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 endogenously expressed Fc receptor gamma chain, a protein with limited expression to immune cells, mostly myeloid cells[1-3]. Here, we present that intravenous infusion of lipid-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 GPC3 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 GP75 targeted Fcα Receptor Fusion Construct was also associated with the initiation of broad systemic immune responses, characterized by tumoral 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

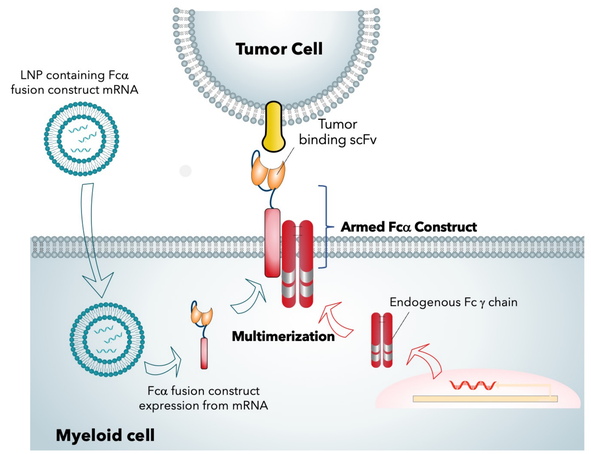

Figure 1. Schematic of Fcα fusion construct delivery and expression method. Fcα fusion constructs are designed by fusing a tumor binding scFv with the human Fc alpha receptor α-chain (CD89). mRNA encoding the construct is formulated in LNP and delivered directly in vivo. Fcα fusion construct forms a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

Results

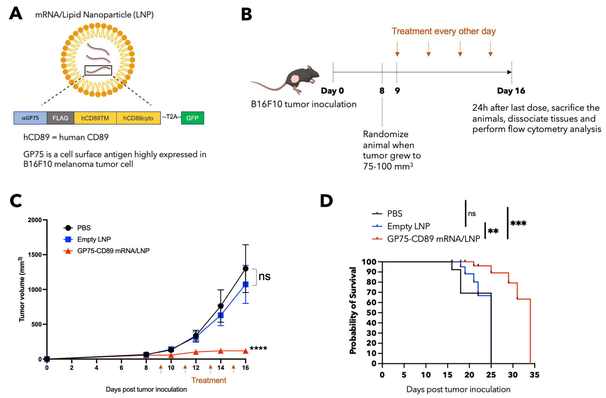

Figure 2: GP75 targeted Fcα fusion construct shows anti-tumor activity in syngeneic B16 melanoma model. (A) Diagram of GP75 targeted Fcα fusion construct (GP75-CD89) . FLAG tag within construct and GFP enables determination of construct expression and LNP delivery, respectively. (B) Animal study and treatment design. LNP was dosed every other day at 2 mg/kg body weight. (C) Treatment with GP75-CD89 LNP significantly reduced tumor growth compared to empty LNP and PBS controls. (D) Treatment with GP75-CD89 LNP significantly improved animal survival.

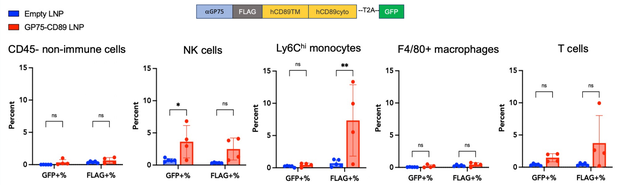

Figure 3: LNP mediated delivery and expression of GP75 targeted Fcα fusion construct in tumor leukocytes. 24 h after dosing of tumor bearing animal with GP75-CD89 LNP (Day 16 in Figure 1C), animals were sacrificed and tumor were isolated and dissociated into single cell suspension, which was stained with fluorescent antibodies. Percentage of expression of GP75-CD89 receptor (FLAG) and LNP (GFP) were analyzed by flow cytometry. Non-immune cells are CD45-; NK cells are CD45+ NK1.1+; Monocytes are CD45+ CD11b+ Ly6Chi; Macrophages are CD45+ CD11b+ F4/80+; T cells are CD45+ CD3+

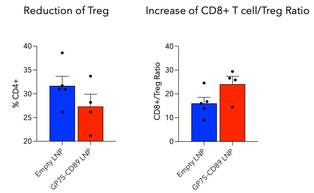

Figure 4: GP75-CD89 treatment was associated with reduced regulatory T cells in tumor microenvironment. 24 h after dosing of tumor bearing animal with GP75-CD89 LNP (Day 16 in Figure 1C), animals were sacrificed and tumor were isolated and dissociated into single cell suspension, which was stained with fluorescent antibodies. Treg are CD45+ CD3+ CD4+ CD25+Foxp3+; CD8 T cells are CD45+CD3+CD8+.

