

Review Article

Prostate epithelial stem and progenitor cells

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Abstract: The classic androgen ablation and replacement experiment demonstrates that prostate epithelia possess extensive regenerative capacities and implies the existence of the prostate stem/progenitor cells. These cells may serve as the cells of origin for prostate cancer and their intrinsic property may dictate the clinical behaviors of the resulting diseases. Therefore, detailed characterization of these cells will potentially benefit disease prevention, diagnosis and prognosis. In this review, we describe several major *in vitro* and *in vivo* approaches that have been employed in the studies of the prostate stem cell activities, summarize the major progress that has been made during the last two decades regarding the identity of prostate stem/progenitor cells and their niches, and discuss some remaining outstanding questions in the field.

Keywords: Prostate stem cells, lineage hierarchy, prostate regeneration, lineage tracing, plasticity, niche

Introduction

The prostate is an exocrine gland that surrounds the urethra at the base of the bladder. The human prostate is organized as central, transitional and peripheral zones while the mouse prostate consists of dorsal, lateral, ventral, and anterior lobes. During embryonic development the prostatic epithelium is derived from the endodermal urogenital sinus (UGS). It is first visible by the presence of buds in the urogenital sinus epithelium at embryonic day 17.5 [1]. There are three types of epithelial cells in the prostate based on their morphology and intracellular antigens: the luminal cells that directly surround the lumen produce secretory proteins and express cytokeratin 8 (K8), K18, prostate specific antigen (PSA), and high levels of the androgen receptor (AR), the basal cells are aligned between the luminal cells and the basement membrane and express markers such as K5, K14, and P63 [2]. Neuroendocrine cells are very rare and secrete neurotrophic factors [3]. In addition, intermediate or transit-amplifying cells that express both the basal and luminal lineage markers are detectable during the developmental stage, under pathological conditions in adult, or when prostate

epithelial cells were cultured *in vitro* [4-9]. Somatic stem cells are defined as the cells that possess the capacities for multilineage differentiation and self-renewal, while progenitors are the more committed cells that may only generate one type or some types of the cells within an organ [10]. This review aims to present an overview of the current knowledge on prostate epithelial stem/progenitor cells, the assays and approaches that identify prostatic stem/progenitor cells as well as the prostate stem cell niche. The role of the stem/progenitors as the cells of origin for prostate cancer has been extensively reviewed elsewhere [11-14, 100] and is not the major topic of this review.

Evidence of stem and progenitor cells in the prostate

The concept of a prostate stem cell was originally conceived almost four decades ago [15]. Early studies demonstrated that adult murine prostate tissues are capable of undergoing numerous cycles of involution and regeneration in response to alternating androgen ablation and replacement, implying the existence of cells that possess the two essential features of

stem cells: self-renewal and multi-lineage differentiation. It has been hypothesized that prostate stem cells are localized in the basal cell layer because basal cells are independent of androgen for their survival and express many stem cell associated genes such as bcl-2, telomerase and p63 etc. [15-18]. On the other hand, the fact that not all luminal cells undergo androgen ablation-induced apoptosis implicates that some of the luminal cells may also possess the progenitor activity to sustain the luminal cell lineage.

Characterizing prostate stem/progenitor cells using *in vitro* and *ex vivo* functional assays

Early studies in prostate stem/progenitor cell biology have been hindered due to a lack of functional assays enabling quantitative measurement of the activity of prostate stem cells. Firstly, *in vitro* two-dimensional (2D) or three-dimensional (3D) culture systems are applied to identify multi-potential progenitor cells in murine or human prostate tissues [4, 5, 17, 19-22]. However, very few studies demonstrated that the cultured putative progenitor cells possess long-term self-renewal capacity and could be serially passaged efficiently [21].

