Riboswitch-controlled delivery of therapeutic hormones for gene therapy

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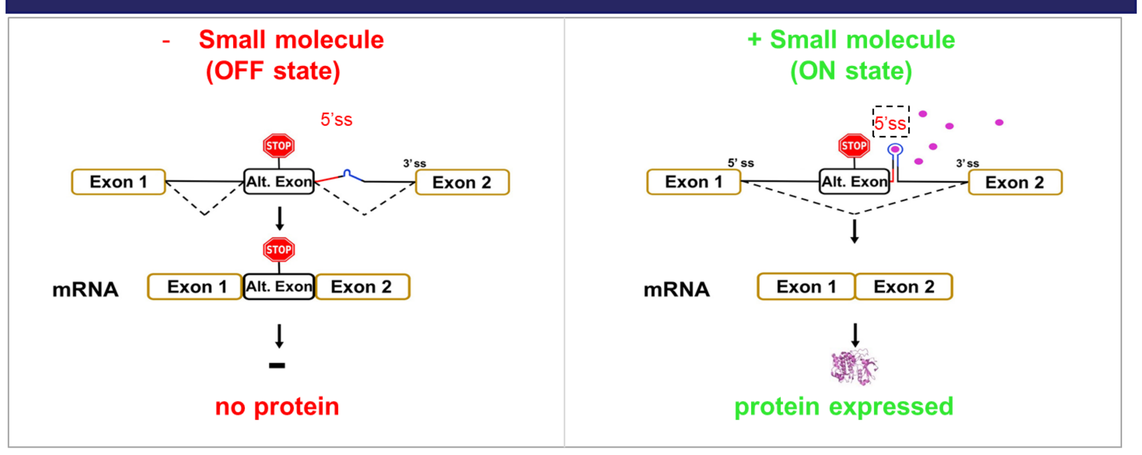
Abstract

AAV-mediated gene transfer is a promising therapy for many diseases. However, excessive amounts of transgene—especially hormones or growth factors—from unregulated vectors may not be desirable and may limit the success of treatment. A genetic switch or gene control mechanism would provide a gene therapy approach that can be safely controlled and is applicable to a broader range of disease areas. Here, we present the development of regulated hormone expression, e.g., erythropoietin (Epo) and parathyroid hormone (PTH), whose expression is controlled by our synthetic mammalian riboswitch via bespoken small molecule inducers. In contrast to previously reported gene regulation systems that use exogenous protein components, our riboswitch platform utilizes RNA element that contains an aptamer as a sensor for small molecule ligand/inducer. In our aptamer riboswitch system, aptamer/ligand binding alters transgene splicing, turning the gene on or off in a dose dependent fashion. In the absence of the small molecule inducer, Epo or PTH gene with the riboswitch does not express protein. In the presence of the small molecule inducer, Epo or PTH is robustly produced in an inducer dose-dependent manner. When Epo gene with riboswitch was delivered in AAV via a 1x IM injection to mice with kidney disease-associated anemia, no change was seen in hematocrit in the absence of the small molecule inducer. Following oral delivery of the small molecule-inducer Epo was produced in precise dose response to the dose of the oral small molecule. This orally induced Epo resulted increased hematocrits – again in a dose response fashion, to normal levels. Demonstrating that the induced transgene expression reaches efficacious therapeutic levels. Similarly, when PTH gene with riboswitch was delivered in AAV via a 1x IM injection to mice, oral small molecule treatment induced dose responsive PTH expression and increased blood calcium levels. Our data provide evidence that our riboswitch platform is effective for precise small molecule-controlled delivery of therapeutic hormones and peptides.

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

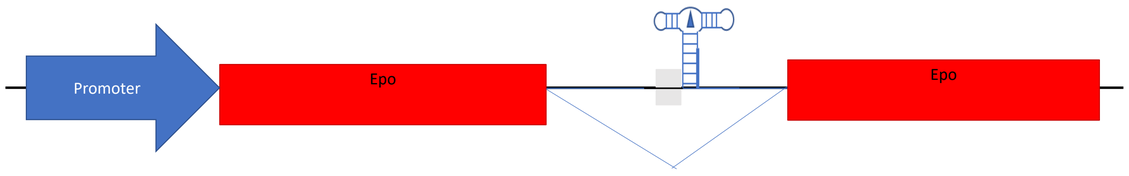
In the absence of small molecule (Left panel):

