Combining scRNA-seq and Digital Spatial Profiling to decode the cellular pathogenesis of human bladder cancer

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Abstract

Bladder cancer is the fourth most common cancer in men and the fifth most common malignancy overall. Bladder cancer presents phenotypically as papillary, muscle invasive, and carcinoma *in situ* (**Figure 1**), each having different behavior and response to therapy. A deeper understanding of the cellular and molecular composition of bladder tumors could provide much needed biomarkers to aid the clinician in treatment choices as well as in the development of new therapies. **Single cell RNA sequencing** (scRNA-seq) is a powerful new technology that provides a transcriptomic profile of every cell in the tumor. However, to understand the transcriptional changes associated with cancer, a proper control for every cell type is needed. The Strand laboratory is the first to produce a fully annotated and validated cellular atlas of the normal human bladder using fresh specimens from young organ donors aged 18-35 (**Figure 3**).

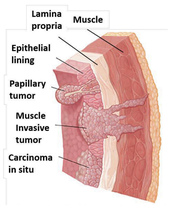


Figure 1. Bladder anatomy and tumor subtypes

While scRNA-seq is a robust tool for determining the identity of cell types, the requirement for fresh tissue and digestion into a cell suspension limits our ability to link discrete pathological features with individual cell type molecular profiles.

To identify the spatial location and relationships of cells within the bladder, we used NanoString's GeoMx Digital Spatial Profiler (DSP) and the novel Human Whole Transcriptome Atlas (HuWTA). Using cell type-specific markers identified by scRNA-seq, we analyzed the whole transcriptomes of epithelial cells, myofibroblasts, and fibroblasts in normal bladder, invasive tumors, and surrounding tumor-invaded tissue in FFPE tissue sections. In normal bladder, expression profiles from the selected cell types correlated well with those from scRNA-seq. Furthermore, DSP data allowed cell types identified by scRNA-seq to be localized spatially. In bladder cancer, we identified cell type-specific gene expression changes in tumors, as well as between normal and tumor adjacent normal tissue. Bladder tumors were both morphologically and transcriptionally heterogeneous, and we identified transcriptional signatures that vary both between tumors and spatially within an individual tumor. These data provide a map of spatial gene expression in bladder tumors, and will help guide the identification of cell types and biomarkers altered in cancer.

scRNA-seq and GeoMx DSP methods

Human Tissue: Bladder tumors were collected from pathology at UTSW. Normal human bladders were obtained from organ donors aged 18-35 after consent at the Southwest Transplant Alliance according to approved IRB protocols.

scRNA-seq: Full thickness sections of fresh bladder tissue from 4 individuals were separately digested into single cell suspensions in 2mg/ml collagenase for 1.5 hours for encapsulation and barcoding using the 10x Genomics Chromium controller. Libraries were sequenced to ~50k reads/cell and analyzed using Seurat.

DSP: Freshly cut FFPE sections of normal bladder and bladder cancer were stained by RNAScope with probes for anchor genes marking epithelia (pan-CK), fibroblasts (MFAP5) and myofibroblasts (F3). Sections were hybridized with GeoMx HuWTA reagents. ROIs were selected for analysis and segmented by the marker genes. Released tags were quantified on an Illumina next-generation sequencer.

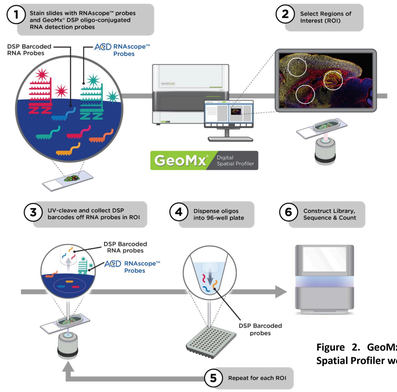


Figure 2. GeoMx Digital Spatial Profiler workflow.