In an effort to define murine prostate stem/progenitor cells, a dissociated prostate cell regeneration assay was developed based on a classic tissue fragment recombination assay [23-26]. By mechanism, this dissociated prostate cell regeneration assay is very similar to the hematopoietic reconstitution assay or the cleared fat pad assay for the mammary gland. Briefly, adult murine prostate tissues are mechanically and enzymatically dissociated into single cells. Dissociated single cells are combined with embryonic urogenital sinus mesenchymal (UGSM) cells and grafted under the kidney capsule of immunodeficient male host mice. UGSM cells play a critical inductive role for the morphogenesis of prostatic epithelial glandular structures during development [27, 28]. The exact mechanisms for this induction are unknown, but it has been speculated that androgen works directly on UGSM cells to induce the secretion of andromedins, which in turn stimulate the proliferation and differentiation of prostate stem cells to regenerate glandular structures *de novo* [29, 30]. Regenerated glandular structures are microscopically reminiscent of

adult murine prostate tissues. They are composed of a single layer of epithelial cells surrounding a lumen filled with protein secretions [24]. All three major epithelial cell types are detectable based on IHC staining for lineage markers [31-33]. When regeneration experiments were performed using a mixture of fluorescent protein-marked prostatic epithelial cells, all individual glandular structures were derived from cells of a single donor as indicated by glands of a single color. These data clearly demonstrate the existence of single cells within adult murine prostate epithelia that possess multi-lineage differentiation capacity [33]. These studies lay the basis for this method being used as an assay to measure prostate stem cell activity. Since single cells are used in this assay, it is possible to quantitatively compare the regenerative capacity of murine prostate cells from different genetic backgrounds or of different ages. Most importantly, prostate epithelial cells can be FACS fractionated into subpopulations based on their surface antigenic profiles and the regenerative capacities of these groups can be directly compared. This technique was the original process through which murine prostate stem cells were identified [31-33]. Prostate-regenerating cells also possess the capacity for self-renewal, another key feature of stem cells. Primary regenerated tissues can be serially passaged 2-3 times, but the size of the secondary and tertiary regenerated tissues decrease substantially though same numbers of the cells were grafted each time [34]. In an alternative approach, prostate cells from a transgenic mouse strain that expresses the luciferase transgene specifically in the prostate were used for regeneration. Bioluminescence imaging demonstrated that regenerated tissues underwent several cycles of involution and regeneration in response to deprivation and replacement of androgen stimulation [35].

Since using these *in vivo* methods to measure the self-renewal capacity of prostate stem cells is time-consuming and technically challenging, an *in vitro* prostate sphere assay was developed as a simplified surrogate assay [9]. The prostate sphere assay is very similar to the neurosphere and mammosphere assays used for the study of the neural and mammary gland stem cells [36-38]. In this assay, a small fraction of prostate cells are capable of forming

spheroids when cultured in 3D matrigel. When the prostate sphere assay was performed using a mixture of different fluorescent protein-marked prostate epithelial cells, all the formed spheres were monomeric demonstrating that they were derived clonally [9]. Finally, these spheroid structures can be serially passaged in bulk or individually. Overall, these data demonstrates that sphere-forming cells possess the self-renewal capacity that characterizes stem cells. It should be noted that prostate sphere cells in this assay are not functionally equivalent to stem cells since they rarely generate prostate glandular structures when stimulated by UGSM cells in the regeneration assay. This also raises the question whether the sphere-forming cells *in vitro* and the prostate-regenerating cells *in vivo* are exactly the same population. Two other independent groups have also reported similar but slightly technically different assays for the study of the prostate stem cells [39, 40]. In these studies, cultured prostate sphere cells are able to efficiently regenerate prostate glandular structures *in vivo* when stimulated by the UGSM cells.

With these assays established, major breakthroughs have been made towards the identification of murine and human prostate stem/progenitor cells by many independent groups during the past decades. The basic idea is to fractionate prostatic epithelial cells based on their surface antigenic profiles and then determine which fractions possess prostate stem cell activity using these functional assays. Independent studies from the laboratories of Owen Witte and Lynnette Wilson showed that Sca-1 enriches for the stem cell activity as measured by the *in vivo* prostate regeneration system and the *in vitro* prostate sphere assay [33, 41]. Subsequent studies showed that additional markers such as CD49f, Trop2 and CD166 can further enrich for the stem cell activity among the Sca-1⁺ cells [9, 31, 32, 42]. Leong et al reported successful regeneration of prostatic tissues from single murine prostate-derived cells that display a surface antigenic profile of Lin⁻Sca-1⁺CD44⁺CD133⁺CD117⁺ and a very recent study showed that slow cycling stem/progenitor cells that localized in the prostate ducts proximal to the urethra were enriched in this populations [43, 44]. All these FACS-sorted cells display a basal cell phenotype, corroborating the hypothesis that basal cells possess the

