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Prostate Luminal Progenitor Cells in Development and Cancer

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Abstract

Prostate cancer (PCa) has predominantly a luminal phenotype. Basal cells were previously identified as a cell-of-origin for PCa, but increasing evidence implicates luminal cells as a preferred cell-of-origin for PCa, as well as key drivers of tumor development and progression. Prostate luminal cells are understudied compared to basal cells. This review describes the contribution of prostate luminal progenitor (LP) cells to luminal cell development and their role in prostate development, androgen-mediated regeneration of castrated prostate, and tumorigenesis. The potential value of LP transcriptomics to identify new targets and therapies to treat aggressive PCa is also discussed. Finally, we propose future research directions focusing on molecular mechanisms underlying LP cell biology and heterogeneity in normal and diseased prostate.

Keywords

Luminal progenitors; Prostate stem cells; Prostate cancer; Cancer stem cells; Castration resistance

Prostate Luminal Cells

Prostate cancer (PCa) is the most frequently diagnosed malignancy and the second leading cause of cancer-related mortality among men [1]. The prostate is an exocrine gland that sits around the urethra at the base of the bladder and functions to nourish and protect sperm by secreting a slightly alkaline fluid that constitutes roughly 30% of the semen. The adult prostate contains basal and luminal epithelial cells, together with rare neuroendocrine (NE) cells, organized as a pseudo- stratified epithelium surrounded by stromal cells (Box 1). Luminal cells line the lumen and produce secretory proteins such as human PSA (prostate-

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specific antigen) or mouse probasin (Pb), express cytokeratin 8 (CK8) and androgen receptor (AR), and are androgen-responsive. Basal cells are aligned between the basement membrane and the luminal cell layer and express CK5, CK14, and the stem cell (SC) transcription factor p63. NE cells, the least characterized population, are androgen-independent and are identified by expression of NE differentiation markers such as chromogranin A and synaptophysin [2]. Basal cells, much like the myoepithelial/basal cells in the mammary gland, are the main pool of prostate stem cells (PSCs), whereas luminal cells are generally viewed as differentiated cells with limited stem/progenitor cell capacity [3, 4].

In the adult prostate, secretory luminal cells are the functional epithelial cell type that is self-maintained by luminal progenitor cells (LPs) (Box 1) [5]. Pathologically, the benign prostate hyperplasia (BPH) is a common disorder that arises from aberrant proliferation of both prostate epithelial and stromal progenitor cells and is frequently associated with inflammation [6]. PCa displays a predominantly luminal phenotype with loss of basal cells, leading many to speculate that luminal cells and LPs may be the cell-of-origin for PCa and the driving force for tumor progression, respectively. This calls for a better dissection of the role of LPs in prostate homeostasis and tumorigenesis.

When confined to the organ, primary PCa is treated by radical prostatectomy and/or radiation with a good prognosis. Androgen deprivation therapy (ADT), which blocks androgen synthesis (e.g., abiraterone) or AR function (e.g., bicalutamide, enzalutamide), is the main therapeutic regimen for patients with locally advanced and metastatic PCa. However, the majority of patients develop castration-resistant prostate cancer (CRPC). There is currently no effective therapy for metastatic CRPC, and tumor cell heterogeneity is a major challenge to clinical management. PCa contains many phenotypically and functionally distinct subsets of cells, including a relatively small population of cancer cells with stem-like properties, often termed cancer stem cells (CSCs) [7, 8]. CSCs resemble the normal stem/progenitor cells of the prostate, preexist in primary PCa and become enriched in CRPC, and may mediate tumor aggressiveness [8]. Since PCa has a luminal phenotype, conceivably, prostate CSCs should exhibit LP-like properties. Therefore, in-depth understanding of the luminal cell lineage hierarchy and biology of LPs may help develop new therapeutic strategies to treat aggressive PCa

Despite various methods developed to identify (preferentially basal) stem/progenitor cell populations in the prostate (Box 2), LPs remain poorly defined due to relatively few studies [9, 10]. Many *ex vivo* PSC studies have been constrained by culture conditions that promote basal, but not luminal, cell propagation [11], since luminal cells are extremely sensitive to tissue dissociation and generally fail to survive in explant cultures or in grafts [10]. Recent lineage-tracing studies revealed the existence of LPs [5, 12], and advances in 2D and 3D culture systems allow LPs to survive and proliferate [11, 13, 14]. Due to technical improvement, many studies that focus on prostate epithelial cell hierarchy, particularly on luminal cell biology, are emerging (Box 1) [3, 5, 11, 12, 15–18]. Consequently, several markers that potentially identify and/or enrich for LPs in human and mouse prostates under diverse conditions have been reported (Table 1). In this review, we summarize the current knowledge in the hierarchy and contribution of luminal cell lineage in the normal and diseased prostate, and present evidence to establish LPs as the key cell population that

mediates prostate development and cancer progression. We also discuss how transcriptomics of LPs may lead to the identification of new targets and therapeutic strategies to treat aggressive PCa.

LPs in Prostate Development and Regeneration

Herein, we define the LPs as luminal cells that exhibit functional stem-like properties in biological assays. Many studies have proven the existence of LPs and their contributions to prostate development and regeneration (Box 1 and 2). Although prostatic acini are macroscopically organized similarly at the cellular level, prostate glands differ significantly between species. We thus describe, below, the studies performed in human and animal systems separately.

Studies in the mouse prostate

Lineage-tracing studies [3, 16, 17] revealed that basal stem/progenitor cells, the main pool of PSCs, contribute significantly to the generation of embryonic luminal cells, as the organ is originated from basal SCs. However, during early postnatal development (i.e., a phase of luminal cell expansion in the first ~2 weeks), the contribution of basal cells to luminal cells via differentiation through LPs becomes limited, and after establishment of the luminal cell layer in puberty, differentiated luminal cells are self-sustained by LPs during homeostasis and androgen- mediated regeneration from castration (Box 1) [5, 15, 16]. Since luminal cells significantly outnumber basal cells in the mouse prostate, it is conceivable that LPs play a central role in epithelial differentiation and expansion during postnatal development. Similarly, it is anticipated that LPs are drivers of regeneration, as not all luminal cells die from castration.

