Poster # 464

MEIRAGTx

Riboswitch-regulated chimeric antigen receptor (RiboCAR) enhances CAR-T cell anti-cancer efficacy

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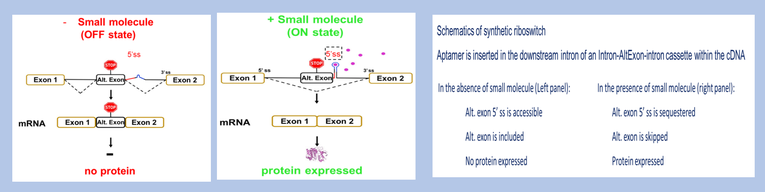
ABSTRACT

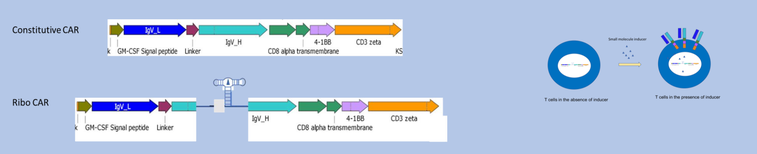
Chimeric antigen receptor (CAR)-T cell therapy is a promising therapy against cancer. However, the uncontrolled CAR expression causes severe CAR-T cell-associated toxicity and CAR-T cell exhaustion, limiting the success of this living drug. Here, we present the development of RiboCAR, a mammalian synthetic riboswitch-regulated CAR expression via small molecule inducer. Unlike previously reported regulatable CAR platforms that utilize viral protease or chemical-induced protein dimerization, RiboCAR contains an RNA ON riboswitch in the coding sequence of a CAR transgene, in which the aptamer functions as a sensor for a specific novel small molecule inducer. The expression level of the CAR gene with the riboswitch completely depends on the presence of the riboswitch inducer, with undetectable CAR in the absence of the small molecule and significant CAR expression that is higher than constitutively active CAR upon maximal small molecule induction. The induced CAR expression diminished after withdrawal of the small molecule inducer. Further, CAR expression is titratable in response to the levels of the small molecule inducer. Consistent with small molecule induced expression of the CAR molecule, CAR triggered-activation of CAR-T cells is also controlled by the small molecule inducer. More importantly, T cells with RiboCAR showed delayed exhaustion during expansion in the absence of small molecule inducer and enhanced target cell-stimulated T cell activation and anti-cancer cytotoxicity in the presence of small molecule inducer, when compared with T cells constitutively expressing CAR. With a bioavailable small molecule inducer, the RiboCAR-T cell activity can be precisely tuned and "remotely" controlled *in vivo*, thus improving the efficacy and safety of CAR-T cell therapy.

Schematics of aptamer-modulated alternative splicing riboswitch and RiboCAR

A. Novel synthetic mammalian riboswitch modulates alternative splicing via small molecule and aptamer binding



B. Riboswitch-regulated expression of chimeric antigen receptor



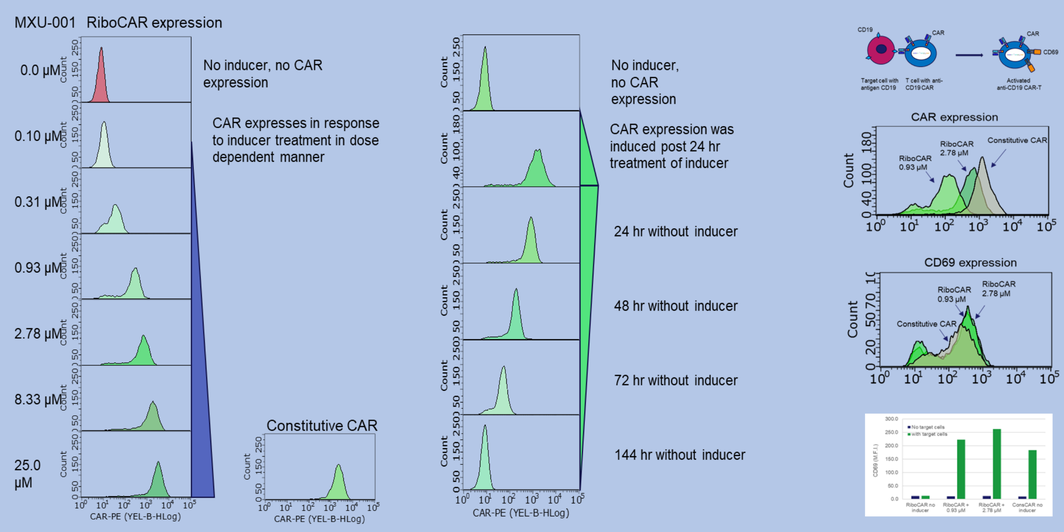
A. Schematics of synthetic aptamer riboswitch
B. Schematics of the RiboCAR gene: riboswitch cassette is inserted in the coding sequence of CAR gene. In the absence of the small molecule inducer, CAR is not expressed on the cell surface. In the presence of the small molecule inducer, CAR is expressed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

