least one AON was found to be effective for each gene. With these candidate AONs, transfection was repeated to assess the effect of exon skipping on protein level as well. After 48 h, proteins were isolated and downregulation of binding proteins and its effect on the downstream proteins, which are involved in IGF-1 signaling cascade, were confirmed by Western blot. Using exon skipping mediated knockdown of IGFBP1 and IGFBP3, we confirmed in vitro that IGF-1 signaling can be enhanced, making it a potential therapy to be tested in vivo. As next step, these AONs will be used in mdx mice to see whether this treatment will improve muscle quality and function.

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**EP.88**

**Inflammasome inhibitors for the treatment of muscular dystrophies**

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Duchenne muscular dystrophy (DMD) is the most frequent inherited human myopathy and one of the most devastating muscular dystrophies. Although dystrophin mutations represent the primary cause of DMD, it is the secondary processes involving persistent inflammation that likely exacerbate disease progression. Our group previously described the involvement of the NLRP3 inflammasome as having a major role in the deleterious inflammatory process worsening the dystrophic phenotype. Recently, MCC950 was discovered as an extremely potent, selective, small molecule inhibitors of NLRP3 and could thus be promising in muscle diseases with an inflammatory component. Four-week-old mdx mice (n = 6 per group) were orally treated with MCC950 (mdx-MCC950) 80mg/kg for 8 weeks and compared with untreated (mdx) mice and to control mice. In vivo functional tests were carried out to measure the global force and endurance of mice. Ex vivo biochemical and molecular analyses were performed to evaluate the pathophysiology of the skeletal muscle. Mdx-MCC950 mice exhibited enhanced physical performance with an increase in both muscle force and endurance versus mdx mice (2.5-fold for wire test, P = 0.01; and 1.5-fold for grip test, P = 0.04). In addition, MCC950 reduced oxidative stress (-20%, P = 0.048 for HNE) and inflammation (-25%, P = 0.047 for IL-1β). Finally, necrosis, embryonic myosin (a marker of muscle regeneration) and the number of small sized myofibers were reduced. MCC950 improved significantly mice performances in vivo, counterbalanced excessive inflammatory and oxidative responses, mitigated necrosis and slowed down the myofibers degradation/regeneration turnover. This molecule could thus offer promising therapeutic prospect for managing DMD or other muscle and inflammatory disorders.

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**EP.89**

**TRPC1 and TRPC3 involvement in DMD physiopathology and as potential targets for treatment in complement to rAAV-microdystrophin**

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Duchenne muscular dystrophy (DMD) is an inherited and lethal disease leading to the lack of expression of dystrophin and muscle degeneration for which there is no curative treatment. Current life expectancy of DMD patients is 20 to 40 years with final cardio-respiratory complications. Clinical trials are ongoing using rAAV-microdystrophin (rAAV-MD) gene therapy. The aim being a shift from DMD toward a milder dystrophinopathy, the Becker muscular dystrophy (BMD). However, BMD patients still exhibit muscle decline and die before the age of 60. In DMD patients, as in animal models, muscle cell necrosis is triggered by an increased Ca+ influx (SPCA). This may rely on a deregulation of the activity and/or expression of some ions channels, in particular the Transient Receptor Potential channels (TRP). In this study, we evaluated the involvement of TRPC1 and TRPC3 Ca2+ channels in the pathogenesis of DMD in skeletal muscle from the DMDmdx rats, an animal model that faithfully mimics human DMD disease. We demonstrated early [Ca2+]i, and SPCA increases in EDL muscle fibers from DMDmdx rats compared to wild type ones. This was accompanied by an increase of TRPC3 protein level in dystrophic rats. TRPC1 mRNA and protein levels increased only 7 months after birth in DMDmdx rats. The subcellular localization of TRPC1 and TRPC3 were not modified. We found that the molecular weight of TRPC3 was slightly higher in DMDmdx rats, due to post-translational changes. The SPCA increase in DMDmdx rats was related to a Pyr10-sensitive Ca2+ influx, MD expression via rAAV-MD injection induced only partial prevention of alterations in Ca2+ homeostasis, muscle strength and TRPC3 overexpression. TRPC3 thus represents a good therapeutic target for the development of a treatment for DMD, as a complement or alternative to rAAV-MD gene therapy.

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**A novel AA8V8-based gene therapy for Duchenne muscular dystrophy: preclinical studies in the mdx mouse**


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RGX-202, a recombinant adenov-associated virus of serotype 8 (AAV8) with an optimized human microdystrophin transgene under the control of a muscle specific promoter (spc5-12), is a potential gene therapy candidate for the treatment of DMD. The microdystrophin includes 4 rods and 3 hinges and an extended coding region of the carboxyl terminal (CT) domain, known to bind ß-dystrobrevin and ß-syntrophin. Enhanced association of ß-syntrophin may help recruiting neuronal nitric oxide synthase (nNOS) at the sarcolema, facilitating vasodilation during exercise and protecting muscle against ischemic stress. The protein coding sequence was codon-optimized and depleted of CpG dinucleotides. RGX-202 was administered intravenously to male mdx mice and evaluated over 26 weeks. RGX-202-treated animals exhibited significant increases in muscle strength and normalization of gait as shown by fine kinematic analysis. Using T2 magnetic resonance imaging (MRI) to probe muscle damage characteristic of mdx, dose-dependent reductions of the hyper intense lesions were measured, consistent with an improvement in dystrophic pathology. High levels of RGX-202 vector DNA and microdystrophin protein were detected in the skeletal and cardiac muscles, and membrane assembly of the Dystrophin Associated Protein Complex (DAPC) was observed by immunostaining of muscle sections. Alongside staining for ß-syntrophin, dystrobrevin, and ß-dystroglycan, there was also evidence for nNOS recruitment at the sarcolema. RGX-202 treatment resulted in a reduction in the number of activated Pax7 positive satellite cells, further suggesting an improvement in abnormal muscle regeneration. Our data showed that treatment with RGX-202 resulted in the expression of an active microdystrophin which was able to improve muscle function and reduce dystrophic pathology in mdx mice. We are currently developing RGX-202 as a candidate for microdystrophin gene therapy in DMD patients.

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**Oral treatment with AdipoRon attenuates cardiomyopathy in an aged mouse model for Duchenne muscular dystrophy**

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