Myeloid-Derived Suppressor Cells Inhibit T Cell Activation in Prostate Cancer through Nitrating LCK

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Background: Patients with advanced prostate cancer (PCa) benefit from androgen deprivation therapy initially, but often later develop lethal metastatic castration-resistant prostate cancer (mCRPC). Immune checkpoint blockade (ICB) using antibodies against CTLA4 or PD1/PD-L1 generates durable therapeutic responses in a variety of cancer types. However, mCRPC showed overwhelming de novo resistance to ICB. Our previous publications established that myeloid-derived suppressor cells (MDSCs) are the key player in PCa immune evasion, and targeting MDSCs with multikinase inhibitors or CXCR2 inhibitors could synergize with ICB to elicit potent anti-mCRPC effect (*Cancer Discovery, 2016; Nature, 2017*). More effective and specific MDSC inhibition relies on deeper mechanistic understanding of MDSCs. One main mechanism for MDSCs to induce T cell tolerance is through secretion of reactive nitrogen species (RNS, e.g. peroxynitrite). However, this mechanism remains poorly understood and very few nitrated proteins are known.

Methods: We developed a new nitroproteomic approach for the identification of nitropeptides from cells and tissues (*JoVE.* 2019. PMID: 31259896). The biological models we used for target identification and functional validation are *Pten/p53/Smad4* PCa transgenic model and syngeneic Lewis Lung Carcinoma (LLC) model.

Results: We identified that lymphocyte-specific protein tyrosine kinase (LCK), an initiating tyrosine kinase in the T cell receptor signaling cascade, is nitrated at Tyr394 by MDSCs. LCK nitration inhibited T cell activation, leading to reduced interleukin-2 production and proliferation. In human T cells with defective endogenous LCK, wild type, but not nitrated LCK, rescued IL2 production. We documented elevated 3-nitrotyrosine signals in clinical samples of CRPC. In both the Pten/p53/Smad4 mCRPC model and LLC model, we showed that ICB therapy (PD1 and CTLA4 antibody cocktail) elicited strong anti-tumor efficacy when combined with a RNS neutralizing agent.

Conclusions: We have identified a previously unknown mechanism of T cell inactivation by MDSC-induced protein nitration and illuminated a clinical path hypothesis for combining ICB with RNS-reducing agents in the treatment of mCRPC. Our study has been recently published (Feng, et al. *PNAS.* PMID: 30232256)

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