#123 Spatial Transcriptomic Profiling of Prostate Cancer Reveals Zone Specific Androgen Receptor Signaling and Tumor Immune Microenvironment

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Abstract

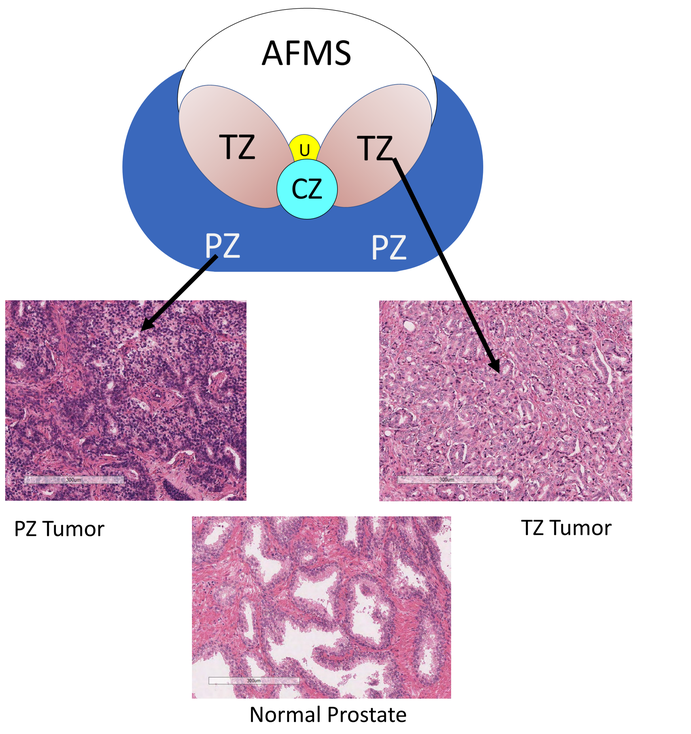
Background: Transitional zone prostate cancer (PCa) accounts for approximately 30% of disease, tends to have higher PSA and larger size, but lower odds of seminal vesicle invasion, extra-capsular extension, recurrence rates compared with peripheral zone (PZ). Underlying mechanisms for these differences are poorly understood. We performed spatial transcriptomic profiling to elucidate the biology of TZ and PZ PCa.

Methods: With samples from three PCa patients after radical prostatectomy (one each with PZ, TZ, and both PZ and TZ tumors), we used NanoString's Whole Transcriptome Atlas on the GeoMx® Digital Spatial Profiler (DSP) to assess gene expression in multiple regions of interest (ROI) per patient (42 total tumor ROIs). Morphology markers SYTO13 (nucleus), PanCK (epithelium), SMA (stroma), and CD45 (immune cells) were used to select ROIs. Raw counts for 17,128 genes were imported to R for downstream data analyses. Counts were Q3 normalized and scaled. We performed differential expression analysis using a linear model fit by empirical Bayes moderation, gene set enrichment analysis (GSEA) by cancer hallmarks, XCell gene sets for pathway enrichment and immune cell deconvolution using CIBERSORT.

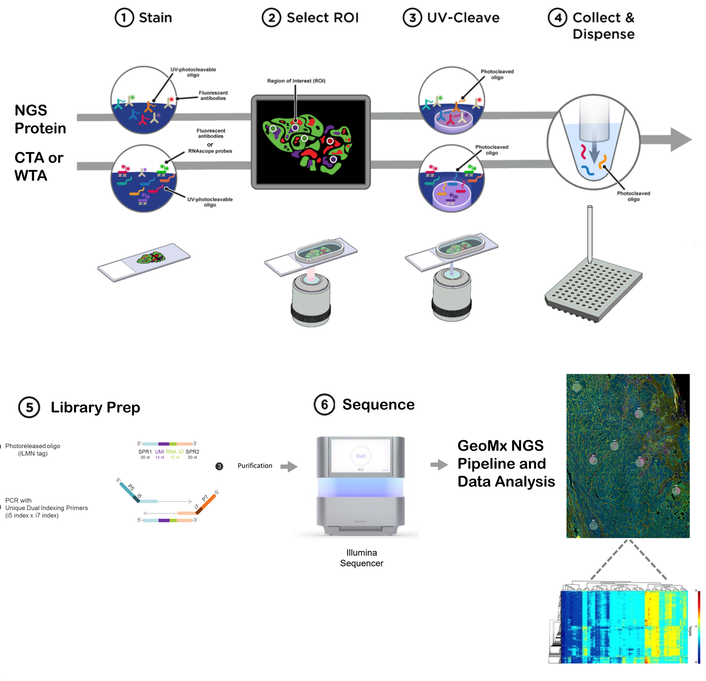
Results: There were grade group (GG) 4 (n=10) and 5 (n=10) tumors in PZ and GG 3 (n=10), 4 (n=11) and 5 (n=1) in TZ . We observed distinct gene expression profile between PZ (n = 20) and TZ (n=22) tumors. Androgen receptor (AR) signaling was significantly higher in TZ PCa ROIs compared to PZ in both GSEA (FDR < 5%) and the androgen subcomponent of the genomic prostate score (p<0.001), regardless of grade or cellular composition. CIBERSORT's absolute immune signature scores were computed for GG4 tumors and were significantly higher in PZ GG4 tumors compared to TZ GG4 tumors. Notably, CD4+ memory T cells were significantly higher in PZ GG4 regions compared to TZ GG4 tumor regions (p<0.05).

