Heterogenous activation of immune suppressive pathways revealed by scRNAseq may underscore resistance to PD-1 therapy in metastatic castration resistant prostate cancer

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Metastatic castration resistant prostate cancer (mCRPC) is a deadly disease that until recently, was thought to be resistant to checkpoint blockade. A recent clinical trial revealed that pembrolizumab when given in combination with the androgen deprivation therapy (ADT) drug enzalutamide, achieved an unprecedented 18% complete biochemical response rate in mCRPC patients. Though promising, ~80% of these patients failed to respond and ultimately died of their disease and the mechanisms of resistance remain unclear. The immunological landscape of mCRPC is largely unknown and revealing this heterogeneity is difficult due to limited amounts of patient biopsy material. To better understand the underlying mechanisms of response or resistance in this patient population, we evaluated the expression of PD-L1 by immunohistochemistry in patient biopsies and observed no significant expression. Therefore, to reveal alternative pathways of resistance, we developed a protocol for isolating leukocytes from fresh core-needle biopsies taken from enzalutamide resistant metastatic lesions prior to and after exposure to pembrolizumab. Biopsies underwent gentle enzymatic and mechanical dissociation prior to leukocyte enrichment and single cell gel emulsion capture, cell and gene barcoding, and reverse transcription. Libraries were sequenced at greater than 10,000 reads per cell and computational algorithms applied to identify leukocyte subsets.

The abundance of tumor-infiltrating leukocytes was on average less than 1% of total cells independent of biopsy location. After leukocytes underwent single-cell RNA sequencing, we developed a leukocyte gene signature of enzalutamide resistant metastatic prostate cancer and used this to deconvolute bulk RNA sequencing data from prior biopsies. Using this assay, we have analyzed more than 25,000 leukocytes from more than 10 patients including responders and non-responders to pembrolizumab. Computational approaches revealed the diverse cellular heterogeneity of mCRPC across patients. Consistent with our protein analysis of PD-L1, minimal PD-L1 mRNA was detected on leukocytes or tumor cells in most patients despite some patients responding to anti-PD-1 therapy. To determine if the absence of PD-L1 was restricted to the metastatic lesions, we obtained hormone therapy naïve primary prostate cancer core needle biopsies for single-cell RNA sequencing. Consistent with the metastatic niche, minimal PD-L1 mRNA was detected in the primary disease. In contrast, inhibitory pathways beyond PD-1 were revealed by our single-cell analysis. We developed orthotopic mouse models of mCRPC to validate these targets in mice treated with ADT and to reveal new clinical therapies to enhance anti-tumor responses in patients with mCRPC.

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