and N.L. Lister, Cancers (Basel) 2021, **13** , 634. Wang, R., J. Xu, L. Juliette, A. Castilleja, J. Love, S.Y. Sung, H.E. Zhau, T.J. Goodwin, and L.W. Chung, Semin Cancer Biol 2005, **15** , 535. Ravi, M., V. Paramesh, S.R. Kaviya, E. Anuradha, and F.D. Solomon, J Cell Physiol 2015,**230** , 136. Pereira, B.A., N.L. Lister, K. Hashimoto, L. Teng, M. Flandes-Iparraguirre, A. Eder, A. Sanchez-Herrero, B. Niranjan, and A. Melbourne Urological Research, Biomaterials 2019, **197** , 37. Eder, T., A. Weber, H. Neuwirt, G. Grunbacher, C. Ploner, H. Klocker, N. Sampson, and I.E. Eder, Int J Mol Sci 2016, **17** , 938. Neuwirt, H., J. Bouchal, G. Kharaishvili, C. Ploner, K. Johrer, F. Pitterl, A. Weber, H. Klocker, and I.E. Eder, Cell Commun Signal 2020, **18** , 139. Kato, M., V.R. Placencio-Hickok, A. Madhav, S. Haldar, M. Tripathi, S. Billet, R. Mishra, B. Smith, K. Rohena-Rivera, P. Agarwal, F. Duong, B. Angara, D. Hickok, Z. Liu, and N.A. Bhowmick, Oncogene 2019,**38** , 540. Paindelli, C., N. Navone, C.J. Logothetis, P. Friedl, and E. Dondossola, Biomaterials 2019, **197** , 41. Bock, N., A. Shokoohmand, T. Kryza, J. Rohl, J. Meijer, P.A. Tran, C.C. Nelson, J.A. Clements, and D.W. Hutmacher, Bone Res 2019, **7** , 42. Bock, N., T. Kryza, A. Shokoohmand, J. Rohl, A. Ravichandran, M.L. Wille, C.C. Nelson, D.W. Hutmacher, and J.A. Clements, Sci Adv 2021, **7** , 2743. Aisenbrey, E.A. and W.L. Murphy, Nat Rev Mater 2020, **5** , 744. Mosquera, M.J., S. Kim, R. Bareja, Z. Fang, S. Cai, H. Pan, M. Asad, M.L. Martin, M. Sigouros, F.M. Rowdo, S. Ackermann, J. Capuano, J. Bernheim, C. Cheung, A. Doane, N. Brady, R. Singh, D.S. Rickman, V. Prabhu, J.E. Allen, L. Puca, A.F. Coskun, M.A. Rubin, H. Beltran, J.M. Mosquera, O. Elemento, and A. Singh, Adv Mater 2022, **34** , 245. Zhang, Z., W.R. Karthaus, Y.S. Lee, V.R. Gao, C. Wu, J.W. Russo, M. Liu, J.M. Mota, W. Abida, E. Linton, E. Lee, S.D. Barnes, H.A. Chen, N. Mao, J. Wongvipat, D. Choi, X. Chen, H. Zhao, K. Manova-Todorova, E. de Stanchina, M.E. Taplin, S.P. Balk, D.E. Rathkopf, A. Gopalan, B.S. Carver, P. Mu, X. Jiang, P.A. Watson, and C.L. Sawyers, Cancer Cell 2020, **38** , 246. Ebbing, E.A., A.P. van der Zalm, A. Steins, A. Creemers, S. Hermsen, R. Rentenaar, M. Klein, C. Waasdorp, G.K.J. Hooijer, S.L. Meijer, K.K. Krishnadath, C.J.A. Punt, M.I. van Berge Henegouwen, S.S. Gisbertz, O.M. van Delden, M. Hulshof, J.P. Medema, H.W.M. van Laarhoven, and M.F. Bijlsma, Proc Natl Acad Sci U S A 2019,**116** , 647. Schnalzger,

