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Review Article

Cellular plasticity and tumor ecosystem dynamics in prostate cancer: insights from single-cell and spatial transcriptomics

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Abstract: Prostate cancer is a heterogeneous disease shaped by evolving cellular states within a spatially organized tumor ecosystem. Advances in single-cell RNA-sequencing, single-nucleus sequencing, and spatial transcriptomics have facilitated high-resolution dissection of epithelial lineage hierarchies, tumor-associated luminal states, and microenvironmental remodeling. These approaches have revealed that progression from androgen receptor-dependent adenocarcinoma to castration-resistant prostate cancer and the development of neuroendocrine phenotypes reflect lineage plasticity and epigenetic reprogramming rather than simple linear genetic progression. Single-cell studies have indicated that immunosuppressive niches, cancer-associated fibroblast heterogeneity, endothelial activation, and metabolic adaptation collectively contribute to therapeutic resistance. The integration of multi-omics data with spatial context has begun to redefine prostate cancer taxonomy based on cellular state composition and ecosystem architecture. In this review, we summarize recent discoveries from single-cell and spatial analyses, discuss their implications for biomarker development and treatment stratification, and outline ongoing technical challenges, including standardization, reproducibility, and clinical scalability. Overall, this review provides valuable insights for the development of a state-informed framework to understand prostate cancer progression and guide precision oncology strategies.

Keywords: Prostate cancer, single-cell RNA sequencing, spatial transcriptomics, tumor microenvironment, lineage plasticity

Introduction

Prostate cancer (PC) is a major global health burden and currently the second most commonly diagnosed malignancy among men worldwide, with an estimated 1.46 million new cases and approximately 396,000 deaths reported annually in 2022 [1]. Nearly one in eight men is diagnosed with PC during their lifetime, underscoring its profound impact on public health. Although localized disease is often effectively managed with radical prostatectomy or radiotherapy [2], improving outcomes in advanced and metastatic settings remains a major clinical challenge.

Androgen deprivation therapy is the cornerstone of advanced PC treatment, reflecting the central roles of androgen receptor (AR) signaling in tumor growth and survival. However, within 2-3 years of treatment initiation, a substantial proportion of patients progress to castration-resistant PC (CRPC) [3]. Despite the introduction of next-generation AR pathway inhibitors, taxane-based chemotherapy, and poly (ADP-ribose) polymerase inhibitors, metastatic CRPC remains largely incurable. Precision oncology approaches based on genomic alterations, such as homologous recombination repair defects including BRCA1/2 mutations, facilitate biomarker-driven treatment of selected patient populations [4, 5]. However, durable responses remain limited, with therapeutic resistance ultimately emerging in most cases. These clinical realities underscore the urgent need to elucidate the biological mechanisms driving disease progression and treatment failure in PC.

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A defining feature of the above-mentioned challenges is the profound heterogeneity of PC. Tumor heterogeneity exists both between patients and within individual tumors, where multiple cellular populations with distinct transcriptional states, differentiation programs, and functional properties coexist. Conventional classification systems based on histopathology and bulk genomic profiling capture dominant molecular alterations but fail to resolve this underlying diversity. Consequently, critical subpopulations that may drive disease progression, metastasis, and therapeutic resistance remain unclear. PC evolution is not solely governed by the linear accumulation of genetic alterations but instead reflects dynamic transitions between cellular states and lineage programs [6-10].

Central to this dynamic model is lineage plasticity, a key mechanism linking tumor heterogeneity to therapeutic resistance. Treatment-emergent neuroendocrine PC (NEPC) is a prototypical example of lineage reprogramming. AR-independent neuroendocrine phenotypes arise from pre-existing adenocarcinoma clones via transcriptional and epigenetic reprogramming rather than de novo tumorigenesis [7]. This process is frequently associated with the loss of TP53 and RB1, activation of developmental transcriptional programs, and large-scale remodeling of the epigenetic landscape. These reports indicate that tumor progression is driven by both genetic alterations and reversible transitions in cellular identity, highlighting the importance of cellular state dynamics in PC biology.

Importantly, cellular state transitions do not occur in isolation but are tightly coupled to the tumor microenvironment (TME). The TME, which is composed of epithelial, immune, stromal, and vascular components, actively shapes tumor behavior via complex and context-dependent interactions. For example, cancer-associated fibroblasts (CAFs) contribute to extracellular matrix (ECM) remodeling, growth factor secretion, and the establishment of physical and biochemical barriers that limit immune cell infiltration and drug delivery. Immunosuppressive cell populations, including regulatory T cells (Tregs), tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MDSCs), further reinforce immune evasion and contribute to immunotherapy resistance. Additionally, hypoxic niches within tumors activate hypoxia-inducible factor 1 subunit alpha (HIF1A)-driven transcriptional programs, promoting metabolic reprogramming, stem-like phenotypes, and therapeutic resistance.

The above-mentioned observations support a model in which tumor cells and their surrounding microenvironment co-evolve, forming adaptive ecosystems characterized by lineage plasticity, AR pathway bypass, and microenvironmental remodeling across diverse PC subtypes [7, 11]. Within this framework, tumor heterogeneity, microenvironmental remodeling, disease progression, and therapeutic resistance are not independent processes but represent interconnected manifestations of a continuously evolving system. Therefore, PC is not a static disease defined by fixed histological categories but a dynamic state-driven ecosystem shaped by both intrinsic cellular programs and extrinsic microenvironmental influences.

Complex and multi-layered biological processes cannot be adequately resolved using conventional bulk genomic or transcriptomic approaches. Bulk analyses inherently average signals across heterogeneous cell populations, obscuring lineage hierarchies, transient cellular states, and spatially organized cell-cell interactions. Consequently, key drivers of disease progression and therapeutic resistance, including rare subpopulations and microenvironmental niches, may remain undetected.

Recent advances in single-cell RNA-sequencing (scRNA-seq) and spatial transcriptomics have overcome these limitations by enabling high-resolution dissection of tumors at the level of individual cells and their spatial context. These technologies allow simultaneous characterization of cellular states, reconstruction of lineage relationships, and mapping of spatially organized interactions in intact tumor tissues [12, 13]. Importantly, they provide a framework for cataloging cellular diversity and understanding the dynamic interplay between tumor cells and their microenvironment, thereby offering new insights into the mechanisms underlying disease heterogeneity, progression, and therapeutic resistance.

