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Discussion

With several clinical trials ongoing aiming to restore dystrophin synthesis, more attention has been drawn to answering the most tempting question; how much dystrophin is needed to delay and/or prevent disease pathology and improve muscle function in DMD patients? The definite answer to this is rather complex as many factors need to be considered. This thesis covers many of these aspects, although several more still remain to be answered.

First of all, the amount of dystrophin needed depends on whether the newly synthesized dystrophin protein will be full-length, which might be achieved with e.g. stem cell therapy, or whether it is an internally truncated dystrophin obtained by e.g. gene therapy or AON-mediated exon skipping. In mice, full-length dystrophin is more effective than truncated dystrophin. Differences in efficacy between different truncated dystrophins are also expected, as the phenotype of BMD patients expressing different truncated dystrophin also varies between individuals.

Second, the way in which dystrophin will be expressed differs between the different types of treatment. Stem cell, PMO-mediated exon skipping and gene therapy result in dystrophin expression in a mosaic manner, whereas gentamycin administration and 2OMePS-mediated exon skipping result in a more uniformly expressed dystrophin pattern. Additionally, although stem cell therapy results in fibers expressing dystrophin at wild type intensities, the other therapies will likely result in intensities that are significantly lower and that might differ from muscle to muscle. Both the type and levels of dystrophin expression largely influences its ability to protect fibers against pathology. Especially when dystrophin is expressed in a mosaic manner, the distribution of dystrophin within a fiber will probably largely influence its protective effect. Large dystrophin negative clusters might still be prone to injury and are likely to be replaced by either smaller dystrophin negative clusters or more homogenous dispersion. Nevertheless it seems that dystrophin positive fibers create a beneficial environment for dystrophin negative ones and thereby enhance their survival. There might however be a threshold to the number of dystrophin positive parts within a fiber needed to ensure such an environment. Interestingly, limited studies in mdx mice have shown that a uniform expression will probably protect fibers better than when similar amounts of total protein are expressed in a mosaic manner. Extensive studies addressing these issues are lacking. In the same line, it is also interesting to know how much dystrophin can migrate over the membrane away from its transcription side.

The levels of dystrophin needed might also vary between different skeletal muscles. It is likely that slow fibers require less dystrophin than fast fibers to ensure protection against pathology. Likewise, muscles undergoing great workloads might be more demanding.

So far, the heart appeared to be very challenging to target. Dystrophin restoration in skeletal muscle potentially results in higher activity levels in DMD patients, which subsequently increases the workload for the heart. As DMD patients already suffer from dilated cardiomyopathy this might worsen its progression. The few publications available on this topic showed that 50% of dystrophin is sufficient to prevent heart failure, but based on our study we know that levels <22% already improve heart function significantly. Others have shown that low
dystrophin levels in the diaphragm also partly prevent against heart failure in mice. With the studies described in this thesis and those performed by others more insight has been obtained regarding the protective effect of low dystrophin levels in several mouse models for DMD. So far, conclusions based on mouse studies show strong similarities with the limited amount of human case reports available and with results obtained from gentamycin upregulation. This indicates that results obtained in mice are probably useful in translation to conclusions with respect to the dystrophin levels needed in humans. It is however unknown which effect size in mice is needed (e.g. improvement of 30% in the hanging wire test) to obtain clinically relevant effects in DMD patients. It might be that specific improvements obtained with low dystrophin levels in mice do have a less benefit in the larger muscles of the human body. Undoubtedly, the timing of initiation of dystrophin expression will largely influence the efficacy of the therapy. Ongoing clinical trials will probably gives more insight in these matters.