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Novel riboswitches regulate AAV-delivered transgene expression in mammals via oral small molecule inducers

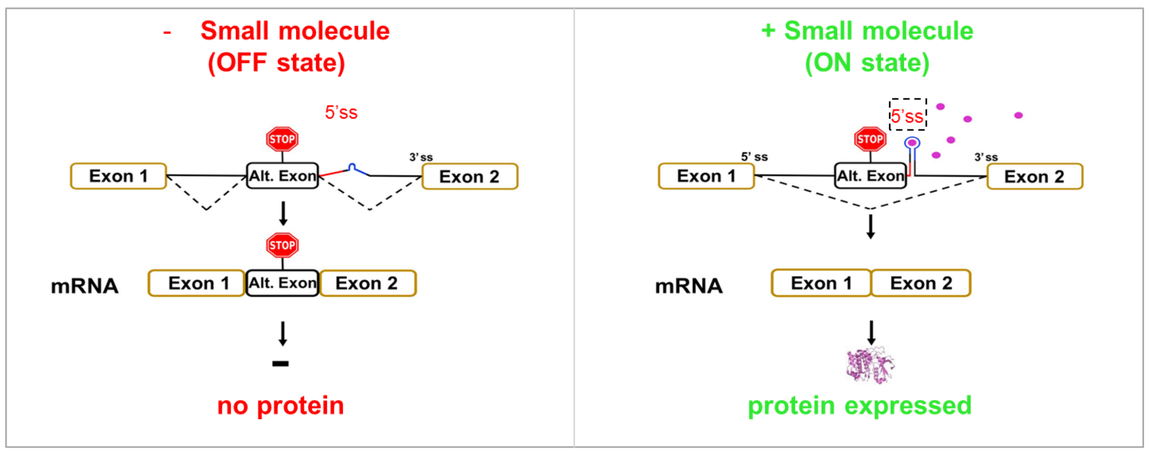
Xuecui Guo[1], Zhaojing Zhong[1], Jae Gyun Oh[1], George Wang[1], Zhikai Zhang[1], Pier Taruc[1], Irina Lu[1], Kevin Liu[1], Jean Kim[1], Jeegar Patel[1], Meredith J. Zeller[2], Kelin Li[3], Jeffery Aubé[3], Kevin M. Weeks[2], Samuel D. Waksal[1], Alexandria J. Forbes[1]

[1]Gene Regulation, MeiraGTx New York [2]Department of Chemistry, University of North Carolina at Chapel Hill [3]Division of Chemical Biology and Medicinal Chemistry, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill

Abstract

Controlled expression of delivered transgenes may be critical for optimized, safe and effective genetic medicines. Here, we report that by linking a synthetic aptamer to an alternative splicing gene expression platform, we have created a robust, synthetic mammalian riboswitch cassette that regulates gene expression tightly and dynamically in response to small molecule inducers. The splicing-based expression platform creates an “on” switch in the presence of the small molecule by sequestering a splice site of an alternative exon. This switch has an exceptionally high dynamic range which has allowed us to screen, identify and modify novel aptamers that bind and respond to novel small molecules. With these riboswitches, we were able to tightly regulate expression in vivo, using oral small molecules, of hormones such as human growth hormone, growth factors such as erythropoietin (Epo), therapeutic antibodies such as anti-PD1 and anti-HER2 antibodies, chimeric antigen receptors (CARs), and nucleases such as CasRx protein. Riboswitches that respond to these novel small molecule inducers regulate transgene expression with high dynamic range in a dose-dependent manner. When delivered through an AAV vector to the liver or the muscle in mice, the engineered riboswitches reversibly regulate transgene expression via an orally delivered small molecule inducer, providing precise control of transgene expression. Thus, our potent gene regulation system provides the first synthetic aptamer riboswitch that is capable of controlling therapeutic gene expression with precise dose control through orally available small molecule inducers. This platform enables precise temporal and spatial control of gene expression for gene and cell therapies.

Design of Aptamer modulated alternative splicing Riboswitch

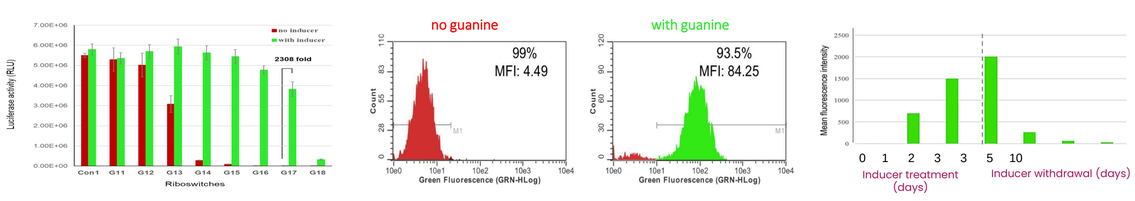


Schematics of synthetic riboswitch
Aptamer is inserted in the downstream intron of an Intron-AltExon-Intron cassette within the cDNA

In the absence of small molecule (Left panel):
- Alt. exon 5’ ss is accessible
- Alt. exon is included
- No protein expressed

