New Directions in Biology & Disease of Skeletal Muscle 2008

The Westin at Canal Place New Orleans
April 27- April 30, 2008

Meeting Support

Muscular Dystrophy Association
National Institutes of Health
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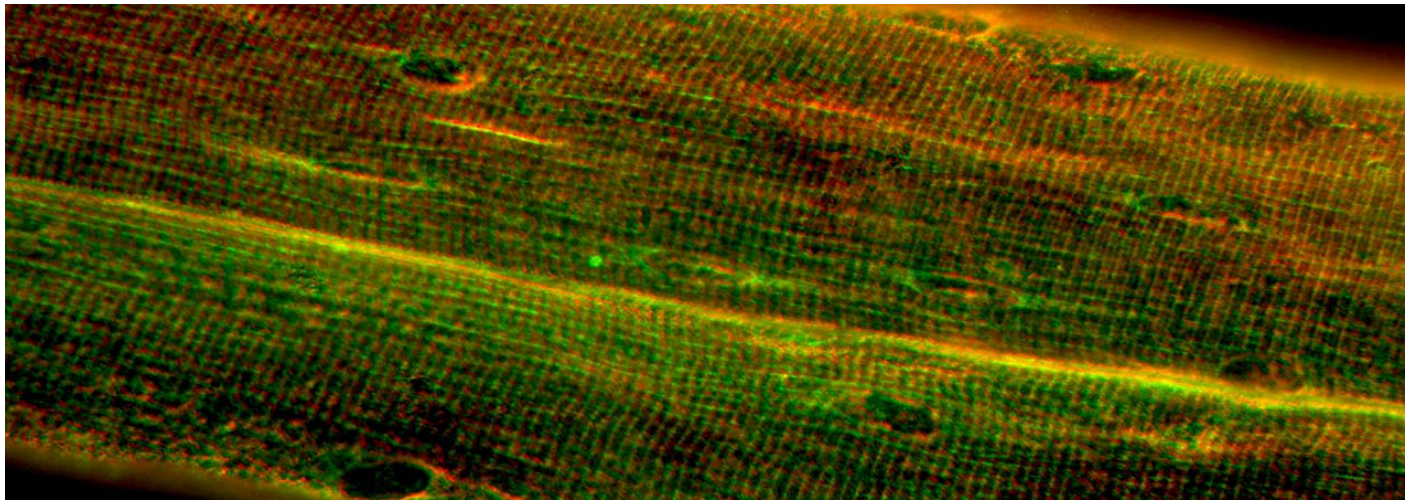

Meeting organizers

H. Lee Sweeney
University of Pennsylvania

Elizabeth McNally
University of Chicago

Richard Lymn
The Lymn Foundation, Bethesda

Program Committee

Rachelle Crosbie
University of California Los Angeles

Carsten Bönnemann
University of Pennsylvania

Stephen Tapscott
Fred Hutchinson Cancer Research Center

Katie Bushby
University of Newcastle upon Tyne

Maurice Swanson
University of Florida

The goal of this meeting is to facilitate research progress with the goal of translation to therapy for muscular diseases. This meaning brings together basic, clinical and translational researchers from distinct venues including academia and industry to address the challenges of understanding muscle disease including the muscular dystrophies and to form collaborations to meet these challenges. The first meeting was held in 2004 in San Diego and the second meeting was held in 2006 in Dallas. The current session will provide the opportunity for new collaborations and relationships to form and will allow young scientists to be exposed to muscle disease biology. This meeting is self-organized and grew from the need to provide a forum for investigators interested in muscle disease and treatment to gather.

MEETING PROGRAM April 27 – 30, 2008 Westin New Orleans at Canal Place

SUNDAY AFTERNOON: REGISTRATION (2 PM - 7 PM)

SUNDAY EVENING SESSION: TARGET IDENTIFICATION 3:00 -6:00 PM

Lee Sweeney	Univ. of Pennsylvania	"Industry Efforts Towards Therapeutic Development in Muscular Dystrophy"
Thomas Meier	Santhera Pharmaceuticals	"Idebenone in DMD for cardiomyopathy and MD"
Ellen Welch	PTC Therapeutics	"Identifying new treatments for neuromuscular disease"
Carl Morris	Wyeth	"Myostatin in preclinical models of muscle disease"
Jon Tinsley	Summit plc	"Compounds for utrophin transcription"
Jeremy Caldwell	Novartis Research Foundation	"High throughput cellular technologies for target and drug discovery"
TBA	Genzyme	"Discovery for muscle disease"

EVENING RECEPTION (7:00 - 9:00 PM)

MONDAY AM: MUSCLE SYSTEMS BIOLOGY (S. TAPSCOTT, CHAIR)

8:30-9:15	Eric Olson	KEYNOTE "MicroRNAs in muscle"
9:15-9:45	Lou Kunkel	Profiling microRNAs in diseased muscle
9:45-10:15	Peter Bram 't Hoen	Computational Modeling of Regulatory Networks in Muscle
10:15-10:45	BREAK	
10:45-11:15	Stephen Tapscott	Regulation of skeletal muscle gene expression
11:15-11:35	*Gopal Patel	miRNA-mediated translational regulation of utrophin-A
11:35-11:55	*Alan Beggs	Selenoprotein N deficiency affects redox state
11:55-1:30	**BREAK FOR LUNCH and SET UP POSTERS**	

MONDAY PM: REPEATS AND CONTRACTIONS IN MUSCULAR DYSTROPHY (M. SWANSON, CHAIR)

1:30-1:55	Maury Swanson	Modeling pathogenic mechanisms for repeat diseases
1:55-2:20	W. Kryzyzosiak	Triplet repeats function
2:20-2:45	Mani Mahadevan	Reversible phenotypes in myotonic dystrophy
2:45-3:10	Tom Cooper	Disrupted developmentally regulated splicing in DM
3:10-3:30	BREAK	
3:30-3:55	Laura Ranum	Multisystemic mouse models of CCUG toxicity
3:55-4:20	Rabi Tawil	Mechanisms of disease in FSHD
4:20-4:40	*Sara Winokur	Transcription in FSHD
4:40-5:00	*Jane Hewitt	Functional studies of the mouse Dux4 homologue
5:00-5:20	*S. van der Maarel	Specific sequence variations in 4qter are associated with FSHD

MONDAY EVENING: POSTER SESSION I (7:00 -10:00 PM) even numbered posters

TUESDAY AM: THE NUCLEUS IN MUSCULAR DYSTROPHY (E. MCNALLY, CHAIR)

8:30-8:50	Elizabeth McNally	Nuclear membrane proteins in heart and muscle disease
8:50-9:20	Nicholas Levy	New insights on the laminopathies: genes to therapy.
9:20-9:50	Bob Goldman	The Nuclear Lamins in Health and Disease
9:50-10:15	BREAK	

10:15-10:45	Jeanne Lawrence	Nuclear organization of gene expression
10:45-11:15	Larry Gerace	Nuclear membrane proteins involved in myogenesis
11:15-11:45	Patrick Dion	Molecular events with expanded PABPN1 toxicity in OPMD
11:45-12:05	*Frederic Magdinier	CTCF couples insulation and perinuclear localization of D4Z4

12:05-1:30 BREAK FOR LUNCH

TUESDAY PM: MODELS AND THERAPY FOR MUSCLE DISEASE (K. BUSHBY, CHAIR)

1:30-2:00	Katie Bushby	Moving to human trials- the TREAT-NMD approach
2:00-2:30	Anna Maria De Luca	Conducting trials in the mdx mouse
2:30-3:00	Patrick Dreyfus	Exon skipping therapy in the dystrophic dog
3:00-3:30	Judith Van Deutekom	Preclinical/clinical development of oligonucleotides for DMD
3:30 to 4:00	BREAK	
4:00-4:30	Tom Rando	Muscle satellite cells in muscular dystrophy
4:30-4:50	*James Dowling	A Zebrafish Model of Myotubular Myopathy
4:50-5:10	*Jane Seto	Altered muscle function in Actn3 knockout mice
5:10-5:30	*Melissa Spencer	Trim32 deleted mouse replicates features of LGMD2H

TUEDAY EVENING: POSTER SESSION II (7:00-10:00 PM) odd numbered posters

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WEDNESDAY AM: MATRIX AND STEM CELLS IN MUSCLE (C. BONNEMANN, CHAIR)

9:00-9:30	Carsten Bönnemann	Collagen VI in myopathy
9:30-10:00	Terry Partridge	Satellite and reserve cells in muscle
10:00-10:30	*Kathryn Mitchell	Myogenic precursors
10:30-10:50	*Justin Fallon	Systemically administered biglycan in MD
10:50-11:10	Jianming Liu	Integrin $\alpha 7\beta 1$ and the DGC
11:10-11:30	**Announcement of Young Investigator Poster Awards**	

11:30-1:30 BREAK FOR LUNCH

WEDNESDAY PM: THE DYSTROPHIN COMPLEX AND THERAPY (R. CROSBIE, CHAIR)

1:30-2:00	Rachelle Crosbie	Sarcospan in muscle disease
2:00-2:30	Eric Hoffman	Profiling/systems biology of muscle disease
2:30-2:50	*Dongsheng Duan	AAV mediated therapy in the dystrophic dog
2:50-3:15	BREAK	
3:15-3:45	Kevin Campbell	Muscular Dystrophy as a Complex Disease: Insights from Mouse Models
3:45-4:15	Jeff Chamberlain	Viral gene therapy for Duchenne MD
4:15-5:00	Lee Sweeney	An update on PTC124: limitations and hurdles of the emerging therapies for MD and Closing Remarks.

BANQUET (7PM-10PM)

Natchez Steamboat cruise. Be at the dock at 7 PM.
Please pay $25 at the registration desk to attend this event.

"NEW AND NOTABLE"

ABSTRACT LIST

40. Extraocular Muscle: Identification of a novel anatomical niche with unique stem cell content (Pacheco-Pinedo)
41. Defective skeletal muscle regeneration in a mouse model for alpha7 integrin congenital myopathy (Burkin, Dean J)
42. Regulation of MMP-2 by CTGF and its relationship with fibrosis (Droppelmann, Cristian A)
43. Transplantation of uncultured mononuclear cells from skeletal muscle retain long term regenerative capacity (Wallace, Gregory Q)
44. Endoplasmic reticulum retention of collagen type VI in Ullrich Congenital Muscular Dystrophy (Allamand, Lainé)
45. Regulation of TGF-beta dependent signaling by decorin and LRP-1 in skeletal muscle cells (Cabello-Verrugio, Claudio)
46. Multipotency of adult human myoendothelial cells demonstrated by single-cell-derived clonal populations (Zheng, Bo)
47. Isolation Of Distinct Myogenic Progenitor Cells From Pax7 Deficient Skeletal Muscle Based On Adhesion Characteristics (Lu, Aiping)
48. Muscle-derived stem cells regenerate skeletal muscle and peripheral nerve, but undergo microenvironment-induced transformation (Lavasani, Mitra)
49. Prospective isolation of skeletal muscle stem cells with a Pax7 reporter (Bosnakovski, Darko)
50. The use of follistatin to improve the transplantation of muscle-derived stem cells (Zhu, Jinhong)
51. Optimization of cell therapy for recessive muscular dystrophies (Cossu, Giulio)
52. Osteopontin And Skeletal Muscle Myoblasts: Expression And Function (Uaesoontrachoon, Kitipong)
53. The homeodomain transcription factor Barx2 regulates satellite cell-mediated muscle growth and repair (Helen Makarenkova)

TARGETS FOR THERAPY (pg. 25)

54. Novel Enzyme Immunoassays for the Detection of Slow and Fast Isoforms of Skeletal Troponin I: Clinical Characterization of Skeletal Muscle Injury Using Troponin I Isoforms (Moussazadeh, Mitra)
55. Novel role for calpain-3 in protein complex regulating calcium release in muscle (Kramerova, Irina)
56. Cardiac Pathology In A Case Of Limb-Girdle Muscular Dystrophy Type 2I (LGMD-2I) (Moore, Steven A)
57. Diagnostic Testing For Congenital Muscular Dystrophies In A Cohort Of USA Patients (Moore, Steven A)
58. Regulation of cell death by the Bax/Ku70 system in normal and laminin-alpha2-deficient muscle (Vishnudas, Vivek K)
59. The Endocytic Recycling Protein EHD2 Interacts with Myoferlin to Regulate Myoblast Fusion (Demonbreun, Alexis)
60. Investigating the role of cytoplasmic gamma-actin in dystrophic muscle (Prins, Kurt W)
61. Functional exchange of sarcomeric alpha-actin by cytoplasmic gamma-actin (Jaeger, Michele A)
62. Blocking TGF-β receptors I and II affect C2C12 myoblast fusion, in a Smad-independent pathway (Rebeca, Droguett)
63. Involvement of Ozz-E3, a muscle-specific ubiquitin ligase, in muscle regeneration (Zanoteli, Edmar)
64. Skeletal muscle cells express and respond to connective tissue growth factor (CTGF/CCN2), a profibrotic cytokine (Vial, Cecilia)
65. Regulatory mechanisms of TGF-beta signaling attenuation during myogenesis. (Brandan, Enrique)
66. Proteomic identification of the LIM domain protein FHL1 as the gene-product mutated in reducing body myopathy (Schessl, Joachim)
67. Calpain 3 Mutations Causing Lgmd2a That Do Not Impair Proteolytic Function, Cause Its Destabilization And Degradation By Other Proteases (Spencer, Melissa J)
68. IGF-I E-peptides are active in vitro and in vivo (Barton, Elisabeth R)
69. Genetic loci that modify muscular dystrophy (Heydemann, Ahlke)
70. A Novel nNOS Signaling Pathway Regulates Skeletal Muscle Size and Strength (Percival, Justin M)
71. Examination Data at Enrollment for 510 Subjects in the United Dystrophinopathy Project (Soltanzadeh, Payam)
72. Morphologic and Genetic Assessment of Patients with Congenital Fiber Type Disproportion and Tropomyosin 3 Mutation (Lawlor, Michael)
73. Dysferlin in T cell apoptosis (Jamieson, Christina A)
74. Cardiac Ankyrin Repeat Protein is a biological marker of atrophy in dystrophic skeletal muscles (Danièle, Nathalie F)
75. A Founder Allele in the DMD Gene is Associated with a Mild Becker Phenotype (Weiss, Robert B)
76. Immunofluorescent quantification of low level expression in the dystrophin protein (Taylor, Laura E)
77. The myogenic transcription factor pax3 is phosphorylated by casein kinase II in vitro (Dietz, Kevin N)
78. Identification of the sites of phosphorylation on the myogenic transcription factor Pax3 (Hollenbach, Andrew D)
79. Identification of Novel Potential Target Genes Involved in Muscle Aging (GRENIER, Guillaume)
80. Collagen VI related myopathies: The Ullrich-Bethlem spectrum of disorders (Bonnemann, Carsten G)
81. Proteolytic cleavage modulates alpha7beta1 integrin function (Liu, Jianming)

THERAPIES FOR MUSCLE DISEASE (pg. 32)

82. Inhibition of smad3 phosphorylation by halofuginone prevents muscle fibrosis and improves muscle performance in muscle dystrophies (Halevy, Orna)
83. Hematopoietic cell transplantation provides an immune tolerant platform for myoblast transplantation in dystrophic dogs (Parker, Maura H)
84. Antagonism of myostatin ameliorates the dystrophic phenotype in mdx mice (Senna Salerno)
85. Antagonism of myostatin as a treatment for muscle wasting conditions (Senna Salerno)
86. Readthrough-inducing ointment: the new approach for the treatment of genetic disorders caused by nonsense mutations (Shioizuka, Masataka)
87. Systemic AAV-9 delivery in normal dog leads to high-level persistent transduction in whole body skeletal muscle (Yue, Yongping)
88. Functional resolution of fibrosis in mdx mouse dystrophic heart and skeletal muscle by halofuginone (Anderson, Judy E)
89. Cardiac specific expression of the ΔH2-R19 mini-dystrophin gene only partially restored heart function in aged mdx mice (Brian, Bostick)
90. Systemically-administered biglycan upregulates utrophin, counters dystrophic pathology and improves muscle function in mdx mice: a novel pharmacological approach for DMD therapy (Amenta, Alison R)
91. Non-invasive monitoring of skeletal muscle therapy protocols by 1H-NMR imaging (Baligand, Céline)
92. Imatinib mesilate (Gleevec®) ameliorates the dystrophic phenotype in exercised mdx mice (Bizario, João)
93. Treatment with a soluble activin receptor type IIb results in increased muscle mass in marmosets (Lachey, Jennifer L)
94. A novel myostatin inhibitor attenuates muscle loss and improves muscle function in response to hypoxia (Pistilli, Emidio E)
95. The six minute walk test as a clinical trial outcome measure in Duchenne muscular dystrophy (DMD): Reliability and correlation with disease progression and clinical timed function testing (McDonald, Craig M)
96. Quantitative characterisation of dystrophic muscle in GRMD dogs by NMR imaging (Thibaud, Jean-Laurent)
97. TAT-Utrophin crosses cell barriers to combat dystrophin deficiency (Sonnemann, Kevin J)
98. Prednisolone and Inactivity Effects on Recovery Following Eccentric Injury in mdx Mice (Baltgalvis, Kristen A)
99. Suppressing DMD frameshift mutations (Anderson, Christine B)
100. Delivery of Mini-dystrophin: A head-to-tale of two recombinant adeno-associated viral constructs (Odom, Guy L)
101. Osteopontin Is A Modulator Of Early Necrotic And Late Fibrotic Processes In Mdx Muscle (Spencer, Melissa J)
102. The Relationship Between Regional Body Composition and Quantitative Strength in Facioscapulohumeral Muscular Dystrophy (FSHD) (Skalsky, Andrew J)
103. Assessment of Regional Body Composition with Dual Energy X-Ray Absorptiometry in Duchenne Muscular Dystrophy: Correlation of Lean Body Mass and Strength (Skalsky, Andrew J)
104. Stable Genome Alteration of the Dystrophin Gene for DMD Due to Frame-Shift Mutations Using Oligonucleotide-Mediated Exon Skipping (Bertoni, Carmen)
105. Leucine increases muscle stress output of MDX mice (Voelker, Kevin A)
106. LMP-420: A Transcriptional Inhibitor of TNF with Therapeutic Potential for Muscle Diseases (Costa, Maria Cristina)
107. Reduced muscle necrosis and long-term benefits in dystrophic mdx mice after cV1q (blockade of TNF) treatment (Radley, Hannah G)
108. siRNA Mediated Allele Specific Silencing of Dominant Negative COL6A3 Mutation Causing UCMD (Zou, Yaqun)
109. Beyond the bench and towards the bedside: Resources designed to facilitate clinical studies of the burdens of muscle disease (Lymn, Ph.D., Richard W)
110. Moving to human trials- the TREAT-NMD approach (Bushby, Katie M)
111. Progression of physical limitations of myotonic dystrophy type 1 (DM1) patients enrolled in the NIH Registry and discussion of endpoint measures in clinical trials (Hilbert, M.S., James E)
112. Optimization of therapeutic antisense-mediated exon skipping for Duchenne muscular dystrophy (Aartsma-Rus, Annemieke)
113. Preclinical Drug Studies In Mdx Mice: A Challenge To Speed Up Pharmacotherapy Of Duchenne Muscular Dystrophy (De Luca, Annanaria)
114. Myostatin blockade in a murine knockout model of limb girdle muscular dystrophy type 2G (LGMD2G) (Markert, Chad D)
115. siRNA Mediated Allele Specific Selective Silencing of a Dominant Negative COL6A3 Mutation Causing UCMD (Zou, Yaqun)
116. Pre-clinical and clinical development of 2OMePS antisense oligonucleotides for Duchenne Muscular Dystrophy (J.C.T. van Deutekom)
117. Muscle function recovery in dystrophic dog after exon skipping gene therapy (Adeline Vulin)

NUCLEAR MEMBRANE (pg. 41)

118. Deletion of lamin A/C lysine 32 is responsible for abnormal muscle maturation associated with proliferation and differentiation defects in mice (Bertrand, Anne T)
119. Lmo7, an emerin-binding transcription activator, regulates C2C12 myoblast differentiation (Holaska, James M)
120. Mice Expressing Mutant Nesprin-1 Display Emery Dreifuss Muscular Dystrophy (Puckelwartz, Megan)

REPEATS AND CONTRACTIONS (pg. 42)

