Future of biotherapeutics: Harnessing mRNA to enhance elastin expression

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Organs that are subjected to repeated stretching such as the skin, lungs, and vasculature rely on elasticity provided by the extracellular matrix (ECM) for structural integrity. Tissue elasticity is dependent on elastic fibers composed of a protein core of insoluble elastin that is assembled onto a microfiber scaffold. Tropoelastin (TE), the basic building block of elastin, is secreted as a soluble monomer that undergoes self-aggregation and cross-linking to form insoluble elastin. The process of elastogenesis is developmentally regulated and occurs early in life, with components of the elastin machinery, including TE, subsequently downregulated in adulthood. Once formed, elastic fibers are highly resilient and have a long half-life of 74 years in humans.[1] However, both reduced expression of TE during development and accelerated degradation of preformed elastic fibers can alter tissue elasticity. In genetic disorders such as supravalvular aortic stenosis, mutations in the elastin gene (*ELN*) results in functional *ELN* haploinsufficiency. Inflammatory fragmentation of elastic fibers in the lung is commonly seen in chronic obstructive pulmonary disease (COPD), and disruption of elastic fibers in the medial wall of the aorta contributes to aortic aneurysmal dilation. In the skin, the loss of elastic fibers because of direct injury or burns can result in scarring and loss of elasticity. In these disease states, increasing elastin expression may be important to restore tissue homeostasis. In a recent issue of *Molecular Therapy – Nucleic Acids*, Golombek et al. describe a novel strategy to augment TE production in the skin using synthetic mRNA.[2] The authors show that synthetic TE encoding mRNA increased TE protein expression both in cell culture systems and *in vivo* in a porcine skin model.
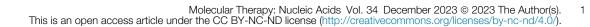
Recombinant TE and genetic strategies using viral vectors have been used to augment TE expression. Administration of recombinant human TE increased elastic fiber formation in skin patches both *ex vivo* and *in vivo*.[3] Adenoviral vectors carrying a recombinant TE gene increased elastin mRNA and protein expression by aortic vascular smooth muscle cells and facilitated reconstruction of elastic fibers *in vivo* in an experimental model of abdominal aortic aneurysm.[4] mRNA therapies offer multiple advantages over recombinant proteins or viral vector–based treatment strategies; they are relatively simpler to synthesize *in vitro*, they are easier to deliver, and because mRNA transcripts do not enter the cell nucleus, there is no risk of the delivered nucleic acid integrating with the host genome. It is therefore exciting that Golombek et al. now report encouraging results regarding the effectiveness of structure-optimized synthetic mRNA variants in inducing TE protein expression. The authors found that although mRNA codon optimization improved TE protein expression, nucleotide modifications resulted in both improved TE translation and greater cell viability. Overall, mRNA variants with both codon optimization and base modifications induced the highest protein expression.[2]

Although the potential for mRNA biotherapeutics has been recognized for over 3 decades, approval and widespread use of mRNA vaccines during the coronavirus disease 2019 (COVID-19) pandemic placed mRNA-based therapies in the limelight. Recent scientific advances have enabled the development of additional mRNA-based therapeutics that are in early clinical trials for diseases as diverse as heart failure, hereditary amyloidosis, and cystic fibrosis.[5] Much

of this progress is attributed to better understanding of strategies to address two major hurdles with mRNA therapeutics: the need for high translational efficiency and concerns about mRNA-induced activation of innate immune defenses through Toll-like receptors (TLRs). Each component of mRNA can be optimized to enhance protein expression. 5′ cap and 3′ poly (A) tail analogs can be synthesized to maximize mRNA stability, and the composition of the 5′ and 3′ UTR can be customized to the target cell to increase the tissue specificity of translation. Furthermore, degeneracy of the genetic code allows for codon optimization to overcome the limitations associated with species-specific differences in codon usage and tRNA abundance, thereby enhancing translational efficiency. A breakthrough in the optimization of mRNA therapeutics came from seminal work from Katalin Kariko and Drew Weissman, winners of the Nobel Prize in Physiology or Medicine in 2023. They found that certain nucleotide modifications in mRNA dampened its inflammatory response and abolished mRNA-mediated dendritic cell activation.[6] Additional studies from their group demonstrated that *in vitro* transcribed mRNA with pseudouridine (Ψ) nucleotide modification was less immunogenic and enhanced the translational capacity and stability of mRNA.[7] The modification of mRNA nucleotides also reduces the activation of protein kinase R, which is responsible for the phosphorylation of the translation initiation factor 2α, thereby increasing translational efficiency.[8] Thus, mRNA nucleotide modifications have the potential to increase protein expression without the adverse effects of increased immunogenicity.

