Single Cell Analysis of Normal Basal Cells Reveals Cellular Heterogeneity and Link to Aggressive Prostate Cancer Phenotypes

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Background
The signaling pathways and functional characteristics central to normal stem cells are often observed in aggressive malignancies. We have shown that histological subtypes of advanced prostate cancer vary in their enrichment of a normal prostate basal stem cell signature with the highly aggressive, small cell neuroendocrine carcinoma being the most stem cell-like. It is unclear if the human prostate basal stem cell population is composed of distinct subpopulations and if a specific subpopulation is driving this similarity with aggressive prostate cancer.

Methods
We isolated benign Trop2+ CD49f Hi basal stem cells from human prostates obtained after radical prostatectomy. A suspended basal cell solution was deposited into a 96-chamber microfluidic chip and inserted into a Fluidigm C1 for single cell capture and cDNA synthesis. Single cell RNA-seq compatible barcoded libraries were synthesized and subjected to high-throughput RNA sequencing. Unsupervised clustering was performed on single cell RNA-seq gene expression data to identify subpopulations within the Trop2+ CD49f Hi basal stem cell population. We further performed differential expression analysis, gene set enrichment analysis, and MARINa to define subpopulation specific cell surface markers, gene networks and transcription factors. Using a microfluidic barcode chip assay, we measured the protein expression of 10 assay-compatible cell surface markers at the single cell level. FACS analysis was also performed to confirm the cell surface expression of differentially expressed markers. Gene expression datasets of human prostate cancer were mined to identify cell surface markers differentially expressed in prostate stem cell subpopulations and aggressive cancer phenotypes.

Results
We isolated and performed RNA-seq on over 150 benign human prostate Trop2+ CD49f Hi basal stem cell single cells. Unsupervised clustering identified multiple subpopulations within the bulk basal stem cell population. Bioinformatic analyses revealed each subpopulation to be associated with distinct gene networks, transcription factors, and cell surface markers. Surface marker protein expression on over 750 single basal stem cells from 2 human prostates confirmed the existence of subpopulations within the bulk basal cell population. Molecular interrogation of human prostate cancer samples showed that neuroendocrine prostate cancer was enriched for a number of differentially expressed cell surface markers from the single cell data.

Conclusions
Our results suggest that the human prostate basal stem cell population is composed of molecularly distinct subpopulations. Further, targeting molecular features such as cell surface markers common to normal stem cells and aggressive disease may be a novel approach for treating advanced prostate cancer.

Conflict of Interest
None

Funding Acknowledgements
This work was supported by an American Cancer Society postdoctoral fellowship PF-16-082-01-TBE and Prostate Cancer Foundation Young Investigator Award (to B.A.S.), the Howard Hughes Medical Institute, and a Stand Up To Cancer/American Association of Cancer Research/Prostate Cancer Foundation Prostate Dream Team Translational Research Grant SU2C-AACR-DT0812 (J.M.S. and O.N.W.).