121. Functional studies of the mouse Dux4 homologue (Hewitt, Jane E)
122. CTCF couples insulation and perinuclear localization of the D4Z4 subtelomeric element in FSHD but not in control cells (Magdinier, Frédérique)
123. MBNL3 inhibits muscle differentiation by disrupting MEF2D beta-exon splicing (Wang, Edith H)
124. Specific sequence variations in 4qter are associated with FSHD (van der Maarel, Silvere M)
125. Regulation of TNF mRNA stability by CUGBP1 in muscle cells and myotonic dystrophy (Lee, Jerome E)
126. Looking for the Elusive FSHD-Determining Sequence Proximal to D4Z4 Repeats at 4q35: High-Resolution DNaseI Hypersensitivity Profiling with Tiled Microarrays (Tsumagari, Koji)
127. In vivo study of alternative splicing factors using rAAV mediated gene transfer (Shin, Jihae)
128. Viral-mediated gene transfer to analyze RNA splicing during muscle development (Shin, Jihae)
129. A DNA repeat linked to facioscapulohumeral muscular dystrophy has a high density of potential guanine quadruplexes, Non-B DNA structures (Chen, Desheng)
130. Therapeutic application for a cell culture model of myotonic dystrophy (Koshelev, Misha V)
131. Severe skeletal muscle wasting in a tissue-specific, inducible mouse model for myotonic dystrophy, type 1 (Orengo, James P)
132. High similarity of global muscle splicing defects in two mouse models of DM1 (Du, Hongqing)
133. Nuclear organization of gene expression in relation to skeletal muscle development and diseaes (Lawrence, Jeanne)

BASIC MUSCLE BIOLOGY (pg. 45)

134. Structural basis of familial hypertrophic cardiomyopathy caused by myosin point mutation R403Q (Hanein, Dorit)
135. Subtle skeletal muscle defects in mice that lack alpha7 integrin and utrophin (Wesler, Jennifer V)
136. Altered muscle function in Actn3 knockout mice: mechanistic insights into the association between alpha-actinin-3 deficiency and human athletic performance (Seto, Jane T)
137. Deregulated PKA activity and localization in muscular dystrophy (Naya, Frank J)
138. Ozz-E3 ubiquitin ligase targets sarcomeric thick filaments to regulate the embryonic isoform of myosin heavy chain during skeletal muscle development (Campos, Yvan)
139. Extraocular muscles buffer calcium better than limb muscle: implications for preferential sparing in Duchenne's Muscular Dystrophy (DMD) (Zeiger, Ulrike)
140. Structural and functional evaluation of branched myofibers in young and old mdx mice (Lovering, Richard M)
141. Characterization of the mammalian ferlins in myogenesis (Posey, Avery D)
142. Characterization And Expression Of Alpha-Actinin Family Members During Zebrafish Development (Gupta, Vandana)
143. miRNA-mediated translational regulation of utrophin-A (UtrnA) mRNA by elements in its 5' and 3'-UTRs (Patel, Gopal P)
144. Studies into the contribution of the FKHR (FOX01a) DNA binding domain to Pax3-FKHR DNA binding (Johanson, Kelly E)
145. Novel flanking DNA sequences enhance FKHR (FOX01a) binding affinity but do not alter DNA bending (Sidhu, Alpa)
146. Sarcoplasmic Reticulum Calcium Uptake And Speed Of Relaxation Are Depressed In Nebulin-Free Skeletal Muscle (Ottenheijm, Coen A)
147. Titin Kinase (Tk) Region Differentially Modulates Excitation-Contraction Coupling In Skeletal Vs Cardiac Muscle (Ottenheijm, Coen A)
148. Identification of a novel Z-band associated protein complex (Blanco, Gonzalo)

MUSCLE SYSTEMS BIOLOGY (pg. 49)

149. Rescue of mechanical function, morphology and signaling by desmin plasmid transfection into muscles from desmin knockout mice (Lieber, Richard L)
150. Expression of the muscle glycogen phosphorylase gene in patients with McArdle disease: the role of nonsense-mediated mRNA decay (Nogales-Gadea, Gisela)
151. Altered gene expression profiles in spastic muscle from wrist flexors and extensors in children with cerebral palsy determined from genechip analysis (Smith, Lucas R)
152. Distinctive patterns of microRNA expression in extraocular muscles (EOMs) (Zeiger, Ulrike)

153. Identification of the neuromuscular junction (NMJ) transcriptome of extraocular muscles (EOM) by laser capture microdissection (LCM) (Ketterer, Caroline)
154. Consequences of dysferlin deficiency in human muscle tissue (Kang, Peter B)
155. Duration of untreated juvenile dermatomyositis (JDM) at diagnosis: Impact on muscle biopsy (MBx) gene expression profiles, comparisons with polymyositis (PM) (Pachman, Lauren M)
156. Coupled Bistable Switches Control Cellular Memory and Developmental Plasticity in Human Skeletal Muscle Progenitors (Tse, William T)
157. Gene Expression Profiling of Muscle Samples from Patients Carrying SEPN1 Mutations (Agrawal, Pankaj B)
158. The fallacy of foci: A heterodox view of mutant RNA in myotonic dystrophy (Junghans, Richard P)
159. Distinctive patterns of miRNA expression in human muscular disorders (Iris, Eisenberg-Loebl)
160. A model for efficacy of methylprednisone: re-synchronization of the tissue repair process (Hoffman, Eric P)
161. Microarray expression profiling reveals gene expression associated with the dystrophin-glycoprotein and alpha7beta1 integrin complexes (Liu, Jianming)
162. Computational Modeling of Regulatory Networks in Muscle Differentiation ('t Hoen, Peter A)
163. Alternative splicing and miRNA regulation during myogenesis (Hall, Megan P)
164. miRNAs in diseased muscle (Kunkel, Louis M)
165. Essential role for microRNAs during skeletal muscle development (O'Rourke, Jason R)
166. The transcriptional and post-transcriptional regulation of skeletal muscle gene expression (Tapscott, Stephen J)
167. Elucidating alternative splicing misregulation in myotonic dystrophy type 1 (DM1) (Cline, Melissa S)

ABSTRACTS

MODELS FOR MUSCLE DISEASE

1. A rostrocaudal muscular dystrophy caused by a defect in choline kinase beta

Sher, Roger B (The Jackson Laboratory, Bar Harbor, Maine 04609)
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Muscular dystrophies are a genetically heterogeneous group of disorders that together affect 1:2000 births worldwide. Rostrocaudal muscular dystrophy (rmd) is a new recessive mouse mutation that causes a progressive muscular dystrophy. The rmd mutation is a 1.6-kb intragenic deletion within the choline kinase beta (Chkb) gene. CHKB is one of two mammalian choline kinase (CHK) enzymes that catalyze the phosphorylation of choline to phosphocholine in the biosynthesis of the major membrane phospholipid phosphatidylcholine. While mutant rmd mice show a dramatic decrease of CHK activity in all tissues, the dystrophy is only evident in skeletal muscle tissues in an unusual rostral-to-caudal gradient. Severe membrane disruptions are not evident as determined by creatine kinase levels, Evans Blue infiltration, and unaltered levels of dystrophin-glycoprotein complex proteins. Evidence also indicates that muscle membrane repair is unaffected. The presence of massively enl arged mitochondria in affected muscle tissues suggests that mitochondrial disruption may be a consequence of the mutation. Injection of Danio rario embryos with a morpholino targeted to the zebrafish Chkb homologue results in severely altered muscle development and mitochondrial morphology. The rmd mutant mouse offers a unique model for understanding mechanisms of muscle degeneration and potentially the role of membrane phospholipids in mitochondrial stability.

2. Trim32 knock-out mouse replicates features of LGMD2H

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LGMD2H is a hereditary skeletal muscle disorder caused by mutations in Trim32 (T32). We previously identified T32 to be an E3 ubiquitin ligase that binds to myosin and ubiquitinates actin. To date 4 different T32 mutations in the NHL domains have been linked to LGMD2H, while a 5th mutation in the B-box of T32 causes a completely different disorder called Bardet Biedl Syndrome (BBS11) characterized by obesity, renal abnormalities, and other diverse features. It is not understood how allelic mutations in T32 can create such diverse phenotypic outcomes. To create a tool for elucidating the complex in vivo functions of T32, we generated a T32 knock-out (T32KO) mouse. T32KO animals gain more weight than WT littermates, replicating a BBS feature. Histochemical analysis of T32KO skeletal muscles revealed a dystrophic phenotype. Electron microscopy showed areas lacking mitochondria, disorganized myofilaments, and vacuoles. The rate of fusion and differentiation was reduce d in primary cultures from T32KO muscles likely due to delayed cell cycle withdrawal. These findings confirm that T32 plays an important role in normal skeletal muscle development and confirm that the T32KO is a valid model for the study of the vivo function of T32.

3. Generation of a model mouse for Fukuyama congenital muscular dystrophy carrying a retrotransposal insertion in the 3' UTR in the fukutin gene

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Fukuyama congenital muscular dystrophy (FCMD) is characterized by severe congenital muscular dystrophy with brain anomaly. FCMD is caused by mutations in fukutin and the major mutation is a retrotransposal insertion in the 3' untranslated region. In FCMD, dystroglycan (DG), a cell surface receptor for matrix proteins, is hypoglycosylated and loses the laminin-binding activity. To understand the pathogenesis and develop a therapeutic strategy, we generated a model mouse carrying the retrotransposal insertion. The targeting vector was generated using mutant lox system. Exon 10 of mouse fukutin was substituted by exon 10 of human patient's fukutin with the retrotransposal insertion. Mice homozygous for the mutation show no typical sign of muscular dystrophy. Western blotting analysis shows that the majority of DG species are hypoglycosylated but functionally glycosylated forms are also present. Solid-phase binding assays indicated that ~50% of laminin-binding activity remained in the mutant skeletal muscle. Finally, we show that abnormally glycosylated DG species were restored after adenoviral gene transfer of the fukutin gene. Taken together, these data demonstrate that the presence of only a small population of functionally glycosylated DG is sufficient to prevent disease progression, suggesting a possibility of glycotherapy to a group of congenital muscular dystrophy.

4. Mishandling of aggregate prone proteins in p97/VCP associated inclusion body myopathy

Weihl, Conrad C (Dept. of Neurology, Washington University, St. Louis, MO)
Ju, Jeong-Sun (Dept. of Neurology, Washington University, St. Louis, MO)
Miller, Sara E (Dept. of Neurology, Washington University, St. Louis, MO)

Protein aggregate myopathies are an emerging group of muscle disorders. These myopathies are due to mutations in proteins that make them prone to aggregate or lose their protein folding activity as seen with the myofibrillar myopathy causing proteins desmin and alphaB-crystallin, respectively. We suggest that another mechanism causing abnormal protein aggregation in muscle is due to impaired degradation of misfolded proteins. Mutations in the ubiquitin-proteasome system (UPS) chaperone, p97/VCP cause an autosomal dominant inclusion body myopathy, IBMPFD. Patients and transgenic mice expressing mutant p97/VCP have prominent amyloid and ubiquitin containing inclusions. This increase in ubiquitinated proteins is due to impairment in UPS function that occurs prior to the onset of muscle weakness or overt pathology in these animals. In addition, cells expressing IBMPFD mutant p97/VCP fail to sequester misfolded proteins into large inclusion bodies that are normal ly degraded via autophagy. This results in an increase in toxic aggregate prone proteins. Our studies emphasize the necessary interplay between both the UPS and autophagic systems. We suggest that IBMPFD is due to mishandling of misfolded proteins that accumulate and aggregate with age.

5. RNA CCUG repeats change protein turnover in DM2 patients

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Schneider-Gold, Christiane (Univ. of Gottingen, Gottingen, Germany)
Wang, Guo Li (Baylor Col. of Medicine, Houston, TX 77030)
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Myotonic Dystrophy 2 (DM2) is a multisystemic skeletal muscle disease caused by an expansion of CCTG repeats, transcription of which results in accumulation of un-translated CCUG RNA. DM2 is similar but not identical to Myotonic Dystrophy 1, caused by an expansion of CUG repeats. To address the mechanisms responsible for specific features of DM2, we have searched for specific CCUG-binding proteins. These studies have identified multiprotein complexes as new targets which are affected by expanded RNA CCUG repeats. Purification of these complexes and mass spectroscopy analysis showed that CCUG repeats interact with the 20S proteasome and with the complex containing CUGBP1 and eukaryotic translation factors 2, eIF2. Consistent with biological functions of the 20S proteasome and the CUGBP1-eIF2 complexes, the proteasome-dependent degradation of short-lived proteins and translation of known CUGBP1 targets are altered in DM2 cells. We have found that expression of CCU G repeats in normal myoblasts reproduces DM2-like changes of the protein misregulation. These new data demonstrate that the expanded RNA CCUG repeats target multiprotein complexes, including the 20S proteasome and the CUGBP1-eIF2 complexes. The interaction of the CCUG repeats with these complexes changes protein turnover in DM2 patients. This work is supported by grant AR052791 (NIAMS).

6. Dysregulation of CUGBP1 RNA-binding activity in DM1 myogenesis

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Myotonic Dystrophy 1 (DM1) is a multisystemic muscle disease caused by accumulation of untranslated RNA CUG repeats which increase levels of CUGBP1 protein. The increase of CUGBP1 in mouse models is associated with muscular dystrophy, myotonia, and a delay of muscle development and differentiation, features of the congenital form of DM1. Since CUGBP1 transgenic mouse model is the only model with symptoms of congenital DM1, we have studied the detailed mechanism by which misregulation of CUGBP1 causes a delay of DM1 myogenesis. We report that CUGBP1 specifically binds to many muscle RNAs and that its RNA-binding affinity is regulated by PI3K-Akt and cyclinD-cdk4 pathways through phosphorylation of CUGBP1 at different residues. In DM1 myoblasts, CUGBP1 is activated by Akt leading to high levels of cyclin D1 and to increased proliferation of DM1 cells. In DM1 myotubes, cyclin D3 and cdk4 levels are significantly lower than those in normal myotubes causing a reducti on of CUGBP1 affinity to p21 mRNA and a reduction of p21 synthesis. We have shown that the normalization of cyclin D3-cdk4 in DM1 cells leads to a partial correction of the differentiation of DM1 myocytes through CUGBP1-dependent manner. Identification of signal transduction pathways regulating CUGBP1 activity will help to develop approaches for reversing DM1 symptoms associated with the CUGBP1 increase. This work is supported by grant AR44387 (NIAMS).

7. Altered Expression of Alpha7 Integrin and Aggregation of Sodium Channel SCN8A In Scapuloperoneal Muscular Dystrophy

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Scapuloperoneal muscular dystrophy (SPMD) is a dominant disease that maps to the long arm of human chromosome 12. The genes encoding the alpha7 integrin chain and sodium channel SCN8A are also located in this chromosomal region thus we studied these as candidates for SPMD in two independent families. SPMD skeletal muscle exhibited reduced levels of the alpha7A integrin, an increase in dystrophin, and normal levels of laminin. SCN8A was localized by immunofluorescence to T-tubules in the Z-line region, but in SPMD muscle it forms aggregates containing dystrophin, desmin and actin. These aggregates stain with Congo red, indicating their amyloid-like properties. No mutations were detected in the ITGA7 or SCN8A genes or cDNAs. SPMD muscle cells grew normally in culture but formed protein aggregates under stress. These results suggest that SPMD is a complex disorder affecting the expression, localization and stability of several critical muscle proteins.

8. Preservation of Muscle Force in Mdx3cv Mice Correlates with the Low-level Expression of a Near Full-length Dystrophin Protein

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Complete absence of dystrophin causes Duchenne muscular dystrophy (DMD). Dystrophin restoration at ≥ 20% level reduces muscle pathology and improves muscle force. Levels lower than this are considered therapeutically irrelevant. Interestingly, less than 20% dystrophin expression is seen in some Becker muscular dystrophy (BMD) patients. To understand the role of low-level dystrophin expression, we compared muscle force and pathology in mdx3cv and mdx4cv mice. Dystrophin was eliminated in mdx4cv mice. But mdx3cv mice expressed a near full-length dystrophin protein at ~5% of the normal level. Consistent with previous reports, we found dystrophic skeletal muscle pathology in both strains. Surprisingly, mdx3cv extensor digitorium longus (EDL) muscle showed significantly higher tetanic force and it was also more resistant to eccentric contraction-induced injury. Furthermore, mdx3cv forelimb grip force was stronger. Immunostaining revealed utrophin up-regulat ion and detectable dystrophin-associated glycoprotein complex assembly on the sarcolemma in both strains. Our results suggest that a sub-therapeutic level expression of a near full-length membrane-bound dystrophin may have contributed to muscle force preservation in mdx3cv mice. This finding may help to explain the benign clinical phenotype in some BMD patients. (Supported by NIH and MDA).

9. Destabilization of the dystrophin-glycoprotein complex without functional deficits in alpha-dystrobrevin null muscle

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Alpha-dystrobrevin is a component of the dystrophin-glycoprotein complex (DGC) and is thought to have both structural and signaling roles in skeletal muscle. Mice deficient for alpha-dystrobrevin (adbn-/-) exhibit extensive myofiber degeneration and neuromuscular junction abnormalities. However, the biochemical stability of the DGC and the functional performance of adbn-/- muscle have not been characterized. Here we show that the biochemical association between dystrophin and beta-dystroglycan is compromised in adbn-/- skeletal muscle, suggesting that alpha-dystrobrevin plays a structural role in stabilizing the DGC. However, despite muscle cell death and DGC destabilization, costamere organization and physiological performance is normal in adbn-/- skeletal muscle. Our results demonstrate that myofiber degeneration alone does not cause functional deficits and suggests that more complex pathological factors contribute to the development of muscle weakness in muscular dystrophy.

10. Selenoprotein N deficiency alters redox state and is associated with increased ryanodine receptor channel activity in myogenic cells

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SEPN1 (selenoprotein N) and RYR1 (ryanodine receptor, RyR1) gene mutations both cause multiminicore disease (MmD), a rare congenital myopathy. Selenoprotein N is a ubiquitously expressed protein of unknown function, residing in the membrane of the endoplasmic reticulum. We studied murine myogenic C2C12 cells in which we knocked down (KD) the expression of Sepn1 by RNA interference. Since most selenoproteins are enzymes involved in redox reactions, we investigated the redox state in Sepn1-KD cells. We observed increased levels of oxidized glutathione, reflecting a more oxidizing environment. Activities of both glutathione and thioredoxin reductases were increased, while glutathione peroxidase levels were unchanged. Higher levels of oxidation affect the functions of many proteins including the sarcoplasmic reticulum (SR) calcium channel, RyR1, which has been shown to be redox-sensitive. In preliminary experiments, we observed a 3-fold increase in the affinity of RyR 1 for ryanodine in Sepn1-KD cells, reflecting a more active channel, possibly resulting in chronic calcium leakage from the SR. Further studies to confirm this finding and to determine if these changes reflect altered redox state of the channels are underway. These results suggest a common pathogenic mechanism for both SEPN1 and RYR1 mutations whereby alterations in RyR1 channel activity lead to weakness due to abnormal muscle excitation/contraction coupling.

11. Genetic Deletion of trkB.T1 Results in Enhanced Neuromuscular Performance

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Neurotrophins modulate plasticity, differentiation and survival of motor neurons and muscle fibers. Thus they represent an attractive candidate for therapeutic modulation within the neuromuscular axis. The neurotrophin ligands BDNF and NT-4/5 exert their effects via the cell surface receptor tropomyosin-related kinase B (trkB) of which there are several alternatively-spliced isoforms including a full-length, catalytically active receptor (trkB.FL) and a truncated isoform trkB.T1. TrkB.T1 is expressed in motor neurons and muscle fibers, yet little is known about the role of this receptor isoform in neuromuscular function. In our recently generated trkB.T1 null we have found a significant enhancement of neuromuscular function (increased grip strength, voluntary running, and nerve-evoked contractility) with mild alterations in NMJ morphology. Examination of ex vivo muscle function revealed a significant increase in muscle specific contractility in the trkB.T1 null i n the absence of fiber type alterations. TrkB.T1 null myofibers did however exhibit significant increase in electrically evoked calcium release. We conclude that genetic deletion of trkB.T1 results in increased neuromuscular performance with novel postsynaptic, muscle-specific contributions. This gain-of-function phenotype is possibly due to removal of dominant negative inhibition of trkB.FL signaling. (C. W. W. (AR002177, AR053318), S. G. D. (NR009672), R. M. W. (NS047777),R. B.-G. (NS046490),intramural NCI Center for Cancer Research to L. T.)

