Unlocking the tumor-immune microenvironment by integrating bulk EV-RNAs with single-cell RNA-seq in liquid biopsies

Edgar Gonzalez-Kozlova[1#], Tzu-yi (Tina) Chen[2#], Taliah Solemani[2], Ash Tewari[3], Carlos Cordon-Cardo[3], Gustavo Stolovitzki[4], Navneet Dogra[2,3]

# Equal contributions

Corresponding authors: Edgar Gonzalez-Kozlova & Navneet Dogra.

Emails: edgar.gonzalez-kozlova@mssm.edu, navneet.dogra@mssm.edu

Departments of Oncological Sciences[1], Genetics and Genomics[2] and Pathology[3] at Icahn School of Medicine, Mount Sinai. IBM[4]

Introduction

Extracellular vesicles (EVs) have brought great momentum to the non-invasive liquid biopsy procedure for the characterization, detection, and monitoring of cancer. While our fundamental understanding of EVs exponentially increases, the mechanisms for cargo loading, inter-cellular communication, organ homeostasis, and association with disease remain unknown. Despite differences in the transcriptomes of cells and EVs, we can carefully map RNA signatures to quantify tumor and immune infiltration from EVs.

Methods

- Tissues and biofluids were collected from Mount Sinai cohorts: 1) Prostate cancer (n=10) (tissue, serum and urine); 2) Prostatectomy resistant (n=8) (serum) and 3) Tumor and adjacent normal tissue for single cell RNAseq (n =1).
- EV-Transcriptomes were aligned to Hg38 with BWA, Bowtie2 and Excerpt.
- Single cell analysis were done using Seurat, Monocle, MAGENA and scDissector.
- Computational and statistical analysis were done using R. Key packages include Cybersort, scMapper, dream, edger and ggplot2

Results

- We identified RNA signatures associated with immune response, apoptosis and prostate cancer in cells and EVs. Signatures in EVs scored higher than their cellular counterparts. (Fig. 1B, C & D)
- While corresponding to similar biological pathways, RNA signatures in EVs were different compared to cells. (Fig. 1E)
- Single cell RNAseq from tumor and adjacent normal cells allowed to identify cell clusters corresponding to immune cells, luminal and basal tissues in a single prostate cancer patient. (Fig. 2 A & B)
- Tumor cells were enriched in KLK2, 3 and NKX3-1, identified in both tumor and adjacent normal tissues. (Fig. 2 C)
- Integration of single cell RNAseq was possible using mixed effect linear models and correlative approaches. (Fig. 1A & 2D)
- Using immune cell type reference signatures allowed to identify these cells in bulk tissue and EV transcriptomes. (Fig. 3 & 4)
- Comparing two methods for cell type deconvolution revealed cell types in EV-RNAs identified by 2 independent analysis. (Fig. 3A)
- Comparing immune cell types between single cell transcriptomes and deconvoluted EV revealed a correlation of ~0.9 between major cell types. (Fig. 3B)
- Serum was the biofluid that reflected more accurate the cellular proportions identified in single cell transcriptomes. (Fig 3B)
- Plasma, CD4+ , myeloid and dendritic cells were the best represented in EV transcriptomes. (Fig. 4)

Discussion

Despite the limitations from our sample size, this proof-of-concept study provides novel insights for using EV-RNAs as minimally invasive tools to characterize the phenotype of the tumor microenvironment. While isolating single EVs or cell specific EVs may be challenging, cleaver use of molecular signatures, single cell experiments, deconvolution methods and careful statistical modeling may be sufficient for using EVs are tools that can report on the composition and states of tumor-immune compartments fro tumors using minimally invasive liquid biopsies.

Blood derived **extracellular vesicle transcriptomes** are **informative** of **tumor** and **immune** phenotypes.

