In Vivo Suppressor tRNA Mediated Readthrough Therapy for Nonsense Mutations

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Genetic diseases are caused by a variety of mutations and changes to the genome. A nonsense mutation introduces a premature termination codon (PTC) in the mRNA and accounts for ~11% of mutations associated with human diseases. Since the PTC stops translation before a full-length protein is produced, the truncated protein may be degraded or not be as effective. PTC readthrough is a method to restore protein expression at the translational level.

Figure 1: Modified tRNAs that can decode a stop codon are known as suppressor tRNAs (stRNAs), and have therapeutic potential as readthrough agents. Suppressor tRNAs differ from natural tRNAs by one nucleotide in the anticodon and insert an amino acid during protein elongation.

However, the in vivo therapeutic efficacy of stRNAs has been understudied compared to other readthrough agents such as aminoglycosides and PTC124. In this study, we aim to develop an in vivo suppressor tRNA therapy delivered by AAV vectors for a lysosomal storage disease known as mucopolysaccharidosis type I (MPS-I) and evaluate its safety profile.

Figure 2: We first screened a panel of suppressor tRNAs for their UAG stop codon readthrough activity in HEK293 cells using a GFP reporter with a Y39X (UAC-->UAG) mutation, and a dual-luciferase reporter with a nonsense mutation (UGG-->UAG) underlying MPS-I, respectively. After triple-transfection with a plasmid expressing each stRNA, cells were imaged and assayed for luminescence. Suppressor tRNAs E, F, and G efficiently induced and restored reporter protein expression.

Figure 3: stRNA F was packaged into a lentiviral vector co-expressing GFP, and this vector was used to infect fibroblast cells from an MPS-I patient with homozygous IDUA^{W402X} mutation (UGG-->UAG) that abrogates iduronidase (IDUA) activity. GFP was used to assess the infection efficiency of the fibroblast cells. IDUA activity was restored to 1.7% of the normal level, which is above the targeted therapeutic threshold of 0.5%.

Figure 4: We packaged stRNA F into an AAV9 vector and systemically delivered 4E12 GC to a mouse model of MPS-I harboring the *Idua*^{W392X} mutation (UGG-->UAG) analogous to the most common human mutation. Sustainable restoration of serum and liver IDUA activity of 1-5% of the normal level was observed up to 23 weeks post-injection and counting.

Figure 5: Two new constructs expressing two and four copies, respectively, of stRNA F driven by a Pol III promoter were packaged into AAV9. One-fourth of the original dosage was delivered to the same MPS-1 mouse model. Only female mice were injected for this experiment. After 10 weeks, the mice injected with the vector harboring two copies of stRNA F demonstrated the highest serum IDUA activity level.

Figure 6: HEK293 cells were treated with 0.1mg/ml G418, a widely used readthrough compound, for 24 hours. Ribosome profiling is an established platform to provide a global snapshot of the positions of mRNA-bound ribosomes. In short, ribosomes are frozen in place during protein synthesis and ribosome-protected fragments are isolated for sequencing. In G418-treated cells, ribosomes were localized to both the CDS and 3' UTR with fewer ribosomes stalled at the natural stop codon, compared to the untreated cells. This platform will be used for stRNA-treated cells and tissue to assess global readthrough at normal stop codons.

The functional suppressor tRNAs identified in this study were able to read through PTC mutations in two reporter assays, primary patient cells, and a mouse model of MPS-I syndrome. stRNA F could be potentially used to treat other diseases caused by a UAG PTC. Compared to gene replacement and CRISPR-based gene editing, the small gene size of suppressor tRNA is highly amenable to AAV vector delivery, and lack of foreign protein expression has a favorable immunological profile.

Further assessment of the therapeutic efficacy is ongoing to determine the long-term effect of stRNA treatment for MPS-1. In addition to serum and tissue IDUA activity, other markers will be assessed such as glycosaminoglycan levels, tissue histology, and behavioral assessments. The safety profile of suppressor tRNAs will be investigated through platforms such as ribosome profiling and tRNA-seq. tRNA-seq could reveal the effect of stRNAs on the endogenous tRNA pool, as changes could lead to deleterious cellular effects. Additional stRNAs will be developed to target UGA and UAA codons as well as insert other amino acids. Delivery of stRNAs is not limited to AAV and lentivirus; other methods such as synthetic tRNAs could prove useful for the treatment of other diseases.