12. Respiratory phenotypes in the mdx mouse model of Duchenne's Muscular Dystrophy (DMD)

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Respiratory failure contributes significantly to the morbidity and mortality of neuromuscular diseases and is the commonest cause of mortality in DMD. Despite its critical importance, basic respiratory physiology and effect of hypoxia on muscle is poorly studied in humans and the mdx mouse. To evaluate mdx respiration, we custom-built a plethysmograph (with 1 microlitre sensitivity) and exposed awake adult mdx and control mice to graded levels of hypoxia and hypercapnia to determine RR, VT, and VE, of HVR and HCVR. Additionally, measurements were made by intratracheal intubation and phrenic nerve discharges recorded using electrodes from anesthetized mice to understand the integrated responses of central and peripheral chemoreceptors. Significant abnormalities were found in mdx mice respiratory parameters during normoxia as well as a blunting of the reflexes. Additionally, arterial and venous blood Po2, Pco2, pH and HCO3 measured from adult mdx mice revealed abnormalities in blood gases. These observations support our hypotheses that mdx dystrophic mice have respiratory abnormalities and that hypoxia itself may contribute, as a "second-hit', to muscle patho-physiology in DMD. Funding: MDA, WADA

13. Muscle physiology, membrane structure, and susceptibility to injury in animals lacking keratin 19 and/or desmin

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Intermediate filaments, composed of desmin and of keratins, play important roles in linking contractile elements to each other and to the sarcolemma in striated muscle. We reported that the tibialis anterior muscles (TAs) of mice lacking keratin 19 (K19) generate lower specific tension than controls. TAs from K19 mice also lost costameres and accumulated mitochondria below the sarcolemma. Here we compare the function and morphology of fast-twitch skeletal muscle from normal mice, and mice lacking K19, desmin, or both proteins (DKO). The TA was isolated in situ to measure contractile characteristics (i.e., twitch, tetany, and fatigue). Dorsiflexor torque was measured before and at several time points after animals sustained an injury induced by several high strain lengthening contractions. We evaluated membrane damage by measuring serum CK and counting Evans blue dye labeled fibers, assessed structural changes with confocal and electron microscopy, and determ ined functional changes on a treadmill. The specific tension was reduced in K19 null, desmin null, and DKO mice, compared to controls (reduced 16%, 21%, and 38%, respectively, $P<0.05$), but only the DKO was more susceptible to injury, measured by loss of torque (48% in DKO compared to 39% in normal mice post injury) and increased membrane damage. Recovery was also delayed in the DKO mice, as all other groups recovered 80% torque by day 3, while the DKO recovered only 66% torque. The tension and torque findings were paralleled by changes in overall exercise tolerance, which diminished significantly in the DKO. Our results suggest that knock-out of K19 or desmin in fast-twitch skeletal muscle disrupts the organization of muscle fibers and compromises contractile force and recovery from injury, especially when both are missing.

14. Real-time interstitial oxygen content measurements reveals novel mechanism for degeneration of skeletal muscles in dystrophin-deficient mdx mice.

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Although the primary cause of dystrophy is the lack of dystrophin, the mechanisms by which dystrophin-deficiency causes progressive muscle damage and respiratory-cardiac failure remains obscure. Several theories exist to explain degeneration of dystrophin-deficient muscles including; disorder of the microcirculation and vascular impairment, ischemia, impaired mitochondrial function and oxidative stress.
We have determined interstitial oxygen pressure by a non-invasive, real-time oxygen-dependent quenching of phosphorescence method. Surprisingly, at rest the oxygen pressure in the interstitial space of mdx muscle is substantially higher than normal muscle. Further, mdx mice did not show as much hypoxic- and exercise-dependent oxygen depletion as occurs in normal mice, suggesting decreased oxygen uptake and/ or vascular function in mdx muscle. We hypothesize that later stages of the dystrophic phenotype is characterized by the loss of vascular barrier integrity/ function accompanied by increased oxidative stress due to decreased oxygen utilization in mdx muscle.
Funding: MDA and NIH.

15. Rescue of the human alpha-sarcoglycan R77C recurrent mutation

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Limb girdle muscular dystrophy type 2D (LGMD2D, OMIM600119) is a genetic progressive myopathy that is caused by mutations in the human alpha-sarcoglycan gene (SGCA). We have introduced in mice the most prevalent LGMD2D mutation, R77C, and we observed an absence of LGMD2D-like phenotype at histological or physiological level. Using a heterologous cellular model of the sarcoglycan complex formation, we showed that the R77C allele encodes a protein that fails to be delivered to its proper cellular localization in the plasma membrane, consequently to the disappearance of a positively charged residue. Subsequently, we were able to pharmacologically rescue the R77C protein from endoplasmic reticulum-retention by blocking the protein quality control at the level of the proteasomal degradation or endoplasmic reticulum-routing.

16. Antioxidant supplementation enhances muscle recovery from contusion injury in a rat model

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Grape seed extract (GSE) contains free radical scavenging agents, proanthocyanidins, which inhibit oxidative tissue damage better than vitamins C and E or β-carotene in mice. We investigated the effect of Oxiprovin™, a GSE supplement, on skeletal muscle recovery following contusion injury in rats. 40 Wistar rats were divided into 2 groups, orally gavaged with either Oxiprovin™ (Oxi, 20 mg/kg/d) or vehicle (P, saline 1 ml/kg/d) starting 2 weeks prior to injury. Control animals (C, n=8) were sham-prepared. The remaining animals (Inj, n=32) were anaesthetised and injured non-invasively on one gastrocnemius using the drop-mass technique (200g from 50cm). Muscle was harvested at 4 hr, 3, 7 and 14 days later, fixed and embedded in paraffin wax. Sections were stained using 3 satellite cell (SC) markers (including CD34, CD56 and m-cad) and foetal myosin heavy chain (MHCf) (marking regenerating fibers). Oxi-Inj had significantly more CD56+ SC in the border zone soo n after injury (Oxi-C: 0.025 ± 0.006 vs Oxi-Inj 4 hr post: 0.233 ± 0.035 SC/myofiber, $p<0.001$). Increases were not seen in P-Inj until day 3 (0.101 ± 0.006 SC/myofiber, $p<0.001$). MHCf positive fibres were significantly higher in Oxi-Inj by day 3 (vs Oxi-C or P-Inj day 3, $p<0.001$), but not until day 7 in P-Inj ($p<0.001$). Chronic Oxiprovin™ supplementation significantly increased satellite cell activation and mobilisation to the injury site early after injury and reduced the time to myofiber regeneration.

17. Characterization of a tet-repressible muscle-specific Pitx1 transgenic mouse as a model of FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disorder linked to contractions of the D4Z4 repeat array in the subtelomeric region of chromosome 4q35. We recently showed that paired-like homeodomain transcription factor 1 (PITX1) was specifically up-regulated in patients with FSHD comparing to 11 other neuromuscular disorders by expression profiling. In addition, the double homeobox 4 (DUX4) gene located within the D4Z4 repeat unit could directly regulate PITX1 expression. In this study, we hypothesized that up-regulation of Pitx1 in muscles activated molecular pathways involved in muscle atrophy. Tet-repressible muscle-specific Pitx1 transgenic mice were generated by crossing Pitx1 transgenic mice (TRE-Pitx1) with transgenic mice expressing tetracycline activator driven by mouse creatine kinase promoter (mCK-tTA). The TRE-Pitx1/mCK-tTA mice were kept on doxycycline (200µg/ml) until 3 weeks old. Over-expression of Pitx1 was induced b y withdrawing doxycycline, the mice showed significant weight loss, muscle weakness, reduced muscle mass, reduction of vertical and horizontal movements comparing to their single transgenic siblings. Over-expression of Pitx1 was confirmed by immunohistochemistry in myonuclei of skeletal muscles, while it was not observed in other tissues except few positive nuclei in the heart muscle. Hematoxylin and Eosin staining showed angular atrophic myofibers, necrotic myofibers and mild inflammation infiltration. The results suggest that the gene may play an important role in muscle atrophy and in FSHD.

18. A novel SNaPshot® assay to detect the mdx mutation

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The mdx mouse is an animal model for Duchenne Muscular Dystrophy (DMD). To evaluate possible treatments and carry out genetic studies, it is essential to distinguish between mice that carry the dystrophic (mutant) or wild type (wt) allele(s). The current Amplification-Resistant Mutation System (ARMS) assay requires two separate reactions to determine which allelic variant is present, is labor intensive, and employs the mutagenic agent ethidium bromide. An alternate assay based on single nucleotide primer (SNP) extension technology (i.e., SNaPshot®) is described. The SNaPshot® assay has been optimized to identify both wt and mutant alleles providing a robust, automatable assay for high-throughput analysis. A primer pair designed using Autoprimer (Beckman Coulter, Inc, Brea, CA) was used at concentrations as low as 0.1 µM in a 25 µl sample with a range of DNA concentrations for the initial PCR. The SNP extension primer was diluted to 0.4 µM and the full PC R product was used in the SNaPshot® reaction. The optimized SNaPshot® assay was used to quickly genotype mdx mice without false positives or negatives. This approach is potentially automatable and high-throughput. This research was supported by NIH RO1AR049881 (RWG).

19. Knockdown of myotubularin gene in differentiating C2C12 cells is associated with upregulation of doublecortin, a microtubule associated protein.

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Mutations in the MTM1 gene encoding myotubularin result in X-linked myotubular myopathy (XLMTM), histopathologically characterized by the presence of misplaced nuclei and mitochondria in a higher than expected proportion of myofibers. Following gene expression profiling we found, (i) up-regulation of one of the microtubule-associated proteins (MAPs), doublecortin (Dcx), in well-differentiated C2C12 cell myotubes with knocked down (KD) expression of Mtm1 (using antisense RNA). Real-time quantitative PCR throughout the C2C12 proliferation and differentiation period revealed elevated levels of Dcx mRNA in: (ii) Mtm1 KD cells and, (iii) in cells over-expressing a catalytically inactive Mtm1. Moreover, (iv) doublecortin, previously thought to be expressed exclusively in neurons, was found in desmin-positive human fetal (week 14) myoblasts. In addition, (v) immunostaining revealed that DCX co-localizes with microtubules in myoblasts. Microtubule arrays during myogenesis are completely rearranged from radial centrosomal arrays at myoblasts to noncentrosomal microtubules arranged in parallel to the long axis of multinucleated myotubes. We hypothesize that lack of myotubularin results in compensatory changes in microtubule-associated proteins like doublecortin, which as a regulator of microtubule stability, may have an impact on the distribution of organelles.

20. Titin mutation impairs developmental muscle hypertrophy in mdm mice

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Muscular dystrophy with myositis (mdm) is a recessive mouse mutation with severe and progressive muscular degeneration caused by a deletion in the N2A domain of titin. Mdm mutant mice have small diameter muscle fibers consistent with a delay in muscle maturation and/or an impaired hypertrophy response. In order to identify the onset of the mdm disease, we have compared fiber number, size and fiber-type composition of mdm and wt skeletal muscle at timepoints prior to visible motor impairment. To investigate the hypertrophic response of mdm muscles, we have treated mdm and wt animals with a soluble receptor (RAP-031) that blocks the myostatin pathway. Myostatin is well known as a negative regulator of muscle growth; knockout of the myostatin gene or inactivation of its receptor (ActIIB) results in increased muscle mass. Wild-type animals treated with RAP-031 had increased muscle mass. In contrast, mdm mice showed no significant difference in muscle mass or muscle pe rformance as a result of RAP-031 treatment. In addition, we are creating mdm-myostatin knockout mice in order to analyze the potential role of myostatin during early muscle development. Our data suggests that mdm muscular dystrophy disease mechanism may involve a delay in myofiber maturation and lack of hypertrophic response. This work was supported by NIH grant R01AR049043 from NIAMS to G.A.C., K.A.H. was supported by a development grant from MDA.

21. Quantification of muscle pathology and inflammation during the early disease time course of mdx mice

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Duchenne muscular dystrophy is a genetic disorder characterized by severe muscle wasting and early death in affected boys. The primary cause of this disease is mutations in the dystrophin gene resulting in the loss of the dystrophin protein from muscle fibers. In the absence of dystrophin, muscles undergo massive degeneration and inflammation. Inflammation contributes substantially to dystrophic muscle pathology; however, the time course of this process has not been clearly characterized before and during acute disease onset. In mdx mice age 21 days, 3.5% ($P \leq 0.05$) of the tibialis anterior (TA) muscle cross sectional surface was degenerating; by age 28 days, 30% ($P \leq 0.05$) of the muscle surface was regenerating. Immune cell infiltration peaked at age 28 days and covered 3-4% ($P \leq 0.05$) of the TA muscle cross sectional surface. Flow cytometry revealed skeletal muscle from mdx mice age 35 days contained a higher proportion of CD11b+ F4/80+ macrophages compared to w ild type, but macrophages did not appear to express more chemokine receptor (CCR) 2 on their surface. Although there was no increase in CCR2, expression of monocyte chemoattractant protein-1 (MCP-1) was elevated in mdx TA muscles at age 21 days. Inflammation is a significant feature of dystrophic muscle pathology that appears to be regulated by the interaction of cytokines and immune cells. Supported by NIH RO1AR049881 (RWG).

22. Ringed muscle fibers protect the sarcolemma from contraction-induced injury

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The ability of skeletal muscle to endure injury is essential for the most basic functions of life. Using dystrophin deficient mdx mice expressing a truncated microdystrophin (deltaR4-R23), we show that tears in the Achilles myotendinous junction (MTJ) and tendon led to a complete remodeling of the peripheral myofibrils into rings to protect the muscle membrane from contraction-induced injury. These muscles over-expressed various spectrin based cytoskeletal and focal adhesion proteins and changed the direction of costameres lengthways down the muscle. These muscles were smaller and weaker than wild-type muscles, but retained specific force and did not degenerate. We found that the tears in the MTJ and tendon correlated with the presence of hinge 2 in deltaR4-R23. Switching hinge 2 with hinge 3 led to a highly functional microdystrophin with greater potential to treat DMD using recombinant adeno-associated virus. These results indicate that skeletal muscle end ures certain types of injury by remodeling into ringed fibers.

23. Molecular consequences of “missense” mutation in the Selenocysteine Redefinition Element.

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Mutations in the SEPN1 gene have been implicated in a spectrum of clinically related congenital myopathies, collectively referred to as SEPN1-related myopathies. Selenocysteine incorporation in Selenoprotein N (SelN) occurs through a dedicated decoding pathway which redefines an in frame UGA codon to encode selenocysteine. Efficient insertion during decoding of SelN requires two cis-acting RNA structural elements: the Selenocysteine Insertions Sequence (SECIS) located in the 3’ UTR, and the Selenocysteine Redefinition Element (SRE) located immediately downstream of the UGA codon. Here we report the effect of four point mutations identified in the SRE element on selenocysteine insertion efficiency. Three mutations that maintain base pairing potential by complementary nucleotide changes have no significant effect on UGA-SEC decoding. The missense mutation c.1397G>A, which disrupts the RNA secondary structure of the SRE element, significantly reduces selenocysteine insertion efficiency in vitro and in cultured mammalian cells. In cultured fibroblasts and muscle from patients with this mutation, SelN expression is negligible. This further highlights the importance of the SRE element during normal SelN expression and illustrates a molecular mechanism by which this missense mutation can potentially lead to SEPN1-related myopathy. This work was supported by NIH/NIGMS R01 GM077462 (MTH) and NIH/NINDS R01 NS432644 (KMF).

24. A Zebrafish Model of Myotubular Myopathy

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Myotubular Myopathy is one of the most severe muscle disorders of childhood. It is defined by pathomnemonic muscle biopsy findings that include abundant centralized nuclei surrounded by haloes of disorganized organelles. It results from mutations in myotubularin (MTM1), a dual specificity phosphatase that modifies phosphoinositide residues. In vitro, MTM1 is a critical regulator of membrane trafficking pathways. However, despite the discovery of gene mutations 10 years ago and a wealth of cell culture data, it is still unclear how and why abnormalities in MTM1 result in the development of muscle disease. This has been a significant barrier to the development of treatment modalities. In order to elucidate the pathomechanisms underlying Myotubular Myopathy, we have developed a zebrafish model of the disease. Using a morpholino knockdown strategy, we have generated fish with clinical and pathologic characteristics consistent with those observed in human patients. We are now utilizing this powerful system to test several essential questions about MTM1 function. In particular, we are defining whether MTM1 has phosphatase activity in vivo, and whether such activity is critical for the development of disease. We are also determining what role myotubularin plays in membrane trafficking in the living organism. In all, we feel that by employing our zebrafish model we will make significant advances in the understanding of MTM1 function and dysfunction.

25. Breast cancer has differential effects on skeletal muscle function in the presence and absence of the dystrophin glycoprotein complex

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Breast cancer (BC) induces cachexia (e.g., loss of muscle mass). In BC cells, the dystrophin glycoprotein complex (DGC) is modified. In mdx mice, which lack the DGC in all tissues, breast tumors are not prolific, suggesting regulation of tumor formation and growth may depend on the presence of the DGC. Mdx mice demonstrate increased rather than decreased mass in limb muscles, but stress output is depressed. We therefore tested the idea that the complete absence of the DGC would blunt tumor growth but further depress muscle stress output. Female WT and mdx mice were injected with E0771 murine BC cells subcutaneously (5000 cells/site, 4 sites/mouse). After three weeks, tumor mass was 83% smaller in mdx mice. Compared to WT and mdx non-injected mice, BC decreased extensor digitorum longus (EDL) muscle stress output of WT EDLs by 10-16%, but increased stress output of mdx EDLs by 17-32%. These results suggest that both tumor growth and effects on skeletal muscle function in BC may depend on the presence of the DGC. Funding: NIH RO1AR049881 (RWG), Virginia Tech (YHJ).

26. C. Elegans as a model system for Limb-Girdle muscular dystrophy 2B (LGMD2B)

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Dysferlin gene mutations cause LGMD2B, but the mechanisms underlying pathogenesis are yet to be fully defined. The founding member of the dysferlin gene family C. elegans (C.e.) fer-1, is essential for sperm vesicle membrane fusion but has not been thought to be expressed or have any role in (C.e.) muscle. Here, we present three lines of evidence that fer-1functions in (C.e.) muscle. First, RT-PCR of fer-1 mRNA from FACS sorted (C.e.) muscle cells shows that fer-1 is expressed in muscle. Second, microarray profiling from fer-1 mutants shows enrichment for genes involved in worm locomotion, suggesting that loss of fer-1 alters the expression of genes involved in muscle function. Third, fer-1 mutants have defects in acetylcholine and GABA based neurotransmission. Pharmacological studies indicate that fer-1 functions post-synaptically in muscle to regulate Ach signaling. Our findings create the opportunity to exploit the significant genetic advantages of (C.e.) to better understand molecular patho-physiology, as well as provide a novel platform for drug discovery for LGMD2B. Supported by: Pennsylvania Muscle Institute and NIH Grant T32 AR-053461.

27.In-vivo monitoring of disease progression in mdx mice

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Purpose: To develop MRI as a valid measure of disease progression in mdx mice. Methods: mdx mice (5-48 wks old) underwent MRI (4.7T) of their lower hindlimbs. Muscle volumes of the posterior and anterior compartment muscles were computed from high resolution, 3D T1-weighted, transaxial MR images (21.5x42.9x375 μm3) at 5, 9, 13 and 17 wks of age. At 17wks, hindlimb muscles were excised and wet weights determined. Muscle damage was quantified in the gastroc (GAS) and tibialis anterior (TA) from T2-weighted images. Magnetization transfer (MT) imaging was used to examine fibrosis in both young and old mdx mice and compared to histological sections. Results: An increase in muscle volume was seen using MRI from 5-17wks of age (total volume=0.17 ± 0.02 cm3 (mean±SEM) at 5 wks, 0.28 ± 0.01 at 17 wks; p=0.006). There was a significant correlation between muscle volume and wet weights (r=0.70-0.85). Muscle damage was highest at 8 wks of age in the GAS (16.4±6.1%) and 7 weeks of the age in the TA (13.8±6.3%), decreasing between 9 to 11 weeks (5.8±0.4% and 3.1±0.7%, respectively). There was an age-dependent reduction in MT of young vs. old mdx mice (GAS: 0.79±0.02 vs 0.76±0.03; TA: 0.81±0.02 vs. 0.78±0.004), which corresponded to greater fibrosis. Conclusion: MRI is a non-invasive tool which can be used to track disease progression in mdx mice, including changes in muscle volume, % damage and fibrosis. MRI may be applied in studies examining therapies for muscular dystrophy. Funding: Funding provided by the Muscular Dystrophy Association (MDA4170), the Senator Paul Wellstone Muscular Dystrophy Center Grant (1U54AR052646-01A1).

28. VCP transgenic mice showed muscle weakness and aggregates of endoplasmic reticulum.

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Hereditary dominant inclusion body myopathy with Paget disease and Frontotemporal Dementia (IBMPFD) is caused by mutation of valosin-containing protein (VCP). VCP plays essential roles in many cellular processes including membrane trafficking and protein degradation. We studied the VCP R155P mutation in the original IBMPFD family, and cloned the whole human genomic sequence of VCP gene with and without the R155P mutation into transgenic mice. The transgenes were confirmed by DNA sequencing and southern blot analysis. VCP R155P mice showed muscle weakness compared to wild type VCP mice on Rotarod test. Muscle pathology showed atrophy, denervation and abundant rimmed vacuoles which were mainly present in type I fibers. EM identified the vacuoles as sarcoplasmic reticulum aggregates. Confocal analysis showed VCP accumulation in vacuoles. Vacuoles were also positively stained for Ryanodine Receptor, Inositol 1,4,5-Triphosphate Receptor 1, and calreticulum, verifyi ng the vacuoles are ER-related and the pathogenesis of IBMPFD may be related to the disruption of ER assembly and function. TDP-43 also accumulated in VCP R155P muscles, implicating its role in non-neuronal pathology. Additional insight in the pathogenesis of IBMPFD can be revealed with this VCP transgenic model, which can possibly lead to identification of pathways for intervention.

29. A new view of the roles of SepN and RyRs in muscle disease and development

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Genes that appear to be unrelated have been linked to a number of congenital myopathies with overlapping phenotypes, indicating the genes might function in a common molecular pathway. However, variability in the details of disease presentation (heterogeneity of phenotype) and the prevalence of non-null disease alleles (heterogeneity of genotype) has made it difficult to discern if the disease-associated genes contribute to a common function. Our approach has been to identify the first cellular events perturbed by complete loss of gene function. We have analyzed in the zebrafish embryo the primary cellular and biochemical defects that arise from complete loss-of-function of genes linked to muscle disorders. We find Selenoprotein N (SepN) and Ryanodine Receptor intracellular calcium release channels (RyRs) work together to control intracellular calcium release in a number of developmental contexts. First we demonstrate that SepN regulates RyR function. In humans, complete loss-of-function mutations in SepN1 or missense alleles of the skeletal muscle RyR1 gene cause congenital myopathies involving disruption of myofibril organization. Loss of SepN or RyRs in the zebrafish embryo causes similar muscle defects. Further, the two genes appear to interact functionally in the zebrafish. We show SepN and RyRs are physically associated in vivo and both are required for regulating intracellular calcium levels in the embryo. As complete loss of SepN but not RyR is tolerated in humans, we postulated SepN is a modifier of RyR channel activity. We demonstrate that RyR channel properties are altered in SEPN1-mutant diseased human muscle and SepN-depleted zebrafish embryos. Second we demonstrate that RyR function is required for numerous signal transduction events that regulate cell identity and cell differentiation of muscle and other tissues. RyR function is traditionally associated with regulating excitation-contraction coupling in muscle function and sarcomere assembly in muscle differentiation. Analysis of embryos deficient for either SepN or RyRs uncovered novel biological roles for the intracellular calcium release channels: Loss of RyR channel function also disrupted L/R patterning and the ability of muscle and non-muscle precursor cells to respond to growth factor signaling directing their differentiation. Our work indicates how animal modeling can simplify conceptualization of congenital disorders.

30. Calcium as the central mediator of degeneration in muscular dystrophy

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Muscular dystrophy (MD) is a muscle wasting disease, which typically results in death by the second or third decade of life. Unfortunately, there are no cures for the disease and current therapeutic treatments available only extend lifespan by a few years. Many forms of muscular dystrophy are caused by a mutation in proteins that reside within the dystrophin-glycoprotein complex (DGC). The DGC serves as a structural attachment apparatus that spans the underlying contractile units to the basal lamina outside the cell, ultimately providing support for the cell membrane. Without a completely functional DGC the integrity of the muscle cell membrane is compromised, allowing for contraction-induced membrane tears. Ca2+ entry via tears in the membrane, along with dysregulation of Ca2+ channels, results in an increase in cytosolic Ca2+ levels in dystrophic myotubes. Increased cytosolic Ca2+ can cause degeneration through a number of downstream effectors, including i ts effect on degradative enzymes and cell death proteins. Thus, we hypothesize that Ca2+ is the central mediator of skeletal muscle degeneration in MD. To date this hypothesis has not been rigorously tested in vivo and results of these studies could result in novel therapeutic approaches. To investigate the role of Ca2+ in skeletal muscle degeneration we generated three independent skeletal muscle specific transgenic mice that overexpress membrane calcium/cation channels. One transgenic (total of 3 lines) expresses the sodium/calcium exchanger (NCX1), which typically transports Ca2+ out and Na+ into the cell, although our preliminary studies determined that NCX TG fibers have increased baseline Ca2+ levels and Ca2+ transients. Remarkably, the medium and high expressing lines exhibited skeletal muscle disease as judged by histological analysis (central nuclei, fiber area, fibrosis), while the low expressing line did not display overt disease. Furthermore, the quadriceps muscle of 15 month old NCX TG (high line) mice had a large amount of fat replacement, indicating loss of regenerative capacity. We have crossed the low and high NCX TG with a model of MD, which lacks delta-sarcoglycan (Scgd-/-, a component of the DGC), and, preliminarily, no benefit was observed at 6 weeks or 6 months of age from the low line cross. As another model of altered Ca2+ levels we have also generated mice overexpressing transient receptor potential channel of the canonical subclass (TRPC3, one line). Similarly, these mice also exhibit disease at 2 months of age as muscle weights are reduced compared to NTG and gross histology is abnormal. Compared to the NCX TG mice, TRPC3 TGs have more skeletal muscle disease (3.8% of quadriceps myocytes contain central nuclei in high line of NCX TG and 26.6% in the same muscle of the TRPC3 TGs) possibly because the pathway involving TRPC3 is a true influx pathway. We are currently also crossing the TRPC3 TGs with the δ-sarc oglycan MD model. Finally, we have generated one line of TG mice overexpressing Plasma Membrane Calcium ATPase (PMCA4b). Young animals did not display skeletal muscle disease, however there was some disease at 6 months of age and we are currently crossing these mice with our MD model to test whether MD pathogenesis can be reduced through greater calcium clearance. Overall, our preliminary results indicate altered Ca2+ handling can cause disease without a mutation in the DGC and our future results should allow for analysis of whether enhanced removal of intracellular Ca2+ benefits MD pathogenesis or even enhances it by altering trans-sarcolemmal flux.

31. Validation of a New Immobilization Method for Skeletal Muscle Atrophy Induction in Mice

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Skeletal muscle atrophy is a serious concern for the rehabilitation of patients afflicted with nerve injury or immobilized after surgery. Although it is known that muscle atrophy results from an imbalance in protein degradation and synthesis, the molecular events leading to this condition are poorly understood. We describe a new hind-limb immobilization procedure that induces skeletal muscle atrophy by using a surgical staple to impede one hind leg. This method is simple, cost effective and less invasive than other methods currently used. The tibialis anterior (TA) muscle of mice, in which one hind-limb was immobilized with a surgical staple, was compared to contra-lateral unstapled limbs (control). Results indicated that TA weight and myofiber cross sectional area diminished drastically and rapidly as compared to the control TA. Furthermore, known molecular markers for skeletal muscle atrophy were significantly regulated in the TAs of stapled hind-limbs compared to their control TAs. In conclusion, we validated a new method for studying skeletal muscle atrophy through immobilization. This model will be useful for determining molecular processes and will be beneficial for the development of effective therapies.

32. An experimental model of acute quadriplegic myopathy: Underlying mechanisms and specific intervention strategies

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Acute Quadriplegic Myopathy (AQM) is considered a consequence of modern treatment in anesthesiology and intensive care. AQM was initially regarded as a rare condition, but we now know that neuromuscular dysfunction is found in up to 30% of the general intensive care unit (ICU) population and 70 - 80% of certain sub-groups. This potentially lethal condition prolongs the recovery of critical care patients, thereby, increasing the median ICU treatment costs three-fold and additional substantial costs are associated with the subsequent extended rehabilitation requirements and drastically impaired quality of life. With appreciation of the frequency of acquired myopathies in ICU patient, there has been growing interest in their risk factors and underlying pathophysiological mechanisms. Mechanical ventilation, post-synaptic block och neuromuscular transmission (NMB), muscle unloading, sepsis, circulating active factors and/or steroids have been proposed as triggering f actors. The understanding of basic mechanisms underlying AQM in the clinical setting is poor in part due to the fact the generalized muscle weakness is complicated by the co-existence of more than one factor underlying muscle paralysis in the ICU patients. Different primary diseases, large variability in pharmacological treatment, and collection of muscle samples several weeks after admission to the ICU and exposure to causative agents are other factors complicating mechanistic studies of AQM in the clinical setting. There is, accordingly, compelling need for an experimental animal model mimicking the ICU conditions, including long-term exposure to NMB agents, high-dose systemic corticosteroids, mechanical ventilation and muscle unloading. In an attempt to mimic the ICU condition, we have used a novel experimental rat ICU model in which the animal is paralyzed by NMB, mechanically ventilated and extensively monitored for periods varying from hours to three weeks. This mod el offers a unique possibility to study regulation of myofibrillar protein synthesis and degradation, regulation of muscle contraction, muscle structure, muscle size and the effects of interventions on a large number of muscles over an extended period of time. The model effectively mimics ICU conditions. The ability to follow animals over an extended time is of specific interest in studies on AQM where myofibrillar proteins with very slow turnover appear to be selectively affected. Specific pharmacological and mechanical loading intervention strategies are presently being evaluated in this experimental animal model. Preliminary results demonstrate a significant intervention-induced reduction of the muscle wasting associated with ICU treatment.

33. A new mouse model for 'dystroglycanopathies' associated with mutations in Fukutin Related Protein (FKRP)

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Mutations in Fukutin related protein (FKRP) underlie a form of congenital muscular dystrophy (MDC1C) and limb girdle muscular dystrophy (LGMD2I). In addition allelic mutations of FKRP gene are associated with Walker-Warburg Syndrome (WWS) and Muscle-Eye-Brain disease (MEB). Whilst the precise functional role of FKRP remains unclear, there is a clear correlation between severity of the condition affecting patients and the depletion of glycosylated alpha-dystroglycan (ADG), suggesting a role for FKRP in the post-translational modification of this protein. We have generated a mouse model that displays a significant reduction in FKRP expression which presents a clear phenotype in muscle as well as defects in the eye and brain resembling MEB disease. These animals provide a unique model with which to investigate the pathogenesis associated with severe mutations in FKRP.

34. A C.elegans model of muscular dystrophy

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The nematode C. elegans is the simplest model organism that possesses most components of the dystrophin complex. Mutations in genes encoding components of the complex cause a characteristic locomotory deficit and can cause muscle degenerative phenotype. Previously we established a genetic screen to identify mutants that exhibit the same locomotory deficit as that of dystrophin mutant. So far we have molecularly characterized a few mutations that may represent novel components of the dystrophin complex or interact with the dystrophin complex. We conclude that the dystrophin complex in C. elegans provides a critical link to signaling molecules. These signaling molecules are responsible for preventing muscle overexcitation (thus calcium overload) by exerting an inhibitory role in muscle excitation. Currently we are trying to identify additional molecular components and to understand possible biological significance of these molecular components. Additionally, study on the detailed degenerative mechanism is under way using genetic approaches such as genome wide RNAi analysis. This C. elegans genetic study will provide us a unique opportunity to identify novel genes responsible for neuromuscular diseases, to understand the molecular function of the identified genes, and to help to explore new therapeutics and diagnostics. Funded by NIH (H.K.) and MDA (S.M.).

35. VCP disease: Inclusion body & vacuolar myopathy, Paget and Frontotemporal dementia

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Mutations in p97/VCP cause inclusion body myopathy (IBM) with Paget's disease (PDB) and frontotemporal dementia (FTD)(IBMPFD). VCP is an AAA-ATPase gene associated with a variety of cellular activities, including cell cycle control, membrane fusion and ubiquitin (Ub)-proteasome mediated Endoplasmic Reticulum-Associated Degradation (ERAD) pathway. Most of the mutations are located in the ubiquitin binding domain and potentially interact with each other (p.Arg155-p.Asn387, p.Arg159-p.Ala232 and p.Arg191-p.Leu198), suggesting that these residues may have a similar and specific function within the VCP homohexamer. The phenotype has been expanded based on findings in affected individuals from 27 families from North and South America and Europe. Systematic immunohistochemical examination of 10 patient muscle biopsies (4 families; 2 different mutations) revealed that 6 had marked evidence of rimmed vacuoles both sarcoplasmic and subsarcolemmal while 2 had none. The inclusions seen in 7 patients were sarcoplasmic and myonuclear in IBMPFD muscle fibers and present in vacuolated and non-vacuolated fibers. Electron microscopy demonstrated disordered myofibrils, vacuolation, and tubulofilamentous inclusions within myonuclei and myofibers. PDB frequently diagnosed because of elevated alkaline phosphatase typically involves the spine, hips, scapulae and skull in >50% of affected individuals is successfully treated with bisphosphonates. Electron microscopy of PDB osteoclasts identifies characteristic nuclear and cytoplasmic paired helical filaments (PHF) inclusions ~15 nm diameter also seen in the nuclear and cytoplasmic inclusions of muscle. FTD diagnosed at a mean age of 55 y. is characterized by dysnomia, dyscalculia, comprehension deficits, relative preservation of memory, and in later stages by inability to speak, auditory comprehension deficits for even one-step commands, alexia, and agraphia. A systematic analysis of the neuropathologic changes in eight persons with IBMPFD and VCP mutations revealed Ub-positive neuronal intranuclear inclusions, dystrophic neuritis, and rare intracytoplasmic inclusions. The Ub pathology was abundant in the neocortex, less robust in limbic and subcortical nuclei, and absent in the dentate gyrus. Only rare inclusions were detected with antibodies to VCP. Analysis of the APOE e4 genotype status of 231 members of 15 families suggests a link with the incidence of FTD. Our cell and animal models will characterize the common molecular pathways and help develop treatments for this multifaceted disorder.

MATRIX AND STEM CELLS

36. PW1 interstitial cells (PICs): a new source of myogenic progenitors

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Non-satellite cells possess myogenic potential and can participate in muscle regeneration, however, their precise position, origin and relationship to satellite cells remain unclear. During postnatal growth, PW1/Peg3, a gene co-expressed with Pax7 in satellite cells, is also expressed in a sub-population of muscle resident interstitial cells, termed PICs (PW1 interstitial cells). In primary cultures, we readily obtain PW1+/Pax7+/MyoD+ cells (myoblasts) but fail to obtain PW1+/Pax7- cells (PICs). We therefore sought to isolate PICs by FACS using stem cell markers. We obtain a population of Sca+/CD34+ cells that is highly enriched for PICs, distinct from the Sca1-/CD34+ satellite cells. In culture, PICs predominantly acquire a smooth muscle identity but do show a low spontaneous skeletal myogenic conversion. When PICs are co-cultured with satellite cells, they become PW1+/Pax7+/MyoD+, indistinguishable from satellite cells. In the Pax7 mutant, we note a marked increase in the PIC population. In culture, purified Pax7 mutant satellite cells show pronounced myogenic capacity. In contrast, Pax7 mutant PICs cannot spontaneously convert to the myogenic lineage, suggesting that Pax7 is required to recruit PICs to the satellite cell pool.

37. Functional skeletal muscle regeneration from differentiating embryonic stem cells

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Little progress has been made towards the use of embryonic stem cells (ES) to study and isolate skeletal muscle progenitors. This is in part due to the rarity of skeletal muscle precursors within the EB, as well as the lack of reliable identification and isolation criteria. We have developed an ES cell line in which expression of Pax3, the master regulator of the embryonic myogenic program, can be induced by doxycycline (dox). Our results show that expression of Pax3 during EB differentiation enhances paraxial mesoderm, and cells with myogenic potential within this population. However, transplantation of Pax3-induced cells lead to teratoma formation, indicating the presence of residual undifferentiated cells. By sorting for PDGFalphaR, a paraxial mesoderm marker, and absence of Flk-1, a lateral plate mesoderm marker, we show that early muscle precursors with significant potential for muscle regeneration can be isolated from differentiating ES cell cultures. Purifi ed cells demonstrated significant potential for muscle regeneration as observed upon transplantation into immuno-deficient mice. Intramuscular and systemic transplantation of these cells into dystrophic mice results in extensive engraftment of adult myofibers with enhanced contractile function. These data demonstrate the therapeutic potential of ES cells in muscular dystrophy.

38. Pw1: a potential marker of multiple stem cell populations

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Pw1/Peg3 is a maternally imprinted gene, expressed during skeletal myogenesis. Pw1 mediates cell stress (p53/TNF) and pro-survival (NFkB) pathways in vitro, as well as muscle atrophy and stem cell number in vivo. To characterize further the role of Pw1, we generated reporter mice carrying lacZ or GFP in the Pw1 locus. As expected, we observe broad reporter expression during embryogenesis. In postnatal mice, we detect reporter expression in muscle stem cells as well as strong expression in epidermal hair follicle bulge cells revealing that Pw1 expression defines resident stem cell populations in at least two different lineages. To define the function of Pw1 in skin stem cells, we performed a depilation-injury in PW1-lacZ mice. During hair follicle regeneration, b-galactosidase positive cells begin to proliferate, followed by expression in the new hair shaft as well as in the developing inner and outer root sheath during the growing phase, suggesting that Pw1 is inv olved in the de novo formation of the hair follicle. We are presently addressing if Pw1 is functionally involved in the regenerative potential of these stem cells as well as the potential of purified skin stem cells to participate in non skin lineages.

39. “Myospheres” can be used to maintain and isolate primitive muscle cells

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Our understanding of stem and progenitor cells has greatly expanded in recent years and the potential for their being used for clinical applications has become more apparent. However there are still many unanswered questions about how to best isolate and expand these very important cells. Here we take a cue from the isolation of neural stem cells, which can be grown in culture as neurospheres, and have isolated similar structures from adult skeletal muscle. In this study we were able to generate free-floating muscle-derived aggregates, which we refer to as “myospheres”, using the same techniques used in the formation of neurospheres. As neurospheres can be used to maintain neural stem cells in culture, we propose that the formation of myospheres can be used in a similar manner to maintain and expand primitive muscle-derived cells. Myosphere cultures were generated from the hindlimbs of adult (6-8 weeks) C57BL/6 mice, which were minced, dissociated using dispase/collagenase, and then triturated through progressively smaller fire polished glass pipettes to disrupt cells larger than 10uM. The dissociated muscle was plated in neural stem cell media (DMEM:F12 with B27 supplement, no serum) supplemented with 20ng/ml bFGF, 20ng/ml EGF, and 2ug/ml heparin. To monitor the growth of these myospheres we transduced the initial cultures with a lentiviral vector that expressed yellow fluorescent protein (YFP). This was done because lentiviral vectors integrate directly into the genome allowing us to monitor sphere growth through YFP expression of the initially transduced cells as well as in their progeny. Free-floating myospheres were observed within 15 days of the initial isolation (sizes ranged 50-100uM), muscle-derived spheres were maintained in culture for at least three months. Immuno-fluorescence studies o f the myospheres showed that they were Sca 1 positive (stem cell marker) but CD 29 (B1 integrin, neurosphere marker) and CD 31 (endothelial marker) negative. After two months in culture myospheres derived from adult hindlimb muscle were dissociated by trypsin and then these cells were plated using the preplating technique of Qu-Petersen, et al. (J Cell Biol 2002) in myoblast media (F10 media with 20%FCS) supplemented with 5.0ng/ml bFGF. Two weeks after plating, colonies of small rounded myoblast-like cells formed and could be passaged as adherent cells (similar to primary myoblast). Immunofluorescence staining of these cells showed that they were desmin +, Myo D+, Sca 1 + (4-26%), CD31-, and CD34-. To show that the myosphere cells could function in a similar manner as primary myoblasts, these cells were plated on matrigel in DMEM containing 2%HS and allowed to differentiate into myotubes. Five days after plating these cells fused forming multinucleated tubes. Here we d emonstrate a new technique that can be used to isolate

and maintain primitive muscle cells in culture. Future plans include to further investigate these myosphere-derived cells, it is expected that like their neurosphere counterparts, they will also be able to form multi-lineages, and thus myosphere-derived cells could serve as a possible source of stem cells with the potential to repair multiple components of injured muscle. This research was supported by a grant from NIH-NIAMS, Grant Number 1 KO1 AR052372-01A2.

40. Extraocular Muscle: Identification of a novel anatomical niche with unique stem cell content

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The extraocular muscles (EOMs) are a distinct muscle allotype, enigmatically spared in Duchenne muscular dystrophy (DMD). We hypothesized that increased and distinct stem cells may underlie the sparing. Both, the committed satellite cells (SCs) and uncommitted side population (SP) stem cells were isolated and studied. EOMs had a 3x higher SC and 15x higher SP cell content compared to limb muscles. Expression profiling revealed 348 transcripts that define the EOM-SP transcriptome. Over 92% of transcripts were SP-specific, as they were absent in previous whole-muscle microarray studies. Cultured SC and EOM-SP cells revealed superior in vitro proliferative and myogenic capacity, respectively. We suggest a model wherein an unique EOM stem cell niche underlies continual myogenesis. We believe the greater numbers of stem cells and the greater inherent capacity for efficient myogenesis of EOM stem cells contributes to their clinical sparing in DMD and offers clues for no vel cell-based therapeutic strategies.

41. Defective skeletal muscle regeneration in a mouse model for alpha7 integrin congenital myopathy

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Mutations in the alpha7 integrin gene cause congenital myopathy characterized by delayed developmental milestones and impaired mobility. Loss of the alpha7 integrin in dystrophin-deficient mice results in severe muscle pathology and premature death. Previous studies in dystrophic mice suggest the alpha7beta1 integrin may be critical for muscle repair. To investigate the role alpha7beta1 integrin plays in muscle regeneration, cardiotoxin was used to induce muscle damage in alpha7 integrin null mice. Unlike wild-type muscle, which responded rapidly to repair damaged myofibers, alpha7 integrin deficient muscle exhibited defective regeneration. Muscle damage in alpha7 integrin null muscle resulted in increased membrane fragility, fewer centrally located nuclei and hypotrophic muscle fibers. Analysis of BrdU incorporation and Pax7 and MyoD expression revealed a profound delay in myogenic repair cell activation and proliferation in alpha7 integrin null animals. Our data demonstrate a crucial role for the alpha7beta1 integrin in satellite cell activation and myoblast differentiation during muscle regeneration.

