Prostate luminal progenitor cells: from mouse to human, from health to disease

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Abstract | Stem and progenitor cells of the adult prostate epithelium have historically been believed to reside mainly or exclusively within the basal cell compartment and to possess basal-like phenotypic characteristics. Within the past decade, evidence of the existence of luminal epithelial cells exhibiting stem/progenitor properties has been obtained by lineage tracing and by functional characterization of sorted luminal-like cells. In 2020, the boom of single-cell transcriptomics led to increasingly exhaustive profiling of putative mouse luminal progenitor cells and, importantly, to the identification of cognate cells in the human prostate. The enrichment of luminal progenitor cells in genetically modified mouse models of prostate inflammation, benign prostate hypertrophy and prostate cancer, and the intrinsic castration tolerance of these cells, suggest their potential role in prostate pathogenesis and in resistance to androgen deprivation therapy. This Review bridges different approaches that have been used in the field to characterize luminal progenitor cells, including the unification of multiple identifiers employed to define these cells (names and markers). It also provides an overview of the intrinsic functional properties of luminal progenitor cells, and addresses their relevance in mouse and human prostate pathophysiology.

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https://doi.org/10.1038/s41585-021-00561-2

Prostate diseases such as benign prostatic hyperplasia (BPH)[1–3] and prostate cancer[4,5] are associated with increased age. Therefore, the global burden of these diseases is expected to increase as a result of rising life expectancy[3,5]. Although current medical therapies provide transient benefit to patients, treatments are usually not curative and disease progression is frequently observed. As a result, many patients with BPH ultimately need to undergo surgical ablation of the adenoma, and most patients with metastatic prostate cancer will die from their disease, despite the ability to cure localized tumours with surgery or radiation therapy[6]. Understanding the mechanisms underlying pathological conditions of the prostate is required to improve treatments. Identification of the cell types responsible for disease initiation, progression and/or drug resistance is important, as their molecular profile could lead to the identification of relevant therapeutic targets.

The prostate is a branched epithelium comprising the glandular epithelial compartment and its underlying mesenchymal stromal part; reciprocal interactions between these components have a central role in prostate morphogenesis and pathophysiology[1,7–9]. The stroma contains several cell types, including smooth muscle cells (surrounding the glands), fibroblasts, endothelial cells, resident immune cells, nerve cells and adipocytes. The glands contain mainly luminal and basal cells organized in a pseudostratified epithelium in which very rare neuroendocrine cells are interspersed. These various cell types can be discriminated based on the expression of typical markers, including cytokeratin 5 (CK5), CK14 and p63 for basal cells, CK8 and CK18 for luminal cells, and synaptophysin (SYP) and chromogranin A (CgA) for neuroendocrine cells[10,11]. So-called intermediate (or transit-amplifying) cells are luminal-committed basal cells that co-express basal and luminal cytokeratins[11]. Whether these intermediate cells represent a distinct cell type or correspond to a transition state between basal and luminal cells remains unclear[12].

The function and principles of prostate biology are globally conserved across species; however, murine and human prostates display anatomical and histological differences[13–15] (BOX 1). The absence of spontaneous development of prostate proliferative diseases (BPH and cancer) in rodents might be a result of these species-specific features, possibly including differing stem and/or progenitor cell populations and/or properties. Despite these differences in disease

Key points

- LSC^{med} defines a prototypic population of epithelial cells discovered in 2014 in the mouse prostate that exhibit luminal and stem-like features and were therefore called luminal progenitor cells. Their signature includes *Krt4* and *Psca*.
- Phenotypically similar cells have been identified in the human prostate. In both species, these cells reside mainly, but not only, in proximal regions of the gland (peri-urethral area and/or central transition zones).
- In the adult mouse prostate in situ, luminal progenitor cells are unipotent stem-like cells that contribute to post-castration prostatic tissue regeneration in cooperation with alternative cell-driven mechanisms.
- Luminal progenitor cells are amplified in various pathological states (such as inflammation, benign prostatic hyperplasia and cancer). The underlying molecular and cellular mechanisms are virtually unknown but might involve complex circuitry with inflammatory and stromal cells.
- Luminal progenitor cells are more castration-tolerant than differentiated luminal cells. Thus, they could contribute to resistance to androgen-targeting therapeutic strategies (5α-reductase inhibitors and androgen deprivation therapy) and promote disease progression.

development, mice remain the preferred animal model in which to study prostate pathophysiology owing to extensive evidence that genetic alterations identified in human prostate cancer trigger tumorigenesis when engineered in the mouse prostate[15,16]. In any case, even though this Review discusses the potential involvement of luminal progenitor cells in human prostate diseases in light of lessons from relevant mouse models, extrapolating findings from mice to humans should be done with caution.

The seminal observation that the rodent prostate epithelium has the capacity to fully regenerate several times after successive cycles of castration followed by androgen replacement[17] suggested that the rodent adult prostate harbours castration-resistant cells exhibiting stem-like capacity. As massive apoptosis of androgen-dependent luminal cells was detected following castration, the tissue-regenerating capacity observed after androgen replenishment has been hypothesized to reside mainly within the basal cell layer, which is much less affected by androgen deprivation[18]. Intermediate cells residing in the basal layer have been proposed to house prostatic cells exhibiting stemness[19,20]. Fluorescence-activated cell sorting (FACS)-enriched basal cells were subsequently shown to form spheroids in vitro and to regenerate glandular structures when engrafted into host mice[21,22]; by contrast, luminal cells were much less efficient in these stem cell functional assays (BOX 2). However, studies involving light microscopy autoradiography (^{3}H-thymidine) analyses of rat prostates indicated that mitotic cells were also observed in situ in the secretory (luminal) epithelium during both normal growth[23] and androgen-induced regeneration of prostates in castrated animals[18,24]. These findings pioneered the idea that basal and secretory (luminal) cells might be self-replicating cell compartments, and that basal cells might function as a 'reserve' population only stimulated into the proliferative cycle by tissue damage, for example, castration[18,24]. A key observation supporting the existence of luminal stem and/or progenitor cells was provided by a 2009 study reporting that, in castrated mice, expression of the androgen-regulated *Nkx3.1* homeobox gene persisted in a rare population of luminal cells exhibiting stem-like properties; this cell population was referred to as castration-resistant NKX3.1-expressing cells (CARNs)[25]. Further in vivo lineage tracing studies showed that basal and luminal cell compartments are self-sustained, and that luminal epithelium regeneration largely involved unipotent, androgen-resistant luminal progenitor cells[26,27].

Although fluorescently labelled CARNs could be isolated to demonstrate their stem-like capacity in reconstitution assays (BOX 2), their molecular identity was not elucidated[25]. As previously achieved for the cell population referred to as basal/stem[22,28], cell-sorting enrichment of a discrete luminal cell population exhibiting progenitor-like capacity was instrumental to uncovering the molecular identity of such putative luminal progenitor cells; elucidating their functional characteristics ex vivo; and identifying markers enabling their tracking in prostate tissues[29–31]. Within the past 2 years, the increased use of single-cell transcriptomics has highlighted the unsuspected heterogeneity of the luminal cell lineage in the healthy prostate and confirmed that a population of luminal progenitor cells identified based on the expression of luminal and stem-related genes represent a transcriptionally distinct cell entity in the mouse prostate[32–36]. However, despite using similar technological approaches and mouse models, unrelated nomenclatures were used in the different studies. Alongside these studies, evidence has accumulated to support the involvement of luminal progenitor cells in hyperproliferative prostatic diseases (BPH and cancer) in mice and possibly in humans.

Box 1 | **Rodent versus human prostate**

Despite a conserved physiological role in reproduction across species, the prostate gland exhibits various species-specific features at various levels[13–15].

Anatomy

The healthy adult human prostate has the size and shape of a walnut, and harbours three distinct histological zones (central, transition and peripheral). By contrast, the rodent prostate comprises four paired lobes (dorsal, lateral, ventral and anterior). The dorsolateral prostate is often considered to be analogous to the human prostate peripheral zone. Recent single-cell RNA sequencing analyses[34] support the fact that lateral rather than dorsal mouse prostate is similar to the human prostate peripheral zone.

Histology

In both species, the prostate exhibits a ductal–acinar histology with similar pseudostratified epithelium. The basal cell layer is continuous in humans compared with discontinuous in mice, and neuroendocrine cells are rarer in mice than in humans. The fibromuscular stroma is much more prominent in the human prostate than in the mouse prostate and, in mice, its density varies depending on the lobe and between proximal versus distal areas.

Molecular

Prostate-specific antigen (PSA) is the typical marker of fully differentiated luminal epithelial cells and prognostic marker of prostate cancer in humans. The PSA gene is not present in the murine genome.

Pathology

Benign prostatic hyperplasia and, to a lesser extent, prostate cancer, are frequent diseases in ageing men. By contrast, rodents never develop spontaneous prostate tumours. One of the hallmarks of human prostate cancer is the disappearance of basal cell markers. This is not the case in genetically modified mouse models of prostate cancer, in which basal cells can in some instance be amplified. There is no firm evidence that any specific murine prostate lobe is more valid as a model for human prostate cancer, and models have been described involving every lobe and, frequently, multiple lobes.

Box 2 | **Experimental assessment of stem/progenitor properties**

Stem cells are clonal cells characterized by their ability to self-renew and generate terminally differentiated cells. In contrast to pluripotent embryonic stem cells that can differentiate into all cell types in an organism, adult stem and progenitor cells differentiate into a limited number of cell types within a given organ. Depending on the diversity of cell types they can become, they are referred to as multipotent, bipotent or unipotent.

Assessing stemness properties in vitro

Three-dimensional cell models called prostaspheres and organoids involve the culture of dissociated prostatic cells in specific media and semi-solid conditions. As these structures are clonal, their number is used to reflect the number of stem and/or progenitor cells present in the initial cell pool. The stem and/or progenitor cell potency (unipotent, bipotent or multipotent) is determined based on the presence of one or more cell types in the prostaspheres or organoids (using, for example, immunohistochemistry), and their self-renewal capacity by replating cells dissociated from these 3D structures (a step called passage).

Assessing stemness properties in vivo

The tissue regeneration (or reconstitution) assay measures the capacity of dissociated cells to reconstitute prostatic-like glandular structures when engrafted into host mice. This assay requires prostatic cells to be embedded within urogenital sinus mesenchyme (UGSM) cells[39]. Lineage tracing[72,133] follows genetically fluorescent-labelled cells and their progeny in the organ in situ, for example, in castration-regeneration experiments.

Pros and cons

All assays can be applied to healthy and cancer cells and/or models[13]. Although prostaspheres, organoids and tissue regeneration are increasingly used as surrogates for organ structure investigation, disease modelling and drug testing[79,134], these assays are called facultative as their experimental conditions might favour stem-like properties that overcome those naturally occurring in vivo. Although lineage tracing avoids this bias, pitfalls of this approach include labelling efficiency (for example, endogenous promoter activity in cells that are not fully differentiated might not be strong enough to turn on the transgene) and the actual cell-type specificity and/or temporally restricted activity of artificial promoters used to control Cre-mediated fluorescent reporter expression[72].

The tremendous knowledge on luminal progenitor cells gained over the past couple of years has highlighted the importance of bridging the conclusions of independent studies regarding the characterization of this newly identified prostatic cell population.

In this Review, we first portray the molecular profile of luminal progenitor cells, on the basis of which we discuss the potential overlap between the many cells that have been referred to as 'luminal progenitor cells' using various experimental approaches. We then examine the functional characterization of luminal progenitor cells and their involvement in prostate homeostasis and pathogenesis in mice and, potentially, in humans. Certain aspects of luminal progenitor biology (for example, epithelial lineage hierarchy, development and ageing) outside the scope of this Review are reviewed in detail elsewhere[11,37,38].

Identifying luminal progenitor cells

Key studies initially reported the existence of a previously undefined mouse prostate epithelial cell population in cell sorting experiments, which led to the identification and molecular profiling of luminal progenitor cells. Based on biomarker expression and spatial localization within the prostatic tissue, this cell population has shown similarities with putative luminal progenitor cells identified by single-cell transcriptomics analysis of mouse and human prostates, or by in vivo tracking using single-gene-based approaches such as label retaining or lineage tracing. In this section, we propose the unification of multiple identifiers that have been used in single-cell studies to define the luminal progenitor cell cluster.

Molecular profiling

In mice, basal, luminal and stromal cells (referred to as lineage-negative (Lin^-) cells owing to negative staining for CD31, CD45 and Ter119 lineage markers[39]) can be separately enriched by cell sorting (FACS) of fresh prostates using established cell surface markers. CD49f (also known as integrin α6) combined with stem cell antigen 1 (SCA-1; also known as Ly6a) is one of the most popular sorting strategies for Lin^- cells[28,29,39–41] (FIG. 1a; TABLE 1). Luminal cells express no SCA-1 and moderate levels of CD49f (FIG. 1a). Cells expressing high levels of SCA-1 and CD49f (called LSC^{high} for $Lin^-SCA\text{-}1^+CD49f^{high}$) have been shown to express basal markers and to possess enriched stem-like capacities[22,28]; thus, this cell population is also referred to as basal/stem[28,31]. In addition, a fraction of basal cells exhibits a $SCA\text{-}1^{low}$ or $SCA\text{-}1^-$ profile, especially in the distal prostate[22].

Analyses of prolactin-induced premalignant mouse prostates[42] by our group using a CD49f/SCA-1 sorting strategy have revealed the emergence of a previously unidentified cell population. This population displays levels of CD49f expression similar to those of luminal cells (referred to as medium[29,31] or low[30,43] depending on studies) and high SCA-1 expression similar to that of basal cells (FIG. 1b); following the naming convention of LSC^{high} cells, we named this newly discovered cell population LSC^{med} ($Lin^-SCA\text{-}1^+CD49f^{med}$)[29]. LSC^{med} cells represent only ~5% of healthy mouse prostate epithelium (FIG. 1a), which explains why they were not identified as a distinct cell population in previous cell sorting studies. Based on their $CK8^+CK5^-p63^-$ profile, LSC^{med} cells were identified as luminal cells[30,31]. Preliminary functional characterizations showed that FACS-enriched LSC^{med} cells have the capacity to generate prostaspheres and to differentiate into $SCA\text{-}1^-$ luminal cells upon androgen stimulation in vitro, which led us to suggest that this cell population could contain luminal progenitor cells[29].

