Generation and Characterization of a *Dmd*^{EGFP} Reporter Mouse as a Tool to Investigate Dystrophin Expression

Mina Petkova

Dystrophin is a rod-shaped cytoplasmic protein that physically links the cytoskeleton to the ECM through the dystrophin-associated protein complex (DAPC), thereby providing sarcolemmal stability. Mutations in the dystrophin encoding *DMD* gene cause the severe X-linked disorder Duchenne muscular dystrophy (DMD). DMD is characterized by progressive muscle wasting and fibrosis, impairing notably skeletal and heart muscle function as well as to various degrees cognitive, visual and gastrointestinal function due to missing dystrophin in the respective tissues. In this work a novel *Dmd*^{EGFP} reporter mouse that expresses a fluorescently labelled endogenous dystrophin – EGFP fusion protein was generated and characterized. The protein was tagged at the C-terminus that is present in the most dystrophin isoforms. To date, no dystrophin reporter mice exist, thus imaging is only possible by indirect antibody-mediated processing *ex vivo*. For the generation of transgenic mice a targeting vector containing a FLAG-EGFP coding sequence inserted in-frame after the last modified exon 79 of the murine *Dmd* gene was constructed. Following the EGFP sequence a lox*P* flanked *neomycin* cassette was inserted into the 3'UTR. The vector was used for modification of the X-chromosomal *Dmd* locus in embryonic stem cells and germline transmission of the modified allele. After removal of the *neomycin* selection cassette in the F1 generation *Dmd*^{EGFP} mice and their wildtype littermates were characterized.

Strong natural EGFP expression was observed in skeletal and smooth muscles, heart, brain and the eye and EGFP fluorescence co-localized with dystrophin at all sites suggesting proper tagging of the major dystrophin isoforms. In skeletal muscle, dystrophin as well as other proteins of the DAPC were expressed in normal quantity at correct sarcolemmal/subsarcolemmal localization. Skeletal muscle maintained normal tissue architecture, suggesting a correct function of the dystrophin-EGFP fusion protein. Isolated myofibers as well as satellite-cell derived myotubes expressed EGFP *in vitro*. Thus, the novel dystrophin reporter mouse provides a valuable tool for direct visualization of dystrophin expression. Furthermore, the model can be used to investigate dystrophin re-expression *in vivo* or *ex vivo* after various gene therapy protocols that are aimed at the reestablishment of the dystrophin open reading frame or in naturally occurring revertant fibers.