248. *In Vivo* Delivery of Suppressor tRNA Overcomes a Pathogenic Nonsense Mutation in Mice

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A nonsense mutation introduces a premature termination codon (PTC) in mRNA that prevents full-length protein synthesis, and accounts for ~11% of human pathogenic mutations. Engineered tRNAs that can decode a termination codon and readthrough a PTC are known as suppressor tRNAs (stRNAs), and have long been considered as potential therapeutic agents targeting PTCs. However, their *in vivo* therapeutic efficacy and safety have been understudied compared to other readthrough agents such as aminoglycosides. In this study, we aim to develop an *in vivo* stRNA therapy delivered by AAV vectors as platform therapeutics for nonsense mutations, such as the *IDUA-W402X* mutation found in the majority of Hurler syndrome patients. We first screened a panel of stRNAs for readthrough activity in HEK293 cells and Hurler syndrome patient fibroblasts that carry homozygous *IDUA-W402X* (TGG>TAG) nonsense mutation; this mutation abrogates the lysosomal enzyme iduronidase (IDUA) activity. One stRNA was able to restore IDUA enzymatic activity above the targeted therapeutic threshold of 0.5% of normal level. This stRNA gene was packaged as recombinant AAV9 (rAAV9) and systemically delivered to a mouse model of Hurler syndrome harboring the *Idua-W392X* mutation (TGG>TAG) analogous to the human mutation. At 10 weeks post-treatment, serum IDUA activity was restored up to 6% of wildtype (WT) level, accompanied by 70% reduction in urine glycosaminoglycans (GAGs), the primary substrates accumulated due to IDUA deficiency. Furthermore, IDUA activity was restored to 9% and 27% of WT levels in the liver and heart, respectively, which led to significant decrease in tissue GAGs (95% and 66% reduction in the liver and heart, respectively). Elevation of other lysosomal enzymes triggered by GAG accumulation, a hallmark of several lysosomal disorders including Hurler syndrome, was almost completely normalized. Local but not systemic rAAV9.stRNA delivery to the brain and skeletal muscle achieved significant IDUA activity restoration, which correlates with stRNA gene delivery efficiency. These results demonstrate that rAAV9.tRNA could alleviate whole-body disease burden and tissue pathology caused by the *Idua-W392X* nonsense mutation. Ribosome profiling of patient fibroblasts revealed that, whereas G418 (the benchmark readthrough aminoglycoside) induced high global readthrough at normal stop codons and strong perturbation in translation elongation, stRNA had a much milder impact and only acted on the UAG stop codon. Similarly, the rAAV9-stRNA-treated mouse liver exhibited UAG-restricted global readthrough and unperturbed translation elongation, indicating a better safety profile than aminoglycoside. We did not observe gross abnormalities nor any changes of comprehensive serum clinical chemistry in the treated mice. tRNA sequencing is ongoing to assess endogenous tRNA homeostasis following stRNA treatment. The functional stRNAs identified in this study could be potentially used to treat a range of diseases caused by a UAG PTC. Compared to gene replacement and CRISPR-based gene editing, the small gene size of stRNA is highly amenable to AAV vector delivery, and lack of foreign protein expression has a favorable immunological profile. *These authors contributed equally to this work. [a]Co-corresponding authors.

249. The 3´tsRNAS Are Aminoacylated Further Implicating Their Role in Ribosome Biogenesis during Tissue Homeostasis and Cancer

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Emerging evidence indicates that tRNA-derived small RNAs (tsRNAs) are involved in fine-tuning gene expression and become dysregulated in various cancers. There are at least 6 major types of tsRNAs derived from the mature or precursor tRNA genes in the genome. We recently showed that the 22nt LeuCAG tsRNA from 3´ end of the mature $tRNA^{Leu}$ is required for efficient translation of two ribosomal protein mRNAs and ribosome biogenesis. Inactivation of this tsRNA induced apoptosis in rapidly dividing cells and in patient-derived orthotopic hepatocellular carcinomas implanted in mouse liver (Kim et al., Nature 2017). Here we used bioinformatic algorithms to establish the expression profiles of the more than 150, 3'-tsRNAs present in human normal versus tumor paired tissues. We found a number of 3'tsRNAs that are up or down regulated in nine human cancers evaluated to date suggesting they may play a role in the oncogenic process perhaps by regulating the cell's protein synthetic capacity. The mechanism involved in the generation of the 3´-tsRNAs has remained elusive and it is unclear if the 3´-ends of 3´-tsRNAs are aminoacylated. Here we report an enzymatic method utilizing exonuclease T to determine the 3´charging status of tRNAs and tsRNAs. Our results showed that the LeuCAG 3´-tsRNA is fully charged and originated solely from the charged mature tRNA. When the leucyl-tRNA synthetase was knocked down, less tsRNA was generated while the mature tRNA was not. Because the aminoacyl-tRNA synthetases form a complex on actively translating ribosomes, our results support a model where the tsRNAs are generated during translation and finely tune ribosomal protein synthesis, and thus regulate ribosome biogenesis during homeostasis and altered growth conditions including cancer. These small RNAs represent novel targets for treating cancer.