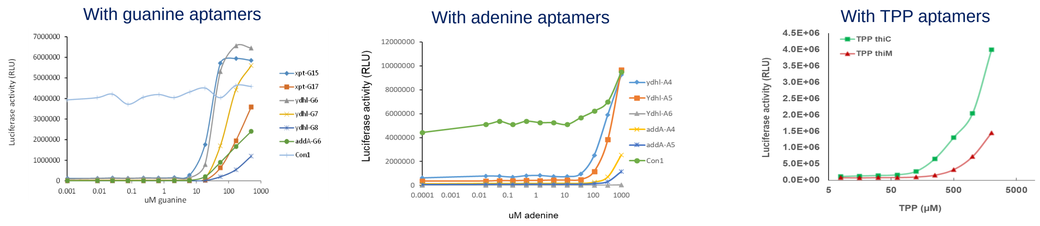
In the presence of small molecule (right panel):
- Alt. exon 5’ ss is sequestered
- Alt. exon is skipped
- Protein expressed

Synthetic Guanine riboswitch regulates gene expression in response to guanine treatment with high dynamic range



HEK 293 cells were transfected with Luciferase constructs (left) or with EGFP construct (middle and right) with riboswitches with guanine aptamer. Transfected cells were treated with the aptamer ligand guanine for 20 hr or for the indicated time (right).

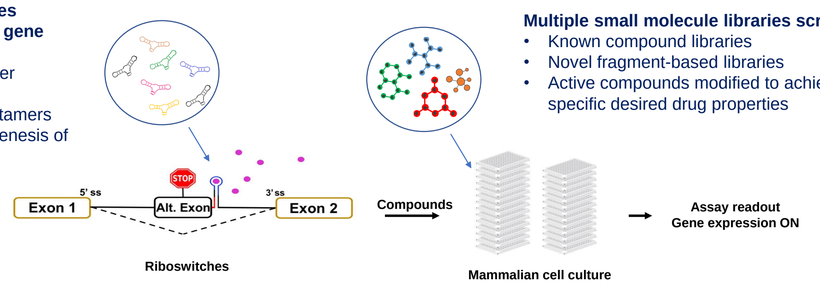
Different Aptamer Sequences can be used interchangeably in the regulation Cassette



HEK 293 cells were transfected with Luciferase constructs with riboswitches containing guanine aptamers (left), adenine aptamers (middle) or TPP aptamers (right). Transfected cells were treated with the aptamer ligands at the indicated doses .The graphs show the dose responses of the switch to different aptamer binders. Con 1 is the control construct with no riboswitch cassette.

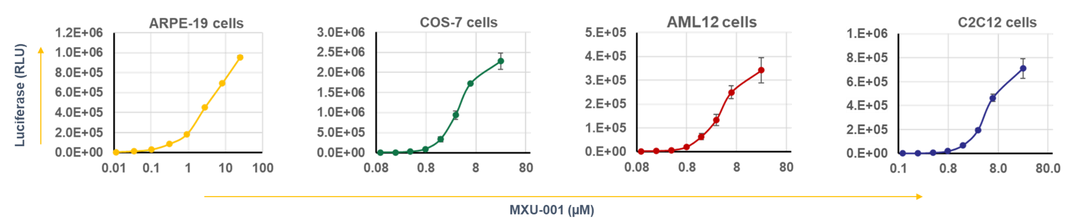
High dynamic range gene regulation cassette enables robust screening for riboswitch in mammalian cells

Large aptamer libraries incorporated into the gene expression cassette
- Randomized aptamer sequence
- Selected natural aptamers
- Site directed mutagenesis of active aptamers



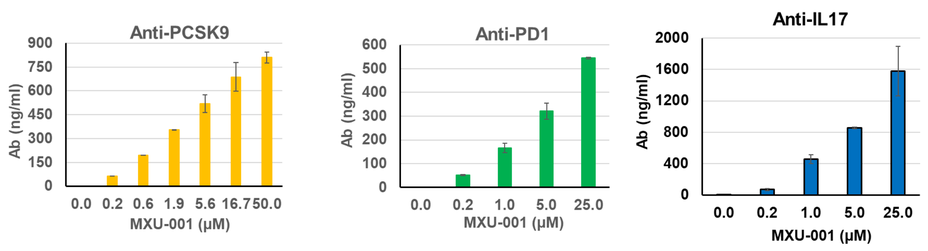
Multiple small molecule libraries screened
- Known compound libraries
- Novel fragment-based libraries
- Active compounds modified to achieve different specific desired drug properties

Riboswitch functions in multiple mammalian cell types



ARPE-19 cells, COS-7 cells, AML 12 and mouse myoblast cell line C2C12 were transfected with Luciferase constructs containing riboswitch. Transfected cells were treated with the novel synthetic small molecule at the indicated concentration, and luciferase activity was measured 20 hours after treatment.

