Engineered Suppressor tRNA Mitigates Nonsense-Mutated Duchenne Muscular Dystrophy in mice

Yuxuan Shen[1], Yuelin Yang[1], Liangzhen Dong[1], Zhetao Zheng[1], Bangyan Kong[1], Zhiyang Dou[1], Qing Xia[1]*

[1] State Key Laboratory of Natural and Biomimetic Drugs, Department of Chemical Biology, School of Pharmaceutical Sciences, Peking University, Beijing, China.

Transfer RNAs (tRNAs) facilitate the transport of amino acids from mRNA to the ribosome, playing an essential role in translation, the process of protein synthesis guided by genetic instructions. Suppressor transfer RNAs (stRNAs), derived from natural tRNA through anticodon mutation, enable the recognition of three distinct premature termination codons (PTCs): UAA, UAG, and UGA. They hold significant potential as nucleic acid therapeutics for treating nonsense mutation diseases. Here, we report the identification of 18 codons encoding 10 disease-associated amino acids in nonsense mutation diseases, as well as the construction of a library consisting of 155 stRNAs. This library was generated by introducing a single base mutation in the anticodon of natural tRNA to enable decoding of PTCs. To better screen stRNAs with high PTC readthrough efficiency, we conducted a systematic study on the impact of base mutations in different regions of the stRNA, as well as amino acid substitutions in aminoacyl tRNA synthetase (aaRS) that interact with stRNA. Additionally, we evaluated the effects of stRNA combined with corresponding amino acids at varying concentrations on the readthrough rate. Our investigation revealed key sites in both stRNA and aaRS that influence readthrough efficiency. In a cellular model carrying a mutated Dp71 gene with three PTCs, we observed that $stRNA^{Gln}$ demonstrated superior efficiency in reading the mRNA. Furthermore, we explored the potential of a combination therapy using stRNAs to target different mutated sites of the Dp71 gene, aiming to establish a universal therapeutic approach for nonsense mutation diseases. Notably, we demonstrated the effective restoration of dystrophin expression and improved muscle function in the TA muscle of the *mdx* mouse model through in situ electroporation and intramuscular delivery of $stRNA^{Gln}$ via AAV. In conclusion, our

comprehensive investigation has elucidated the factors influencing stRNA readthrough efficiency, confirming the potential of stRNAs as therapeutic agents for pathogenic nonsense mutations. Future efforts will focus on optimizing stRNA combinations and expanding their application to diverse disease models.