

Title

Spatial Immunophenotyping of Matched Prostate Cancer Primary Tumors and Involved Lymph Nodes

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Background. Prostate cancer is a leading cause of cancer-specific mortality in men, and nodal metastasis is associated with distant progression and death. Prostate cancer is immunologically cold and resistant to checkpoint blockade, though newer immunotherapies show promise. The immune architecture of tumor-involved lymph nodes, and how it differs from the primary tumor, is poorly defined, including how regulatory T-cell infiltration and loss of cytotoxic T cells vary between patients. Clarifying this immune landscape has direct implications for immune-modulatory therapy in men with node-positive disease. Here we characterize the spatial immune landscape of matched primary tumors and involved lymph nodes.

Methods. Matched primary tumor and tumor-involved lymph node from 6 men with clinically localized prostate cancer treated with radical prostatectomy and lymphadenectomy were profiled with a 36-marker multiplex immunofluorescence panel (CODEX). After segmentation, marker intensities were scaled and cells were clustered into broad compartments, then sub-clustered to resolve individual cell types. A support vector classifier then assigned cell types across whole-slide images. Dendritic cell-CD8-CD4 triads were defined by proximity of all three cells within 25 μm , and each triad's distance to the nearest tumor cell was measured. Cellular neighborhoods were defined from each cell's 20 nearest neighbors and assessed relative to a kernel-density tumor boundary.

Results. Clustering resolved 13 immune, stromal, and tumor lineages, and a classifier extended these calls to the full dataset of 10.7 million cells. Immune infiltration varied widely in both the primary tumor (median 8.8%; range 2.4–14.5%) and involved lymph nodes (median 45.8%; range 12.9–59.8%). Dendritic cell-CD8-CD4 triads also varied markedly, particularly within involved nodes (median 254; range 69–3,475). Based on the distance from each triad to the nearest tumor cell, involved nodes separated into two phenotypes. An immune-excluded phenotype ($n=3$) had a mean triad-to-tumor distance of 1,245 μm (range 656–2,325 μm), whereas a non-excluded phenotype ($n=3$) had a mean distance of 32 μm (range 25–47 μm). Cellular neighborhood analysis stratified by phenotype showed that excluded nodes were enriched for regulatory T cells (mean 2,464 vs 571) and depleted of cytotoxic CD8 T cells (mean 24 vs 230).

Conclusions. Spatial immunophenotyping of matched primary tumor and involved lymph node shows that nodal metastases in prostate cancer are not uniformly immune-excluded. In these exploratory data, a subset of involved nodes show an immune-excluded phenotype, with tumor foci depleted of immune effectors, including dendritic cell-CD8-CD4 triads and CD8 T cells, and enriched for regulatory T cells; others show greater effector infiltration and fewer regulatory T cells. This heterogeneity may help explain the divergent outcomes of men with node-positive disease, and recognizing the excluded phenotype could identify patients who may benefit from immune-modulatory therapy. These findings are limited by small sample size and are being validated in an expanded cohort.

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