42. Regulation of MMP-2 by CTGF and its relationship with fibrosis

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In order to understand the fibrotic process observed in muscular pathologies like Duchenne Muscular Dystrophy (DMD) we analyzed the participation of Connective Tissue Growth Factor (CTGF), a profibrotic factor overexpressed in fibrotic wounds which induce connective tissue synthesis, and metalloproteinase type 2 (MMP-2 or gelatinase A), an important extracellular matrix remodeling enzyme. We analyzed the expression of MMP-2 in 3T3 fibroblasts in response to rCTGF using relative quantitative PCR and zymography analyses. We observed that CTGF regulates the MMP-2 expression and gelatinase activity in 3T3 fibroblasts in conditioned medium. We evaluated through western blot the presence of fibronectin (FN), a marker of fibrosis, in response to rCTGF in presence of metalloproteinase inhibitor GM-6001 and in a stable kock-down cell line for MMP-2. In the GM-6001 treated and knock-down cells an increase in the amount of FN relative to control was viewed. Moreover, in thes e cells we observed a decrease in amount of FN in response to CTGF. This behavior is analogous to the observed in fibroblasts of mdx dystrophic mice. These results suggest that regulation of the expression and activity of MMP-2 can play an important role in the fibrosis genesis. Fondap-CRCP, MIFAB, MDA 3790 and Fondecyt 306091.

43. Transplantation of uncultured mononuclear cells from skeletal muscle retain long term regenerative capacity

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Satellite cells are muscle repair cells that are activated after damage or disease to regenerate muscle fibers. Thus, isolating and transplanting these cells is an attractive strategy for treating muscle diseases, but have been limited by their decreased regenerative capacity when exposed to culture conditions. Additional challenges for stem cell-based therapies include maintaining long-term survival of transplanted cells and avoiding immune responses. We have isolated adult muscle mononuclear cells (AMMCs) from normal, strain-matched adult mice and injected them directly into muscles of immunocompetent, delta-sarcoglycan null mice. AMMCs were 35 times more efficient at restoring sarcoglycan than primary myoblasts that had been expanded in culture. AMMC-derived muscle fibers expressed sarcoglycan protein throughout their entire length consistent with enhanced migratory ability. Moreover, recipient fibers resisted muscle degeneration and exercise-induced damage. Donor-derived fibers increased with time after transplantation, suggesting that AMMCs survive for at least six months to augment the satellite cell pool. Interestingly, AMMCs regenerated large clusters of sarcoglycan-positive muscle fibers in aged dystrophic muscle. Supported by MDA.

44. Endoplasmic reticulum retention of collagen type VI in Ullrich Congenital Muscular Dystrophy

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Ullrich congenital muscular dystrophy (UCMD) is characterized by a marked distal joint hyperlaxity associated with multiple joint contractures. Early signs may be neonatal, such as arthrogryposis, torticollis and hip dislocation. Respiratory insufficiency often develops in the first decade of life. Autosomal recessive and dominant de novo mutations in the 3 genes encoding type VI collagen (COL6) cause UCMD. COL6 is composed of 3 chains (alpha1, alpha2 and alpha3) which associate intracellularly prior to secretion and alignment to form microfibrills in the extracellular space where it provides a structural link between basement membranes and the surrounding matrix. By immunolabelling of COL6 on muscle biopsies and cultured skin fibroblasts, we identified 6 UCMD patients presenting a striking intracellular staining in both conditions. In 4 of them a marked diminution in the mRNA levels of the alpha2 or alpha3 chains was detected by a chain-specific quantitative RT-PCR, and mutations inducing premature termination codons were identified in the corresponding genes. In the other 2 patients, mutations that do not lead to mRNA degradation were identified in the COL6A2 and COL6A3 genes. Most interestingly, electronic microscopy demonstrated that COL6 was retained into the endoplasmic reticulum of patients' fibroblasts, suggesting the involvement of the unfolded protein response in the subsequent degradation of the unassembled chains. Funding sources: INSERM, AFM, GIS-Institut des Maladies Rares, Assistance Publique- Hôpitaux de Paris.

45. Regulation of TGF-beta dependent signaling by decorin and LRP-1 in skeletal muscle cells

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Skeletal muscle differentiation is strongly inhibited by TGF-beta. Decorin is one of the proteoglycans can bind TGF-beta and regulate its activity. This soluble proteoglycan is endocytosed in skeletal muscle cells by low-density lipoprotein receptor-related protein (LRP-1). Interestingly, decorin is essential for TGF-beta dependent inhibition of myogenesis. The aim of this work is to know how decorin favors TGF-beta activity. Studies using myoblasts lacking decorin, in which the levels of LRP-1 were diminished by means of siRNA, suggest that decorin favors TGF beta dependent signaling through a mechanism that involves to LRP-1. At the intracellular level, the TGF-beta Smad dependent signaling pathway was not altered by the absence of decorin and/or LRP-1, which suggests the participation of a non canonical signaling pathway. Using pharmacologic inhibitors we established that PI3K is needed to regulate TGF-beta signaling by decorin through LRP-1. At the extracellular level an increase of the interaction between decorin and LRP-1 occurs when TGF-beta is present, which suggests the formation of a complex among them. These results show a novel regulatory mechanism of TGF-beta signaling in skeletal muscle cells. (Supported by FONDAP, MIFAB, MDA 3790).

46. Multipotency of adult human myoendothelial cells demonstrated by single-cell-derived clonal populations

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We have previously reported that murine muscle-derived stem cells (mu-MDSCs) can differentiate into endothelial cells, blood cells, bone and cartilage cells In addition, we have recently reported a novel myoendothelial cells in human skeletal muscle that display similarity with mu-MDSCs and exhibit a high muscle regeneration potential in skeletal and cardiac muscles. In the present study, we investigated whether clonal population of myoendothelial cells has the equivalent multipotent features observed with mu-MDSCs. Our study demonstrated that myoendothelial cell clones isolated by FACS expressed markers of myogenic, endothelial, perivascular and mesenchymal stem cells (MSCs). Interestingly, these myoendothelial clones expressed markers of MSCs (CD29, CD44, CD90 and CD105). Furthermore, these cells can spontaneously differentiate into endothelial cells, smooth muscle cells, pericytes, and neural cells in vitro. More importantly, these clonal cells also differenti ated into adipocytes, chondrocytes, osteoblasts and cardiac myocytes under specific conditions both in vitro and in vivo. Our results suggest that myoendothelial cells may represent the human counterpart of mu-MDSCs and are likely related to other mesenchymal stem cells.

47. Isolation Of Distinct Myogenic Progenitor Cells From Pax7 Deficient Skeletal Muscle Based On Adhesion Characteristics

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The myogenic potential of Pax7-/- muscle cells has long been debated. Herein, we report the use of a preplate technique to isolate 2 populations of myogenic progenitor cells with different myogenic capacities. One population was rapidly-adhering cells (RAC), displayed a high myogenic index and were desmin, c-Met, M-cadherin, CD56 and Pax3 positive. They were also capable of myogenic differentiation in vitro and in vivo, but were rapidly lost to terminal differentiation when continuously replated. We posit that this population represents a satellite cell population, and the second population represents a stem cell population because they were slowly-adhering cells (SAC), displayed a low myogenic index and were Sca-1 and CD34 positive. The SAC population also required Pax7 transduction in order to restore its' myogenic capacity. We believe that these two populations of cells may help explain the discrepancies presented in the literature concerning the presence of myogenic progenitor cells found in Pax7-/- mice. The original report that myogenic progenitor cells could not be isolated from Pax7-/- muscle could refer to a population that was similar to our SAC which were capable of long-term proliferation, but possessed very limited myogenic potential. The contradictory reports that myogenic progenitor cells could be isolated from Pax7-/- muscle may have alluded to a cell population similar to the RAC which are highly myogenic both in vitro and in vivo.

48. Muscle-derived stem cells regenerate skeletal muscle and peripheral nerve, but undergo microenvironment-induced transformation

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Here, we describe a population of stem cells isolated from skeletal muscle of female newborn mice using a preplate technique that can differentiate into muscle, neuronal, and glial cells in vitro and in vivo. These cells were able to form myosin heavy chain positive myotubes in vitro, and regenerate muscle fibers 17 weeks post-implantation in mdx mice. These cells had a high predilection towards expressing both neuronal and glial cell markers and were able to generate neurospheres in neurogenic media. When implanted into sciatic nerve defects the cells enhanced the rate of nerve regeneration and restored function. However, several weeks after regenerating the sciatic nerve, neoplastic growths formed. The resulting tumors were malignant Triton tumors that expressed myogenic, neurogenic, and glial markers. While the stem cells used in this study were not oncogenic in nature (no tumors were observed when implanted subcutaneously or injected intravenously for more tha n 1 year), the neoplasias were composed almost entirely of donor cells. Furthermore, cells isolated from the tumors generated were serially transplantable. We posit that the progenitor cells used were transformed in a time- and microenvironment-dependent manner, when they received concomitant neurogenic and myogenic signals. Interestingly, this transformation could be abrogated by further differentiation of the cells toward the neurogenic lineage prior to implantation.

49. Prospective isolation of skeletal muscle stem cells with a Pax7 reporter

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Muscle regeneration occurs through activation of quiescent satellite cells whose progeny proliferate, differentiate and fuse to make new myofibers. We use a transgenic Pax7-ZsGreen reporter mouse to prospectively isolate stem cells of skeletal muscle by flow cytometry. We show that certain markers (CD34 and CD29) label Pax7-expressing cells invariably, while others including CXCR4 label a subpopulation of Pax7+ cells. In response to injury, Pax7+ cells reduce CD34, CD29, and CXCR4 expression, increase in size, and acquire Sca-1. When directly isolated and cultured in vitro, Pax7+ cells display the hallmarks of activation and proliferate, initially as suspension aggregates, and later distributed between suspension and adherence. Pax7+ satellite cells expanded exclusively in suspension for two weeks can engraft and contribute to regeneration in mdx-/- mice. These results establish a novel animal model for the study of muscle stem cell physiology, and a novel c ulture system for expansion of engraftable muscle progenitors.

50. The use of follistatin to improve the transplantation of muscle-derived stem cells

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Myoblast transplantation has been proposed as a potential treatment for Duchenne muscular dystrophy (DMD), but has been hindered by numerous limitations (eg. poor survival). We have observed that the injection of muscle-derived stem cells (MDSCs) displays a greater muscle regeneration capacity than myoblasts, but the success of stem cell transplantation remains limited. Myostatin (MSTN) is a key inhibitor of muscle growth and may represent a potential target to improve the success of cell transplantation. We recently observed that MDSCs isolated from MSTN-/- muscle displayed better regeneration in host muscle than wide-type (WT) MDSCs, suggesting that blocking of MSTN signaling pathway enhances the efficiency of cell transplantation. Follistatin (FLST) is a potent antagonist of MSTN and may represent an approach to improve cell transplantation. We found that FLST significantly stimulated myogenic differentiation of myoblasts, as well as expressions of myoD, Myf5, and myogenin in myoblasts. FLST overexpressing (OE) transgenic mice showed significantly increased muscle regeneration and decreased fibrosis after injury, in contrast to WT mice. Moreover, we have been able to isolate MDSCs from FLST/OE mice, and compare their regeneration index with WT-MDSC after injected into the skeletal muscles of mdx/scid mice. Dystrophin immunostaining revealed that FLST/OE-MDSCs regenerated a larger number of dystrophin-positive muscle fibers than WT-MDSCs. Our results suggest that a combination of stem cell therapy with gene therapy (eg. FLST) may provide an effective treatment for DMD. (Supported by NIH).

51. Optimization of cell therapy for recessive muscular dystrophies

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Mesoangioblasts are recently characterized stem cells that are associated with the vasculature and can differentiate in different types of solid mesoderm including skeletal muscle (Minasi et Development 129, 2773,. 2002). When both wild type or dystrophic, genetically corrected, mesoangioblasts were delivered intra-arterially to dystrophic muscle of alpha-sarcoglycan null mice (a model for limb girdle muscular dystrophy), they resulted in a dramatic functional amelioration of the dystrophic phenotype (Sampaolesi et al. Science 301, 487, 2003).
Intra-arterial or systemic delivery of wild type, non DLA matched mesoangioblasts resulted in a partial recovery of muscle morphology and function, dystrophin expression and clinical amelioration, which persisted for a few months after removal of immune suppression. Delivery of autologous mesoangioblasts expressing human micro-dystrophin did not cause a comparable amelioration, despite widespread micro-dystrophin expression (Sampaolesi et al Nature 444, 574. 2006). These results show efficacy of cell therapy in a large, immuno-competent animal and set the rationale for a future clinical trial, using donor cells from an HLA-matched donor under immune suppression. Moreover, human adult mesoangioblasts were isolated and expanded in vitro from muscle biopsies: they were shown to correspond to a subset oh human pericytes and, more importantly to differentiate spontaneously in skeletal muscle with high efficiency (Dellavalle et al. Nature Cell Biol. 9, 255, 2007). Based on these results a first clinical trial with donor mesoangioblasts is planned for the near future. Problems still facing this approach and possible strategies to overcome them will be discussed.

52. Osteopontin And Skeletal Muscle Myoblasts: Expression And Function

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Osteopontin (OPN), a secreted glycoprotein, that can act as either a constituent of the extracellular matrix or as a growth factor. To investigate the role of this molecule in muscle repair and development, we sought to characterize OPN expression by muscle cells in vitro, the functional effects of OPN on myoblasts and its expression during muscle regeneration in vivo. Using PCR and ELISA, we have demonstrated that myoblasts express and secrete OPN. OPN staining was detected in proliferating myoblasts but not in myotubes in vitro or mature undamaged muscle fibers in vivo. QPCR and ELISA analysis indicated that OPN mRNA and protein levels were down-regulated during muscle differentiation in vitro, but OPN mRNA expression by cultured myoblasts was upregulated by FGF-2, IL-1-beta, TGF-beta or thrombin. In vivo, OPN staining was observed in areas of muscle regeneration in the muscles of mdx mice associated with desmin-positive myogenic cells, and OPN transcript levels were 14-25-fold higher in affected muscles of mdx mice than in muscles from control mice. OPN-coated plastic substrata were found to promote cell adhesion and differentiation but not proliferation or migration. Conversely, in solution OPN was found to significantly increase adhesion to fibronectin and proliferation but inhibit differentiation. These observations suggest that osteopontin released by myoblasts may assist in controlling the response of myoblasts to injury.

53. The homeodomain transcription factor Barx2 regulates satellite cell-mediated muscle growth and repair.

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Homeobox genes play critical roles in embryonic development; however, with the exception of Pax7, the roles of homeoboxes in postnatal muscle development and repair are not well characterized. Here we report that the Barx2 homeobox factor is important for muscle growth and repair and that it interacts with other muscle-expressed transcription factors. Barx2 expression overlaps with Pax7 in embryonic and postnatal muscle and is strongly upregulated after muscle injury, indicating that it is expressed in satellite cells. Barx2 null mice show reduced body and muscle mass, as well as defective repair after cardiotoxin-induced muscle injury. In addition, loss of Barx2 in dystrophic *mdx* mice (Barx2/mdx double null) leads to a much more severe muscle phenotype than either parental strain alone. These data are consistent with satellite cell dysfunction in Barx2 null mice. In support of this idea, cultured Barx2 null satellite cells show reduced proliferation and differentiation as well as downregulation of differentiation markers such as MyoD and myogenin. Moreover, we have found that Barx2 directly controls the expression of muscle-specific genes in cooperation with MyoD and SRF. Together these observations suggest that Barx2 is an important new regulator of satellite cell function.

TARGETS FOR THERAPY

54. Novel Enzyme Immunoassays for the Detection of Slow and Fast Isoforms of Skeletal Troponin I: Clinical Characterization of Skeletal Muscle Injury Using Troponin I Isoforms

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The diagnosis of skeletal muscle injury has been a challenge since the common serum markers for this condition namely, myoglobin, lactate dehydrogenase, and creatine kinase (CK), lack specificity to skeletal tissue. Therefore, the need for skeletal-specific biomarkers is clear. In recent years, skeletal troponin I (sTnI) has gained wide attention as a serum biomarker of skeletal muscle damage. sTnI is a myofilament regulatory protein and exists in two isoforms, ssTnI and fsTnI, associated with the slow (type I) and the fast (type II) twitch muscle fibers, respectively. Depending on the origin of muscle injury, one or both of these isoforms may be released into the blood stream.

We have developed enzyme immunoassays which specifically react with the fast and the slow isoforms of sTnI. These ELISAs are very sensitive with LLOQ of <1ng/mL and do not cross-react with cardiac troponin I. Using these assays, we have analyzed serum specimen from hospitalized sepsis, rhabdomyolysis, muscular dystrophy, and ventilator patients, as well as a set of serum specimen from random volunteers, for the presence of fsTnI and ssTnI isoforms. Results demonstrate that on average, sTnI isoforms are present at very low (<4ng/ml) to undetectable levels in normal healthy subjects; whereas, they are widely detectable in varying ratios, in sepsis, muscular dystrophy, and ventilator patients ranging between 5 – 100 ng/mL, and in statin-induced rhabdomyolysis, ranging from 50 to 2200 ng/mL.

We have observed disease-specific ratios of ssTnI and fsTnI in serum with no direct correlation to CK. We believe that circulating sTnI isoforms may serve as important indices of skeletal muscle damage and may aide in determining the origin and severity of muscle injury, as well as specific diseases or clinical conditions associated with the injury.

55. Novel role for calpain-3 in protein complex regulating calcium release in muscle

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Calpain 3 (CAPN3) is a non-lysosomal cysteine protease that is necessary for normal muscle function, as mutations in CAPN3 result in limb girdle muscular dystrophy (LGMD2A). To elucidate the biological roles of CAPN3 in skeletal muscle, we performed a search for potential substrates and interacting partners and identified glycolytic enzyme aldolase A (AldoA) as a binding partner for CAPN3. In co-expression studies CAPN3 degraded AldoA; however, no accumulation of AldoA was observed in CAPN3 deficient (C3KO) muscles suggesting that AldoA is not an in vivo substrate of CAPN3. Instead, we found CAPN3 to be necessary for recruitment of AldoA to triads, which are structural components of muscle fibers responsible for calcium transport and excitation-contraction coupling. Decreased AldoA in C3KO triads correlated with a reduced number of calcium release channels, ryanodine receptors. We measured calcium transients and found calcium release significantly reduced in fiber s from C3KO muscles. Together, these data suggest that CAPN3 acts as a structural component of the triad-associated protein complex that includes glycolytic enzymes, and that the structural integrity of this complex is important for calcium release in skeletal muscles.

56. Cardiac Pathology In A Case Of Limb-Girdle Muscular Dystrophy Type 2I (LGMD-2I)

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LGMD-2I is caused by mutations in fukutin-related protein (FKRP) that lead to abnormal glycosylation of alpha-dystroglycan in skeletal muscle. Here, we report cardiac pathology in a 17-year old boy who initially presented with progressive proximal leg weakness exacerbated by illness or exertion. The skeletal muscle biopsy showed a mild muscular dystrophy with normal staining for dystrophin, sarcoglycans, utrophin, merosin, and beta-dystroglycan. Staining with alpha-dystroglycan glycoepitope antibodies IIH6 and VIA4-1 varied from absent to nearly normal in a mosaic pattern characteristic for secondary dystroglycanopathies. Direct sequencing of the FKRP gene detected the common point mutation c.826C>A (p.L276I) in both alleles, thus establishing the diagnosis of LGMD-2I. At the age of 17, the patient developed severe congestive heart failure and underwent cardiac transplantation. The heart showed biventricular hypertrophy and dilatation with interstitial fibrosis an d extensive fatty replacement. Immunoperoxidase staining demonstrated a complete absence of IIH6 staining - a pattern similar to, but more severe than, the one observed in the patient's skeletal muscle. While clinical cardiac involvement is common in LGMD-2I, this is one of the first direct demonstrations of cardiac pathology and severe impairment of heart alpha-dystroglycan glycosylation in this disease. Supported by NS053672.

