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Insulin-like growth factor-I and interleukin-8 augment myocilin levels and protein kinase B/Akt phosphorylation in rat primary skeletal myoblasts

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Introduction: The aim of this study was to examine and compare potential effects of IGF-I, an anabolic factor in skeletal muscle, and IL-8, a cytokine released in contracting myofibers, on expression of myocilin and phosphorylation of its downstream signaling element, protein kinase B (PKB)/Akt in rat skeletal myoblasts.
Materials and methods: Primary skeletal muscle cells (RSkMc), isolated from the limbal skeletal muscle of neonatal rats, and subjected to 11 days of differentiation were used for the study. The cells were exposed to IGF-I (final concentration – 25 nmol/l), IL-8 (final concentration – 1 ng/ml), or combination of both factors, in the entire period of differentiation. Transcript levels of myocilin were examined using qPCR technique, with Gapdh and beta-actin, serving as housekeeping genes. The cellular content of myocilin, total and phopshorylated PKB/Akt proteins were assessed by immunoblotting. Student t-test was used for comparison the results.
Results: IGF-I or IL-8 did not modify cell viability. Myotube formation in RSkMc cultures was increased by IGF-I (by 39%, $p < 0.05$), and unaltered by IL-8 acting alone or in combination with the growth factor. Myocilin transcript levels were appr. 2-fold higher under IGF-I, IL-8 and growth factor-cytokine treatment, in comparison to control values. As a consequence, cellular contents of myocilin protein were also augmented and similar in all experimental groups. IGF-I, IL-8 or both these factor combined did not affect PKB/Akt protein levels. However, PKB phosphorylation levels were markedly increased, especially in the presence of IL-8 (acting both alone and in combination).
Conclusion: IGF-I and IL-8 augment cellular content of myocilin, a protein involved in regulation of skeletal muscle size, via transcriptional mechanism. This effect can result in enhancement of PKB activity, manifested by an increased kinase phosphorylation, however it is not sufficient in stimulation of myotube formation.

Session 06 Muscle cytoskeleton

Oral presentations

S06.O1

Structural and functional roles of the giants of the striated muscle sarcomere-titin and nebulin

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Titin and nebulin are mega-dalton sized proteins that form myofilaments located in the striated muscle sarcomere. Nebulin is embedded in the Z-disk and is coextensive with the thin filament. Recent work from several laboratories has provided evidence that nebulin does not span all the way to the thin filament pointed end, bringing into question nebulin's earlier proposed role in thin filament length regulation. These findings will be discussed as well as recent work on muscles in which the size of nebulin has been altered and the effect of nebulin on thin filament length is studied. The structural and functional effects of conditionally deleting nebulin from skeletal or cardiac muscle will also be addressed. Titin is a giant protein that spans in the sarcomere from Z-disk to M-band. It's I-band spanning region functions as a molecular spring that contributes to passive stiffness of the heart with multiple stiffness adjustment mechanisms that exist within this molecular spring. Importantly, recent work from multiple laboratories has shown that these stiffness adjustment mechanisms are deranged in heart failure with preserved ejection fraction (HFpEF). Our recent studies in mice with HFpEF-like symptoms support that manipulating titin's splicing machinery through targeting the splicing factor RBM20 greatly improves diastolic function.

S06.O2-255

Ablation of palladin in adult cardiac muscle causes cardiac dilation and systolic dysfunction

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Introduction: Palladin (PALLD) belongs to the PALLD/myopalladin (MYPN)/myotilin (MYOT) family of immunoglobulin-containing proteins in the sarcomeric Z-line associated with the actin cytoskeleton. While MYPN and MYOT are expressed in striated and skeletal muscle, respectively, PALLD is ubiquitously expressed in several isoforms. The longest PALLD isoform is expressed predominantly in striated muscle and shows high structural homology to MYPN. However, while MYPN gene mutations have been associated with human cardiomyopathies, the role of PALLD in the heart has remained unknown, partly due to embryonic lethality of PALLD knockout (KO) mice. To study the role of PALLD in the heart, we generated constitutive (cPKOc) and inducible (cPKOi) cardiac specific PALLD KO mice as well as constitutive double KO mice for PALLD and MYPN (cPKOc/MKO).
Methods and Results: While echocardiographic analyses revealed development of dilated cardiomyopathy (DCM) of MYPN KO (MKO) mice starting from 4 months of age, cPKOc mice exhibited no cardiac phenotype either at basal conditions or following transaortic constriction. MKO/cPKOc mice exhibited a similar phenotype as MKO mice, suggesting that MYPN and PALLD do not have overlapping functions. In contrast, induction of PALLD KO in adult cPKOi mice resulted in progressive cardiac dilation and systolic dysfunction, associated with reduced cardiomyocyte contractility (Ionoptix analysis), increased resting tension (mechanics on isolated single myofibrils), abnormal intercalated disc ultrastructure, upregulation of markers of cardiac pathological remodeling, ERK activation, and fibrosis.
Conclusions: The development of a DCM phenotype in cPKOi mice induced at adult stage demonstrates that PALLD is essential for normal cardiac function and propose PALLD as a candidate gene for cardiomyopathy. In contrast, cPKOc mice exhibited no cardiac

phenotype, likely due to compensatory mechanisms, which do not appear to involve its closest homologue MYPN.

