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Congenital muscular dystrophy

Mini-agrin delivers in mice

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In a recent issue of PNAS, Qiao *et al*.[1] present the first study of a successful somatic gene therapy to treat laminin α2-deficient congenital muscular dystrophy (MDC1A) in a mouse model.

The researchers from the University of Pittsburgh used a state-of-the-art adeno-associated viral (AAV) vector to systemically deliver mini-agrin, whose efficacy for the treatment had been demonstrated earlier in transgenic mice.[2,3] Their work impressively demonstrates the feasibility of systemic gene delivery for long-term transduction of skeletal and cardiac muscle and subsequent substantial amelioration of the disease without any additional pharmacological intervention. This work points the way to a new approach to gene therapy for patients suffering from MDC1A.

MDC1A is an autosomal recessive muscle wasting disease that often leads to death in early childhood. It is caused by mutations in *LAMA2*, the gene encoding laminin α2, which assembles with the β1 and the γ1 chain to laminin-2, the main laminin isoform present in the basement membrane of muscle fibers and peripheral nerves (Figure 1a). Basement membranes are highly structured sheets of extracellular matrix molecules that surround many cells. Although other laminin isoforms are synthesized in the muscle of MDC1A patients, they do not form a proper basement membrane that is connected to the muscle sarcolemmal membrane (Figure 1b). Hence, the chain of proteins linking the actin cytoskeleton via the sarcolemma to the basement membrane is inter-