57. Diagnostic Testing For Congenital Muscular Dystrophies In A Cohort Of USA Patients

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The congenital muscular dystrophies (CMD) are a genetically and phenotypically heterogeneous group of neuromuscular and developmental diseases. Since the 1995 report that LAMA2 mutations are responsible for merosin-deficient CMD, twelve additional CMD genes have now been identified. Diagnostic testing for CMD began at The University of Iowa in 1997 with an immunostaining panel of 4 antibodies that has now expanded to include 15 antibodies. Molecular genetic testing using direct DNA sequencing of the CMD genes responsible for dystroglycanopathies has been added since 2004. 147 muscle biopsies and 62 DNA samples have now been evaluated for patients with a clinical diagnosis of CMD; 33 cases had a biopsy and DNA. Immunostaining abnormalities were diagnostic in 63% of biopsies (53 dystroglycanopathy, 19 merosin-deficient, 21 collagen VI-deficient). Gene sequencing confirmed the diagnosis in 25 of the dystroglycanopathy patients across the clinical spectrum from WWS to MDC1C (3 POMT1, 5 POMT2, 1 POMGnT1, 7 FCMD, and 9 FKRP). Analysis of fibroblast culture collagen VI biosynthesis and cDNA has confirmed COL6 mutations in 6 patients. These data indicate that presumptive or definitive diagnoses can be made in ~70% of CMD patients. While the data suggest that dystroglycanopathies (as a group) are the most common form of CMD, a population-based study will be needed to determine the true incidence of CMD subtypes in the USA. Supported by NS053672.

58. Regulation of cell death by the Bax/Ku70 system in normal and laminin-alpha2-deficient muscle.

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Mutations that lead to deficiency of laminin-alpha-2 (Lama2) cause Congenital Muscular Dystrophy Type 1A (MDC1A. Previously,our laboratory demonstrated that increased apoptosis through the Bcl-2/Bax pathway is a significant contributor to pathology in the Lama2-null mouse model of MDC1A.In this work, we show that Bax activation and translocation to mitochondria and induction of apoptosis in muscle cells is regulated by interaction of Bax with the mulitfunctional protein Ku70.In the cytoplasm, Ku70 normally binds to Bax, thereby retaining Bax in the cytoplasm.Upon induction of apoptosis,however, the Bax binding domain of cytoplasmic Ku70 is acetylated and Bax is released for translocation to mitochondria.Following previous reports, we synthesized cell permeable pentapeptides--termed Bax Inhibiting Peptides (BIPs)–which are based on the Bax binding domain of Ku70. We found that BIPS,but not scrambled peptides,inhibited staurosporine-induced cell death in C2C12 ce lls.We also found that BIPs inhibited the translocation of Bax out of the cytoplasm upon staurosporine treatment.Finally,we found that the acetylated form of Ku70–an indicator of increased apoptosis--was more abundant in Lama2-null than in normal mouse muscles.These results identify the Bax/Ku70 pathway as a therapeutic target in MDC1A.Funded by MDA, NHLBI, NIAMS.

59. The Endocytic Recycling Protein EHD2 Interacts with Myoferlin to Regulate Myoblast Fusion

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Mutations in dysferlin lead to muscular dystrophy, and the loss of dysferlin leads to defective repair of skeletal myofibers. Myoferlin is highly related to dysferlin in that both are membrane-anchored, multiple C2 domain-containing proteins. Myoferlin is highly expressed in myoblasts that are undergoing fusion to multinucleate myotubes while dysferlin is more highly expressed in multinucleate myotubes and myofibers. Myoferlin is required for efficient myoblast fusion to myotubes. Myoferlin null muscle has smaller myofibers and in culture form smaller myotubes. Myoblast fusion also contributes to muscle repair after damage, and consistent with this, myoferlin is highly upregulated in damaged muscle. We found that myoferlin binds directly to the eps 15 homology domain protein, EHD2. Members of the EHD family have been implicated in endocytic recycling of membrane receptors. EHD2 binds directly to the second C2 domain of myoferlin and EHD2 is reduced in myof erlin null myoblasts. Introduction of dominant negative EHD2 into myoblasts leads to the sequestration of myoferlin and inhibition of myoblast fusion. The interaction of myoferlin with EHD2 identifies molecular overlap between the endocytic recycling pathway and the machinery that regulates myoblast membrane fusion. Supported by NIH NS047726.

60. Investigating the role of cytoplasmic gamma-actin in dystrophic muscle

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Recently, we documented a ten-fold increase in cytoplasmic gamma-actin expression in dystrophin-deficient muscle, which led to the hypothesis that increased gamma-actin expression is acting in a compensatory manner. To explore this idea, we generated muscle-specific double knockout (ms-DKO) mice by breeding the conditional Actg1 allele to the mdx background. Surprisingly, mdx and ms-DKO mice presented with comparable levels of myofiber necrosis, membrane instability, and deficits in muscle function. The lack of an exacerbated phenotype in the ms-DKO mice indicates that gamma-actin and dystrophin function in a common pathway. While both mouse models showed similar increases in utrophin expression when compared to wild type mice, we did not detect differential plectin expression in any of the mice examined. Moreover utrophin, and not plectin, was found to interact with beta-dystroglycan, which suggests that utrophin compensates for the loss of dystrophin throug h gamma-actin independent mechanisms.

61. Functional exchange of sarcomeric alpha-actin by cytoplasmic gamma-actin

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Although cytoplasmic gamma-actin is expressed at very low levels in skeletal muscle, its importance has been demonstrated by conditional knock-out mice which exhibit a novel progressive myopathy (Sonnemann et al. Dev. Cell, 2006). Here we report the characterization of mice transgenically overexpressing gamma-actin ~2000-fold above wild-type levels in skeletal muscle. In wild-type muscle gamma-actin localizes to costameres, blood vessels and nerves, while in transgenic mice gamma-actin also incorporated substantially into thin filaments. Quantitative western blot analysis showed gamma-actin comprised 40% of the total actin population in transgenic skeletal muscle with a concomitant 40% decrease in alpha-actin levels, further demonstrating that total actin levels in skeletal muscle are tightly regulated. Surprisingly, gross over-expression of gamma-actin had no detrimental effects, as transgenic muscle was histologically and ultrastructurally identical to wild- type muscle. Furthermore, transgenic mice exhibited normal physiological performance, indicating that gamma cytoplasmic-actin is functionally capable of replacing alpha-actin within the contractile apparatus. These data suggest cytoplasmic gamma-actin upregulation as a therapeutic approach for nemaline-myopathies caused by alpha-actin deficiency.

62. Blocking TGF-β receptors I and II affect C2C12 myoblast fusion, in a Smad-independent pathway

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TGF-beta, strong inhibitor of myogenesis, signals through RIbeta and RIIbeta. However, during myogenesis, these receptors increase on the cell surface, therefore we evaluated their relevance during this process. Inhibiting RIbeta kinase activity with SB431542, myogenin induction increases, but expression of late differentiation markers, such as creatine kinase and myosin are decreased. Using SB431542 or a dominant negative form of RIIbeta, myoblast fusion is strongly affected, and integrin beta1 and N-cadherin, critical molecules in myotube formation decrease. Overexpression of Smad7 (strong inhibitor of Smad proteins pathway) did not affect myoblast fusion, suggesting that receptors signaling is Smad-independent. Among non-canonical pathways we evaluated Akt and p38 pathways, but these were unaffected. Finally, TGF-beta1, 2 and 3 seems to be not the ligand that activated these receptors during myogenesis, because blocking antibody of these factors did not affect myoblasts fusion. These results suggest that cell signaling through the TGF-beta receptors, and activation of a Smad-independent pathway, are essential for myoblast fusion. (Supported by FONDAP, MIFAB, MDA 3790).

63. Involvement of Ozz-E3, a muscle-specific ubiquitin ligase, in muscle regeneration

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Ozz, an E3 ligase specific for striated muscle, is a member of the Suppressor of Cytokine Signaling (SOCS) family of proteins. Ozz expression during myogenesis coincides with myofiber formation and growth. The induction of necrosis, by injecting cardiotoxin (ctx) in skeletal muscle of mice, showed a reduction of Ozz protein expression up to 3 days after lesion. Ozz levels normalized around day 4; increased progressively up to day 10 and went back to normal at 21 days. Analysis of Ozz mRNA revealed a progressive up-regulation of the Ozz up to day 4 after lesion, followed by normalization. Immunohistochemical study showed an increased Ozz expression throughout the cytosol, but especially in regions juxtaposed to the nuclei, 3 days after lesion. At 5-7 days post injury, the expression of Ozz in the cytosol became more diffuse. A number of positive nuclei for Ozz expression were also seen in the developing myotubes. The overall morphology of the Ozz–/– skeletal mu scle did not seem to differ from that of wild-type muscle after ctx treatment. However, at the ultrastructural level there were few developing fibers in Ozz–/– muscle with distinct sarcomeric alterations. The expression pattern of Ozz during muscle regeneration and the alterations seen in Ozz–/– fibers support the notion that Ozz-E3 plays a role in myofiber maturation and growth during regeneration. This research was funded by NIH grant AR049867, Cancer Center Core Grant CA021765, and the American Lebanese Syrian Associated Charities (ALSAC).

64. Skeletal muscle cells express and respond to connective tissue growth factor (CTGF/CCN2), a profibrotic cytokine

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Muscular dystrophies are characterized by a progressive wasting of the musculature ending up in an extensive fibrosis with an increased extracellular matrix (ECM) deposition. Connective tissue growth factor (CTGF) is involved in several fibrosis, however, its role in skeletal muscle is unknown. Here we show that myoblasts and myotubes synthesize CTGF in response to transforming growth factor-beta and lysophosphatidic acid. In myoblasts and myotubes CTGF induced several ECM molecules such as fibronectin, collagen type I and III. It had an inhibitory effect on muscle differentiation evaluated by the nuclear translocation of the muscle regulatory factor myogenin, and of myosin expression. CTGF treatment of myoblasts induced their dedifferentiation, downregulating MyoD and desmin, two markers of committed myoblasts, together with a strong reorganization of actin filaments, but not tubulin filaments. Finally we show that CTGF effect in myoblasts depends on ERK1/2 signa ling pathway. These results provide novel evidence for the underlying mechanisms and participation of skeletal muscle cells in the synthesis and role of CTGF inducing fibrosis, inhibiting myogenesis and dedifferentiating myoblasts. Supported by FONDAP, MIFAB, MDA3790

65. Regulatory mechanisms of TGF-beta signaling attenuation during myogenesis.

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TGF-beta (TGFb) is a strong inhibitor of myogenesis. It binds to transducing receptors (TGFbR I and II) on the cell surface, activating a Smad-dependent pathway. To understand how successful skeletal muscle formation occurs in vivo, in the presence of TGFb, we have studied three mechanisms of TGFb signaling attenuation. i) A decrease of Smad protein levels together with a decrease of TGFbeta dependent Smad2 phosphorylation and Smad4 nuclear translocation. ii) At extracellular milieu, the proteoglycans decorin, biglycan and betaglycan all diminished the TGFb availability decreasing its biological activity and, iii) TGFb dependent signaling is regulated by electrical activity in rat primary myotubes; the inhibition of electrical activity increases TGFbR I levels, and the promotion of electrical activity in myotube cultures causes TGFbR I to decrease diminishing signaling. Altogether, these findings support three novel regulatory mechanisms acting on TGFb signaling c ascade in myogenesis. Fondap-CRCP, MIFAB, MDA 3790.

66. Proteomic identification of the LIM domain protein FHL1 as the gene-product mutated in reducing body myopathy

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Reducing body myopathy (RBM) is a rare progressive myopathy defined by characteristic aggresome-like inclusions in muscle. Traditional gene identification in this disease has been complicated by the frequently sporadic occurrence of patients and small family sizes. As an alternative approach we used laser microdissection of the intracytoplasmic inclusions followed by nanoflow LC-MS/MS coupled with proteomic analysis, to identify FHL1 (SLIM1), encoded on Xq26.3, as the most prominent component of the inclusions. FHL1 belongs to the family of four and a half LIM domain proteins (FHL1-5); it is predominantly expressed in skeletal but also in cardiac muscle. Mutational analysis up to now identified 7 different FHL1 mutations in patients with RBM, including severely affected sporadic female patients as well as familial cases with severely affected boys and more mildly affected mothers. Mutations were found exclusively in the 2nd LIM domain, changing invariant in the zi nc-coordinating histidine and cysteine residues. Transfection of mutant FHL1 induced the formation of inclusions with aggresomal features in COS-7 and skeletal muscle C2C12 cells, incorporating both mutant and wild type FHL1 as well as trapping other proteins in a dominant negative manner. Thus, a novel laser microdissection/proteomics approach has helped identify both inherited and de-novo mutations in FHL1 thereby defining a new X-linked protein aggregation disorder of muscle.

67. Calpain 3 Mutations Causing Lgmd2a That Do Not Impair Proteolytic Function, Cause Its Destabilization And Degradation By Other Proteases

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Mutations in the gene encoding the proteolytic enzyme calpain 3 (capn3) result in limb girdle muscular dystrophy type 2A (LGMD2A). Most pathogenic capn3 mutations are known to abolish proteolytic activity; however, there are some mutations that retain activity (i.e. R448H and D705G), but interfere with binding to titin. To understand why these two mutations are pathogenic in vivo, we created transgenic mice that express R448H and D705G mutants on the CAPN3 knock out (C3KO) background. Initial biochemical experiments comparing the mutant transgenes to a WT transgene suggest that these two mutations destabilize CAPN3 and cause it to degrade faster than the WT protein. In addition, our studies illuminate a difference between the current investigation and previous studies by suggesting that CAPN3 is more stable in intact muscle vs. a muscle extract. This destabilization is not only due to autolysis, as previously believed, but also due to other proteases acting upon C APN3. Furthermore, our studies validate that these are the pathogenic mutations as they were not able to rescue the C3KO phenotype. Taken together these studies suggest that anchorage to titin and autolysis may not be the only factors influencing CAPN3 stability in vivo.

68. IGF-I E-peptides are active in vitro and in vivo

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Insulin-like growth factor I (IGF-I) is a strong therapeutic candidate for muscle disease because it aids in the repair and maintenance of tissue health. The igf1 gene not only produces IGF-I, but also generates multiple E-peptide extensions through alternative splicing. The E-peptides might modulate the actions of IGF-I, or they might have independent activity. C2C12 cells were exposed to synthetic E-peptides to determine if the possessed inherent bioactivity. Both EA and EB peptides caused a significant increase in cell number at physiologically relevant doses. Proliferation was enhanced in the presence of serum, suggesting that co-factors may modulate the effects of the E-peptides. Viral administration of IGF-IA, IGF-IB, and IGFStop (a construct that lacks any E peptide) into muscles of C57 mice was utilized to determine mechanisms responsible for E-peptide activity. Microarray analysis revealed responses that were driven by increased IGF-I regardless of the p resence of E-peptide, such as cell survival. In contrast, distinct expression patterns were observed for each IGF isoform, which included matrix remodeling genes. These studies provide evidence that the igf1 gene produces multiple biologically active proteins that may work in concert to enhance muscle repair.

69. Genetic loci that modify muscular dystrophy

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Muscular dystrophy patients with the identical gene mutation often have a range of phenotypes that affect age of onset and severity of muscle weakness. For example, the identical gamma-sarcoglycan gene mutation is associated with both mild and severe forms of disease. Mice lacking gamma-sarcoglycan (Sgcg null) also display phenotypic variability dependent on genetic background. We utilized two disease hallmarks, membrane permeability to Evans Blue dye and fibrosis, to measure disease severity in this murine model of Limb Girdle Muscular Dystrophy type 2. When the Sgcg null mutation was bred onto four different mouse strains, we identified the DBA/2J strain as most susceptible to disease, and the 129SvJ strain as more resistant to the disease process while CD1 and C57/Bl6 mice had intermediate disease progression. This disparity of phenotypes establishes the presence of genetic modifier loci that affect muscular dystrophy disease progression in mouse models. We now analyzed the Sgcg null F2 generation from an intercross between the parental strains Sgcg-129SvJ and Sgcg-DBA. Testing by whole genome SNP analysis of the gF2 animals with a range of disease now identified a single dominant locus and six minor loci (DMOD1 – DMOD7) that are responsible for the modification of disease progression. Supported by NIH 61322.

70. A Novel nNOS Signaling Pathway Regulates Skeletal Muscle Size and Strength

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NO (nitric oxide) is synthesized by neuronal nitric oxide synthase mu isoenzyme (nNOSmu) in skeletal muscle. Increased NO levels substantially improve the dystrophic phenotype of different mouse models of human muscular dystrophy by mechanisms that appear largely independent of nNOSmu function. This suggests the existence of additional NO signaling pathways in muscle. We present evidence for this contention by identifying a novel nNOS signaling pathway in skeletal muscle. We identified a splice variant of nNOS called nNOSbeta localized to the Golgi complex. Loss of nNOSbeta activity resulted in reduced muscle mass with muscles exhibiting lower force-generating capacity. Furthermore, the absence of nNOS decreased the ability of muscle to resist exercise-induced fatigue. In summary, we have identified a novel nNOS-signaling pathway that regulates skeletal muscle size and strength. We provide the first evidence that differential targeting of nNOS isozymes creates functionally distinct nNOS signaling pathways by facilitating localized synthesis of an otherwise directionless free radical gas. The activation of this novel pathway may account for the beneficial effects of NO on murine dystrophic muscle. These data make nNOSbeta of relevance to a wide range of muscle diseases where preventing the loss of muscle bulk and strength is of clinical importance.

71. Examination Data at Enrollment for 510 Subjects in the United Dystrophinopathy Project

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Introduction: Mutations in the DMD gene, encoding dystrophin, are responsible for the dystrophinopathies. These include Duchenne, Becker, and Intermediate Muscular Dystrophies (DMD, BMD, and IMD); X-linked dilated cardiomyopathy; and manifesting carrier states. The United Dystrophinopathy Project (UDP) is a large multicenter consortium which has established a genotype/phenotype database and patient registry, using recent advances in molecular diagnostic methods to determine genotypes. Here we provide an overview of the phenotypic features of patients at the time of enrollment into UDP. Methods: Dystrophinopathy patients are enrolled through muscular dystrophy clinics at each of the 7 participating centers, and clinical subtype is determined by the expert clinician at each center. Historical data is recorded, and most patients undergo a yearly standardized physical examination. Genotyping is performed by a central laboratory. Results: Currently, 849 patients are enrolled, of whom 510 (including 383 DMD and 101 BMD) have undergone the standardized examination. We present baseline data regarding manual muscle scores, timed functional tests, functional grades, and pulmonary function tests in this large cohort. Conclusion: Data from the UDP will help to establish the natural history of the dystrophinopathies in an era of modern clinical practice, and will provide cohorts of characterized patients for clinical trials. Support: NIH (R01NS043264)

72. Morphologic and Genetic Assessment of Patients with Congenital Fiber Type Disproportion and Tropomyosin 3 Mutation

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Congenital fiber type disproportion (CFTD) is a rare congenital myopathy characterized by hypotonia, muscle weakness, and type I fiber hypotrophy without other notable findings on biopsy. Mutations in genes encoding skeletal muscle α-actin (ACTA1) and selenoprotein N 1 (SEPN1) have been found in a few cases of CFTD and in other congenital myopathies, suggesting that other congenital myopathy genes may be involved in CFTD. The muscle tropomyosin 3 gene, TPM3, is mutated in rare cases of nemaline myopathy with type 1 fiber hypotrophy. Using genomic PCR and DNA sequencing, we screened TPM3 in patients with a clinical diagnosis of CFTD, nemaline myopathy, or undefined congenital myopathy. Mutations in TPM3 were identified in 5 of 12 patients with CFTD, as well as in one case of nemaline myopathy. These mutations included autosomal dominant, recessive, and de novo mutations, and the nemaline myopathy patient had the same mutation as one of the CFTD patients. Review of muscle biopsies from patients with TPM3 mutations all displayed marked type I smallness. Our results illustrate the genetic heterogeneity seen in CFTD and further support the overlap between CFTD and other congenital myopathies.

73. Dysferlin in T cell apoptosis

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Mutations in the dysferlin gene, DYSF, cause dysferlinopathy, the rare form of muscular dystrophy also known as Limb-Girdle Muscular Dystrophy Type 2B (LGMD2B) and Myoshi Myopathy (MM). In dysferlinopathy there is a marked increase in death of muscle fibers and inflammation in the muscles of patients. Dysferlin is required to repair damage to the muscle membrane. In the absence of dysferlin it is thought that this damage accumulates, triggers inflammation and leads to progressive, permanent muscle loss. The class of steroid hormones known as glucocorticoids is often used to decrease inflammation and immune function in a wide range of diseases including muscular dystrophies and muscle inflammatory diseases. Surprisingly, glucocorticoid treatment is not effective in reducing muscle damage in LGMD2B/MM patients and can make damage worse. Using genome-wide DNA microarrays we found that glucocorticoid treatment induced dysferlin gene expression in mouse T lymphocyt es during apoptosis. Synaptotagmins were also induced. We verified these gene expression changes using quantitative RT-PCR. We are investigating a novel role for dysferlin in apoptosis in T cells of the immune system and skeletal muscle. Our overall objective is to understand how this role of dysferlin in cell death may relate to inflammation and loss of muscle tissue in dysferlinopathy.

74. Cardiac Ankyrin Repeat Protein is a biological marker of atrophy in dystrophic skeletal muscles

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Muscular dystrophies (MD) are a group of genetic diseases characterized by progressive muscle degeneration and weakness. Muscle atrophy is a very common clinical feature of these pathologies. In an attempt to identify potential therapeutic targets for the correction of muscle wasting in muscular dystrophies, the expression of several pivotal proteins involved in protein metabolism was investigated in 4 MD animal models. Amongst all the proteins considered, the expression of CARP, a regulator of transcription factors, appears to be the only one systematically rising. CARP forced over-expression in muscle fibres fails to induce an atrophic phenotype, indicating that CARP per se cannot initiate the phenomenon. CARP is also persistently up-regulated in a condition leading to atrophy (definitive sciatic nerve denervation), whereas MAFbx and MURF1, two E3 ubiquitin ligases known to be involved in muscle wasting, are only transiently over-expressed. These results suggest that CARP might be of particular importance in the late phase of atrophy. Finally, we conclude that CARP is a major marker of muscular pathologies and therefore propose its down-regulation as a potential therapeutic target.

75. A Founder Allele in the DMD Gene is Associated with a Mild Becker Phenotype

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Identical mutations within the DMD gene in unrelated individuals can arise in two ways: hot-spot or founder mutations. A recurrent p.Trp3X mutation was first ascertained in a proband with no symptoms until age 20 and who walked until the age of 62. Three unrelated individuals carrying a p.Trp3X mutation were all ascertained in childhood incidental to elevated creatine kinase (CK) levels detected in the context of other illnesses. Family history assessments failed to detect relatedness between these four probands or their relatives. To determine whether the p.Trp3X mutation represents a hot-spot or founder mutation, we used Affymetrix SNP arrays to detect the pattern of allele sharing throughout the genome and at the DMD gene. We observed an identical 3.6-megabase haplotype overlapping the DMD gene, which is consistent with the Trp3X mutation being identical by descent in these individuals. The size of this founder haplotype suggests that the minimal age of this mutation is greater than 7 generations. The discovery of a founder mutation associated with a mild Becker phenotype suggests that the prevalence of hypomorphic dystrophin mutations can be re-examined with the use of improved genomic analysis. Funding source: National Institutes of Health grant R01 NS43264-04.

76. Immunofluorescent quantification of low level expression in the dystrophin protein

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Quantification of low dystrophin levels in muscle sections presents a technical challenge. This is particularly true for the detection of what may be significant changes in levels of expression where the absolute levels still remain low, as might be expected in proof of principle drug trials. We have developed a method for the reliable immunofluorescent quantification of low level expression. Muscle sections (9 or 11 micron) are double-stained using monoclonal spectrin and dystropin antibodies. Fields for imaging are randomly selected under bright field microscopy, and confocal 12-bit fluorescent images obtained for analysis in Metamorph using a custom script. Contiguous 3x3 pixel areas of signal define spectrin positive sarcolemmal membrane, and dystrophin signal is analyzed only from spectrin-positive pixels, eliminating non-specific dystrophin signals. Revertant fibers can be excluded from this analysis by setting an upper limit to the dystrophin intensity level. The average intensity of dystrophin over the whole image is reported. Our preliminary studies DMD and BMD control tissues show consistent measures of intensity levels between serial sections of the same biopsy, and between specimens prepared on multiple days. Reproducible elevations in dystrophin intensity levels detected in BMD as compared to DMD images suggest that this quantification method can be used to distinguish between the two phenotypes. Studies comparing quantification of IF results to an improved Western blot quantification protocol are underway. Support: NIH/NINDS R01 NS432644 (KMF).

77. The myogenic transcription factor pax3 is phosphorylated by casein kinase II in vitro

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Pax3, a member of the paired class homeodomain family of transcription factors, plays a role in early myogenesis and is associated with alveolar rhabdomyosarcoma (ARMS), a solid muscle tumor. ARMS, which occurs in the trunk and extremities of older adolescents and is associated with a poor prognosis, is characterized by a t(2:13)(q35;q14) chromosomal translocation which fuses the gene for Pax3 to the gene for FKHR (FOXO1a) to form the oncogenic fusion protein Pax3-FKHR. Pax3-FKHR contributes to the deregulation of muscle development and the formation of ARMS. FKHR is known to be regulated by phosphorylation. However, although a majority of the sites of FKHR phosphorylation are present on Pax3-FKHR, phosphorylation of these sites do not regulate Pax3-FKHR. Therefore, it is possible that phosphorylation of the Pax3 region of Pax3-FKHR may play a role in the regulation of Pax3-FKHR. Previously, we identified Ser201, 205, and 209 as the sites of phosphoryl ation on Pax3. Therefore, the goal of this present study is to identify the kinase(s) involved in order to better understand the possible regulatory mechanism of Pax3 and Pax3-FKHR. By using in vitro kinase assays, we demonstrate that Pax3 is phosphorylated by CKII. Further, CKII phosphorylation of Ser205 is required for efficient phosphorylation of Ser201 and Ser209. Future studies aim to determine the role of phosphorylation in the regulation of Pax3 and Pax3-FKHR.

78. Identification of the sites of phosphorylation on the myogenic transcription factor Pax3

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Alveolar Rhabdomyosarcoma (ARMS), the more aggressive form of Rhabdomyosarcoma (RMS), is most often characterized by a t(2:13)(q35;q14) chromosomal translocation, which results in the fusion of two myogenic transcription factors, Pax3 and FKHR. The resulting oncogenic fusion protein Pax3-FKHR contributes to the deregulation of muscle development and the formation of ARMS. Posttranslational modifications such as phosphorylation are common mechanisms for the regulation of transcription factors. At present, the sites of phosphorylation on Pax3 and if these sites are used on Pax3-FKHR are unknown. Therefore, the goal of this study was to identify the sites of phosphorylation on Pax3. We established a valid in vitro kinase assay to assist with the identification of these sites. Deletion mutant analysis determined that phosphorylation occurred in the region of Pax3 immediately adjacent to the octapeptide domain. Subsequent point mutant analyses conclusively identi fied Ser201, Ser205, and Ser209 as the sites of Pax3 phosphorylation in vitro. Finally, we demonstrate that Ser205 and Ser209 are phosphorylated in vivo in proliferating mouse primary myoblasts. Future studies include determining if these sites are utilized on Pax3-FKHR and identifying a biological function for these phosphorylation events in muscle development and the promotion of ARMS.

79. Identification of Novel Potential Target Genes Involved in Muscle Aging

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Sarcopenia is a degenerative process that occurs in skeletal muscle during aging. It results in decreased mobility and increase the risk for falls and bone fractures. Because the elderly population is growing, it is imperative to understand the mechanisms underlying sarcopenia. For this purpose, we used two complementary gene analysis methods to identify new potential targets involved in sarcopenia. DNA chip analyses from young (4 weeks) and aged (24 months) mouse muscles show a down-regulation of 131 genes in aged muscles, mostly involved in protein modifications, cell signalling, cell growth/differentiation and muscle development/function. In addition, high stringent subtractive library permitted to clone genes specifically expressed in young and aged muscles. The expression of these genes was confirmed by qPCR. Of note, some of these clones are described for the first time and may represent novel target genes involved in sarcopenia. Further overexpression and k nock-down assays will be necessary to confirm their role in sarcopenia. These results may lead to preventive and therapeutic approaches aimed at slowing down skeletal muscle degeneration observed during aging.

80. Collagen VI related myopathies: The Ullrich-Bethlem spectrum of disorders

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Collagen type VI is a microfibrillar component of the extracellular matrix (ECM), composed of three alpha chains encoded by three different genes on chromosomes 21 and 2. The three alpha chains assemble into a heterotrimeric monomer followed by dimerization and tetramerization within the cell. Secretion of the tetramer is followed by microfibrillar assembly in the ECM. Mutations in the three alpha chains of collagen type VI underly two principal clinical phenotypes: the milder Bethlem myopathy (BM) and the severe congenital muscular dystrophy type Ullrich (UCMD). In addition to muscle weakness there also is a significant connective tissue component to the phenotype consisting of joint hyperlaxity as well as of progressive contractures. BM is inherited as a dominant, whereas mutations in UCMD may be recessive as well as de-novo heterozyote acting in a dominant negative fashion. These severe de-novo mutations frequently are in-frame deletions in the N-terminal part of the triple helical domains of the respective chains allowing the mutant chains to be incorporated into the collagen VI tetramer followed by secretion into the ECM. Missense substitutions of the invariant glycine of the triple helical collagenous Gly-X-Y amino acid triplet constitute another important type of mutation acting in a dominant fashion associated with a wide spectrum of severity. The accumulating genotype/phenotype data increasingly emphasizes the existence of a spectrum of collagen VI related phenotypes extending form Ullrich disease to the mild end of the Bethlem phenotype, as well as the potential for considerable clinical variability associated with a given mutation. The patho-mechanisms of collagen VI deficiency in skeletal muscle are still incompletely understood. Disease associated mutations in collagen VI cause a loss of the normally close interaction of collagen VI with the basement membrane around muscle cells. A mouse model of collagen VI deficiency as well as experiments in human cell culture suggest the occurrence of mitochondrially mediated myofiber apoptosis as a consequence of collagen VI mutations, while observations in human biopsies also suggest myofiber atrophy as a prominent component of the disease. Treatment approaches close to clinical trials are primarily concerned with pharmacological strategies designed to interfere with the myofiber apoptosis. In a much more experimental stage are strategies aiming at allele specific elimination of mutations that act in a dominant negative fashion. CGB is supported by MDA USA (MDA3896) and NIH/NIAMS (R01AR051999).

81. Proteolytic cleavage modulates alpha7beta1 integrin function

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The dystrophin-glycoprotein complex and the alpha7beta1 integrin are two major linkage systems that connect the cytoskeleton within muscle fibers with laminin in the extracellular matrix. Functional diversity of this integrin is generated by alternative RNA splicing and post-translational modifications. We have identified a species-specific site in the alpha7 chain, at amino acid 603RRQ605 that undergoes an intra-peptide cleavage. This cleavage is mediated by a serine protease, the urokinase plasminogen activator (uPA). Mutation of RRQ to GRQ at this site prevents cleavage. This RRQ sequence in the alpha7 chain is highly conserved among vertebrates with exception of the mouse. Protein structure modeling indicates the cleavage site is located in an open region between the beta-propeller and thigh domains of the alpha7 chain. Compared to non-cleavable alpha7 chain, the cleavable form enhances cell adhesion and spreading on laminin. Upon myogenic differentiation, cle avage of the alpha7 chain is significantly elevated, as is uPA activity. Our results suggest that proteolytic cleavage is a novel mechanism that regulates alpha7 integrin function in skeletal muscle, and, that the generation of such cleavage may play an important role during muscle regeneration, disease and aging. Supported by the NIH and MDA.

THERAPIES FOR MUSCLE DISEASE

82. Inhibition of smad3 phosphorylation by halofuginone prevents muscle fibrosis and improves muscle performance in muscle dystrophies

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Fibrosis is a characteristic feature of the muscle in patients with DMD and CMD, leading to cardiorespiratory failure and delayed motor development respectively. In the present study, we evaluated the efficacy of Halofuginone (Halo), an anti-fibrotic compound, in reducing muscle fibrosis in mdx and Dy2J/Dy2J mice, animal models of DMD and CMD, respectively. Halo prevented the age-dependent increase in collagen synthesis in the diaphragm of young mdx mice and the exacerbation of fibrosis and myopathy in mdx mice treated with cyclosporine A. A similar effect was observed in the gastrocnemius and tibial cranialis of Dy2J/Dy2J mice. The mitigation of diaphragm fibrosis in mdx mice was associated with a decrease in the number of central myonuclei, suggesting a reduction or a delay in the progression of muscle damage and regeneration. Moreover, enhanced motor coordination and balance, and a major improvement in cardiac muscle function were observed. Phosphorylation of S mad2/3, a key signaling molecule downstream to TGF-beta and myostatin, was inhibited by Halo in vivo and in cultures of C2 cell line and of primary mouse myoblasts derived from the mdx and Dy2J/Dy2J mice. This inhibition was partially due to Smad2/3 association with phosphorylated Akt. Since the inhibition of TGF-beta-dependent fibrosis by Halo results in a major improvement in muscle performance, Halo has strong potential as a novel anti-fibrotic drug for muscular dystrophies.

83. Hematopoietic cell transplantation provides an immune tolerant platform for myoblast transplantation in dystrophic dogs

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Duchenne Muscular Dystrophy (DMD) is the most common and severe form of muscular dystrophy in humans. The goal of myogenic stem cell transplant therapy for DMD is to increase dystrophin expression in existing muscle fibers and provide a source of stem cells for future muscle generation. Although syngeneic myogenic stem cell transplants have been successful in mice, allogeneic transplants of myogenic stem cells were ineffective in several human trials. To determine whether allogeneic muscle progenitor cells can be successfully transplanted in an immune tolerant recipient, we induced immune tolerance in two DMD affected (xmd) dogs through hematopoietic cell transplantation (HCT). Injection of freshly isolated muscle-derived cells from the HCT donor into either fully or partially chimeric xmd recipients restored dystrophin expression up to 6.72% of wild-type levels, reduced the number of centrally located nuclei, and improved muscle structure. Dystrophin expression w as maintained for at least 24 weeks. Taken together, these data indicate that immune tolerance to donor myoblasts provides an important platform from which to further improve myoblast transplantation, with the goal of restoring dystrophin expression to patients with DMD. Research supported by NIH grants HD47175, CA15704, CA78902, DK56465, and MDA Research Development grant 4332.

84. Antagonism of myostatin ameliorates the dystrophic phenotype in mdx mice

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In dystrophic conditions there is constant activation of satellite cells due to repeated cycles of muscle degeneration and subsequent regeneration. Recently we showed that myostatin is a powerful inhibitor of satellite cell activation and myoblast proliferation and differentiation. Here we have investigated the therapeutic potential of novel recombinant myostatin antagonists in ameliorating dystrophic muscle loss in the mdx mouse model. When tested for their ability to modulated myoblast growth in vitro, the recombinant antagonists (Mstn-ant1, 2 and 3) showed the capacity to significantly increase myoblast proliferation. Furthermore, in competition assays, they were able to overcome the anti-proliferative effect of exogenous myostatin in a dose dependent manner. Three trials of varying duration were performed to assess the ability of the recombinat proteins to antagonize myostatin in vivo: an 8 week trial with Mstn-ant1 and 2; a 4 week trial with Mstn-ant3; and a 10 day trail with Mstn-ant1. A variety of markers were used to determine the efficacy of the antagonists in ameliorating the dystrophic phenotype. High levels of serum creatine kinase (CK) is considered a marker for muscle fiber damage. Serum CK levels were reduced in the antagonist treated mice in all trials when compared to saline controls. A morphological improvement, with an increase in the regenerating area and concomitant reduction in the necrotic area, was also observed for the muscles of Mstn-Ant1, 2 and 3 treated mice when compared to controls, with an accompanying increase in both Pax7 and MyoD protein expression. However, while individual hind limb muscles from mice treated with Mstn-ant1 and 2 weighed significantly less than muscles from mice treated with saline in the 8 week trial, no weight reduction was observe in the muscles from mice in the 4 week or the 10 day trial. This suggests that a minimum treatment period is required for the decrease in muscle weig ht due to inflammation and cell infiltration. The results presented here thus suggest that peptide antagonists of myostatin are a viable option for ameliorating the dystrophic phenotype in mdx mice.

85. Antagonism of myostatin as a treatment for muscle wasting conditions

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Muscle satellite cells are a distinct lineage of myogenic progenitors responsible for postnatal muscle growth. When required, satellite cells are activated to proliferate and differentiate into myoblasts that contribute to the formation of new and existing myofibers. Myostatin is a transforming growth factor-beta superfamily member that functions as a potent inhibitor of muscle growth. Myostatin has been shown to inhibit satellite cell activation in mice, which could have significant consequences during muscle wasting and regeneration conditions such as sarcopenia, cachexia, wound healing and muscular dystrophy. We have previously demonstrated that myostatin has an active role in the regulation of myogenesis. More recently, we have conducted studies to test the therapeutic value of a myostatin antagonist during a variety of muscle wasting conditions. Antagonism of myostatin following notexin injury in aged mice led to satellite cell activation, increased Pax7 and My oD protein levels, and greater myoblast and macrophage cell migration resulting in enhanced muscle regeneration. Aged mice treated with the antagonist also displayed a 12% increase in grip strength as compared to the control group. We have also investigated the therapeutic potential of myostatin antagonists in ameliorating dystrophic muscle loss in the mdx mouse model. Treatment of mdx mice with a myostatin antagonist led to decreased CK serum levels, increased MyoD and Pax7 expression, and a morphologically improved dystrophic muscle, due to a reduction in necrotic areas and a concomitant increase in regenerating areas. Using the same antagonists in a Dexamethasone induced cachexia model, we have shown a significant increase in both Pax7 and MyoD expression, as well as a tendency for increased muscle mass in the antagonist treated mice. We therefore propose that the antagonism of myostatin has significant therapeutic potential in the alleviation of sarcopenia, the amelio ration of cachexia symptoms and the dystrophic phenotype, and in muscle regeneration following injury.

86. Readthrough-inducing ointment: the new approach for the treatment of genetic disorders caused by nonsense mutations

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The ability of aminoglycoside antibiotics, such as gentamicin (GM), to promote readthrough of premature termination codons (PTC) has attracted interest in these drugs as potential therapeutic agents in nonsense mutations. In order to measure readthrough efficiency with quantitative accuracy, we have established transgenic mouse strain containing dual-reporter gene with PTC. The objective of this study was to evaluate the transdermal drug delivery (TDD) potential for treatment of genetic diseases caused by nonsense mutations. Here, we show that skin permeability can be increased through the use of chemical enhancer. It was found that the readthrough activity by transdermal administration of GM resulted similar to those by subcutaneous injection. In vivo permeation was verified by LC-MS/MS and the reduction in the intercellular adhesiveness was observed in prickle cell layer and basal layer by electron microscopic analysis. These readthrough therapy on TDD may provide significant value in preventing genetic diseases associated with nonsense mutations.

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87. Systemic AAV-9 delivery in normal dog leads to high-level persistent transduction in whole body skeletal muscle

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Adeno-associated virus (AAV) is a promising vector for muscle disease gene therapy. The dog model represents an ideal intermediate system prior to human trials. Previous attempts to deliver AAV directly to canine muscle have largely failed to achieve efficient transduction because of a strong immune response. In this study, we evaluated systemic AAV-9 gene delivery in newborn dogs by local and systemic delivery. Transgene expression was examined at different time points after AAV infection by biopsy or whole body necropsy. In contrast to the previous reports of low expression and strong immune reaction in dog muscle, we observed efficient transduction at 4 weeks following intramuscular gene delivery. Importantly, systemic gene delivery resulted in impressive whole body skeletal muscles transduction for up to 6 months. In more than 20 different muscle groups (including head, neck, chest, abdominal, thoracic and pelvic limbs), we observed ≥ 80% transduction in the majority of muscles throughout the entire muscle length. Taken together, our results provide the first evidence that systemic AAV delivery can reach multiple muscles in a large animal and that body size is not a barrier to intravascular AAV gene transfer. Our results raise the hope of whole body correction for many muscle diseases such as Duchenne muscular dystrophy. (Supported by NIH and MDA).

88. Functional resolution of fibrosis in mdx mouse dystrophic heart and skeletal muscle by halofuginone

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The effect of halofuginone (Halo) on established fibrosis in older mdx dystrophic muscle was investigated. Mice (8-9-months) treated with Halo (or saline) for 5, 10 or 12 weeks were assessed weekly for grip strength and voluntary running. Echocardiography was performed at 0, 5, and 10 weeks. Respiratory function and exercise-induced muscle damage were tested. Heart, quadriceps, diaphragm and tibialis anterior muscles were collected to study fibrosis, collagen I and III expression, collagen content using a novel collagenase-digestion method, and cell proliferation. Hepatocyte growth factor (HGF) and alpha-smooth muscle actin (SMA) proteins were assayed in quadriceps. Halo decreased expression of collagens I and III, muscle collagen 1α(I) and SMA content. Muscle-cell proliferation increased at 5 weeks, and HGF increased by 10 weeks treatment. Halo markedly improved function, including cardiac function, reduced muscle damage, improved respiratory function, and impro ved recovery from exercise. Halo reduced the impact of dystrophy by partly resolving pre-existing fibrosis and reducing collagen synthesis in cardiac and skeletal muscles. Marked improvements in vital-organ functions implicate Halo as a strong candidate drug to reduce morbidity and mortality in DMD.

89. Cardiac specific expression of the ΔH2-R19 mini-dystrophin gene only partially restored heart function in aged mdx mice

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Heart failure is a leading cause of death in Duchenne muscular dystrophy (DMD). Effective treatments for DMD cardiomyopathy are lacking. A 6 kb ΔH2-R19 mini-dystrophin gene has been shown to completely rescue skeletal muscle pathology and restore skeletal muscle force. The potential of this minigene for heart rescue is unknown. To address this issue, we developed a series of transgenic mouse lines expressing the ΔH2-R19 minigene in the heart. We evaluated heart function after these mice were backcrossed to the congenic mdx background. Transgenic ΔH2-R19 minigene expression significantly strengthened the sarcolemma integrity and reduced Evans blue dye uptake in the heart. Surprisingly, ECG defects were only partially normalized. Closed-chest Millar catheter assay revealed a normal systolic function. Stroke volume and cardiac output were improved but did not reach those of the normal mice. Taking together, our results provide critical insight towards the understanding of the dystrophin structure-function relationship in the heart. It also calls for developing novel therapeutic strategies to treat DMD cardiomyopathy. (Supported by NIH and MDA).

