Titratable and reversible control of CAR-T cell receptor and activity by riboswitch (RiboCAR) via oral small molecule

P399

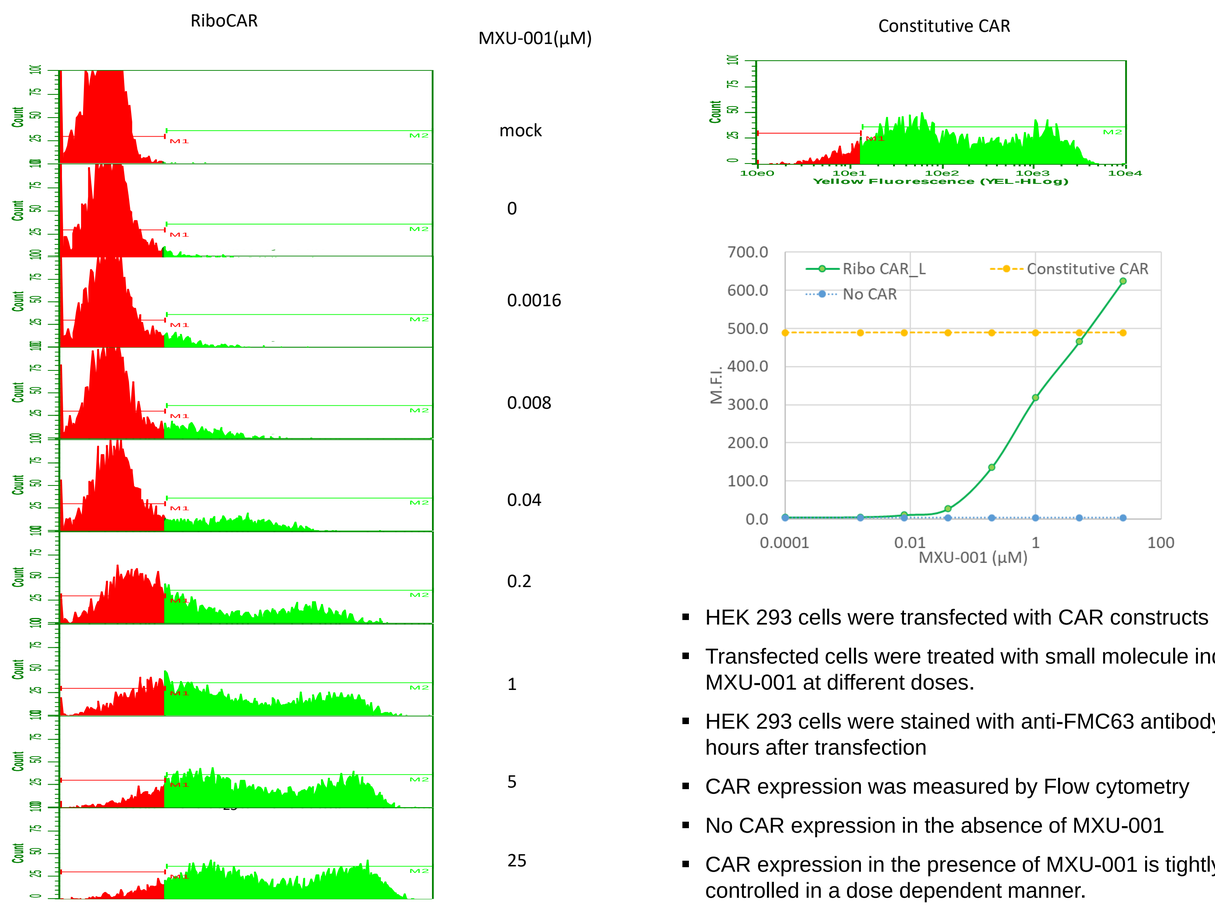
Xuecui Guo, Zhaojing Zhong, George Wang, Zhikai Zhang, Samuel D Waksal, Alexandria J Forbes

