Autophagy is crucial in the turnover of cell components both in constitutive conditions and in response to starvation. Defects of this degradative system play a role in various diseases, but little is known about autophagy in muscular dystrophies. Mutations in any of the three genes coding for collagen VI cause several muscle diseases in humans, including Ulrich congenital muscular dystrophy (UCMD), Bethlem myopathy (BM) and Congenital myosclerosis. Collagen VI null (Colba1−/−) mice display an early onset myopathic phenotype characterized by organelle defects, mitochondrial dysfunction and spontaneous apoptosis, leading to myofiber degeneration. We found that persistence of abnormal organelles and apoptosis are caused by defective autophagy. Indeed, skeletal muscles of Colba1−/− mice displayed an impairment of autophagic flux, which matched the lower induction of Beclin 1 and Bnip3 and the lack of autophagosomes after starvation. Notably, reactivation of autophagy by genetic, dietary and pharmacological approaches restored myofiber survival and ameliorated the dystrophic phenotype of Colba1−/− mice. Furthermore, muscle biopsies from patients affected by UCMD and BM showed reduced levels of Beclin 1 and Bnip3. These findings indicate that defective activation of the autophagic machinery plays a pathogenic role in congenital muscular dystrophies.

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Zebrafish as a model system for GNE mediated hereditary inclusion body myopathy (HIBM)


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Hereditary inclusion body myopathy (HIBM) is a unique neuromuscular adult onset disease characterized by slowly progressive distal and proximal muscle weakness, caused by missense recessive mutations in GNE, the gene coding for UDP-GlcNAc 2-epimerase/ManNAc kinase. Recently zebrafish have been recognized as a potent tool for the study of development and of various human disorders, such as neuromuscular disease. In particular, the GNE gene and protein are well conserved between zebrafish and mammals, and especially with the human ortholog. In order to unravel new insights in the pathophysiology of HIBM we are using the zebrafish system towards the elucidation of the fundamental mechanisms of GNE in normal muscle and in disease. Through in situ hybridization of whole mounted embryos we have shown that GNE is expressed mostly in brain and in muscle. We have also evaluated the effects of GNE knockdown on muscle development and organization by injecting specifically designed morpholinos in zb embryos. Knockdown of GNE expression results in a variety of dose-dependent phenotypes with marked muscle defects and disorganized thick filaments. In addition we have cloned the zebrafish GNE CDNA under the zebrafish GNE promoter and have generated transgenic fish carrying a GNE promoter driven Enhanced Green Fluorescent Protein (EGFP) reporter gene. This experiment is intended to show the feasibility of transgenic EGFP fish by the transposon methodology and to confirm GNE expression in fish tissues. The gne:EGFP transgenic zebrafish exhibits a maternal and ubiquitous pattern of GFP expression in fertilized eggs and early stage embryos. Moreover, the pattern of GFP expression recapitulates that of GNE mRNA in zebrafish embryos, indicating that the gne:EGFP transgenic line can be used as a model to study the molecular regulation of GNE gene expression.

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A mouse model that recapitulates features of facioscapulohumeral muscular dystrophy


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Facioscapulohumeral muscular dystrophy (FHSID) is a progressive muscle disorder which affects 1,200,000 people worldwide. FSHID is most often caused by contraction of the D4Z4 repeat on chromosome 4q35, but only if the contraction occurs in specific permissive genetic backgrounds of this chromosome. The contraction of the repeat is associated with D4Z4 chromatin relaxation and transcriptional derepression of the DUX4 gene encoding for a toxic double homoeodomain protein. Only on permissive chromosomes the DUX4 transcript stabilized by a polyadenylation signal outside the repeat resulting in high amounts of DUX4 protein in 1:1000 myonuclei in cell culture. DUX4 is a conserved retrogene suggesting that it has a functional role in normal cells. Indeed, DUX4 is expressed at high levels in the germ line. This supports a model in which the DUX4 retrogene has a function in the germ line, and is epigenetically silenced in somatic cells with incomplete repression in FSHD myonuclei. However, the exact function of DUX4 in germ line and its dysfunction in FSHD muscle is not known. As DUX4 is very toxic, attempts to generate transgenic mice expressing (inducible) DUX4 have proven difficult.

