Systemic administration of CpG-STAT3 antisense oligonucleotides induces regression of two bone-localized prostate tumor models

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Background: Signal Transducer and Activator of Transcription 3 (STAT3) is an oncogenic transcription factor, which plays important role in both prostate cancer progression and in sustaining immune-suppression in the tumor microenvironment. We previously demonstrated that Toll-like Receptor 9 (TLR9) ligands allow for targeted delivery of oligonucleotides to $TLR9^+$ cells in prostate tumors, such as cancer stem-like cells and tumor-associated myeloid immune cells.

Methods: Here, we describe new strategy to deliver nuclease-resistant STAT3 antisense oligonucleotides (ASO) to bone-localized prostate cancer. Tethering TLR9 agonist (CpG-ODN) to STAT3 ASO permits internalization of the CpG-STAT3ASO conjugate by $TLR9^+$ human and mouse cells without transfection reagents.

Results: We demonstrate that CpG-STAT3ASO is internalized by polymorphonuclear myeloid-derived suppressor cells (PMN-MDCSs) derived from blood of prostate cancer patients, as well as human (DU145, PC3) and mouse (Myc-CaP, RM9) prostate cancer cells. Compared to the STAT3ASO alone, CpG-STAT3ASO had improved potency and accelerated kinetics of target gene knock down at mRNA and protein levels. The biodistribution studies in mice showed that systemic *i.v.* injections of CpG-$STAT3ASO^{Cy3}$ effectively targeted prostate tumor-associated myeloid cells, such as dendritic cells and macrophages. For efficacy studies, we used two genetically different models of mouse castration-resistant prostate tumors implanted intratibially: Ras-/Myc-driven RM9 and *Pten*$^{pc-/-}$*Smad4*$^{pc-/-}$*Trp53*$^{c-/-}$ (PST). Repeated *i.v.* injections of unformulated CpG-STAT3ASO (5 mg/kg/every other day), but not the unconjugated CpG ODN or STAT3ASO alone, induced regression of bone-localized tumors in the majority of treated mice independently from cancer genetics. Antitumor effects of CpG-STAT3ASO resulted primarily from the potent immune responses and thus were not observed in immunodeficient NSG mice. In immunocompetent mice, CpG-STAT3ASO treatment reduced STAT3 activity in both cancer cells and in tumor-associated immune cells, thereby reducing PD-L1 levels on $CD11b^+Gr1^+$ MDSCs together with the percentage of $CD4^+FoxP3^+$ regulatory T cells. Our *ex vivo* studies on CRPC patients' derived PMN-MDSCs support translational potential of this approach. In contrast to CpG ODN and STAT3ASO alone, CpG-STAT3ASO conjugate reduced immunosuppressive potential of primary PMN-MDSCs, thereby restoring proliferation and activity of co-cultured T cell.

Conclusions: By targeting STAT3 mainly in the prostate tumor microenvironment, CpG-STAT3ASO provides broader and more efficient strategy for the treatment of genetically diverse metastatic prostate cancers.

The authors declare no conflict of interests.

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