Conclusions: We identified higher AR signaling in TZ tumors, rationalizing historically observed higher PSA levels compared to PZ. We also observed increased immune infiltration within PZ microenvironment compared to TZ. Further studies will identify robust biomarkers to distinguish PZ from TZ and shed light on PCa immunomodulation, with potential immunotherapy implications.

Zones of Human Prostate and Prostate Function



GeoMx® Digital Spatial Profiling Technology Workflow With NGS Readout



Targeted Whole Transcriptome Profiling using GeoMx® DSP on Peripheral and Transitional Zone Tumors

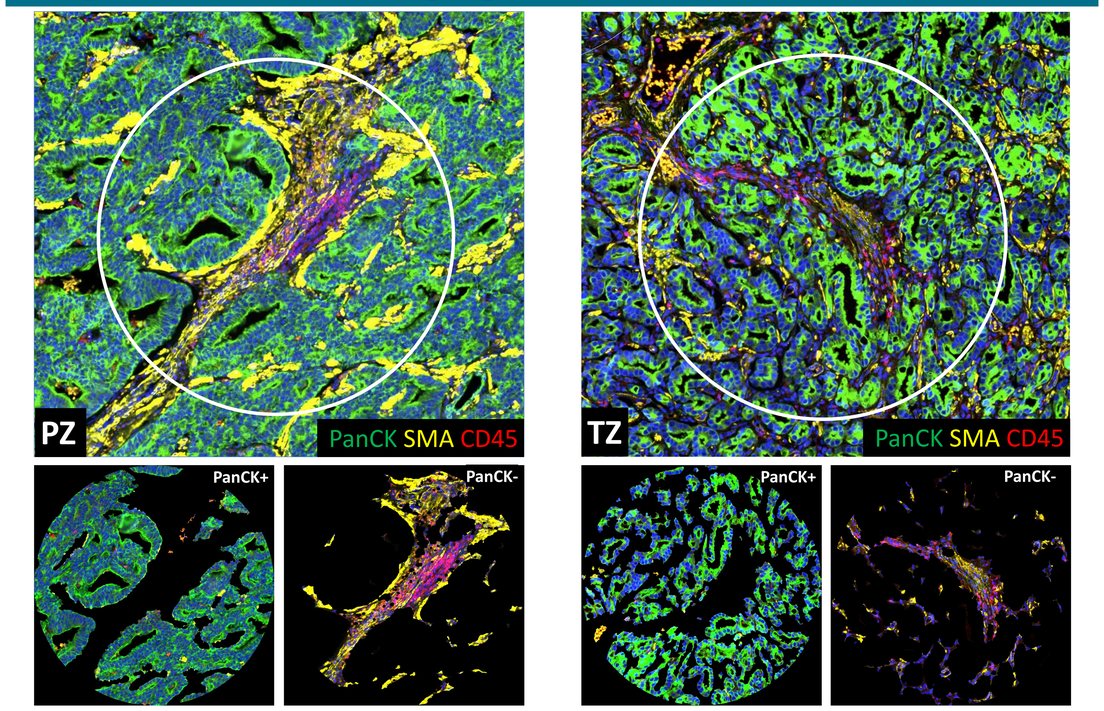


Figure 1. PZ and TZ grade 4 tumor and microenvironment. Representative ROIs (660 µ diameter) in the PZ and TZ tumors. Nuclei were visualized with Syto13 (blue). Tissue sections were labeled with Pan-cytokeratin (PanCK, green), α-SMA (yellow), and CD45 (red). ROIs were segmented into PanCK+ (tumor) and PanCK- (microenvironment with stroma and immune cells).

