Poster #1433

MEIRAGTx

AAV-mediated, small molecule-riboswitch-controlled delivery of growth hormone rescues growth in GH-deficient *B.little* mice

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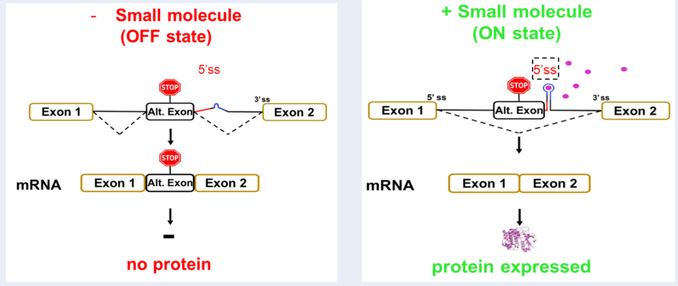
ABSTRACT

Controlled expression of delivered transgenes may be critical for optimized, safe and effective genetic medicines. AAV-mediated gene transfer is a promising therapy for many diseases. However, excessive amounts of transgene from unregulated vector may limit the breadth of applicability of gene therapy. A specific and precise mechanism for gene control via orally delivered small molecules with high dynamic range and gene expression at least as high as unregulated genes would provide a safe and effective gene therapy approach to a broad range of disease areas. Excessive amounts of therapeutic protein—especially hormones or growth factors—expressed from unregulated vectors may result in unwanted side effects and a narrow therapeutic window, as well as limiting efficacy of gene therapy. Here, we present the development of an optimized vectorized human growth hormone gene, whose expression is specifically and precisely in dose response to a bespoke oral small molecules inducer via a synthetic mammalian riboswitch. Different from previously reported gene regulation systems that involve using exogenous protein components, our gene expression platform utilizes a riboswitch which is an RNA element that contains an aptamer as a sensor for a small molecule ligand/inducer. The riboswitch has been built for activity in mammalian cells and results in activation of gene expression from a very low or undetectable basal level in the absence of the small molecule, to therapeutic levels generally exceeding the levels achieved with constitutive expression, in a tight dose response to the orally delivered small molecule. In the absence of the small molecule inducer *in vitro*, the growth hormone (GH) gene containing the riboswitch does not express growth hormone protein. In the presence of the small molecule inducer, growth hormone is robustly produced in a precise inducer dose-dependent manner. When the growth hormone gene with riboswitch was delivered in AAV into the muscle of growth hormone deficient *B.little* mice via local intramuscular injection, the oral small molecule inducer treatment resulted in increased body weight and body length of the mice. The improvement of the animal growth in *B.little* mice indicates that the induction of expression of growth hormone achieves therapeutic level in these animals and demonstrates for the first time rescue of GH deficiency via the delivery of a small molecule inducer of a locally delivered gene therapy rather than repeated injection of exogenously produced synthetic growth hormone. Our data provide evidence that our riboswitch platform provides efficacious and safe platform for delivering GH via gene expression control.

Aptamer-modulated alternative splicing riboswitch

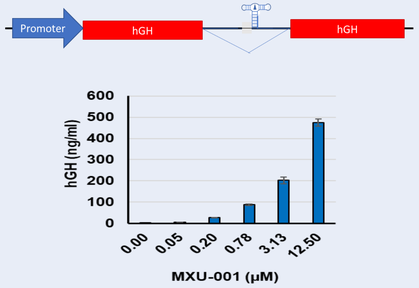


Schematics of synthetic riboswitch

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

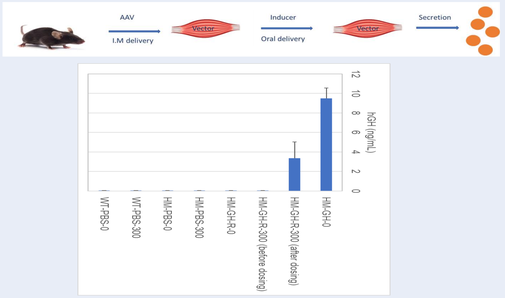
In the absence of small molecule (Left panel):	In the presence of small molecule (right panel):
Alt. exon 5′ ss is accessible	Alt. exon 5′ ss is sequestered
Alt. exon is included	Alt. exon is skipped
No protein expressed	Protein expressed

