#102 Spatial Characterization of the Tumor Microenvironment in Pancreatic Cancer with Whole transcriptome profiling and high plex single cell imaging.

Youngmi Kim[1], David T. Ting[2,3], Patrick Danaher[1], Amaya Pankaj[2], Eunae You[2], Sean Kim[1], Michael Leon[1], Khrystyna North[1], Sarah Murphy[1], Vikram Devgan[1], and Sarah Warren[1]

1 NanoString Technologies, Seattle, WA
2 Mass General Cancer Center, Boston, MA
3 Harvard Medical School, Boston, MA

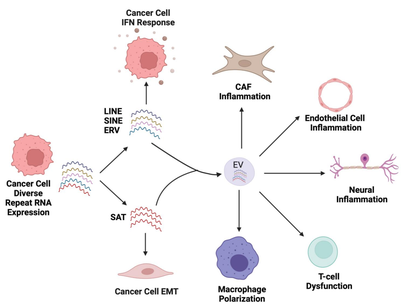
NanoString Technologies
530 Fairview Avenue North, Seattle, WA 98109

Summary

In this study, we used the GeoMx® Whole Transcriptome Atlas and RNAscope to visualize repeat elements and characterize the impact of repeat elements-presenting regions of pancreatic cancer. Then, we used CosMx® SMI to collect gene expressions of spatially-annotated single cells in both repeat elements-positive and repeat elements-negative regions. SMI uses fluorescent molecular barcodes to enable in-situ measurement of biological targets on an intact tissue sample, such as formalin-fixed paraffin-embedded tissues using a 1000+-plex RNA panel. Using the RNA expression data from SMI, we were able to spatially visualize how EVs encapsulating repeat elements impact the surrounding CAFs and other cells in the microenvironment. Using cell type deconvolution algorithms, we characterize the changes in gene expression within cell populations using the GeoMx as well as individual cells using SMI. Using nearest neighbor analyses, we are able to characterize expression profile changes between CAFs and immune cells that are close and distant from repeat expression tumor cells.

These results have provided a new lens in viewing the impact of repeat element tumor expression and mechanistic role in the microenvironment via EVs, which have the opportunity to identify novel therapeutic avenues and biomarkers in pancreatic cancer.

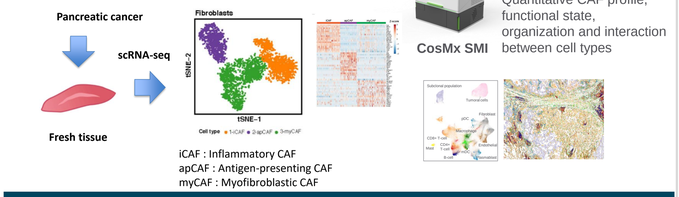
Introduction

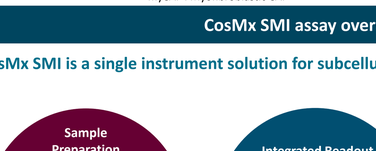


Repeat sequences have been shown to play a role in wide range of diseases including cancer, chronic viral infection, and neurodegeneration. A recent Phase II clinical trial of pancreatic cancer indicating that expression of certain repeat RNAs are correlated with response to combined immune checkpoint blockade and sublethal radiation therapy. More mechanistic studies have now revealed that these repeat elements are transcribed and often packaged in extracellular vesicles (EVs) which can be released from the tumor cells and interact with cells in the tumor microenvironment including cancer-associated fibroblasts (CAFs). Thus, it is important to understand the spatial distribution of repeat RNAs found in tumor cells and the surrounding CAFs that can provide mechanistic insight into their consequence in shaping the cellular response to tumors. Of particular interest is the impact they have on CAF subtypes that have been defined by single cell RNA-seq (scRNA-seq), which include the inflammatory (iCAF) or myofibroblastic (myCAF) phenotypes. Given the limitations of bulk RNA-seq and scRNA-seq in understanding the precise spatial distribution of this phenomenon, we used the NanoString GeoMx and Spatial Molecular Imager (SMI) to visualize these repeat elements and study their impact on the surrounding cells.