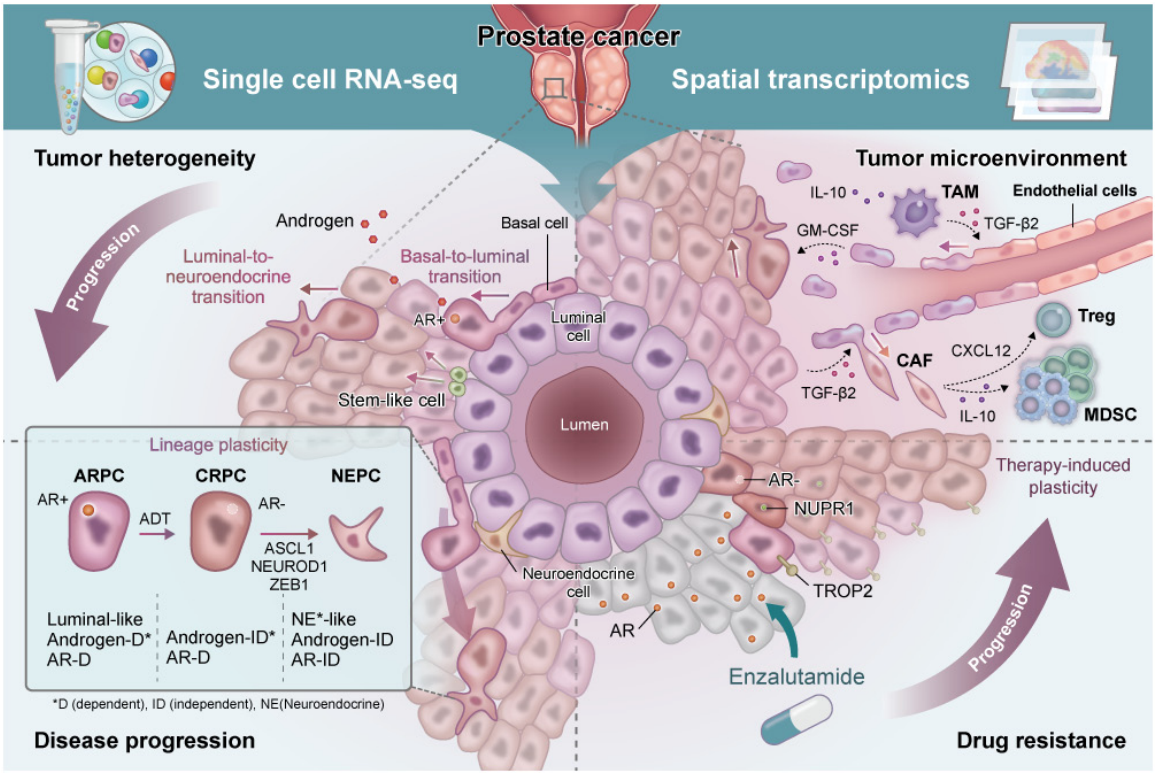

Figure 1. Conceptual framework of prostate cancer progression revealed via single-cell and spatial analyses. This schematic highlights four key concepts: 1: Tumor heterogeneity, including luminal, basal, and stem-like epithelial states. 2: Lineage plasticity depicting transitions from androgen receptor (AR)-dependent adenocarcinoma to castration-resistant prostate cancer (CRPC) and neuroendocrine phenotypes. 3: Tumor microenvironment (TME) interactions, including immune, stromal, and vascular components shaping disease behavior. 4: Therapy-induced resistance driven by cellular state transitions under treatment pressure. Abbreviations: AR, androgen receptor; ARPC, androgen receptor-positive prostate cancer; CRPC, castration-resistant prostate cancer; NEPC, neuroendocrine prostate cancer; CAF, cancer-associated fibroblast; TAM, tumor-associated macrophage; Tregs, regulatory T cell; MDSC, myeloid-derived suppressor cell.

In this review, we summarize the recent advances in single-cell and spatial analyses of PC, focusing on tumor heterogeneity, TME dynamics, disease progression, and therapeutic resistance mechanisms. Moreover, we discuss the ways in which the integration of multi-omics and spatially resolved approaches can redefine the biological taxonomy of PC and inform next-generation precision oncology strategies. The conceptual framework underlying these processes is illustrated in **Figure 1**.

Single-cell analysis methods

An overview of single-cell and spatial transcriptomic workflows is provided in **Figure 2**.

scRNA-seq workflow

scRNA-seq facilitates transcriptomic profiling at the resolution of individual cells through a multi-step experimental and computational workflow.

The process begins with the acquisition of high-quality biological material. In PC research, samples may be derived from radical prostatectomy specimens, needle biopsies, transurethral resections, circulating tumor cells, or experimental models. Solid tissues are mechanically dissociated and enzymatically digested, commonly using collagenase and related proteolytic enzymes, to generate a viable single-cell suspension while minimizing RNA degradation.

Early scRNA-seq approaches relied on plate-based protocols capable of profiling individual cells with high sensitivity [14, 15]. Subsequent advances introduced droplet-based microfluidic systems, such as Drop-seq, and later com-