Following these observations, a separate group used the same sorting strategy to show that wild-type LSC^{med} cells (termed $SCA\text{-}1^+$ luminal cells in their study) were able to generate organoids and to form glandular structures in reconstitution assays (BOX 2), confirming their progenitor capacities[30]. This group also discovered that LSC^{med} cells are androgen independent, as they proportionally enrich in situ following mouse castration[30]. Accordingly, LSC^{med} cells enriched from healthy mouse prostate were shown to exhibit intrinsically low levels of androgen signalling[31] despite transcriptomic levels of androgen receptor expression similar to those of secretory luminal cells[30,31]. Transcriptomic profiling analysis of the three epithelial cell populations (basal, LSC^{med} and luminal) (FIG. 1a) identified a 111-gene signature that established LSC^{med} cells as a distinct epithelial cell entity[31]. According to their above-mentioned characteristics, LSC^{med} cells express luminal (*Krt8*, *Krt18*, *Krt19*, *Tmprss2* and *Cd24*) and stem-like (*Tacstd2* (encoding TROP2 protein), *Sox2*, *Cd44* and *Sca-1*) markers but not basal

(*Krt5*, *Krt14* and *Tp63*) and secretory (*Pbsn* and *Msmb*) genes[31,44]. Importantly, *Krt4* (encoding CK4 protein) was validated as a specific marker for the detection of mouse LSC^{med} cells at the mRNA and protein levels, which enabled LSC^{med} cells to be tracked on tissue sections[31]. CK4 immunostaining analyses[31] revealed that, in the mouse prostate, LSC^{med} cells primarily localize within the proximal (periurethral) region of the gland (that is, where stem and progenitor cells have been suggested to preferentially reside based on the results of facultative stem assays[45–47]), are amplified post-castration (in agreement with FACS observations[30,31]) and exhibit low levels of nuclear androgen receptor (in agreement with intrinsically low androgen signalling). These phenotypic and functional characteristics are largely shared with mouse TROP2^{+} luminal cells, which express several typical LSC^{med} cell markers (for example, *Krt4*, *Psca* and *Cyp2f2*) and are enriched in progenitor cells[43,48], suggesting strong overlap between the two cell populations.

Single-cell profiling

The prostate epithelial hierarchy is traditionally defined based on anatomical features (for example, pseudostratified epithelium) and histological markers that distinguish basal, luminal, intermediate and neuroendocrine cells[10,49] (see Introduction). In the past 3 years, several single-cell RNA sequencing (scRNA-seq) studies of healthy mouse prostates have provided unprecedented evidence that the molecular diversity of the prostate epithelium far exceeds the classical histological description[32–36]. Despite the largely different number of cells sequenced in each scRNA-seq study[50], a unique cluster of basal cells was identified in all of them, suggesting a relative molecular homogeneity. By contrast, luminal cell heterogeneity ranged from three to six distinct clusters (TABLE 2), which most probably reflects methodological differences in bioinformatic processes (for example, parameters used for cell clustering). Each study used a different nomenclature to describe luminal lineage heterogeneity (TABLE 2). Typical luminal markers such as *Ar* (encoding the androgen receptor) and *Krt8* were generally found to be expressed in all luminal cell populations. Quantitatively, the major cluster (or clusters) corresponds to differentiated luminal cells exhibiting a secretory phenotype and characterized by the expression of typical markers, for example, *Nkx3.1*, *Spink1* and *Pbsn*. Of the five scRNA-seq studies, three observed that the various differentiated luminal cell clusters were highly lobe segregated and particularly enriched in distal parts of the prostate tissue[32,34,36].

All mouse prostate studies identified one population of non-secretory luminal cells exhibiting phenotypic characteristics compatible with progenitor cells, including their enrichment in stemness-related genes and Gene Ontology functions (for example, developmental process and epithelial cell differentiation[31,32,35]), the expression of specific markers, and their spatial localization in the prostate gland (see Spatial distribution). Each publication named this cell population differently (TABLE 2). To investigate the actual overlap between these cell clusters, we reanalysed the scRNA-seq transcriptomic data[32–36] to identify the top biomarkers of these putative luminal progenitor cells in each individual study and compare their expression level in each cell population across studies[50]. Although one study claimed that the non-secretory luminal cell cluster was actually of urethral origin[36,37] (see Spatial distribution), their scRNA-seq data were included in the comparison given the obvious molecular proximity of these 'urethral' luminal cells with the putative luminal progenitor cells (herein used as the generic term for this cluster) referred to as prostatic cells by all other groups[32–35]. This analysis identified a signature of 15 genes common to all studies (TABLE 3). Strikingly, several of them, including the LSC^{med} cell marker *Krt4*, were previously identified in the 111-gene mouse LSC^{med} cell signature[31] (TABLE 3). Accordingly, a significant enrichment of the latter signature was observed in each scRNA-seq dataset for the sole population identified as luminal progenitor cells[50] (FIG. 2a–d). Together, these bioinformatic analyses

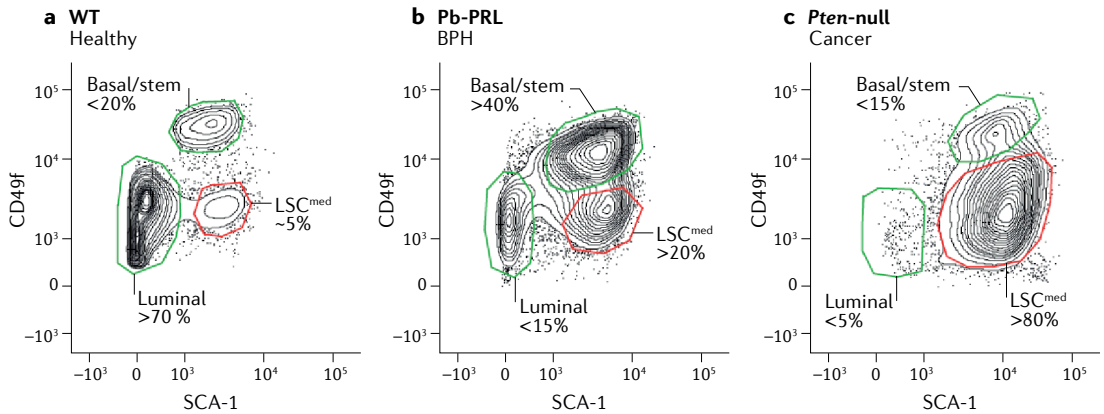


Fig. 1 | **Enrichment of mouse prostate luminal progenitor cells by cell sorting.** Representative SCA-1/CD49f cell sorting profiles of lineage-negative (Lin^{-}) cells recovered from wild-type (WT) (part **a**), Pb-PRL (part **b**) and *Pten*-null (part **c**) mouse prostates[30]. These panels illustrate the amplification of lineage-negative (Lin^{-})SCA-1^{+}CD49f^{med} (LSC^{med}) cells at the expense of secretory luminal cells in three mouse models of healthy prostate, benign prostatic hyperplasia (BPH) and prostate cancer. Data from Sackmann Sala et al.[31].

Table 1 | **Cell-sorting strategies used to enrich LSC^{med}-like luminal progenitor cells from wild-type mouse prostate**

Study	Identifier of the luminal progenitor cell population enriched	Cell sorting enrichment strategy
Sackmann Sala et al.[29,31]	LSC^{med}	Lin$^-$/CD49f^{med}/SCA-1$^+$
Kwon et al.[30,44]	SCA-1$^+$ luminal	Lin$^-$/CD49f^{low}/SCA-1$^+$/CD24$^+$ (luminal)
Guo et al.[32]	Luminal-C	EpCAM$^+$ (epithelial)/TMPRSS22–YFP$^+$ (luminal tracing)/TROP2$^+$
Crowley et al.[34]	Lum-P	Lin$^-$/TROP2$^+$/SCA-1$^+$/CD66a$^+$
Karthaus et al.[33]	L2	Lin$^-$/CD49^{low}/CD24$^+$ (luminal)/CD26^{low}/SCA-1$^+$
Crowell et al.[43]	TROP2$^+$	EpCAM$^+$ (epithelial)/CD49f^{low}/TROP2$^+$

LSC^{med}, lineage-negative (Lin$^-$)SCA-1$^+$CD49f^{med}. LSC^{med}-like luminal progenitor cells exhibit similar levels of CD49f to mature luminal cells, which have been referred to as low[30,43] or medium[29,31] depending on the study. Hence, sorting profiles identified as CD49f^{med} and CD49f^{low} should not be seen as reflecting distinct cell populations regarding CD49f expression.

demonstrate that the various luminal progenitor cell populations identified by scRNA-seq in the healthy mouse prostate not only show high overlap with each other but also with LSC^{med} cells previously enriched by cell sorting[30,31]. A unification of the different progenitor cell identifiers is presented in TABLE 2. 'LSC^{med}-like cells' is used to refer to these largely overlapping luminal progenitor cell populations. According to their molecular proximity with LSC^{med} cells, several markers of TROP2$^+$ luminal cells are part of the 15-gene signature of LSC^{med}-like cells (for example, *Krt4, Psca, Cyp2f2, Krt19, Anxa3* and *Ppp1r1b*). Finally, the core phenotypic signature of LSC^{med}-like luminal progenitor cells appeared to persist with age[38,43], after castration[31,33,35] and in tumours[31,32], suggesting that their molecular hallmarks can be used to track them in various pathophysiological contexts.

In parallel with these mouse studies, scRNA-seq data for the human benign prostate has been published by four groups[32–34,36,51]. Experimental samples were obtained from young male organ donors[32,36,51] or from patient specimens[33,34] (radical prostatectomy or cystoprostatectomy). As in mice, the luminal compartment of the human prostate displayed higher diversity than the basal cell compartment, as all but one study[33] identified a unique basal cell cluster. In addition to secretory luminal cells, pioneering research by one group identified two unprecedented luminal cell types that share molecular similarity with two pulmonary epithelial progenitor cell types called Club and Hillock cells[37,51]. The existence of Club and Hillock cell populations in the human prostate has also been reported in other studies[33,34]. In the prostate, the transcriptional profiles of these two cell types display some similarity. Both are enriched in *PSCA*, a typical stemness-related gene, suggesting that they might display stem and/or progenitor properties[51]. The similarity with lung Club cells includes the expression of *SCGB1A1* (REFS[33,36,51]), a secretoglobin family gene member. Prostate Hillock cells are distinguished from Club cells by *KRT13* expression[33,36,51] and exhibit a more basal phenotype (KRT5$^+$). In keeping with these characteristics, prostatic Hillock cells were classified as one of the two basal cell clusters identified in one study[33]. Whether prostatic Hillock cells are related to previously identified intermediate (CK5$^+$ and CK8$^+$) cells is unknown. Interestingly, an independent study previously identified KRT13 as a human prostate stem cell marker and a key contributor to stem cell maintenance[52]. Overall, scRNA-seq studies of human prostate suggested the existence of at least one cluster of putative luminal progenitor cells[32–34] (TABLE 2). To our knowledge, no consensus signature of the putative human prostatic luminal progenitor cells based on bona fide bioinformatic reanalysis of available scRNA-seq datasets has yet been reported.

Although no secretoglobin gene expression could be detected in mouse LSC^{med}-like cells[36], the LSC^{med} cell signature was enriched only in Club and Hillock cells of the human prostate ($P<2e\text{-}16$)[50] (FIG. 2e). These data strongly suggest that mouse LSC^{med}-like luminal progenitor cells correspond to Club and Hillock cells in the human prostate. Low CD38 expression was the first phenotypic biomarker proposed for human luminal progenitor cells[53]. These CD38^{low} cells were subsequently shown to share several features with mouse TROP2$^+$ luminal progenitor cells[43], themselves identified as LSC^{med}-like cells (see above). Accordingly, the human CD38^{low} luminal progenitor signature[53] contains several markers shared with the 15-gene consensus signature of mouse LSC^{med}-like cells (for example, *PSCA, WFDC2, KRT4* and *PPP1R1B*; TABLE 3) and, logically, even more with the 111-gene signature of LSC^{med} cells[31] (for example, *PSCA, WFDC2, KRT4, PPP1R1B, ARL14, C5, KCNK5, LTF, TNFAIP6* and *CXCL17*).

Altogether, these scRNA-seq studies reveal that the molecular heterogeneity of luminal cells far exceeds that of basal cells, especially in mice, and includes one (mouse) or potentially two (human) populations of cells that have a strong molecular overlap with LSC^{med} luminal progenitor cells.

Spatial distribution

In both mouse and human adult prostate, luminal progenitor cells comprise ~2% of prostatic cells (or ~5% of epithelial cells) as determined by cell sorting[31], scRNA-seq[32,33,36] and label retaining[45,54]. All mouse studies agree that luminal progenitor cells are particularly enriched in the prostatic ducts localized in proximal (peri-urethral) regions of all prostate lobes[30–32,35,36,43,44,55]. In mice, this localization seems to be established during early prostate development[35,36] and to be highly conserved across all ages. Morphologically, luminal progenitor cells display areas of high mitochondrial density, complex membrane interdigitation and no vesicles[34]. According to their spatial localization, two studies highlighted the molecular similarity of LSC^{med}-like cells with prostatic periurethral cells[34] and urethral luminal cells[36]. In agreement with the strong CK4 immunostaining observed in the mouse urethral epithelium, cells referred to as prostatic luminal progenitor cells by various groups[31,32,34,38,43] have been proposed to actually be urethral luminal cells that expand from the urethral epithelium into the epithelium of the proximal prostatic ducts[36,37]. Indeed, cells displaying this molecular

profile are enriched in the urethra[36]. By contrast, Lum-P (LSC^{med}-like) luminal progenitor cells (TABLE 2) have been suggested to be true prostatic cells based on NKX3.1 promoter-driven lineage tracing, which marks prostatic but not urethral cells[34]. Further studies are necessary to elucidate whether LSC^{med}-like cells might include different cell subsets that share highly similar molecular profiles (TABLE 3) despite potential different embryonic origins.

