Title: Deciphering Human Prostate Carcinoma-Associated Fibroblast Heterogeneity using scRNA-seq

Background: Carcinoma-associated fibroblasts (CAF) are a heterogenous component of the prostate tumor microenvironment and have been demonstrated to regulate prostate cancer growth and progression in a variety of ways. The extent of CAF heterogeneity in prostate cancer tissues has not been well described.

Methods: These studies expand upon our recent description of the heterogeneity of cultured CAF by isolating fibroblasts directly from human prostate cancer and matched normal tissues for single-cell mRNA-sequencing (scRNA-seq) analysis. These studies evaluated several digestion conditions for isolation of prostatic fibroblasts. Sorting of primary fibroblasts was conducted by excluding CD45 (immune), CD200 (endothelial), and EpCAM (epithelial) cells. Prostate cancer-containing peripheral zone (PZ) and matched cancer-free transition zone (TZ) tissues were digested and sorted for viable CD45-CD200-EpCAM- cells for downstream scRNA-seq analysis using the 10X Chromium System. Normal fibroblasts from young, healthy donors were used as a comparison. CellRanger and Seurat were used for cell clustering and differential gene expression analysis.

Results: Successful isolation of fibroblasts requires longer tissue digestion protocols than for immune or epithelial cells. Clustering of fibroblasts from cancer-containing PZ or cancer-free TZ identifies a more heterogeneous population of fibroblasts from the PZ compared to the TZ of the same patient. Although nearly all of these cells express vimentin, there is heterogeneous expression of other fibroblast markers. When all fibroblasts from the same patient are clustered together, a subset of PZ fibroblasts form isolated cell clusters. Finally, prostate fibroblasts freshly isolated from a cancer patient cluster separately from cultured CAF and also from normal prostate fibroblasts isolated from a young, healthy donor.

Conclusions: These studies suggest prostate CAF are more transcriptionally heterogeneous than normal fibroblasts within the same patient. These results will initiate further investigation of the unique CAF subpopulations present in prostate cancer tissue which aid in prostate cancer progression or limit therapeutic potential.