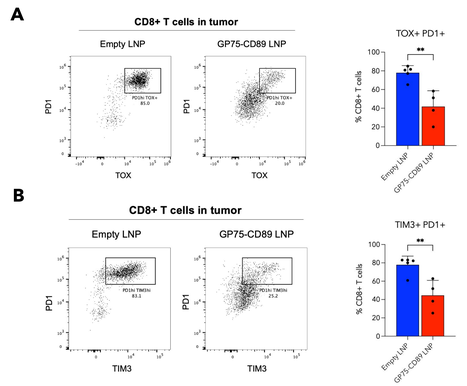

Figure 5: Treatment with GP75-CD89 LNP was associated with a reduction of the amount of exhausted CD8 T cells in tumor. (A) Treatment with GP75-CD89 LNP significantly reduced the percentage of exhausted CD8 T cells which are PD1^{hi} TOX+ (B) Treatment with GP75-CD89 LNP significantly reduced the percentage of exhausted CD8 T cells which are PD1^{hi} TIM3+

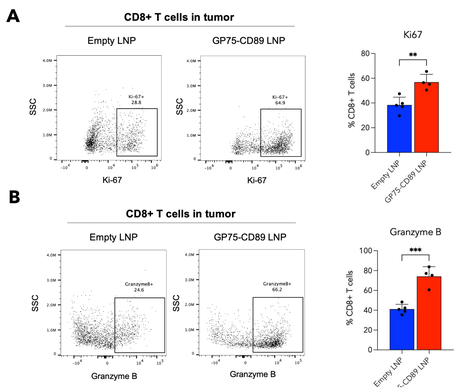

Figure 6: Treatment with GP75-CD89 LNP was associated with increased expression of markers of CD8 T cells activation in tumor. (A) Treatment with GP75-CD89 LNP significantly increased the percentage of activated CD8 T cells (Ki67+ CD8+) (B) Treatment with GP75-CD89 LNP significantly increased the percentage granzyme B positive CD8 T cells, indicative of higher cytolytic function.

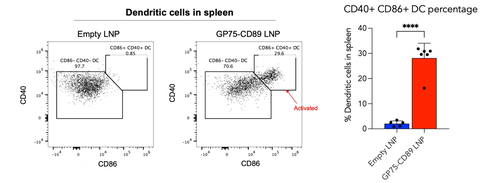

Figure 7: Treatment with GP75-CD89 LNP was associated with increased frequency of activated dendritic cells in the spleen. Splenocytes were isolated and analyzed by flow cytometry. DC were CD45+CD11c+MHCII+. CD40 and CD86 expression indicates activation of dendritic cells in spleen.

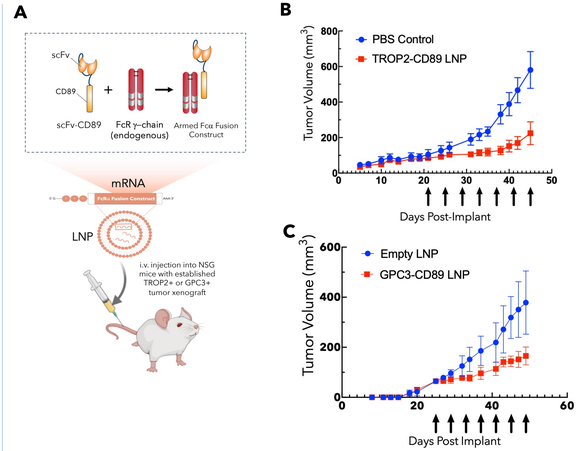

Figure 8: Fcα fusion construct delivered via LNP shows anti-tumor efficacy in liver and breast cancer xenograft models (A) scFv against cancer associated antigen TROP2 or GPC3 were fused to CD89 and formulated into mRNA/LNP for delivery via i.v. injection (B) HCC1964 cells were subcutaneously inoculated to NSG mice. 21 days later when tumor volume reached 50-190 mm3, TROP2-CD89 LNP was administered at 2 mg/kg, i.v. A total of 7 treatments were made every 4 days. (3) HepG2 cells were subcutaneously inoculated to NSG mice. 24 days later when tumor volume reached 50-190 mm3, GPC3-CD89 LNP was administered at 2 mg/kg, i.v. A total of 7 treatments were made every 4 days

Conclusions

- We designed a novel chimeric antigen receptor by fusing a tumor binding scFv domain with the human Fc receptor α chain (CD89). Stable expression of the scFv-CD89 fusion requires endogenous Fc receptor γ-chain which is only present in immune cells, particularly myeloid & NK cells.
- Intravenous delivery of GP75-Fcα 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 GPC3+ hepatocellular carcinoma and TROP2+ triple negative breast cancer, delivery of mRNA/LNP encoding GPC3 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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