Early BrdU pulse-chase experiments suggested the existence of a population of quiescent luminal cells capable of retaining labels in the proximal normal prostate [21]. Employing a similar label- retention strategy, we recently developed a bigenic mouse model, in which a histone 2B (H2B)- GFP fusion protein is driven by the promoter of a preferential luminal-lineage gene *Probasin* (*Pb*). This model allowed us to specifically identify, isolate, and characterize the luminal slow-cycling label retaining cells (LRCs) as functional LPs [22]. LRCs are inherently resistant to castration, can generate large colonies and organoids *in vitro,* and can form prostatic glands in tissue regeneration assays *in vivo*. Luminal LRC-derived organoids and prostatic glands contain both basal and luminal cells, suggesting bipotency. These findings reveal the functional properties of normal LPs. Importantly, analysis of a spectrum of phenotypic markers previously linked to epithelial cell stemness in luminal LRCs reveals heterogeneity in normal LP populations [22].

Several rare LP populations that contribute to prostate regeneration have been reported in mice, including castration-resistant NKX3.1-expressing (CARN) [12], Sca-1^+ [23], castration-resistant Bmi1-expressing (CARB) [24] and $Lgr5^+$ cells [25]. CARNs are castration-induced and exhibit multipotentiality in single-cell transplantation assays [12]. A distinct population of Sca-1^+ luminal cells is identified in castration-regressed prostates, which express AR but are androgen- independent and can form organoids *in vitro* and prostate glands *in vivo* containing both cell lineages, demonstrating bipotency [23]. Also,

Bmi1 marks a rare population of castration-resistant LPs (CARBs) enriched in the proximal mouse prostateand capable of tissue regeneration and self- renewal, which, interestingly, are distinct from CARNs [24]. The WNT target gene Lgr5 is a SC marker in multiple adult tissues. Recent lineage tracing studies reveal that $Lgr5^+$ cells are primarily luminal, long-lived stem/progenitor cells in regressed prostates, and are required for efficient adult prostate regeneration [25]. Therefore, the identification of LPs in castrated prostates suggests an independence of AR signaling, although luminal cells are generally AR^+ by immunostaining. Several studies employing genetic AR-deletion strategies support this idea. For instance, it was shown that AR is dispensable for the survival and proliferation of luminal sphere-forming cells *in vitro* [26]. Consistently, the LP properties of CARNs are unaffected by *AR* deletion during prostate regeneration [27]. *AR* deletion suppresses tumor formation upon *Pten* loss in CARNs [27], suggesting differential requirements for AR in LPs during regeneration versus tumor initiation. Moreover, indirect evidence, counter-intuitively, demonstrates that *AR* deletion does not affect luminal cell survival but induces transient over-proliferation; strikingly, these AR- luminal cells exhibit a stem-like phenotype [28]. We have also observed an AR^{low} phenotype in LPs [11, 22]. These data together reveals intrinsic castration resistance in LPs, and thus understanding this property may shed light on mechanisms of ADT resistance.

Studies in human systems

There is only a limited number of studies on LPs in the human prostate. 3D organoid assays [14] and 2D culture systems [11] have been used to enrich and propagate LPs and stem-like cells in human prostate [14]. To date, there is only one study reporting $CD38^{low}$ as a potential phenotypic marker for human LPs in that $CD38^{low}$ cells, compared to $CD38^{hi}$ cells, display higher SC activities in biological assays [29]. Notably, CD38 was initially reported as a general marker of luminal cells in human prostate [30]. Since differential expression of CD38 enables isolation of two distinct luminal subsets, other luminal cell markers (e.g., CK8, CK19, AR) with heterogeneous expression may also prove useful to fractionate and dissect the luminal cell subpopulations.

Plasticity of LPs

There is a debate regarding the plasticity of LPs, as bi- or multi-potency is frequently observed in SC-related *in vitro* (e.g., 2D and 3D cultures) and *ex vivo* (e.g., prostate regeneration) assays, but not in *in-situ* lineage tracing studies. Particularly, LPs are always unipotent *in situ*, albeit extremely rare bipotent LPs, such as CARNs, are reported in regeneration assays [12]. There has been no direct evidence for the ‘dedifferentiation’ or ‘transdifferentiation’ of luminal cells into basal or NE cells, respectively, in lineage tracing studies under physiological conditions (although this dedifferentiation has been observed in the context of cancer progression). This may suggest that current *in vitro* SC-related assays (in both mouse and human prostates) are inductive of facultative functions of luminal cells, as is the case for basal cells [3]. As in other tissue SCs, LPs are heterogeneous and different studies have reported distinct phenotypes of putative LPs (Table 1). The precise relationship between these subpopulations and whether they reside at different LP “plasticity states” remains unclear.

LPs as the Cell-of-Origin for PCa

The cell-of-origin for cancer is a cell within the tissue that initially becomes tumorigenically transformed. Understanding the nature of these cells helps disease prevention, diagnosis and prognosis [44]. The cancer cell-of-origin may or may not be tissue SCs, but studies have strongly suggested that normal stem/progenitor cells are the cells of origin for many human cancers due to their proliferative potential and longevity that could allow accumulation of oncogenic mutations. For decades, luminal cells have been hypothesized as the cell-of-origin for PCa. However, early work in mouse models revealed that basal cells, relative to luminal cells, display higher efficiency for cancer initiation and can produce luminal-like disease in experimental settings [45]. Similarly, studies using freshly purified bulk human prostate epithelial cells coupled with tissue regeneration assays reported that only the basal cells can undergo tumorigenic transformation (Figure 1A) [46]. However, the same group later demonstrated that human LPs enriched by organoid culture (Box 2) [14], just like basal SCs, can also initiate PCa when overexpressing oncogenes [47]. Likewise, we have demonstrated that 2D culture-enriched human LPs can function, as efficiently as cultured basal cells, as a cell-of-origin for PCa (Fig. 1A) [11]. The discrepancy between studies using bulk vs. LP-enriched luminal cells could potentially be reconciled by the rarity of the transformation target, LPs. Importantly, multiple recent lineage-tracing studies [3, 5, 12, 18, 48, 49] provided definitive evidence of PCa initiation from both basal and luminal cells upon lineage-specific *Pten* deletion (Figure 1B). In fact, luminal cells are actually favored as cells of origin for PCa in many experimental conditions [18]. Additionally, a recent report indicated that, in response to oncogenic transformation, the human $CD38^{low}$ LP population can initiate PCa *ex vivo* [29].

LPs Drive PCa Development and Progression

Recent advances shed light on the role of LPs in PCa development and progression. We define PCa LPs as the cancer cells that functionally resemble normal LPs without expression of basal cell markers. We summarize below how LPs drive tumor progression in both mouse and human systems.