The induced and reversible expression of CAR on cell surface of Jurkat-T cells

A. The inducibility of RiboCAR

B. The reversibility of RiboCAR

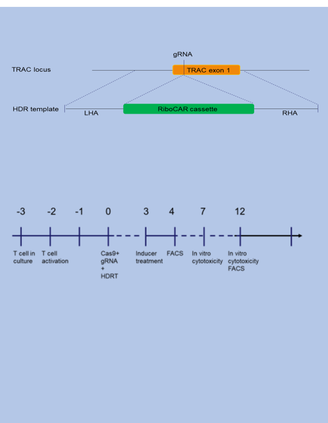
C. RiboCAR-mediated cell activation



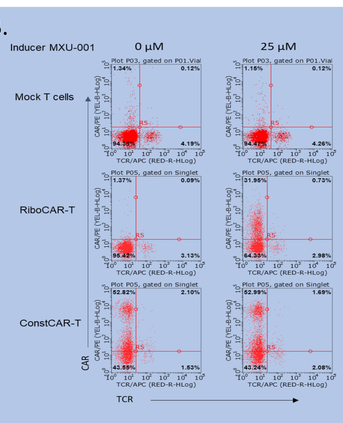
A. Jurkat T cells with RiboCAR knocked into the TRAC locus were treated with a novel small molecule inducer MXU-001 at various concentrations for 48 hours.
B. 24 hours post MXU-001 treatment, RiboCAR transfected Jurkat T cells were withdrawn from MXU-001 treatment and CAR expression was monitored every 24 hours.
C. Jurkat T cells with RiboCAR or constitutive CAR knocked into the TRAC locus were co-cultured with CD19+ Raji cells at 1:1 ratio in the presence of MXU-001 at the indicated concentrations for 24 hours.

Induced and tuned expression of RiboCAR in primary human T cells

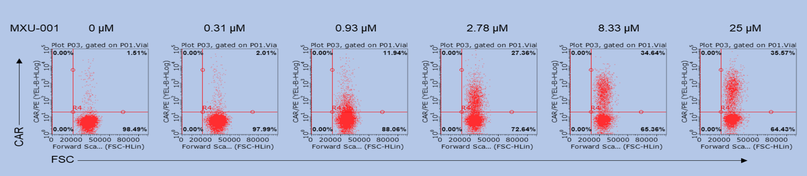
A.



B.



C.

