CAR-T cells engineered to express a Fas-CD40 chimera display superior persistence and tumour cytotoxicity

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Introduction

Engineered T cells can have remarkable efficacy against haematological cancers, however activity against some tumours, particularly solid cancers, are limited by inhibitory receptors expressed by the tumour or its microenvironment. One key inhibitory receptor is FasL. Upon FasL binding to the death receptor, Fas/CD95, on the T cell, an intracellular cascade is triggered committing that cell to die by apoptosis. FasL is expressed by most cancers, regulatory T cells, endothelial cells, myeloid-derived suppressor cells and cancer-associated fibroblasts. Notably, activated T cells constitutively express Fas and are as such highly susceptible to FasL-mediated apoptosis. We add to the literature of strategies to engineer T cells to be resistant to FasL-mediated killing by testing a set of Fas-TNFR chimeric proteins.

Results

We first investigated over-expression of truncated Fas which lacks an endodomain. This completely rescued FasL-mediated apoptosis. However, truncated Fas significantly reduced CD3/28-induced T-cell proliferation and when co-expressed with a chimeric antigen receptor (CAR) resulted in reduced cytotoxicity. Given that Fas is known to have a dual role, we hypothesized that reduced T cell function was due to loss of Fas activatory signalling. Subsequently, we engineered a set of chimeric proteins that fused the ectodomain and transmembrane domain of Fas to the endodomain of pro-survival tumor necrosis factor receptors (TNFRs). We screened 17 Fas-TNFR chimeric proteins by incubating with immobilised FasL and assessed for their ability to rescue FasL-mediated apoptosis and induce proliferation. All the Fas-TNFRs except the Fas-LTβR, Fas-TROY, Fas-DcR2 and Fas-RELT chimeras rescued FasL-induced T-cell apoptosis, and T-cells expressing Fas-CD40, Fas-Fn14, Fas-BCMA and Fas-CD27 displayed the greatest induction of proliferation with fold-increases relative to truncated Fas of 10.7, 8.2, 6.8 and 5.7 respectively, which also correlated with secretion of IFN-γ.

Fas-TNFRs were co-expressed with a GD2 targeting CAR and were subjected to restimulation by target cancer cells expressing either GD2 or GD2/FasL, where Fas-CD40 displayed superior proliferation and cytotoxicity by serially killing targets up to 10 stimulations compared to CAR-T cells alone or CAR-T cells expressing the other Fas-TNFR chimeras, which could only kill targets up to 7 rounds of stimulation. Importantly, CAR-T cells expressing Fas-CD40 did not proliferate autonomously in the absence of target cell stimulation. Furthermore, upon multiple rounds of target cell stimulation, CAR-T cells expressing Fas-CD40 maintained a less differentiated memory phenotype, evidenced via a reduction in CD45RA+CD62L+ expression, and CD4+ CAR-T cells expressing Fas-CD40 expressed fewer exhaustion markers (PD1, TIM3 and LAG3) compared to CAR-T cells expressing the other Fas-TNFRs.

Conclusions

We have identified a chimeric Fas-CD40 protein that is able to not only rescue FasL-mediated T-cell apoptosis, but also elicit superior proliferation and anti-tumour cytotoxicity in the presence of FasL.

Figure Legend

PBMCs expressing CAR alone or with Fas-TNFRs were cultured with immobilised FasL for five days, at which point cell count was analysed by flow cytometry.