LPs Drive Mouse PCa Development Initiated from Luminal Cells

PCa initiated from *Pte*n deletion in luminal cells driven by luminal-cell-specific promoters (e.g., *Ck8*, *Pb*, and *Nkx3.1*) has a luminal phenotype (Figure 1B). Considering that these promoters can capture LPs, we speculate that either the normal LPs are initially transformed to become tumor- initiating cells or mature luminal cells regain LP-like properties upon transformation to propagate the tumor. It was reported that activation of PI3K/AKT signaling upon *Pten* loss is critical for prostate CSC maintenance and viability [50]. Though it is hard to discriminate whether differentiated luminal cells or normal LPs are the initial cells-of-origin, it is easy to infer that transformed cells exhibiting LP properties are the cells that mediate PCa initiation and tumor cell expansion. In support, the PCa with luminal-specific *Pten* deletion was initiated by a population of $pAKT^{+}$ LPs overexpressing Ck19 and Sca-1, and subsequently maintained, at least in part, by the accumulation of LPs [48]. Additionally, a recent study investigating the stem/progenitor properties of cancer cells from the aggressive *Pten*/*Trp53*-null mouse PCa demonstrated the co-existence of two classes of

LPs [51]. One is the previously described multipotent LPs [14] that can form organoids containing both basal and luminal cells, and the other is the committed LPs that only generate luminal cell-containing organoids. Surprisingly, these two types of LPs can initiate two distinct graft phenotypes: multipotent LPs give rise to adenosquamous tumors and adenocarcinoma with variable sarcomatoid morphology, whereas committed LPs only produce adenocarcinomas [51]. Importantly, these committed LPs constitute a major population in tumors and are castration-resistant [51], suggesting that PCa LPs can maintain tumor development and mediate treatment resistance. Consistently, a recent report demonstrated that a $Lin^{-}/Sca\text{-}1^{+}/CD49f^{med}$ (LSC^{med}) LP population pre-exists in various mouse models of androgen-sensitive PCa, is inherently castration-resistant, and becomes dominant in CRPC [52]. Overall, these studies highlight that LPs represent the driving force behind androgen-independent progression of luminal cell-originated PCa.

LPs also Drive Mouse PCa Development Initiated from Basal Cells

Lineage-tracing studies [5, 49, 53] demonstrated that a basal cell-specific deletion of *Pten* invariably results in a first step of basal-to-luminal differentiation followed by appearance of luminal-like tumors (Figure 1B). This basal-to-luminal differentiation seems to represent a critical event, as basal cells *in situ* are relatively resistant to direct oncogenic transformation and prostate basal cell carcinoma is rare [5]. In support, the first few $pAKT^{+}$ transformed luminal cells differentiated from *Pten*-deleted basal cells are all proliferative and responsible for later cancer cell expansion [5, 49]. We thus reason that these $pAKT^{+}$ luminal cancer cells possess LP-like properties and probably are, at least functionally, PCa LPs that drive tumor growth and progression. Interestingly, bacteria-induced inflammation and prostatitis also promote basal-to-luminal differentiation and accelerate initiation of prostatic intraepithelial neoplasia (PIN) in a basal- cell-origin PCa mouse model (Figure 1B) [53]. Consistently, inflammation decreases AR expression and endows luminal cells with an AR^{low} stem-like phenotype, which may promote tumorigenesis [6]. These observations, combined with our knowledge on the prostate cell hierarchy (Box 1), suggest that, regardless of the cell-of-origin, PCa development always converges on LPs.

Plasticity of LPs Drives Mouse PCa Progression

After progression to lethal CRPC stage, PCa still mainly presents as luminal-like adenocarcinomas, though up to 25% of ADT-failed patients may develop neuroendocrine PCa (NEPC). The transgenic adenocarcinoma mouse prostate (TRAMP) model, driven by a rat luminal gene *Pb* promoter-mediated SV40-Tag oncogene expression develops NEPC at late stages [54]. Similarly, prostate-specific loss of *Rb1* and *Trp53* mediated by *Pb*-Cre4 facilitates PCa lineage plasticity and therapeutic resistance by developing NEPC [55]. From a luminal differentiation phenotype to a stem-like NE phenotype, these findings reveal the plasticity of luminal cancer cells during tumor progression and under treatment pressure. A study that tracked the response of a genetic mouse model of PCa initiated by deletion of *Pten* and *Trp53* in luminal cells to abiraterone revealed that these tumors are intrinsically abiraterone-resistant and progress to NEPC [56]. Importantly, NEPC is originated from transdifferentiation of luminal cells as revealed by lineage tracing [56]. These data collectively suggest that luminal cells acquire stem-like lineage plasticity (i.e., LP-like) as a mechanism of treatment resistance and tumor progression. It is worth noting that this

transdifferentiation of luminal into NE cells has never been observed in normal mouse prostate development and experimental regeneration.

LPs Drive Human PCa Progression

Studies in human model systems have also implicated LPs in PCa maintenance and progression. For example, we identified a population of $PSA^{-/lo}$ PCa cells with LP characteristics in luminal cell lines and xenografts (e.g., LNCaP, LAPC4, LAPC9), which gradually increase in number from low- to high-grade tumors and are significantly expanded in CRPC [57, 58], suggesting that LPs preexist in untreated tumors and contribute to cancer progression. This notion is supported by the establishment of 3D luminal organoids from primary and circulating tumor cells isolated from CRPC patients [59]. Studies using a bone metastasis-derived and androgen-dependent PCa xenograft model, BM18, reported that LP-like cells survive castration and reinitiate CRPC [60], implicating LPs as the CRPC-initiating cells. This is further strengthened by observations that PCa originating in basal cells progresses to adenocarcinoma by propagation of luminal-like cells [61]. Furthermore, the frequency of $CD38^{low}$ LPs is positively correlated with PCa progression and poor outcome [29]. Of note, consistent with animal studies, luminal cell plasticity is also observed in human PCa. Previous studies analyzing the *TMPRSS2-ERG* fusion breakpoints in both adenocarcinoma and NEPC samples indicated a potential transdifferentiation of PCa into NEPC [62]. A recent study in AR^{+} PCa models shows that these tumors can develop resistance to antiandrogens via a phenotypic shift from luminal cells to AR- basal- and NE-like cells upon deletion of *Rb1* and *Trp53* [63]. Altogether, these data highlights the critical role of LPs in human PCa development, progression, and therapy resistance, and establish LPs as the ‘core’ target to prevent and/or eliminate CRPC. Therefore, future focus on defining signaling pathways that drive LP cell differentiation and self-renewal, with an aim to identify ‘actionable’ targets, holds clinical significance. Studies of LPs in mammary gland validate this idea (Box 3).

