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AAV-dependent targeting of myostatin function

Follistatin strikes back at muscular dystrophy

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Muscular dystrophies are muscle-wasting diseases due to mutations of proteins, which modulate skeletal muscle function and, in the case of dystrophin, form a link between the cytoskeleton and the basal lamina. Dystrophin-deficient myopathies are the most common genetically determined skeletal muscle diseases and, although a clinically effective therapy is still lacking, two new therapeutic approaches are being investigated in animal models of the disease. In one case, the objective is to reconstitute dystrophin gene expression either by cell therapy, gene transfer or endogenous gene repair by exon skipping. The latter can be achieved by adeno-associated viral (AAV) vectors expressing small nuclear ribonucleoproteins that target the mutated exons and form a shorter but in-frame transcript that is translated into functional dystrophin.[1,2] The other approach consists in targeting molecular pathways downstream of the primary genetic defect, that is, the dystrophin mutation. As an example, pharmacological administration of histone deacetylase inhibitors[3] or nitric oxide donors[4,5] have been shown to counteract the progression of muscular dystrophy in the *mdx* mouse model of duchenne muscular dystrophy (DMD). The beneficial effect of these treatments relies, at least in part, on the transcriptional activation of the myostatin antagonist follistatin. Previous studies have shown the role of myostatin, a member of the transforming growth factor-beta family, as a negative regulator of muscle development, thus indicating this molecule and its interactors as possible new therapeutic targets. Deletion of the myostatin gene from *mdx* mice significantly enlarged the muscle mass and increased strength and performance.[6] Notably, the development of antibodies that specifically target and inhibit myostatin led to functional improvement of dystrophic muscle in mice[7] raising the possibility that myostatin blocking therapies may represent an alternative to gene replacement.

Very recently, the identification of myostatin-binding proteins capable of regulating myostatin activity further expanded the number of potential therapeutic targets in muscular dystrophy. In this regard, the study published in PNAS by Haidet *et al*.[8] describes the beneficial effect of different myostatin-binding proteins delivered by one-time postnatal intramuscular injection of AAV vectors to *mdx* mice. Specifically, the authors expressed known secreted myostatin-inhibiting genes, that is, the growth and differentiation factor-associated serum protein 1, the follistatin-related gene and the follistatin splicing variant FS-344. Each and all these molecules increased the muscle body mass and led to functional improvement. In this experiment, the FS-344 isoform showed the greatest efficacy during a 2-year follow-up and the injected animals displayed muscle fibers hypertrophy, reduced connective tissue deposition and increased muscle strength compared to controls. This beneficial effect could be achieved in young as well as aged animals, a result that could have particular relevance for older DMD patients with significantly compromised muscle structure and regenerative capacity.

It is noteworthy that Follistatin generates the FS-344 and FS-317 isoforms by alternative splicing. By subsequent cleavage FS-344 and FS-317 give rise to FS-315 and FS-288 polypeptides respectively; FS-315 is found in the circulation, whereas FS-288 exhibits high tissue affinity and may decrease reproductive ability because it locates in the gonads and interferes with pituitary follicle-stimulating hormone. Interestingly, in the study by Haidet *et al*.[8] the expression of the FS-344 isoform and its derivative FS-315 did not affect fertility neither were found histological alterations in the gonads. In addition, an increase in the muscle mass was observed both in the directly injected muscles and in surrounding tissues, suggesting a potential paracrine effect of the circulating isoform. Unfortunately, it was not determined whether the FS-344 follistatin isoform had any positive effect on heart function; this is a relevant issue as currently more then 30% of all deaths among DMD patients results from cardiac involvement. Further, it remains to be established the molecular mechanism of action of FS-344, including its ability to inhibit activins, proteins that regulate myostatin function, the level of muscle maturation as well as the possible side effects in distal organs, such as the liver and brain.

Haidet *et al*.[8] used an AAV vector to deliver the myostatin inhibitor proteins cDNAs. AAV are in general safe and efficient tools for gene transfer and maintain for a prolonged time, even years, high levels of *in vivo* transgene expression in the absence of significant inactivating immune responses. In this regard, Haidet *et al*.[8] clearly showed that a single injection of the myostatin inhibitor proteins encoding AAVs enhanced locally the muscle mass for approximately 2 years. However, local intramuscular injection is not a feasible strategy for a disease in which the majority of muscles have to be reached by the therapeutic agent. Thus, AAV delivery into the systemic circulation, it is recent the discovery that AAV serotype 8 is more efficient than other serotypes for gene delivery in skeletal and cardiac muscles,[9] and the use of skeletal and cardiac muscle-specific promoters need to be examined to move these interesting results to clinical evaluation. In addition, it has to be kept in mind that early clinical studies with AAV vectors have shown complications related to unexpected immune responses against the vectors,[10] moreover AAV might not be administered to patients who are already immunized against the natural virus.[10]

In conclusion, Haidet *et al*.[8] provide new and valuable data about a novel and potentially clinically relevant strategy for muscle regeneration in DMD. A number of issues,

however, remain to be addressed to move their results from the bench to the bedside. ■

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