Quantitative FACS analysis of mouse anterior prostate indicated ~10-fold lower frequency of SCA-1$^+$ luminal cells (that is, luminal progenitor cells) in distal than in proximal ducts[30]. Accordingly, several studies investigating mouse prostate epithelium heterogeneity[30,32–34,43], but not all[36], identified small clusters of immuno-labelled luminal progenitor cells spread in distal parts of the gland, especially in the ventral prostate[33,34]. These cells are preferentially located in invagination tips[33,34], where rapidly dividing progenitor-like cells during organogenesis and regeneration were also identified[56]. An abrupt transition between luminal cell subtypes residing in proximal (enriched in progenitor cells) and distal (enriched in secretory cells) regions has been observed by cell-type-specific immunostaining[33–36]. In human

Table 2 | **Luminal cell compartment heterogeneity in mouse and human prostates as determined by scRNA-seq**

Study	Cell subsets analysed	Number and identifiers of luminal cell clusters		Identifier of the luminal progenitor cluster	
		Mouse	Human	Mouse	Human
Henry et al.[51]	All prostate cells	–	*n* = 3 Luminal (LE) Club (OE1) Hillock (OE2)	–	Club[a] and Hillock[a]
Joseph et al.[36]	All prostate + urethral cells	*n* = 4 AP Lum DLP Lum VP Lum Ur Lum (+SV/ED Lum)	*n* = 3 Luminal (LE) Club (OE1) Hillock (OE2)	Ur Lum[a]	
Guo et al.[32]	All prostate cells	*n* = 3 Luminal-A (VP) Luminal-B (DLP, AP) Luminal-C (All lobes)	*n* = 4 Luminal-A Luminal-B Luminal-C Luminal-undefined	Luminal-C	h-Luminal-C
Crowley et al.[34]	All prostate cells (aggregated and lobe-specific analyses)	*n* = 5 Lum-A (AP) Lum-D (DP) Lum-L (LP) Lum-V (VP) Lum-P (proximal) (+PrU)	*n* = 3 Lum acinar Lum ductal PrU-like	Lum-P	Luminal ductal
Karthaus et al.[33]	All prostate cells	*n* = 3 L1 (96%) L2 (3%) L3 (1%) (% of luminal cell pool)	*n* = 2 L1-like L2/Club-like (Hillock cells classified as KRT13$^+$ basal cells)	L2	L2/Club-like
Mevel et al.[35]	Prostate epithelial cells (EpCAM$^+$) Individual lobes, intact and castrated	*n* = 6 Lum-A (intact) Lum-B (intact) Lum-C (intact) Lum-D (intact) Lum-E (castrated) Lum-F (castrated)	–	Lum-D	–

–, not applicable; AP, anterior prostate; DLP, dorsolateral prostate; DP, dorsal prostate; ED, ejaculatory duct; LE, luminal epithelia; LP, lateral prostate; OE, other epithelia; PrU, periurethral; scRNA-seq, single-cell RNA sequencing; SV, seminal vesicle; Ur, urethra; VP, ventral prostate. [a]Cells referred to as urethral luminal by Strand lab.

prostate, although all cell subpopulations identified by scRNA-seq have been reported to contain cells from the transition, central and peripheral zones[32], Club and Hillock cells have been proposed to be more specifically enriched in the transition and central zones[51], reminiscent of the preferential proximal (periurethral) localization of LSC^{med}-like cells in the mouse prostate ducts.

Other experimental approaches

Together, studies in mice and humans have identified LSC^{med}-like luminal progenitor cells as an unprecedented cell population of the prostate epithelium. Transcriptomic profiling of these cells at cell-population and single-cell levels identified phenotypic biomarkers that, alone (for example, CK4 (REF.[31])) or in combination (for example, SCA-1 and CK8 (REF.[30])), have been used to identify putative luminal progenitor cells in prostate sections. As all cells are unlikely to express all markers at a given moment, validation of the gene products listed in TABLE 3 should broaden the panel of useful markers. Meanwhile, we can rely on the updated molecular profiling of mouse prostatic luminal progenitors to retrospectively address the extent to which putative luminal stem and/or progenitor cells characterized by a restricted number of phenotypic markers in previous studies might correspond to the well-defined LSC^{med}-like luminal progenitor cells.

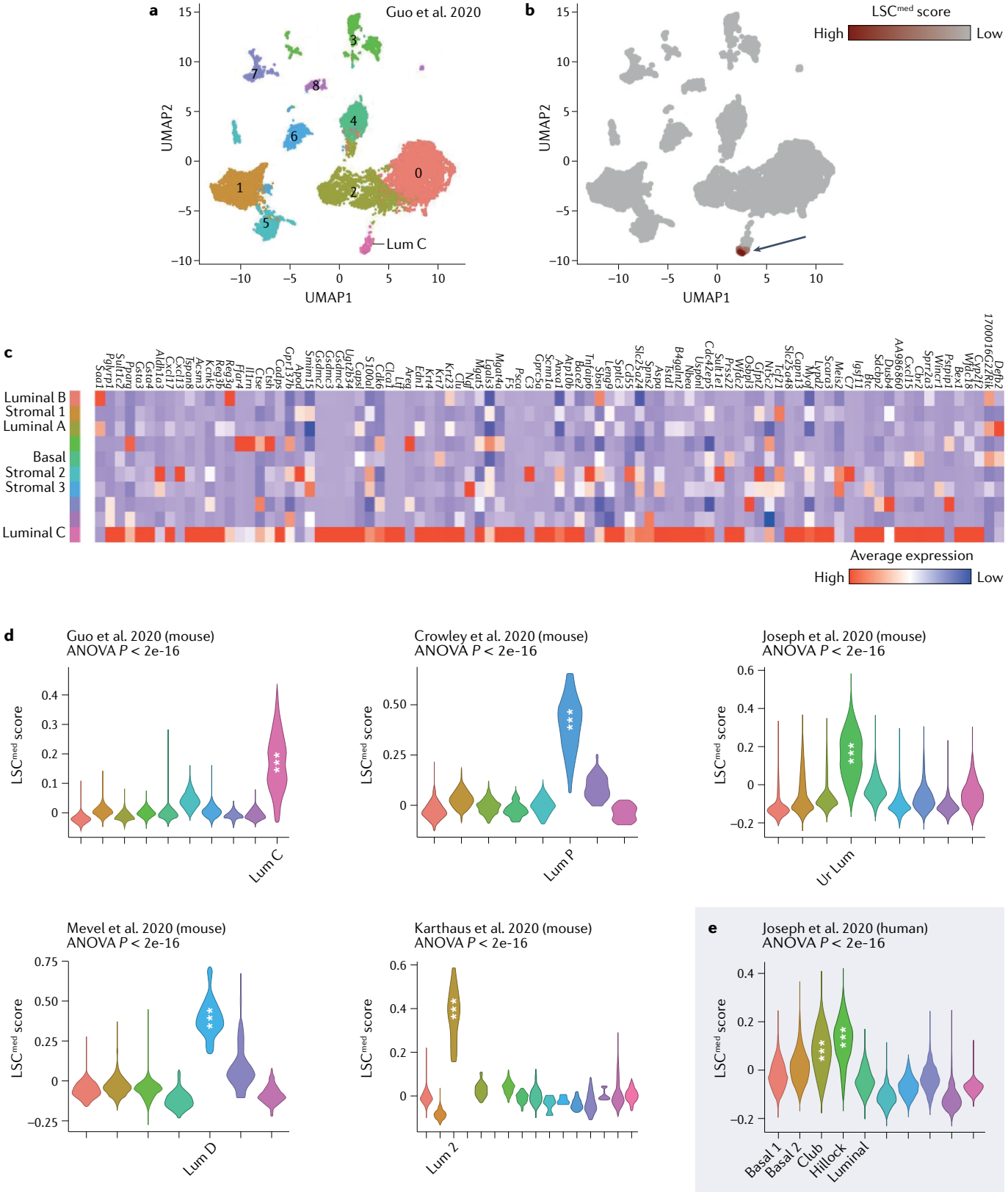
CARNs are rare castration-resistant stem/progenitor cells characterized by persisting *Nkx3.1* expression in androgen-deprived mouse prostates[25]. Castration-resistant *Bmi1*-expressing cells (CARBs) were identified in lineage-tracing experiments as a distinct, *Nkx3.1*-negative luminal progenitor cell population[57]. Both CARNs and CARBs have been shown to be competent for prostate gland regeneration and tumour initiation[25,57,58], thereby assessing their stem/progenitor properties (see Prostate pathophysiology). *Nkx3.1* has been reported to be expressed at extremely low[32,33,35,53] or undetectable[34] levels in mouse LSC^{med}-like luminal progenitor cells, suggesting that the latter cells are distinct from CARNs. Although LSC^{med}-like cells and CARBs both express *Sox2* (REFS[31,58]), *Bmi1* expression is low in the former and does not discriminate them from basal and luminal cell populations[31,33,59]. A study in mice reported that *Bmi1* is expressed in 2.5% of CK14^{+} and 2.1% of CK5^{+} basal cells[57]; whether these labelled basal cells are lost upon castration or differentiate into luminal cells cannot be easily assessed with lineage tracing and tissue section analysis. The initial labelling of basal cells with *Bmi1* promoter might explain why any overlap of CARBs with the LSC^{med}-like luminal progenitor cell population might involve only a few luminal cells. Accordingly, none of the scRNA-seq studies identified *Bmi1* as a gene typically expressed in luminal progenitor cells.

A stem/progenitor cell population defined by the expression of *Kit/Cd117* (stem cell factor) has also been identified in the mouse prostate[60]. After enrichment using four stem cell markers (*Cd117*, *Cd133*, *Cd44* and *Sca1*) this cell population was able to regenerate prostatic gland in reconstitution assays. Although *Cd133/Prom1* has been proposed as a marker of human prostate epithelial stem cells[61] and of mouse luminal progenitor cells[62], *Cd44* and *Sca1* are highly expressed in basal cells. A subsequent study[63] showed that all cells enriched using these four markers were positive for *Cd49f*, without discriminating LSC^{high} (basal) from LSC^{med}-like cells (FIG. 1a). As this *Kit*$^{+}$ population expresses both basal and luminal markers[60], the possibility that the luminal cells contained in this mixed cell population partially overlap with LSC^{med}-like luminal progenitor cells cannot be excluded. Rare castration-resistant, long-lived *Lgr5*$^{+}$ progenitor cells able to regenerate prostatic tissue after mouse castration have been identified in the basal and luminal cell layers[64]. Intriguingly, although *Lgr5*$^{+}$ cells are mostly luminal (~20% are basal cells[64]), scRNA-seq data[33] suggest that *Lgr5* is expressed by L1 (intrinsically) and L3 (only after castration) luminal populations, but not by L2 luminal progenitor cells (TABLE 2). Another group reported that FACS-enriched CD49f$^{med/lo}$/PROM1^{+} luminal cell population exhibits stem/progenitor properties in facultative stem cell assays[62]. Although *Prom1* is expressed in both LSC^{med}-like and differentiated luminal cells[31], the latter population exhibits very low progenitor-like properties (see Stem cell functional assays), suggesting that CD49f$^{med/lo}$/PROM1^{+} cells partly overlap with LSC^{med}-like cells. Other mouse studies identified, in the proximal prostate, castration-resistant luminal progenitor cells based on label-retaining capacity (slow-cycling cells)[54] and expression of *Ly6d*[65] or *Sox2* (REF.[66]). Although none of these features is restricted to LSC^{med}-like cells, they match their documented properties, suggesting potential overlap. In particular, the signature of CD38^{low} human luminal progenitor cells was highly enriched in label-retaining cells[54]. Finally, a pioneering report[67]

Table 3 | **Transcriptomic signature of mouse LSC^{med}-like luminal progenitor cells[50]**

scRNA-seq	LSC^{med}
Krt4	++
Psca	++
Wfdc2	++
Cyp2f2	++
Gsta4	++
Cbr2	++
Tspan8	++
Clu	++
Krt19	+
Klf5	+
Anxa3	+
Ppp1r1b	+
S100a11	↔
Krt8	↔
Atp1b1	↔

++, fold change >1.5 ($P < 0.05$); +, fold change >1.2 ($P < 0.05$); ↔, expressed at a similar level to that in basal and/or luminal cells; LSC^{med}, lineage-negative (Lin^{-})SCA-1^{+}CD49f^{med}; scRNA-seq, single-cell RNA sequencing.



identified putative *Clu*$^{+}$*Tacstd2*$^{+}$*Sca1*$^{+}$ luminal progenitor cells based on transcriptomic and immunostaining studies[67]; although their stem/progenitor properties in normal adult prostate were not experimentally assessed, their expansion in *Pten* deletion-driven mouse prostate tumours is similar to reported properties for LSC^{med} cells[31] (see Prostate cancer). Additional information on methods and markers used to identify the various luminal progenitor cells reported to date is available in other reviews[11,37].

Fig. 2 | **Transcriptomic similarity between FACS-enriched LSC^{med} cells and putative luminal progenitor cell clusters identified by scRNA-seq analyses of wild-type mouse and healthy human prostates. a–c** | The transcriptomic correspondence between lineage-negative (Lin$^-$)SCA-1$^+$CD49f^{med} (LSC^{med}) cells and prostate luminal progenitor cells identified by single-cell RNA sequencing (scRNA-seq) is illustrated using the dataset of Guo et al.[32] as a representative example (see Puig Lombardi et al.[50] for technical details, including the number of cells sequenced in all the studies compared). **a** | UMAP projections based on linear dimensionality reduction by principal component analysis (PCA) for 19,503 single-cell transcriptomes. **b** | A single subpopulation matched LSC^{med}-like cells, as shown by high calculated LSC^{med} gene signature scores. **c** | Heatmap representation of average expression levels per cluster of LSC^{med} signature genes found in the dataset of Guo et al.[32]. **d**,**e** | The violin plots show the calculated LSC^{med} scores per cluster for 5 mouse[32–36] (part **d**) and 1 human[36] (part **e**) prostate scRNA-seq studies (***, Tukey multiple comparisons of means $P_{adj} < 0.001$ for all pairwise comparisons performed). Mouse Ur Lum and human Club and Hillock cells were referred to as urethral by the authors. FACS, fluorescence-activated cell sorting. Data from Puig Lombardi et al.[50].

Prostate pathophysiology

The functional characterization of prostatic luminal progenitor cells in health and diseases (FIG. 3) and their relevance and amplification in pathophysiological contexts have been documented in mice and, for some, in humans (FIG. 4). LSC^{med}-like cells are currently the only prostatic luminal progenitor cell population that has been exhaustively profiled at the population and single-cell levels.

