T.O. 5

Lack of myostatin results in satellite independent muscle fibre hypertrophy and mitochondrial depletion

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At the 9th WMS congress in Gothenburg, we reported that despite having a larger muscle mass, myostatin knockout mouse were not stronger compared to wildtypes. In fact they exhibited a significantly decreased specific force when maximum tetanic force generation was expressed as a function of muscle size. Here, we show that muscle from mstn−/− mouse enlarged predominantly through an increase in individual fibre size. Interestingly, individual muscle fibres from mstn−/− mouse, although being bigger, contained a similar number of myonuclei compared to wildtype fibres, indicating no increased recruitment of satellite cells during hypertrophic growth. We determined the number of satellite cells attached to single myofibres and found no increase in satellite cells per myofibre from mstn−/− mouse. We investigated the ability of satellite cells to proliferate by performing single myofibre explant cultures from mstn−/− and wildtype muscles. Satellite cells from mstn−/− mouse fibres did not proliferate faster than from wildtype mouse. In a complimentary experiment, myofibres from wildtype mouse were treated with recombinant myostatin protein, which had no effect on satellite cell proliferation. Thus, excessive muscle growth in lack of myostatin did not result from increased satellite cell activity and nuclear density of myostatin depleted fibres was decreased. In extensor digitorum muscle from mstn−/− mouse, we revealed a fibre type conversion towards fast glycolytic fibres and loss of oxidative fibres. However, staining for the activity of mitochondrial enzymes revealed a decrease in SDH activity that could not be accounted for by sole fibre type conversion. We investigated the ratio of the number of mtDNA (MT-CO1) copies per single copy nuclear gene (Ndufv1) using quantitative real-time PCR and found an about 50% decrease in extensor digitorum longus and soleus muscle from mstn−/− muscle that was unrelated to fibre type composition. Hence, the reduction of the mtDNA/myonucleus ratio in muscle from mstn−/− mouse truly reflects a mitochondrial depletion. The effect of the decreased mtDNA/myonucleus ratio is likely to be enhanced due to the decreased nuclear to cytoplasmic ratio in muscle from mstn−/− mouse resulting in a greatly reduced number of mitochondria per cytoplasm. We discuss that myostatin limits muscle fibre size and stimulates proliferation of mitochondria and that such effect optimises aerobic metabolism and force output.

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T.O. 6

Systemic delta-sarcoglycan gene transfer into cardiomyopathic BIO14.6 hamsters by AAV

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The delta-sarcoglycan (delta-SG) deficient Syrian hamster strain BIO14.6 is one the most used animal model for human primitive cardiomyopathy and muscular dystrophy. The main goal of our project is a rescue of cardiac and muscular functionality of BIO14.6 hamsters using adeno-associated viral (AAV) vectors injection. We observed that therapy could be much more successful in younger animals (16 days old BIO14.6 hamsters), in which we injected 2×10^{12} GC of the AAV2/8 vector expressing the human delta-SG. After four months from the first intraperitoneal injection, we performed a second intravenous injection. We used 3×10^{12} GC in a different serotype (AAV2/1). One month later, we observed a very high expression of delta-SG in the muscular and cardiac tissues and, moreover, an improvement in their muscular functionality under exercise. These results are promising because all the injected animals are viable and to date do not show overt cardiomyopathy. Recently, we used double-stranded (ds) AAV vector for more rapid and efficient AAV-mediated transgene expression. We injected 1×10^{12} GC in younger (10 day-old) BIO14.6 hamsters and the treatment is under study.

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NEW DEVELOPMENTS ACROSS THE NEUROMUSCULAR FIELD: ORAL PRESENTATIONS

G.O. 7

Pharmacological chaperones as an alternate treatment for Pompe disease

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Pompe disease (GSD-II) is caused by a deficiency of acid alpha-glucosidase (GAA) activity. Juvenile- and adult-onset are the most common forms of the disease, characterized by skeletal muscle (limb girdle) weakness and respiratory failure. The underlying cause of muscle wasting is still unclear since glycogen accumulation does not correlate with muscle weakness. Interestingly, mistrafficking of membrane stabilizing proteins, such as alpha-dystroglycan, sarcoglycans and dystrophin, and their presence in inclusion bodies also containing various UPR-related heat shock proteins have been observed in muscle biopsies. These results suggest that global protein mistrafficking and accumulation may contribute to muscle wasting and disease progression. To test this hypothesis, we characterized various patient fibroblasts for GAA accumulation and global protein mistrafficking. Our results show that these cells indeed have lysosomal proliferation, massive accumulation of mutant GAA in LAMP-1 positive compartments, and formation of perinuclear inclusion bodies that stain positive for GAA and negative for LAMP-1. The number of inclusion bodies and density of lysosomal-like structures increased significantly as the cells reached confluence. XBP-1 splicing assays demonstrate that patient cells are less responsive to the ER stress inducer tunicamycin than wild-type cells, suggesting that disease fibroblasts may have adapted to a state of chronic stress. To determine whether the GAA enzyme activity and protein trafficking can be rescued, fibroblasts were treated with various concentrations of a pharmacological chaperone. Our results show that chaperone treatment yields 4–8-fold increases in GAA activity in several infantile- and adult-onset cell lines. To test for in vivo effectiveness, wild-type mice were treated with increasing doses of chaperone to assess drug accessibility to disease relevant tissues, including skeletal muscles, heart, brain and liver. Our results show an increase in GAA activity in all tissues