T.E., M.H. de Groot, C. Zhang, M.H. Mosa, B.E. Michels, J. Roder, T. Darvishi, W.S. Wels, and H.F. Farin, EMBO J 2019, **38** , 1248. Öhlund, D., A. Handly-Santana, G. Biffi, E. Elyada, A.S. Almeida, M. Ponz-Sarvise, V. Corbo, T.E. Oni, S.A. Hearn, E.J. Lee, Chio, II, C.I. Hwang, H. Tiriac, L.A. Baker, D.D. Engle, C. Feig, A. Kultti, M. Egeblad, D.T. Fearon, J.M. Crawford, H. Clevers, Y. Park, and D.A. Tuveson, J Exp Med 2017, **214** , 349. Puca, L., R. Bareja, D. Prandi, R. Shaw, M. Benelli, W.R. Karthaus, J. Hess, M. Sigouros, A. Donoghue, M. Kossai, D. Gao, J. Cyrta, V. Sailer, A. Vosoughi, C. Pauli, Y. Churakova, C. Cheung, L.D. Deonarine, T.J. McNary, R. Rosati, S.T. Tagawa, D.M. Nanus, J.M. Mosquera, C.L. Sawyers, Y. Chen, G. Inghirami, R.A. Rao, C. Grandori, O. Elemento, A. Sboner, F. Demichelis, M.A. Rubin, and H. Beltran, Nat Commun 2018, **9** , 150. Xue, Y., B. Wang, Y. Tao, J. Xia, K. Yuan, J. Zheng, W. Zhai, and W. Xue, Precis Clin Med 2022, **5** , 451. DiMarco, R.L., J. Su, K.S. Yan, R. Dewi, C.J. Kuo, and S.C. Heilshorn, Integr Biol (Camb) 2014, **6** , 252. Li, X., L. Nadauld, A. Ootani, D.C. Corney, R.K. Pai, O. Gevaert, M.A. Cantrell, P.G. Rack, J.T. Neal, C.W. Chan, T. Yeung, X. Gong, J. Yuan, J. Wilhelmy, S. Robine, L.D. Attardi, S.K. Plevritis, K.E. Hung, C.Z. Chen, H.P. Ji, and C.J. Kuo, Nat Med 2014,**20** , 753. Neal, J.T., X. Li, J. Zhu, V. Giangarra, C.L. Grzeskowiak, J. Ju, I.H. Liu, S.H. Chiou, A.A. Salahudeen, A.R. Smith, B.C. Deutsch, L. Liao, A.J. Zemek, F. Zhao, K. Karlsson, L.M. Schultz, T.J. Metzner, L.D. Nadauld, Y.Y. Tseng, S. Alkhairy, C. Oh, P. Keskula, D. Mendoza-Villanueva, F.M. De La Vega, P.L. Kunz, J.C. Liao, J.T. Leppert, J.B. Sunwoo, C. Sabatti, J.S. Boehm, W.C. Hahn, G.X.Y. Zheng, M.M. Davis, and C.J. Kuo, Cell 2018, **175** , 754. Esser, L.K., V. Branchi, S. Leonardelli, N. Pelusi, A.G. Simon, N. Klumper, J. Ellinger, S. Hauser, M.A. Gonzalez-Carmona, M. Ritter, G. Kristiansen, H. Schorle, M. Holzel, and M.I. Toma, Front Oncol 2020, **10** , 55. Gunti, S., A.T.K. Hoke, K.P. Vu, and N.R. London, Jr., Cancers (Basel) 2021,**13** , 456. Franchi-Mendes, T., R. Eduardo, G. Domenici, and C. Brito, Cancers (Basel) 2021, **13** , 1857. Hsiao, A.Y., Y.S. Torisawa, Y.C. Tung, S. Sud, R.S. Taichman, K.J. Pienta, and S. Takayama, Biomaterials 2009, **30** , 1658. Padmyastuti, A., M.G. Sarmiento, M. Dib, J. Ehrhardt, J. Schoon, M. Somova, M. Burchardt, C. Roennau, and P.C. Pinto, Sci Rep 2023, **13** , 159. Mulholland, T., M. McAllister, S. Patek, D. Flint, M. Underwood, A. Sim, J. Edwards, and M. Zagnoni, Sci Rep 2018, **8** , 160. Payne, M.C., S. Ho, T. Hashimoto, S. Imboden, J.A. Diaz, B.S. Lee, M.J. Rupert, N.Y. Cai, A.S. Goldstein, and N.Y.C. Lin, Biotechnol J 2023, **18** , 661. Park, S.E., A. Georgescu, and D. Huh, Science 2019, **364** , 644462. Sung, B., Math Biosci 2022, **352** , 63. Verbruggen, S.W., J. Nolan, M.P. Duffy, O.M.T. Pearce, C.R. Jacobs, and M.M. Knight, Adv Sci (Weinh) 2023, 64. Wang, Y., D.B. Gunasekara, M.I. Reed, M. DiSalvo, S.J. Bultman, C.E. Sims, S.T. Magness, and N.L. Allbritton, Biomaterials 2017, **128** , 65. Shirure, V.S., Y. Bi, M.B. Curtis, A. Lezia, M.M. Goedegebuure, S.P. Goedegebuure, R. Aft, R.C. Fields, and S.C. George, Lab Chip 2018, **18** , 2366. Homan, K.A., N. Gupta, K.T. Kroll, D.B. Kolesky, M. Skylar-Scott, T. Miyoshi, D. Mau, M.T. Valerius, T. Ferrante, J.V. Bonventre, J.A. Lewis, and R. Morizane, Nat Methods 2019, **16** , 367. Perez, L.M. and L. Nonn, Front Oncol 2022,**12** , 68. Parrish, A.R., K. Sallam, D.W. Nyman, J. Orozco, A.E. Cress, B.L. Dalkin, R.B. Nagle, and A.J. Gandolfi, Cell Biol Toxicol 2002, **18** , 369. Papini, S., A. Rosellini, D. Campani, A. DeMatteis, C. Selli, and R.P. Revoltella, Prostate 2004, **59** , 470. Maund, S.L., R. Nolley, and D.M. Peehl, Lab Invest 2014,**94** , 271. Centenera, M.M., J.L. Gillis, A.R. Hanson, S. Jindal, R.A. Taylor, G.P. Risbridger, P.D. Sutherland, H.I. Scher, G.V. Raj, K.E. Knudsen, T. Yeadon, B. Australian Prostate Cancer, W.D. Tilley, and L.M. Butler, Clin Cancer Res 2012, **18** , 1372. Zhang, W., W.M. van Weerden, C.M.A. de Ridder, S. Erkens-Schulze, E. Schonfeld, T.G. Meijer, R. Kanaar, D.C. van Gent, and J. Nonnekens, Prostate 2019,**79** , 473. Drost, J., W.R. Karthaus, D. Gao, E. Driehuis, C.L. Sawyers, Y. Chen, and H. Clevers, Nat Protoc 2016, **11** , 274. Karkampouna, S., F. La Manna, A. Benjak, M. Kiener, M. De Menna, E. Zoni, J. Grosjean, I. Klima, A. Garofoli, M. Bolis, A. Vallerga, J.P. Theurillat, M.R. De Filippo, V. Genitsch, D. Keller, T.H. Booij, C.U. Stirnimann, K. Eng, A. Sboner, C.K.Y. Ng, S. Piscuoglio, P.C. Gray, M. Spahn, M.A. Rubin, G.N. Thalmann, and M. Kruithof-de Julio, Nat Commun 2021, **12** , 175. Guo, C., I. Figueiredo, B. Gurel, A. Neeb, G. Seed, M. Crespo, S. Carreira, J. Rekowski, L. Buroni, J. Welti, D. Bogdan, L. Gallagher, A. Sharp, M.D. Fenor de la Maza, P. Rescigno, D. Westaby, K. Chandran, R. Riisnaes, A. Ferreira, S. Miranda, B. Cali, A. Alimonti, S. Bressan, A.H.T. Nguyen, M.M. Shen, J.E. Hawley, A. Obradovic, C.G. Drake, C. Bertan, C. Baker, N. Tunariu, W. Yuan, and J.S. de Bono, Eur Urol 2023, **83** , 3