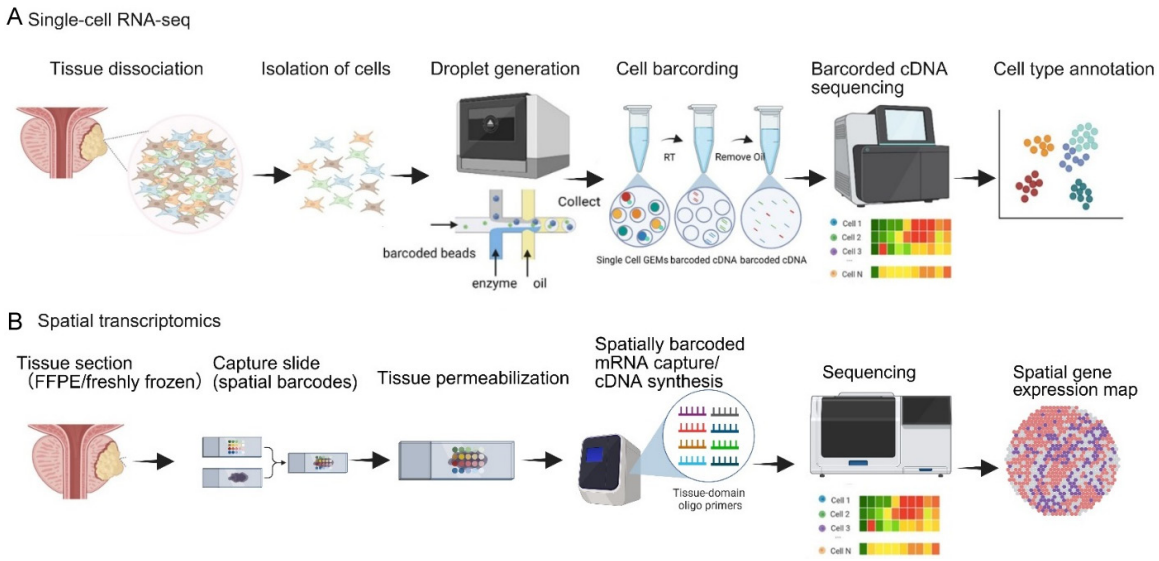

Figure 2. Overview of single-cell and spatial transcriptomic workflows in prostate cancer research. A: Workflow of single-cell RNA-sequencing (scRNA-seq). Prostate tissue is dissociated into a single-cell suspension, followed by droplet-based barcoding, cDNA synthesis, and high-throughput sequencing to generate transcriptomic profiles of individual cells. B: Workflow of spatial transcriptomic profiling using the Visium platform. Tissue sections are placed on spatially barcoded capture slides, followed by tissue permeabilization, mRNA capture, cDNA synthesis, and sequencing to generate spatially resolved gene expression maps while preserving the tissue architecture. The figure was created using BioRender (http://biorender.com). Abbreviations: scRNA-seq, single-cell RNA sequencing; RNA-seq, RNA sequencing; cDNA, complementary DNA; mRNA, messenger RNA; FFPE, formalin-fixed paraffin-embedded; RT, reverse transcription; GEMs, gel beads-in-emulsion.

mercial platforms, including the 10x Genomics Chromium system, enabling massively parallel transcriptomic profiling of thousands of cells in a single experiment [16, 17]. Simultaneously, combinatorial indexing strategies, such as single-cell combinatorial indexing-RNA-seq developed by the Shendure group, enabled scalable single-cell transcriptomic profiling without physical cell isolation [18, 19]. Additional innovations, including barcoding-based multiplexing approaches such as cell hashing, further increased experimental flexibility and throughput in single-cell experiments [20]. Alternative high-throughput single-cell transcriptomic strategies include microwell-based platforms, such as Seq-Well, and probe-based chemistries, including protein interaction profile-seq, which enable scalable single-cell profiling without relying on specialized droplet microfluidic platforms [21].

Upon cell lysis in each reaction compartment, the released mRNA molecules hybridize to oligo-dT primers containing both a cell-specific barcode and unique molecular identifiers, enabling accurate transcript quantification and correction of amplification bias.

Reverse transcription converts mRNA into complementary DNA (cDNA), which is amplified and processed into sequencing libraries via adapter ligation and indexing. High-throughput sequencing generates millions of short DNA sequence reads per experiment.

Bioinformatics processing includes quality control filtering, elimination of cell doublets, alignment to a reference genome, unique molecular identifier collapse, normalization, dimensionality reduction, clustering, and differential gene expression analysis. These computational steps facilitate the identification of transcriptionally distinct cell populations and inference of cellular states in heterogeneous tumor tissues.

Although highly informative, scRNA-seq is influenced by technical variables, particularly during tissue dissociation, which may lead to the selective loss of fragile cell populations or induction of stress-related transcriptional programs. Therefore, careful optimization of sample preparations and analytical pipelines is essential for accurate biological interpretation.

Single-cell isolation of PC specimens

Single-cell isolation from PC tissues presents unique technical challenges owing to the glandular architecture, abundant stromal components, poor viability of prostate epithelial cells, and dense ECM. Therefore, careful optimization of dissociation protocols is necessary to preserve cellular viability and transcriptomic integrity. Biological material can be obtained from radical prostatectomy specimens, needle biopsies, transurethral resections, and experimental models [10, 22-24].

Rapid autopsy programs have emerged as important sources of advanced and metastatic PC tissues. Rapid autopsy enables systematic sampling of multiple metastatic sites within a short postmortem interval, preserving RNA quality and facilitating comprehensive profiling of therapy-resistant and neuroendocrine diseases, which are often difficult to access during life [7, 25].

Tissue processing typically involves mechanical mincing, followed by enzymatic digestion using collagenase, dispase, or related proteolytic enzymes to disrupt ECM structures. The resulting suspension is filtered to remove debris and aggregates, yielding single-cell preparations for downstream capture. Additional processing steps may include red blood cell lysis and viable cell enrichment. In some protocols, fluorescence-activated cell sorting is used to isolate specific populations or remove dead cells. Surface markers such as epithelial cell adhesion molecule and CD45 are frequently used to enrich epithelial and immune compartments, respectively, depending on the study objectives [27, 28]. Notably, dissociation conditions influence cellular representation and may preferentially affect fragile stromal or immune subsets. Therefore, standardization of isolation strategies and documentation of processing parameters are critical for reproducibility and cross-study comparisons.

Sample preservation and experimental enhancements

The feasibility of single-cell profiling in clinical PC research depends on sequencing platforms, sample handling, and experimental design. Preservation of viable and transcriptionally intact cells is critical. Cryopreservation protocols largely maintain tumor heterogeneity with minimal distortion in downstream transcriptomic analyses [29]. Although a modest induction of stress-related gene signatures may occur in some contexts, the preserved samples remain suitable for high-resolution profiling and integrated multi-omics applications, expanding the practical applicability of single-cell technologies to clinical cohorts where immediate processing is not always feasible.

Single-cell transcriptomics is being increasingly combined with complementary modalities, including chromatin accessibility profiling (e.g., single-cell assay for transposase-accessible chromatin using sequencing) and surface protein detection. These integrated approaches allow simultaneous investigation of transcriptional states, regulatory architecture, and protein expression at the level of individual cells [12]. Collectively, these methodological developments have facilitated the broader implementation of single-cell analysis in translational research settings and contributed to more standardized and clinically adaptable study designs.

