Drug Selection of γδ T cells using miRNA target sequences

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Abstract

Gamma delta T cells constitute a small subset of circulating T lymphocytes in peripheral blood, and recognize antigens in an MHC-independent manner through their γδ T cell receptor. We sought to selectively expand ablatable γδ T cells using an inducible suicide switch, drug and miRNA selection strategies. Drug resistant plasmid encoding an inducible caspase 9 (iC9) suicide switch and a double mutant human dihydrofolate reductase (DHFR) gene was generated by ligating FLAG-tagged double mutant DHFR, T2A, and Myc-tagged iC9 (dDiC9) sequences. In order to selectively expand γδ T cells, 4 copies of miRNA10a target sequences were inserted in the 3' UTR of the dDiC9 construct and the plasmids dDiC9-miRT10a3' and dDiC9-miRT10a5' were generated to hybridize with the miRNA10a in the 3' and 5' direction, respectively. PBMC cells were electroporated with dDiC9 constructs using the Sleeping Beauty transposase system. Electroporated cells were expanded on K562 Clone 4 artificial antigen presenting cells with IL-2, and under the selection of methotrexate (MTX) for 14 days. Analysis by flow cytometry determined that selective expansion of γδ T cells of >3-fold was achieved with the dDiC9-miRT10a5' construct. However, drug selection conditions allowed for competition between γδ and αβ T cells, instead of the expected competition between NK cells and γδ T cells. Nonetheless, we were able to selectively expand both γδ and αβ T cells in the presence of MTX. Analysis to test effectiveness of the caspase 9 suicide switch revealed that cells electroporated with dDiC9 constructs were effectively ablated after induction of apoptosis with a chemical inducer. To conclude, we have been successful in selectively expanding ablatable T cells using a suicide switch, drug and miRNA selection strategies.

Introduction

Among the circulating T cells, 1-5% express the γδ T cell receptor (TCR) and are known as γδ Tcells. Their TCR is composed of one γ and one δ chain, and unlike the αβ T cells, γδ T cells do not recognize antigens as peptides presented by MHC molecules, but instead seem to recognize their target antigens directly. These cells have been shown to have endogenous cytotoxic activity towards tumor cells including solid tumors and hematopoietic malignancies, therefore γδ T cells are currently being targeted as tools for novel cancer immunotherapy strategies.

The side effects observed in the setting of adoptive therapy with gene-engineered T cells include on-target and off-target toxicity, and conditioning toxicity. Strategies to deal with unexpected toxicities of the infused T cells include the addition of a suicide switch into the engineered T cells.

One of the major side effects of most anticancer chemotherapeutic drugs is myelosupression. Methotrexate (MTX) is a drug that is commonly used in combination chemotherapy to treat a wide range of malignancies. It depletes reduced folate pools and also inhibits dihydrofolate reductase (DHFR), which decreases DNA and RNA synthesis thus inhibiting cell growth. Previous studies have shown that by inserting a mutated human DHFR gene into hematopoietic cell lines MTX resistance was developed, indicating that the use of gene transfer of mutated DHFR can limit MTX myelotoxicity in cancer patients. In this study we have focused on developing selection strategies for ablatable γδ T cells using an inducible suicide switch, drug and microRNA selection strategies.

Hypothesis

Selective expression of double mutant dihydrofolate reductase-2A-inducible caspase 9 (dDiC9) transgene in γδ T cells by miRNA selection can allow for rapid selection of ablatable γδ T cells.

Methods

Generation of dDiC9 DNA plasmids

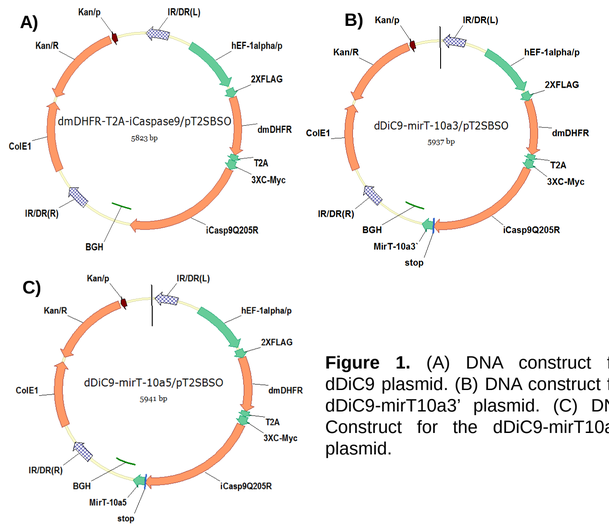

Figure 1. (A) DNA construct for dDiC9 plasmid. (B) DNA construct for dDiC9-mirT10a3' plasmid. (C) DNA Construct for the dDiC9-mirT10a5' plasmid.

Expansion and Selection of **γδ** T cells

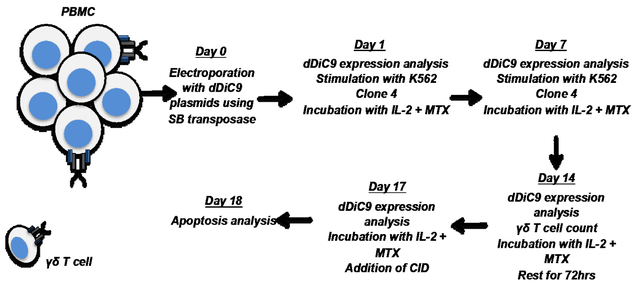

Figure 2. Human PBMC were electroporated with dDiC9 constructs (Figure 1) and stimulated with K562 aAPC in presence of IL-2 and MTX. To determine functionality of iC9 transgene a chemical inducer of dimerization was used and apoptosis was measured.

