HHS Public Access

Author manuscript

Neuromuscul Disord. Author manuscript; available in PMC 2017 January 17.

Published in final edited form as:

Neuromuscul Disord. 2013 February ; 23(2): 188–191. doi:10.1016/j.nmd.2012.06.353.

Report on the Myomatrix Conference April 22–24, 2012, University of Nevada, Reno, Nevada, USA

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Abstract

The Myomatrix 2012 conference held April 22–24th, 2012 at the University of Nevada, Reno convened 73 international participants to discuss the dynamic relationship between muscle and its matrix in muscular dystrophy with a specific focus on congenital muscular dystrophy. Seven sessions over 2½ days defined three central themes: (1) the role of extracellular matrix proteins and compartments in development and specifically in congenital muscular dystrophy (CMD) (2) the role of extracellular matrix signaling and adhesion to membrane receptors and (3) the balance and interplay between inflammation and fibrosis as drivers of altered matrix stiffness, impaired regeneration and progressive dystrophy. This report highlights major conference findings and the translational roadmap as defined by conference attendees.

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Competing interests

None.

Authors contributions

AR and DJB wrote the conference report; CB, SB, ST, JD, MAR, MLM, DG, RHC, GK, KN, MG edited the manuscript.

Keywords

Conference report; Congenital muscular dystrophy; Translational Research Road Map; MDC1A; DMD; Dystroglycanopathy; Collagen VI related myopathy; Muscular dystrophy; Extracellular matrix; Fibrosis; Laminin; Integrin

1. Background

The congenital muscular dystrophies (CMDs) are a group of rare genetically defined multi-organ disorders, classified into four major groups based on gene involvement, predicted protein function and localization: (1) abnormalities of *O*-mannosyl glycosylation of the matrix receptor α-dystroglycan (αDG-related dystrophy, αDG-MD), (2) abnormalities of extracellular matrix proteins laminin α2 (*LAMA2*-related muscular dystrophy, *LAMA2*-MD), collagen type VI (Collagen VI-related myopathy), (3) abnormalities of nuclear proteins, lamin A/C (*LMNA*-related muscular dystrophy) and (4) abnormalities of the sarcoplasmic reticulum and T-tubule triad (selenoprotein N1 (*SEPN1*) and ryanodine receptor 1 (*SEPN1*-related myopathy, *RYR1*-related myopathy). The myomatrix describes the specialized extracellular matrix (ECM) of muscle. ECM and thus myomatrix involvement are prominent in CMD, and impacts every aspect of the disease. This ranges from ECM changes following typical dystrophic mechanisms of sarcolemmal instability, which then become disease drivers in their own right, to novel primary matrix pathology originating from mutations in matrix components. Both primary and secondary ECM involvement may further impair regeneration, and drive degeneration, fibrosis and apoptosis as the disease progresses. The clinical phenotype in the CMDs with prominent ECM involvement often involves prenatal onset of the muscle pathology with clinical manifestations at birth or during the first 2 years of life, while the ongoing postnatal disease activity and resulting perturbations of muscle and its matrix commonly lead to progressive weakness, contractures, scoliosis, nutritional deficiencies and respiratory insufficiency. Although the genetic basis and pathogenesis of many CMDs are partially understood, there are currently no effective treatments.

2. Myomatrix in development and muscular dystrophy

Interactions between muscle and its connective tissue play an important role in muscle development regulating both myogenesis and muscle morphogenesis. In particular, different laminin isoforms, key components of post-natal muscle ECM, are critical for the establishment of the myotome. Early in axial muscle morphogenesis, laminin disassembles followed by a re-establishment of a laminin framework after myotomal cells have reached their final orientation. Tenascin and fibronectin appear to play a tendon-like role in the segmented myotome and possibly guide muscle morphogenesis while cell-cell interactions between muscle and muscle connective tissue fibroblasts (which presumably produce much of the myomatrix) regulate myogenesis. In the limb, Tcf4+ connective tissue fibroblasts modulate muscle fiber type and maturation. Such interactions between Tcf4+ fibroblasts and myogenic cells are important later in the adult for regulating the expansion of adult muscle

stem cells, satellite cells, during muscle regeneration, warranting further investigation into their involvement in various disease scenarios, including in the CMDs.