GSEA Analysis using MSigDB Cancer Hallmark Pathways plus Xcell Gene Sets

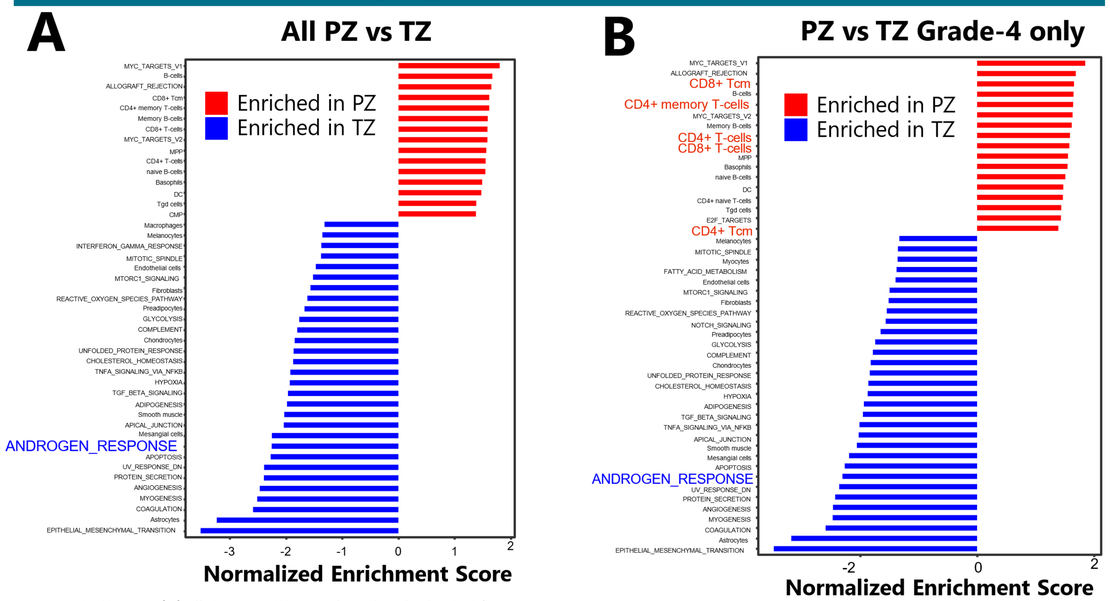
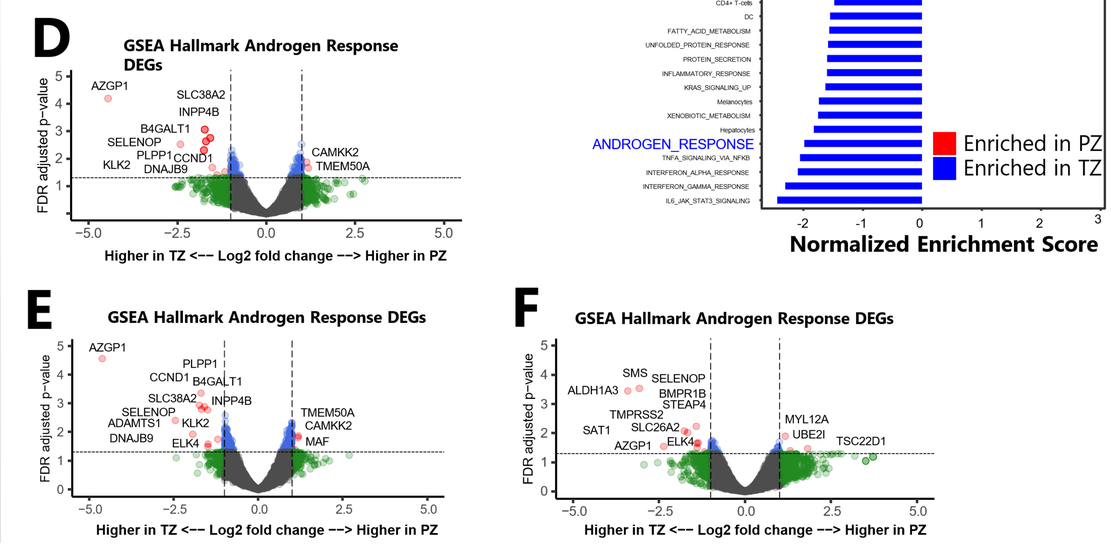


Figure 2. GSEA in **(A)** all PZ vs TZ ROIs combined and adjusted for grade; **(B)** PZ Grade-4 vs TZ Grade-4 ; and **(C)** PZ vs TZ within the same patient adjusted for grade. Androgen response pathway was significantly enriched in TZ across all three comparisons. Additionally, CD4+ memory and CD8+ T cells were also enriched in all PZ vs TZ and Grade-4 comparisons.