Experimental design

Generate multi-omics data sets to investigate roles of repeat RNAs in PDACs

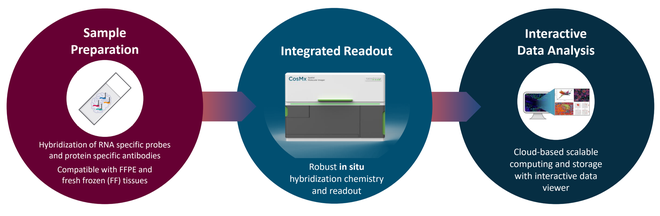


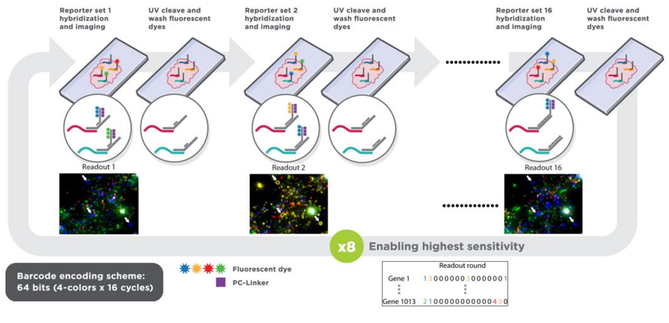


iCAF : Inflammatory CAF
apCAF : Antigen-presenting CAF
myCAF : Myofibroblastic CAF

CosMx SMI assay overview

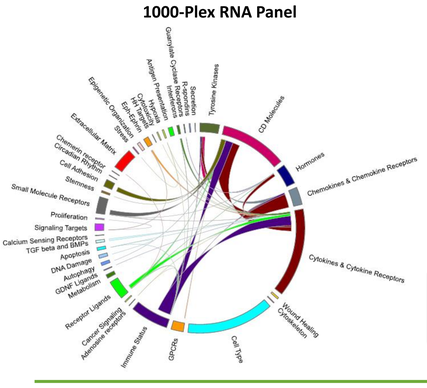
CosMx SMI is a single instrument solution for subcellular spatial analysis





High plex CosMx SMI panel

1000-Plex RNA Panel



Applications	# of genes
Cell typing and mapping	243 genes
Cell state and function	269 genes
Cell-cell interaction (ligand-receptor)	435 genes
Hormone activities	46 genes

Custom spike-in genes

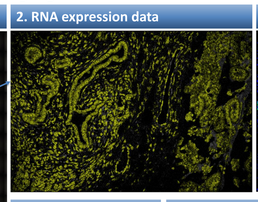
Repeat RNAs: HERV-H, HERV-K, LINE1_orf1, LINE1_orf2 , HSATII

CosMX SMI visualizes 965 gene targets in Pancreatic Cancer tissues

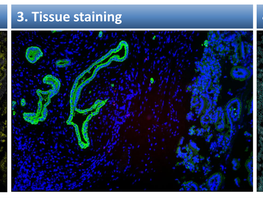
CosMx SMI spatially resolve 965 target genes across 3 PDAC tissues and 1 normal pancreatic tissue.

