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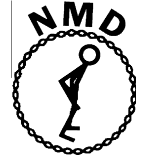
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Meeting report

6th Dysferlin Conference, 3–6 April 2013, Arlington, Virginia, USA

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Abstract

The 2013 Dysferlin Conference, sponsored and organized by the Jain Foundation, was held from April 3–6, 2013 in Arlington, VA. Participants included 34 researcher speakers, 5 dysferlinopathy patients and all 8 members of the Jain Foundation team. Dysferlinopathy is a rare disease that typically robs patients of mobility during their second or third decade of life. The goals of these Dysferlin Conferences are to bring experts in the field together so that they will collaborate with one another, to quicken the pace of understanding the biology of the disease and to build effective platforms to ameliorate disease. This is important because the function of dysferlin and how to compensate for its absence is still not well understood, in spite of the fact that the dysferlin gene was identified more than a decade ago. The objective of this conference, therefore, was to share and discuss the newest unpublished research defining the role of dysferlin in skeletal muscle, why its absence causes muscular dystrophy and possible therapies for dysferlin-deficient muscular dystrophy patients.
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Session 1: Exploring the function of dysferlin

This session explored the potential functions of the dysferlin protein.

Robert Bloch (USA) used immunoflorescent labeling and a pH sensitive GFP moiety linked to the C-terminus of dysferlin to show that dysferlin is localized to the transverse tubules both *in vivo* and *in vitro*. This localization of dysferlin to t-tubules suggests a role for dysferlin in maintaining t-tubule integrity. To test this hypothesis, t-tubules in both dysferlin deficient A/J and wild-type muscle were subjected to injury *in vivo* and *in vitro* and outcomes were assessed. In the first injury model, the hindlimb muscles *in vivo* were subjected to large strain eccentric injury (LSI) and torque loss and recovery, t-tubule disruption, necrosis, and inflammation were measured. While the initial loss of contractile strength was similar in both wild-type and A/J muscles, the t-tubule structure was maintained in wild-type but highly disrupted in the dysferlin null muscle soon after LSI, as observed by the level of DHPR disruption. In a separate injury experiment, FDB myofibers were subjected to osmotic shock (OSI) in culture. Measurements of t-tubule disruption, changes in Ca2+ transients and baseline, and changes in the efflux of the impermeant dye sulforhodamine B (SulfB) from the t-tubule lumen, were then performed. OSI rapidly caused dysregulation of Ca2+ homeostasis, disruption of DHPR organization and a slowing of SulfB efflux from the t-tubules of dysferlin-null myofibers, consistent with a disruption of the t-tubule structure. Expression of full-length dysferlin protected against the changes seen after OSI, indicating that these effects are dysferlin dependent. Taken together, these results show that dysferlin plays an important role in stabilizing the structure and function of t-tubules in myofibers both *in vivo* and *in vitro* and that these injury assays could be used in evaluating potential therapies for dysferlinopathy.

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Aurelia Defour (USA) used several different murine and human cell lines to characterize the subcellular defects present when dysferlin is absent. The dysferlin deficient muscle cell lines evaluated included C2C12 shRNA dysferlin, H2 K-A/J myoblasts derived from a cross between the dysferlin deficient A/J line and the immortomouse, and an immortalized human dysferlin deficient cell line generated from the myoblasts of a dysferlinopathy patient. Evaluation of these lines showed that while lack of dysferlin did not affect the ability of these cells to proliferate or differentiate, it did affect their repair capability using two independent methods for cell membrane injury and repair [1]. In addition, because lysosomal exocytosis is associated with repair of injured cell membrane, she went on to test if dysferlin deficiency affects lysosomal exocytosis. Using several assays for lysosomal exocytosis such as cell membrane LAMP1 staining, live lysosomal trafficking and docking with TIRF, she and her colleagues showed that there are fewer lysosomes at cell membranes and that there is a delay and reduction in injury-triggered lysosome exocytosis. These findings demonstrate a role of dysferlin in cell membrane repair and the docking and calcium triggered fusion of lysosomes. These effects likely contribute to poor healing of dysferlin deficient muscle cells and could help explain some of the underlying pathophysiology of dysferlinopathy.