Single-nucleus RNA-sequencing (snRNA-seq)

Compared to scRNA-seq, which measures transcript levels in both the cytoplasm and nucleus, snRNA-seq uses isolated nuclei instead of whole cells for gene expression profiling and primarily measures the levels of transcripts in the nucleus or those associated with the nuclear membrane. The development of snRNA-seq has advanced the assessment of single-cell transcriptomes using archived frozen biospecimens that are not amenable to intact cell dissociation. Dissociation may impair some sensitive cells in scRNA-seq, and snRNA-seq is suitable for gene expression profiling in difficult-to-isolate cells (e.g., adipocytes and neurons) and cryopreserved tissues. Furthermore, isolating individual cells for scRNA-seq requires long-term incubation and protease digestion, whereas the nuclei needed for snRNA-seq can be quickly and easily obtained from fresh, lightly fixed, or frozen tissues, allowing researchers to obtain transcriptomes that are less likely to be destroyed during isolation. Consequently, snRNA-seq is a

more flexible and less intrusive option for specific types of tissues and cells, whereas scRNA-seq provides a more comprehensive view of the cellular transcriptome when conditions permit.

snRNA-seq is roughly comparable to scRNA-seq and may be a more cost-effective approach as it provides comparable gene detection with reduced dissociation bias and maintains cellular representativeness while minimizing cell number requirement [30, 31]. However, snRNA-seq primarily measures nuclear transcripts and cannot sequence cytoplasmic RNAs (e.g., gene isoforms and mitochondrial or chloroplast RNAs). The choice between these methods depends on the specific requirements of the study, cell types involved, and condition of the tissue samples.

Spatial single-cell profiling

A limitation of scRNA-seq and snRNA-seq is the loss of cellular context with respect to the spatial relationships among cell types within a tissue ecosystem. Recent advances in spatial gene expression analysis at the single-cell level have led to the development of technologies such as Multiplexed Error-Robust Fluorescence in Situ Hybridization, spatial transcriptomics/Visium, and in situ imaging platforms, including 10x Genomics Xenium and NanoString CosMx [13, 32, 33]. Xenium In Situ enables the spatial detection of hundreds to thousands of RNA targets together with multiplexed proteins, facilitating comprehensive molecular profiling at subcellular resolution. Additionally, data from each tissue section can be visualized immediately after instrument operation. The NanoString CosMx Spatial Molecular Imager enables the highly multiplexed spatial detection of more than 5,000 RNA transcripts together with more than 100 proteins in intact tissue sections [33]. Compatible with both fresh-frozen and formalin-fixed paraffin-embedded tissue specimens, it facilitates quantitative visualization of gene and protein expression at single-cell and subcellular resolutions. Multimodal analysis, including protein imaging with CosMx Spatial Molecular Imager, enables improved cell segmentation and high-plex in situ analysis.

PC single-cell research findings

Tumor heterogeneity

PC is characterized by profound clinical and molecular heterogeneity, reflecting diversity in cellular origin, transcriptional states, and interactions within the TME. The adult prostate epithelium consists primarily of luminal, basal, and neuroendocrine cell types, with substantial heterogeneity in both luminal and basal compartments [34-46]. Both basal and luminal populations exhibit unipotent and bipotent progenitor capacities during tissue repair and regeneration [37, 42-46]. However, the precise lineage hierarchy and plasticity of these epithelial subsets in human PC remain unclear.

Spatial transcriptomics has further refined the baseline epithelial organization of the adult human prostate. A spatially resolved transcriptomic atlas has shown distinct anatomical distribution of epithelial subtypes and regional transcriptional programs, providing a high-resolution reference framework for interpreting tumor-associated states [47]. These reports support that tumor heterogeneity arises in a pre-existing spatially organized epithelial hierarchy rather than from a uniform luminal compartment.

The advent of scRNA-seq, spatial transcriptomics, and integrated multi-omics approaches has enabled the investigation of PC heterogeneity at unprecedented resolution. Rather than viewing tumors as homogeneous luminal expansions, these technologies have revealed diverse epithelial states, transitional phenotypes, and spatially restricted subclonal architectures. Henry et al. performed scRNA-seq of more than 34,000 cells from normal human prostates and identified two previously unrecognized epithelial populations, club and hillock cells, enriched in the prostatic urethra and proximal ducts [48]. Their findings refined the baseline cellular taxonomy of the prostate and suggested that the epithelial hierarchy is more complex than previously considered. Building on this foundation, Song et al. applied scRNA-seq to prostate biopsies, prostatectomy specimens, and patient-derived organoids from localized PC cases [27]. They identified tumor-associated epithelial states characterized by

enhanced androgen signaling and observed enrichment of club-like cells in tumors. ERG-negative tumor cells exhibited transcriptional profiles resembling those of adjacent normal luminal cells, suggesting lineage convergence and phenotypic continuity rather than discrete transformation.

Studies on human and mouse tissues have identified multipotent luminal progenitor-like populations (LumP and PrU cells). Their cross-species comparison revealed the important differences between human and murine epithelial compositions, raising concerns regarding direct extrapolation from mouse models to human PC [28]. A study further dissected intratumoral diversity and identified three distinct luminal subpopulations, including a malignant type-1 luminal subtype enriched in hepsin expression, in primary tumors [49]. This work suggested that malignant transformation preferentially involves specific luminal programs rather than uniform luminal expansion. Subsequent analyses of treatment-naïve PC specimens identified four major transcriptional subtypes: Basal, luminal, stromal, and neuroendocrine-like [50]. In epithelial compartments, stem-like and differentiated states coexisted, and lineage trajectory analysis suggested evolutionary transitions linking epithelial diversity to subclonal tumor architecture. Stem-like states were associated with therapeutic resistance and adverse clinical features, highlighting the functional relevance of transcriptional heterogeneity. The integration of scRNA-seq, spatial transcriptomics, and bulk assay for transposase-accessible chromatin using sequencing further refined these observations, identifying an $SOX9^{high}/AR^{low}$ stem-like club cell subset enriched following neoadjuvant androgen deprivation therapy and providing evidence for therapy-associated state remodeling [51]. Spatial analyses have also revealed localized cell-cell communication networks, particularly between epithelial cells and stromal or immune compartments. Macrophage and neutrophil populations exhibit diverse activation states associated with tumor progression. An immunosuppressive microenvironment characterized by Treg infiltration, potentially mediated by FAP^{+} fibroblasts, has been reported in advanced disease. An in-depth synthesis of emerging single-cell omics findings in PC further emphasized the importance of spatial heterogeneity, CAF diversity, and immune ecosystem complexity [52]. Data integration of epigenetic profiling and computational modeling, including deep learning approaches, has been used to reconstruct tumor ecosystems beyond static cell classification. Complementing human data, murine studies have demonstrated strain- and lobe-specific transcriptional differences in the mouse prostate epithelium using scRNA-seq [53]. The identification of rare epithelial populations and distinct fibroblast subsets underscores the intrinsic tissue heterogeneity in experimental models.