1. Select regions of interest

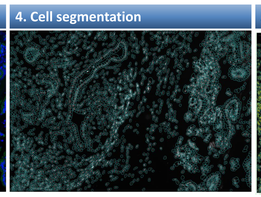
2. RNA expression data



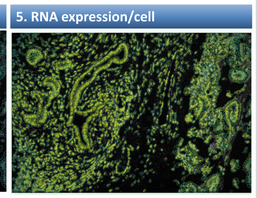
3. Tissue staining



4. Cell segmentation



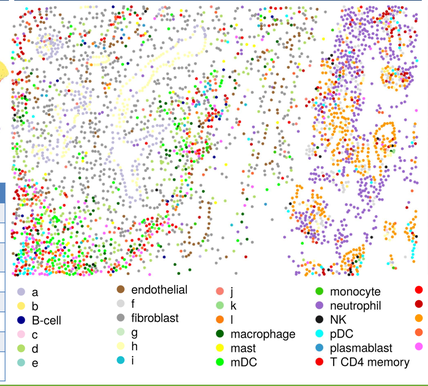
5. RNA expression/cell



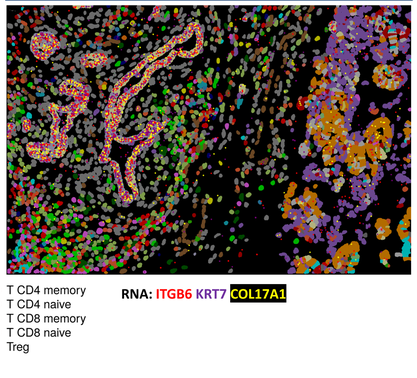
6. Cell typing analysis

4 PDAC tissues	
Total number of cell detected	337,969
% cells (>20 RNA counts, %)	98
Total number of transcripts detected (count)	114,966,012
% transcript assigned to cell (%)	98
Total FOV number	98
Mean transcript/cell (count)	263
Mean transcript/cell/target (count)	263
Mean neg probe/cell/target (count)	0.85

7. Spatial single cell display



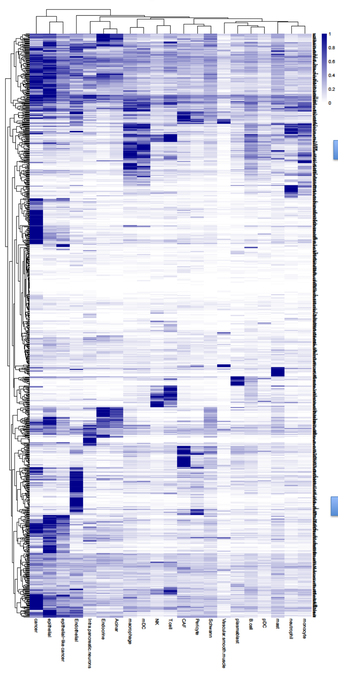
8. Marker genes



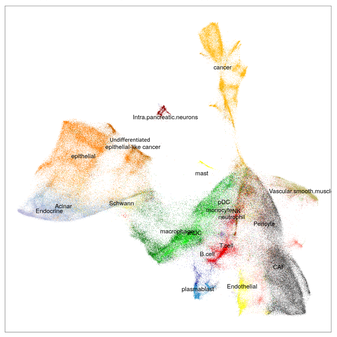
RNA: ITGB6 KRT7 COL17A1

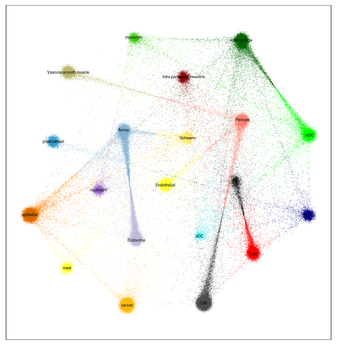
Customized CosMx cell typing analysis identifies 21 cell types of PDAC tissues and visualize their spatial locations

Gene expression signatures specific to cell types are defined using scRNA-seq data.

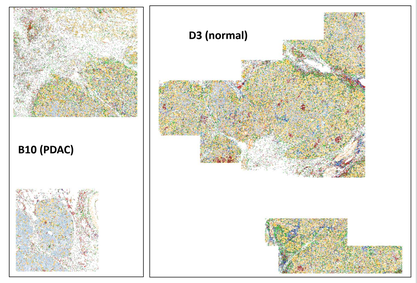


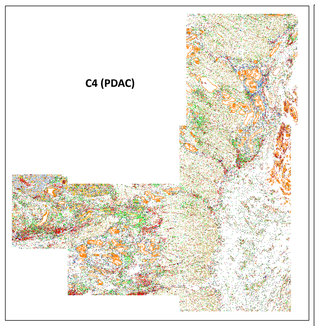
Nanostring cell typing algorithm determines cell types matching to a tissue type.

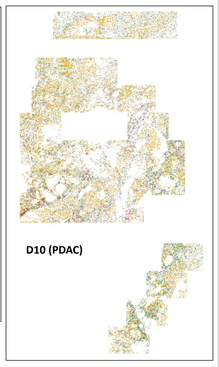




CosMx SMI retains x,y coordinates of cells unlike dissociated scRNA-seq data.