Therapeutic Targeting of LPs

Gene expression is a key determinant of cellular phenotype. We have annotated the transcriptomic features of LPs by interrogating available gene expression datasets associated with potential LP populations (Box 4). This analysis links gene signatures of LPs to aggressive PCa and to poor patient survival outcome [11, 22, 29, 52], suggesting that LP-gene signatures may be developed as a “biomarker” for patient stratification. In addition, the transcriptomics of LPs can be interrogated in-depth to nominate new therapeutic targets. Further studies on dynamic roles of LPs during PCa development and progression are warranted to generate clinically relevant LP signatures. Developing therapeutics to target PCa LPs is of clinic importance. As current ADT mainly eliminates differentiated and AR-dependent cells, combined targeting of LPs should provide better therapeutic efficacy. Below we propose three provocative strategies to therapeutically target LPs and treat aggressive PCa.

An inflammation gene signature is repeatedly identified across multiple LP datasets (Box 4). We thus hypothesize that a combination of current ADT and anti-inflammatory drugs may

benefit patients with CRPC. Non-steroidal anti-inflammatory drugs (NSAIDs), especially low-dose aspirin, have been associated with reduced cancer incidence and mortality [73]. Studies reported that chronic aspirin use is associated with a decreased overall PCa risk at the population level [74], although other studies from the same group later demonstrated that PCa patients had elevated cancer mortality in the Finnish Prostate Cancer Screening Trial [75]. This discrepancy may be explained by the fact that the patient cohorts are mixed populations without stratification. Alternatively, we suggest that inhibitors targeting the NF-κB pathway and critical cytokine/chemokine-mediated pathways (e.g., IL-1, IL-4, IL-6) might be better anti-inflammatory candidates than NSAIDs. These molecules are upregulated in multiple LP populations. Encouragingly, recent preclinical studies indicate that targeting NF-κB by Artesunate restores sensitivity of CRPC cells to anti-androgens by decreasing AR-variant expression [76, 77].

A second strategy invokes targeting the ‘stemness’ signature of advanced PCa cells. Preclinical studies in colorectal cancer showed that treatment of patient-derived xenografts (PDXs) with a small-molecule BMI1 inhibitor abolishes BMI1-driven self-renewal of CSCs and inhibits tumor growth [78]. Interestingly, $BMI1^{+}$ cells mark a population of castration-resistant LPs in regressed mouse prostate [24]. In addition to BMI-1, BCL-2 is frequently overexpressed in SCs and CRPC resulting in chemo-resistance and poor prognosis [79]. BCL-2 is significantly upregulated in human $CD38^{low}$ and PSA^{low} cells. In 2016, the first BCL-2 inhibitor Venetoclax (ABT-199) was approved to treat patients with chronic lymphocytic leukemia, and is now in multiple clinical trials for different cancers. We thus envision that combination of ADT and BMI1 or BCL2 inhibitors will produce a better prognosis compared with single agents alone. In support, our work on preclinical xenograft models indicates that inhibition of BCL-2, in combination with enzalutamide, dramatically impairs the development of CRPC. Moreover, epithelial-to-mesenchymal transition (EMT) is emerging as a promising target to suppress stemness, as EMT in general promotes CSC properties as well as cancer metastasis and therapeutic resistance [80]. Particularly, androgen deprivation promotes the SC-like features of both normal mouse prostate and human PCa via EMT [81]. EMT suppression is thus expected to attenuate the stemness of CSCs and inhibit CRPC. Various small- molecule agents that interfere with EMT signaling (e.g., Bufalin targeting TGF-β, Moscatilin targeting Snail/Slug, RO4929097 targeting Notch) abolish EMT features and inhibit progression of multiple cancers [82].

Finally, the neurogenesis program (Box 4) may represent a ‘new’ target for aggressive PCa. The concept that neural fibers play important roles in cancer development and progression is not novel, as cancer cells can exploit neurotropic factors released by the nerve fibers to generate a favorable microenvironment for survival and invasion [83]. For instance, formation of autonomic nerve fibers in the prostate gland regulates tumor development and dissemination in mouse models, and the density of nerve fibers in clinical specimens is associated with poor clinical outcomes [84]. However, our knowledge regarding cell-autonomous biological functions of neurogenesis genes in PCa development and progression is just beginning to emerge. Studies demonstrated that autocrine cholinergic signaling (ACS) mediated by CHRM3 functionally promotes PCa growth and castration resistance via phosphorylation of AKT [85]. Furthermore, CHRM3 is upregulated in a large subset of human BPH relative to normal prostate tissues and CHRM3 activation promotes

proliferation of prostatic (presumably luminal) progenitor cells [86]. Together, these data establish that blockade of ACS by targeting cholinergic receptors, CHRM3 in particular, may represent a new therapeutic approach to manage aggressive PCa.

Concluding Remarks

Human PCa is largely luminal-like. However, luminal cells are understudied compared with basal cells, mainly due to technical challenges: (1) current SC-related assays have a tendency to bias towards basal cells, and (2) it is still unfeasible to culture and expand differentiated primary prostate luminal cells expressing AR and PSA. Although a few human PCa cell lines are available, these lines are not representative of most stages of disease progression, and establishing PDX models remains challenging [10]. Recent development of *in vitro* 2D and 3D cultures and lineage- tracing approaches has greatly advanced our understanding of luminal cell biology. However, the precise phenotypic identity of LPs remains ill-defined as most of the LP markers (Table 1) are also expressed in basal cells. Furthermore, tracking LP fates is not rigorously done in lineage-tracing studies due to a lack of known LP-specific gene promoters, and more in-depth studies using human systems are needed.