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

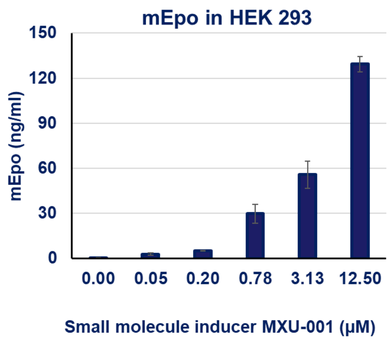
In the presence of small molecule (Right panel):

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

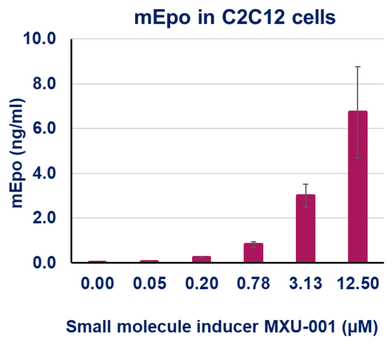
Riboswitch-regulated erythropoietin (Epo) expression *in vitro*



Schematics of Erythropoietin expression constructs with riboswitch cassette being inserted in the coding sequence

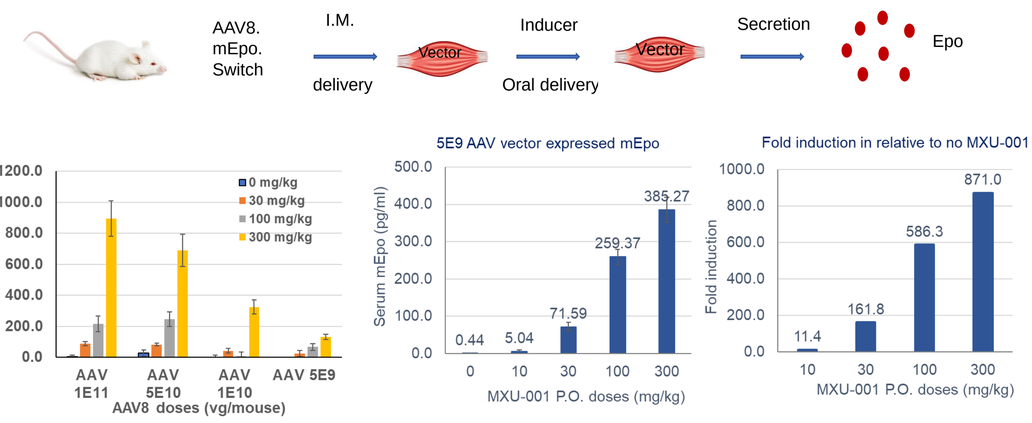


- HEK 293 cells were transfected with construct containing mEpo gene with riboswitch
- mEpo expression was induced by riboswitch inducer MXU-001 in dose dependent manner



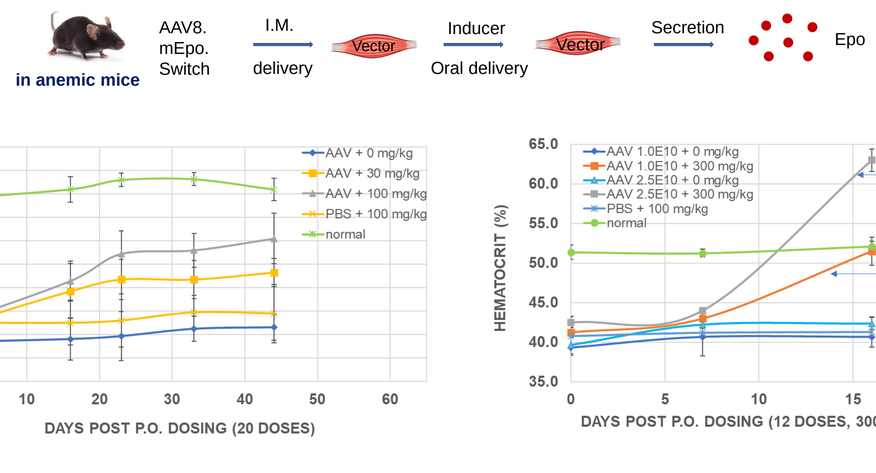
- C2C12 cells were transfected with construct containing mEpo gene with riboswitch.
- mEpo expression was induced by riboswitch inducer MXU-001 in dose dependent manner

Riboswitch regulated expression of Epo via orally dosed inducer *in vivo*



- AAV vector AAV8.mEpo.Switch was injected into the muscle of Balb/c mice at different doses
- 4 weeks post AAV delivery, mice were dosed with MXU-001 orally with different doses
- Serum samples were collected 16 hours post MXU-001 oral dosing
- mEpo expression was induced in MXU-001 dose dependent manner, in every AAV dose group.

