template the aggregation of normal desmin. This pathomechanism may be amenable to therapies aimed at decreasing protein aggregate transmission and propagation from myofiber to myofiber.

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NEW INSIGHTS INTO MUSCLE FUNCTION, IMAGING, THERAPY AND PREVENTION

NI.O.23

Sh3kbp1 involvement during skeletal muscle fibers formation: a new candidate for centronuclear myopathies

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Centronuclear myopathies (CNM) are a group of congenital myopathies characterized by skeletal muscle weakness, fatigability and atrophy. In affected muscles, myonuclei are abnormally located at the center of mature muscle fibers since they never migrate to the cell periphery as in healthy muscles. Initially considered as a consequence of the pathology, myonuclear mispositioning has recently emerged as one of the possible causes of muscle functionality defects observed in CNM. So far, the entire molecular machinery responsible for myonuclear positioning during myofibers formation is not known but recent clues involve cytoskeleton proteins. To discover new actors of myonuclear positioning, we performed a siRNA screen on potential cytoskeleton regulators and identified Sh3kbp1 as a new key regulator protein. Sh3kbp1 is ubiquitously expressed, but is up regulated upon muscle differentiation *in vitro* and specifically reactivated during *in vivo* muscle regeneration in mice. In *in vitro* conditions (C2C12 and primary muscle cells) and in mouse muscle *in vivo* (*Tibialis anterior),* Sh3kbp1 accumulates progressively both around myonuclei and at triads. Sh3kbp1 knockdown using siRNA in primary myoblasts or stable shRNA C2C12 cells reveals that the protein is not essential for the activation of myogenesis, but is required for normal myoblasts fusion and muscle fibers maturation. Indeed Sh3kbp1 downregulation increases fusion capacity and induces defects in T-tubules establishment and myonuclear positioning, which are typical features of CNM. Additionally, we demonstrated that Sh3kbp1 is interacting with Dynamin2 and is upregulated in a *Dynamin2* mouse model of CNM. Further experiments are currently on-going to elucidate the involvement of the two partners in the CNM phenotype. Altogether our results show that Sh3kbp1 is a new key regulator of both T-tubules maturation and myonuclear positioning, and identify Sh3kbp1 as a central player to better understand CNM physiopathology.

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NI.O.24

Centronuclear myopathy-causing mutations in dynamin-2 impair actin-dependent trafficking in muscle cells

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Dynamin-2 is a large GTP-ase that mediates membrane remodeling and actin dynamics in different cell types. It is composed by five highly conserved domains: a GTP-ase domain, a middle structural domain, a PH domain that binds phosphoinisitides a GTP-ase effector domain and a proline-enriched-domain that binds SH3-containing partners. Mutations mainly localized in the middle and PH domains of dynamin-2 cause dominant centronuclear myopathy (CNM), a congenital disorder characterized by progressive weakness and atrophy of skeletal muscles. However, how these mutations affect the role of dynamin-2 in muscle cells is still unclear. In the present work, we demonstrate that dynamin catalytic activity is required for *de novo* actin polymerization and to promote actin-mediated trafficking of the GLUT4 glucose transporter in muscle cells. These dynamin functions are impaired in myoblasts expressing dynamin-2 constructs carrying CNM-linked middle domain mutations. Similar effects were observed in mature muscle fibers isolated from a mouse-animal model of CNM. Furthermore, GLUT4 displays aberrant perinuclear accumulation in biopsies from CNM patients carrying middle-domain mutations in dynamin-2 suggesting intracellular trafficking defects. Together, these data present dynamin-2 as a key regulator of actin remodeling and GLUT4 trafficking in muscle cells. In addition, these findings support a model in which an impaired actin-dependent trafficking could contribute to the pathological mechanism in dynamin-2-associated CNM.

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NI.O.25

Dynamic assessment of muscle perfusion, deoxymyoglobin and phosphorylated metabolites concentrations through fast interleaved NMR acquisitions with a clinical 3T scanner

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NMR allows to quantify *in vivo* multiple aspects of physiological parameters such as regional perfusion, blood and tissue oxygenation, intracellular pH or high-energy phosphate metabolism. Classical NMR acquisition schemes rarely explore more than a few biological parameters during a dynamic paradigm, such as exercise or leg ischemia, and thus multiple separate experimental sessions are required at the expense of adding experimental variability on biological processes which are already multifactorial, increased length of scan time and patient discomfort. We present an interleaved NMR pulse sequence simultaneously measuring a perfusion image, ^{1}H deoxy-myoglobin (dMb) and ^{31}P MR spectra on a standard 3T Prisma scanner which was successfully evaluated in healthy subjects either in the ischemic calf muscle (8-mins cuff on the thigh) or in the exercising quadriceps muscle (8-mins of quadriceps contraction by lifting a load placed on the foot every 3 s). Using a dual-tuned ^{1}H/^{31}P surface coil, one ^{31}P and 80 ^{1}H dMb non-localized MR spectra were acquired during the evolution time (820 ms) of a pulsed-ASL (SATIR) sequence. Images were acquired using FLASH (radial read-out, 256 points, 128 spokes). Data sets were generated every 2 seconds (ischemia) or 3 seconds (exercise) during the 20-min experiment. The biochemical responses in both paradigms were in agreement with the literature although peak perfusion values during hyperaemia were lower (25.2 mL/min/100g at peak versus 0.22 ± 2.80 mL/min/100 g during ischemia), likely due to the reduced ASL tagging efficacy of the surface coil. This work shows the feasibility of dynamic interleaved measurements with a high temporal resolution in a clinical setting and with different experimental paradigms. This setup opens new possibilities to investigate non-invasively complex or subtle alterations of the coupling between microcirculatory regulation and muscle energetics in NMDs, and in particular in DMD, glycogenoses or even centronuclear congenital myopathies.

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