Which cells resist castration and mediate subsequent tumor relapse is a central question in PCa research. A long-standing hypothesis is that stem/progenitor cells may preferentially survive ADT and subsequently repopulate CRPC. It remains to be determined whether LPs may be the cell-of- origin for CRPC or LPs represent the CSCs in human PCa (see Outstanding Questions). The transdifferentiation of luminal cells into basal- and/or NE-like cells is emerging as a new research direction to dissect the underlying mechanisms of NEPC development. Molecular signatures of prostate epithelial cell types can instruct our understanding of the biological heterogeneity present within PCa, and may offer new therapeutic insights. Obviously, more defined markers of normal LPs and prostate CSCs in CRPC are needed to fully understand the evolutionary trajectory of PCa and CRPC. An unbiased way to reveal the heterogeneity, as well as the LP-specific markers, in normal and diseased prostate is to perform single-cell RNA-seq analysis to dissect cell-to-cell variation within the luminal cell compartment under both physiological and castration conditions. In this review, we have enumerated several promising targets based on the analysis of transcriptomic features of LPs derived from available datasets. Accordingly, combinatorial therapies that co-target differentiated (e.g., antiandrogens) and LP (anti-LP treatments) cell populations in aggressive PCa patients are expected to have superior clinical outcomes. Immunotherapy is another potential therapeutic strategy, as immunity-related pathways are frequently enriched in LPs (Box 4). Although stem/progenitor cells are generally immune-privileged, experimental modulation of their immunogenicity is feasible and effective targeting of CSCs via immunotherapy have been reported in several cancer types [72]. Therefore, the interplay between LPs and immune system represents a new direction of future exploration.

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Glossary

Stem cells (SCs):
are cells capable of self-renewal while being able to differentiate into multiple cell lineages that constitute the organ (multipotency).

Progenitor cells:
are early descendants of SCs with a commitment to differentiate into more differentiated and functionally specialized cells (unipotency).

Epithelial cell hierarchy:
describes the cellular architecture of mammalian tissues that are composed of cells organized in a hierarchical manner, including SCs, committed progenitors, differentiating cells, and terminally differentiated cells. Like a pyramid, SCs sit at the apex and differentiated mature cells at the base with progenitors representing the transitional stage of differentiation between them. The SC hierarchy is a continuum as the differentiation process is dynamic and continuous. Little is known about most tissue SC lineages and we often name the subsets of cells in a specific tissue with certain self-renewal and differentiation abilities simply stem/progenitor cells.

Facultative function of SCs:
referring to the state of cells that normally exhibit unipotency but gain the capacity to function as multipotent SCs under certain conditions

Dedifferentiation:
describes the process whereby differentiated cells lose their specialized characteristics and revert to a less differentiated state (e.g., terminally differentiated AR^{+} luminal epithelial cells back to $AR^{-/lo}$ progenitor state).

Transdifferentiation:
describes the process of a cell changing its lineage identity (e.g., luminal to NE cells).

Epithelial plasticity:
describes the ability of cells to dynamically switch between different phenotypic and functional cellular states in response to environmental changes

Prostate regression and regeneration:
describes the phenomenon of the mouse prostate atrophy upon surgical castration (e.g., via removal of testicle and epididymis or cauterization of seminar vesicles) and subsequent prostate regrowth in response to androgen re-administration. The mouse prostate can undergo many rounds of regression and regeneration following androgen ablation and restoration, respectively.

Androgen deprivation therapy (ADT) and castration:

ADT is a treatment to block androgen/AR signaling in PCa patients, although ADT-resistance eventually develops. Castration in animals mimics the effect of ADT in patients, thus is often used to study the mechanisms of castration resistance.

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Box 1.

Hierarchy of Prostate Epithelial Cells

In the adult mouse, the prostate possesses a significant regenerative potential and can undergo multiple rounds of castration-induced regression and androgen-induced regeneration, testifying the existence of stem/progenitor cells. The hierarchy of the prostate epithelial cell lineages is emerging from recent lineage-tracing studies [3, 5, 15–17], though the identity of many proposed stem/progenitor cell populations in both basal and luminal cell layers (Figure IA and IB) remains to be fully defined.

The mouse prostate originates from a $p63^+$ basal SC population at the embryonic stage [17] and the massive epithelial expansion starting after birth is driven by progenitors. During early postnatal development, multipotent basal SCs differentiate into basal, luminal and NE cells, and into unipotent basal and luminal progenitors (Figure IB) [16]. An intermediate cell population that localizes in the basal cell layer (about 15% of basal cells) and express both basal and luminal cell markers (e.g., $CK5^+CK8^+$) was reported in early postnatal development [16]. Notably, these intermediate bipotent basal cells are primarily luminal-committed progenitors (Figure IB). During adult prostate homeostasis and regeneration *in vivo*, basal and luminal cells are mainly self- sustained by their corresponding unipotent progenitors [5, 15], although rare bipotent basal progenitors have been reported to contribute to homeostasis and regeneration (Figure IC) [3]. These bipotent basal cells seem to be extremely scanty within the overall population (about 0.02% in homeostasis and 0.04% in regeneration) [3] and have not been observed in independent studies [5]. The outlined prostate lineage hierarchy (Figure IB and IC) is largely corroborated by analysis of cell division modes in epithelial cells [19], which shows that basal cells can display both symmetric and asymmetric division leading to different cell fates, whereas luminal cells only exhibit symmetrical division.

A thorough analysis of the lineage hierarchy within the luminal cell compartment is warranted. How are LPs maintained? What is the inter-relationship between heterogeneous LP populations identified by different strategies and markers (Table 1) and between LPs and the basal stem/progenitor subsets? How do LPs contribute to prostate development and tumorigenesis? Due to difficulties in performing studies in human tissue, the human prostate epithelial cell hierarchy is much less delineated. Caution is required when extrapolating the findings in animal studies to the human prostate, since there are anatomic, molecular and physiologic differences between species [20]. Nonetheless, the principle of prostate cell biology is generally conserved between mouse and human.

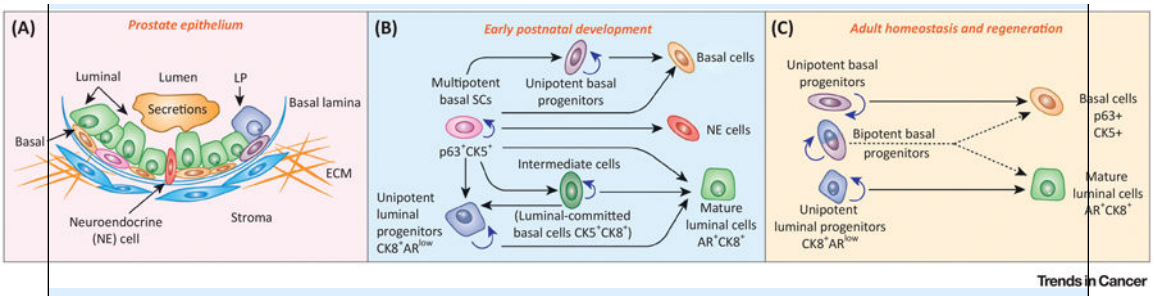

Figure I in Box 1. Hierarchy of prostate epithelial cell lineages

(A) A simplified cartoon illustrating the epithelial cell composition and glandular structure of the adult prostate.

