Antagonizing the SIRPα–CD47 pathway is gaining traction as an effective and novel approach to immune manipulation as design of immunotherapeutics broadens to include blockade of innate immune checkpoints. Recently, the combination of tumor-targeting antibodies with SIRPα–CD47 blockade has provided promising clinical results, suggesting that increased phagocytosis of cancer cells is clinically relevant for treatment of hematologic cancers.1 However, the ability for this combination to enhance phagocytosis in the context of solid tumors may be remarkably diminished for several reasons including reduced expression of "eat-me" signals like SLAMf7, increased immune suppression in the tumor microenvironment (TME), and the physical size of tumor cells when adhered in a complex heterogeneous environment. To achieve efficacy in solid tumor indications, it is reported that therapies blocking the SIRPα–CD47 axis also potentiate adaptive immune mechanisms and not solely phagocytosis.

METHODS

Subcutaneous mouse tumor models and a mouse bone marrow-derived dendritic cell (BMDC) cross-presentation assay were used to assess the efficacy of SIRPα blockade in solid tumors. Here we demonstrate the immunomodulatory effects of SIRPα blockade. In an in vitro assay with tumor cell suspension, SIRPα blockade increases the cellular uptake of tumor cells by matured peritoneal macrophages as a monotherapy and to a greater degree when combined with a tumor targeting antibody. Using a mouse BMDC cross-presentation assay, we also demonstrate that the blockage of SIRPα results in increased T cell expansion, supporting a role for SIRPα blockade in enhancing DC skewing and function. We further demonstrate that DC phenotype is shifted towards the cross-presenting DC1 phenotype in this assay. Additionally, SIRPα blockade functions to modify the myeloid compartment in the TME of solid tumors. In the mouse mammary tumor model 4T1, we demonstrate that SIRPα blockade skewes the DC population towards the cross-presenting DC1 phenotype and increases the CD8 expression on DC2 in the spleen and lymphnodes. Lastly, in the subcutaneous tumor models 4T1 and CT26, SIRPα blockade combined with PD-1 blockade reduces tumor burden and increases overall survival.

RESULTS

A) In vitro cross presentation assay setup. BMDC were elicited with FLT-3-supplemented media for 7 days. On Day 7, MC38-Ova tumor cells, along with antibodies blocking SIRPα, CD47, and PD-1 were added. On Day 8, OT-1 T cells were enriched, CFSE labelled, and added to the culture. Controls for this assay showed that OT-1 T cells do not persist in the absence of antigen, nor do they expand without APC presentation.

B) Representative FACS plots and summary graph of T cell expansion after 72 hours coculture with MC38-Ova and matured APC.

C) In a similar culture, we analyzed the addition of MC38 WT+ soluble SIRPβ2, peptide, rather than MC38-Ova to understand if the increase in T cell expansion was due to increased cross-presentation.

Figure 3. SIRPα Blockade Increased T Cell Expansion in vitro

A) Percentage composition and absolute numbers of cells in the MC38 Ova (A+B) and MC38 WT + SLB (C,D) cultures from Figure 3 D.

Figure 4. SIRPα Blockade Increases DC1 Abundance in vitro

Figure 5. SIRPα Combination with PD-1 Modulates T Cell Responses

A) Mouse bearing 4T1 tumors were treated with isotype, 180ug anti-SIRPα, +/- 200ug anti-PD-1, then sacrificed for immune analysis.

B) After 1 treatment, CD8 expression increase in the spleen in the DC2 (SIRPα+ CD103+) population.

C) After 2 treatments, the % DC1 (SIRPα- CD103+) of CD11c+ cells in the tumor is increased.

Figure 6. SIRPα Increases Changes in the DC Compartment in vivo

Figure 7. Combination of SIRPα + aPD-1 Reduces Tumor Burden in Syngeneic Tumor Models

A) To diminish the effects of Fc binding, we altered the anti-SIRPα and anti-PD-1 antibodies to include the CD65A mutation, which nullifies Fc binding.

B) Biweekly intratumoral injections of 180ug anti-SIRPα +/- weekly injections of 200ug PD-1 (RMP1-14, D265A) were initiated 5 days after tumor implantation and sustained for 3 weeks.

C) Biweekly IT injections of 200ug anti-SIRPα or weekly injections of 200ug PD-1 (RMP1-14) were initiated 8 days after implantation and sustained for 3 weeks.

CONCLUSIONS

Together, these data suggest that antagonizing SIRPα-CD47 results in multiple functional consequences in myeloid cells. In suspension tumor cultures, SIRPα blockade results in an increase in uptake of tumor cells by macrophages. In an in vitro cross presentation assay, SIRPα blockade results in more DC1 phenotype DCs in culture and higher expansion of CD8+ T cells. In vivo, SIRPα blockade leads to multiple changes of cell phenotype and cell numbers in the tumor microenvironment. Mice treated with SIRPα blockade express increased tumor load as well as higher CD8 expression on DC2 phenotype cells. We hypothesize that these cellular changes are the driving force behind the combinational efficacy that we see when SIRPα agents are combined with PD-1 blockade. These data continue to add to the clinical development of ADU-185, a pan-alek, anti-human SIRPα antibody.

references: