Molecular Therapy
Nucleic Acids
Commentary

Messenger RNA brings gene editing a step closer to treat muscular dystrophies

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Muscle tissue is composed of elongated and multinucleated contractile cells that reside within a highly organized connective tissue matrix. Muscle fibers are enveloped by a membrane termed the sarcolemma, in which are embedded proteins with structural and signaling roles. Myopathies are so called because they result in muscular weakness caused by dysfunction of diverse constituents of this highly specialized system.[1] A large group of muscle diseases, termed muscular dystrophies, of which the best known is Duchenne muscular dystrophy (DMD), are monogenic diseases caused by numerous autosomal dominant, recessive, and X-linked mutations that predominantly affect muscle-protein-encoding genes. The *SGC* genes encode the sarcoglycan protein complex that is embedded in the sarcolemma and are mutated in limb girdle muscular dystrophy (LGMD). Although individually rare, when considered together, there is a large unmet clinical need, as available treatments do not reverse progressive muscle weakness. In a proof-of-principle study, Stadelmann et al. bring the potential of gene editing to restore muscle function caused by mutations in sarcolemmal proteins a step closer. By using messenger RNA as a platform to deliver the gene-editing effectors into cells, very high rates of gene correction were achieved that avoided the safety concerns of plasmid-mediated delivery. In future, to inform clinical trials, further studies using suitable animal models are needed to test whether gene-corrected muscle cells provide enduring reversibility of muscle weakness.

Skeletal muscle contains normally quiescent skeletal muscle stem cells, also termed satellite cells, that reside adjacent to the sarcolemma. In a process that recapitulates embryonic development, satellite cells undergo asymmetric division to replace terminally differentiated muscle fibers that are damaged in muscular dystrophies and other myopathies. Concurrently, this mode of cell division serves to replenish the pool of stem cells. Satellite cells can be obtained from muscle biopsies, a relatively simple procedure, and then readily expanded *ex vivo*. These cells are endowed with superior repair properties compared with muscle progenitors derived from current *in vitro* human-induced pluripotent stem cell (hiPSC) differentiation protocols.[2]

Novel gene therapies and related treatments have started to become available for muscular dystrophies, but they still depend on endogenous mechanisms of satellite cell repair. Gene silencing using antisense oligonucleotides signaled the advent of gene therapies for muscular dystrophies, exemplified by the archetypal disease, DMD.[3] A drawback of the latter approach is the modest therapeutic effect and frequent dosing that is required. Alongside antisense oligos, viral-mediated gene-replacement therapy has received approval by the US Food and Drug Administration (FDA) for the treatment of another form of muscle weakness, spinal muscular atrophy (SMA). However, this procedure potentially has greater risks, and comprehensive data on the long-term safety of using viral vectors, commonly adeno-associated viral (AAV) vectors, will take time to gather.[4] A third strategy aims to prevent the degradation of mRNAs that harbor premature STOP codons through nonsense-mediated decay (NMD). In DMD, the truncated mutant protein products are partially functional and so this mechanism contributes significantly to the deficiency of the *DMD* gene product, dystrophin. Small-molecule drugs that interfere with NMD have been approved for the treatment of DMD in the European Union.[5]

The monogenic nature of most muscular dystrophies makes this group of diseases amenable to CRISPR-Cas9 gene editing to correct causative genetic mutations.[6] By installing permanent changes in the sequence of the affected gene, the hope is that this approach will eventually offer a lasting cure with a "one-off" treatment. Cas9 induces double-strand breaks in DNA at genomic sites targeted by a complementary guide RNA (gRNA). Repair of these double-strand breaks then ensues by non-homologous end-joining or homology-directed repair. A complementary approach targeting single nucleotides uses a modified version of Cas9 devoid of DNA-cutting activity as a component of DNA base editors. Adenine base editors can produce an A to G nucleotide change (T to C on the opposite strand) within a targeting window several nucleotides long.

Delivery of the CRISPR or base editing effectors to cells or tissues is a key consideration. For human therapies, plasmid-mediated transfer is not an option because of the potential risks of insertional mutagenesis and biosafety. These adverse effects can be mitigated by delivering Cas9 as mRNA in a complex with gRNA. Moreover, the transient nature of Cas9 expression resulting

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from mRNA delivery reduces off-target genome editing.

Stadelmann et al. used mRNA to encode an adenine base editor and introduced it into satellite cells from multiple donors *ex vivo*, with the aim of correcting a disease-causing point mutation in an *SGC* gene.[7] To test their gene-editing approach, they first targeted a single nucleotide in the *NCAM1* coding sequence achieving an on-target editing rate of 90%. They then targeted *SGCA* and reported a similar editing efficiency. In an earlier study, they could not detect off-target effects within the activity window of the base editor for this gene and for this cell type.[8] Furthermore, satellite cells nucleofected with the base editor mRNA retained their viability and remained functionally intact, and high rates of on-target editing could be achieved without enrichment. Together with the relatively short duration in culture, this makes for an efficient protocol to obtain sufficient gene-corrected satellite cells for downstream use. These findings strengthen support for potential early-phase human clinical trials.

The study of Stadelmann et al. contributes significantly to the groundwork needed to bring this technology into clinical trials. mRNA delivery of gene-editing effectors is clearly feasible, efficient, and safer than plasmid delivery. However, before this work can be extended in human clinical trials, a thorough unbiased evaluation of off-target effects will be necessary. Furthermore, data on the efficiency and specificity of correcting a broader range of mutations should be evaluated. From a translational viewpoint, important next steps are to leverage mammalian models of muscular dystrophies to determine the minimum number of gene-edited satellite cells that should be transplanted into damaged muscle to restore function. The relationship between functional improvement, the type of muscular dystrophy, and the targeted muscle will need to be determined.

An improvement on the current step of culturing satellite cells *ex vivo* would be desirable, and to this end, the possibly complementary approach of *in vivo* base or gene editing could transform the therapeutic landscape. To realize this goal, better delivery systems are first needed. In this respect, lipid nanoparticles (LNPs) hold great potential. At present, however, LNP delivery systems have been tested successfully in compact organs, such as the liver, rather than in skeletal muscle.[9] Targeting specific muscles, for example, proximal limb muscles, might be more appropriate in restoring important functions, such as ambulation. Beyond focusing on repair of muscle, a considerable therapeutic challenge is posed by multi-system involvement in muscular dystrophies. An exciting decade of translational research lies ahead, which should suggest the best gene therapeutic option to repair dystrophic skeletal muscle, with the treatment of DMD likely to be at the forefront.

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