Stem cell functional assays

The proximal region of the mouse prostate is known to be enriched in epithelial cells exhibiting stem-like capacity[45,60]. SCA-1 expression was initially shown to occur in at least a proportion of these cells[68], but the SCA-1$^+$ epithelial cell population has now been shown to encompass both basal and LSC^{med}-like luminal progenitor cells (FIG. 1). For luminal progenitor populations that could be enriched by cell sorting — that is, LSC^{med} (REF.[29]), SCA-1$^+$ luminal[30], Lum-P[34], TROP2$^+$ luminal[43], L2 (REF.[33]) and Luminal-C[32] (TABLE 1) — their stem-like properties were experimentally demonstrated through their ability to generate prostaspheres and/or organoids in vitro, and/or to reconstitute prostatic glands when engrafted into host mice (BOX 2).

Approximately 1.8% of dissociated prostate cells are capable of generating organoids of 20–30 µm in size[30]. Depending on the study, the organoid formation capacity of LSC^{med}-like luminal progenitor cells was ~2–4-fold lower than that of basal cells, but ~2–6-fold higher than that of differentiated (secretory) luminal populations[30,32–34,53]. Importantly, luminal progenitor cells enriched from distal ducts formed twice the number of organoids compared with their proximal counterparts[32]. This feature could reflect higher quiescence of stem-like cells within the proximal prostate regions[45], which has been suggested to be maintained by non-canonical WNT/ROR2 and TGFβ signalling[69]. Reminiscent of pioneering studies involving LSC^{med} cell-generated prostaspheres[29], organoids generated from LSC^{med}-like luminal progenitor cells were larger than those generated from basal cells[30,34,43], indicating higher cell proliferation[43]. The organoids contained both basal and luminal cells[30,32,53], and their number, size and morphology were not affected by the presence or absence of androgens[30]. Importantly, the ability of LSC^{med}-like progenitor cells to self-renew was assessed by serial organoid passages[30]. Of note, luminal progenitor cells defined as CD49f$^{med/lo}$/PROM1$^+$ also generated rare single-layered organoids with limited self-renewal capacity[62]; the progenitor cells involved might not overlap with the well-defined LSC^{med}-like cells. In reconstitution assays, the capacity of LSC^{med}-like luminal progenitor cells to regenerate prostatic glands from a small number of cells was, as for organoids, lower and higher than that of basal and luminal cells, respectively[30,34]. The regenerated tubular structures contained a lumen surrounded by CK8$^+$ cells encapsulated by a discontinuous layer of CK5$^+$ basal cells[30,34]. *Sox2* was demonstrated to be essential for this property[44]. Remarkably, TROP2$^+$ luminal progenitor cells were found to reside in the invagination tips of reconstituted glands, as observed in situ[32]. Thus, the capacity of enriched luminal progenitor cells to generate both basal and luminal cells indicates bipotency in these 'facultative' functional assays (BOX 2).

The heterogeneity of the FACS-enriched LSC^{med}-like cell population was evidenced at least at two levels. First, the population's organoid formation capacity (meaning the percentage of cells able to generate an organoid) ranged from ~4%[30,34,53] to ~10%[33]. The fact that only a small fraction of LSC^{med}-like cells harbours stem-like properties is an important notion to emphasize as the term 'luminal progenitor' used to define this cell population might be confusing regarding the actual properties of each individual cell. Second, the actual progenitor cells contained in this population generated at least three types of organoid differing in size, shape and phenotypic markers[30]. In particular, one type of organoid lacked expression of basal markers. This is similar to observations from reconstitution assays using LSC^{med} and CD49f$^{med/lo}$/PROM1$^+$ cells enriched from *Pten*-deficient[31], and *Pten*- and *Tp53*-deficient[62] prostate tumours, respectively. These observations suggest that distinct luminal progenitor cells might exhibit different stem-like potency (unipotency versus bipotency), at least in these in vitro functional assays.

Prostate development and homeostasis

Lineage tracing experiments with *Krt5* and *Krt8* promoters, which mark basal and luminal cells, respectively, revealed that during the first 2 weeks of postnatal development, multipotent basal progenitor cells give rise to unipotent luminal progenitor cells that contribute to luminal lineage expansion[70–72]. Analysis of the orientation of cell division showed that luminal progenitor cells usually undergo symmetrical divisions generating two luminal daughter cells at this stage of prostate development[71]. As soon as puberty begins, basal progenitor cells restrict their capacity to generate luminal cells and only unipotent luminal progenitor cells sustain the lineage expansion[73]. This model suggests that during the early phase of growth, basal cells display bipotent properties and act as a pool of luminal progenitor cells[70,73]. In parallel, another study showed that CK8$^+$ luminal progenitor cells can also exhibit bipotent properties during the first week of postnatal development (P1–P7) owing to the low level of androgen receptor expression. At the onset of puberty, androgen receptor expression increases in

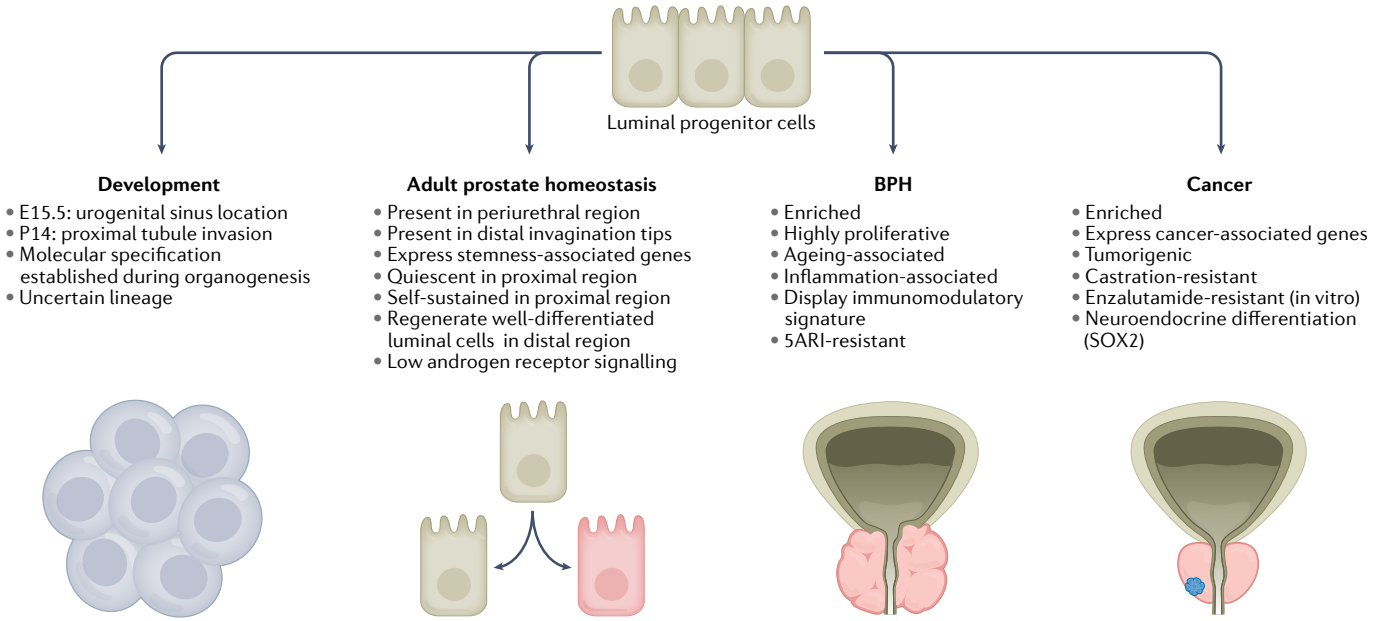


Fig. 3 | **Overview of luminal progenitor cell properties in prostate pathophysiology.** The main features of luminal progenitor cells in prostate development, homeostasis, benign prostatic hyperplasia (BPH) and cancer as determined in mouse studies are summarized. See Prostate pathophysiology for a discussion of their potential relevance in human diseases. 5ARI, 5α-reductase inhibitor; E, embryonic day; P, postnatal day.

luminal progenitor cells at the time when unipotency is established[74]. Importantly, the molecular characterization of embryonic and postnatal luminal progenitor cells was recently assessed by scRNA-seq[35]. Urogenital sinus mesenchyme (UGSM) explant analysis uncovered the emergence of a luminal cluster that, in addition to classical luminal marker expression (CK8, CK18), presents elevated levels of progenitor markers[35] (TABLE 3). CK8^{high} progenitor cells of embryonic prostates are also characterized by increased *Runx1* expression and absence of *Nkx3.1* (REF.[35]). Lineage tracing demonstrated that luminal *Runx1*$^{+}$ cells are localized at the proximal-periurethral prostate and are marked by CK4 expression. *Runx1*$^{+}$-traced cells expand while also generating Nkx3.1^{+} cells, which are distally located to a small extent[35]. The features of these traced cells combined with the marker expression highlighted by scRNA-seq imply that the spatial and molecular specification of luminal progenitor cells seem to be already established during prostate organogenesis. Additional discussion can be found elsewhere[37].

Unlike tissues such as the intestine, the adult prostate epithelium undergoes extremely slow turnover during homeostasis. The limited proliferation in the adult mouse prostate primarily involves luminal cells[41]. Lineage tracing experiments using *Krt8*-CreER^{T2} and *PSA*-CreER^{T2} showed that in adult mice, the luminal lineage is self-sustained by unipotent luminal progenitor cells[26,27]. The lineage-specific stem cell features of the adult luminal compartment indicated that luminal bipotency is an acquired capacity when cells are subjected to assay manipulations (BOX 2), rather than a physiological property of the intact tissue. Label retention studies using H2B-GFP or BrdU pulse–chase experiments showed the presence of slow cycling cells, a property shared by some epithelial stem and/or progenitor cells, in the proximal ducts, close to the urethra, and which correspond to 2% of the whole luminal population after 6 months of chase[45,54,75]. Interestingly, these quiescent luminal stem and/or progenitor cells display phenotypic similarity with LSC^{med}-like cells, including high expression levels of *Sca-1* and *Trop2* (REFS[30,43,44,75]). Higher level of non-canonical WNT signalling induced by the stromal cells surrounding the proximal prostate has been proposed to regulate the unique features of *Sca-1*$^{+}$ luminal progenitor cells via interactions with basal and stromal cells within this region[69].

Using mitochondrial DNA mutations to follow up clonal expansion of cells during human adult prostate homeostasis, Moad and colleagues suggested that bipotent basal epithelial progenitor cells are the main contributors to epithelium maintenance through the feeding of epithelial flows traversing the branching gland network, whereas a minor luminal unipotent stem/progenitor cell pool maintains the most proximal luminal cells[76]. These stem/progenitor cells reside in the main trunks of the juxta-urethral ducts, which was recently shown to be predominantly composed of Club and Hillock cells[36,51]. The potential overlap between these cells' subsets has yet to be investigated. This proximal location could also account for a role of luminal progenitor cells as prostate safeguards. By analogy to pulmonary progenitor Club cells, which are predominantly found in the proximal trachea[77], exhibit regenerative properties and express antimicrobial and anti-viral proteins and chemokines[78], prostatic luminal progenitor cells could act as the first line of defence against pathogen infection from the urethra in a retrograde contamination way[37], thereby further contributing to tissue homeostasis.

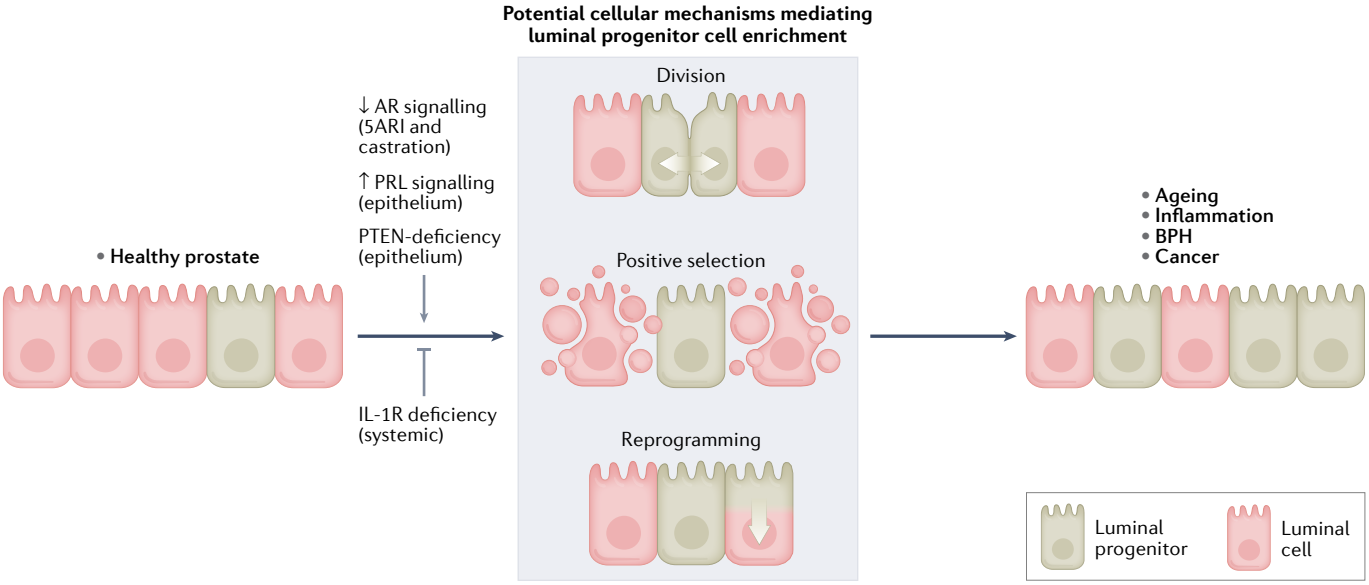


Fig. 4 | **Regulation of disease-associated luminal progenitor cell amplification.** Schematic representation of the enrichment of luminal progenitor cells observed in various pathophysiological contexts in mouse and/or in human. Four identified molecular pathways contributing to the regulation of luminal progenitor cell prevalence in mice are indicated. Three potential, non-mutually exclusive mechanisms of luminal progenitor cell enrichment are depicted. 5ARI, 5α-reductase inhibitor; AR, androgen receptor; BPH, benign prostatic hyperplasia; IL-1R, interleukin-1 receptor; PRL, prolactin.

