

# Stem Cell Lineage Hierarchy by Keratin Profiling in Normal Human Prostate Epithelial Cells and Prostate Cancer

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**Background:** Keratins are intermediate filament proteins that perform both mechanical and non-mechanical functions. Several specific keratins have been implicated in stem cells and cancers. Using a sphere-based label retention assay, we recently isolated and characterized prostate stem cells and cancer stem-like cells from mixed progenitor populations (PMID 28651114), identifying keratin 13 (KRT13) as a specific prostate stem cell marker regulating self-renewal. Herein, we utilize detailed keratin profiles to further clarify the human prostate epithelial lineage hierarchy and identify prostate cancer stem-like cells.

**Methods and Results:** Primary prostate epithelial cells were 3D cultured (5 days) to form prostaspheres (PS) followed by PS-based long-term label retention and FACS to separate stem cells from progenitors. In normal prostate tissues from 3 healthy donors, RNA-seq revealed enrichment of KRT13, 23, 80, 78, 86 and 4 in CFSE<sup>High</sup> label-retaining prostate stem cells while KRT6, 17, 14, 5, 8, 18 and P63 were enriched in CFSE<sup>Low</sup> non-retaining progenitors. Immunostaining confirmed increased proteins of KRT13, 23, 80, 78, 4 and 19 in CFSE<sup>High</sup> prostate stem cells. We next used Fluidigm C1 captured single cell RNA-seq and identified three major clusters in the label-retaining stem cell population; *Cluster I* represents quiescent stem cells (KRT13, 23, 80, 78, 4 enriched), while *Clusters II & III* contain active stem cells and bipotent progenitors, respectively (KRT16, 17, 6 enriched). GSEA analysis found stem cell and cancer related pathways enrichment in *Cluster I*. Three additional clusters were identified in non-retaining progenitor cells, with *Cluster IV* representing unipotent basal progenitor cells (KRT5, 14, 6, 16 enriched) and *Clusters V & VI* as early & late stage unipotent luminal progenitors (KRT8, 18, 10 enriched). Cancer stem-like cells were similarly isolated from three prostate cancer specimens and RNA-seq with MetaCore pathway analysis found enrichment of cytoskeleton remodeling Keratin filaments. Interestingly, in addition to normal stem cell keratins (KRT13, 23, 80, 78, 4), other keratins (KRT10, 19, 6, 75, 16, 79, 3, 82) were enriched in cancer stem-like cells. Surprisingly, stem-like cells from patient-matched benign regions revealed a similar keratin profile, suggesting a cancer field effect for stem-like cell populations.

**Conclusion:** Taken together, using gene profiling with an emphasis on keratin patterns, we have delineated the lineage hierarchy of human prostate stem cells originating from the activation of quiescent stem cells to bipotent progenitors that give rise to unipotent basal and luminal progenitor cells. We have identified common keratins enriched in stem cells from normal prostate and cancer/benign tissues, as well as keratins unique in stem-like cells from prostate cancer. This clarification of the stem cell lineage hierarchy and keratin profiling of human prostate stem cells and cancer stem-like cells may provide enhanced opportunities for translational studies that target therapeutic-resistant cancer stem-like cells.

**Keywords:** Prostate, Stem cell, Single cell analysis, RNA-seq, Keratin.

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