stem cell activity. Finally, other markers have also been utilized to characterize the stem cell activity in the prostate. For example, the side population cells that can efficiently efflux fluorescent vital dye Hoechst 33342 produced more spheroids and generated more ductal growth and gland numbers than those of the non-side population [45, 46]; expression of the cytoplasmic aldehyde dehydrogenase (ALDH) is enriched in a subset of stem/progenitor cells which also express other stem cell antigens like CD9, Bcl-2, CD200, CD24, CD133, Oct 3/4, ABCG2, and Nestin [47].

Similar conclusions were made in the human prostate. Goldstein et al showed by the prostate regeneration assay that the prostate stem cell activity is also enriched in the CD49f⁺Trop2⁺ human prostate basal cells [48, 49]. Interestingly, Garraway et al showed that the basal prostate cells that form prostate spheres may not be equivalent to the cells that regenerate tubular structures in the prostate regeneration assay [50]. They showed that Epcam⁺CD44⁺CD49f^{hi} cells are the tubule regenerating prostate stem cell population while Epcam⁺CD44⁺CD49f^{hi} cells are the sphere-forming cells [51]. CD133 is another marker that has been reported to enrich prostate stem cell activity. Richardson et al showed that when mixed with human prostatic stromal cells and incubated subcutaneously in immunodeficient host mice, integrin $\alpha 2\beta 1^{\text{high}}$ CD133⁺ cells from human prostate tissues can reconstitute prostatic-like acini [52]. Two separate studies showed that CD133⁺ murine and human prostate epithelial cells can give rise to branching structures *in vitro* and regenerate stratified human prostate glands *in vivo*, respectively [53, 54]. However, Yamamoto et al showed that CD133 may not serve as a marker for human prostate stem cells [55].

Characterizing prostate stem cells using the *in vivo* lineage tracing approach

A major limitation of the dissociated prostate regeneration assay is that prostate cells were taken out of their native microenvironment and stimulated by the embryonically derived UGSM cells that have strong reprogramming capacity [56, 57]. It was not clear if the regenerative capacity that basal cells displayed in this assay contributes to the maintenance of prostate epi-

thelia substantially *in vivo*. One way to overcome this limitation is to specifically label basal cells or luminal cells with fluorescence proteins and then track the fates of the labeled cells *in vivo* in their native microenvironment. This approach has been utilized by several groups including ours during the last few years to investigate the prostate epithelial lineage hierarchy. Transgenic mice are generated so that they express the tamoxifen-responding CreER transgene driven by prostate lineage specific promoters. These mice were bred with the fluorescent reporter that can enable the expression of green or yellow fluorescent proteins upon tamoxifen induced Cre-LoxP mediated homologous recombination. Experimental mice were either aged or underwent induced-epithelial turnover by alternating androgen ablation and replacement. Subsequently, prostate tissues were examined by immunohistochemical analysis to determine the fate of the fluorescently labeled prostate basal cells or luminal cells [58-62].

The promoters that are utilized to label prostate basal cells are those of cytokeratin 5 and 14, while the promoters of cytokeratin 8, 18 and prostate stem antigen were utilized to label prostate luminal cells [58-63]. In these studies prostate basal cells or luminal cells in adult mice were labeled with EYFP or EGFP. These studies have reached the consensus that prostate basal and luminal cells are mostly independently sustained in adult mice. However, minor discrepancies exist among these studies. For examples, two of the studies showed that an extremely small percentage of prostate basal cells are able to generate luminal cells during lineage tracing [60, 61]. This difference could be due to the different choices of the promoters in these studies. Alternatively, the differential labeling efficiencies for the prostate basal cell lineage among these studies may also account for the difference. In spite of this, it is generally agreed from these studies that adult murine prostate basal cells and luminal cells are independently sustained.

