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Commentary

Therapeutic potential for mRNA-based IGF-I regenerative therapy

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Recent advances in messenger RNA (mRNA) therapeutics have led to the development of alternative nonviral gene transfer methods with the potential for enhanced therapeutic efficacy.[1,2] In a recent issue of *Molecular Therapy Nucleic Acids*, Antony and colleagues investigated the regenerative potential of an insulin-like growth factor-I (IGF-I) delivery approach incorporating localized administration of *in-vitro*-transcribed (IVT) mRNA compounds (Cpds.).[3] IGF-I expression from modified mRNA Cpds. was evaluated in both mouse myotoxic skeletal muscle injury and rabbit spinal disc herniation models, and increased protein expression, secretion, and recovery were observed with treatment. These findings support the regenerative potential of chemically modified IGF-I mRNA therapeutics as a nonviral-based alternative for treating various musculoskeletal and neurodegenerative diseases.

Viral-based gene therapies, including adeno-associated viruses (AAVs), comprise the majority of clinical gene therapy trials worldwide, in comparison to nonviral therapies such as plasmid DNA- and RNA-based transfers.[4] Recombinant AAV (rAAV) technologies emerged in the 1980s, leading to the first rAAV application in humans in 1995.[5] Currently, rAAVs are the most prominent vector platform used for delivering therapeutic transgenes *in vivo*, with much higher efficiency compared to nonviral vectors.[6] Recent advances in rAAV technologies have led to US Food and Drug Administration (FDA) approval for gene therapies to treat several genetic disorders including retinal dystrophy, spinal muscular atrophy, hemophilia A and B, and Duchenne muscular dystrophy. Amid these considerable milestones, important challenges remain for rAAV-based technologies including high manufacturing costs, patient accessibility and affordability, extensive quality control measures and standardization, and immunological challenges for achieving efficient delivery and persistent gene expression.[6]

Advances in nucleic acid therapeutics for gene therapies have rapidly evolved from conventional plasmid DNA-based vectors to new platform technologies incorporating nucleic acid conjugates, mRNA, and gene-editing therapeutics.[7] Furthermore, our understanding of the mechanisms underlying RNA modifications and their effects on cellular processes has led to clinical advances in RNA therapeutics and the ability to manipulate RNA to achieve increased levels of protein expression.[1] The rapid development of mRNA-based COVID-19 vaccines has further enhanced interest in mRNA applications for gene delivery, protein replacement, and regenerative therapies.[8] When compared to other viral and DNA-based technologies, IVT mRNA therapeutics offer several advantages: it only requires cytoplasmic delivery, it utilizes endogenous translation mechanisms to transiently express proteins, and it poses low risks for genomic integration.[2,7,9] However, disadvantages of IVT mRNA therapeutics include mRNA instability, inefficient delivery, extracellular and intracellular barriers, and immunogenicity.[2] To address these limitations, many advances in IVT mRNA technologies utilize mRNA base modifications to minimize immune stimulatory effects, increase transcript stability, and augment translation.[2] Delivery systems such as lipid nanoparticles (LNPs) are also frequently used with modified mRNA Cpds. to enhance stability and achieve targeted delivery.[8]

Antony et al. compared IVT IGF-I mRNA Cpds. engineered with different signal peptide (SP) sequences designed to enhance protein secretion to native IGF-I mRNA after local intramuscular injection in the tibialis anterior (TA) in mice and local intraspinal delivery in rabbits. The researchers hypothesized that local delivery of naked IGF-I mRNA could achieve more abundant IGF-I translation in specific regions that are difficult to transfect, including skeletal muscle and vertebral discs. A combined mRNA delivery approach with LNPs was not evaluated in this study, supported by the researchers' rationale of avoiding potential LNP-induced systemic effects as well as the larger volumes of LNP-mRNA required to achieve therapeutic efficiency in the smaller localized anatomical regions being investigated.[3]

IGF-I plays a central regulatory role in skeletal muscle growth and repair and connective tissue remodeling.[10] IGF-I deficiency is implicated in a number of musculoskeletal, neurodegenerative, cardiovascular, and growth-related disorders. IGF-I replacement therapies may provide muscle regenerative and neuroprotective effects but are limited by short half-life and dosing insufficiencies of systemically delivered recombinant human (rh)IGF-I proteins.[11] Aside from these limitations, there have been recent advances in (rh)IGF-I therapeutics including the 2023 FDA approval of an IGF-I-based synthetic peptide, trofinetide, for treating the rare developmental disorder Rett syndrome by enhancing IGF-I expression in the brain.[12] In a viral-based approach, overexpression of IGF-I in skeletal muscle using rAAVs was shown to attenuate muscle damage, increase muscle size and function, and improve regeneration in mouse models of muscle injury and peripheral artery disease-associated hindlimb ischemia.[10,13] As with many cellular and gene-based therapies, achieving therapeutic efficacy in regenerative applications has challenges including target site specificity, dosage and delivery optimization,

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immune response activation, and other potential off-target systemic effects.