scRNA-seq defines anchor genes used for DSP in human bladder

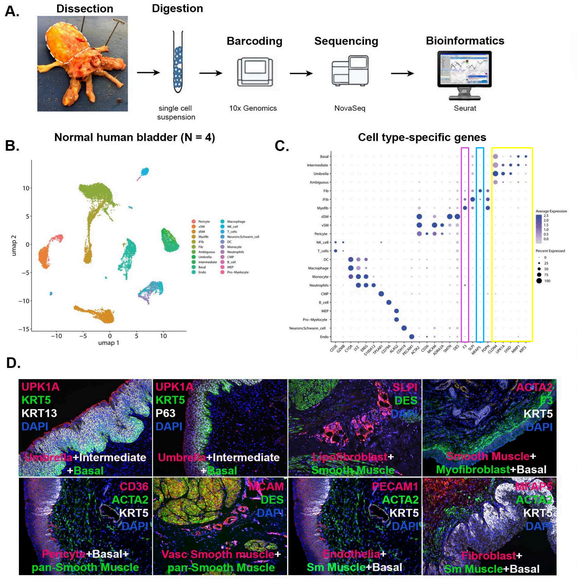


Figure 3. A cellular atlas of the normal human bladder. (A) Normal human bladder from young organ donors is collected fresh, digested into a cell suspension for single cell RNA sequencing. (B) Bioinformatics analysis clusters like cells in 2D space for an objective characterization of cell types. (C) Differentially expressed genes for each cell type are displayed by dot plot. Anchor genes used as segmentation markers for DSP are highlighted. (D) Antibodies against proteins exclusively expressed by each cell type were used to validate their location.

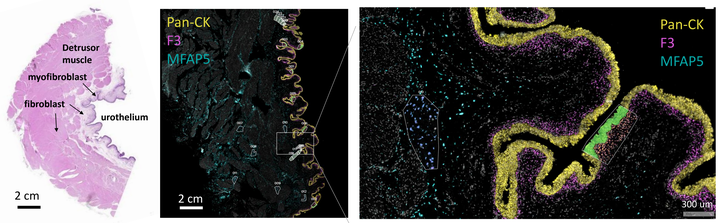


Figure 4. Anchor gene markers highlight 3 cell types of interest for DSP analysis. (Left) Bladder from young healthy men was fixed in formalin and embedded in paraffin. Sections were mounted on glass slides for hybridization with a pan-epithelial marker (pan-cytokeratin), a myofibroblast marker (F3), and a fibroblast marker (MFAP5). (Right) Regions of interest (ROIs) shown above were selected for capture of RNA and segmented by each of the three anchor genes.

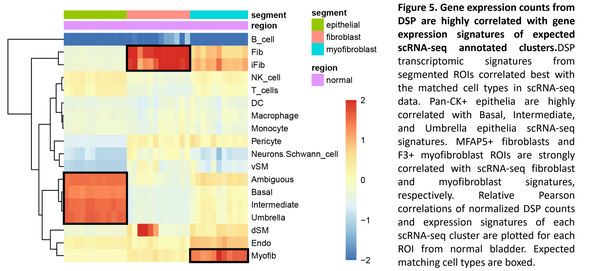


Figure 5. Gene expression counts from DSP are highly correlated with gene expression signatures of expected scRNA-seq annotated clusters. DSP transcriptomic signatures from segmented ROIs correlated best with the matched cell types in scRNA-seq data. Pan-CK+ epithelia are highly correlated with Basal, Intermediate, and Umbrella epithelia scRNA-seq signatures. MFAP5+ fibroblasts and F3+ myofibroblast ROIs are strongly correlated with scRNA-seq fibroblast and myofibroblast signatures, respectively. Relative Pearson correlations of normalized DSP counts and expression signatures of each scRNA-seq cluster are plotted for each ROI from normal bladder. Expected matching cell types are boxed.

Cell type deconvolution of normal bladder tissue

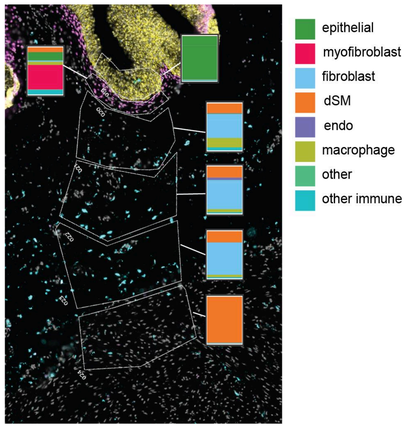


Figure 9. Cell type deconvolution on unsegmented regions of normal bladder tissue reveals the expected cell composition in each tissue layer, with contributions predominantly from epithelial cells, myofibroblasts, fibroblasts, and smooth muscle. Deconvolution was run using the SpatialDecon package with gene expression signatures of each cluster from normal bladder scRNA-seq data.