(B, C) Hierarchy of epithelial cell lineages during early prostate development (B) and during homeostasis and regeneration (C), revealed by genetic lineage-tracing studies. Blue curved arrows denote self-renewal and dashed arrows indicate inconsistent results reported by different groups.

Box 2.

Methods to Identify and Markers of LPs

Stem cells (SCs) possess unique biological properties- such as quiescence, detoxification, unique gene expression profile, self-renewal and asymmetric cell division, and the ability for tissue regeneration *in vivo*- that allow experimental identification and purification [8, 9, 31]. Various methodologies were developed over the past decades to identify and characterize the stem/progenitor cell populations in the prostate (Table I). However, a context-related interpretation of these results is needed, as some approaches do not strictly identify stem/progenitor cells in a given context. For example, label retention only identifies slow-cycling cells, but both fast and quiescent progenitors coexist in several rapidly renewing tissues such as the small intestine and the blood [31]. In support, H2B-GFP label retention is not specific for hematopoietic SCs when used as a single parameter [32]. Likewise, both the side population (SP) and Aldefluor assays rely on the preferential expression of detoxification genes (e.g., ABCG2 in SP and ALDH1A1 in Aldefluor) in putative SCs [8]. However, the SP may not be specific for a CSC phenotype in glioblastoma multiforme [33], and ALDH activity does not select for cells with enhanced aggressive properties in melanoma [34]. Interestingly, we have previously shown that SP, but not ABCG2 expression, can enrich CSCs in PCa models [35]. Recently developed 2D and 3D culture systems, that allow survival and proliferation of LPs, facilitate the dissection of luminal cell biology [11, 14]; but we need to keep in mind that these culture-enriched LPs are, unlikely, functionally equivalent to LPs *in situ,* as they are taken out of their niche and placed under selective pressure rendered by the culture media. Collectively, these studies indicate that the strategies (Table I) for identification and enrichment of normal and cancer stem/progenitor cells are likely applicable in a tissue/tumor- dependent manner.

In the context of prostate LPs, there still lack well-established markers, and, in this review, we define the LPs based on their functional stem-like properties. Several markers that enrich for human or mouse stem-like luminal cells in normal, castration-regressed, or diseased prostates with or without treatments have been reported (Table 1). These markers, however, are not exclusive to the LPs, and the majority (e.g., Sca-1, $PSA^{-/low}$, $AR^{-/low}$, CD44, α2β1, ALDH, Nanog) preferentially identifies basal/stem cells. The phenotypic markers unique to, and restricted to, LPs in the human and/or mouse prostate remain largely unknown.

Table I.

Several Primary Methods to Identify LPs

Method	Principle	Key points	Refs
Label retention	Slow cycling of somatic SCs	Identify label-retaining cells (LRCs) in many, but not all, tissues that display stem-like properties. BrdU-LRCs identify potential prostate LPs, but BrdU does not allow live cell purification for functional validation. H2B-GFP allows cell purification and GFP^{+} LRCs identify mouse prostate LPs.	[8, 21, 22, 31, 36]

Method	Principle	Key points	Refs
Tissue regeneration (or recombination)	PSCs can regenerate a prostate structure when mixed with inductive embryonic urogenital sinus mesenchyme (UGM) and transplanted under the kidney capsule or skin of immunodeficient male mice	A 'gold-standard' assay to determine the putative SC properties of prostate cell populations *in vivo*. Studies using bulk purified populations show that only basal cells are regenerative. When enriched for LPs, luminal cell populations are also regenerative.	[11, 12, 14, 22, 23, 37]
SP and ALDH	Enhanced cellular detoxification machinery in SCs	Each assay identifies stem/progenitor cells in many, but not all, tissues and tumors. SP enriches for PSCs in both cancer cell cultures and human primary PCa specimens.	[33–35, 38, 39]
		$ALDH^{hi}$ cells identify a subset of luminal cells resided in mouse proximal prostate ducts with tissue regeneration ability *in vivo*. The identity and functional properties of these SP and $ALDH^{hi}$ luminal cells are unknown.	
FACS	Cells expressing known SC-associated surface markers are potentially stem-like	Depends entirely on the use of known SC markers, thus has limited use in understudied luminal cell lineages. The majority of widely used SC markers preferentially identify prostate basal but not luminal stem/progenitor cells.	[40–43]
Lineage Tracing	Uses cell lineage-specific promoter to drive the expression of fluorescence protein to allow cell labeling and subsequently fate tracking in development and tumorigenesis *in situ*	Uses general luminal cell-specific gene promoters to establish the existence of LPs, but cannot pinpoint which precise cell(s) is the LP. Reveals the dynamic change of luminal cell layer maintained by LPs during prostate development, regeneration and tumorigenesis. LP-specific gene promoter is needed to rigorously track the fates of LP *in vivo*.	[5, 12, 20, 44]
In Vitro Cultures	The stem/progenitor cells, relative to differentiated cells, preferentially survive and display proliferation and differentiation ability when removed out of microenvironment	Easily manageable compared to *in vivo* methods that are time-consuming and technically challenging. Previously established 2D culture systems preferentially support basal-like cell growth. A new 2D cell culture is developed to enrich LPs from human prostate luminal isolates. Recently reported 3D systems supports long-term expansion of primary mouse and human prostate organoids that recapitulate in vivo glandular morphology.	[11, 13, 14]

Box 3.

Lessons Learned from Mammary LP Studies

The prostate and breast organs share many biological similarities and the majority of both human PCa and breast cancer (BCa) exhibit luminal epithelial differentiation. In general, our understanding in PCa is not as advanced as in BCa; lessons can therefore be learned from studies in BCa. Based on mammary transplantation and lineage tracing studies, a hierarchy of stem and progenitor cells similar to that in the prostate (Box 1) has been found. In terms of the role of LPs in BCa, it is shown in animal studies that *Brca1*-deficient basal-like BCa actually originates from LPs and exhibits an overall LP-like gene expression profile [64]. Consistently, analysis in human specimens indicates that BCa tissue with *BRCA1* mutation harbors an expanded LP population that highly expresses c-Kit and shows B27-independent growth *in vitro* [65]. Notably, gene expression profiling reveals a molecular similarity between *BRCA1*-mutant basal BCa and normal LPs [65], establishing LPs as the possible target cells for transformation and propagator of BRCA1-associated basal tumors. Intriguingly, these LPs fail to undergo luminal differentiation and generate luminal tumors, but instead retain features of undifferentiated myoepithelial cells leading to a basal-like BCa. Thus, the genetic background of patient population (heterozygous *BRCA1* mutation in this case), in addition to affecting BCa incidence rates, significantly impacts progenitor fate commitment and tumor phenotype [66]. Interestingly, EMT factor SNAI2 is overexpressed in $BRCA1^{mut/+}$ cells resulting in suppression of luminal lineage commitment and differentiation [66].