Riboswitch-regulated expression of hGH *in vitro*



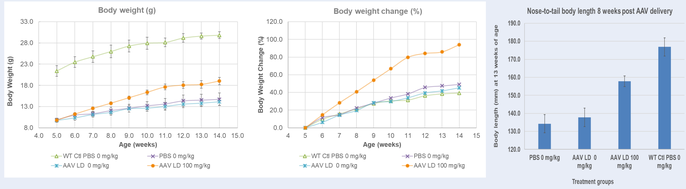
- Schematics of human growth hormone (hGH) expression construct with riboswitch cassette being inserted in hGH coding sequence.
- Antibody constructs were transfected into HEK 293 cells.
- Transfected HEK 293 cells were treated with small molecule inducer MXU-001 at indicated concentrations.
- hGH from HEK 293 culture was measured 24 hours post transfection by ELISA.

Riboswitch-regulated expression of human growth hormone via orally dosed inducer *in vivo*



- AAV.hGH vector with or without riboswitch was delivered I.M. to homozygous B. little mice. Mice were dosed orally with MXU-001 for 12 weeks.
- 47 weeks post AAV I.M. delivery, blood samples were collected from AAV injected mice before and after MXU-001 oral administration.
- hGH was assayed using human hGH-specific ELISA.

Increase in body weight and body length by AAV-delivered, small molecule-induced hGH *in B.little* mice



- Homozygous B6-little mice (C57BL/6J-Ghrhr<lit>/J mice) at age of 5 weeks were injected intramuscularly (I.M.) with low dose (LD) AAV.hGH vector (n=6) or PBS (n=6).
- Mice were dosed orally with indicated doses of MXU-001 for 8 weeks.
- Body weight was monitored weekly post AAV injection.
- Nose-to-tail body length was measured every 4 weeks.

Increases in tissue weights by AAV-delivered, small molecule-induced hGH in *B.little* mice

	BW	Length	Heart	Liver	Kidney	Spleen	Lung	Femur	Tibia
WT-PBS-0	39±5	192±5	166±28	1862±361	251±55	101±28	229±40	16.8±0.3	19.5±0.5
WT-PBS-300	36±5	189±2	169±35	1629±216	251±55	86±15	210±28	16.5±0.6	18.8±0.6
HM-PBS-0	22±7	146±5	70±5	633±76	117±8	39±9	120±18	11.8±0.5	15±0.4
HM-PBS-300	19±2	144±0	63±4	666±158	100±14	31±2	114±10	10.8±0.3	15±0.0
HM-GH-R-0	26±4	157±4	81±14	778±113	124±31	48±11	131±16	12.8±0.6	16.1±0.2
HM-GH-R-300	30±7	173±3	99±18	1006±218	149±21	53±6	143±18	14.5±0.5	17.5±0.4
HM-GH-0	35±6	177±4	137±25	1377±116	197±47	187±133	167±15	16±1.0	18.7±1.5

- AAV.hGH vector containing riboswitch was delivered I.M. to homozygous B. little mice and were dosed with MXU-001 orally for 12 weeks.
- Tissue of normal (WT) and growth hormone deficient (HM) mice injected with PBS or AAV9 encoding hGH with/without riboswitch was collected 47 weeks post AAV I.M. delivery. Data are expressed as the mean ± S.D.

Summary

- Our data demonstrate that our synthetic mammalian riboswitch works efficiently both *in vitro* and *in vivo* in regulating growth hormone expression via a riboswitch small molecule inducer.
- Growth hormone expression was precisely controlled via orally available small molecule inducer *in vivo*.
- The induced human growth hormone achieves efficacious level in promoting growth in growth hormone deficient animals.
- Our data supports a safe and efficacious therapeutic approach in treating hormone deficiency.