PTEN/P53 Altered Prostate Cancer Has Unique Role for Tumor-Associated Macrophages Upon Androgen Signaling Inhibitor Resistance

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Background

Metastatic Castration-Resistant Prostate Cancer (mCRPC) is incurable and inevitably develops resistance after treatment of androgen signaling inhibitors (ASIs). This resistance and subsequent poor outcome in patients are frequently associated with genetic alteration in two or more well-known tumor suppressors such as PTEN, P53, and/or RB1. "Aggressive Variant Prostate Cancer (AVPC)", which occupies over 20% of mCRPC and is characterized by high lethality, defined by possession of PTEN, RB1, or TP53 alteration. RB1/TP53 mutant tumors have been explored variously; however, PTEN/TP53 mutant tumors are less well characterized despite their greater prevalence. We hypothesized that two genetically different prostate cancers have distinct mechanisms in tumor development and ASI resistance. We organized an *in vitro* mouse organoids model and an *in vivo* organoids-injected mouse model to examine mechanism differences in cancer cell-intrinsic and extrinsic levels through single-cell RNAseq analysis.

Methods

We derived normal prostate organoids from FVB mice and modeled genetic alterations by double CRISPRn knockout ($Pten^-/Trp53^-$ or $Rb1^-/Trp53^-$). These "pre-injected organoids" were injected subcutaneously into syngeneic immunocompetent FVB mice, resulting in tumor development. After detecting tumors, half were treated simultaneously with degarelix and apalutamide as ASIs. RNA was extracted from both the pre-injected organoids and the corresponding tumors for further single-cell RNAseq (scRNAseq) analysis.

Results

The results of scRNAseq analysis indicated that $Pten^-/Trp53^-$ and $Rb1^-/Trp53^-$ tumors had distinct cell type populations through pre-injected organoid cells, non-immune tumor cells, and immune cells. After re-clustering these three different cell populations, we realized genetically enriched sub-clusters in cancer cells and pre-injected organoid cells, confirming their mutually exclusive characteristics. The exclusiveness between genotypes in pre-injected organoid cells and cancer cells suggested that the cell-intrinsic mechanisms in tumorigenesis and ASI resistance occurred differently through $Pten^-/Trp53^-$ or $Rb1^-/Trp53^-$ alteration and manifested in different subtypes of prostate cancer.

We also observed cancer-cell extrinsic effects through immune cells on tumorigenesis and ASI resistance. Macrophages were dominant immune cell types of both $Pten^-/Trp53^-$ and $Rb1^-/Trp53^-$ tumors, and interestingly, some sub-populations of macrophages were highly enriched in ASI-treated $Pten^-/Trp53^-$ tumors. According to the cell-cell interaction analysis, these M2-polarized macrophages with $Cd14^+/Apoe^+/Mrc1^+/MHC\text{-}II^-$ had characteristically inferred ligand-receptor pair interactions to $Pten^-/Trp53^-$ and/or $Rb1^-/Trp53^-$ cancer cells such as APP-TREM2, VEGFA-NRP1/NRP2 and Cd47-SIRPα related to immune evasion phenotypes.

Conclusions

PTEN/TP53 and RB1/TP53 alterations exhibited differences in prostate cancer tumorigenesis and ASI resistance through both cancer-cell intrinsic and immune cell-based effects. The two genetic alterations led to the development of distinct cancer cell subtypes, and tumors with PTEN/TP53 alterations were

significantly enriched in M2-polarized macrophages, which have been reported to play a role in immunosuppression, upon ASI treatment. Some ligand-receptor pair interactions are inferred to influence tumorigenesis and ASI resistance in PTEN/TP53 and/or RB1/TP53 altered prostate cancers, but further investigation is required.

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Conflicts of Interest Disclosure Statement

All authors declare no competing interests.