Collectively, the above-mentioned studies illustrate a shift in the conceptual framework of PC heterogeneity. Instead of cataloging discrete cell types, single-cell and spatial approaches reveal dynamic epithelial states, lineage plasticity, and context-dependent ecosystem interactions. A structured overview of epithelial lineage states identified via single-cell analyses is provided in **Table 1**. This refined view challenges traditional taxonomy based solely on histology and dominant genomic alterations and provides a framework for linking cellular state composition to therapeutic vulnerability and clinical outcomes.

TME

The TME plays central roles in PC development, progression, and therapeutic resistance. In addition to malignant epithelial cells, the TME comprises immune cells, stromal stem cells, endothelial cells, fibroblasts, myofibroblasts, and neural crest-derived cells, which collectively shape tumor behavior via complex bidirectional interactions [34, 48, 51]. These interactions are mediated by chemokines, cytokines, ECM components, and mechanical cues, leading to changes in gene expression and cellular phenotypes in both tumor and stromal compartments [48, 52-54]. Single-cell and spatial transcriptomic approaches have dissected this tumor ecosystem, revealing previously unappreciated heterogeneity and intercellular communication networks [54-59].

Early single-cell analyses highlighted the interplay between epithelial states and immune components, including the identification of tumor-associated epithelial subpopulations with enhanced androgen signaling and shared immune response signatures across ERG-positive

Table 1. Epithelial hierarchy and tumor-associated luminal states in prostate cancer revealed via single-cell analyses

Conceptual Category	Cellular Population Identified	Key Features/Markers	Species	Representative Studies
Basal stem cells	Basal epithelial stem cells	Self-renewal tumor initiation	Mouse/ human	Lawson 2010 [40]; Wang ZA 2013 [43]; Choi 2012 [41]
Luminal progenitors	Luminal progenitor cells	Organoid-forming capacity	Human/ mouse	Chua 2014 [37]; Karthaus 2014 [44]; Karthaus 2020 [38]
Cell of origin models	Basal vs. luminal origin debate	Pten deletion models	Mouse	Goldstein 2010 [39]; Lu 2013 [42]; Kwon 2014 [45]
Club-like epithelial cells	Club and hillock cells	Urethral enrichment	Human	Henry 2018 [48]; Song 2022 [27]
Tumor-associated luminal states	AR-high tumor-enriched club-like cells	Androgen signaling	Human	Song 2022 [27]
Luminal subtype stratification	Type-1 luminal subtype	Early-stage stratification	Human	Ma 2020 [49]
Developmental hierarchy overlay	Stem-like and differentiated states	Subclonal evolution	Human	Ge 2022 [50]
Multi-omic heterogeneity	$SOX9^{high}/AR^{low}$ subset	ADT-associated plasticity	Human	Bian 2024 [51]
Spatial epithelial baseline	Anatomical epithelial distribution	Spatially resolved atlas	Human	Hu 2025 [47]

This table summarizes epithelial cell populations, including basal stem cells, luminal progenitors, tumor-associated luminal states, and spatially defined epithelial subtypes, together with their key features and representative studies.

and -negative tumors [27]. A previous report linked differential gene expression to metastasis and tumor-immune interactions, identifying activated endothelial cells enriched in CRPC that are associated with invasive behavior [10]. Complementing this, characterization of tumor endothelial cells revealed the upregulation of angiogenesis- and ECM-related pathways, including C-X-C motif chemokine ligand 12 (CXCL12) - C-X-C motif chemokine receptor 4 (CXCR4) signaling, suggesting potential prognostic and therapeutic relevance [63].

Recent high-resolution spatial and multi-omics analyses have further refined our understanding of TME organization. Single-cell and spatial transcriptomics have demonstrated a close interaction between club-like epithelial cells and immunosuppressive myeloid populations, particularly MDSCs, suggesting coordinated epithelial-immune remodeling within aggressive tumors [64]. Extending this concept, spatial multi-omics profiling has identified pro-inflammatory chemokine activity and immune cell recruitment patterns associated with aggressive disease phenotypes, highlighting chemokine-driven microenvironmental circuits as potential therapeutic targets [65]. Fibroblast heterogeneity has also emerged as a clinically relevant dimension of the TME. Single-cell sequencing of CRPC-associated fibroblasts has revealed distinct CAF subpopulations linked to prognosis and differential immunotherapy responses, underscoring the contribution of stromal diversity to therapeutic outcomes [66].

Comprehensive ecosystem-level modeling has underscored the relevance of TME heterogeneity. Integrative single-cell and spatial transcriptomic analysis combined with interpretable machine learning has identified a “lethal tumor axis” characterized by chromosomal instability, AR activation, and stemness programs embedded within specific spatial niches, illustrating the prognostic potential of ecosystem-informed stratification [67]. A synthesis of single-cell omics findings emphasized spatial context and multi-omics integration as critical for understanding tumor evolution and treatment vulnerability [26]. These conclusions are supported by studies highlighting the regulation of the TME by immune, vascular, stromal, and microbial components, including potential roles of the microbiome in androgen metabolism and therapeutic resistance [68]. As the TME comprises an evolving ecosystem shaped by tumor-

intrinsic programs and systemic influences [69], the integration of single-cell and spatial analyses is necessary to uncover immune evasion mechanisms and guide personalized therapy, particularly in treatment-refractory diseases [70].

Previous studies have actively explored the therapeutic targeting of TME components, including angiogenesis and immunosuppressive networks [71, 72]. Immune checkpoint blockade exhibits limited efficacy in PC, partly because of the low expression of target molecules in most tumors [73-76]. In phase III trials, ipilimumab did not significantly improve overall survival in metastatic CRPC, although some subsets showed delayed or modest benefits [73, 74]. Reviews have highlighted the need for improved patient stratification and practical combination strategies [75]. Notably, programmed death-ligand 1 expression is rare in primary PC but elevated in small-cell and metastatic CRPC, suggesting that the immune context evolves with disease progression [76].