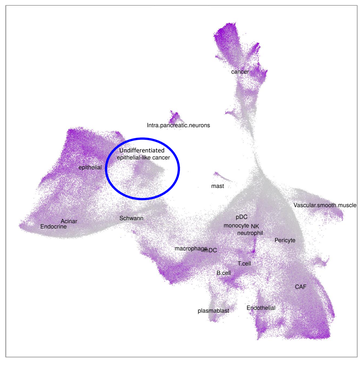




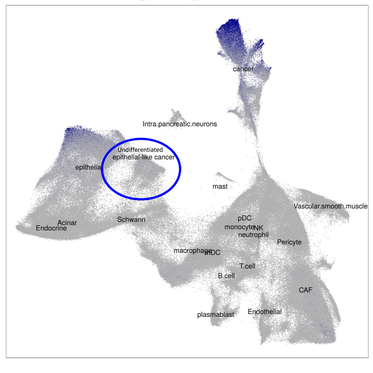


Repeat RNA-expressing cancer cells are losing keratin expressions, suggesting that repeat RNA promotes EMT

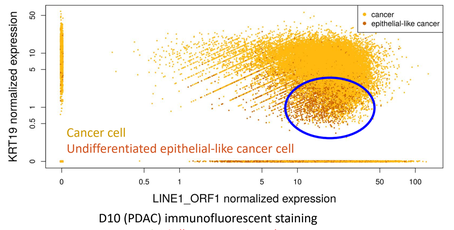
Line_orf1 expression: 0 48+



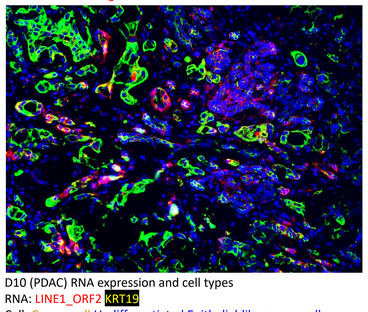
KRT19 expression: 0 20+

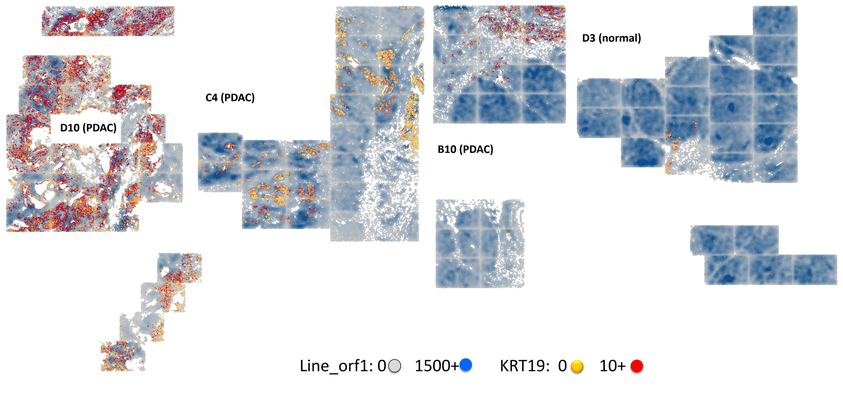


KRT19 expression loss in undifferentiated Epithelial-like cancer cells



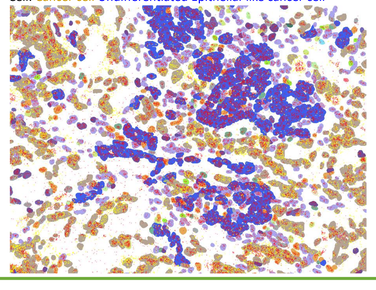
D10 (PDAC) immunofluorescent staining
DAPI PanCK Cell segmentation Ab