Spatial changes in gene expression in heterogeneous bladder tumors

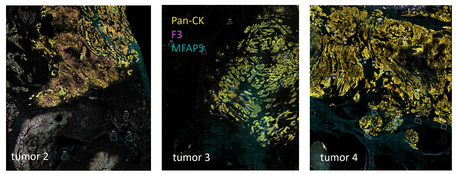


Figure 5. ROI selection in 4 high grade human bladder tumors. Polygon ROIs spanning different morphological regions of tumor and tumor adjacent normal tissue were selected. When possible, ROIs were segmented using epithelial, myofibroblast, and fibroblast markers as in normal bladder.

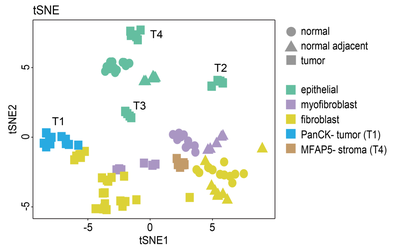


Figure 6. ROIs cluster by region and cell type. Tumor-adjacent normal tissue clusters separately from normal for epithelial and myofibroblasts, highlighting the differences between tumor adjacent normal and true normal bladder. In one tumor sample (T1) the tumor was PanCK-, and ROIs from this tumor cluster more closely with tumor stroma than epithelial tumors.

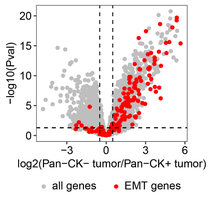
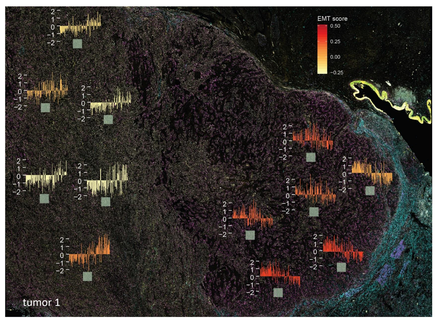


Figure 7. One tumor was PanCK- and heterogeneous across the tumor. Left: Compared to epithelial tumors, this sample highly expresses genes associated with epithelial to mesenchymal transition (EMT). The volcano plot shows differential expression between the PanCK- tumor and the other three tumors. Genes in red are in the GSEA hallmark EMT gene set. Bottom: ROIs from the right side of the PanCK- tumor, which appears more differentiated, have higher expression of most EMT genes than those on the poorly differentiated left side. A subset of EMT genes have higher expression on the left side. Relative expression of each EMT gene is plotted for each ROI and colored by the average EMT gene expression for that ROI. Genes are plotted in the same order for all ROIs, and are ordered by mean expression on the right side of the tumor.



Transcriptional changes in stroma

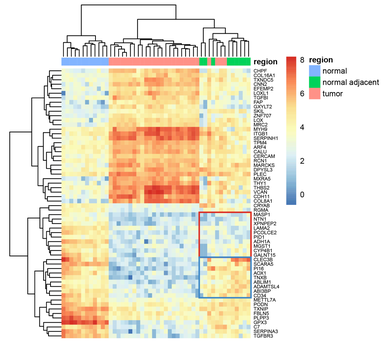


Figure 8. Identifying gene expression changes in fibroblasts in tumor stroma. Normalized log2 DSP counts of the top 30 upregulated and top 30 downregulated genes between normal and tumor are plotted for each fibroblast ROI, out of 2025 significantly differentially expressed genes total. Both genes known to be highly expressed in tumor stroma (such as FAP, CDH11, and TGFB1) and novel genes are differentially expressed. Expression of these DE genes in normal adjacent tissue is generally intermediate between tumor and normal. However, some clusters of genes have much more tumor-like expression in normal adjacent ROIs (red box) while other clusters are more normal-like (blue box).

Conclusions

- scRNA-seq identifies cell type-specific genes in normal human bladder that can be used as marker genes for spatial gene expression profiling by GeoMx DSP
- Whole transcriptomes of cell types profiled by DSP are highly correlated with the expected cell clusters from scRNA-seq, validating both methods
- We identify cell type-specific gene expression changes between normal bladders and bladder cancer, as well as spatial heterogeneity in gene expression signatures within an individual tumor
- Normal appearing regions adjacent to bladder tumors have abnormal transcriptional signatures, highlighting the importance of profiling truly healthy tissue
- Cell type deconvolution of DSP data can classify regions with mixed cell populations and spatially localize cell clusters identified by scRNA-seq

Acknowledgements

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