Exploring the significance of early transient inflammation in dystrophic pathology and the interaction between inflammatory cells, myogenic cells, and connective tissue fibroblasts may best be accomplished in well-characterized congenital onset dystrophic murine models, such as the $dy^{w/w}$ animal model of *LAMA2*-related muscular dystrophy (MDC1A). Mechanisms underlying CMD clinical pathology have been identified through selective pathway monitoring, inhibition of pathways and targeted treatment interventions in this animal model. These studies highlight early postnatal surges in inflammation, in part mediated by toll-like receptors (TLR). This inflammation is partially inhibited through anti-apoptotic strategies, including muscle- specific BCL2 overexpression or systemic BAX-KO and PUMA inhibition, indicating that in *LAMA2*-MD early muscle apoptosis/necrosis and inflammation are tightly linked. Regulation of TLRs or their ligands might provide novel points for intervention in the earliest stages of *LAMA2*-MD disease progression.

3. Myomatrix and signaling, receptors and sarcolemmal membrane

Matrix signaling pathways in dystrophy include developmentally and chronically altered matrix-muscle interactions in skeletal muscle and in some subtypes, in the eye and the central nervous system. From a translational perspective, disease rescue becomes unattainable without targeting both the primary deficiency and the consequently dysregulated matrix. Exacerbation of skeletal muscle pathology following LARGE overexpression in an FKRP knock down murine model highlights the challenges in glycosylation up-regulation without tissue, developmental and disease specific considerations. Furthermore, a review of primary and downstream targets in the $dy^{w/w}$ animal model and their relative efficacy in improving dystrophic pathology and survival, underscores the greater efficacy of addressing primary targets. A lack of total rescue, however, emphasizes the need for combinatorial agents to maximize the therapeutic efficacy of approaches such as possible primary protein replacement therapy with Laminin-111. As an example, glucocorticoids, a mainstay of treatment in Duchenne muscular dystrophy (DMD) with anecdotal efficacy in αDG-MD, may have effects on multiple pathways, possibly including integrin signaling, and may qualify as such a combinatorial agent by conferring a significant, yet transient ameliorating effect on disease progression.

The α7β1 integrin emerges as a key mediator of myomatrix signaling to muscle. The degree of ECM linkage to muscle membrane mediated by laminin isoform interactions with cell surface receptors has implications for the design of therapeutic compounds that display a characteristic affinity profile between remaining laminin isoforms, the integrins, including α7β1 integrin and the utrophin-glycoprotein complex. The novel integrin effector protein, Bit-1, which regulates Bcl-2 is downstream of α7β1 integrin activation and *Bit-1*KO mice exhibit myopathic changes. These findings highlight the role of integrin-mediated signaling in maintaining muscle cell myogenic potential.

Disturbed signaling also affects other intracellular pathways involved in cellular homeostasis such as the proteasome and autophagy. Both increased proteasome activity and autophagy in

the dy$^{3k/3k}$ mouse model can be selectively inhibited demonstrating improvement in fibrosis, apoptosis, muscle histology and survival. Evaluating the role of combinatorial therapy targeting both autophagy and proteasome inhibition, as well as probing *Bit-1* as a potential therapeutic are additional translational priorities but unlikely to provide primary rescue. Another structure potentially affected in CMD is the neuromuscular junction (NMJ) where the agrin receptor complex LRP4/MuSK along with αDG may provide important interactions with the matrix. A re-evaluation of the NMJ in dystrophic pathology as a possibly important player and target seems appropriate.

4. Myomatrix and cell regeneration, inflammation and fibrosis in muscular dystrophy

Regeneration of muscle is a complex process based on the myogenic potential of resident cell populations, which in turn is influenced by inflammation, fibrosis, angiogenesis, and innervation. The involvement of notch and NF-κB signaling pathways provides an opportunity to therapeutically influence these processes. In several muscular dystrophies, NF-κB activation proceeds though the specific classical IκB kinase (IKK) signaling cascade. Selective blockade of IKK assembly using the NEMO binding peptide (NBD) shows preliminary efficacy in dy$^{w/w}$ in concordance with similar positive results in the mdx model of DMD. The role of fibrosis in modulating muscle regenerative potential, the crosstalk of inflammation and fibrosis, and whether a primary satellite cell dysfunction is present in CMD remain unanswered. Key priorities for further inquiry include determining (1) whether fibrosis in muscle tissue is reversible and whether there is a stage of irreversibility (point of no return), (2) whether the main drivers of fibrosis are the same in all dystrophic conditions or whether fibrosis initiated by different primary events evokes distinct fibrotic pathways and (3) whether inflammation and fibrosis, a part of muscle's healthy response to injury-induced regeneration are also partially beneficial in chronic injury and thus, what the effects of long-term down-regulation as therapeutic strategies might be.