The lineage tracing approach has also been utilized to investigate how prostate epithelial lineage is established during early development. Ousset *et al* specifically labeled prostate basal and luminal cells at the postnatal developmental stage [58]. The authors demonstrated that at this stage a fraction of basal cells possess

the stem cell capacity for multiple lineage differentiation and are able to generate basal and luminal cells. In contrast, luminal cells at this stage have already become committed and can only generate luminal cells. Collectively, this study demonstrated that at the postnatal developmental stage, some prostate basal cells possess multilineage differentiation capacities. They may give rise to luminal cells through the generation of unipotent luminal progenitors, or by a linear differentiation scheme through K5 and K8 dual positive intermediate cells. In contrast, luminal cells have largely completed lineage commitment at this stage and only possess unipotent potential. This study, together with the lineage tracing studies of the adult prostate epithelial cells imply a developmental stage-dependent switch of the mechanisms for epithelial maintenance, which has been observed previously in the mammary gland [64-66].

Using a lineage tracing approach, Wang *et al* identified a CARN cell population in castrated mice [67]. They utilized an Nkx3.1-CreER^{T2} mouse strain that expresses the tamoxifen-responding CreER^{T2} under the endogenous promoter of Nkx3.1. In intact mice, Nkx3.1 is mainly expressed by luminal cells but is also expressed in a few basal cells [68]. However, using a fluorescence reporter line, Wang *et al.* showed that in castrated Nkx3.1-CreER^{T2} mice, Nkx3.1 is only expressed in a small fraction of luminal cells, which were termed as the castration-resistant Nkx3.1-expressing (CARN) cells. They showed that CARN cells can generate both basal cells and luminal cells. This study shows that in castrated mice some phenotypically luminal-like cells also possess stem cell activity. It should be noted that despite a luminal phenotype, the origin of the CARN cells is unknown. It is possible that basal cells can adapt a CARN cell phenotype in castrated mice.

Functional plasticity of prostate basal stem cells

Rodent prostate basal epithelial cells are capable of adapting a luminal cell phenotype during *in vitro* culture [69]. Both human and rodent prostate basal cells can generate luminal cells and neuroendocrine cells in the prostate regeneration assay [31-33, 41, 43, 49, 70]. In contrast, in the lineage tracing assay adult murine prostate basal cells mostly only generate basal cells [59-62]. We reasoned that the distinct

behaviors of prostate basal cells in these studies reflect the different experimental conditions of these assays. Prostate tissues are often chopped into small pieces and dissociated into single cells in the regeneration assay. This experimental procedure creates substantial tissue damages. Tissue damage often occurs in the human prostate during certain pathological conditions such as inflammation and prostatitis, which have been suggested to promote the initiation of prostate-related disorders [71]. To determine whether tissue damage induced by inflammation affects the differentiation program of prostate basal epithelial cells, our laboratory further performed the lineage tracing study for the basal cells in the context of a uropathogenic bacteria-induced mouse model for prostatitis [72-74]. We first labeled prostate basal cells in the K14-CreER; mTmG mice with green fluorescent protein via tamoxifen induction. Then we instilled the uropathogenic bacteria CP9 into the bladder of experimental mice transurathrally to induce prostatitis. Our result showed that prostate inflammation drives differentiation of basal cells into luminal cells efficiently [75]. This study directly demonstrates the plasticity of prostate basal cells and suggests that the capacity for multi-lineage differentiation represents a facultative function of prostate basal cells under pathological conditions.

Prostate stem cell niche

Though not always correct, replication-quiescence is considered as one feature for somatic stem cells. Therefore stem cells have been predicted to retain labeling by nucleotide analogues such as BrdU [76-78]. To identify the label-retention cells in the prostate, Tsujimura et al. labeled all the murine prostate cells with BrdU and then performed many cycles of androgen ablation and add-back [79]. They found that long-term label-retaining cells in the prostate localize to the region of the prostate gland that is proximal to the urethra. Functional studies using an *in vitro* colony-forming assay or an *in vivo* prostate regeneration assay corroborated that the cells in the proximal regions are enriched in stem cell activity [34, 79]. Consistently, glands in the proximal region contain more epithelial cells expressing the prostate stem cell marker Sca-1 [33]. Stromal cells in the proximal region seem to act as the func-

tional niche for prostate stem cells. It has been previously shown that there is a regional phenotypic heterogeneity in the stromal cells distributed along the prostatic ductal system [80]. Stromal cells in the proximal region appear as myofibroblasts morphologically and are distinct from the fibroblast cells frequently seen in the stroma distal to the urethra. In contrast to the fibroblastic cells that usually stimulate the proliferation of prostatic epithelial cells, myofibroblastic cells secrete high levels of TGF- β and induce cell quiescence. A transgenic mouse study has also shown that inhibition of TGF signaling in the prostatic stroma by itself is capable of inducing the formation of precancerous lesions possibly by driving stem cells into active cell cycle [81]. Blum et al performed a gene expression profile analysis for the urogenital sinus mesenchymal cells and suggested that many development-associated signaling may be involved in the maintenance of the niche for the prostate stem cells [82].

It was also reported that the vascular density in the proximal region is the highest in the prostate and that endothelial cells can support the growth of immortalized prostate cell lines transplanted under renal capsules of immunodeficient host mice [83, 84]. Given the findings that endothelial cells can serve as the niche for neural stem cells as well as hematopoietic stem cells, it may also be possible that endothelial cells can help maintain the homeostatic status of the "stemness" of the prostate stem cells [85, 86]. Of note, both basal and luminal cells in the proximal region are capable of retaining label [79]. This is consistent with the conclusion from the lineage tracing studies that stem/progenitors cells exist in both prostate basal and luminal cells. It should be noted that observations from several tissue systems have questioned replication-quiescence being a stem cell feature and whether all label-retaining cells are stem cells [87-89].

Conclusions and future directions

Much progress has been made during the last decade in understanding the lineage hierarchy in prostate epithelia. Based on the published studies we have had a basic understanding of how prostate epithelial lineage hierarchy is maintained [12]. It appears that prostate basal cells and luminal cells in adults are indepen-

dently sustained while basal cells exhibit functional plasticity and possess the potential to generate luminal cells and neuroendocrine cells. However, the lineage hierarchy within the individual cell lineages remains uncharacterized. It remains a question whether there are progenitors within individual cell lineages or each lineage is maintained via stochastic cell duplication.

Current studies support that there is a lineage hierarchy in basal cells because Trop2⁺ basal cells appears to be more potent than Trop2⁻ basal cells in terms of regenerative capacities [31]. However it should be noted that this may simply reflect the propensity of cells to dissociation induced apoptosis as treating cells with the ROCK kinase inhibitor substantially enhances stem cell activity in those assays [90].

On the other hand, it remains clueless how the luminal cell lineage is maintained. By modifying the culture conditions, two research groups have reported very recently that a very small percentage (0.3-1%) of luminal epithelial cells is able to survive in vitro and displays stem cell activities [101, 102]. Interestingly, in all current stem cell assays, prostate cells are always dissociated into single cells. Therefore, the low efficiency of putative luminal stem or progenitor cells to survive and expand in these assays may also reflect their strong susceptibility to anoikis. Anoikis is apoptosis induced in cells by insufficient or inappropriate cell-matrix interactions [91]. Compared to the luminal epithelial cells, dissociated basal epithelial cells are likely more resistant to anoikis. This is because they possess various intrinsic molecular signaling that counteracts anoikis, such as expression of Bcl-2 and adhesion-associated membrane receptors and their substrates in extracellular matrix, and protein kinases [92-98] etc. We recently showed that even though Notch signaling can suppress anoikis of prostate luminal cells only a fraction of luminal cells can form prostate spheres in vitro and generate tubular structures in vivo [99]. This observation implies that the sphere-forming luminal cells may be functionally distinct from the non-sphere-forming luminal cells, and supports the existence of a lineage hierarchy in prostate luminal cells. It will be critical to characterize the progenitors in the luminal cell lineage. Fully understanding of the lineage hierarchy in both basal and luminal cells will provide insights into the properties and identities of the cells of ori-

gin for prostate cancer as well as those of the prostate cancer stem cells.

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Disclosure of conflict of interest

The authors have no conflict of interest to declare.

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Prostate stem cells

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Prostate stem cells

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Prostate stem cells

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Prostate stem cells

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