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

Renee E. Vickman[1], Nadia A. Lanman[2], Omar E. Franco[1], Brian T. Helfand[1], Victoria Gil[1], Max Greenberg[1], Yana Filipovich[1], Gervaise H. Henry[3], Douglas W. Strand[3], Susan E. Crawford[1], Timothy L. Ratliff[2],and Simon W. Hayward[1]

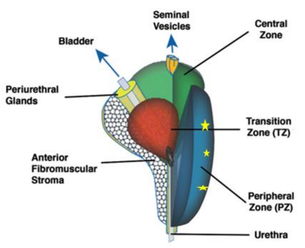
[1]NorthShore University HealthSystem, Evanston, IL, USA
[2]Purdue University, West Lafayette, IN, USA
[3]University of Texas Southwestern, Dallas, TX, USA

Abstract

Carcinoma-associated fibroblasts (CAF) are a heterogeneous 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. 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. Successful isolation of fibroblasts required longer tissue digestion protocols than immune or epithelial cells and was conducted by excluding CD45 (immune), CD200 (endothelial), and EpCAM (epithelial) cells. Digestion of prostate cancer-containing peripheral zone (PZ) and matched cancer-free transition zone (TZ), followed by cell sorting of viable CD45-CD200-EpCAM- cells for scRNA-seq analysis using the 10X Chromium System, identified a more heterogeneous population of fibroblasts from the PZ compared to the TZ of the same patient. Although nearly all of these cells expressed vimentin, there was heterogeneous expression of other fibroblast markers. When all fibroblasts from the same patient were clustered together, a subset of PZ fibroblasts formed 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. These studies suggest that CAF are more transcriptionally heterogeneous than normal fibroblasts from within the same prostate. 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.

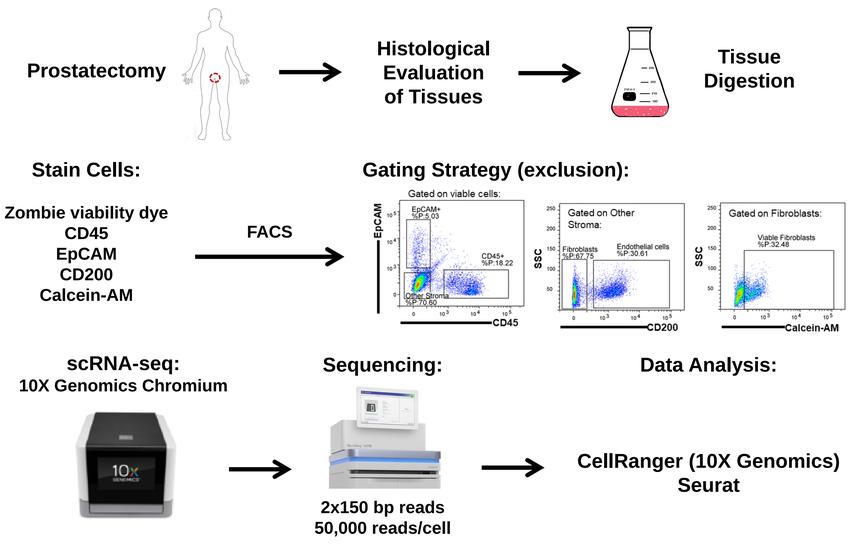
Introduction

The tumor microenvironment (TME) is complex, containing many different cell types. CAF are an important component of the tumor microenvironment and are capable of promoting tumor growth *in vivo*. Heterogeneity of CAF is well known but not thoroughly understood. Normal prostate fibroblasts (NPF) contribute to the development and maintenance of normal prostate structure through growth factor and cytokine secretion, but CAF have been noted to contribute to prostate cancer progression. scRNA-seq can be used to elucidate the heterogeneity of CAF and to help understand their role(s) within the TME. Our previous scRNA-seq studies indicated that cultured CAF derived from prostate cancer tissues have a limited number of subpopulations (Vickman, et. al 2020, *Prostate*).



Here we performed scRNA-seq on fibroblasts directly from digested human prostate tissues, with CAF isolated from the peripheral zone (PZ) containing cancer, and NPF isolated from the cancer-free transition zone (TZ) from the same patient. This allows for direct comparison of CAF to normal fibroblasts from within the same prostate. To do this, PZ and TZ tissues from prostate cancer patients was digested and prepared for fluorescence-activated cell sorting (FACS) of fibroblasts, followed by scRNA-seq analysis. Vimentin is expressed by all fibroblasts, but cannot be used for isolation due to intracellular localization. Even though other markers are often used to identify CAF, including fibroblast specific protein-1 (FSP-1), CD90, podoplanin (PDPN), and alpha-smooth muscle actin (α-SMA), our previous studies indicated that expression of these markers is heterogeneous between and within CAF from patients. Thus, we used an exclusion strategy for fibroblast isolation so as to not bias downstream data. We hypothesize that CAF display greater heterogeneity compared to NPF from the same patient, and that cultured CAF do not maintain the heterogeneity seen in CAF isolated directly from patient tissues. More work is needed to understand and characterize the level and role(s) of CAF heterogeneity within the prostate TME.

