Poster #764

MEIRAGTx

Riboswitch-regulated chimeric antigen receptor (RiboCAR) enhances T cell activity

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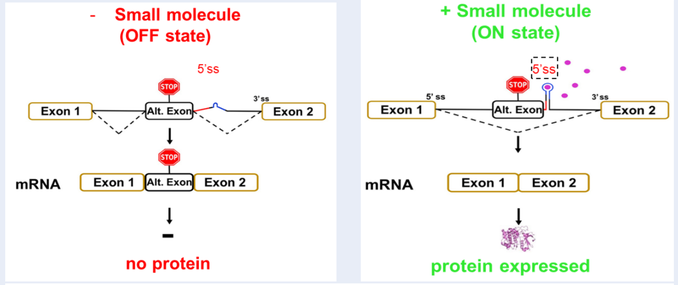
ABSTRACT

Chimeric antigen receptor (CAR)-T cell therapy is a promising treatment for certain cancers. However, it is increasingly evident that the level of CAR molecule expression is important for CAR-T cell activation, durability and anti-cancer activities. Here, we present the development of RiboCAR, a system for precise regulation of CAR expression via orally available small molecule inducers. Unlike previously reported regulatable CAR platforms that utilize viral protease or chemical-induced protein dimerization, RiboCAR contains a synthetic mammalian ON riboswitch in the coding sequence of the CAR transgene, in which the aptamer functions as a sensor for a specific novel small molecule inducer. The expression level of the CAR gene is precisely dependent on the level of the riboswitch inducer. CAR is undetectable in the absence of the small molecule, and a precise dose response in CAR levels is achieved with increasing dose of small molecule, reaching levels higher than constitutively active CAR upon maximal small molecule dose. Induced CAR expression diminishes following withdrawal of the small molecule. Consistent with small molecule induced expression of the CAR molecule, we demonstrate CAR triggered-activation of CAR-T cells is regulated by the small molecule inducer. Additionally, T cells with low levels of CAR expression via RiboCAR show enhanced target cell-stimulated T cell activation, reduced markers of exhaustion and greater cytotoxicity when compared with T cells expressing CAR constitutively. CAR levels can be activated to the most effective levels and can be switched on and off according to the presence of the small molecules. With a bioavailable small molecule inducer, CAR-T activity can be precisely tuned and "remotely" controlled in vivo. This precise control of CAR levels provides a system for improving the efficacy and durability of CAR-T as well as a safety mechanism for CAR-T cell therapy in comparison to current therapies with constitutively active CAR expression..

Aptamer-modulated alternative splicing riboswitch

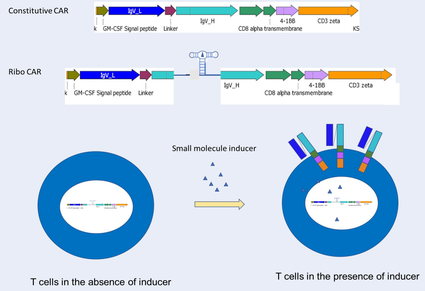


Schematics of synthetic riboswitch

Aptamer is inserted in the downstream intron of an Intron-AltExon-intron cassette within the cDNA

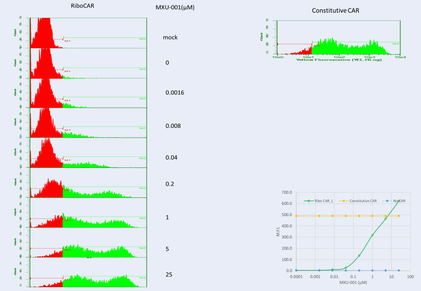
In the absence of small molecule (Left panel):	In the presence of small molecule (right panel):
Alt. exon 5' ss is accessible	Alt. exon 5' ss is sequestered
Alt. exon is included	Alt. exon is skipped
No protein expressed	Protein expressed

