

P3.14**New AAVs for the muscle gene therapy in sarcoglycan deficient animals**

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Recombinant adeno-associated viruses (rAAV) are among the most promising vectors for gene delivery in vivo, because they show widespread and sustained transgene expression after a single administration. In previous experiments we injected into BIO14.6 hamsters the human delta-sarcoglycan cDNA by AAV2/8 by single intraperitoneal injection at two weeks of age. We obtained the body-wide restoration of delta-sarcoglycan expression associated with functional reconstitution of the sarcoglycan complex and with significant lowering of centralized nuclei and fibrosis in skeletal muscle. Motor ability and cardiac functions were rescued. However, we noticed that a high dosage of AAV was required, because part of the vector was captured by liver, especially when the animals were injected intraperitoneally. In hamsters this problem was overcome by increasing the dosage, but such approach is unsuitable for future human applications. In fact, the liver can be damaged by this unwanted AAV exposure and the unfeasibility of huge vector preparations may be critical.

We have now introduced a codon-optimized cDNA sequence of human delta sarcoglycan assembled with an ingenierized AAV2. This was prepared according to recent demonstration of a muscle-specific targeting obtained by replacing the hexapeptide sequence that recognize the heparan sulfate receptor. With this modified vector we aim to scale down the minimal effective systemic AAV dosage to 10[12]–10[13]/kg.

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MYOSTATIN ET AL.; POSTER PRESENTATIONS**P3.15****Crosstalk between myostatin and IGF-1 signalling pathways during myoblast proliferation and differentiation**

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The deficiency of the membrane stabilizing protein dystrophin typical of Duchenne muscular dystrophy increases susceptibility of muscle to contraction-induced damage. One strategy to prevent mechanical stress-induced damage in dystrophic muscle is to promote muscle fiber hypertrophy and regeneration. Postnatal growth and regeneration are regulated by a variety of endogenous growth factors, most notably Insulin-like growth factor-1 (IGF-1) and myostatin.

IGF-1 is a positive regulator in proliferation and differentiation of skeletal muscle cells, while myostatin acts as a negative regulator of skeletal muscle mass. These growth factors exert their antagonist functions activating different transduction pathways mediated by their receptors. Myostatin has been shown to bind to Activin type II receptors, activating a signal transduction pathway which leads phosphorylation of the transcription factors Smad2/Smad3 which

ultimately leads to suppression of myogenesis. By the other hand, almost all biological actions of IGF-1 are mediated by binding to the IGF-1 receptor activating transduction pathways mediated by Phosphoinositide 3-kinase (PI3K) related to muscle proliferation, differentiation, protein synthesis and hypertrophy. Recently experimental data obtained by our group strongly suggest a negative feedback through a cross talk between IGF-1 and myostatin signaling pathways during myogenesis. Using myoblast primary culture and C2C12 cells transfected with siRNA dystrophin, we found that IGF-1 induces myostatin mRNA expression through the activation of signaling pathways PI3K/Akt, Calcineurin/NFAT and MAP kinases MEK/ERK. In addition, myostatin inhibits calcium release induced by IGF-1 in myoblast and the activation of the transduction pathways Calcineurin–NFAT. Considering these findings, we propose that during myoblast differentiation, IGF-1 regulates myostatin gene expression and, in contrast, myostatin inhibits the IGF-1 signaling pathways. Fondecyt 11090274.

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P3.16**Myostatin inhibits differentiation of normal and dysferlin-deficient human skeletal myoblasts – similarities and differences**

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Dysferlin (DYSF) gene mutations cause limb girdle muscular dystrophy 2B due to defects in muscle membrane repair. Myostatin is a potent negative regulator of muscle mass and therefore myostatin pharmacological inactivation is a promising target to ameliorate muscular dystrophies. Elevated myostatin levels have been observed in different pathological situations like muscle disuse and cachexia. Here we delineate the role of myostatin in muscular dystrophies associated with mutations in dysferlin. In our analysis we compared normal and dysferlin-deficient primary human skeletal myoblasts and myotubes that harbor different mutations in DYSF (homozygous: c.4022T>C; c.2810+2T>A; compound heterozygous: c.855+1delG c.895G>A; c.1448C>A c.*107T>A) resulting in the absence or the intracellular aggregation of dysferlin. We found that myostatin (R& D Systems) severely inhibits the expression of early and late myogenic differentiation markers including myogenic transcription factors (MyoD, myogenin), muscle structural proteins (desmin, myosin heavy chain, α actinin) and sarcolemmal proteins (dysferlin, caveolin3). Myostatin is not only able to block the induction of muscle differentiation program but also to elicit a rapid dedifferentiating effect on mature myotubes. However, myostatin differentially affects dysferlin-deficient compared to normal primary human skeletal myotubes. Dysferlin-deficient myotubes are more sensitive to myostatin inhibition of myogenic differentiation.

Myostatin acts not only as negative regulator of skeletal muscle growth but also as a positive regulator of fibrosis. The negative effect of myostatin on differentiation is accompanied by an increase in the expression of fibronectin, a fibrosis-associated extracellular matrix molecule especially in dysferlin-deficient primary human myotubes.

Altogether, myostatin-related effects on muscle differentiation and fibrosis are more pronounced on dysferlin-deficient compared to normal primary human myotubes.

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