Differential Expression of Androgen Response Pathway Genes

Figure 3. Region-specific AR gene expression. Volcano plots display only significant genes from AR pathway across all three comparisons **(D)** – All PZ vs TZ adusted for grade **(E)** – PZ v TZ Grade 4 only; **(F)** PZ vs TZ same patient. AR regulator gene AZGP1 was consistently enriched in TZ in all comparisons. Another AR regulator KLK2 was only enriched in all PZ vs TZ and Grade-4 only comparisons.



Summary of differentially expressed genes by spatial location

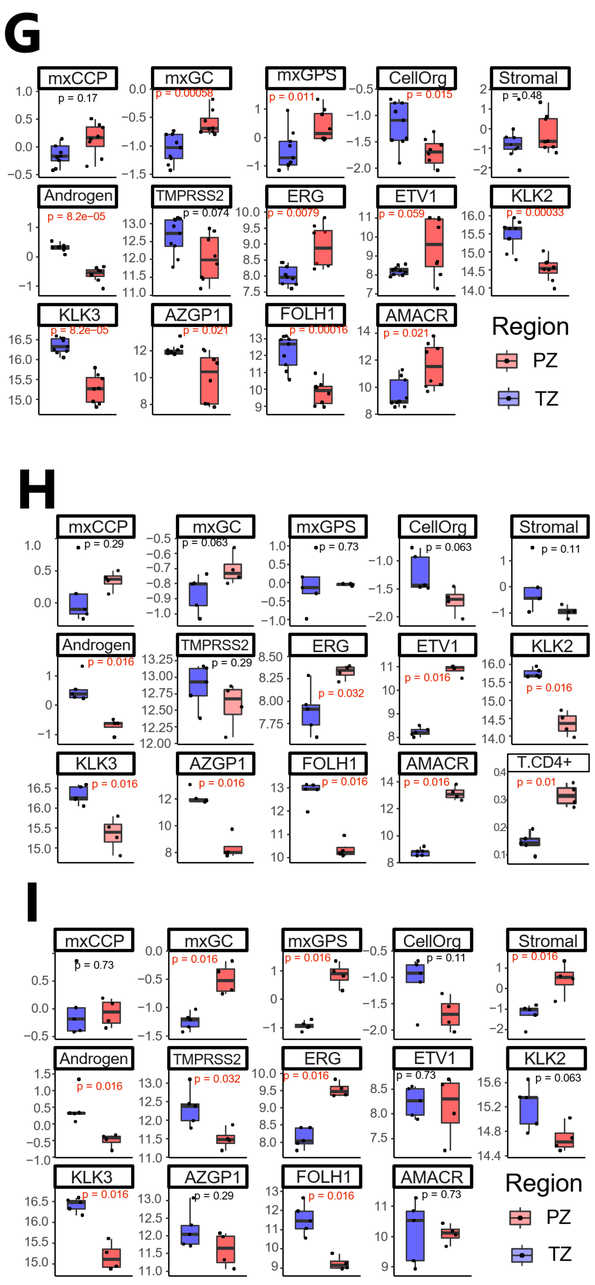


Figure 4. Boxplots display commercially derived prognostic scores (mxCCP, mxGC, mxGPS), mxGPS subcomponent scores (cellular organization, Stroma, Androgen), normalized expression of ETS fusion genes (TMPRSS2, ERG, ETV1), AR related genes (KLK2, KLK3, AZGP1) and PCa related genes (FOLH1, AMACR) between **(G)** All PZ vs TZ adjusted for grade **(H)** PZ v TZ Grade 4 only; **(I)** PZ vs TZ same patient. ETS fusion related genes, AMACR, decipher (mxGC) and mxGPS scores were significantly higher among PZ whereas androgen subcomponent score of mxGPS, FOLH1 and AR related genes were higher in TZ.

Results

1) Androgen Receptor (AR) signaling was significantly higher in TZ PCa ROIs compared to PZ ROIs in both GSEA (false discovery rate < 5%) and the androgen subcomponent of the genomic prostate score (p<0.001), regardless of grade, epithelial, stromal or immune component of the region.

2) CIBERSORT's absolute immune signature scores were found to be significantly higher in PZ GG4 tumors compared to TZ GG4 tumors. Notably, CD4+ memory T cells were significantly higher in PZ GG4 regions compared to TZ GG4 tumor regions (p<0.05).

Conclusions and next steps

- We observed higher AR signaling in TZ cancers and higher levels of immune infiltration on PZ cancers. This is in concordance with prior knowledge that TZ tumors may be associated with higher serum PSA and PZ tumors may be associated with inflammation. Further studies are needed to discern the biological and clinical significance of the different molecular features of PZ and TZ PCa.

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