Identification and therapeutic target of myeloid-mediated mechanisms of immunotherapy resistance in prostate cancer

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Background: Patients with metastatic castration-resistant prostate cancer (mCRPC) are generally resistant to immune checkpoint inhibitors (ICIs)[1,2] due in part to the presence of immunosuppressive myeloid cells within tumors.[3] CSF1R blockade and other broad targeting approaches have faced clinical challenges[4–6] due to the heterogeneous nature of myeloid cells. Therefore, we hypothesize that a comprehensive understanding of distinct immunosuppressive myeloid subsets and their molecular mechanisms at the single-cell level is essential for enhancing treatment efficacy.

Methods: We performed single-cell analysis of patient biopsies from various stages, including localized disease, metastatic hormone-sensitive prostate cancer, or mCRPC. These findings were reverse-translated into a syngeneic mouse model of prostate cancer, where we performed multi-omic single-cell profiling, functional assays, tumor efficacy tests, and mechanistic studies.

Results: Single-cell analysis of patient biopsies identified a distinct subset of tumor-associated macrophages expressing elevated *SPP1* transcripts (*SPP1*^{hi}-TAMs), which were more abundant in mCRPC compared to earlier stages. These macrophages exhibited elevated immunosuppressive molecular programs[7–9] and significantly lower CSF1R transcript levels than other macrophages, potentially explaining the limited clinical efficacy of CSF1R inhibition. Multi-omic profiling of the mouse model revealed an analogous macrophage subset that suppressed CD8$^+$ T cell activity in co-culture. Adoptive transfer of *Spp1*^{hi}-TAMs into CRPC increased the frequency of exhausted CD8$^+$ T cells, leading to resistance to ICIs and worsened survival outcomes. Pathway analysis identified adenosine signaling as a key mechanism of immunosuppression[10,11]. Pharmacologic inhibition of adenosine receptors with ciforadenant reduced *Spp1*^{hi}-TAM-mediated CD8$^+$ T cell suppression *in vitro* and improved the sensitivity of CRPC cells to PD-1 blockade *in vivo*. Notably, adenosine receptor blockade decreased *Spp1*^{hi}-TAM prevalence, suggesting a shift toward a less immunosuppressive myeloid landscape. Moreover, combined blockade of adenosine receptors and PD-1 significantly increased the density of polyfunctional CD8$^+$ T cells within the tumors. Clinical validation demonstrated that mCRPC patients receiving a combination of adenosine receptor inhibition (ciforadenant) and PD-L1 blockade (atezolizumab) exhibited improved anti-tumor efficacy compared to those on monotherapy.

Conclusions: Our studies reveal a significant increase in immunosuppressive *SPP1*^{hi}-TAM prevalence as prostate cancer progresses to lethal stages. These macrophages play a critical role in resistance to ICIs through adenosine signaling. Our findings highlight the potential of targeting these cells and their signaling pathways as promising therapeutic strategies.

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