Single-cell profiling has refined our understanding of immune heterogeneity within the TME. Tregs, exhausted CD8+ T cells, TAMs, and MDSCs are frequently enriched in the microenvironment of tumors in which immunosuppressive phenotypes are associated with adverse outcomes [12, 77-85]. For example, increased immune infiltration has been reported in BRCA2-mutated tumors but with a suppressed immune phenotype, highlighting the complexity in predicting immunotherapy responses [77]. Additional studies have delineated transcriptional programs underlying T cell exhaustion and immune regulation, including networks involving TCF-1 and CX3CR1 [77-80]. Genetic background also shapes the immune ecosystem architecture. Distinct oncogenic drivers generate divergent immune landscapes that influence tumor progression via context-specific mechanisms [86]. These reports further support that immune heterogeneity in PC is not uniform but genetically and transcriptionally structured.

Myeloid-derived cytokine signaling contributes to immune remodeling in advanced PC. Interleukin-23 secreted by tumor-associated myeloid cells drives castration resistance and promotes tumor progression [87]. Re-education of TAMs via CXCR2 blockade induces senescence and inhibits tumor growth, underscoring the plasticity of myeloid populations within the TME [88]. These findings illustrate that immune suppression in PC is not merely a passive phenomenon but is actively sustained via cytokine-driven feedback loops. Mechanistic insights have also emerged from single cell studies on PC-associated immune cell types, which have demonstrated that AR signaling in T cells constrains their effector function and contributes to immunotherapy resistance. AR inhibition enhances interferon-γ production and responsiveness to programmed death protein 1 blockade [81]. Smad7 regulates programmed death protein 1 signaling pathways in immune cells, suggesting additional modulatory axes [82].

High-resolution single-cell analyses have also revealed distinct immunosuppressive niches. Studies on immune cells in localized and metastatic PC have identified tumor-enriched MDSCs, TAMs, and Tregs, demonstrated CCL20-CCR6-mediated crosstalk between myeloid cells and Tregs [89], and characterized a prostate-specific macrophage subset linked to zinc metabolism and favorable outcomes [90]. Kfoury et al. reported lipid-laden TAM populations in bone metastases with immunosuppressive properties [91]. Masetti et al. identified macrophage receptor with collagenous structure-expressing macrophages promoting tumor invasiveness via interleukin-1β-dependent pathways [92]. Spatial analyses have indicated that exhausted CD8+ T cells and immunosuppressive populations are enriched in high-grade PC compared to low-grade tumors [93] and identified angiogenic and suppressive fibroblast niches in aggressive cribriform subtypes [94]. Specifically, Pygo2 deletion reshapes the immune microenvironment and enhances responsiveness to immune checkpoint blockade therapy by modulating the p53/Sp1/Kit/indoleamine 2,3-dioxygenase 1 axis [95].

Taken together, these findings support a conceptual shift in which the PC TME is understood to be an evolving ecosystem rather than a passive background. Immune, stromal, vascular, and microbial components interact with tumor cells to shape disease progression, therapeutic resistance, and clinical outcomes. The major TME programs characterized in PC are summarized in **Table 2**. Overall, high-resolution

Table 2. Tumor microenvironment (TME) programs and cellular interactions in prostate cancer identified via single-cell and spatial analyses

Conceptual Category	Cellular Population Identified	Defining Program/Feature	Species	Clinical/Biological Relevance	Representative Studies
Tumor endothelium	Activated endothelial cells and TECs	CXCL12-CXCR4 axis	Human	Angiogenesis targeting	Chen S 2021 [10]; Heidegger 2022 [63]
Cancer-associated fibroblasts	CRPC-associated CAF subtypes	FAP and TGF-β signaling	Human	Immunotherapy stratification	Qiu 2025 [66]
Myeloid compartment	TAMs, lipid-laden TAMs, and MDSCs	CCL20-CCR6 and MARCO	Human	Immune suppression and metastasis	Hirz 2023 [89]; Kfoury 2021 [91]; Masetti 2022 [92]
Club-like cell-myeloid interaction	Club-like epithelial-MDSC crosstalk	Chemokine signaling	Human	Aggressive PC immune niches	Kiviaho 2024 [64]; Krossa 2025 [65]
T cell states	Tregs and exhausted CD8+ T cells	TCF-1 and CX3CR1	Human	ICB response prediction	Adorno Febles 2023 [93]; Chen Z 2019 [80]
Immune checkpoint context	PD-L1 low primary; high CRPC	AR-immune interplay	Human	Limited ICB efficacy	Haffner 2018 [76]; Guan 2022 [81]
Ecosystem-level malignant axis	"Lethal tumor axis"	Chromosomal instability and stemness	Human	Prognostic stratification	Ge 2026 [67]

This table summarizes major components of the tumor microenvironment, including endothelial cells, cancer-associated fibroblasts, myeloid populations, and T cell subsets, along with their defining molecular features, spatial characteristics, and representative studies. Abbreviations: CAFs, cancer-associated fibroblasts; TAMs, tumor-associated macrophages; MDSCs, myeloid-derived suppressor cells; Tregs, regulatory T cells; PD-L1, programmed death-ligand 1.

molecular profiling enables the integration of cellular composition, spatial architecture, and regulatory networks, providing a foundation for the development of new ecosystem-informed precision oncology strategies.

Insights into PC progression

PC progression from localized neoplastic growth to invasion and metastasis is characterized by progressive phenotypic diversification, with lineage plasticity and therapeutic adaptation playing central roles in treatment resistance and metastatic competence. Single-cell technologies have provided unprecedented resolution to trace these evolutionary trajectories at the cellular state level rather than as static histological categories.

Clinically, disease progression includes treatment resistance, which often involves a transition from AR-dependent luminal adenocarcinoma to CRPC, and, in some cases, NEPC. This shift is not simply a loss of AR signaling but reflects extensive reprogramming of developmental and differentiation pathways [96-101]. Single-cell analyses have revealed that CRPC and NEPC comprise multiple coexisting cellular states defined by distinct transcription factor networks [102]. Subsets characterized by achaete-scute family bHLH transcription factor 1, neuronal differentiation 1, or POU2F3 expression resemble the neuroendocrine programs observed in other epithelial cancers, suggesting convergent lineage trajectories rather than a single deterministic pathway [102-104].