In the study by Antony et al., the authors evaluated three different IGF-I mRNA Cpds., which included an IGF-I mRNA containing the native SP (Cpd. 1) and two IVT mRNAs engineered with the SP variants brain-derived neurotrophic factor (BDNF; Cpd. 2) and neurotrophin-3 (NTF-3; Cpd. 3). The goal of including BDNF and NTF-3 was to incorporate SPs associated with high levels of translation and secretion as an approach for enhancing IGF-I secretion from IGF-I mRNA-transfected cells. In the initial studies, the researchers established the tissue regeneration potential of the native-SP Cpd. 1 in a mouse myotoxic TA muscle injury model. Intramuscular delivery of 10 and 30 μg doses of Cpd. 1 improved functional recovery by 40%–50%, resulted in less injury-induced skeletal muscle fiber atrophy, and accelerated structural regeneration of type II muscle fibers compared to the vehicle control and low-dosage Cpd. 1 treatment groups when assessed 28 days after injury.[3]

The SP-modified Cpds. enhanced expression of IGF-1 and regeneration. First, using *in vitro* models, the authors evaluated BDNF-SP-modified Cpd. 2 and observed 2- to 6-fold enhanced IGF-I secretion in HEK293, C2C12 murine myoblast, and human primary skeletal muscle cells when compared to Cpd. 1. Similarly, in the mouse myotoxic injury model, Cpd. 2 demonstrated 15-fold increased potency compared to dose responses observed for Cpd. 1, supporting a lower dosage strategy for achieving functional recovery using the BDNF-SP-enhanced IGF-I mRNA.[3]

Importantly, in the *in vitro* studies, transfection of the IGF-I-mRNA Cpds. was carried out using cationic-lipid-based reagents including Lipofectamine 2000 and JetMessenger, designed to enhance intracellular delivery. However, *in vivo*, the authors utilized a naked IGF-I mRNA delivery approach, which is typically inefficient since mRNA is subject to rapid extracellular degradation and electrostatically impaired delivery across the negatively charged plasma membrane of target cells, especially without the incorporation of delivery carrier.[14] In an extensive series of *in vivo* experiments, the authors demonstrate that local intramuscular injection of naked IVT IGF-I mRNA containing variant SPs leads to increased IGF-I secretion, accelerates regeneration after skeletal muscle injury, and slows spinal disc degeneration.[3]

To explore specificity and safety, the authors evaluated the pharmacokinetics and pharmacodynamics of IGF-I mRNA and protein expression after intramuscular delivery of the BDNF-SP-modified Cpd. 2. In a second rodent muscle injury model, Cpd. 2 exhibited a dose-dependent 17–25 h half-life in treated TA samples and was detectable for up to 3 days after delivery in specific regions proximal to the injection site. These findings demonstrate that intramuscular delivery of IVT IGF-I mRNA remained spatially localized to the delivery site and did not exhibit leakage into larger surrounding regions of the transfected TA tissues.[3] Biomarkers for IGF-I receptor pathway signaling (pAKT) also demonstrated dose-dependent increases with IGF-I expression in specific regions, indicating activation of downstream signaling pathways. Importantly, therapeutically relevant levels of secreted hIGF-I (up to 18 pg/mg) were observed in regions localized to the injection site.

Skeletal muscle regeneration is regulated by endogenous quiescent muscle stem cells referred to as satellite cells (SCs). Upon muscle injury, SCs are activated to a proliferative state, forming myoblasts in a cascade of events leading to the formation of new skeletal muscle fibers.[15] To explore the regenerative effects of intramuscular treatment, Antony et al. evaluated several biomarkers associated with muscle regeneration after local delivery of Cpd. 2 to the injury site. Increased Pax7 and MYH3 expression was observed at lower doses, suggesting SC activation and muscle fiber regeneration. However, an inhibitory effect among regenerative muscle cell makers resulted from treatment with higher doses, highlighting the importance of optimizing the dosage when evaluating nucleic acid-based therapeutic agents, including IVT mRNA.

The therapeutic potential of localized delivery of SP-optimized IGF-I mRNA was further evaluated in a rabbit intervertebral disc injury model using Cpd. 3 containing the modified NTF-3-SP. *In vivo*, treatment with Cpd. 3 was administered via local intraspinal injection 30 days after the progression of spinal degeneration from induced spinal disc injury. X-ray analysis of the treated spinal regions and histological assessment of specific anatomical regions of the discs surrounding the injury site displayed 60% less tissue deterioration for Cpd. 3-treated discs than the vehicle-treated discs. Based on these findings, the authors conclude that increased IGF-I secretion resulting from SP-optimized IGF-I mRNA treatment surrounding the injury site slowed the progression of disc degeneration. Importantly, further elucidation of the mRNA transfection mechanisms involved in delivery to degenerative tissue and the effects of increased IGF-I secretion in these regions remains an area of interest.

In conclusion, using preclinical models, Antony et al. demonstrate the potential value of an IVT-mRNA-based therapeutic approach for enhancing IGF-I expression and secretion after skeletal muscle and neurodegenerative injuries. Importantly, the cellular uptake mechanisms of the naked IVT mRNA delivery approach used in this study remains unclear. The focus of the investigation was on evaluating the therapeutic efficacy of the localized IVT mRNA delivery approach, and processes involved in transfection of the targeted cells and regions were not explored. This remains an area in need of further investigation with potential comparison to an mRNA carrier approach such as LNPs. In addition, while the IVT mRNA treatments in these studies were administered locally, potential tissue-related and immune stimulatory effects from the direct injection approaches as well as the exogenously modified mRNA Cpds. should be evaluated in future studies, as unmodified mRNA-based delivery has been shown to be associated with immune responses.[2,9] Overall, Antony and colleagues provide an interesting investigation using novel SP-modified IGF-I mRNAs to augment translation, secretion, and localized persistence in targeted regions of muscle and spinal disc

injury. The study further demonstrates that IGF-I mRNA expression remains localized to the targeted delivery site and also establishes optimized IGF-I mRNA dosages, which are important factors for achieving therapeutic efficacy and safety. These findings are valuable for advancing the field of nucleic acid therapeutics and highlight IVT IGF-I mRNA as a potentially translatable treatment for enhancing skeletal muscle regeneration after injury and slowing the progression of neurodegeneration.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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