Investigation of novel modifications and optimization of the mRNA manufacturing process for therapeutic applications

Abstract:

The development of molecular tools based on synthetic mRNA technology has been ongoing for over 30 years. The continuous improvement of methods for producing, storing, and delivering nucleic acids to patient cells has contributed to the widespread use of RNA-based medical procedures, including mRNA vaccines and gene therapies. The main problem that remains unresolved is the low stability of RNA. Under physiological conditions, mRNA is relatively rapidly degraded, with this process typically initiated at the terminal regions of the molecule. Natural mRNA has protective structures at both ends that regulate its degradation and protect the coding regions. Most mature mRNAs in eukaryotes have a cap structure at the 5' end, which protects against 5'-exonucleases and supports translation. At the 3' end, there is a poly(A) tail that protects the molecule from degradation at this end, increasing its stability and participating in the translation process. The interaction between these structures is crucial for efficient translation and the regulation of mRNA stability in eukaryotic cells, which is essential for potential therapeutic applications. In recent years, a number of new mRNA modification methods have been developed, significantly improving its biological activity and laying the foundation for cancer therapies and further research in this field. My doctoral research focuses on the development of these technologies and their potential applications.

The first part of my dissertation involves the development of a biophysical method for studying the interactions of synthetic mRNA with the IFIT1 protein. The technique of microscale thermophoresis enabled precise analysis of molecular interactions, helping to understand the mechanisms of RNA recognition by the human immune system. The study included over forty RNA molecules with various structural modifications at the 5' end. The results allowed the identification of the impact of chemical modifications on interactions with IFIT1 and the characterization of the patterns of these interactions.

The second part of my research focused on developing a method for detecting double-stranded RNA (dsRNA) in mRNA preparations. dsRNA molecules are strong immunogenic factors that can trigger unwanted immune responses in patients. The rapid and sensitive dot-blot immunoassay I developed allowed the assessment of dsRNA content in mRNA preparations, which is crucial for quality control of these therapeutic products.

The third part of my dissertation focused on structural modifications in the poly(A) tail region of mRNA. My research aimed to increase the stability of this sequence at the DNA level and improve the translational potential of mRNA. The genetic constructs designed, containing various heteronucleotide arrangements, were evaluated for their biological activity both in vitro and in vivo, which allowed for the identification of the most promising modifications with therapeutic potential.

The methods and modifications I developed aim to improve mRNA technology, opening new prospects for gene therapies and other medical applications. Their implementation could contribute to enhancing the efficacy and safety of mRNA-based therapies, which is crucial for the future development of molecular medicine.