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Background

Immune checkpoint inhibitors have transformed the treatment of many solid tumors, yet high-risk localized prostate cancer (HRLPC) remains largely refractory to immunotherapy. This limited benefit reflects both a scarcity of predictive biomarkers and an incomplete understanding of prostate cancer immunogenicity. We previously identified a subset of HRLPC tumors with dense lymphocytic infiltration associated with PTEN loss. Here, we sought to define the epithelial programs and immune architecture underlying this inflammatory phenotype and assess their potential relevance to immunotherapy response.

Methods

Single-nucleus RNA and chromatin-accessibility profiles were generated from treatment-naïve prostate cancer biopsies using the 10x Genomics Multiome RNA and ATAC platform under NIH Institutional Review Board protocol 16-C-0010. The spatial organization of PTEN-loss tumors was evaluated using Xenium spatial transcriptomic profiling with the human 5K gene panel on two tissue microarrays containing sixteen 4-mm cores. Cells were annotated with SingleR using integrated public and immune single-cell reference datasets. Multiplex immunofluorescence was performed using the OPAL system with antibodies against CD11c, TCF1, CD4, CD19, CD8, PSA, and ELF3. To functionally evaluate ELF3, retinoic-acid-treated RWPE-1 prostate epithelial cells were transfected with ELF3-targeting or scrambled-control siRNA, followed by quantitative PCR analysis of ELF3 and PIGR expression.

Results

Integrated spatial transcriptomic, single-nucleus multiomic, and multiplex imaging analyses identified ELF3 as a candidate regulator of a club-like epithelial phenotype present in both PTEN-deficient tumor cells and benign club-like epithelium. This ELF3-associated state was linked to increased immune recruitment within the tumor and surrounding microenvironment. Xenium profiling revealed diverse immune populations near club-like epithelial regions, including TCF1+ progenitor-like CD4+ T cells, T helper 17 cells, B cells, and myeloid dendritic cells. These populations were frequently organized into lymphoid aggregates resembling nascent tertiary lymphoid structures. RNA-interference experiments supported a direct or indirect role for ELF3 in regulating the club-like marker PIGR. In an immune checkpoint inhibitor-treated metastatic castration-resistant prostate cancer cohort, elevated PIGR transcript levels or PTEN loss were associated with improved overall and progression-free survival.

Conclusions

These findings identify an ELF3-associated club-like epithelial program linked to immune-cell recruitment and lymphoid organization in prostate cancer. PIGR and PTEN-related signatures may help identify immune-engaged prostate tumors and warrant further evaluation as biomarkers for immunotherapy stratification.

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Conflicts of Interest Disclosure Statement: None