Gene Regulation, MeiraGTx, New York

Abstract

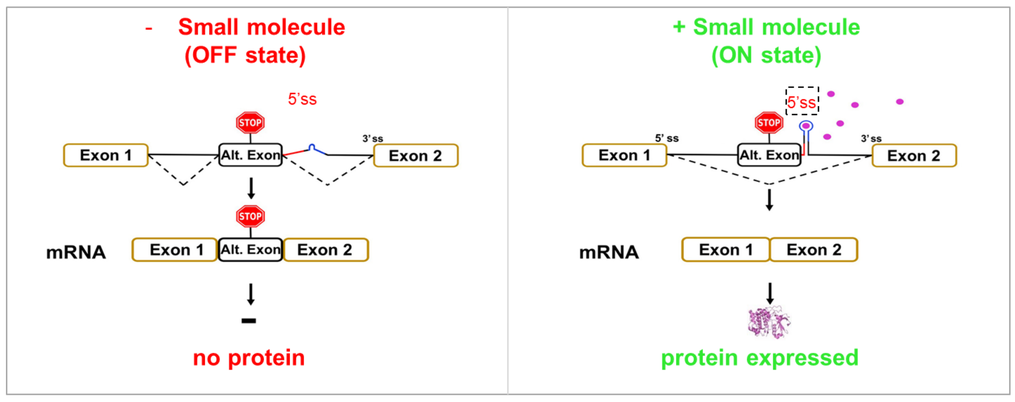
Chimeric antigen receptor (CAR)-T cell therapy is a promising therapy for the treatment of cancer. However, uncontrolled CAR expression, CAR-T expansion and activity causes severe CAR-T cell-associated toxicity and CAR-T cell exhaustion, limiting the success of this living drug. Here, we present RiboCAR, a mammalian synthetic riboswitch-based gene regulation system for regulating CAR expression via small molecule inducer. Unlike previously reported regulatable CAR platforms that utilize viral protease or chemical induced protein dimerization, RiboCAR contains an ON aptamer riboswitch in the coding sequence of a CAR transgene, in which, the aptamer functions as a sensor for a small molecule. Upon binding with the small molecule ligand, the aptamer RNA adopts a conformation change that triggers splicing, resulting in the expression of the intact functional CAR molecule. The CAR gene with the riboswitch only expresses CAR protein in the presence of the small molecule inducer. Further, CAR expression is titratable by titrating the dose of the small molecule inducer. With an optimal dose of small molecule, the induced level of CAR can reach the level of constitutively expressed CAR. Consistent with small molecule induced expression of the CAR, CAR triggered-activation of CAR-T cells and the production of IL-2 and INFγ are also controlled by small molecule inducer in a dose dependent manner. With a bioavailable small molecule inducer, CAR-T activity can be precisely tuned and “remotely” controlled in vivo, both temporally and spatially, thus providing a safety mechanism for CAR-T cell therapy as well as the ability to finely modulate CAR-T activity over time.

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



- HEK 293 cells were transfected with CAR constructs
- Transfected cells were treated with small molecule inducer MXU-001 at different doses.
- HEK 293 cells were stained with anti-FMC63 antibody 48 hours after transfection
- CAR expression was measured by Flow cytometry
- No CAR expression in the absence of MXU-001
- CAR expression in the presence of MXU-001 is tightly controlled in a dose dependent manner.

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

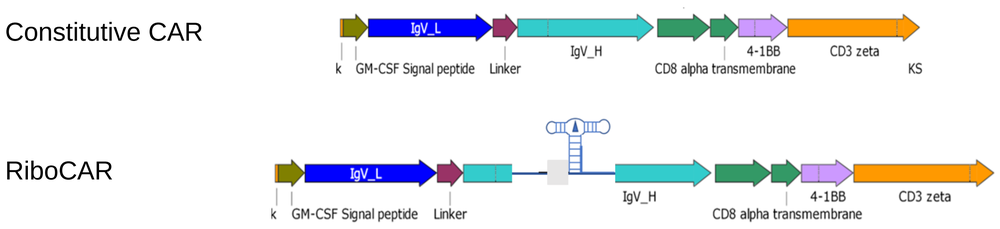
In the absence of small molecule (Left panel):