In their study, Golombek et al.[2] found that TE mRNA transcripts with pseudouridine (Ψ/m5C) and N[1] methylated pseudouridine (me[1]Ψ/m5C and me[1]Ψ/C) induced the highest protein expression *in vitro*. In

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addition, a codon-optimized mRNA variant with me$^1\Psi$/C modification resulted in the highest *de novo* TE synthesis when injected in porcine skin. Ψ, which is derived from uridine via pseudouridylation, a nucleotide-specific isomerization reaction, is a naturally occurring nucleotide found in a variety of RNA. Ψ can be substituted for uridine during the *in vitro* transcription of RNA by replacing uridine-5′-triphosphate (TP) with ΨTP. Methylation of the amine group at the N1 position of Ψ results in me$^1\Psi$, another naturally occurring RNA modification found in many organisms. Although both Ψ and me$^1\Psi$ containing RNA are more stable compared to uridine-containing transcripts, me$^1\Psi$ is less prone to wobble pairing with nonadenine nucleotides.[9] Of note, in both the Pfizer-BioNtech (BNT162b2) and Moderna Therapeutics (mRNA-1273) COVID-19 mRNA vaccines, uridines were replaced by me$^1\Psi$ during *in vitro* transcription, likely contributing (among other modifications) to their demonstrated efficacy.

Although the results from the study by Golombek et al.[2] are intriguing, additional points need to be carefully considered when evaluating the role of synthetic mRNA for augmenting TE expression. First, the immunogenicity of TE mRNA variants needs to be examined thoroughly. mRNA can activate the TLR family of innate immune receptors—namely, TLR3, TLR7, and TLR8—resulting in proinflammatory responses. Furthermore, the activation of TLRs on dendritic cells can result in antigen presentation and enhanced T cell–mediated adaptive responses. Local and systemic reactogenicity have been observed with mRNA vaccines and other mRNA therapeutics in clinical trials,[5] and these reactions are more likely for transcripts requiring serial administration.

Second, it is important to determine whether, along with augmentation of protein expression, synthetic TE mRNA also increases the assembly of mature elastin fibers, the hallmark ECM component required for tissue elasticity. This is important because mRNA codon modifications may alter TE protein confirmation and function. Finally, strategies to effectively deliver TE mRNA transcripts *in vivo* will need to be optimized further. Although the use of isotonic buffers as vehicle may be sufficient in certain conditions, additional approaches such as combining mRNA with lipid nanoparticles may be needed to enhance the efficacy of cellular uptake and the translation of elastin *in vivo*. Furthermore, although transdermal administration may be sufficient for enhancing elastin synthesis in the skin, different strategies will be needed to target other organs in which reduced functional elastin fibers determine pathology, such as the lung in COPD or the vascular wall in aortic aneurysms. These issues need to be addressed in future studies.

The development of COVID-19 vaccines has put mRNA biotherapeutics front and center, and this is an exciting time for the field. Currently, trials using these agents are in various stages of development for applications in immunobiology, oncology, vaccine development, and metabolic diseases. Harnessing this therapy to increase TE expression has the potential for substantial clinical benefit. This could also pave the way for the development of similar strategies to enhance the expression of other ECM components that may be disrupted in disease or may decrease with age.

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DECLARATION OF INTERESTS
The authors declare no competing interests.

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