S06.O4-234

O-GlcNAcylation is a key modulator of cytoskeletal interactome involved in the skeletal muscle sarcomeric organization

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The striated muscle is an intricate, efficient and precise machine, composed of highly specialized myofibrils. While thin and thick filaments interact to generate the force of contraction, other proteins constitute an intricate and interconnected network, termed nowadays sarcomeric cytoskeleton, whose role is the regulation of sarcomere function to ensure its efficient working. We have recently demonstrated that O-GlcNAcylation, a particular glycosylation akin to phosphorylation, is a key modulator of sarcomeric organization since the sarcomere morphometry is modified according to the O-GlcNAcylation level of myofibrillar proteins. In particular, the morphometric changes were accompanied by changes in several protein-protein complexes involving key structural proteins. In particular, we demonstrated that the interaction between desmin and its molecular chaperon alphaB-crystallin, was modulated according to the variation of global O-GlcNAc level. We currently focused on the dynamic of O-GlcNAcylation and phosphorylation of these two key proteins of sarcomeric organization. Furthermore, through a proteomic approach performed on C2C12 myotubes, based on Click Chemistry and mass spectrometry, we have identified several key structural proteins bearing O-GlcNAc moieties, and localized O-GlcNAc sites on some of these proteins. Interestingly, some sites corresponded to mutation sites in neuromuscular disorders, in particular myofibrillar myopathies characterized by a marked disorganization of myofibrils. Taken together, all these data provided new insights for the fine understanding of the complex interactome underlying the organization of sarcomeric structure.

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Trans-complementing immunoglobulin domain folding in the myomesin and obscurin-like-1 complex: implications for M-band protein network organization

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Immunoglobulin-like (Ig) domains are globular domains composed of about 100 residues, and one of most frequently utilized domain by sarcomeric modular proteins, like titin (aka connectin), obscurin and filamin. Although homology between Ig domains is not high (25 to 50%), the structures of sarcomeric Ig domains determined so far are very similar, forming a so-called "beta-sandwich" of two beta-sheets comprised of 7–10 beta strands. Recently, we determined the crystal structure of the human myomesin / obscurin-like-1(obsl1) complex formed by Ig domain 3 of obsl1 (OL3) and the short linker of myomesin between fibronectin type-3 domains 4 and 5 (MyL). The crystal structure of this complex elucidated a novel trans-complementing Ig domain fold, in which the MyL beta-strand participated in one beta-sheet formation of OL3 with beta-strands B, B', D and E via hydrogen bonds and hydrophobic interactions, whereby a complete Ig domain was folded. Mechanical forces required for detachment of the complex was measured by atomic force microscopy (AFM) and shown to be similar to, or higher than for an intact Ig domain. The structure of the complex showed the key residues on the linker of myomesin for incorporation to OL3 were not conserved in other myomesin isoform (Myom2 and 3), and the specificity of Myom1 was validated by binding assay both in vitro and in cellula. In conclusion, the intermolecular complementing Ig-fold formed by myomesin and obsl1 is an integral part of titin-obsl1-myomesin ternary protein network in M-band. Considering its firm mechanical interaction (135pN), compared to that of titin and obsl1 (30pN), the complex might contribute to the essential backbone of the M-band protein network. This work is under review.

Posters

S06.P2-339

Tetranectin labels myofibrillar disruptions after eccentric exercise in humans

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Introduction: Tetranectin is a secretory protein suggested to play a role in tissue healing processes. Here, we suggest that tetranectin is correlated to sarcomere disruption after high-force eccentric exercise and could act as a marker for myofibrillar disruptions.
Materials and methods: Eleven male students performed one bout of 300 unilateral maximal eccentric muscle actions with knee-extensors, and needle biopsies were taken from m. vastus lateralis. Immunostaining on cross-sections showed positive tetranectin labeling within myofibers, evident as early as 0.5 hours after the exercise bout, and the intracellular tetranectin staining remained prominent up to 7 days.
Results: The percentage of positive labeled myofibers in the loaded leg varied considerable between subjects (17 – 100%), compared to <3% in controls. A correlation was observed between the proportion of tetranectin positive fibers and the reduction in force-generating capacity. Positive tetranectin labeling was shown to co-localize with disruptions of sarcomere organization, visualized by double labeling against F-actin and HSP27. At the mRNA level, in situ hybridization revealed pericellular localization of tetranectin mRNA, suggesting that the protein is synthesized outside the myofibers.
Discussion: In summary, we report the novel finding of a rapid and sustained accumulation of tetranectin within damaged myofibers, bound to distinct areas and segments of damaged sarcomeres. Judging from its presence in disrupted areas we suggest a role of tetranectin in remodeling of damaged sarcomeres after overload. Furthermore, the absence of tetranectin in intact sarcomeres enables positive tetranectin staining as a useful marker for the quantification of sarcomere disruptions.