Collectively, the recent advances in the characterization of prostate cells at molecular level as well as the spatial distribution of these markers indicated the existence of a progenitor niche around the urethra that is already defined during early development (FIG. 3). Moreover, these findings shed light on the relationship between the murine and the human prostate, suggesting that the progenitor properties of luminal cells as well as their niche location are conserved among species.

Post-castration prostate regeneration

Although the adult prostate is mostly quiescent under homeostatic conditions, it exhibits incredible plasticity upon injuries. In particular, murine prostate glands are repeatedly able to fully regenerate within a few weeks after regression in response to serial cycles of castration followed by androgen replacement. Evidence exists that, at least in rodents, epithelial cell regeneration occurs locally more than by migration of stem and/or progenitor cells mainly residing within the proximal regions of the prostate gland[33,37,56].

Pioneering studies provided evidence that basal cells are more castration-tolerant than luminal cells[18,24]. Accordingly, androgen withdrawal in culture medium of organoids has been reported to lead to atrophy of the luminal compartment[79,80]. However, lineage tracing of CK8-expressing luminal epithelia demonstrated that prostate luminal cells are largely derived from unipotent luminal progenitor cells after castration–regeneration cycles[25–27]. According to their regenerative properties, CARBs were shown to be ~4-fold enriched following castration[57]. Regarding LSC^{med}-like luminal progenitor cells (FIG. 4), 3–9-fold enrichment post-castration was also reported for LSC^{med} (REF.[31]), SCA-1$^+$ luminal[30], Luminal-C[32] and TROP2$^+$ urethral luminal[36] cells. In situ, lineage tracing of *Krt4*$^+$ cells following androgen restoration showed ribbons of marked cells emanated from glandular invagination tips, suggesting the contribution of a clonal mechanism to epithelial regeneration in distal prostate[32]. This phenotype gradually increased at each regression–regeneration cycle[32]. TROP2$^+$ distal Luminal-C cells were suggested to both self-renew to maintain their population and to differentiate into TROP2$^-$/NKX3.1$^+$ (secretory) daughter luminal cells[32].

Although androgen deprivation is dogmatically linked to massive luminal cell apoptosis, FACS and single-cell analyses indicated that many luminal cells identified as differentiated or secretory (that is, *Sca1*^{neg} (REFS[30,31]), L1 (REF.[33]) or Luminal-B[32]; TABLE 2) persisted after castration. In line with previous findings suggesting that most adult epithelial cells result from stochastic cell divisions[81], a subsequent study used lineage tracing to show that *Sca-1*$^+$ and *Sca-1*^{neg} luminal cell lineages (that is, LSC^{med}-like and secretory cells, respectively) were independently sustained in castration–regeneration experiments, suggesting a progenitor-independent mechanism of luminal cell regeneration[44]. Accordingly, another study[33] showed that, at early stages of prostate regeneration induced by androgen replenishment, L1 secretory luminal cells were more proliferative than L2 luminal progenitor cells in situ, and their respective capacity to generate organoids in vitro no longer differed, in contrast to cells harvested from intact mice. Remarkably, 28 days after castration, the transcriptional profile of L1 secretory cells resembled that of L2 luminal progenitor cells, which was in part due to abrogation of androgen signalling in L1 secretory cells, as this feature is a major discriminator of both cell populations in intact mice[31,33]. This transcriptional proximity was transient and rapidly diluted after androgen addback[33].

Together, these observations suggest that differentiated and/or secretory L1 cells actively contribute to post-castration prostate regeneration where they reside — that is, in the distal prostate — and that their regenerative potential might be facilitated by the transient acquisition of stem/progenitor-like features through partial dedifferentiation into luminal progenitor-like cells[33,82]. Of interest, a separate study[35] reported that the Lum-D luminal progenitor population did not amplify, and even regressed, after castration, while retaining its molecular identity (*Trop2*$^+$, *Krt4*$^+$, *Runx1*$^+$). These authors raised the hypothesis that some Lum-D cells might undergo partial reprogramming into another luminal population, Lum-E (TABLE 2), that concomitantly emerged in castrated mice. This Lum-E population is appropriately enriched in developmental processes[35].

In summary, at least four non-mutually-exclusive mechanisms might cooperate to promote post-castration regeneration of the prostatic epithelium in the distal prostate: proliferation and clonal expansion of CK4$^+$ luminal progenitor cells followed by differentiation into luminal (TROP2$^-$) daughter cells[32]; transcriptomic reprogramming of luminal progenitor cells towards a specific post-castration state[35]; partial and transient dedifferentiation of secretory luminal cells into a luminal progenitor-like profile[33]; and stochastic divisions of independently sustained cell lineages[44]. In these processes, the LSC^{med} state, perhaps more than LSC^{med} cells, might constitute a functional hub for castration tolerance, a prerequisite for prostate regeneration.

Benign prostatic hyperplasia

BPH is a very frequent disease that will affect one in four men over their lifetime[3]. BPH is caused by the progressive growth of the prostate transition zone and periurethral area, which ultimately constricts the urethral opening[1,83,84] and results in lower urinary tract symptoms (LUTS) that are particularly prevalent in the ageing population[1–3]. The hyperplasia underlying BPH is driven by the abnormal growth of the stromal and the glandular epithelium[84], and both phenotypes often coexist within the same hypertrophied prostate[2]. Based on the role of androgens in promoting developmental and post-pubertal prostate growth, 5α-reductase inhibitors (5ARIs) are, to date, the only aetiological treatment used in BPH[2,85]. These drugs block intraprostatic conversion of testosterone into its active metabolite dihydrotestosterone, a reaction thought to take place mainly in stromal cells. 5ARIs are used for the treatment of large (>40 cc) BPH[2]. As a result, prostate shrinkage is clinically observed after 6–9 months of treatment[1] which is assumed to reflect apoptosis of androgen-dependent luminal cells of the glandular epithelium. Although LUTS improvement is initially observed in most treated patients, a substantial number (~20%) will report clinical progression of BPH-related symptoms after only 4 years, and 5% will ultimately undergo surgical removal of the adenoma[86,87]. Surgery can result in sexual adverse events such as retrograde ejaculation in more than 70% of cases, as well as urinary sepsis (10–15%), severe bleeding complication (3–10%) or delayed urethral stenosis (2–3%)[88].

LSC^{med} cells were discovered owing to their expansion in a mouse model of BPH[29] (FIG. 1b; FIG. 4). In these mice, called Pb-PRL, prostate hyperplasia is triggered by prolactin (PRL) overexpression in prostate secretory luminal cells using the prostate-specific probasin (Pb) promoter[89]. This model was developed to mimic PRL expression observed in the human prostate[90] (PRL is not detected in mouse prostate) and to investigate its possible role in promoting human prostate carcinogenesis[42]. However, Pb-PRL mice did not develop cancer but instead developed many features of human BPH, including epithelial and stromal hyperplasia, inflammation and an altered urine flow pattern reminiscent of LUTS[89,91–93]. The marked amplification of LSC^{med} luminal progenitor cells compared with wild-type littermates was assessed by cell sorting, and CK4 immunostaining demonstrated that these cells had spread throughout the whole prostatic epithelium, including distal regions in which they are normally sparse[29,31]. Their highly proliferative profile suggested their contribution to the disease[29].

The involvement of stem and/or progenitor cells in pathological conditions of the human prostate, including BPH, has been long suspected, and adult prostate hyperplastic growth has been suggested to result from reactivation of developmental cellular and molecular processes (embryonic reawakening)[1,38,83,94–97]. Studies over the past 3 years[51] showed that Club cells were enriched in the transition and periurethral zones of the human prostate, that is, where BPH develops. In the mouse, ageing was shown to be associated with a luminal progenitor signature (for example, *Psca*$^+$, *Krt4*$^+$ and *Pigr*$^+$) matching marked expansion of TROP2$^+$ luminal progenitor cells in old mouse prostates[38,43]. Using PSCA as a marker of luminal progenitor cells, the luminal cell compartment has been suggested to be amplified with age in humans, and further amplified in BPH[38,43]. These findings were supported by single-cell analyses showing that, as observed for LSC^{med} cells in Pb-PRL versus wild-type mice, prostate Club (but not Hillock) cells were ~5-fold amplified in BPH compared with healthy human prostates[36]. Whether Club cells exhibit progenitor properties and, if so, whether they are maintained in ageing as assessed for their mouse prostate counterparts[38,43], remains to be assessed.

The mechanisms underlying luminal progenitor amplification in BPH are still poorly understood[1]. Both systemic and local signals might be involved, and inflammation-related circuits are emerging as relevant candidates. Massive immune cell infiltration is a typical feature of BPH, and chronic prostatic inflammation has been associated with LUTS severity[98,99]. Concomitant growth of LSC^{med}-like progenitor and intra-prostatic inflammatory cell compartments has been observed in mouse and human BPH phenotypes[29,43,91,92,98]. Like pulmonary Club cells, which can serve as progenitor cells for tissue repair in inflammatory contexts[77,78], prostatic Club cells are enriched in immunomodulatory programmes and signatures[51]. Similar findings have been reported for the closely-related LSC^{med}, TROP2$^+$ (mouse) and CD38^{low} (human) luminal progenitor cells[31,43,53]. These findings suggest that the expansion of luminal progenitor cells in BPH might occur in response to

signals from inflammatory cells present in hyperplastic prostate tissue. In agreement, CD38^{low} luminal progenitor cells were shown to expand in proximity to stromal regions enriched in inflammatory cells in benign human prostates[53]. Some studies have shown that the inflammatory microenvironment per se promoted luminal progenitor expansion irrespective of any BPH context: for example, in a mouse model of autoimmune inflammation, in which amplification of the LSC^{med}-like cell population, in addition to that of basal (LSC^{high}) cells stressed by the authors, was obvious in SCA-1/CD49f cell sorting profiles of inflamed prostates[100]. Similarly, sixfold expansion of KIT^{+} luminal progenitor cells was reported in a model of bacterially induced prostate inflammation, including neutrophils, lymphocytes and macrophages[63]. Interestingly, interleukin-1 receptor (IL-1R)-deficient mice exhibited an expansion rate of those particular luminal progenitor cells half that of IL-1R^{+} mice, highlighting the role of IL-1R signalling in this process (FIG. 4). As prostatic inflammation was unaffected in IL-1R-deficient mice, KIT^{+} luminal progenitor amplification was suggested to require functional IL-1R signalling at the epithelial–stromal interaction level[63]. Accordingly, a subsequent study showed that IL-1 production by macrophages was induced in response to androgen receptor signalling disruption in luminal epithelial cells, resulting in androgen receptor-independent, inflammation-mediated epithelial cell proliferation[101]. The IL-1R pathways have a critical role in prostate development by activating insulin-like growth factor signalling, in line with the reawakening hypothesis, suggesting that developmental signalling pathways might have a role in the hyperplastic reaction to inflammation[83]. Many other cytokines and chemokines (for example, IL-1, IL-6, IL-8, IL-15, IL-17, TNF, etc.) are also present in the BPH microenvironment[85,98,99,101], some of which presumably also contribute to promoting prostatic cell stemness[69,102].

The impact of both age-related and therapeutic decline of androgen levels and signalling on non-cancerous prostatic luminal progenitor amplification should also be addressed (FIG. 4). First, the intrinsic androgen independence of luminal progenitor cells predisposes them to survive androgen lowering and deprivation therapies more efficiently than androgen-dependent luminal cells. Mouse KIT^{+} luminal progenitor cells were even shown to proliferate post-castration[103]. Second, castration triggers rapid infiltration of immune cells inside the prostatic tissue[102], as do epithelial cell-specific *Ar* gene deletion[101] and systemic treatment of Pb-PRL mice with finasteride[91], the prototypic 5ARI. These two mechanisms together might contribute to the expansion of LSC^{med}-like cells in castrated compared with intact mice[30,31], and of Club cells in patients with BPH treated with 5ARIs[36].

Regarding how luminal progenitor cells could contribute to epithelial hyperplasia, LSC^{med}-like cells are more proliferative, exhibit higher organoid-forming capacity and form larger prostaspheres and organoids than differentiated luminal counterparts[29,43]. Importantly, these features were maintained in old mice[43], contrary to the general assumption that adult stem cell functions decline in ageing[38,104]. Two studies showed that stem/progenitor cells enriched from inflamed prostates exhibited a higher proliferative profile[63] and generated bigger spheres than cells harvested from age-matched controls[63,100]. These findings indicate that inflammation further promotes the intrinsically elevated growth properties of luminal progenitor cells. Together, these observations suggest that luminal progenitor cell expansion should contribute to prostate epithelial hyperplasia in the transition and peri-urethral area and directly contribute to BPH-associated obstructive LUTS by compressing the urethra. As LSC^{med}-like luminal progenitor cells express many pro-inflammatory cytokines and chemokines (for example, *Tnfa*, *Il6*, *Il8*, *Ccxl1*, *Cxcl3*, *Ccl20*, *Il1a*, *Ifng*)[31,43,53], reciprocal interactions between luminal progenitor cells, intra-prostatic inflammatory cells and stromal cells should take place, resulting in a vicious circle, ultimately promoting stromal and epithelial hyperplasia. We speculate that treatments interfering with androgen signalling (such as with 5ARIs) could progressively enhance such a circuitry through promoting inflammation, which might ultimately counterbalance their initial beneficial growth-inhibitory effects.

Prostate cancer

The identity of the cells of origin of prostate cancer has been long debated[12]. Both basal and luminal cells have been shown to be able to initiate prostate tumours in both facultative assays and in vivo lineage-traced cancer models[12,26,28,40,41,105–107] (BOX 2). Intriguingly, the relative tumorigenicity of basal cells compared with luminal cells appears to be largely assay-dependent. In reconstitution assays, transformed basal cells embedded in UGSM are much more potent than their luminal counterparts at initiating prostate tumours in host mice[22,28,39,108]. However, in experiments involving tumorigenesis in situ, luminal cells were shown to be the preferred cell of origin of cancer[26,41,107]. Indeed, tumours of basal origin developed more slowly than those of luminal origin, despite basal-to-luminal differentiation being observed[26,28,39,41,108,109]. A reasonable explanation for this apparent discrepancy is that the specific conditions of facultative stem cell assays (for example, the inductive properties of UGSM) might mimic those encountered in organogenesis and tissue damage and repair, which could artificially boost the plasticity of basal cells and promote their responsiveness in these assays[12,41]. In addition, we speculate that the definition of luminal cells in the two types of assay could be an important confounding factor. Indeed, cells used in reconstitution assays must first be enriched by flow cytometry[39]. Based on the expression of cell surface markers classically used to discriminate luminal from basal cells (for example, SCA-1, CD49f, TROP2), enriched luminal cells (SCA-1^{-}/CD49f$^{low/med}$/TROP2^{-}) will lack the LSC^{med}-like progenitor cell pool (SCA-1^{+}/CD49f$^{low/med}$/TROP2^{+}) (FIG. 1). By contrast, as luminal progenitor cells express many typical luminal genes whose promoters are used to drive luminal-specific expression of oncogenes or of fluorescent reporter genes in lineage-tracing experiments (such as *Krt8*)[26,107], luminal progenitor cells will be included in cells defined as 'luminal' in such experimental settings.