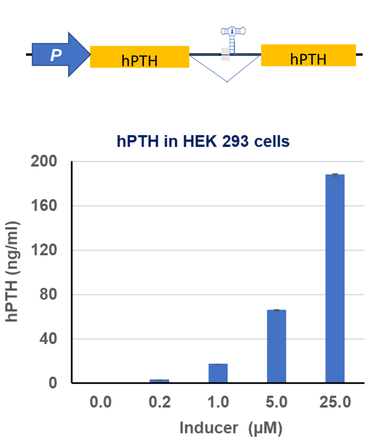
Riboswitch Controlled Epo Restores Hematocrit in Chronic Kidney Disease (CKD) Associated Anemia



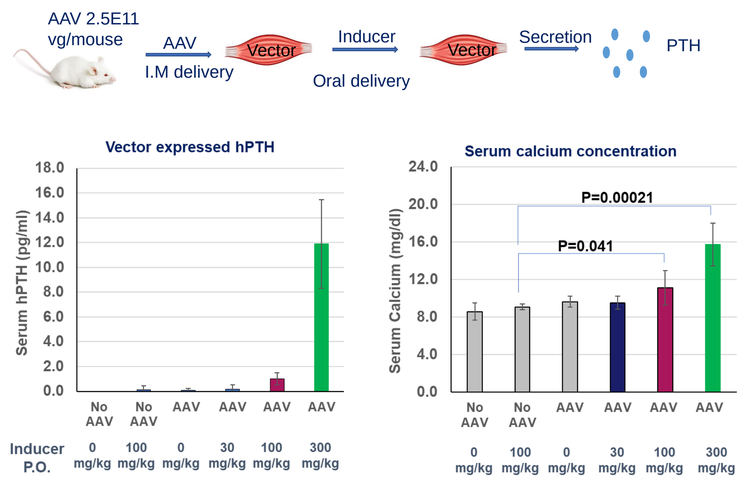
- AAV vector at $2.5x10^{10}$ vg/mouse was injected into the muscle of C57BL/6 mice
- 1 week post AAV, mice were injected with adenine to induce anemia.
- 4 weeks post AAV delivery, mice were dosed with MXU-001 or control, orally with different doses
- Hematocrit was monitored over the course of dosing.

- AAV vector at $2.5x10^{10}$ or $1x10^{10}$ vg/mouse was injected into the muscle of C57BL/6 mice
- 1 week post AAV, mice were injected with adenine to induce anemia.
- 4 weeks post AAV delivery, mice were dosed with MXU-001 orally with 300 mg/kg (indicated in the graphs) daily.
- Hematocrit was monitored over the course of dosing.

Riboswitch regulated expression of hPTH via orally dosed small molecule inducer *in vivo*



- Schematics of hPTH expression construct with riboswitch cassette
- HEK 293 cells were transfected hPTH expression constructs
- hPTH expression was induced by riboswitch inducer MXU-001 in dose dependent manner



- AAV9.hPTH.Switch were delivered to the muscle of the mice.
- Small molecule inducer was dosed orally.
- Blood samples were collected post 3 doses of MXU-001 treatment
- hPTH and serum calcium were evaluated
- Riboswitch inducer MXU-001 induced AAV delivered hPTH expression in mice in dose dependent manner
- Induced hPTH increased serum calcium level

Summary

- Riboswitch regulates gene expression post transcriptionally
- Riboswitch comprises no exogenous protein components
- Riboswitch controls gene expression by small molecule inducer
- Hormone expression was tightly controlled by riboswitch in vitro and in vivo
- Therapeutic hormone expression was induced by small molecule inducer in dose dependent manner via orally available small molecule inducer
- Induced hormone reached efficacious level *in vivo*
- Riboswitch enables precise control of therapeutic hormone expression by controlling the dose of inducer