Recent single-cell transcriptomic profiling of NEPC has further refined this heterogeneity. Distinct pathological entities, including focal neuroendocrine differentiation, amphicrine tumors, and classical small-cell phenotypes, harbor transcriptionally divergent programs despite overlapping histological features [24]. These findings suggest that neuroendocrine differentiation in PC represents a spectrum of molecularly distinct states rather than a uniform terminal phenotype.

The integration of scRNA-seq with chromatin accessibility profiling has further clarified the temporal dynamics of therapy-induced state transitions. AR-targeted therapy promotes the expression of transcription factors, such as zinc finger E-box-binding homeobox 1, achaete-scute family bHLH transcription factor 1, and neuronal differentiation 1, which are associated with epithelial-mesenchymal transition (EMT)-like programs and neuroendocrine differ-

entiation [105]. Importantly, these transitions may arise not solely from genomic mutations but also via reversible epigenetic remodeling, suggesting that phenotypic plasticity precedes or accompanies clonal selection.

Therapeutic resistance is associated with cellular state diversity. The levels of trophoblast cell-surface antigen 2 (TROP2; also known as TACSTD2), which is highly expressed in CRPC, remain elevated even in AR-independent cell populations [106]. The persistence of TROP2-positive cells suggests that distinct transcriptional states are maintained despite the loss of canonical AR signaling, providing a rationale to develop antibody-drug conjugate strategies targeting non-AR-dependent tumor compartments.

Collectively, these findings support a model in which PC evolution follows multiple branching paths during disease progression rather than a linear path toward AR independence. Therefore, CRPC and NEPC should be regarded as heterogeneous state-defined populations with diverse cellular origins and therapeutic sensitivities. Strategies relying on single biomarkers are inherently limited. Profiling cellular states at single-cell resolution is essential to refine molecular targeting.

Experimental models have corroborated the above-mentioned plasticity. Stepwise overexpression of the ETS transcription factor ETV4 drives neoplastic transformation in the prostate epithelium; single-cell analysis has revealed the emergence of luminal clusters exhibiting cell cycle activation, senescence signatures, and EMT features [107].

Microenvironmental cues further influence tumor progression. Vitamin D signaling suppresses Wnt pathways and promotes epithelial differentiation, thereby limiting tumor progression [108]. In phosphatase and tensin homolog (Pten)-deficient models, treatment with the vitamin D analog Gemini-72 induces apoptosis in precancerous lesions and modulates stromal remodeling and MDSC infiltration [109]. Neural-epithelial interactions have also been implicated, with perineural invasion linked to CXCL/CCL-mediated communication between basal/intermediate epithelial cells and neural elements [110].

Treatment-induced senescence is another adaptive state that potentially influences invasion, metastasis, and treatment resistance. Senescent tumor cells depend on Mcl-1, and targeting this pathway reduces metastatic progression [111]. Genetic perturbations also reshape lineage identity. Tripartite motif-containing 28 deletion alters luminal progenitor programs and accelerates invasive progression [112]. AR hyperactivation synergizes with insulin-like growth factor-1 and Wnt signaling to drive tumorigenesis in Osr1-lineage cells [113]. Hypoxia-induced HIF1A signaling promotes immune-evasive luminal states and malignant progression in prostatic intraepithelial neoplasia lesions [114]. Transcriptional regulators such as MYC and ERG further modulate lineage programs. MYC overexpression induces transcriptional reprogramming by promoting promoter-proximal pausing of RNA polymerase II and indirectly suppressing AR signaling [115]. Conversely, ERG maintains AR co-regulator complexes, and ERG loss markedly reduces AR transcriptional output [116]. Under sustained AR inhibition, PC cells undergo adaptive transcriptional reprogramming that destabilizes luminal lineage identity and promotes alternative differentiation trajectories [117-119]. These adaptive responses may culminate in AR-indifferent phenotypes, including NEPC and double-negative CRPC [97-99, 120-123]. This plasticity is frequently associated with MYCN and aurora kinase A overexpression [123], loss of RB1, TP53, and PTEN [124-126], and epigenetic reprogramming driven by the enhancer of zeste homolog 2 [98, 99]. Epigenetic remodeling and DNA methylation changes are also implicated in radioresistance [100], underscoring the convergence of plasticity and treatment adaptation.

The origin cell of NEPC remains controversial. Some reports suggest differentiation from neuroendocrine cells [127], whereas others indicate the involvement of basal or luminal-derived reprogramming pathways [104, 128]. NE tumor cells exhibit a subset of luminal epithelial characteristics [103]. Defined oncogenic driver combinations reprogram human basal epithelial cells into small-cell neuroendocrine carcinoma, suggesting convergent molecular routes across tissues and cell types [129]. Clinical single-cell analyses have reinforced these concepts. Extensive transcriptional heterogeneity

has been reported in individual cells within CRPC and NEPC tumors, with the expression of targetable surface antigens, such as PSMA, STEAP1/2, and delta-like canonical Notch ligand 3, varying across cellular states [102]. Zinc finger E-box-binding homeobox 1-positive intermediate tumor cell populations exhibit EMT, stemness, and neuroendocrine features, linking metabolic reprogramming and histone lactylation to enhanced plasticity [104]. In-depth analyses of PC composition using single-cell technologies and parallel studies in other tumor types have highlighted the importance of the interplay of genetic, epigenetic, and hormonal signaling alterations in shaping cancer progression [106].

Taken together, these studies redefine PC progression as a process of cellular state transition rather than fixed lineage replacement. Therapeutic pressure does not merely select pre-existing clones but may also induce reversible transcriptional and epigenetic reprogramming, enabling tumors to traverse lineage boundaries. In this context, single-cell and multi-omics analyses provide a conceptual framework to understand plasticity-driven resistance and design strategies targeting cellular states rather than static tumor classifications.

Understanding drug resistance

Therapeutic resistance in advanced PC is not merely a consequence of accumulated mutations but a dynamic reconfiguration of cellular states under treatment pressure. Single-cell analyses are important for distinguishing between clonal selection of pre-existing subpopulations and therapy-induced transcriptional reprogramming.