Riboswitch regulated therapeutic antibody expression



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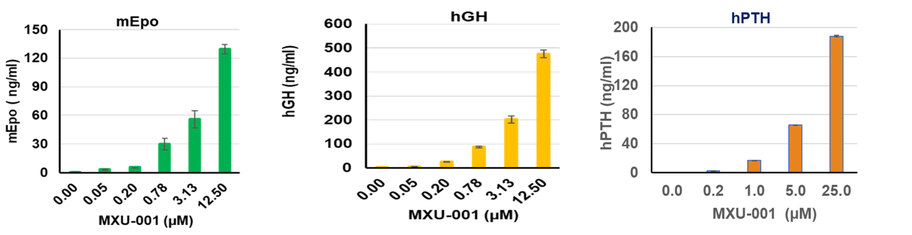
- Antibody constructs were transfected into HEK 293 cells.
- Antibody expression was induced in response to riboswitch inducer in dose dependent manner

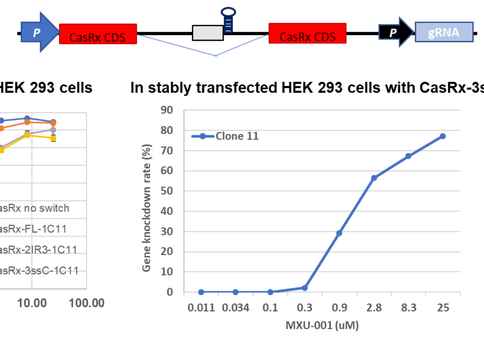
Riboswitch controlled therapeutic hormone expression



Poster 561

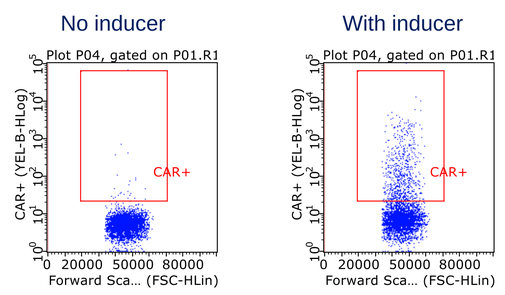
- Hormone constructs were transfected into HEK 293 cells.
- Hormone expression was induced in response to riboswitch inducer in dose dependent manner

Riboswitch regulated CRISPR/CasRx gene editing



- Riboswitch cassette FL-1C11, 2IR3-1C11 and 3ssC-1C11 have different intron sequences that give rise to different splicing activity, therefore to different basal level of CasRx activity.
- RNA knockdown is induced by MXU-001 in dose dependent manner
- CasRx-3ssC-1C11 has the lowest basal activity of CasRx (left)
- Zero basal activity of CasRx in cells stably expressing the CasRx-3ssC-1C11 in the absence of inducer (right)

Riboswitch controlled Chimeric antigen receptor (CAR) expression in Jurkat T cells



RiboCAR

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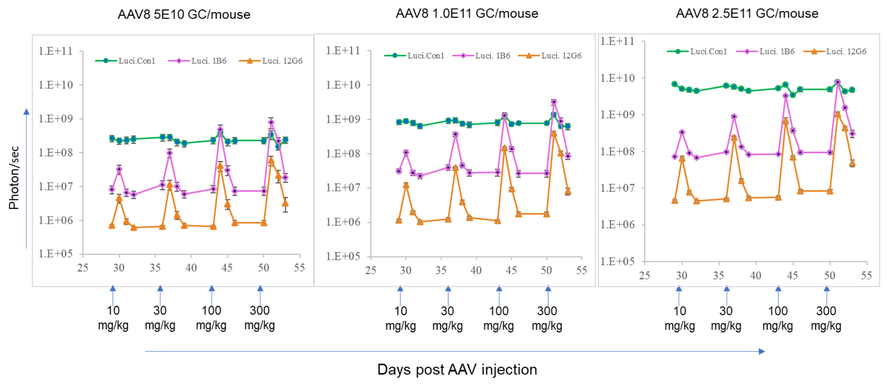
- Jurkat T cells were transfected with CAR construct Ribo CAR
- Transfected cells were treated with 25 µM MXU-001
- No CAR molecule was detected in the absence of inducer.
- Small molecule inducer treatment induced CAR expression on Jurkat T cells.

Riboswitch Regulates Transgene Expression *in vivo* via orally administered inducer



- AAV8 containing luciferase transgene with riboswitch were injected intravenously into Balb/c mice (n=5)
- 4 weeks after AAV delivery, mice were subjected to bioluminescence imaging before (0 hr) and 6 hr, 24 hr and 48 hr post a single oral small molecule dose at the indicated dose level
- Following each dose of oral small molecule activation of the transgene is seen in a dose responsive fashion in each AAV dose group

Summary

- Rationally designed synthetic riboswitches activate transgene expression via a splicing based mechanism
- Novel synthetic riboswitches are highly dynamic in regulating gene expression in mammalian cells
- Riboswitch regulate therapeutic genes with high dynamic range
- AAV delivered transgene expression is precisely regulated in a dose dependent fashion *in vivo* via orally available small molecule inducers
- Our riboswitch gene regulation system provides an unprecedented platform for precise spatial and temporal control of gene therapy for potentially treating a wide range of disorders