As secretory luminal cells have low intrinsic stem-like properties, the presence or absence of luminal progenitor cells within so-called luminal cells depending on the assays is likely to affect their apparent biological properties.

Prostate cancer initiation. *PTEN* is a tumour suppressor gene encoding a lipid phosphatase that counteracts PI3K–AKT signalling. *PTEN* deletion is one of the most frequent genetic alterations observed in human prostate cancer[110], and various models of temporally and/or spatially controlled deletion of *Pten* have been developed to investigate the molecular and cellular mechanisms of prostate tumorigenesis[40,62,111–114]. In a model of biallelic *Pten* deletion driven by the androgen-responsive probasin promoter, LSC^{med} cells comprised ~80% of the epithelial compartment of prostate tumours at 6 months of age as determined by cell sorting[31]. As expected, *Pten*-null bulk tumours were highly enriched in LSC^{med} cell markers both at the mRNA[31,115] and protein[115] levels, confirming that their core identity was maintained in a cancer context. The predominant prevalence and high proliferation rate of these luminal progenitor cells strongly suggested their major contribution to prostate tumorigenesis in this model[31], in agreement with their intrinsic Gene Ontology properties (tumorigenesis, proliferation, motility, angiogenesis, inflammation and stemness-related function)[31,32,43,54]. Their tumorigenicity was confirmed by the ability of FACS-enriched *Pten*-null LSC^{med} cells to initiate tumours in reconstitution assays[31]. The occurrence of tumoural glands of different phenotypes as revealed by monitoring cytokeratin expression suggested that FACS-enriched LSC^{med} cells contained cells displaying different stem-like properties, as observed for wild-type luminal progenitor cells (see Stem cell functional assays). In keeping with these findings, up to eight LSC^{med}-like cell subclusters have been reported by scRNA-seq analysis of prostates from a *Pten*-deficient model[116]. In addition, multipotent CD49f^{med}/PROM1^{+} luminal progenitor cells were shown to generate adenosquamous tumours and their unipotent counterparts generated pure adenocarcinomas[62]. Finally, in tumours generated from *Pten*-null LSC^{med} cells, a microinvasion phenotype was associated with the absence of a basal layer, as observed in human prostate cancer[117]; notably, these glands were highly enriched in LSC^{med} cells (CK4^{+} and CK8^{+})[31].

Reconstitution assays might enhance facultative properties that are not relevant in the native prostatic microenvironment. However, two studies strongly suggest that LSC^{med} cells can initiate prostate tumours in situ. Analysis of the early steps of prostate tumorigenesis induced by *PSA*-driven biallelic deletion of *Pten* showed that hyperplastic epithelial cells positive for phosphorylated (p)AKT were enriched in *Tacstd2*, *Sca1, Ppp1r1b, Wfdc2, Krt19* and *Clu*[67], all identified as hallmarks of LSC^{med}-like luminal progenitor cells (TABLE 3). As early as 4–5 weeks of age — that is, when the *PSA* promoter just starts to be activated by androgens — single pAKT^{+}CLU^{+}TROP2^{+} hyperplastic cells could be identified in the luminal (CK8^{+}) epithelium of distal prostates. Progenitor-like cells sharing the same phenotypic profile were highly prevalent in the proximal prostate, in agreement with the enrichment of luminal progenitor cells in proximal regions. However, these progenitor-like cells were negative for pAKT and not hyperplastic, suggesting that distal hyperplastic foci do not originate from cells of the proximal region. Instead, the rare progenitor-like cells identified in the distal prostate epithelium of aged-matched wild-type mice were proposed to serve as tumour-initiating cells once *Pten* deletion has occurred in these cells[67]. This model is in agreement with the findings of another study[32] that showed that *Pten* deletion specifically targeting luminal progenitor cells using *Krt4* promoter-driven Cre recombinase expression could initiate prostate tumorigenesis, and that distal luminal-C progenitor cells (TABLE 2) were more efficient than proximal progenitor cells at initiating prostate cancer[32]. Luminal-C cells formed small, isolated clusters at the glandular invagination tips in the distal prostate, that is, where CK4^{+} luminal progenitor cells are also localized in healthy distal prostate[32].

Together, these results obtained using various preclinical models and experimental settings indicate that luminal progenitor cells can efficiently initiate prostate tumours and clonally amplify. This does not preclude alternative mechanisms to also contribute to the enrichment of LSC^{med}-like cells observed in *Pten*-deficient tumours. As both SCA-1^{+} (luminal progenitor) and SCA-1^{neg} (secretory luminal) cells can generate prostate tumours in mice[55], we could speculate that partial dedifferentiation of the latter into the former could take place in cancer contexts as this has been reported during prostate regeneration[33] (FIG. 4).

Castration-resistant prostate cancer. A major therapeutic challenge for patients with prostate cancer is the progression of their disease towards castration-resistant prostate cancer (CRPC) while they are still under androgen deprivation therapy (ADT). One of the unsolved questions has been the identification of the cell types that drive CRPC. Although basal cells are classically referred to as the most castration-resistant cells in the prostate epithelium[18], they are absent in human prostate cancer — this feature is used by pathologists to diagnose malignancy[117,118]. This absence of basal cells suggests that cancer relapse more likely involves particular subsets of luminal cells that exhibit both castration tolerance and stem and/or progenitor properties. In mice, CARNs[25] and CARBs[58] have been proposed as potential candidates. However, these luminal cells are defined based on a single marker (*Nkx3.1* and *Bmi1*, respectively), making any correlation with human counterparts elusive. In patient-derived tumour xenografts (PDXs) of treatment-naive prostatic tumours, a luminal-like (CK8^{+}, CK18^{+}) population of castration-tolerant cells expressing stem-like markers (CD44, NANOG and ALDH1) was also shown to survive androgen withdrawal and to regenerate tumours upon restoration of androgens[119]. Their actual identity beyond these few markers remains undetermined.

In contrast to the above-mentioned cells, mouse LSC^{med}-like luminal progenitor cells have been exhaustively profiled, and their proximity to human prostate

Club and Hillock cells has been assessed based on similar transcriptomic profiles (see Single-cell profiling and FIG. 2e). The intrinsic castration-tolerant, stem-like and tumorigenic properties of mouse LSC^{med}-like luminal progenitor cells makes them relevant candidates as CPRC drivers. Importantly, one study reported similar properties for human CD38^{low} luminal progenitor cells and suggested their association with human prostate cancer progression and poor outcome[53]. In *Pten*-null mice, although prostates shrunk after castration as expected[55,58], LSC^{med} cells remained highly prevalent and large clusters of CK4$^+$/Ki67$^+$ cells were detected in regressing prostates, indicating that some LSC^{med} cells not only survive castration, but also proliferate[31]. In agreement, prostate tumours originating from *Sca1*$^+$ luminal progenitor cells are castration-resistant[55]. Finally, the formation of organoids from *Sca1*$^+$ luminal progenitor cells was unaffected by treatment with enzalutamide[30], a second-generation anti-androgen drug efficient at the CRPC stage of the disease[120]. Together, these data suggest that luminal progenitor cells could actively contribute to cancer progression towards CRPC.

Neuroendocrine prostate cancer. Although second-generation anti-androgen therapies (abiraterone, enzalutamide, apalutamide and darolutamide) increase survival of patients with CRPC, and are even now proposed at the hormone-sensitive stage of the disease, their success has been hampered by the emergence of new mechanisms of resistance[121–125]. Prolonged anti-androgen therapy has been shown to promote cellular plasticity, leading to dedifferentiation of prostate tumour cells through epithelial-to-mesenchymal transition, neurodifferentiation and increased stem-like features[126]. Neuroendocrine prostate cancer (NEPC) is an aggressive variant of CRPC that is diagnosed by the expression of neuroendocrine markers (SYP and CgA) in the evolution of up to one-quarter of patients with CRPC[126]. NEPCs exhibit only a few genetic alterations (*TP53*, *MYCN* and *RB1*), suggesting that cancer cell reprogramming to a neuroendocrine state is mainly driven by epigenetic dysregulation and reactivation of developmental processes[126]. Overexpression of the epigenetic modifier enhancer of zeste homologue 2 (*EZH2*) and of the transcription factor *SOX2* was shown to contribute to lineage plasticity enabled by *TP53* and *RB1* loss of function promoting NEPC[126–128]. Remarkably, mouse *Sca1*$^+$ luminal progenitor cells express high levels of *Sox2*, and tumours derived from these cells are more prone to become NEPC after castration than tumours derived from *Sca1*^{neg} luminal cells, suggesting the functional contribution of luminal progenitor cells to this phenotypic shift[55]. In keeping with these findings, NEPC in *Pten*-null mice arises specifically in the proximal prostate, that is, where luminal progenitor cells[30–32,35,36,43,44,55] as well as castration-resistant *Sox2*$^+$ cells[55,66] predominantly reside. Moreover, slow-cycling luminal progenitor cells, also mainly located in proximal prostate, were shown to be enriched in neurogenesis Gene Ontology functions[54]. *Sox2* is mandatory, but not sufficient, for castration-induced neurodifferentiation of prostate adenocarcinomas derived from *Sca1*$^+$ luminal progenitor cells[55].

Relevance of luminal progenitor hallmarks in prostate cancer progression. Like their mouse *Sca1*$^+$ counterparts, human prostate luminal progenitor cells (*TACSTD2*$^+$) express high levels of *SOX2* and are prone to neuroendocrine differentiation in tumour organoids induced by AKT1 and MYC activation[129]. Knocking down *TACSTD2* and *SOX2* expression has been shown to attenuate this property[129]. In human prostate cancer specimens, expression of *TACSTD2* is associated with biochemical recurrence, and its gene product TROP2 was shown to be a driver of metastatic prostate cancer with neuroendocrine phenotype through a circuitry involving upregulation of PARP1 expression[130]. *SOX2* expression was also more elevated in NEPC tumours[127]. By contrast, CD38 expression was the lowest in NEPC compared with adeno-CRPC and localized prostate cancer. Low CD38 expression marks inflammation-associated human prostate luminal progenitor cells[53]. Of interest, lower CD38 expression in NEPC was inversely correlated with promoter methylation[131], suggesting another level of epigenetic regulation of luminal progenitor biology. Finally, *PSCA* expressed by circulating tumour cells was identified as one of eight markers significantly associated with resistance of metastatic prostate cancer to second-generation anti-androgens[132].

Together, these observations suggest that prostatic luminal progenitor cells contribute to CRPC and are intrinsically inclined to promote further progression towards NEPC. Their plasticity might be enhanced by prolonged ADT or earlier addition of second-generation anti-androgen therapies as *SOX2* is an androgen-repressed gene whose expression was shown to be upregulated by androgen deprivation in LNCaP human prostate cancer cells[58].

Conclusion

Luminal progenitor cells are emerging as potentially important contributors to prostate pathophysiology (FIG. 3). Various preclinical models have provided evidence that these cells are amplified in, and could contribute to, several contexts affecting prostate health, such as ageing, inflammation, BPH and cancer. Lowering androgen signalling in situ (5ARIs) or systemically (ADT) is the primary medical strategy for treating patients with large BPH and advanced prostate cancer, respectively. Prostatic luminal progenitor cells are more resistant to androgen deprivation than most mature luminal cells, which suggests that they could contribute to the resistance to these treatments and, thus, promote disease progression. However, our understanding of the molecular mechanisms regulating luminal progenitor cells in these contexts remains extremely poor, as only a couple of candidate pathways have been documented in mouse models of human prostate diseases (FIG. 4).

The primary objective of this Review is to bridge the current knowledge on prostate luminal progenitor cells. One clear limitation in this perspective is the actual identity of cells referred to as luminal progenitor cells. These cells have been defined by authors based on various observations involving different functional approaches (lineage tracing, label-retaining capacity and

facultative stem assays), different phenotypic hallmarks (one or a few selected protein markers used in immunohistochemistry or immunofluorescence, cell-sorting profiles and scRNA-seq in silico clustering), different species (mouse and human) and, ultimately, different nomenclatures (TABLE 2). Bioinformatic approaches support the notion that LSC^{med} cells and luminal progenitor cells identified by scRNA-seq correspond to the same epithelial cell population in the mouse prostate. The transcriptomic signature of this cell population confirmed in several studies suggests that it represents a distinct epithelial cell entity in addition to other luminal and basal cell populations. However, functional stem cell assays demonstrate that only a few cells within this luminal progenitor population exhibit progenitor properties. Determining the molecular identity of these particular stem-like cells is one of the future challenges as they might constitute therapeutic targets in diseased prostate. Not only does the identification of cognate Club and Hillock cells in the human prostate consolidate the translational relevance of past and future discoveries made in preclinical models but it should also contribute to elucidating their pathobiology. One missing piece of the puzzle is whether prostate Club and/or Hillock cells exhibit progenitor properties, as shown for their mouse prostate counterparts.

Despite the rapidly evolving knowledge regarding LSC^{med}-like luminal progenitor cells, several questions remain to be elucidated. First, their embryonic origin — prostatic versus urethral — is a matter of debate. Second, although their progenitor properties have been mainly demonstrated in facultative assays, evidence that these properties are maintained in the adult prostate in situ need to be further ascertained. Third, whether proximal (frequent) and distal (rare) LSC^{med}-like cells represent distinct cell subsets that might respond to different rules (for example, embryonic origin, stemness and/or castration-tolerance), and whether this correlates with various prostate anatomy-associated cellular processes (such as locoregional regeneration and zonal incidence of disease) is currently unknown. Finally, in light of the evidence that various mechanisms involving different cell types seem to cooperate during prostate regeneration, the balance between luminal progenitor-dependent and progenitor-independent processes promoting prostate diseases needs to be carefully investigated.