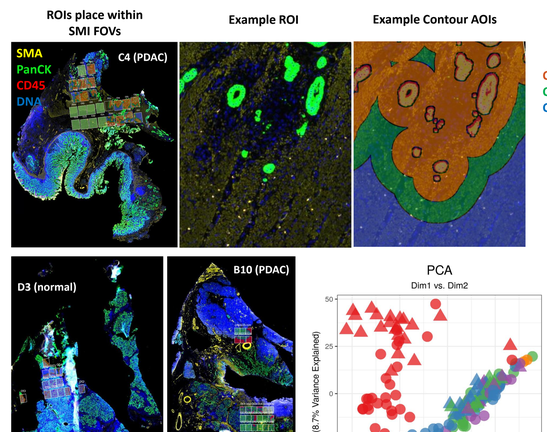


Line_orf1: 0 1500+ KRT19: 0 10+

D10 (PDAC) RNA expression and cell types
RNA: LINE1_ORF2 KRT19
Cell: Cancer cell Undifferentiated Epithelial-like cancer cell

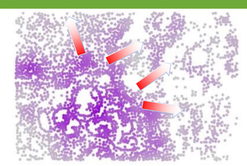


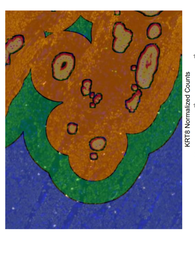
GeoMx DSP profiles whole transcriptome expressions in different tissue compartments

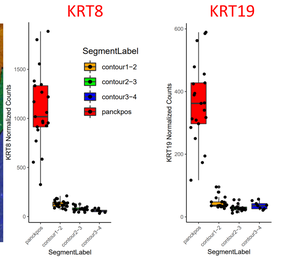


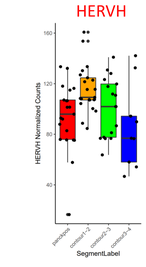
Contour1-2 has the highest repeat RNA expressions

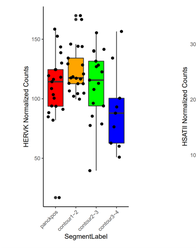
Repeat RNA is secreted from tumor cells and delivered to neighboring cells

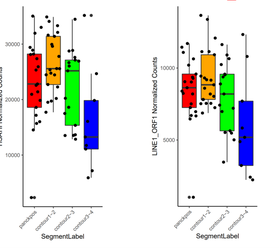


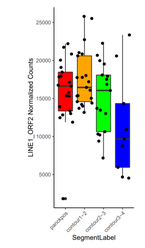










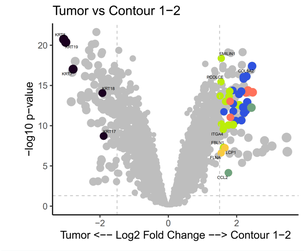


High-repeat-RNA tumor-microenvironment regions have upregulated genes which are associated with cytokine and inflammatory signaling

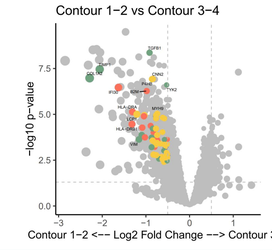
Pathway Names
- Cytokine Signaling in Immune system
- ECM proteoglycans
- Extracellular matrix organization
- Interferon gamma signaling
- Interleukin-4 and Interleukin-13 signaling
- KRT
- Non-integrin membrane-ECM interactions

Pathway Names
- Cytokine Signaling in Immune system
- Interferon gamma signaling
- Interleukin-4 and Interleukin-13 signaling

Tumor vs Contour 1-2



Contour 1-2 vs Contour 3-4



Conclusions

- CosMx SMI is a single instrument solution for subcellular spatial analysis and provides sub-cellular resolution of 1000+-plex transcriptomic information.
- Using the RNA expression data from CosMx SMI and GeoMx DSP, we were able to spatially visualize how repeat elements impact cancer cells and their potential delivery via EVs in affecting surrounding cells in the microenvironment.
- Using pathway analysis, we characterize the changes in gene expression within cell populations using the GeoMx and provide single cell resolution validation using CosMx SMI.
- Cellular subsets with gene expression profiles defined using single-cell sequencing-based multiomics can be spatially resolved using SMI to uncover novel cell-cell interactions.
- These results have provided a new lens in viewing the impact of repeat element tumor expression and mechanistic role in the microenvironment via EVs, which have the opportunity to identify novel therapeutic avenues and biomarkers in pancreatic cancer.

Reference

Elyada et al., Cancer Discovery (2019)
Sodergren et al., J Cancer Res Clin Oncol (2020)

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