Schematics of RiboCAR gene and its expression



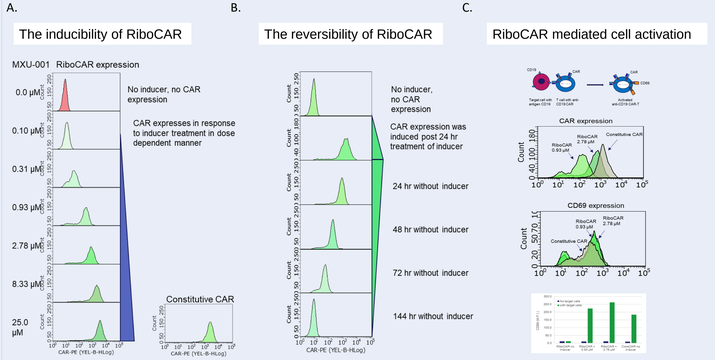
- Schematics of the RiboCAR gene: riboswitch cassette is inserted in the coding sequence of CAR gene.
- In the absence of the small molecule inducer, CAR is not expressed on cell surface.
- In the presence of the small molecule inducer, CAR is expressed on the cell surface.

Riboswitch-controlled CAR expression in HEK 293 cells in response to inducer treatment



- HEK 293 cells were transfected with CAR constructs and treated with MXU-001 at different concentration.
- HEK 293 cells were stained with anti-FMC63 antibody 48 hours after transfection.
- CAR expression in the presence of MXU-001 in dose dependent manner.

Jurkat T cells with RiboCAR express higher level of CD69 in response to target antigen stimulation

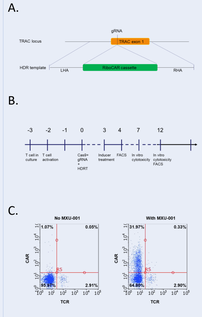
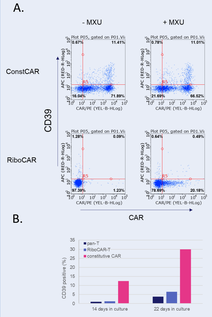
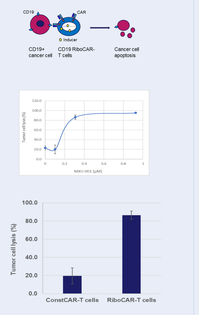


A. Jurkat T cells stably transfected with RiboCAR into the TRAC locus were treated with small molecule inducer MXU-001 at various concentrations for 48 hrs.

B. 24 hours post MXU-001 treatment, Jurkat T cells stably transfected with RiboCAR were withdrawn from MXU-001 treatment and CAR expression was monitored every 24 hours.

C. Jurkat T cells with RiboCAR or constitutive CAR were co-cultured with CD19+ Raji cells at 1:1 ratio in the presence of MXU-001 at the indicated concentrations for 24 hours.

RiboCAR-T cells are more potent than ConstCAR-T cells in anti-cancer activity in cell culture

RiboCAR in primary T cells	RiboCAR-T cells not exhausted	RiboCAR-T cells more cytotoxic
		

A. Schematics of RiboCAR gene targeting to TRAC locus in primary T cells. B. Timeline of RiboCAR targeting to primary T cells. C. Induced expression of CAR in primary T cells in response to MXU-001 4 days post targeting.	A. Expression of CD39 was measured by FACS 12 days or 20 days post transfection. B. CD39 expression in pan-T cells, RiboCAR-T cells and constitutive CAR-T (ConstCAR) cells 12 days or 20 days post transfection.	A. Schematics of RiboCAR-mediated cancer cell killing in the presence of inducer. B. Cytotoxicity of RiboCAR-T cells in inducer dose dependent manner. C. RiboCAR-T cells in the presence of 0.31 μM MXU-001 are more cytotoxic to cancer cells than ConstCAR-T cells

Summary

- CAR expression is tightly regulated by riboswitch via a small molecule inducer in dose dependent manner.
- Riboswitch fine-tunes the level of CAR expression and the activity of CAR-T cells via small molecule inducer.
- RiboCAR-T cells appear to have reduced markers of exhaustion in culture compared to ConstCAR-T cells.
- RiboCAR-T cells appear more potent in cancer cell killing activity in cell culture.
- Riboswitch regulated CAR potentially provides more efficacious CAR-T cell therapy.
- Riboswitch regulated CAR potentially provides tightly regulated CAR-Ts which have safety benefits in addition to the potential for increased potency