Institutionen för fysiologi och farmakologi

Functional role of cytoskeletal, contractile and regulatory proteins in muscle disease

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ABSTRACT

The function of skeletal muscle is an essential component of animal physiology and daily life. Hereditary muscle diseases are comparatively rare in humans, but often very severe. The disease causes are heterogeneous, defects in nerves, intracellular components, structural proteins and the contractile apparatus can be involved. Genetic linkage analyses have revealed associations between some genetic defects and muscle diseases. Causal relationships have been established in some animal models, but the exact function of several of the genes/proteins in the muscle and their roles in pathogenesis of the diseases have not been fully explored. The general aim of the thesis was to develop and analyze muscle disease models in the zebrafish larvae using a combined genetic and physiological approach, focusing on cytoskeletal/structural proteins and to explore different therapeutic options. In Paper I, desmin, which is a key intermediate filament protein, was knocked down in larval muscles by about 50 % using morpholino antisense oligonucleotide injection. This knockdown model had a significant impairment in muscle structure and decreased active force. X-ray diffraction analysis revealed swelling of the filament lattice after desmin knockdown, suggesting a role of desmin in the lateral anchoring of the contractile apparatus. Moreover, the vulnerability to eccentric contractions was lower after desmin knockdown suggesting that desmin is involved in lateral force transmission in the muscle cells. In Paper II, the zebrafish dystrophin null Sapje mutant, a model for Duchenne Muscular Dystrophy (DMD), was characterized. These mutant larvae had structural changes and compromised cell membranes, observed early during development (3 days post fertilization, dpf). Active force was significantly lower (about 50 % of that in the normal siblings). Two-day treatment with Ataluren, a compound causing read through of premature stop codons, partially restored the protein expression of dystrophin in the Sapje mutants. This was accompanied by a significant improvement of muscle structure and active force. The effect of Ataluren on active force revealed a bell-shaped dose dependency, similar to that suggested in initial clinical trials. The pathogenesis of DMD is complex, several factors can be involved. The contribution of the mechanical linkage provided by dystrophin/dystrophin-glycoprotein complex in the disease development of muscular dystrophin was examined in Paper III. Sapje mutants were fully immobilized by BTS (an actomyosin inhibitor) from 18 hours after fertilization until 4 dpf. The structural damage, as assayed by birefringence, was completely abolished by immobilization. To further validate the concept in another dystrophic model, Candyfloss mutants that were laminin-α2 chain null, were treated using the same protocol. These mutants which had significantly impaired structure were also rescued by immobilization. Following washout of BTS and active swimming, structural damage developed supporting mechanical contractions as a primary factor in the development of structural changes in muscular dystrophy. In Paper IV, the role of a sarcomeric protein, myosin binding protein C (MyBPC) was examined in the skeletal muscle. The skeletal isoforms, were knocked down in larvae. Partial removal of the fast type (MyBPC-2) resulted in a severe form of skeletal myopathy with activated degeneration/regeneration processes. Significant alterations were observed in the sarcomeric structure suggesting that MyBPC-2 is required for normal sarcomere assembly. The active force was significantly lower and the maximal shortening velocity was increased after MyBPC-2 knockdown, indicating that the close interaction between MyBPC-2 and the contractile filaments affects cross-bridge interaction. It is expected that a human myopathy associated with MyBPC-2 alterations, if present, would be severe with significant alterations in skeletal muscle structure and function. In general, the papers included in the thesis show that models for human muscle disease in the zebrafish can be analyzed with a clinically relevant functional read out, that novel therapeutic options can be examined in these models and that possible new muscle diseases associated with altered expression of structural muscle proteins can be identified.