Published online 25 January 2022

1. Devlin, C. M., Simms, M. S. & Maitland, N. J. Benign prostatic hyperplasia — what do we know? *BJU Int.* **127**, 389–399 (2021).
2. Strand, D. W., Costa, D. N., Francis, F., Ricke, W. A. & Roehrborn, C. G. Targeting phenotypic heterogeneity in benign prostatic hyperplasia. *Differentiation* **96**, 49–61 (2017).
3. Lee, S. W. H., Chan, E. M. C. & Lai, Y. K. The global burden of lower urinary tract symptoms suggestive of benign prostatic hyperplasia: a systematic review and meta-analysis. *Sci. Rep.* **7**, 7984 (2017).
4. Bell, K. J., Del Mar, C., Wright, G., Dickinson, J. & Glasziou, P. Prevalence of incidental prostate cancer: a systematic review of autopsy studies. *Int. J. Cancer* **137**, 1749–1757 (2015).
5. Gandaglia, G. et al. Epidemiology and prevention of prostate cancer. *Eur. Urol. Oncol.* **4**, 877–892 (2021).
6. Neal, D. E. et al. Ten-year mortality, disease progression, and treatment-related side effects in men with localised prostate cancer from the ProtecT randomised controlled trial according to treatment received. *Eur. Urol.* **77**, 320–330 (2020).
7. Cunha, G. R., Hayward, S. W. & Wang, Y. Z. Role of stroma in carcinogenesis of the prostate. *Differentiation* **70**, 473–485 (2002).
8. Hayward, S. W., Rosen, M. A. & Cunha, G. R. Stromal-epithelial interactions in the normal and neoplastic prostate. *Br. J. Urol.* **79** (Suppl. 2), 18–26 (1997).
9. Cunha, G. R., Donjacour, A. A. & Sugimura, Y. Stromal-epithelial interactions and heterogeneity of proliferative activity within the prostate. *Biochem. Cell Biol.* **64**, 608–614 (1986).
10. Abate-Shen, C. & Shen, M. M. Molecular genetics of prostate cancer. *Genes Dev.* **14**, 2410–2434 (2000).
11. Zhang, D., Zhao, S., Li, X., Kirk, J. S. & Tang, D. G. Prostate luminal progenitor cells in development and cancer. *Trends Cancer* **4**, 769–783 (2018).
12. Li, J. J. & Shen, M. M. Prostate stem cells and cancer stem cells. *Cold Spring Harb. Perspect. Med.* **9**, a030395 (2019).
13. Rycaj, K. & Tang, D. G. Cell-of-origin of cancer versus cancer stem cells: assays and interpretations. *Cancer Res.* **75**, 4003–4011 (2015).
14. Ittmann, M. Anatomy and histology of the human and murine prostate. *Cold Spring Harb. Perspect. Med.* **8**, a030346 (2018).
15. Roy-Burman, P., Wu, H., Powell, W. C., Hagenkord, J. & Cohen, M. B. Genetically defined mouse models that mimic natural aspects of human prostate cancer development. *Endocr. Relat. Cancer* **11**, 225–254 (2004).
16. Shappell, S. B. et al. Prostate pathology of genetically engineered mice: definitions and classification. The consensus report from the Bar Harbor meeting of the Mouse Models of Human Cancer Consortium Prostate Pathology Committee. *Cancer Res.* **64**, 2270–2305 (2004).
17. Bruchovsky, N., Lesser, B., Van Doorn, E. & Craven, S. Hormonal effects on cell proliferation in rat prostate. *Vitam. Horm.* **33**, 61–102 (1975).
18. English, H. F., Santen, R. J. & Isaacs, J. T. Response of glandular versus basal rat ventral prostatic epithelial cells to androgen withdrawal and replacement. *Prostate* **11**, 229–242 (1987).
19. Wang, Y., Hayward, S., Cao, M., Thayer, K. & Cunha, G. Cell differentiation lineage in the prostate. *Differentiation* **68**, 270–279 (2001).
20. Taylor, R. A., Toivanen, R. & Risbridger, G. P. Stem cells in prostate cancer: treating the root of the problem. *Endocr. Relat. Cancer* **17**, R273–R285 (2010).
21. Goldstein, A. S., Stoyanova, T. & Witte, O. N. Primitive origins of prostate cancer: in vivo evidence for prostate-regenerating cells and prostate cancer-initiating cells. *Mol. Oncol.* **4**, 385–396 (2010).
22. Lawson, D. A., Xin, L., Lukacs, R. U., Cheng, D. & Witte, O. N. Isolation and functional characterization of murine prostate stem cells. *Proc. Natl Acad. Sci. USA* **104**, 181–186 (2007).
23. Evans, G. S. & Chandler, J. A. Cell proliferation studies in rat prostate. I. The proliferative role of basal and secretory epithelial cells during normal growth. *Prostate* **10**, 163–178 (1987).
24. Evans, G. S. & Chandler, J. A. Cell proliferation studies in the rat prostate: II. The effects of castration and androgen-induced regeneration upon basal and secretory cell proliferation. *Prostate* **11**, 339–351 (1987).
25. Wang, X. et al. A luminal epithelial stem cell that is a cell of origin for prostate cancer. *Nature* **461**, 495–500 (2009).
26. Choi, N., Zhang, B., Zhang, L., Ittmann, M. & Xin, L. Adult murine prostate basal and luminal cells are self-sustained lineages that can both serve as targets for prostate cancer initiation. *Cancer Cell* **21**, 253–265 (2012).
27. Liu, J. et al. Regenerated luminal epithelial cells are derived from preexisting luminal epithelial cells in adult mouse prostate. *Mol. Endocrinol.* **25**, 1849–1857 (2011).
28. Lawson, D. A. et al. Basal epithelial stem cells are efficient targets for prostate cancer initiation. *Proc. Natl Acad. Sci. USA* **107**, 2610–2615 (2010).
29. Sackmann-Sala, L. et al. Prolactin-induced prostate tumorigenesis links sustained stat5 signaling with the amplification of basal/stem cells and emergence of putative luminal progenitors. *Am. J. Pathol.* **184**, 3105–3119 (2014).
30. Kwon, O. J., Zhang, L. & Xin, L. Stem cell antigen-1 identifies a distinct androgen-independent murine prostatic luminal cell lineage with bipotent potential. *Stem Cell* **34**, 191–202 (2016).
31. Sackmann Sala, L. et al. A rare castration-resistant progenitor cell population is highly enriched in Pten-null prostate tumors. *J. Pathol.* **243**, 54–64 (2017).
32. Guo, W. et al. Single-cell transcriptomics identifies a distinct luminal progenitor cell type in distal prostate invagination tips. *Nat. Genet.* **52**, 908–918 (2020).
33. Karthaus, W. R. et al. Regenerative potential of prostate luminal cells revealed by single-cell analysis. *Science* **368**, 497–505 (2020).
34. Crowley, L. et al. A single-cell atlas of the mouse and human prostate reveals heterogeneity and conservation of epithelial progenitors. *eLife* https://doi.org/10.7554/eLife.59465 (2020).
35. Mevel, R. et al. RUNX1 marks a luminal castration-resistant lineage established at the onset of prostate development. *eLife* **9**, e59465. (2020).
36. Joseph, D. B. et al. Urethral luminal epithelia are castration-insensitive cells of the proximal prostate. *Prostate* **80**, 872–884 (2020).
37. Joseph, D. B., Turco, A. E., Vezina, C. M. & Strand, D. W. Progenitors in prostate development and disease. *Dev. Biol.* **473**, 50–58 (2021).
38. Freeland, J., Crowell, P. D., Giafaglione, J. M., Boutros, P. C. & Goldstein, A. S. Aging of the progenitor cells that initiate prostate cancer. *Cancer Lett.* **515**, 28–35 (2021).
39. Lukacs, R. U., Goldstein, A. S., Lawson, D. A., Cheng, D. & Witte, O. N. Isolation, cultivation and characterization of adult murine prostate stem cells. *Nat. Protoc.* **5**, 702–713 (2010).
40. Mulholland, D. J. et al. Lin$^-$Sca-1$^+$CD49f^{high} stem/progenitors are tumor-initiating cells in the *Pten*-null prostate cancer model. *Cancer Res.* **69**, 8555–8562 (2009).
41. Wang, Z. A. et al. Lineage analysis of basal epithelial cells reveals their unexpected plasticity and supports a cell-of-origin model for prostate cancer heterogeneity. *Nat. Cell Biol.* **15**, 274–283 (2013).
42. Goffin, V., Hoang, D. T., Bogorad, R. L. & Nevalainen, M. T. Prolactin regulation of the prostate gland: a female player in a male game. *Nat. Rev. Urol.* **8**, 597–607 (2011).
43. Crowell, P. D. et al. Expansion of luminal progenitor cells in the aging mouse and human prostate. *Cell Rep.* **28**, 1499–1510.e6 (2019).
44. Kwon, O. J. et al. The Sca-1$^+$ and Sca-1$^-$ mouse prostatic luminal cell lineages are independently sustained. *Stem Cell* **38**, 1479–1491 (2020).