Docetaxel is the cornerstone of metastatic CRPC treatment [130, 131]; however, a substantial proportion of patients exhibit primary resistance or relapse within several years. Early genomic analyses have demonstrated that metastatic lesions may arise from subclones already present within primary tumors [132], suggesting that resistant lineages predate systemic therapy. This observation aligns with emerging single-cell evidence that minor transcriptionally distinct populations persist through treatment and subsequently dominate recurrent disease [133]. Mechanistically, taxane resistance is associated with alterations in microtubule dynamics and anti-apoptotic signaling [134-138]. However, bulk analyses cannot determine whether these features are uniformly distributed or restricted to specific cellular states. Genome-wide clustered regularly interspaced palindromic repeat (CRISPR)/CRISPR-associated protein 9 screens in CRPC models have identified regulators such as developmentally regulated GTP-binding protein 1 and RP2, which influence sensitivity to docetaxel and cabazitaxel via modulation of the AR and mechanistic target of rapamycin complex 1 pathways [139]. Single-cell analyses have further identified stress-response regulators such as nuclear protein 1 as modulators of docetaxel resistance that are not apparent in bulk profiling [140].

Resistance to AR-targeted therapies also reflects cellular state plasticity. Single-cell chromatin and transcriptomic profiling has indicated that pre-existing chromatin configurations predispose tumors to enzalutamide resistance [141]. Particularly, a minor $AR^{low/-}$ population present before treatment exhibits intrinsic resistance and expands under therapeutic pressure [133]. Additionally, HIF1A signaling is implicated in castration resistance in PTEN-deficient tumors, and pharmacological inhibition restores treatment sensitivity in experimental models [142].

In addition to individual drug mechanisms, therapeutic resistance is closely linked to cellular state diversity. The levels of TROP2 (TACSTD2), which is highly expressed in CRPC, remain elevated even in AR-independent cell populations [105]. The persistence of TROP2-positive cells suggests that distinct transcriptional states are maintained despite the loss of canonical AR signaling, providing a rationale for the development of antibody-drug conjugate strategies targeting non-AR-dependent tumor compartments. Reviews on mCRPC resistance mechanisms have further underscored the convergence of adaptive signaling, epigenetic remodeling, and microenvironmental influences in shaping therapeutic outcomes [71, 143].

Collectively, these data indicate that drug resistance in PC is best conceptualized as a state-dependent phenomenon. Treatment does not simply eliminate sensitive clones while leaving the resistant ones; rather, it reshapes the cel-

Table 3. Lineage plasticity and therapy-associated state transitions in prostate cancer revealed via single-cell and multi-omics analyses

Conceptual Category	Cellular Population Identified	Defining Program/ Feature	Species	Clinical/Biological Relevance	Representative Studies
Luminal-to-CRPC transition	AR-low/AR-indifferent luminal states	AR suppression and chromatin remodeling	Human	ARSI resistance	Xue 2022 [133]; Taavitsainen 2023 [141]
Luminal-to-NE transition	ASCL1+, NEUROD1+, and POU2F3+ NE-like states	MYCN, RB1 loss, and EZH2 activation	Human/ PDX	Hormone-indifferent phenotype	Quezada Urban 2025 [24]; Brady 2021 [121]
Therapy-induced intermediate states	ZEB1+ EMT-like populations	EMT program and epigenetic reprogramming	Human	Adaptive resistance	Wang 2023 [105]; Asberry 2022 [120]
Epigenetic reprogramming axis	EZH2-driven lineage switching	Histone modification and AR repression	Human	Targetable epigenetic vulnerability	Berger 2019 [99]; Imamura 2023 [106]
Metabolic plasticity	Zeb1-associated glycolytic states	Lactate-histone lactylation	Human	NEPC progression	Wang 2023 [105]
Hypoxia-associated adaptation	HIF1A-driven luminal immune-evasive states	Hypoxia signaling	Mouse/ Human	Radio- and therapy resistance	Terzic 2023 [142]
Chemotherapy resistance states	NUPR1+ or TROP2+ resistant clones	Anti-apoptotic programs	Human	ADC targeting and taxane resistance	Schnepp 2022 [140]; Xue 2022 [133]

This table summarizes cellular state transitions associated with disease progression and therapeutic resistance, including luminal-to-castration-resistant prostate cancer transition, neuroendocrine differentiation, epigenetic reprogramming, metabolic adaptation, and chemotherapy-resistant subpopulations, together with representative studies. Abbreviations: CRPC, castration-resistant prostate cancer; AR, androgen receptor; ARSI, androgen receptor signaling inhibitor; ADC, antibody-drug conjugate.

lular landscape, enriching phenotypes capable of surviving under new selective pressures. A structured overview of lineage plasticity and therapy-associated resistance states identified via single-cell analyses is presented in **Table 3**. Single-cell and multi-omics approaches provide a framework to anticipate resistance by identifying primed subpopulations and regulatory programs before clinical progression becomes evident.

Clinical implications and future directions

Single-cell analysis has substantially refined our understanding of PC heterogeneity, revealing that tumor progression and therapeutic resistance are shaped by distinct cellular states rather than static histological entities. These insights have substantial clinical implications. First, single-cell approaches provide a framework to better characterize intratumoral heterogeneity. The identification of AR-high and -low populations, stem-like programs, and neuroendocrine-associated states may explain differential treatment responses within the same tumor. This information may further complement conventional pathological grading and genomic profiling. Second, single-cell and spatial analyses have improved our understanding of the TME. These technologies facilitate the refinement of patient stratification for immunotherapy and combination treatments by resolving immunosuppressive niches, endothelial activation states, and fibroblast-mediated signaling networks. Third, the integration of single-cell profiling with spatial transcriptomics may enhance the interpretation of tissue architecture and cell-cell interactions in clinical specimens, including archived formalin-fixed paraffin-embedded samples. This integration may facilitate the development of ecosystem-informed therapeutic strategies.

Future directions include continued standardization of analytical pipelines, improved integration of transcriptomic, epigenomic, and proteomic modalities, and expansion of single-cell profiling to longitudinal and multi-site clinical samples. Although technical and computational challenges remain, ongoing methodological advances are steadily improving reproducibility and scalability. Overall, single-cell and spatial transcriptomic technologies can complement existing molecular diagnostics and contribute to more precise state-aware therapeutic strategies for PC management.

Conclusion

PC progression is increasingly driven by cellular plasticity and dynamic tumor ecosystem interactions rather than linear genetic evolution. Single-cell and spatial transcriptomic analyses have revealed diverse cellular states and micro-

environmental networks underlying heterogeneity and therapeutic resistance, challenging conventional classification systems based on bulk profiling. A state-based framework integrating cellular hierarchy and spatial context may better explain disease behavior. Such approaches can advance precision oncology and improve patient stratification in PC.

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Disclosure of conflict of interest

None.

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