In vivo programming of myeloid cells by mRNA mediated delivery of novel Fcα fusion receptor activates anti-tumor immunity

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Abstract

Immunotherapy has revolutionized cancer treatment. However, for the majority of patients with advanced solid tumors, sustained clinical benefit has yet to be achieved. Myeloid cells such as monocytes and macrophages readily accumulate in tumors, in some cases contributing up to 75% of the tumor mass. Reprogramming circulating and tumor associated myeloid cells to activate their ability to elicit anti-tumor adaptive immunity by phagocytosis, cytokine secretion and antigen presentation is an attractive approach to harness and orchestrate systemic anti-tumor immunity. It remains challenging to specifically target and activate myeloid cells in vivo. To overcome this hurdle, we have developed a novel in vivo myeloid cell engineering platform: Fcα Receptor Fusion Constructs. Unlike other chimeric antigen receptors (CARs), the construct was engineered by fusing a tumor recognition scFv with the alpha chain of human Fc receptors. The stable expression and function of these receptors requires endogenerously expressed Fc receptor gamma chain, a protein with limited expression to immune cells, mostly myeloid cells1-3. Here, we present that intravenous infusion of liposome-nanoparticle (LNP) encapsulating the Fcα Receptor Fusion Construct mRNA results in the uptake and expression of the construct by myeloid cells. In immunodeficient xenograft models of hepatocellular carcinoma and triple negative breast cancer, delivery of LNP mRNA encoding for GP3 or TROP2 targeted Fcα Receptor Fusion Constructs resulted in tumor killing, confirming the ability of this approach to program myeloid cells. Furthermore, in the B16 syngeneic melanoma model, treatment with the melanoma antigen GPT5 targeted Fcα Receptor Fusion Construct was also associated with the initiation of broad systemic immune responses characterized by tumor accumulation of activated CD8+ T cells, reduced tumor associated Tregs and activation of antigen presenting cells in spleen. Together these studies highlight the potential of Fcα Receptor Fusion Construct delivered directly in vivo to program myeloid cells to recognize and kill cancer.

Introduction

Figure 1. Schematic of Fcα fusion construct delivery and expression method. Fcα fusion constructs are designed by fusing a tumor binding scFv with the human Fc receptor α chain (CD89). mRNA encoding the construct is formulated in LNP and delivered directly in vivo. Fcα fusion construct forms a multi-chain complex with endogenerously expressed Fcα chain in myeloid cells, which is also required for its stable cell surface expression. This fully assembled Fcα fusion construct can now recognize tumor cell surface target and activate myeloid cells’ anti-tumor activity.

Results

Figure 3. ULP mediated delivery and expression of GPT5 targeted Fcα fusion construct in tumor model. ULP was injected into B16 tumor bearing mice. At 24 h after dosing of tumor bearing animal with GPT5 ULP (Day 16 in Figure 3B), animals were sacrificed and tumor were isolated and dissociated into single cell suspensions, which was stained with fluorescent antibodies. Percentage of activated CD8+ T cells (CD45+ CD3+ CD8+ IFNγ+ CD25+), NK cells and myeloid cells (CD11b+ MHCII+). Monocytes are CD11b+ CD45+ CD11c+; Macrophages are CD45+ CD11c+ F4/80+; T cells are CD45+ CD3+ CD8+.

Figure 4. GPT5-CD89 treatment was associated with reduced Treg frequency in tumor microenvironment. A) After dosing of GPT5 CD89 ULP i.v., CD4+ T cells were isolated and analyzed for frequency of FoxP3+ Tregs. B) Treatment with GPT5-CD89 ULP significantly reduced the percentage of CD8+ T cells which are PD1+ CD103+. C) Treatment with GPT5-CD89 ULP significantly reduced the percentage of activated CD8+ T cells (CD45+ CD3+ CD8+ IFNγ+ CD25+) and macrophages (CD45+ CD11c+ F4/80+).

Figure 5. Treatment with GPT5-CD89 ULP was associated with a reduction of the amount of exhausted CD8 T cells in tumor. A) Treatment with GPT5-CD89 ULP significantly reduced the percentage of exhausted CD8 T cells (CD45+ CD3+ CD8+ PD1+ CD103+) in tumor. B) Treatment with GPT5-CD89 ULP significantly reduced the percentage of exhausted CD8 T cells which are PD1+ TIM3+.

Figure 6. Treatment with GPT5-CD89 ULP was associated with increased expression of markers of CD8+ T cells activation in tumor. A) Treatment with GPT5-CD89 ULP significantly increased the percentage of activated CD8 T cells (CD45+ CD3+ CD8+ IFNγ+ CD25+), macrophages (CD45+ CD11c+ F4/80+) and exhausted CD8 T cells, proliferation of higher cytolytic function.

Conclusions

• We designed a novel chimeric antigen receptor by fusing a tumor binding scFv domain with the human Fcα receptor α chain (CD89). Stable expression of the scFv-Fcα fusion requires endogenerous Fc receptor α-chain which is only present in immune cells, particularly myeloid & NK cells.
• Intravenous delivery of GPT5-FoS mRNA-LNP resulted in potent anti-tumor activity in the B16 melanoma mouse model. Specific expression of Fcα fusion construct in tumor myeloid and NK cells was observed.
• Delivery of Fcα fusion construct resulted in significant changes in tumor microenvironment, including reduced frequencies of Tregs and exhausted CD8 T cells. Conversely an increase of activated cytolytic CD8 T cells was also observed, suggesting a robust anti-tumor CTL response. These data together shows the ability of in vivo programmed myeloid cells to orchestrate broad immune responses.
• In immunodeficient xenograft models of GP3+ hepatocellular carcinoma and TROP2+ triple negative breast cancer, delivery of mRNA-LNP encoding GP3 or TROP2 targeted Fcα Receptor Fusion Constructs showed significant anti-tumor activity, showing the direct activity of in vivo programmed myeloid cells to kill tumors.

References

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