Muscle Regulation

2288-Pos Board B307
MST1, a Key Player, in Enhancing Fast Skeletal Muscle Atrophy
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Skeletal muscle undergoes rapid atrophy upon denervation and the process of denervation-induced skeletal muscle atrophy is complicated. MST1 (mammalian sterile 20-like kinase 1) is identified as a central component of Hippo signaling pathway. In the present study, we identified that MST1 kinase was expressed widely in skeletal muscles and found that it was dramatically up-regulated in fast- but not slow-dominant skeletal muscles immediately upon denervation. The results of our histological and biochemical studies demonstrated that deletion of MST1 significantly attenuated denervation-induced skeletal muscle content loss and decreased expression level of Atrogin-1 and LC-3 genes in mice. Further studies indicated that MST1, but not MST2, remarkably increased FOXO3a phosphorylation level at Ser207 and promoted its nuclear translocation in atrophic fast-dominant muscles. Thus, we, in our knowledge for the first time, demonstrated that MST1 kinase plays an important role in regulation of denervation-induced skeletal muscle apotrophy, namely, the upregulated MST1 kinase promoted progression of neurogenic atrophy in fast-dominant skeletal muscles through activation of FOXO3a transcription factors.

2289-Pos Board B308
Evaluating Cellular Repair Potential - Lessons from Skeletal Muscle
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The ability to repair disruptions in the plasma membrane is critical for maintaining cellular integrity and health; this is especially true for cells that routinely experience membrane damage as a result of activity such as skeletal muscle. Dysferlinopathies are a class of late onset muscle wasting diseases associated with the absence or truncation of the protein dysferlin. Dysferlin is postulated to have a role in membrane repair and the absence of dysferlin in both humans and animal models is associated with muscle damage. We are developing techniques to evaluate the repair potential of dysferlin null skeletal muscle fibers and myotubes using fluorescence assays of dye-leakage and calcium activity. In isolated muscle fibers or myotubes containing fluorescent dye and subjected to IR laser wounding, both the rate and duration of dye-leakage are measured by confocal microscopy. Upon wounding repair-competent fibers a ''puff'' of dye is released at the wound site and dye leakage ceases within 60 seconds. Under conditions where resealing is inhibited (i.e. low extracellular [Ca^{2+}]) a large amount of dye is lost; leakage continues for >10 minutes. Changes in intracellular [Ca^{2+}]free are measured using a combination of the calcium indicator dyes fluo-4 and Fura Red. Upon wounding, a rise in [Ca^{2+}]free is observed at the wound site and either returns to the pre-wound level following successful resealing, or in the absence of resealing remains high and spreads through the entire fiber or mytube. Dysferlin null muscle, is repair competent but may differ in the developed calcium load, contractile response, and wounding threshold compared to dysferlin positive muscle. Since our overall goal is to develop interventions that boost cellular repair potential, quantifiable metrics of this complicated cellular process are required when testing the efficacy of repair-targeted treatments.

2290-Pos Board B309
Troponin T3 Regulates Cacna1 Gene Transcription
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Troponin T (TnT) plays a major role in striated muscle contraction. We recently demonstrated that the fast skeletal muscle TnT3 is localized in the muscle nucleus, and either its full-length or COOH-terminus leads to muscle cell apoptosis. Here, we further explored the mechanism by which it enters the nucleus and promotes cytotoxicity and investigated its role in nuclear signaling pathway. Amino acid truncation and substitution showed that its COOH-terminus contains a dominant nuclear/nucleolar localization sequence (KLKRQK). Deleting this domain or substituting lysine and arginine residues (KLAAQK) resulted in a dramatic loss of TnT3 nuclear and nucleolar localization. In contrast, the GATAKGKVGGRWK domain-DsRed construct localized exclusively in the cytoplasm, indicating that a nuclear exporting sequence is possibly localized at the COOH-terminus. Additionally, we identified a classical DNA-binding Leucine Zipper Domain (LZD). Deletion of LZD or KLKRQK sequence significantly reduced cell apoptosis compared to full-length TnT3. shRNA knocking down of TnT3 in C2C12 myotubes down-regulated the expression of calcium channel α1s (CaVα1s) but not β1a subunit. Luciferase activity analysis further verified that knocking down TnT3 in C2C12 muscle cells inhibited while overexpression of TnT3-DsRed in mouse muscle in vivo increased the Cacna1 promoter activity. Additionally, chromatin immunoprecipitation using TnT3 antibody cocktails pulled down Cacna1 promoter fragments from C2C12 myotubes. In contrast, pre-rRNA processing in the nucleolus was not affected by TnT3 depletion. We conclude that TnT3 contains both a nuclear localization signal and a DNA binding domain, which may mediate its nuclear signaling in muscle cell apoptosis and gene expression regulation.

