

Muscular dystrophies are characterized by weakness and wasting of skeletal muscle tissues and treating wasting muscle is one of the biggest issues in the neuromuscular field. Several drugs targeting the myostatin pathway have been used in clinical trials to increase muscle mass and function but so far, most drugs had no or limited effects in improving function in neuromuscular patients. In our study, the expression levels of different actors of the myostatin network were analysed at mRNA and protein levels in neuromuscular patients' sera and skeletal muscle specimens. In muscle wasting or atrophying diseases, a strong down-regulation of the whole myostatin pathway was observed with a decrease of myostatin and activin receptor, and an increase of the myostatin antagonist, follistatin. We also provide *in vivo* evidence in the congenital myopathy *mtm1* mouse model that a down-regulated myostatin pathway can be reactivated by correcting the underlying gene defect using AAV-mediated gene therapy. Importantly, we show that the MTM1 restoration effect on muscle mass can be further enhanced by anti-myostatin therapy. Our data may explain the poor clinical efficacy of anti-myostatin approaches in clinical studies and the apparent contradictory results in mice regarding the efficacy of anti-myostatin approaches and may profoundly affect patient selection and stratification for future trials.

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EC.O.5

The multi-systemic protection against age-related tissue function decline in progeric mice through the attenuation of myostatin/activin signalling

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A dominant principle underpinning our understanding of the ageing process is that DNA damage induces a stress response that shifts cellular resources from growth towards maintenance. A contrasting and seemingly irreconcilable view is that prompting growth of, for example skeletal muscle, results in systemic benefit. To investigate the robustness of these axioms, we induced muscle growth in a murine progeric model. Here we show that the muscle of *Ercc1Δ*/progeric mice undergoes an extremely severe form of wasting which can be protected through an intervention that attenuates myostatin/activin signalling. Significantly we found that treated progeric mice not only maintained muscle activity but also kidney function, protected against the development of liver abnormalities and osteoporosis. This study fundamentally challenges the notion that tissue growth and the maintaining tissue function during ageing are incompatible mechanisms. As importantly, it highlights the potential of therapies based on myostatin/activin blockade to promote healthy ageing.

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EC.O.6

Abolition of the NLRP3 inflammasome improves the dystrophic phenotype in a murine model of Duchenne muscular dystrophy

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Assembly of the NLRP3 inflammasome leads to caspase-1 activation and mediates the cleaving and release of several inflammatory cytokines. The NLRP3 inflammasome can amplify inflammatory responses and thus worsen several diseases. Duchenne muscular dystrophy (DMD) is one of the most devastating muscle disease and it is known to harbour a severe inflammation. We have recently shown that NLRP3 was more expressed in skeletal muscle fibers of mdx mice (a murine model of DMD) than in Wild-Type (WT) mice. Adiponectine (ApN) is a hormone known to possess powerful anti-inflammatory effects on skeletal muscle. Interestingly, transgenic mdx mice that overexpress ApN exhibited lower muscle inflammation/damage as well as higher globular muscle force/endurance when compared to regular mdx mice. These beneficial effects of ApN were associated with a reduction in NLRP3 expression in skeletal muscle. In this study, we

investigated the effects of the absence of NLRP3 on the dystrophic phenotype by crossing mdx mice with NLRP3-knockout (NLRP3-KO) mice. First, functional *in vivo* studies (grip test, wire test and treadmill exercise) were performed on 4 groups of mice: WT, NLRP3-KO, mdx and NLRP3-KO-mdx. Compared to WT, mdx mice presented a strong decrease of global force and endurance that was partially restored in NLRP3-KO-mdx mice. In addition, NLRP3-KO-mdx mice also exhibited a significant decrease in muscle damage, oxidative stress and inflammation as well as a reduction in caspase-1 activation, when compared to regular mdx mice. Furthermore, satellite cells obtained from control and DMD subjects were cultured and differentiated into myotubes. We found that NLRP3 basal expression was 3.5-fold higher in DMD myotubes than in control myotubes. This expression was then reduced after ApN treatment. These novel data show that NLRP3 is implicated in DMD where it plays a key pathogenic role, thus opening new therapeutic perspectives to control muscle inflammation and damage.

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EC.O.7

Necroptosis, a programmed form of necrosis, participates in muscle degeneration in Duchenne muscular dystrophy

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Efforts to treat Duchenne muscular dystrophy (DMD) mainly focus on strategies aimed at increasing dystrophin expression, or enhancing muscle regeneration/growth. However, the process of cell death in muscle wasting disorders has been largely overlooked. In DMD, fibres die with a necrotic morphology, making myonecrosis a central process in its pathogenesis. Inflammation and oxidative stress play a significant part in muscle loss, but how inflammation induces myonecrosis is still unknown. Lately, there has been a conceptual revolution in the cell death field, with the discovery of regulated forms of necrosis. In particular, necroptosis, a RIPK3-dependent programmed cell death, plays a major role in cell death following inflammation-induced injuries in several tissues and is commonly initiated by ligands to the TNF Receptor superfamily members. As a programmed cell death mode, necroptosis can be pharmacologically prevented. Its involvement in skeletal muscle degeneration has not yet been reported. We are currently investigating the involvement of necroptosis in inflammatory-induced myonecrosis, and more specifically in the pathogenesis of DMD. *In vitro*, we found that TNF α can trigger necroptosis in C2C12 cell line, suggesting that muscle cells can undergo necroptosis upon inflammatory challenge. *In vivo*, we found evidence of necroptosis in human and mouse dystrophin-deficient muscles. By depleting RIPK3 in mdx mice, we significantly decreased myonecrosis. Together, our data demonstrate that the necroptotic machinery is involved in DMD pathogenesis and that its prevention could represent a new therapeutic target.

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EC.O.8

Epigenetic regulation of a mitochondrial apoptosis mediator, harakiri in maintaining muscle membrane stability in autoimmune myositis

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Currently, the cause of myositis is unknown, but disease onset sometime has been associated with exposure to environmental agents such as viral infections. Although attempts to identify viruses in myositis skeletal muscle have failed, several studies have shown that a viral signature (e.g., Type 1 interferon) is present in myositis muscle. To investigate this, we hypothesized that certain virus alters DNA methylation in the promoter regions of genes, leading to their aberrant expression in target tissues and disease phenotype in susceptible