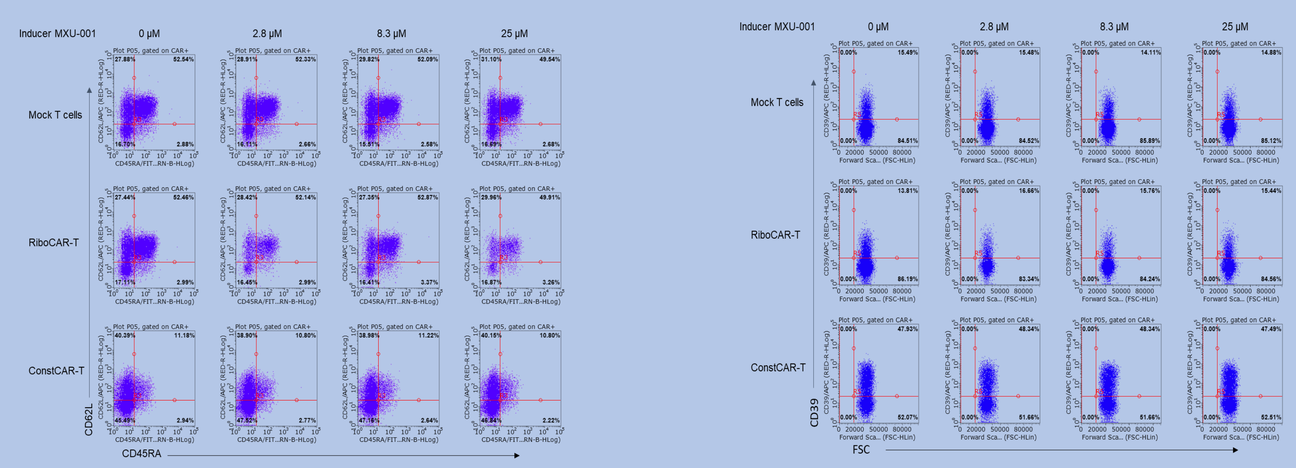


A. Schematics of RiboCAR gene targeting to TRAC locus in primary T cells using CRISPR/cas9 and AAV6 as HDR donor vector, and the timeline of RiboCAR targeting to primary T cells.
B. Engineered T cells were treated with riboswitch small molecule inducer 3 days post gene targeting to induce the expression of CAR in RiboCAR-T cells.
C. RiboCAR-T cells were treated with MXU-001 at the indicated concentrations to induce CAR expression.

RiboCAR-T cells are enriched in naïve/stem cell-like memory T cells

A. RiboCAR-T cells are less differentiated and exhibit indistinguishable phenotype from Mock T cells

B. RiboCAR does not increase the expression of inhibitory exhaustion marker

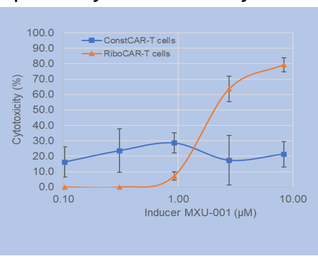


A. RiboCAR-T and ConstCAR-T cells were treated with or without MXU-001 at the indicated concentrations 6 days post CRISPR/cas9 transfection and AAV transduction. 24 hours after MXU-001 treatment, cells were stained with anti-human CD62L and CD45RA antibodies and subjected to flow cytometric analysis.

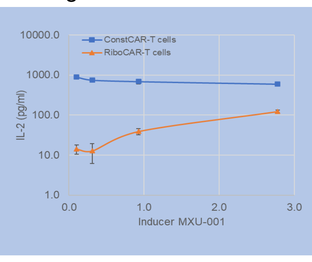
B. RiboCAR-T and ConstCAR-T cells were treated with or without MXU-001 at the indicated concentrations 6 days post CRISPR/cas9 transfection and AAV transduction. 24 hours after MXU-001 treatment, cells were stained with anti-human CD39 antibody and subjected to flow cytometric analysis.

RiboCAR-T cells are more potent than ConstCAR-T cells in anti-cancer activity *in vitro*

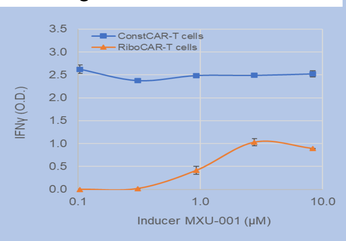
A. RiboCAR-T cells have small molecule dose dependent, superior cytotoxic activity



