BLOCKADE OF SIRPα ON MACROPHAGES TO ENHANCE CAR T ACTIVITY AGAINST GLIOBLASTOMA

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Background The tumor microenvironment in glioblastoma (GBM) is highly immune suppressive, largely due to tumor-associated macrophages (TAMs) signaling. TAMs utilize several checkpoint inhibitors including SIRPα to regulate T cell function through the induction of apoptotic signaling and reduced activation. These also pose a challenge for engineered T cell therapies such as chimeric antigen receptor (CAR) T cells. While previous work has investigated blockade of either SIRPα or its ligand CD47, showing a temporal increase in trafficking of endogenous T cells to the tumor, understanding of the potential impact of such treatments on CAR T cells is lacking. We hypothesize that blocking the interaction of SIRPα with CD47 can improve the trafficking and functional activity of CAR T cells against glioblastoma.

Methods HER2-specific second generation CAR T cells were generated from primary human T cells using a retroviral transduction method. Primary human monocytes were harvested from peripheral blood and differentiated in vitro using M-CSF and IL-4 into M2-skewed macrophages. Macrophages, HER2+ GBM cell lines, and CAR Ts were co-cultured together with and without an anti-human SIRPα blocking monoclonal antibody. Cell surface expression of phenotypic macrophage markers, T cell activation markers, and SIRPα were assessed at baseline and post-treatment by flow cytometry. Supernatant from co-cultures was assessed for the presence of chemokines and cytokines using a custom multiplex panel and analyzed for differences in immunosuppressive signaling. CAR T cytolytic activity was determined by live-cell imaging using GFP-containing LN229-GBM.

Results We observed that TAM-like macrophages altered the cytokine release in CAR T and GBM co-cultures, promoted tumor cell growth, and increased CAR T apoptotic signaling resulting in delayed tumor cell lysis. In the presence of a SIRPα blocking antibody, we noted decreased surface expression of endogenous SIRPα and increased expression of M1-related marker CD86 on the macrophages at 24 hours of co-culture. The expression of several chemokines including MCP-1 was increased. At high ratios of CD28ζ CAR T cells, down-regulation of SIRPα induced by the antibody blockade was not sustained.

Conclusions Macrophages cultured with GBM cells in vitro can alter the immune milieu, leading to CAR T cell apoptosis and hinder tumor-directed cytotoxicity. Blockade of SIRPα favorably alters the cytokine and chemokine production and may facilitate lymphocyte trafficking while reducing inhibitory receptor expression on macrophages. However, these effects can be countered by potent pro-inflammatory CAR T cell signaling, underscoring the need for more robust SIRPα disruption strategies.

Ethics Approval This study was approved by Baylor College of Medicine’s Ethics Board; approval number H-15280.