Research Highlight

Expanding luminal epitheliums as cells of origin for prostate cancer

Despite tremendous progress made in human prostate biology over the past few decades, a full picture of prostate lineage hierarchy and its connection to cancer initiation remain to be delineated. Two recent studies published in Nat Genet. [1] and in Science [2] have profiled subpopulations of prostate cells at the single-cell level. Complementary analyses of data from the studies demonstrate self-renewal and differentiation capacities of different luminal epithelial cells, which can serve as cells of origin for prostate cancer. The system of lineage hierarchy controls cell differentiation and tissue formation from stem and progenitor cells, which may initiate cancer development if the process goes awry [3]. Therefore, it is of great interest to identify cells with stemness or progenitor properties and delineate their lineage hierarchy in order to pinpoint the cells of origin for cancer when cellular genetics are altered [4].

The prostate epithelium is composed of luminal, basal and a rare population of neuroendocrine (NE) cells [5]. Previous studies have identified progenitor cells in both luminal and basal populations, which can serve as cells of origin for prostate cancer [6,7]. However, these studies were all carried out in engineered mouse models, where the initiation of murine prostate cancer might be very different from those in humans under pathophysiological conditions. In addition, previous studies mainly used bulk cells of luminal or basal subtypes, which hindered the identification of relatively rare stem or progenitor cells due to the heterogenous nature of cell populations.

The advent of single-cell profiling has facilitated unprecedented stratification of cells within tissues, which can help to identify rare populations of cells [8]. Recently, in two independent studies, Guo et al. [1] and Karthaus et al. [2] used droplet-based single-cell RNA sequencing (scRNA-seq) to profile prostate cells isolated from mice, and each identified three clusters of luminal epithelial cells. Based on the markers used for clustering, the Luminal-A and Luminal-B cells identified by Guo et al. [1] were largely overlapping the L1 population identified by Karthaus et al. [2], as they were all Nkx3.1+/− and localized in the distal regions of murine prostate. The L2 population of cells identified by Karthaus et al. [2] mainly localized in the proximal prostate, which was also revealed in the other study and was designated as Luminal-C cells. The L2/Luminal-C cells are positive for stemness genes Taqstd2 and Psca, and they are apparently the same progenitor cells identified as Sca-1+/−/Nkx3.1− cells from a previous study [9]. While Karthaus et al. [2] only analyzed cells from the anterior prostate (AP), Guo et al. [1] profiled all three prostate lobes: AP, ventral prostate (VP) and dorsal-lateral prostate (DLP), which revealed lobe-specific distributions of the luminal clusters. Notably, Guo et al. [1] also noticed the existence of some Luminal-C cells at the invagination tips of prostate distal region, for which they separately designated these cells as Prox-Luminal-C and Dist-Luminal-C cells (Fig. 1).

Guo et al. [1] then continued to functionally characterize different clusters of prostate luminal cells. Markov-chain entropy analysis showed that both Prox- and Dist-Luminal-C cells had higher stemness potency scores than Taqstd2− luminal cells. Indeed, Taqstd2+ Prox- and Dist-Luminal-C cells isolated from mice formed organoids at efficiencies three and six times higher, respectively, than Taqstd2− luminal cells (Fig. 1). Renal capsule grafting also demonstrated a much higher capacity of Luminal-C cells in the regeneration of prostate glands than Taqstd2− cells. Remarkably, Luminal-C cells generated both luminal and basal cells in these two assays, and Taqstd2− cells still resided at the invagination tips of regenerated prostate glands, which also had newly produced Taqstd2− Luminal-A and Luminal-B daughter cells (Fig. 1). Additionally, lineage-tracing experiment showed consistent localization of Dist-Luminal-C cells at the invagination tips of prostate distal regions. Similarly, in a prostate regression-regeneration assay, Dist-Luminal-C cells not only sustained Taqstd2− cells, but also generated Taqstd2− luminal cells [1].

Karthaus et al. [2] also showed that L2 cells isolated from intact mice (no castration) had a higher organoid-forming efficiency than L1 cells; L2 cells were less sensitive to castration than L1 cells in the organoid formation assay, consistent with a higher stemness potency of L2/
Luminal-C cells. In a lineage-tracing experiment with luminal-specific Krt8 Cre\textsuperscript{ERT2} driver, the authors showed that luminal cells reconstituted prostate glands at their original proximal or distal regions, suggesting each population contains its own progenitor cells. Both L1 and L2 cells isolated from both proximal and distal regions can form organoids that have both luminal and basal epithelial cells. Prostate gland reconstructed from Luminal-C cells by grafting contains both luminal and basal cell markers, and Tacstd2\textsuperscript{+} Luminal-C cells are confined to the invagination tips of the regenerated gland. Tacstd2\textsuperscript{+} Luminal-C cells isolated from prostate distal regions have the capacity of self-renewal and differentiation into Tacstd2\textsuperscript{+} luminal cells. Dist-Luminal-C cells initiate prostate cancer after lineage-specific deletion of the tumor suppressor gene Pten (dashed rectangle). Growth factors secreted by mesenchymal cells support luminal cell growth. Fgf10, fibroblast growth factor 10; Igf1, insulin-like growth factor 1; Nrg2, neuregulin 2.

Luminal-C cells are localized to the invagination tips of prostate distal regions, whereas in the proximal region, they form a continuous luminal layer. Tacstd2\textsuperscript{+} Luminal-C cells isolated from both proximal and distal regions can form organoids that have both luminal and basal epithelial cells. Prostate gland reconstructed from Luminal-C cells by grafting contains both luminal and basal cell markers, and Tacstd2\textsuperscript{+} Luminal-C cells are confined to the invagination tips of the regenerated gland. Tacstd2\textsuperscript{+} Luminal-C cells isolated from prostate distal regions have the capacity of self-renewal and differentiation into Tacstd2\textsuperscript{+} luminal cells. Dist-Luminal-C cells initiate prostate cancer after lineage-specific deletion of the tumor suppressor gene Pten (dashed rectangle). Growth factors secreted by mesenchymal cells support luminal cell growth. Fgf10, fibroblast growth factor 10; Igf1, insulin-like growth factor 1; Nrg2, neuregulin 2.

In the organoid formation and renal capsule grafting assays, Luminal-C cells generated both luminal and basal cells, whereas in the lineage-tracing and regression-regeneration assays \textit{in vivo}, all lineage-marked cells were positive for luminal marker CK8, but negative for basal makers (Fig. 1), indicating that Luminal-C cells responded differently \textit{in vitro} and \textit{in vivo}. It would be useful in the future to define those factors that determine the differentiation commitment of Luminal-C cells, which may explain the predominantly luminal phenotypes of prostate tumors [6]. It is worth noting that a study on airway epithelial cell dedifferentiation [10] indicated that ablation of stem cells provides an intrinsic stimulus for luminal cell to regain pluripotency, which may give a hint as to how luminal prostate cells produce basal cells \textit{ex vivo}.

**Author contributions**

\textit{Study design:} Gonghong Wei.
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\textit{Data analysis:} Yuexi Gu, Gonghong Wei.
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**Conflicts of interest**

The authors declare no conflict of interest.

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