GENERATION OF HYPOIMMUNOGENIC ALLOGENEIC CAR T CELLS BY INACTIVATION OF TRANSCRIPTIONAL REGULATORS OF HLA CLASS I AND II GENES

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Background Autologous CAR T cell therapies have revolutionized the treatment landscape in hematological malignancies. Using the patient’s own T cells for manufacturing, however, poses limitations on the widespread use of these therapies. Off-the-shelf allogeneic CAR T cells could potentially address these issues by using healthy donor T cells as starting material, consistency of product, immediate availability, and cost and convenience of scalable manufacturing. However, expansion and persistence of infused allogeneic CAR T cells may be limited by immune rejection. Immune “cloaking” strategies centered on deletion of β2-microglobulin can avoid rejection by CD8 T cells but may elicit strong NK cell rejection. Moreover, HLA Class II expression can be induced upon T cell activation to increase the risk of CD4 T cell rejection. Here, we propose an alternative approach to immune evasion by selectively targeting NLRC5 and RFX5, transcriptional regulators controlling expression of HLA molecules.

Methods CRISPR/Cas9 technology was used to knockout NLRC5, RFX5, B2M, CIITA, and/or TRAC. Survival of hypoimmunogenic cells was assessed in mixed lymphocyte reaction (MLR) assays with allogeneic T cells, NK cells, or PBMCs. For in vivo evaluation, mice were engrafted with human T cells and Raji tumor cells followed by administration of hypoimmunogenic CD19 CAR T cells, and CAR T cell persistence and tumor growth were monitored over time.

Results Deletion of NLRC5 and RFX5 resulted in substantial and stable downmodulation, but not complete ablation, of HLA Class I expression. RFX5 KO cells also exhibited downregulation of HLA Class II expression. NLRC5 KO and RFX5 KO T cells showed enhanced survival against allogeneic T cells but elicited only minor NK cell reactivity. When co-cultured with HLA-mismatched PBMCs, NLRC5 KO and RFX5 KO cells effectively mitigated rejection, whereas unclad control and B2M KO cells were eliminated by allogeneic T and NK cells, respectively. These findings were replicated in T cells expressing a CD19 CAR. Inactivation of NLRC5 or RFX5 did not impact CAR T cell phenotype or cytotoxic activity. In vivo, hypoimmunogenic CAR T cells demonstrated superior persistence and antitumor efficacy compared to unclad control CAR T cells in the presence of allogeneic T cells.

Conclusions Hypoimmunogenic CAR T cells can be successfully generated by targeted deletion of NLRC5 or RFX5, which reduces T cell rejection without triggering substantial NK cell rejection and does not affect CAR T cell function. The improved persistence of hypoimmunogenic allogeneic CAR T cells may increase the therapeutic efficacy of off-the-shelf products.