Furthermore, luminal-like cells isolated from human BCa are fully capable of initiating tumors in immunodeficient mice, and these pure luminal cells generate larger and more invasive tumors than their basal-like counterparts [67]. Additionally, both human and mouse mammary LPs can display a certain degree of plasticity under experimental conditions, providing a potential explanation for cellular heterogeneity within BCa [68]. Particularly, mammary luminal cells can produce basal cells upon oncogenic activation by either polyoma middle T antigen (PyMT) or ErbB2 signaling, albeit at low frequency (0.21~1.64%) [69]. Notably, this plasticity of LPs has not been observed in prostate tumorigenesis, but has been observed in PCa progression into NEPC upon ADT. Overall, results in breast and BCa studies establish LPs as a critical target for BCa development and treatment. In fact, many studies have unraveled crucial transcriptional regulators of the self-renewal and differentiation of LPs (e.g., Elf5, Gata3, Notch1/3, FoxM1, Stat5) (reviewed in [68]), and investigations that explore these ‘targets’ therapeutically are ongoing.

Box 4.

Transcriptomic Features of LPs

Annotation of the transcriptome of LPs is expected to provide a foundation for discoveries that can impact PCa understanding and treatment. We analyzed six (three mouse and three human) gene- expression datasets associated with potential LP populations. An earlier microarray profiling (Figure IA) was performed in normal prostates and LP-enriched hyperplastic prostates of mice with luminal cell-specific *Pten* deletion [48]. A recent study reported a 111-gene signature specific to LSC^{med} cells (Figure IB), a LP population greatly enriched in mouse CRPC [52]. Additionally, we identified a dormant population of mouse luminal LRCs as LPs and reported their stem-like gene signature by RNA-seq (Figure IC) [22]. Three human datasets are also examined, including a $PSA^{-/lo}$ CSC population isolated from luminal LAPC9 xenografts (Figure ID), 2D culture-enriched LPs from human benign prostate tissues (Figure IE) [11], and a RNA-seq profile of $CD38^{low}$ LPs (compared to $CD38^{hi}$ luminal cells) isolated from human prostate tissues shown to be associated with PCa progression and poor outcome (Figure IF) [29].

Using DAVID [70], we reanalyzed these gene signatures and established a transcriptomic landscape for LPs (Figure I). The pattern of functional categories of GO terms across all datasets reveals similar molecular features shared by LPs from different contexts. Categories of development & SC, cell proliferation (and cell cycle regulation), and cell adhesion and motility represent the top enriched categories, consistent with the stemness, proliferation potential, and the architectural localization of LPs. Genes associated with inflammation and immunity are frequently observed. Inflammation and immune suppression are hallmarks of cancer [71]. Prostate inflammation is proposed as an etiology for BPH and chronic inflammation represents a risk factor for aggressive PCa [29]. Immunotherapy is a new ‘hope’ for cancer treatment; however, like normal SCs, CSCs normally display low immunogenicity, leading to challenges of efficient immnunological targeting [72]. Surprisingly, neurogenesis is significantly enriched in four out of six datasets, implicating a unique molecular feature. The functional significance of a neurogenesis program in regulating LP cell properties and PCa development and progression remains to be elucidated. Collectively, our analysis exposes the molecular features of LPs and nominates potential therapeutic strategies (e.g., targeting inflammation via NF-κB and/or IL-1) to combat advanced PCa.

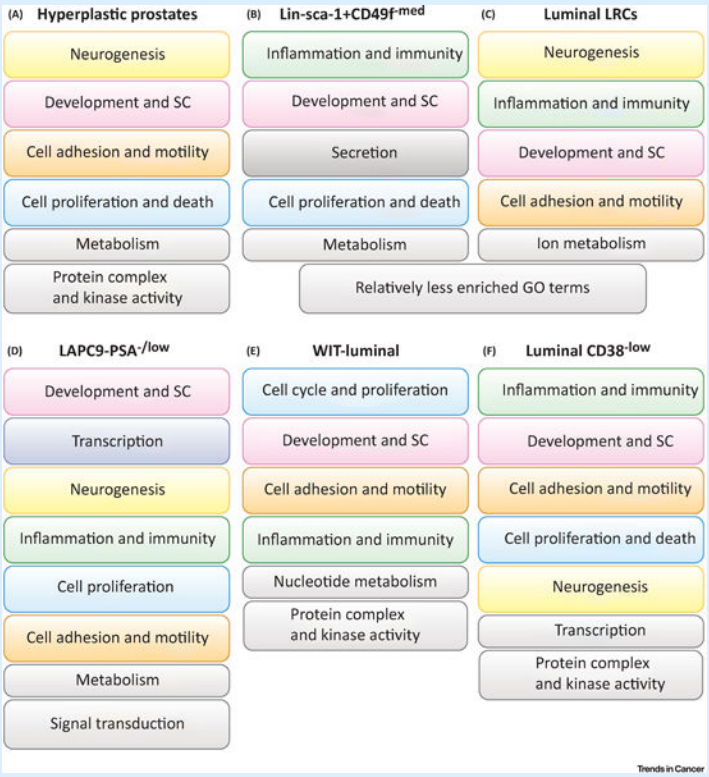

Figure I in Box 4. Transcriptomics of prostate LPs

Three mouse datasets (A [48], B [52], C [22]) and three human datasets (D [58], E [11], F [29]) are used to unravel the molecular features of LPs. DAVID bioinformatics tool is employed to systematically analyze the biological categories of GO terms enriched in genes and signatures overrepresented by reported LP populations. Predominant functional categories are highlighted in color, whereas relatively less enriched categories are uncolored. Technically, the original GO terms from DAVID output were selected with a cutoff of $p<0.05$. For each gene signature, we then pooled the GO terms and re-categorized them by grouping similar GO terms into broad functional categories. For example, GO terms associated with organ process, development, differentiation, and SC-related pathways (e.g., IGF, growth factors) are categorized as “development & SCs”.Similarly, GO terms linked to CNS development and neural/neuronal cell biology will be categorized as “neurogenesis”. Inflammation and immunity encompass GO terms related to inflammatory cell biology and response and immune cell biology and response.

Trends

Luminal cells are understudied but recent advances are shedding light on the functional properties, plasticity and heterogeneity of LPs, and filling an important gap in our understanding of prostate cell biology.

LPs are critical drivers of prostate development, regeneration, cancer initiation and progression.

The drastic expansion of LP-like cancer cells in aggressive PCa suggests that LP gene signatures may have diagnostic and prognostic value in stratifying patients for personalized therapy.

Targeting of PCa LPs represents a promising therapeutic strategy to better treat CRPC.

Outstanding Questions

What are the LP-specific markers?

Are LPs the cell-of-origin for lethal CRPC?

Can LP gene signatures be used as biomarkers to enable personalized treatment?

Are LPs clinically relevant? How can we better target them? Is there any clinical impact of LP- targeting strategies?

Can immunotherapy be developed to efficiently target LPs in CRPC?

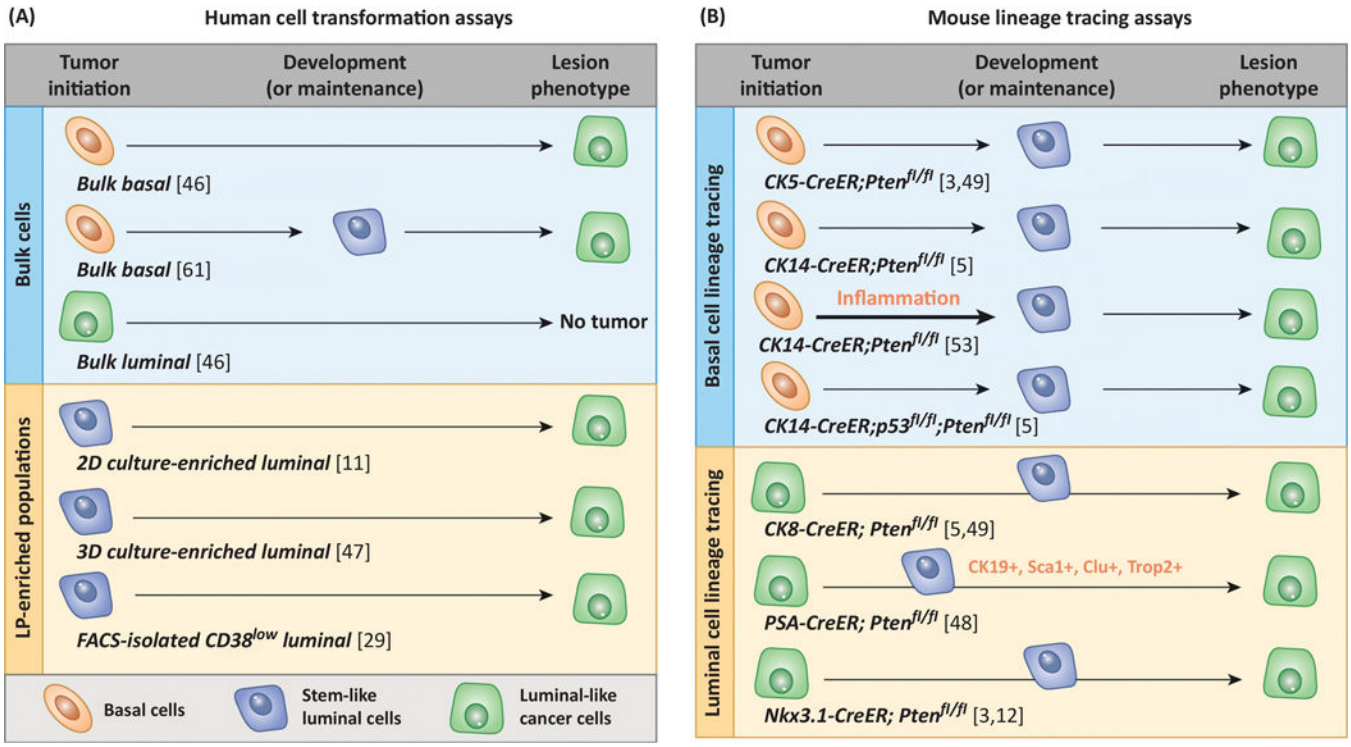

Figure 1. Role of luminal progenitors (LPs) in PCa initiation and development

(A) In the human cell transformation assays using freshly purified bulk prostatic basal and luminal cells and LP-enriched populations (i.e., culture-enriched or FACS-sorted), only basal cells and LPs can be oncogenically transformed to form tumors.

(B) Transformation of basal cells by loss of *Pten* initiates PCa by a first basal-to-luminal differentiation step followed by expansion of stem-like pAKT^{+} and proliferative luminal cells to establish luminal-like tumor (top panel). PCa initiated from luminal-cell-specific loss of *Pten* uniformly manifest a luminal phenotype. One study has characterized the primary *PSA*-CreER;*Pten*$^{fl/fl}$tumor cells and suggested that LPs are the cells initiating and subsequently maintaining the PCa (bottom panel).

Table 1.

Markers to enrich and/or identify the prostate LPs[a]

Conditions	Markers
Mouse prostate	
Normal	LRCs [21, 22], ALDH^{+} [38], Trop2^{+}Sca1^{+}Clu^{+} [48], Sca1^{+}and CK19^{hi} [22], Organoids [14], AR^{low} [22, 26, 28]
Regressed	CARNs [12], CARBs [24], Sca1^{+} [23], Lgr5^{+} [25], LRCs [22]
Cancer	Trop2^{+}Sca1^{+}Clu^{+} and CK19^{hi} [48], Organoids [51], LSC^{med} (Lin^{-}Sca-1^{+}CD49f^{med}) [52]
Human prostate	
Normal	Organoids [14], Culture-enriched luminal cells [11], CD38^{low} [29]
Cancer	Organoids [59], PSA$^{-/low}$ [58], ALDH^{hi}CD44$^{+}\alpha 2\beta 1^{+}$ [57], ALDH^{+}/Nanog^{+}/AR^{low} [60], CD38^{low} [29]

[a]Abbreviations: LRCs, label-retaining cells; ALDH, aldehyde dehydrogenase activity; CARNs: castration-resistant Nkx3–1-expressing cells; CARBs, castration-resistant Bmi1-expressing cells.