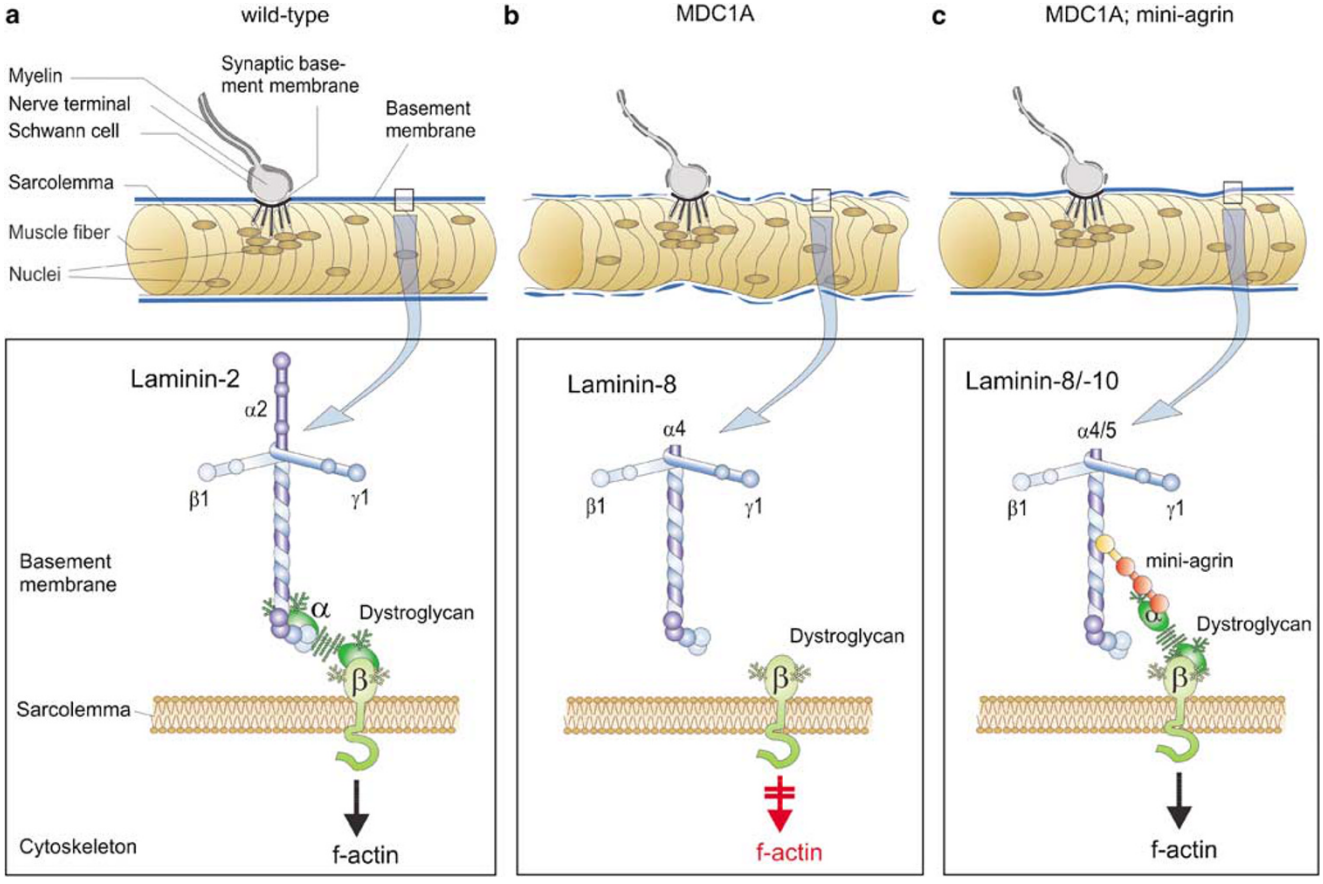

Figure 1 Innervated muscle fiber in (**a**) wild-type, (**b**) MDC1A, and (**c**) MDC1A mice treated with mini-agrin, and the potential mechanism involved in disease progression and treatment (lower panels). (**a**) In wild-type mice, the peripheral nerve is well myelinated and the muscle fibers are healthy. This is based on the linkage of the basement membrane to the cytoskeleton (top panel). This link is likely to be due to the tight connection of the basement membrane component laminin-2 with α-dystroglycan, which in turn is connected to the transmembrane component β-dystroglycan. β-Dystroglycan connects via linker molecules (not shown) to filamentous actin (f-actin). (**b**) Dystrophic MDC1A muscle degenerates and the peripheral nerve is demyelinated (top panel). Mutations in laminin α2 prevent synthesis of laminin-2. Instead, laminin α4 is synthesized in MDC1A muscle to form laminin-8. This isoform cannot link the basement membrane to α-dystroglycan and does not allow the formation of a proper basement membrane (symbolized by the interrupted line in the top panel). (**c**) In MDC1A muscles treated with mini-agrin, both integrity of the muscle and the basement membrane are restored. In contrast, the peripheral nerve is still demyelinated (top panel). Mini-agrin binds via its amino-terminal region to laminin-8 and also stabilizes laminin-10 (α5, β1, γ1). The carboxy-terminal part of mini-agrin connects to α-dystroglycan and restores the connection to the f-actin.

rupted. As a consequence, muscle fibers lose their stability and degenerate. In addition, the regenerative capacity of muscle is substantially lower.[3,4]

Gene therapy has recently suffered from major setbacks because of the death of a participant in a trial due to acute toxicity[5] and the occurrence of leukemia in children who were treated with retrovirus-mediated gene transfer.[6] Gene therapies for muscle dystrophies are also hampered by the fact that more than 600 muscles must be reached to warrant optimal therapy. Thus, local intramuscular injection is definitively not a feasible strategy in a clinical setting. In this case the authors' major achievement came by using AAV1 vectors that allowed long-term (at least 4 months) expression of the transgene in all skeletal muscles examined and in the heart by a single intraperitoneal injection into neonatal mice. Recent methodological advances indicate that AAV6 in conjunction with vascular endothelial growth factor (VEGF)[7] and AAV8[8] might be even more efficient than AAV1.

The second important change was the use of mini-agrin instead of laminin α2. Re-insertion of laminin α2 would be extremely difficult because of the large size of the cDNA (9 kb), which prevents its packaging into AAV vectors. Moreover, laminin α2 must become incorporated into the laminin heterotrimer to be functional. As several domains of laminin α2 contribute to its functionality, it is also unfeasible to generate a miniaturized version without losing its function. Moreover, *de novo* expression of laminin α2 might trigger immune responses in patients. In contrast, the mini-agrin used by Qiao *et al*.[1] has several advantages. Firstly, its cDNA is small enough to be incorporated into AAV vectors. Secondly, because MDC1A patients express agrin endogenously, the immunological rejection of the protein will be minimal.

Agrin, well known for its role in the organization of the nerve-muscle synapse,[9] shares with laminin α2 the ability to bind to α-dystroglycan, a protein that is involved in the linkage of basement membranes to the muscle sarcolemma (Figure 1). Moreover, an amino-terminal domain of agrin confers binding to all laminins. Transgenic overexpression of a mini-agrin consisting solely of the laminin-binding and the α-dystroglycan-binding domain markedly improved the stability, function and regenerative capacity of muscle in mouse models for MDC1A.[2,3] As a consequence, the mice had greatly prolonged lifespan and improved locomotion. As in the transgenic study, Qiao *et al*.[1] restored the structure of the muscle basement membrane, decreased dystrophy-related muscle fibrosis and significantly improved body growth, locomotor functions and lifespan. Mini-agrin expressed in nonmuscle tissue seemed to have no adverse effects during the time window examined. Although mini-agrin was present in the basement membrane of the peripheral nerve, it could not prevent demyelination.[1] This might arise either from insufficient levels of mini-agrin or from the different function of laminin α2 not using α-dystroglycan but integrins as a receptor in peripheral nerve.

In summary, the potential of AAV-mediated, mini-agrin-based gene therapy of MDC1A is high, but all the promising results from the mouse studies must be carefully validated before they can be applied to human patients. Viruses still present a variety of problems for patients since much of our understanding of viral vectors is solely based on studies in mice, which tolerate treatment well. Humans might react differently and the efficacy of vector systems may be markedly different between the two species. Moreover, MDC1A pathology involves also organs other than skeletal and cardiac muscles, and a perfect treatment would also require infection of the peripheral and central nervous system. Since expression of mini-agrin in peripheral nerves failed to prevent neuropathology, it is unlikely that this treatment could alleviate all symptoms. Although the current study by Qiao *et al*.[1] applied mini-agrin at a later stage than the previous transgenic study,[2] it will be important to test the efficacy of mini-agrin that is applied when the symptoms of the dystrophy are apparent. Finally, more detailed information about the molecular mechanisms involved in the beneficial effect of mini-agrin might help to improve the safety and efficacy of MDC1A treatment, especially in combination with treatment using functionally different approaches, such as the prevention of apoptosis.[10,11] ■

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