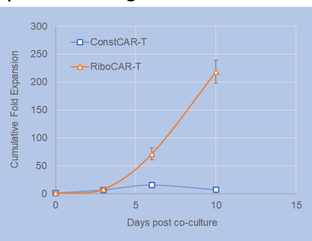
B. RiboCAR-T cells release dose dependent, lower level of IL-2 following tumor cell stimulation



C. RiboCAR-T cells release dose dependent, lower level of IFNγ following tumor cell stimulation



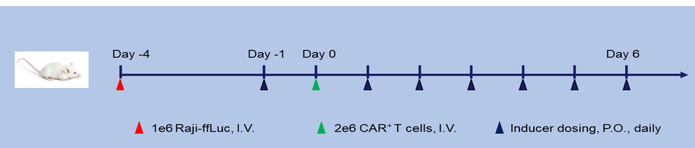
D. RiboCAR-T cells exhibit superior expansion capacity following repeated antigen stimulation



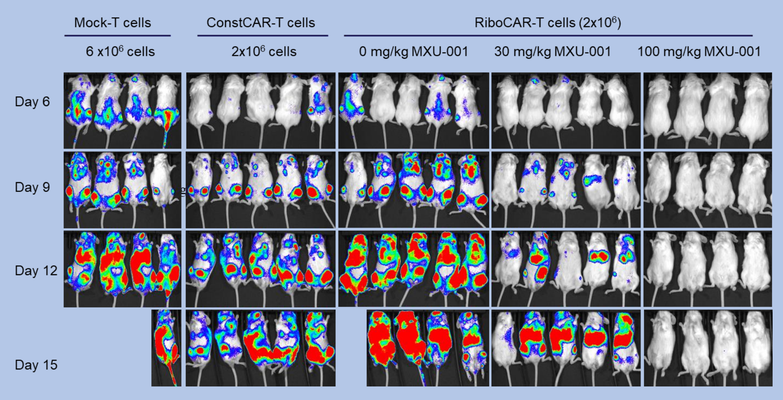
A. RiboCAR-T cells or ConstCAR-T cells were co-cultured with Raji-ffLuc cells at 2:1 E:T ratio in the presence of various concentration of MXU-001 for 48 hours. Luciferase activity was measured for cytotoxicity assessment.
B and C. Supernatants were collected from the cytotoxicity assay in A for IL-2 and IFNγ cytokine ELISA.
D. CAR-T cells were stimulated with MMC-treated Raji cells at 1:1 ratio with the presence of MXU-001 and viable T cells were counted 3 days after stimulation. The process was repeated three times.

RiboCAR-T cells are more potent than ConstCAR-T cells in anti-cancer activity *in vivo*

A.



B. RiboCAR-T cells outperform ConstCAR-T cells *in vivo*



A. Schematic of *in vivo* mouse experiment to evaluate the anti-tumor efficacy of RiboCAR-T cells. Raji-ffLuc cells were injected I.V. into NSG mice. 4 Day post Raji cell inoculation, RiboCAR-T cells were injected I.V., and small molecule inducer MXU-001 was dosed orally and daily at the indicated doses, starting the day before CAR-T cells were intravenously injected.

B. Bioluminescence imaging of tumor growth in mice treated with or without MXU-001 at the indicated doses.

Summary

- CAR expression is tightly regulated by riboswitch via a small molecule inducer in dose dependent manner.
- Small molecule dose can fine-tune the level of RiboCAR expression. This precise control of RiboCAR via small molecule inducer improves the activity of CAR-T cells.
- RiboCAR-T cells appear to have more naïve/stem cell memory T cell phenotype in culture compared to ConstCAR-T cells.
- RiboCAR-T cells appear more potent than ConstCAR-T cells in cancer cell killing activity in cell culture.
- RiboCAR-T cells release lower levels of cytokines following tumor cell stimulation *in vitro.*
- RiboCAR-T cells have higher tumor cell stimulated expansion capacity *in vitro.*
- RiboCAR-T cells have more potent anti-cancer activity than ConstCAR-T cells *in vivo.*
- Riboswitch-regulated CAR provides tightly regulated CAR-Ts which have potential safety benefits in addition to the potential for increased potency.