Molecular Therapy
Editorial

In vitro-Transcribed mRNA Therapeutics: Out of the Shadows and Into the Spotlight

In the 1990s, enthusiasm for gene therapy was high, and the cure of human genetic diseases seemed within reach. However, this excitement halted abruptly when a viral vector used to introduce a therapeutic gene caused the death of a patient. Meanwhile, no therapeutic benefit could be achieved using plasmid vector, which was considered a safer gene delivery tool. In the shadow of DNA-based gene therapy, a few scientists experimented with *in vitro*-transcribed (IVT) mRNA to express a therapeutic protein. Early on, the IVT mRNA was injected into frog oocytes or transfected into cultured cells. Later, translation from the administered IVT mRNA was also achieved in mice. Even at that time, only a few RNA fanatics believed that IVT mRNA could be used as a therapeutic. Instability, immune activation, and difficulty of delivery of the mRNA were identified as major obstacles. During these formative years, the possibility of using the human body to produce therapeutic protein from the delivered IVT mRNA was gradually recognized. Now, over 20 years later, IVT mRNAs are in the spotlight as they are evaluated in preclinical and clinical studies for the treatment of a wide variety of diseases. This newfound enthusiasm has produced a wave of emerging companies to further exploit this technology. In this special issue of *Molecular Therapy*, we review the development of various therapeutic applications of mRNA technology.

It was recognized early on that IVT mRNAs encoding tumor-associated antigens are suitable for therapeutic cancer vaccines due to the transient nature of their translation. For such an application, the immunogenicity of the mRNA is an added benefit by acting as a vaccine adjuvant. Later on, IVT mRNA was developed as a vaccine to prevent infectious disease. Maruggi et al.[1] provide an excellent review of this field, including evaluation of the different mRNA platforms pursued today for prophylactic vaccine development.

Deimmunizing IVT mRNA by incorporating modified nucleosides was a fundamental strategy to broaden the potential applications. The advantage of nonimmunogenic IVT mRNA was first recognized during mRNA-mediated generation of induced pluripotent stem cells. Warren and Lin[2] review this field and give an exquisite account of the development of IVT mRNA encoding key transcription factors for production of human stem cells with the aim of being utilized for personalized regenerative treatments.

At one time, gene therapy was considered to be a process by which a therapeutic gene was permanently introduced into the genome. This approach could, under some circumstances, prove unsafe owing to the insertion of the vector at random locations within the genome. In recent years, a promising new gene therapy approach has emerged in which gene editing enzymes are used to restore or repair a faulty gene. Zhang et al.[3] discuss recent advances in using mRNA encoding zinc-finger, transcription activator-like effector (TALE), or Cas9 nucleases to engineer the genome. The transient presence of the IVT mRNA encoding the nuclease is an important feature to limit off-target effects of these enzymes.

Improvements in mRNA delivery have had a major impact on accelerating the advance of IVT mRNA toward the clinic. The review by Kowalski et al.[4] provides a comprehensive in-depth assessment of the materials and technologies that were developed to deliver mRNA for specific applications. The delivery formulation protects the mRNA cargo in the extracellular milieu and promotes its cellular uptake and release into the cytoplasm. As a result of the continuous advancement of delivery methods, IVT mRNAs are now being tested in clinical trials to provide missing or defective proteins due to genetic disorders or in cases where the delivered protein imparts a therapeutic effect. The most advanced of these treatments is delivery into the liver, a point that Trepotec et al.[5] explore in their review of the use of IVT mRNA to treat hepatic diseases. Introducing mRNA into the heart, however, has proven to be very challenging. Despite the difficulties, several preclinical studies have shown regeneration of cardiac tissue using a variety of IVT mRNA, as illustrated in the review by Magadum et al.[6] Augmenting vascularization of the infarcted heart using mRNA is now under clinical evaluation. Another promising area of IVT mRNA application is in the field of monoclonal antibodies, which is now one of the fastest growing pharmaceutical sectors in the market. Schlake et al.[7] provide an excellent overview of the opportunity to use antibody-encoding mRNA to replace protein-based antibody in the fields of oncology and infectious disease.

In the last three decades, extensive research and technology development in many different fields have contributed to the emergence of IVT mRNA as a therapeutic that has now reached clinical testing. The affordability of gene synthesis services has been important to ensure easy access to optimized genes. It was crucial to generate IVT mRNA by constantly improving cap structures and a long polyA tail to enhance translatability. Incorporation of modified nucleosides into the mRNA and implementing different purification procedures were critical to reduce immunogenicity and further increase the translational capacity. Formulations that protect the mRNA from nucleases and accelerate their cellular uptake combined with improvements to the mRNA molecules were critical advancements for making mRNA a viable therapeutic. Though once regarded as a serious impediment, the transient nature of mRNA technology is now considered a major advantage in making

mRNA therapies safe and, ultimately, a potential game changer in the field of medicine.

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