Single Cell Analysis Reveals Upregulation of Immune Stimulatory Pathways in Quiescent Metastatic Castration Resistant Prostate Cancer Cells

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Background: Quiescent, or G_0 cell cycle phase, cells are common in most prostate cancer (PCa) cases, yet are relatively resistant to many treatments, especially cytotoxic chemotherapy. They also play key roles in tumorigenesis and dormancy and recurrence after curative intent therapy but are difficult to identify when viable.

Methods: To identify characteristics of quiescent prostate cancer cells, we used previously published our FACS methods to isolate cancer cells from bone or bone marrow of eight metastatic castration resistant prostate cancer (mCRPC) patient samples and generated single cell RNA sequencing (scRNA-seq) gene expression data of individually sorted cells. We also generated scRNA-seq data from six PCa cell lines and two normal (cancer free) bone marrow samples as positive and negative controls for cell identification. We eliminated *a priori* any potentially contaminating cells based on tSNE cluster location, expression of a 12 gene panel of PCa markers, and lack of expression of bone marrow markers (*PTPRC* / CD45, and *GYPA* / CD235a), leaving 297 high confidence PCa cells.

Results: To identify characteristics of G_0 cells, we separated the patient PCa cells into quiescent and cycling groups based on expression of *MKI67* (Ki67). GSEA analysis using KEGG pathway groups revealed enrichment of primarily immune related pathways in quiescent cells with the top 3 pathways; "allograft rejection," "autoimmune thyroid disease," and "intestinal immune network for IgA production." Similarly, for GSEA analysis using gene ontology (GO) groups, "beta-2-microglobulin binding" was the second most enriched group in quiescent cells. Type 1 HLA genes were common among multiple pathway groups suggesting potential differences in antigen presentation. To validate these findings, we analyzed an external dataset of scRNA-seq of whole bone marrow from vertebrae of patients with metastatic PCa (Kfoury et al, Cancer Cell 2021). We selected PCa cells using UMAP clustering and the 12 gene PCa signature. Using Ki67 expression to separate cells into quiescent vs. cycling groups and GSEA analysis, we again found increased expression of Mhc and HLA genes in quiescent PCa cells, though in this dataset, HLA class 2 genes were also enriched. In subsequent *in vitro* experiments, induction of quiescence in LNCaP, C4-2B, PC3, and RM1 PCa cell lines by serum deprivation or CDK4/6 inhibition increased cell surface HLA class 1 or Mhc-1.

Conclusions: Overall, these results suggest that antigen presentation is increased in quiescent PCa cells, which may be important for their elimination by the immune system or useful for future immunotherapy approaches.

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