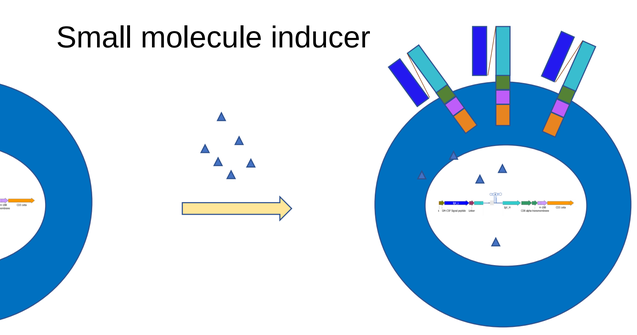
- Alt. exon 5’ ss is accessible
- Alt. exon is included
- No protein expressed

In the presence of small molecule (Right panel):

- Alt. exon 5’ ss is sequestered
- Alt. exon is skipped
- Protein expressed

Riboswitch controlled CAR (RiboCAR)

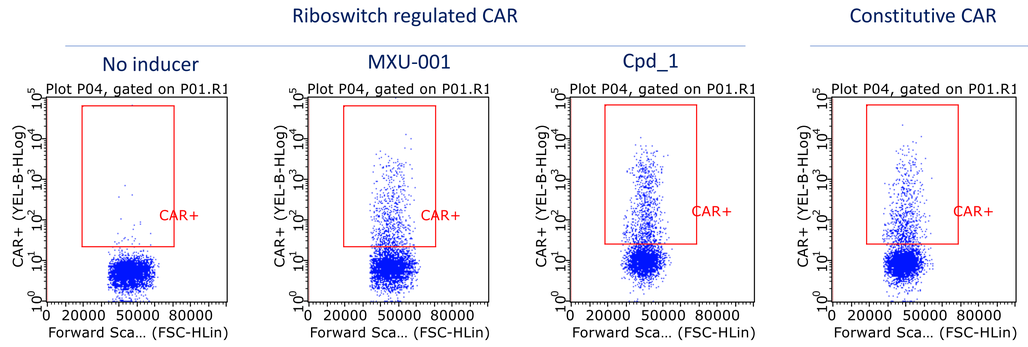


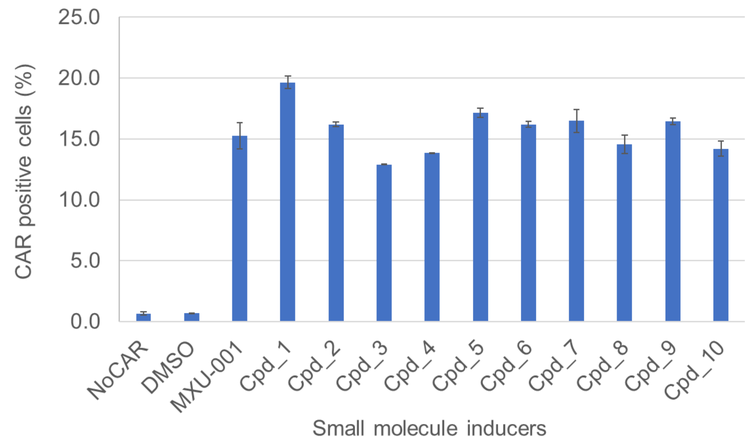


T cells in the absence of inducer

T cells in the presence of inducer

Riboswitch controlled CAR expression in Jurkat T cells in response to inducer treatment





- Jurkat T cells were transfected with CAR construct RiboCAR
- Transfected cells were treated with 25 μM MXU-001 and a selection of alternative small molecule inducers also identified through screening to tightly regulate this aptamer/riboswitch.
- Jurkat T cells were stained with anti-FMC63 antibody 24 hours after transfection
- CAR expression was measured by Flow cytometry
- No CAR molecule was detected in the absence of inducer.
- Small molecule inducer treatment induced CAR expression on Jurkat T cells.
- A number of small molecules identified through screening to activate the riboswitch used in RiboCAR were demonstrated to control CAR expression, some with improved dynamic range over MXU-001

Summary

- ➢ Riboswitch regulates gene expression post transcriptionally
- ➢ Riboswitch comprises no exogenous protein component
- ➢ Riboswitch controls CAR molecule by small molecule inducer
- ➢ CAR expression was tightly controlled by riboswitch
- ➢ CAR expression was induced by small molecule inducer in dose dependent manner
- ➢ Riboswitch controlled CAR potentially provides mechanisms for controlling safety and activity of CAR-T cell therapy