We report on the generation of a transgenic mouse model carrying D4Z4 units from a patient allele that expresses DUX4 and recapitulates all genetic, epigenetic and DUX4 expression attributes of FSHD, including high DUX4 expression levels in the germ line and the characteristic bursts of DUX4 expression in 1:1000 muscle nuclei. These transgenic mice therefore represent a faithful animal model for FSHD and will be a unique model to study the molecular mechanisms underlying FSHD and to test new therapeutic intervention strategies.

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ADVANCES ACROSS THE NEUROMUSCULAR FIELD 2

O.21

Cellular and molecular mechanisms of dysferlinopathy: New insights
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Limb Girdle Muscular Dystrophy Type 2B (LGMD2B) and Miyoshi Myopathy (MM) are caused by mutations in the dysferlin gene, but the role of dysferlin in healthy muscle and the changes that occur when it is mutated or absent are poorly understood. Previous work supported a role for dysferlin in sarcomemmal repair following laser wounding or other in vitro injuries. We study the response of A/J mice, which lack dysferlin, to injury by large-strain lengthening contractions in vivo. We find that dysferlin promotes normal recovery from this physiological injury but is not necessary for sarcomemmal repair. Consistent with this, immunofluorescence microscopic studies of healthy muscle, that we fixed and treated in a hot, mildly acidic solution to expose dysferlin’s epitopes, show that dysferlin is primarily in transverse tubes (TT), not the sarcolemma as previously reported. Furthermore, TT are disrupted when skeletal muscle is injured physiologically, and disruption is much more extensive in A/J muscles than in controls. Studies of FDB myofibers in tissue culture also demonstrate the presence of dysferlin in TT. Brief exposure of control myofibers to hypoosmotic solutions damages TT, in a process dependent upon extracellular Ca^{2+}. As in vivo, A/J myofibers are more extensively damaged by osmotic shock than controls; they are indistinguishable from controls when shocked in Ca^{2+}-free medium, however. Thus Ca^{2+} may promote damage, rather than participate in dysferlin-dependent membrane repair, as previously reported. Our results suggest that the changes in TT following injury in vivo and in vitro are similar, that they require extracellular Ca^{2+}, and that they are much more pronounced when dysferlin is absent. We propose that dysferlin is essential for the integrity of the TT of skeletal muscle, in maintaining this integrity during contraction and relaxation, and in repairing damaged TT following injury. Supported by the Jain Foundation.

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O.22

Dominant inheritance of limb girdle muscular dystrophy type 2A
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Limb girdle muscular dystrophy (LGMD) type 2A is the most common form of LGMD worldwide. Although strict recessive inheritance is assumed, patients carrying a single mutation in the calpain3 gene (CAPN3) are often reported. Such findings are commonly attributed to incomplete mutation screening. We report four families affected by calpainopathy, in whom only one mutation was identified, which segregated with the disease in multiple generations. In three of the families, a 21 bp in-frame deletion (c.643_663del21) was identified in CAPN3. All mutations-carriers had creatine kinase levels above 1000, myopathic muscle histology and mild asymmetric muscle weakness, preferentially of proximal leg and medial gastrocnemius muscles. In a fourth family, a single CAPN3 mutation (p.R572P) resulted in elevated creatine kinase and similar pattern of weakness in a father and son. All mutations were clearly heterozygous on the mRNA level. Also segregating with the mutations in all subjects, was a reduced intensity of calpain 3 on Western blot to about 10-20% of normal levels. The functional calpain 3 molecule is a homodimer. We suggest that the mild LGMD2A phenotype caused by the in-frame 21 bp deletion in three of the families and the p.R572P mutation in the fourth family, probably results from an abnormal protein product that exerts a dominant negative effect on the dimer. Residual calpain 3 activity, thus relies exclusively on two wild-type molecules forming a functional dimer, which would account for the faint protein bands seen on the Western blots of the patients. In conclusion, we provide proof of a new pattern of inheritance of LGMD2A in four unrelated families. This dominantly inherited form of calpainopathy seems to be much milder, and presents later in life than patients with recessive LGMD2A. The disorder could be named as the seventh dominantly inherited LGMD, i.e. LGMD1G.

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O.23

Anoctamin 5 (ANO5) subcellular localization in skeletal muscle
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