Angela Lek (Australia) presented data around the role of dysferlin in repair of membrane injury. Using ballistics induced damage of human skeletal myotubes, she showed that endogenously expressed dysferlin is rapidly mobilized and enriched at sites of injury along with MG53 in a $Ca2+$ dependent manner. However, only a portion of dysferlin appears to be present at the sites of injury as only the C-terminal antibody (Hamlet-1) and not the other more N-terminally located dysferlin antibodies can detect dysferlin at sites of injury. This data is supported by Western blots that show the presence of a 72 k-Da "mini-dysferlin" Hamlet 1 positive band only in myotubes damaged in the presence of calcium. Further, the levels of this 72 k-Da band correlate with the levels of full-length dysferlin, and this "mini-dysferlin" is not produced in myotubes derived from dysferlinopathy patients. Given the size of the band and that it contains the Hamlet-1 epitope, the 72 k-Da band is proposed to contain C2E, C2F, and the transmembrane domain. This makes the fragment look a lot like synaptotagmin. In addition, the formation of the 72 k-Da mini-dysferlin is sensitive to calpeptin, which suggests that this fragment of dysferlin is produced by calpain cleavage. This data suggests that dysferlin could act as a modular protein produced through enzymatic cleavage and that the various functions attributed to dysferlin could be performed by different portions of the protein.

Kevin Sonnemann (USA) utilized recombinant dysferlin proteins to understand how dysferlin modulates membrane fusion events and is recruited to sites of damage. He presented *in vitro* data that demonstrated that dysferlin is able to bind lipids, aggregate vesicles, and stimulate fusion between muscle v-SNARE and muscle t-SNARE containing vesicles in a dose dependent and calcium dependent manner. Using purified recombinant GFP-dysferlin mixed with fluorescent (protein-free) vesicles, he also showed that dysferlin localizes to areas of membrane tubulation upon addition of calcium, suggesting that dysferlin directly modulates membrane lipids by inducing or stabilizing extreme membrane curvature. Lastly, using frog oocytes he showed that dysferlin is recruited approximately 30 s after the wound, that this recruitment requires the presence of the transmembrane domain, and that dysferlin recruitment is prevented when DAG expression is inhibited by injection of SPK609. DAG is enriched at the site of membrane damage and in the absence of DAG recruitment, the membrane fails to repair. Together, these data indicate that dysferlin may perform a primary role in membrane fusion and offers mechanistic insight into the membrane repair deficiency in dysferlinopathies.

Paul S. Blank (USA) presented techniques to evaluate the repair potential of dysferlin deficient skeletal muscle fibers and myotubes using fluorescence microscopy to measure indicator uptake, leakage, and calcium activity. The uptake assay involves membrane wounding and a measurement of the increase in cellular fluorescence. The increase in fluorescence correlates with membrane repair. Paul pointed out that FM-143, a reagent commonly used for this type of assay, may not be appropriate because baseline membrane labeling is affected by the amount of time the cells or fibers are in culture. He demonstrated that the nuclei-staining indicator Sytox Green does not have these problems. When cells containing the indicator calcein red–orange are wounded, a "puff" of indicator is released at the wound site. The leakage amount and time course correlates with membrane resealing. The last technique measured changes in intracellular calcium using a combination of the calcium indicator dyes fluo-4 and Fura Red. Using this assay, an increase in calcium levels is observed at a wound site that returns to the pre-wound level following successful resealing. However, in the absence of resealing, the calcium levels remain high and spread throughout the injured cell. In all three assays, dysferlin deficient muscle was able to reseal in physiological $Ca2+$, but differed in the developed calcium load and wounding threshold, supporting a role for dysferlin in the membrane wounding and repair process.

Jyoti Jaiswal (USA) discussed how the actin cytoskeleton regulates membrane remodeling during cell membrane repair and how dysferlin trafficking is associated with this process. By comparing the cell surface proteome of injured and uninjured cells, he and his colleagues identified various organelles and processes, such as mitochondria, actin polymerization and distribution, and microtubule growth that changed in

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