Another important consequence of an altered ECM is its altered physical properties such as a changed elasticity. Elasticity changes can alter the fate of cellular elements in matrix as well as force transmission via the matrix. 3D matrix modeling can be used to recreate such altered physical properties that may occur in an increasingly stiff dystrophic muscle. Resulting changes in nuclear and cellular elasticity and consequent transcriptional and fate changes in myogenic cells can thus be determined. The dystrophin-glycoprotein complex crucially contributes to lateral force transmission mediated at the costamere. This lateral force transmission accounts for an important component of total force transmission in addition to longitudinal force transmission. Its role and dysfunction in αDG-MD as a mediator of weakness and fatigue warrants further investigation. Developing improved models to evaluate the role of matrix biophysical properties, such as matrix stiffness, may clarify both the influence of the matrix physical properties on force transmission, regeneration and differentiation in various disease states and help define changes in myogenic precursor cell properties (resident or potentially transplanted) required for differentiation and self-renewal in the dystrophic matrix micro-environment.

5. Summary of myomatrix conference

The “myomatrix” refers to the specialized muscle ECM, a compartment in muscle of importance to the CMDs, which are mostly caused by mutations in genes coding for ECM proteins and their receptors, and consequently results in progressive matrix changes. The muscle ECM is also directly involved as a changing disease modifier and driver of muscular dystrophies that are caused by typical sarcolemmal instability with obvious ramifications for therapy development. This conference addressed the myomatrix in the context of normal development and diseased states and highlighted the various roles of the ECM as mediator of signaling, as a major component of the satellite cell niche, modulator of muscle differentiation, and major interactor with both the sarcolemmal membrane and the neuromuscular junction. The conference outlined central themes of a “myomatrix research roadmap” towards translational applications of this research. A key conclusion was that while primary defect correction will deliver a larger effect than downstream therapy targeted at ECM modulation, concomitant treatment of detrimental ECM changes along with amelioration of the primary genetic defect will be required to more fully abrogate dystrophic pathology. Strategies aimed at ameliorating the primary genetic defect include gene replacement, gene repair, and protein replacement. However, the relative efficacy of a late rescue in the presence of advanced dystrophic and matrix pathology remain unknown – i.e. is there a “point of no return” in the development of such secondary pathology. An effective and timely intervention addressing the primary defect, while highly desirable, faces challenges resulting from the onset of CMD pathogenesis already in late fetal development as well as from current diagnostic delays. Thus combinatorial secondary treatment targets will remain highly relevant. Such targets to address along the “myomatrix therapeutic roadmap include: (1) understand the timing and effective management of inflammatory surges and chronic inflammation; (2) enhance repair of damaged sarcolemma; (3) prevent ongoing muscle cell apoptosis and necrosis; (4) identify and modify satellite cell dysfunction to enhance regenerative reserve; (5) fully understand the beneficial and detrimental roles of the muscle interstitial fibroblast population. Delivering clinically effective treatments for the muscular dystrophies will require an improved understanding of pathological triggers of ECM engagement relative to disease subtype and stage, conceptually addressing muscle and matrix as a dynamically interacting unit, the myomatrix.

Acknowledgments

This conference was supported by NIH/NIAMS (R13AR062995) and Muscular Dystrophy Association (MDA219240). Sponsorship for the conference was provided by Genzyme Sanofi, AVIBioPharma, Cellular Dynamics, Cure Congenital Muscular Dystrophy (Cure CMD), LGMD2I Fund, PTC Therapeutics, Santhera Pharmaceuticals and Ultragenyx. The conference organizers wish to thank the University of Nevada, Reno for hosting the Myomatrix conference within the Center for Molecular Medicine and all conference participants. To view the Myomatrix conference program and abstracts: www.curecmd.org/myomatrix.

Abbreviations

αDG-MD	α-dystroglycan related muscular dystrophy
Bax	Bcl-2–associated X protein
Bcl2	B-cell lymphoma 2

CMD	Congenital Muscular Dystrophy
COL6	Collagen 6
ECM	extracellular matrix
FKRP	Fukutin-related protein
IKK	IκB kinase
LAMA2-MD	LAMA2-related muscular dystrophy
MDC1A	Merosin Deficient Congenital Muscular Dystrophy type 1A
MuSK	Muscle Specific Kinase
NBD	NEMO binding protein
NEMO	NF-kappa-B essential modulator
NF-κb	nuclear factor kappa-light-chain-enhancer of activated B cells
PUMA	p53 up-regulated modulator of apoptosis
RYR1	ryanodine receptor 1
SEPN1	Selenoprotein N1
TLR	Toll-like receptors