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Dystrophin knockdown mice suggest that early, transient dystrophin expression might be enough to prevent later pathology

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Abstract

A key issue in Duchenne muscular dystrophy (DMD) gene therapy is whether we need to keep a functional dystrophin expression throughout the entire life span of the patients. Answer to this question will have significant impact on a number of therapeutic approaches, such as oligonucleotide-mediated exon skipping and protein therapy. A recent study by Ghahramani Seno et al provided a clue to this important question (1). The authors applied AAV-mediated RNAi to knockdown dystrophin expression in the tibialis anterior (TA) muscle of 1-m-old C57Bl/10 mice. Dystrophin level was reduced to undetectable levels (by immunohistostaining) four months later. Dystrophin-associated proteins were also reduced. Surprisingly, utrophin was not up-regulated. What is even more surprising is that the authors did not see the characteristic dystrophic muscle pathology after one-year long dystrophin knockdown.

A key issue in Duchenne muscular dystrophy (DMD) gene therapy is whether we need to keep a functional dystrophin expression throughout the entire life span of the patients. An answer to this question would have a significant impact on a number of therapeutic approaches, such as oligonucleotide-mediated exon-skipping and protein therapy. A recent study by Ghahramani Seno et al. provides a clue [1]. The authors applied adeno-associated virus (AAV)-mediated RNA interference (RNAi) to knock down dystrophin expression in the tibialis anterior (TA) muscle of C57Bl/10 mice at one month of age. Dystrophin at the sarcolemma was reduced to undetectable levels (by immunohistostaining) four months later. Dystrophin-associated proteins were also reduced but, surprisingly, utrophin was not up-regulated. What is even more surprising is that the authors did not see the characteristic dystrophic muscle pathology even after one-year of dystrophin knockdown.

The fact that it took several months to reduce dystrophin protein corresponds well with a previous transgenic study in which an inducible promoter was used to control dystrophin expression [2]. Together, these studies suggest that dystrophin is fairly stable once it reaches the sarcolemma.

Compensatory up-regulation of utrophin has been demonstrated in DMD patients and animal models, however, the mechanism that triggers it remains to be fully understood. The lack of up-regulation in Ghahramani Seno et al.'s study suggests that dystrophin deficiency itself is not sufficient, rather, utrophin up-regulation might also depend on dystrophic pathology. Since utrophin expression is not enhanced in sarcoglycan-deficient muscular dystrophies, one could

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speculate that both the loss of dystrophin and a dystrophic environment are required to prompt utrophin up-regulation in DMD.

The most thought-provoking finding coming out of this study is the absence of the dystrophic histopathology in mice that received RNAi knockdown treatment continuously from one month of age for one year. In *mdx* mice, peak muscle degeneration occurs between 2 and 8 weeks. By contrast, following the RNAi knockdown in C57Bl/10 mice, dystrophin levels were not reduced until mice reached 13 weeks of age. This result seems to suggest that the loss of dystrophin is quite tolerable once mice have passed the critical age (~ 2 to 8 weeks).

One of the most promising DMD gene therapy approaches is exon-skipping. A recent phase I clinical trial has demonstrated dystrophin restoration in several DMD patients after local administration [3]. One hurdle to be overcome in moving this exciting therapy forward is the need for frequent re-administration, weekly injections having been used in murine studies [4]. The results from Ghahramani Seno et al. raise the possibility of long-term therapeutic benefit with less frequent injections. Furthermore, one might speculate that a few injections during the critical phase of the disease and/or developmental stages, such as the first few years after birth, could lead to life-long protection. Such an approach would be more appealing to all concerned due to a reduced chance of toxicity and greater convenience. Further study with a single dose of systemic exon-skipping treatment in neonatal *mdx* mice might offer more insights on this tantalizing hypothesis.

Despite the encouraging results, one has to be cautious in over-interpreting the data. First, the TA muscle is only mildly affected in *mdx* mice. It would be interesting to see what happens to the diaphragm after systemic AAV-mediated RNAi knockdown. Second, it is still not clear whether the AAV-mediated RNAi administration has completely eliminated dystrophin expression in the TA muscle. In primary muscle cell culture, dystrophin levels were reduced to less than 10% of those in wild-type cells but there was still residual expression and even very low-level dystrophin might effect some protection. For example, expression of a near full-length dystrophin at 5% of wild type levels has been shown to preserve muscle force [5]. Third, as suggested by this study, transient dystrophin expression (before 13 weeks of age in mice) might protect skeletal muscle. Such an approach, however, would not be expected to prevent dystrophin-deficient cardiomyopathy in human patients. It has been shown that the cleavage of cardiac dystrophin by Coxsackievirus leads to cardiomyopathy [6], which underscores the importance of persistent dystrophin expression in the heart. A loss of cardiac dystrophin, even later in life, is still associated with severe consequences.

Nevertheless, the results from Ghahramani Seno et al. have certainly revealed some intriguing perspectives on our understanding of dystrophinopathy. Their results suggest that the skeletal muscle pathology might be developmentally regulated rather than simply dependent on the absence of dystrophin. If this hypothesis is confirmed, and is able to be translated to humans, then it will definitely be a piece of good news for DMD gene therapy.

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