The influence of low dystrophin levels on disease pathology in mouse models for Duchenne Muscular Dystrophy

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Summary

Duchenne muscular dystrophy (DMD) is the most prevalent neuromuscular disorder, affecting 1:4000 boys. The first symptoms, consisting of difficulties in running, climbing stairs and standing from the ground become apparent by the age of three. Loss of muscle fibers leads to wheelchair dependency from the age of 12 years onwards. Patients die due to respiratory and heart failure around the age of 30. Although symptomatic treatment has significantly improved life expectancy in the Western world, no therapy is available yet.

DMD is caused by mutations in the *DMD* gene which encodes the protein dystrophin. Dystrophin forms a bridge between the sarcolemma and the extracellular matrix and provides stability to muscle fibers during contractions. In the absence of dystrophin, fibers are more prone to exercise-induced damage. This leads to continuous necrosis of muscle fibers which is compensated for by regeneration. Upon exhaustion of the regenerative capacity fibers are replaced by fibrotic and fat tissue, leading to a loss of muscle function.

In healthy individuals the *DMD* gene transcribes dystrophin according to a reading frame in which three mRNA nucleotides contain information for one amino acid (THE DOG BIT THE CAT). Reading frame disrupting mutations, like those found in DMD patients, result in premature termination of protein synthesis (THE DOG BIT HEC AT). In-frame mutations result in transcription of a shortened, but functional protein as both begin and end regions are conserved (THE DOG BIT CAT). These mutations are present in Becker muscular dystrophy (BMD) patients, which express low levels of dystrophin that generally results in a milder phenotype.

Using antisense oligonucleotides (AON) the disrupted reading frame in DMD patients can be restored, allowing synthesis of shorter but partly functional BMD-like dystrophin. At best, this and other clinical interventions result in the expression of low dystrophin levels. Fortunately, expression of wild type levels is not needed, as both humans and mice expressing ~50% of dystrophin do not show pathology. Even expression of ~30% of dystrophin has been found to result in a mild phenotype. Detailed studies on which levels of dystrophin are needed to increase muscle integrity, prevent muscle damage and improve muscle function have been performed in this thesis.

To do so we first had to set up good outcome measures. To assess motor function in *mdx* mice without interfering with the natural course of the disease a functional test regime consisting of four different functional tests was designed. In Chapter 2 we show that this type of exercise indeed did not interfere with disease pathology. Thereupon, we determined motor function in several mouse models for DMD (Chapter 3). We observed that motor function in mice that either did not express utrophin or in a monoallelic manner is significantly impaired compared to that of *mdx* mice. Additionally we identified MMP-9 and TIMP-1 as suitable serum biomarkers to monitor disease progression in both human and mouse samples (Chapter 4). Having good biomarkers is essential as patients need to be repeatedly treated with AONs whereas treatment efficacy cannot be determined on biopsies on a regular basis.

These tools were further validated and used in two new innovative mouse models expressing low levels of dystrophin. The first model was generated by crossing mice with a

mutation in the *Xist* gene (*Xist*$^{\Delta hs}$) with *mdx* mice. In female *mdx-Xist*$^{\Delta hs}$ embryos X-chromosomes that carried the mutated *Xist* gene but expressed intact dystrophin were preferably inactivated. This resulted in the expression of low dystrophin levels in a utrophin positive background (Chapter 5). We observed that dystrophin levels <15% already resulted in significantly improved muscle performance, while histopathology was largely prevented in mice expressing >15% dystrophin. Higher dystrophin levels (>22%) were needed to protect muscles from damage initiated by chronic treadmill exercise. So far, AON delivery to skeletal muscle is much easier to achieve than to the heart which appears to be a very difficult target. In mice, partly restored dystrophin expression in skeletal muscle increases voluntary activities thereby increasing the workload for the heart. The *mdx-Xist*$^{\Delta hs}$ mouse was used to study whether low dystrophin levels in heart can delay or prevent the onset of dilated cardiomyopathy (Chapter 6). Dystrophin levels between 3 and 21% already prevented the development of dilated cardiomyopathy in 10 months old dystrophic mice.

Mdx mice are less severely affected than DMD patients due to over expression of the dystrophin homologue utrophin, which compensates for its absence. Mice lacking both proteins mimic the human phenotype and die before the age of 12 weeks. To determine the effect of low dystrophin levels in these mice *mdx/utrn*$^{+/-}$ mice were crossed with *utrn*$^{-/-}$ *Xist*$^{\Delta hs}$ mice (Chapter 7). Again, based on skewed X-inactivation these mice expressed dystrophin levels <50%. In these mice, <10% dystrophin already significantly improved life expectancy and muscle function. To reduce histopathology >10% dystrophin was needed.

Based on these results it can be concluded that <10% dystrophin already improves the dystrophic phenotype to some extent, but >10% is needed to protect muscles from disease pathology. These findings are encouraging for ongoing and future clinical trails.