Methods



Results

Short-term and long-term digestion methods preferentially isolate different prostatic cell types:

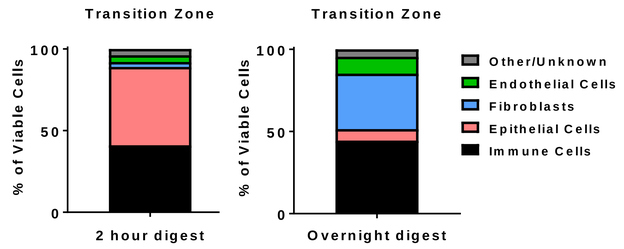


Figure 1. Comparison of short-term *versus* long-term digests indicate a skewed representation of isolated cell types. Human prostate transition zone (TZ) tissue was obtained and immediately digested with Collagenase I + DNAse for either two hours or overnight. Digested samples were washed, stained, and observed by flow cytometry for various cell types, including endothelial cells (CD200+), epithelial cells (EpCAM+), immune cells (CD45+), and fibroblasts (vimentin+).

Sorting fibroblasts using an exclusion strategy successfully isolates vimentin-positive cells:

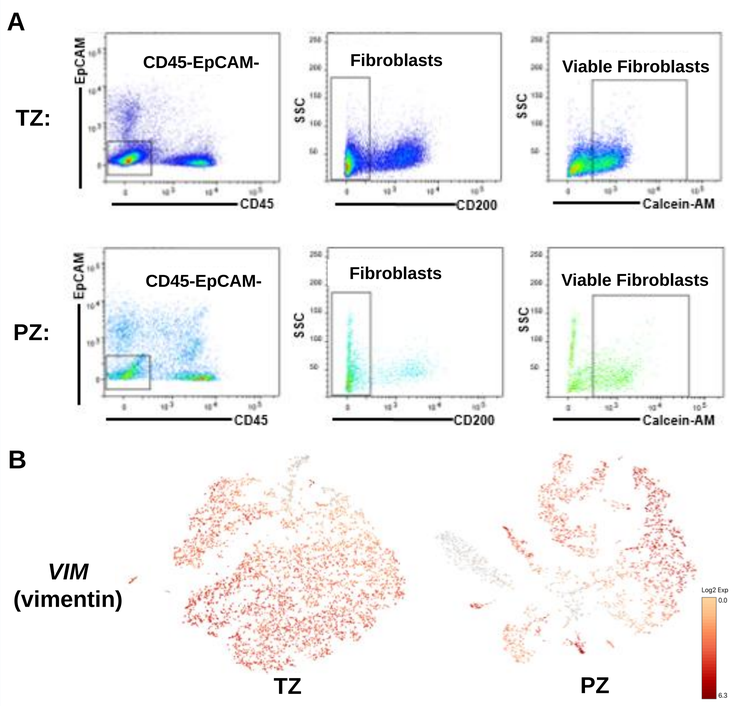


Figure 2. Fibroblasts can be sorted after overnight tissue digestion. Human prostate tissue was obtained from a prostate cancer patient and digested with Collagenase I overnight. PZ was confirmed to contain cancer and TZ was verified to be cancer-free. Digested samples were washed, stained, and sorted viable fibroblasts (CD45-EpCAM-CD200-CalceinAM+) using a BD FACSAria II. Approximately 5,000 fibroblasts were subjected to scRNA-seq analysis. **A)** Flow plots depicting the gating strategy for fibroblast sorting from the PZ and TZ of a single patient. **B)** t-SNE plots indicating Log2 expression levels of *VIM* (vimentin) in TZ and PZ fibroblasts, where red intensity indicates greater vimentin expression in single cells.

Unsupervised clustering indicates greater heterogeneity within PZ fibroblasts compared to TZ fibroblasts:

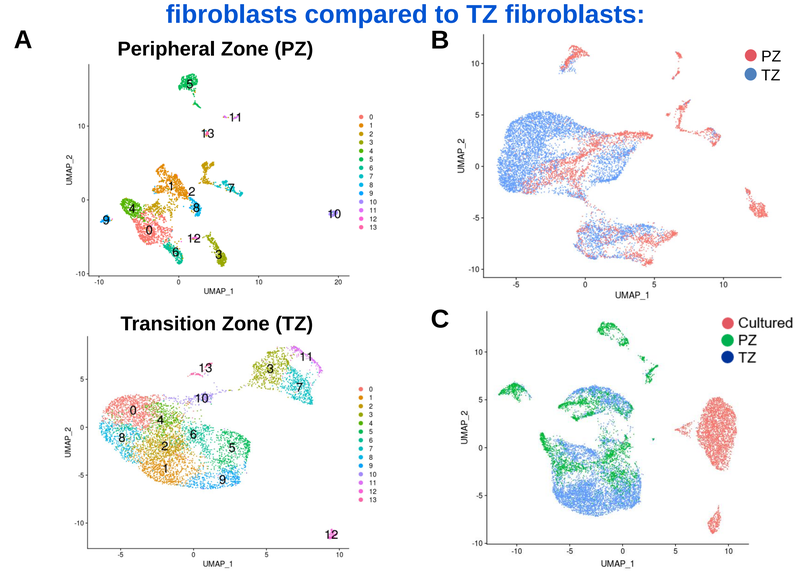


Figure 3. Naïve and combined clustering of PZ and TZ fibroblasts from a prostate cancer patient or cultured CAF. scRNA-seq of human prostate fibroblasts from PZ or TZ tissues and cultured primary CAF were conducted. **A)** Uniform manifold approximation and projection (UMAP) plot of naïve PZ and TZ clustering. **B)** UMAP plots of combined PZ and TZ clustering. **C)** UMAP plot indicating combined clustering of PZ and TZ fibroblasts with cultured fibroblasts from a different cancer patient.

Heterogeneous prostate fibroblasts isolated from a cancer patient cluster separately from those of a young, healthy donor:

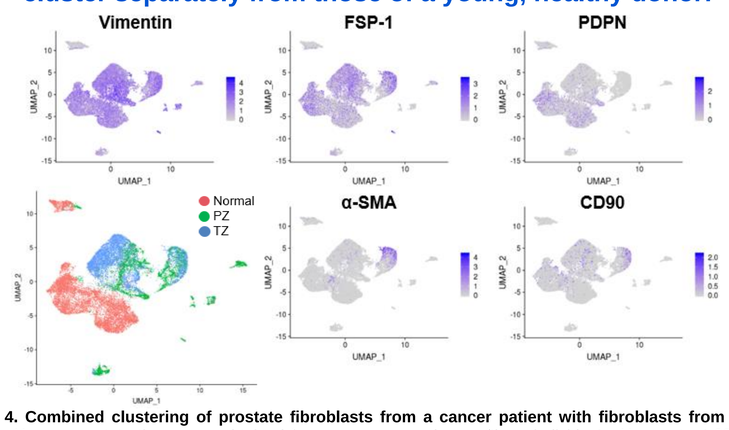


Figure 4. Combined clustering of prostate fibroblasts from a cancer patient with fibroblasts from healthy donors. Human prostate tissue was obtained from young organ donors (three individuals; Henry et. al 2018, *Cell Reports*) or from a prostate cancer patient and scRNA-seq was conducted on digested tissues. Fibroblasts from these patients were combined and subjected to unsupervised clustering and visualized with Seurat. UMAP plots indicate the unsupervised clustering as well as expression of various fibroblast markers vimentin, FSP-1, podoplanin (PDPN), α-SMA, and CD90. Blue intensity indicates relative expression level and gray indicates no detected expression in each cell.

Summary and Future Directions

- The prostate contains numerous cell types and isolation of fibroblasts improves with longer digestion protocols.
- Using an exclusion strategy for cell sorting allows for successful isolation of prostate fibroblasts for downstream applications.
- CAF and NPF isolated from patients express vimentin, but these fibroblasts have variable expression of other markers, such as FSP-1, PDPN, α-SMA, and CD90.
- UMAP plots indicate potentially greater heterogeneity in PZ CAF compared to TZ NPF from the same prostate cancer patient.
- Fibroblasts isolated directly from a prostate cancer patient cluster separately from cultured CAF and normal prostate fibroblasts, and additional patients are being added to validate these findings.

These studies are being expanded to include additional patient samples. Subpopulations of PZ or TZ fibroblasts that cluster separately will be evaluated for biomarkers and with pathway analysis to define the roles of these clusters. The long-term goal of these studies is to characterize subpopulations of CAF which aid in prostate cancer progression through targetable interactions within the TME.

Abbreviations

α-SMA: alpha smooth muscle actin
CAF: carcinoma-associated fibroblasts
FACS: fluorescence activated cell sorting
FSP-1: fibroblast specific protein 1
NPF: normal prostate fibroblasts
PDPN: podoplanin
PZ: peripheral zone
TME: tumor microenvironment
t-SNE: t-distributed stochastic neighbor embedding
TZ: transition zone
UMAP: Uniform Manifold Approximation and Projection
VIM: vimentin

Disclosures

The authors declare no conflicts of interest.

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