Results

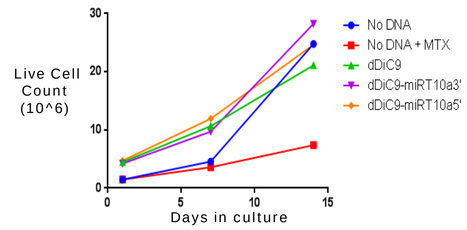

Figure 3. Electroporated cells with dDiC9, dDiC9-mirT10a3', dDiC9-miRT10a5' plasmids and No DNA sham electroporated cells were expanded and stimulated using K562 Clone 4 aAPC over a period of 14 days in the presence of IL-2 and MTX.

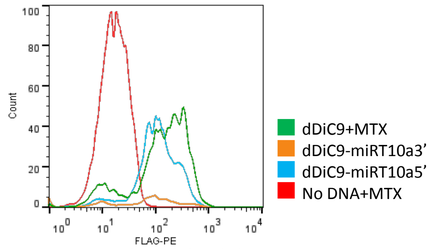

Figure 4. Transgene expression of the dDiC9 transgenes was measured by intracellular staining with anti-FLAG antibody. Results shown only for day 17 of incubation.

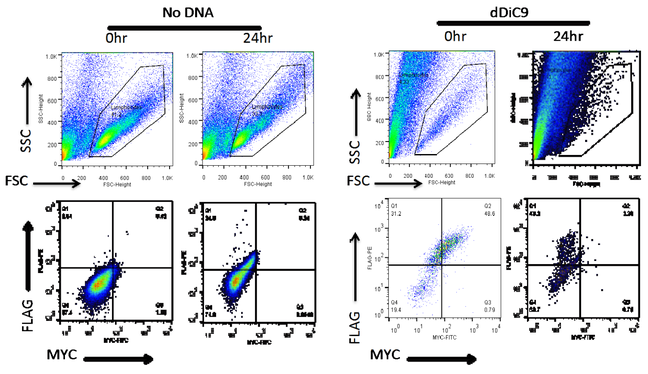

Figure 5. Measurement of induced apoptosis of PBMC by the inducible caspase 9 transgene in the dDiC9 plasmid after 24 hours of incubation with the chemical inducer of dimerization. As measurements of cell viability forward scatter, side scatter and intracellular staining with anti-FLAG and anti- Myc antibodies were used.

Results (cont.)

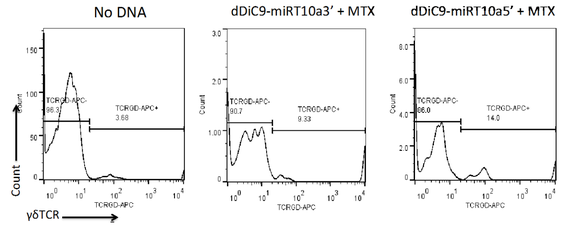

Figure 6. Selection and expansion of γδ T cells by miRNA 10a selection. Total counts for γδ T cells were determined by flow cytometry staining with anti-γδ TCR, after selection of CD3+ cells from the PBMC electroporated with No DNA, dDiC9-miRT10a3' and dDiC9-miRT10a5' plasmids after 14 days of incubation.

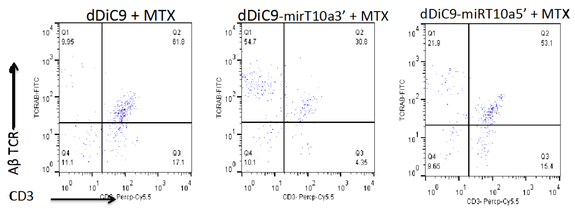

Figure 7. Effect of dDiC9 plasmids on cell type selection in the presence of IL-2 and MTX. Expression of CD3 and αβTCR was measured in cells electroporated with dDiC9 plasmids after 14 days of culture with K562 aAPCs and in the presence of MTX and IL-2.

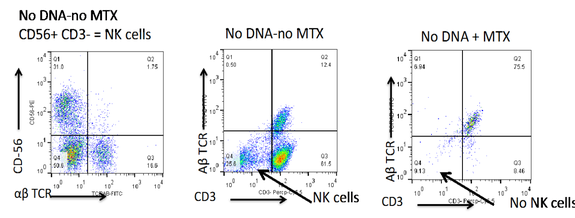

Figure 8. Effect of MTX on culture environment**.** Effects of MTX in cell survival, without genetic manipulation, was determined by monitoring for expression of specific cell markers such as CD3, CD56 and αβ TCR after 14 days of culture in the presence of K562 aAPC and IL-2.

Conclusions

- Drug selection using MTX was successful for selection and expansion of T cells.
- Caspase 9 inducible suicide switch was efficient in causing programmed cell death.
- Observed an increase of >3 fold in γδ T cells numbers when miRNA and drug selection was performed .

References

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