2291-Pos Board B310
Lasp regulates Actin Filament Dynamics in Drosophila Myofibrils Assembly
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The actin cytoskeleton plays a key role in a number of motile and morphogenetic processes. The coordinated assembly and disassembly of actin filaments promotes cell shape changes, mediates motility, contractility, and many other processes. In each case, actin dynamics is finely regulated by a large number of actin-binding proteins (ABPs) that control actin filament polymerization, nucleation and crosslinking.
One group of ABPs is the nebulin family. To date, this family comprises 5 members, each containing from 2 to 185 actin-binding nebulin repeats. In our previous work, we showed that Lasp is the single member of this family in *Drosophila*. Lasp has an N-terminal LIM domain, two nebulin repeats, and a C-terminal SH3 domain. *Lasp* null mutants are homozygous viable, but male sterile. The stem cell niche is not properly anchor and actin cone migration is impaired, resulting in failure of spermatid individualization. Recently, we discovered that Lasp also functions in myofibril assembly. In *Lasp* mutants, sarcomere length is reduced and muscle contractility is weaker than in wild type flies. These results are consistent with a function of nebulin family proteins as scaffolding and actin filament organizing proteins.
Using a double-tagged Lasp, I performed a pull down assay to identify interacting partners. As expected Lasp binds muscle-specific actin isoforms. More surprisingly, most of the isolated proteins are components of thick filaments, suggesting new functions for Lasp. We will report which domain of Lasp regulates actin dynamics, protein interactions and proper localization in sarcomere assembly. By deciphering Lasp function in both Z-disc and A-band, we will obtain further insights into the mechanism of myogenesis.

2292-Pos Board B311
Zasp PDZ Domain Proteins Cooperate in Z-Disc Formation and Myofibril Assembly
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Zasp52 is a multi-domain protein, composed of an N-terminal PDZ domain, ZM motif, and four LIM domains, and is found predominantly in Drosophila muscle. It is part of the PDZ-LIM domain protein family, which comprises Enigma, ENH, ZASP, Alp, CLP36, RIL, and Mystique in vertebrates. In Drosophila embryos, Zasp52 was shown to co-localize with α-actinin at Z-discs and with integrins at myotendinous junctions. Mutations in Zasp52 cause first instar larval lethality with defects in Z-disc assembly and maintenance as well as muscle attachment. During Drosophila indirect flight muscle (IFM) development in the pupa, Zasp52 is present at very early stages of myofibril assembly. It localizes with α-actinin in rudimentary Z-bodies along the assembling myofibril, before other muscle proteins show any periodicity. RNA interference against the last exon encoding the most C-terminal LIM domain results in the depletion of all high molecular weight isoforms. This gives rise to viable but flightless adult animals. IFM sarcomeres show thin and interrupted Z-discs as well as distorted H-zones from early stages of myofibril assembly onwards. These findings suggest that Zasp52 is required for the establishment of normal Z-discs in the IFM and subsequent sarcomere stability after onset of contractility. A closely related PDZ domain containing protein, Zasp66, is also required for Z-disc formation and stability. Simultaneous knock down of Zasp52 and Zasp66 lead to more severe, synergistic myofibril defects, demonstrating that both proteins act together during Z-disc formation, likely because both biochemically bind α-actinin.

2293-Pos Board B312
Regulation of Respiration in Permeabilized Muscle Cells: Apparent KM for ADP Shows the Mitochondrial Outer Membrane Permeability
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