45. Tsujimura, A. et al. Proximal location of mouse prostate epithelial stem cells: a model of prostatic homeostasis. *J. Cell Biol.* **157**, 1257–1265 (2002).
46. Lawson, D. A. & Witte, O. N. Stem cells in prostate cancer initiation and progression. *J. Clin. Invest.* **117**, 2044–2050 (2007).
47. Burger, P. E. et al. Sca-1 expression identifies stem cells in the proximal region of prostatic ducts with high capacity to reconstitute prostatic tissue. *Proc. Natl Acad. Sci. USA* **102**, 7180–7185 (2005).
48. Goldstein, A. S. et al. Trop2 identifies a subpopulation of murine and human prostate basal cells with stem cell characteristics. *Proc. Natl Acad. Sci. USA* **105**, 20882–20887 (2008).
49. Shen, M. M. & Abate-Shen, C. Molecular genetics of prostate cancer: new prospects for old challenges. *Genes Dev.* **24**, 1967–2000 (2010).
50. Puig Lombardi, E., Baures, M., Dariane, C., Guidotti, J.-E. & Goffin, V. In silico computed clusters of prostate luminal progenitors match FACS-Enriched LSCmed cells. Preprint at *bioRxiv* https://doi.org/10.1101/2021.06.16.448624 (2021).
51. Henry, G. H. et al. A cellular anatomy of the normal adult human prostate and prostatic urethra. *Cell Rep.* **25**, 3530–3542.e5 (2018).
52. Hu, W. Y. et al. Isolation and functional interrogation of adult human prostate epithelial stem cells at single cell resolution. *Stem Cell Res.* **23**, 1–12 (2017).
53. Liu, X. et al. Low CD38 identifies progenitor-like inflammation-associated luminal cells that can initiate human prostate cancer and predict poor outcome. *Cell Rep.* **17**, 2596–2606 (2016).
54. Zhang, D. et al. Histone 2B-GFP label-retaining prostate luminal cells possess progenitor cell properties and are intrinsically resistant to castration. *Stem Cell Rep.* **10**, 228–242 (2018).
55. Kwon, O. J., Zhang, L., Jia, D. & Xin, L. Sox2 is necessary for androgen ablation-induced neuroendocrine differentiation from *Pten* null Sca-1^{+} prostate luminal cells. *Oncogene* **40**, 203–214 (2021).
56. Sugimura, Y., Cunha, G. R., Donjacour, A. A., Bigsby, R. M. & Brody, J. R. Whole-mount autoradiography study of DNA synthetic activity during postnatal development and androgen-induced regeneration in the mouse prostate. *Biol. Reprod.* **34**, 985–995 (1986).
57. Yoo, Y. A. et al. Bmi1 marks distinct castration-resistant luminal progenitor cells competent for prostate regeneration and tumour initiation. *Nat. Commun.* **7**, 12943 (2016).
58. Yoo, Y. A. et al. The role of castration-resistant Bmi1+Sox2+ cells in driving recurrence in prostate cancer. *J. Natl Cancer Inst.* **111**, 311–321 (2019).
59. Henry, G. H. & Strand, D. W. Strand Lab analysis of single-cell RNA sequencing. *Zenodo* https://doi.org/10.5281/zenodo.3687064 (2020).
60. Leong, K. G., Wang, B. E., Johnson, L. & Gao, W. Q. Generation of a prostate from a single adult stem cell. *Nature* **456**, 804–808 (2008).
61. Richardson, G. D. et al. CD133, a novel marker for human prostatic epithelial stem cells. *J. Cell Sci.* **117**, 3539–3545 (2004).
62. Agarwal, S. et al. Identification of different classes of luminal progenitor cells within prostate tumors. *Cell Rep.* **13**, 2147–2158 (2015).
63. Wang, L. et al. Expansion of prostate epithelial progenitor cells after inflammation of the mouse prostate. *Am. J. Physiol. Renal Physiol.* **308**, F1421–F1430 (2015).
64. Wang, B. E. et al. Castration-resistant *Lgr5*$^{+}$ cells are long-lived stem cells required for prostatic regeneration. *Stem Cell Rep.* **4**, 768–779 (2015).
65. Barros-Silva, J. D. et al. Single-cell analysis identifies LY6D as a marker linking castration-resistant prostate luminal cells to prostate progenitors and cancer. *Cell Rep.* **25**, 3504–3518.e6 (2018).
66. McAuley, E. et al. Sox2 expression marks castration-resistant progenitor cells in the adult murine prostate. *Stem Cell* **37**, 690–700 (2019).
67. Korsten, H., Ziel-van der Made, A., Ma, X., van der Kwast, T. & Trapman, J. Accumulating progenitor cells in the luminal epithelial cell layer are candidate tumor initiating cells in a Pten knockout mouse prostate cancer model. *PLoS ONE* **4**, e5662 (2009).
68. Xin, L., Lawson, D. A. & Witte, O. N. The Sca-1 cell surface marker enriches for a prostate-regenerating cell subpopulation that can initiate prostate tumorigenesis. *Proc. Natl Acad. Sci. USA* **102**, 6942–6947 (2005).
69. Wei, X. et al. Spatially restricted stromal Wnt signaling restrains prostate epithelial progenitor growth through direct and indirect mechanisms. *Cell Stem Cell* **24**, 753–768.e6 (2019).
70. Ousset, M. et al. Multipotent and unipotent progenitors contribute to prostate postnatal development. *Nat. Cell Biol.* **14**, 1131–1138 (2012).
71. Wang, J. et al. Symmetrical and asymmetrical division analysis provides evidence for a hierarchy of prostate epithelial cell lineages. *Nat. Commun.* **5**, 4758 (2014).
72. Wuidart, A. et al. Quantitative lineage tracing strategies to resolve multipotency in tissue-specific stem cells. *Genes Dev.* **30**, 1261–1277 (2016).
73. Tika, E., Ousset, M., Dannau, A. & Blanpain, C. Spatiotemporal regulation of multipotency during prostate development. *Development* **146**, dev180224 (2019).
74. Shibata, M., Epsi, N. J., Xuan, S., Mitrofanova, A. & Shen, M. M. Bipotent progenitors do not require androgen receptor for luminal specification during prostate organogenesis. *Stem Cell Rep.* **15**, 1026–1036 (2020).
75. Ceder, J. A., Aalders, T. W. & Schalken, J. A. Label retention and stem cell marker expression in the developing and adult prostate identifies basal and luminal epithelial stem cell subpopulations. *Stem Cell Res. Ther.* **8**, 95 (2017).
76. Moad, M. et al. Multipotent basal stem cells, maintained in localized proximal niches, support directed long-ranging epithelial flows in human prostates. *Cell Rep.* **20**, 1609–1622 (2017).
77. Treutlein, B. et al. Reconstructing lineage hierarchies of the distal lung epithelium using single-cell RNA-seq. *Nature* **509**, 371–375 (2014).
78. Rawlins, E. L. et al. The role of Scgb1a1^{+} Clara cells in the long-term maintenance and repair of lung airway, but not alveolar, epithelium. *Cell Stem Cell* **4**, 525–534 (2009).
79. Karthaus, W. R. et al. Identification of multipotent luminal progenitor cells in human prostate organoid cultures. *Cell* **159**, 163–175 (2014).
80. Chua, C. W. et al. Single luminal epithelial progenitors can generate prostate organoids in culture. *Nat. Cell Biol.* **16**, 951–954 (2014).
81. Pignon, J. C. et al. Cell kinetic studies fail to identify sequentially proliferating progenitors as the major source of epithelial renewal in the adult murine prostate. *PLoS ONE* **10**, e0128489 (2015).
82. Shivdasani, R. A., Clevers, H. & de Sauvage, F. J. Tissue regeneration: reserve or reverse? *Science* **371**, 784–786 (2021).
83. Brennen, W. N. & Isaacs, J. T. Mesenchymal stem cells and the embryonic reawakening theory of BPH. *Nat. Rev. Urol.* **15**, 703–715 (2018).
84. Claus, S., Wrenger, M., Senge, T. & Schulze, H. Immunohistochemical determination of age related proliferation rates in normal and benign hyperplastic human prostates. *Urol. Res.* **21**, 305–308 (1993).
85. Izumi, K., Li, L. & Chang, C. Androgen receptor and immune inflammation in benign prostatic hyperplasia and prostate cancer. *Clin. Investig.* **4**, 935–950 (2014).
86. Roehrborn, C. G. et al. The effects of combination therapy with dutasteride and tamsulosin on clinical outcomes in men with symptomatic benign prostatic hyperplasia: 4-year results from the CombAT study. *Eur. Urol.* **57**, 123–131 (2010).
87. McConnell, J. D. et al. The long-term effect of doxazosin, finasteride, and combination therapy on the clinical progression of benign prostatic hyperplasia. *N. Engl. J. Med.* **349**, 2387–2398 (2003).
88. Cornu, J. N. et al. A systematic review and meta-analysis of functional outcomes and complications following transurethral procedures for lower urinary tract symptoms resulting from benign prostatic obstruction: an update. *Eur. Urol.* **67**, 1066–1096 (2015).
89. Kindblom, J. et al. Prostate hyperplasia in a transgenic mouse with prostate-specific expression of prolactin. *Endocrinology* **144**, 2269–2278 (2003).
90. Nevalainen, M. T. et al. Prolactin and prolactin receptors are expressed and functioning in human prostate. *J. Clin. Invest.* **99**, 618–627 (1997).
91. Pigat, N. et al. Combined *Sabal* and *Urtica* extracts (WS® 1541) exert anti-proliferative and anti-inflammatory effects in a mouse model of benign prostate hyperplasia. *Front. Pharmacol.* **10**, 311 (2019).
92. Bernichtein, S. et al. Anti-inflammatory properties of Lipidosterolic extract of Serenoa repens (Permixon®) in a mouse model of prostate hyperplasia. *Prostate* **75**, 706–722 (2015).
93. Lai, K. P. et al. Targeting stromal androgen receptor suppresses prolactin-driven benign prostatic hyperplasia (BPH). *Mol. Endocrinol.* **27**, 1617–1631 (2013).
94. Isaacs, J. T. & Coffey, D. S. Etiology and disease process of benign prostatic hyperplasia. *Prostate Suppl.* **2**, 33–50 (1989).
95. Kwon, O. J., Zhang, L., Ittmann, M. M. & Xin, L. Prostatic inflammation enhances basal-to-luminal differentiation and accelerates initiation of prostate cancer with a basal cell origin. *Proc. Natl Acad. Sci. USA* **111**, E592–E600 (2014).
96. Schalken, J. A. Inflammation in the pathophysiology of benign prostatic hypertrophy. *Eur. Urol. Suppl.* **14**, e1455–e1458 (2015).
97. McNeal, J. E. Origin and evolution of benign prostatic enlargement. *Invest. Urol.* **15**, 340–345 (1978).
98. De Nunzio, C., Presicce, F. & Tubaro, A. Inflammatory mediators in the development and progression of benign prostatic hyperplasia. *Nat. Rev. Urol.* **13**, 613–626 (2016).
99. Bushman, W. A. & Jerde, T. J. The role of prostate inflammation and fibrosis in lower urinary tract symptoms. *Am. J. Physiol. Renal Physiol.* **311**, F817–F821 (2016).
100. Wang, H. H. et al. Characterization of autoimmune inflammation induced prostate stem cell expansion. *Prostate* **75**, 1620–1631 (2015).
101. Zhang, B. et al. Non-cell-autonomous regulation of prostate epithelial homeostasis by androgen receptor. *Mol. Cell* **63**, 976–989 (2016).
102. Yu, Y. et al. Mesenchymal stem cells recruited by castration-induced inflammation activation accelerate prostate cancer hormone resistance via chemokine ligand 5 secretion. *Stem Cell Res. Ther.* **9**, 242 (2018).
103. Shi, X., Gipp, J., Dries, M. & Bushman, W. Prostate progenitor cells proliferate in response to castration. *Stem Cell Res.* **13**, 154–163 (2014).
104. Spehar, K., Pan, A. & Beerman, I. Restoring aged stem cell functionality: current progress and future directions. *Stem Cell* **38**, 1060–1077 (2020).
105. Stoyanova, T. et al. Prostate cancer originating in basal cells progresses to adenocarcinoma propagated by luminal-like cells. *Proc. Natl Acad. Sci. USA* **110**, 20111–20116 (2013).
106. Goldstein, A. S. et al. Purification and direct transformation of epithelial progenitor cells from primary human prostate. *Nat. Protoc.* **6**, 656–667 (2011).
107. Wang, Z. A., Toivanen, R., Bergren, S. K., Chambon, P. & Shen, M. M. Luminal cells are favored as the cell of origin for prostate cancer. *Cell Rep.* **8**, 1339–1346 (2014).
108. Goldstein, A. S., Huang, J., Guo, C., Garraway, I. P. & Witte, O. N. Identification of a cell of origin for human prostate cancer. *Science* **329**, 568–571 (2010).
109. Lu, T. L. et al. Conditionally ablated Pten in prostate basal cells promotes basal-to-luminal differentiation and causes invasive prostate cancer in mice. *Am. J. Pathol.* **182**, 975–991 (2013).
110. Taylor, B. S. et al. Integrative genomic profiling of human prostate cancer. *Cancer Cell* **18**, 11–22 (2010).
111. Wang, S. et al. Prostate-specific deletion of the murine Pten tumor suppressor gene leads to metastatic prostate cancer. *Cancer Cell* **4**, 209–221 (2003).
112. Parisotto, M. et al. PTEN deletion in luminal cells of mature prostate induces replication stress and senescence in vivo. *J. Exp. Med.* **215**, 1749–1763 (2018).
113. Ratnacaram, C. K. et al. Temporally controlled ablation of PTEN in adult mouse prostate epithelium generates a model of invasive prostatic adenocarcinoma. *Proc. Natl Acad. Sci. USA* **105**, 2521–2526 (2008).
114. Mulholland, D. J. et al. Cell autonomous role of PTEN in regulating castration-resistant prostate cancer growth. *Cancer Cell* **19**, 792–804 (2011).
115. Zhang, J. et al. Proteomic and transcriptomic profiling of Pten gene-knockout mouse model of prostate cancer. *Prostate* **80**, 588–605 (2020).
116. Abu El Maaty, M. A. et al. Single-cell analyses unravel cell type-specific responses to a vitamin D analog in prostatic precancerous lesions. *Sci. Adv.* **7**, eabg5982 (2021).
117. Humphrey, P. A. Diagnosis of adenocarcinoma in prostate needle biopsy tissue. *J. Clin. Pathol.* **60**, 35–42 (2007).
118. Shah, R. B., Zhou, M., LeBlanc, M., Snyder, M. & Rubin, M. A. Comparison of the basal cell-specific markers, 34βE12 and p63, in the diagnosis of prostate cancer. *Am. J. Surg. Pathol.* **26**, 1161–1168 (2002).
119. Toivanen, R. et al. A preclinical xenograft model identifies castration-tolerant cancer-repopulating cells

in localized prostate tumors. *Sci. Transl. Med.* **5**, 187ra171 (2013).
120. Scher, H. I. et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. *N. Engl. J. Med.* **367**, 1187–1197 (2012).
121. Ryan, C. J. et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. *N. Engl. J. Med.* **368**, 138–148 (2013).
122. Fizazi, K. et al. Abiraterone plus prednisone in metastatic, castration-sensitive prostate cancer. *N. Engl. J. Med.* **377**, 352–360 (2017).
123. Antonarakis, E. S. et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *N. Engl. J. Med.* **371**, 1028–1038 (2014).
124. Fizazi, K. et al. Nonmetastatic, castration-resistant prostate cancer and survival with darolutamide. *N. Engl. J. Med.* **383**, 1040–1049 (2020).
125. Smith, M. R. et al. Apalutamide treatment and metastasis-free survival in prostate cancer. *N. Engl. J. Med.* **378**, 1408–1418 (2018).
126. Davies, A. H., Beltran, H. & Zoubeidi, A. Cellular plasticity and the neuroendocrine phenotype in prostate cancer. *Nat. Rev. Urol.* **15**, 271–286 (2018).
127. Mu, P. et al. SOX2 promotes lineage plasticity and antiandrogen resistance in TP53- and RB1-deficient prostate cancer. *Science* **355**, 84–88 (2017).
128. Ku, S. Y. et al. Rb1 and Trp53 cooperate to suppress prostate cancer lineage plasticity, metastasis, and antiandrogen resistance. *Science* **355**, 78–83 (2017).
129. Kwon, O. J. et al. De novo induction of lineage plasticity from human prostate luminal epithelial cells by activated AKT1 and c-Myc. *Oncogene* **39**, 7142–7151 (2020).
130. Hsu, E. C. et al. Trop2 is a driver of metastatic prostate cancer with neuroendocrine phenotype via PARP1. *Proc. Natl Acad. Sci. USA* **117**, 2032–2042 (2020).
131. Mottahedeh, J. et al. CD38 is methylated in prostate cancer and regulates extracellular NAD^+. *Cancer Metab.* **6**, 13 (2018).
132. Chung, J. S. et al. Circulating tumor cell-based molecular classifier for predicting resistance to abiraterone and enzalutamide in metastatic castration-resistant prostate cancer. *Neoplasia* **21**, 802–809 (2019).
133. Blanpain, C. & Simons, B. D. Unravelling stem cell dynamics by lineage tracing. *Nat. Rev. Mol. Cell Biol.* **14**, 489–502 (2013).
134. Gao, D. et al. Organoid cultures derived from patients with advanced prostate cancer. *Cell* **159**, 176–187 (2014).

Acknowledgements
V.G. and J.-E.G. are grateful to L. Sackmann Sala for her pioneering work that led to the discovery of LSC^{med} cells. The authors thank the following sources of financial support: Ligue contre le cancer (RS16/75-18, RS17/75-1, RS18/75-48, RS19/75-63, RS20/75-93 and RS21 /75-35), FONCER contre le cancer, Association pour la recherche sur les tumeurs de la prostate (ARTP), Cancéropôle Ile-de-France and Institut National du Cancer (INCa_6672), Inserm and the Université de Paris. M.B. is supported by a fellowship from the Ministry of Research, C.D. by research/mobility fellowships from Inserm, the Association Française d'Urologie and Assistance Publique Hôpitaux de Paris (APHP), and E.T. by a Fonds de la Recherche Scientific (FNRS) fellowship. C.B. is an investigator of WELBIO.

Author contributions
V.G., M.B., C.D., E.P.L. and J.-E.G. researched data for the article. V.G., M.B., C.D., E.T., E.P.L., C.B. and J.-E.G. wrote the article. V.G., M.B., C.D., N.B.D., C.B. and J.-E.G. reviewed/edited the manuscript before submission. All authors made a substantial contribution to discussion of content.

Competing interests
C.D. is a consultant for Janssen, Astellas and Bayer. C.B. is a consultant for Genentech, Nestlé and Chromacure. N.B.D. is a consultant for Koelis and Affluent Medical. The other authors declare no competing interests.

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Review criteria
Most references were retrieved from our personal collection of references, and some were suggested by the peer reviewers. We also searched for original and review articles in PubMed using the following search terms, alone or in combination: prostate, progenitor, stem cells, luminal cells, neuroendocrine, CARN, CARB, Sox2, organoid, lineage-tracing, castration, treatment resistance, recurrence, 5α-reductase, cancer, benign prostate hyperplasia, inflammation, development, single cell, RNA-seq, and biomarkers. All papers identified were full text papers in English. We also searched the reference lists of identified articles for further relevant papers.