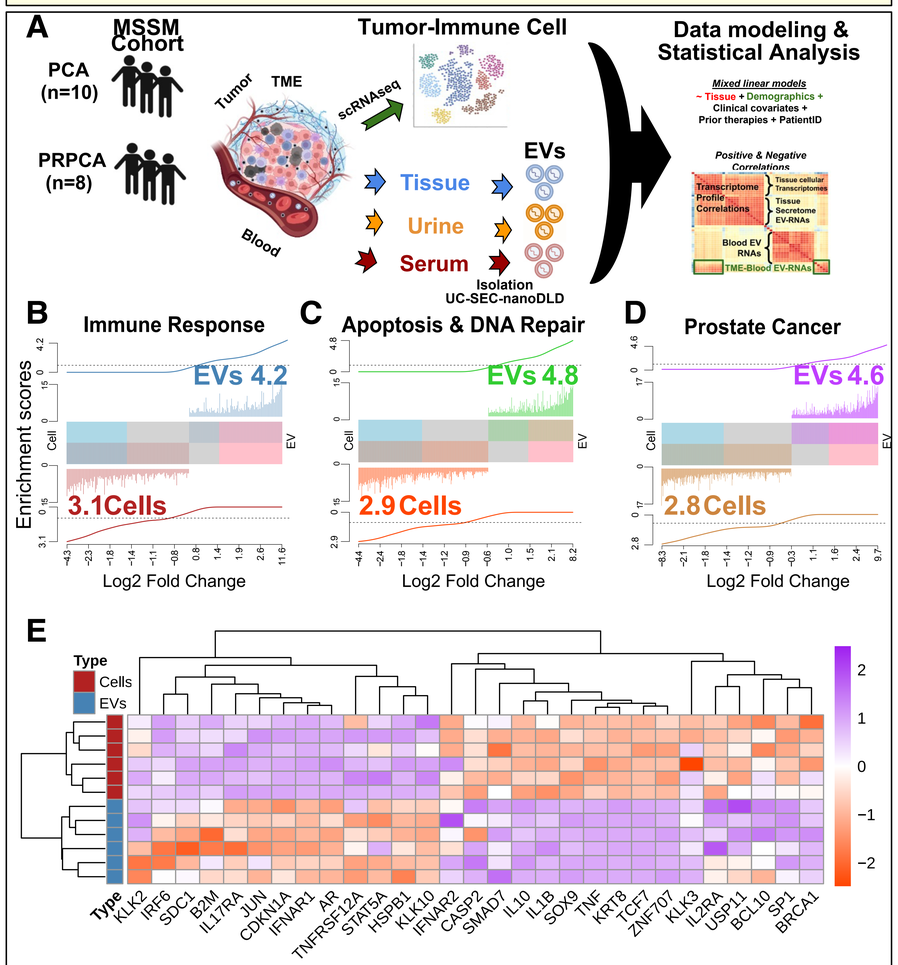

Figure 1. Study overview and molecular signatures between cells and EVs. **A.** Schematics of the 2 cohort of prostate patients (prostate cancer (PCA) and prostatectomy resistant (PRPCA)) tissues and biofluids used to isolate EVs and single cells. High level view of mixed effect model design and correlations used. **B, C & D.** Gene enrichment for immune response, apoptosis and prostate cancer signatures between cells and EVs, respectively. **E.** Heatmap showing the top relative expression differences between cancer signatures identified between cells and EVs.

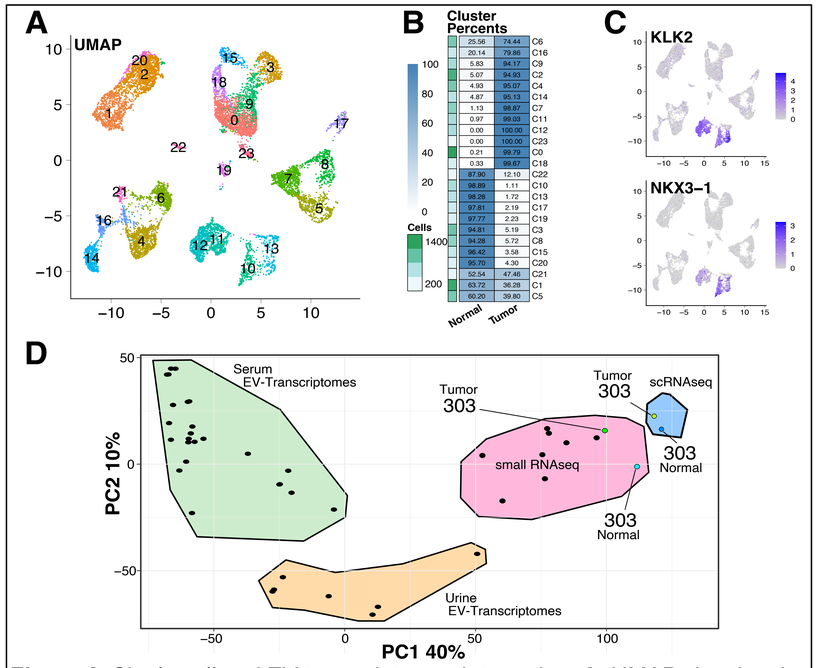

Figure 2. Single cell and EV transcriptomes integration. **A.** UMAP showing the structure of single cell transcriptomes from tumor and adjacent normal tissue. **B.** Percent of cells per cluster based on the tissue's origin. **C.** Clusters with expression of prostate cancer markers KLK2 & NKX3-1. **D.** PCA of cells, EVs and single cell transcriptomes in 2D space.

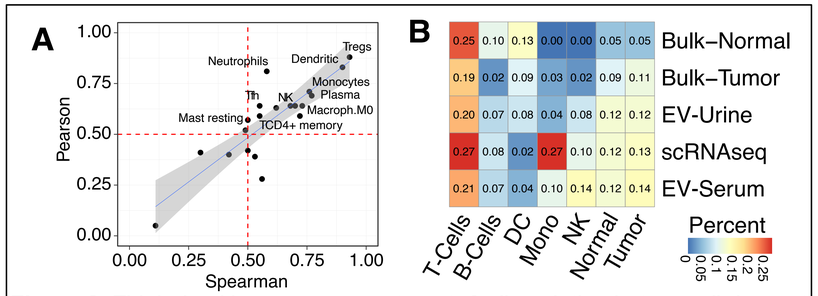

Figure 3. EV-derived immune compartment. **A.** Correlation between Cybersort and scMapper per cell type. **B.** Percent of cells per compartment between tissues, biofluids and single cell transcriptomes.

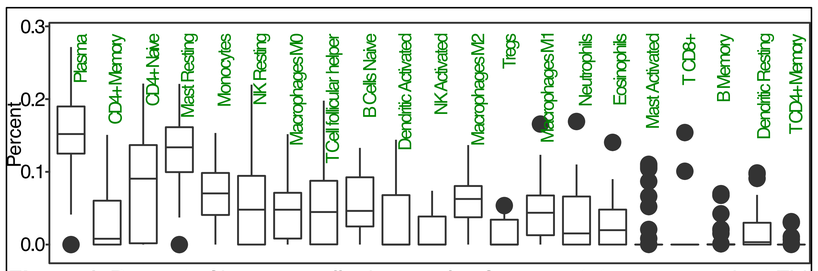

Figure 4. Percent of immune cells deconvolve from prostate cancer patient EV transcriptomes using scMapper. Immune-cell signatures used as reference correspond to dataset LM22 available at Cybersort website.