Table 1 Published articles on the establishment of patient-derived organoids of prostate cancer

Histological type	Tissue collection
Adenocarcinoma	Metastatic lesion Sa
Adenocarcinoma Adenocarcinoma Adenocarcinoma Neuroendocrine Neuroendocrine Adenocarcinoma	CTC Metastatic les

CTC, circulating tumor cell; PDX, patient-derived xenograft; N/A, not applicable.

Table 2 Comparison between simple and complicated PCa models in vitro

	In vitro PCa models	Advantages
Simple model	2D cell lines	Easy accessibility, simple cultivation Stable reproducibility Low cost
	3D tumor spheroids	3D cancer cell growth High throughput
	Patient-derived organoids	3D cancer cell growth Self-organization capacity Genetic heterogeneity r
Complicated model	2D co-culture	Coexistence of cancerous and non-cancerous cells Convenience and ease
	Spheroids based co-culture	3D interaction between heterotypic cells Stable accessibility and cost eff
	Organoids based co-culture	Modeling heterogenic tumors with the presence of other TME compone
	Microfluidic based system	Modeling physiological factors Mimicking peripheral circulation
	Tissue slice culture	Local microenvironment preservation Patient characteristics maintain F
Future perspectives	ALI-PDO	Co-culture of epithelial and non-epithelial cell populations at the beginn
	Organoids-on-a-chip	Pathophysiological factors Modeling interactions between organs

TME, tumor microenvironment; ALI-PDO, air-liquid interface-patient derived organoid.

Figure 1