90. Systemically-administered biglycan upregulates utrophin, counters dystrophic pathology and improves muscle function in mdx mice: a novel pharmacological approach for DMD therapy

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An attractive approach for DMD therapy is the pharmacological upregulation of utrophin, a dystrophin homolog that is prominent in developing muscle. The extracellular matrix protein biglycan is normally present at high levels in immature muscle and regulates the expression of signaling and structural proteins at the sarcolemma including alpha- and gamma- sarcoglycans and the dystrobrevin-syntrophin-nNOS complex. Here we tested whether biglycan treatment can ameliorate pathology and improve function in the muscles of mdx mice, the canonical animal model for DMD. A single systemic injection of recombinant human biglycan protein (rhBGN) improved the health of mdx muscle as indicated by reduced myofiber death and mononuclear cell infiltration up to three weeks later. rhBGN treatment upregulated utrophin expression as judged by immunohistochemistry and western blotting. Studies in mdx:utrophin-/- double mutant mice indicated that the ability of rhBGN to counter mu scle pathology is utrophin-dependent. Repeated rhBGN at 3wk intervals prolongs utrophin upregulation and ameliorates muscle pathology for at least three months. Finally, rhBGN treatment improved muscle function as measured by reduced susceptibility to eccentric contraction-induced muscle damage. We propose that rhBGN could be a therapeutic for DMD.

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91. Non-invasive monitoring of skeletal muscle therapy protocols by 1H-NMR imaging

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High resolution 1H-NMR imaging, combined with NMR contrast agent cell labeling is an emerging methodology for non-invasive monitoring of cell therapy in vivo.
Because of the high sensitivity of iron oxide particulates, most studies have been performed using SPIOs loading protocols. However, potential recapture by macrophages after cell death might be a serious issue and would lead to an overestimation of cell survival. More recently, lanthanide chelates have also been shown to detect grafted cells in vivo. The choice of the appropriate contrast agent is still under consideration.

We investigated SPIO and Gd-DTPA sensitivity and ability to reflect the presence of grafted cells. Taking advantage of the rapid rejection process of xenogenic transplantation, we grafted Gd-DTPA labeled, SPIO labeled and unlabeled human myoblasts into a leg muscle of immunocompetent mice. We compared the time-courses of signal detection and cells disappearance. Label outcome was monitored by NMR imaging and human cells rejection was assessed in parallel by immunohistochemistry, in a 3-month longitudinal study.

As expected, human cells were eliminated from host tissue within 1 week. Quantitative NMR evaluation showed persistence of the Gd-DTPA label in the grafted area for 2 weeks and a total disappearance on day 21. Most importantly, SPIO label was still visible after 3 months, confirming its poor relevance for therapeutic cell monitoring in vivo.

92. Imatinib mesilate (Gleevec®) ameliorates the dystrophic phenotype in exercised mdx mice

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Duchenne Muscular Dystrophy (DMD) is a neuromuscular disorder caused by mutations in the dystrophin gene. It is characterized by progressive skeletal muscle degeneration that leads to weakness and early death by respiratory and cardiac breakdown. There is no specific treatment to DMD. Preclinical tests to find new drugs that can stop or retard DMD progression are usually performed in exercised mdx mouse. One important feature in DMD is the massive muscle infiltration by immune cells and the replacement by fibrous or fatty tissue. Immunomodulators have recently emerged for DMD trials. Imatinib mesilate is a specific inhibitor of tyrosine kinases, such as Bcr-Abl, PDGFR-beta and c-Kit receptors. It also inhibits the profibrogenic activity of TGF-beta. The present study aimed to evaluate imatinib mesilate in mdx mice submitted to treadmill exercise. Four-week old mice were analyzed in the beginning and in the end of a physical activity program during six weeks conside ring histopathological evaluation of gastrocnemius and diaphragm muscles, serum creatine kinase dosage and whole body strength increment. Comparative analyses showed that 0,125 mg/mouse/day resulted in amelioration of the muscular conditions, increased force increment ($p<0.0001$, unpaired t test, n=15 and 21 for the untreated and treated group, respectively) and decreased CK levels ($p=0.0022$, n=7). Histological analyses of the gastrocnemius showed abrupt decreasing of the area occupied by injured myofibers infiltrated by Blue Evans dye ($p=0.0009$, n=3), while diaphragm showed no significant difference. Serum levels of cytokines were evaluated suggesting immunomodulation activity of the drug. Taken together, these data suggest that Gleevec® can ameliorate the dystrophic phenotype in mdx mice, and could be used as potential drug to future clinical tests.

93. Treatment with a soluble activin receptor type IIb results in increased muscle mass in marmosets.

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Myostatin, or GDF-8, is a TGF-beta family member that negatively regulates muscle mass. Myostatin overexpression causes muscle wasting while its inhibition leads to increased muscle. Mice, cows, dogs and humans with genetic removal of myostatin have increased muscle mass thereby demonstrating its function is evolutionarily conserved. Activin receptor type IIb (ActRIIb) is the high affinity receptor for myostatin and other related GDFs. Soluble ActRIIb increases muscle mass in myostatin null mice, suggesting other negative regulators of muscle exist. To study the effects of ActRIIb inhibition in a species closer to humans, common marmosets were treated with a soluble ActRIIb-Fc fusion protein (ACE-031). Weekly administration of ACE-031 at 3 mg/kg resulted in significantly increased lean tissue mass (Echo MRI) at 2 and 4 weeks. At 4 weeks, the treated group had gained 4.3 times more lean tissue (vehicle: +3.45 ± 7.7 g, ACE-031: +14.83 ± 8.5 g) as compared with control animals. These findings show that inhibition of the negative regulators of skeletal muscle with ACE-031 increases total lean tissue in a non-human primate model, suggesting utility in the treatment of muscle disorders in humans. Funded by Acceleron Pharma.

94. A novel myostatin inhibitor attenuates muscle loss and improves muscle function in response to hypoxia

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Reductions in respiratory function contribute significantly to the morbidity and mortality of neuromuscular diseases; respiratory failure being the most common cause of death in DMD. In addition to changes in respiratory function, skeletal muscle function is compromised in DMD, possibly via hypoxia-mediated mechanisms. We have previously demonstrated that hypoxia is a stimulus for the rapid onset of muscle atrophy (Willmann et al., ICNMD 2006). Therefore, we hypothesized that myostatin blockade would attenuate loss of muscle mass & function induced in a hypoxic environment. We treated male C57BL/10 mice with either a soluble ActR-IIB receptor (RAP-031, Acceleron Inc.) or PBS for a 2-week period followed by exposure to hypoxia. Bodyweight of RAP-031 treated mice was 9% greater than control mice following hypoxia. Weights of the EDL, soleus, gastrocnemius, quadriceps, and tibialis anterior muscles were a>20% greater in RAP-031 treated mice following hypoxia, with a concomitant improvement of muscle function in the EDL. These results suggest that myostatin blockade via a soluble ActR-IIB receptor can attenuate body- and muscle weight loses in response to hypoxia. Funding: WADA, Acceleron.

95. The six minute walk test as a clinical trial outcome measure in Duchenne muscular dystrophy (DMD): Reliability and correlation with disease progression and clinical timed function testing

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Background: The six-minute walk test (6MWT) is a commonly used measure of cardiorespiratory endurance. Researchers have begun to use it as a strength-related outcome measure in clinical trials in neuromuscular disease. We evaluated the 6MWTs differentiation between boys with DMD and healthy controls, test-retest variability in DMD boys and correlation of the test with timed functional measures. Methods: We enrolled boys 5-12 years old with DMD (n=15) and without (n=20). Boys with DMD were tested 7 days apart using a modified American Thoracic Society 6MWT and standard clinical timed function testing. Healthy controls underwent testing for one time point only. Results: Distance traveled differed between boys with DMD and healthy controls across all ages. In boys aged 5-6, those with DMD averaged 367 +/- 74M vs. the healthy controls 574 +/- 35M ($p<.01$). In boys aged 7-9, those with DMD averaged 354 +/- 31M vs. the healthy controls 622 +/- 50M ($p<.001$). In boys aged 10-12, those with DMD averaged 265 +/- 146M vs. the healthy controls 646 +/- 49M ($p<.001$). As age increased in the DMD group, % predicted scores decreased from 82% at age 5 to 56% at age 12. Test-retest correlation was high ($r=.92$). 6MWT correlated well in DMD with time to walk 10M ($r=.80$), time to walk 25M ($r=.80$), time to climb 4 standard stairs ($r=.77$). Conclusion: A modified 6MWT in ambulatory boys with DMD is reproducible, differentiates DMD and controls at all ages, and correlates with disease severity.

96. Quantitative characterisation of dystrophic muscle in GRMD dogs by NMR imaging.

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The Golden Retriever Muscular Dystrophy dog lacks dystrophin and shares pathological and clinical similarities with the Duchenne patients. The model, increasingly used in pre-clinical trials, needs to be further characterized. We defined NMR imaging indices of canine dystrophic muscle. Six two-month old control and 6 GRMD dogs were examined at 4 T. Three control and 5 GRMD dogs were examined at 3 T at the age of 2, 4 and 6 months. Standard and fat-saturated T1-weighted images were acquired, followed by T2-weighted images. After Gd-DTPA injection, the time-course of muscle enhancement was monitored with fat-saturated T1-weighted imaging during 2 hours. Extensor carpi radialis and flexor carpi ulnaris were studied. Indices were calculated as follows: T2w/T1w signal ratio (SR= T2w Signal x T1w ref gain)/ T1w Signal x T2w ref gain), T1w and T2w heterogeneity ($H=\sqrt{(SD2- SD2noise/0,655)}$). An exponential decay was fitted to the signal decrease post Gd-DTPA injection; maximal relative enhancement (RE) and time-constant of decay were compared. A three-way analysis of variance was performed. T2w/T1w SR, H2 and RE were found significantly increased in dystrophic muscles at 4 T. These findings were confirmed at 3 T, at all ages. H1 was also found significantly increased in dystrophic muscle at 4 and 6 months at 3 T. These quantitative indices differentiate dystrophic from normal muscles and might be proposed as non-invasive evaluation tools of therapeutic trials. This work was supported by the Association Francaise contre les Myopathies.

97. TAT-Utrophin crosses cell barriers to combat dystrophin deficiency

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Upregulation of the dystrophin homolog utrophin by gene delivery or pharmacological means is thought to provide one possible mechanism to restore membrane integrity and combat the phenotypic effects of dystrophin deficiency. However, despite significant effort, no therapeutic interventions are currently available. Here we identify a novel protein-delivery therapy and show that repeated intraperitoneal injections of cell-penetrating recombinant TAT-utrophin protein into the dystrophin-deficient mdx mouse elevated utrophin levels in all tissues examined, partially restored muscle membrane integrity, reduced the prevalence of muscle fibrosis and degeneration, and improved physiological performance in a dose-dependent manner. These results triple the known size capacity for effective TAT-mediated cell transduction and establish the feasibility of TAT-utrophin as a novel protein-based therapy for the treatment of skeletal muscle and cardiac diseases caused by loss o f dystrophin.

98. Prednisolone and Inactivity Effects on Recovery Following Eccentric Injury in mdx Mice

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Current treatment for DMD is chronic administration of the glucocorticoid prednisone. Prednisone can improve muscle function, but the mechanism is unknown. The purpose of this study was to determine if prednisone treatment in dystrophic mice aids in muscle recovery following a physiological injury. Male mdx mice received a prednisolone pellet (n=16; 1.5 mg) or placebo pellet (n=8) at 4-wks of age; half of the prednisolone mice were restricted in their physical activity. After 6-wks of treatment, the left anterior crual muscles underwent 150 eccentric contractions in vivo. Isometric torque was measured 2 min after the injury protocol and functional recovery was assessed 2 wks later. All groups underwent about a 75% reduction in isometric torque following the injury protocol indicating that prednisolone does not protect muscle from being injured. Two wks later, recovery of in vivo isometric torque, in vitro EDL specific force, and muscle masses were slightly better in the Prednisolone than the Placebo mice. The Prednisolone mice that were restricted in their physical activity recovered the best in terms of EDL specific force (24% higher than Placebo and 12% higher than Prednisolone). These data suggest that while prednisolone may not prevent force loss following eccentric injury, it may help in the recovery process, particularly if physical activity is minimized.

99. Suppressing DMD frameshift mutations.

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We tested the ability of antisense oligonucleotides (AOs) to induce actively translating ribosomes to shift reading frames with the goal of suppressing DMD frameshift mutations. We show that AOs can induce ribosomal frameshifting to high efficiency (>40%) at known frameshift prone sequences. We have also tested human and murine DMD frameshift mutations for suppression by AO treatment. Depending on the mutation, and chemistry of the AO, induced frameshifting varies from <1% to >15%. Optimization of AO design to increase suppression efficiency is underway. As both small and large insertion/deletion frameshift mutations account for ~40% of all DMD cases, AO frameshift suppression therapy could impact a large number of patients. Supported by NIH/NINDS NS051792, and the MDA.

100. Delivery of Mini-dystrophin: A head-to-tale of two recombinant adeno-associated viral constructs

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Recombinant adeno-associated virus (rAAV) gene transfer technology represents a promising therapeutic option for many diseases. However, this system has long been hindered by the small (~4.8 kb) DNA packaging capacity. This limitation results in the exclusion of therapeutically critical regulatory elements and/or larger coding sequences such as the ~14 kb dystrophin cDNA that is mutated in patients with Duchenne muscular dystrophy (DMD). We have demonstrated reconstitution of a highly functional mini-dystrophin protein (ΔH2-R19, 6.2 kb) both in vitro and in vivo following codelivery of two independent rAAV genomes pseudotyped with serotype 6 capsid. In this system, similar to previously reported trans-splicing approaches, one rAAV vector provides the CMV promoter/enhancer region to allow for ubiquitous expression while the second provides the SV40 polyadenylation signal. This design further allows for the separation of transcriptional initiation and processing events, ensuring that only those genomes having undergone the correct intermolecular recombination event will yield the desired protein. These studies provide evidence that functional dystrophin proteins larger than that carried by a single rAAV genome can be reconstituted by codelivery of two rAAV vectors via recombination. Further, the levels of protein attained are consistent with those present in treated dystrophic mouse models demonstrated to have a therapeutic benefit.

101. Osteopontin Is A Modulator Of Early Necrotic And Late Fibrotic Processes In Mdx Muscle

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Leukocytes invade dystrophin-deficient muscles and presumably exacerbate necrosis and promote fibrosis. To understand the nature of the T cell infiltrate in mdx muscles, we isolated leukocytes and analyzed the T cell receptor (TCR) Vbeta usage. T cells bearing the Vbeta8.1/8.2 TCR predominated, which were CD4 / CD8 negative and expressed NK markers such as DX5, suggesting they might be NKT cells. The Vbeta8.1/8.2 cells from mdx muscle expressed Valpha14 but did not bind CD1d, indicating that they are not conventional NKT cells. Treatment of mdx mice with an antibody that inactivates NK and NKT cells was not effective in reducing disease markers. Cell sorting of Vbeta8.1/8.2 cells showed that they express Osteopontin (Opn), a secreted phosphoprotein that can bind to integrins. Ablation of Opn in the mdx mouse (by crossing with the Opn knock out) showed that some disease markers were improved at 4 wks of age, such as serum CK and strength by wire test. These impro vements were not maintained at 6 mo except that the Opn null/mdx had a significant reduction in diaphragm fibrosis. These studies suggest that Opn may be a viable therapeutic target for treating early necrotic and late fibrotic processes in muscular dystrophy.

102. The Relationship Between Regional Body Composition and Quantitative Strength in Facioscapulohumeral Muscular Dystrophy (FSHD)

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Objectives: Assess in Facioscapulohumeral muscular dystrophy (FSHD) and able-bodied controls (1) the regional body composition using a three compartment model of lean tissue mass (LTM), fat tissue mass (FTM), and bone mineral content (BMC) and (2) the correlation of regional LTM and the corresponding regional strength. Design: Cross-sectional, criterion standard, case-control study evaluating eighteen FSHD subjects and eighteen anthropometrically matched controls. A DEXA scanner was used to obtain regional and whole body composition measurements of LTM, FTM, and BMC. A dynamometer determined peak isometric strength of flexion and extension for both the elbow and knee. Results: FSHD showed a significantly increased regional and whole body fat tissue mass (FTM) ($p<0.001$ to 0.017), decreased regional and whole body lean tissue mass (LTM) ($p<0.001$ to 0.010) except for the forearm, and decreased peak isometric strength for all regions measured ($p<0.001$ to 0.020). There was also a significant correlation between the quantitative strength and LTM for both FSHD and controls ($r=0.791$ to 0.906; $p<0.001$). Conclusions: FSHD subjects have higher regional and whole body FTM and lower LTM, and the regional LTM correlates well with strength in both groups.

103. Assessment of Regional Body Composition with Dual Energy X-Ray Absorptiometry in Duchenne Muscular Dystrophy: Correlation of Lean Body Mass and Strength.

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Objectives: Assess (1) the regional body composition especially regional percentage lean mass with dual energy x-ray absorptiometry (DEXA) in Duchenne muscular dystrophy (DMD) and able-bodied controls and (2) the correlation of regional lean mass and the corresponding regional strength. Design: Cross-sectional, criterion standard, case-control study evaluating 23 DMD subjects and 23 controls. A DEXA scanner was used to obtain regional and whole body composition measurements of lean body mass. A dynamometer determined peak isometric strength of flexion and extension for both the elbow and knee. Results: DMD showed a significantly decreased regional lean body mass percentage ($p<0.001$) and increased regional fat mass percentage ($p=0.015$ to 0.064). There was also a significant correlation between the elbow and knee quantitative strength and regional lean body mass for controls ($r=0.832$ to 0.947; $p<0.001$); however, DMD only showed a significant correlation between kne e quantitative strength and regional lean body mass ($r=514$; $p=0.012$). In addition, the knee strength per regional lean body mass trended down with increased age in DMD ($r=-0.180$) but trended up in the controls ($r= 0.359$). Conclusions: DMD subjects have lower regional lean body mass and increased regional fat body mass, and the regional lean body mass correlates with strength.

104. Stable Genome Alteration of the Dystrophin Gene for DMD Due to Frame-Shift Mutations Using Oligonucleotide-Mediated Exon Skipping.

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Somatic gene therapy represents the best option in the treatment of Duchenne muscular dystrophy (DMD). We have explored gene repair of the dystrophin gene in animal models of DMD using oligonucleotides. This type of approach presents several advantages over other non viral mediated technologies. Gene correction is permanent, genes that have undergone repair remain under the control of their own regulatory mechanisms and problems of long term toxicity resulting from continuous administration of the therapeutic agent are reduced.

The designing of oligonucleotides capable to direct the repair process specifically on the genomic sequence has the potential to significantly increase the repair process. We have tested the ability of oligonucleotides containing CpG modifications to activate MBD4, a major protein component of the base excision repair mechanism. The initial studies were performed in vitro and were followed by studies of dystrophin gene repair in vivo using the mdx model for DMD. As a target for the single base substitution we have chosen the splice boundary of exon 23 of the mouse dystrophin gene in order to induced exon skipping to bypass the nonsense mutation and induce expression of internally deleted but functional dystrophin proteins. Dystrophin protein expression was followed over prolonged period of time in treated muscles to asses for stability of repair. This data were complemented by studies at the messenger and genomic level.

105. Leucine increases muscle stress output of MDX mice

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Duchenne muscular dystrophy (DMD) is a lethal, progressive disease, characterized by muscle wasting and weakness. The mechanisms responsible for the onset and progression for the disease are not presently known. Thus, therapeutics to counter loss of muscle function would be advantageous. The branched chain amino acid leucine enhances muscle growth through increased phosphorylation of the translation factors 4E-BP1 and S6K1. We tested the hypothesis that MDX mice provided drinking water with leucine (114mM, MDXL) from age 3-7 weeks would increase extensor digitorum longus (EDL) muscle mass and stress output compared to MDX mice given normal drinking water (MDX). After 4 weeks of treatment, mean body mass (MDXL, 27.0 g; MDX, 25.5 g) and EDL muscle length, mass and cross-sectional area were not different between MDXL and MDX mice. Nevertheless, the MDXL vs the MDX stress–frequency response was increased ($p < 0.05$). Max stress output increased 20% in MDXL comp ared to MDX (30.5 vs 25.4 g/mm2, respectively, $p<0.05$). MDXL muscles demonstrated no changes in stiffness, fatigue, or fatigue recovery compared to MDX. A 4-week treatment with leucine enhanced stress output in mdx skeletal muscle, but not due to increased muscle mass. The mechanism(s) responsible is(are) at present undetermined. This research was supported by NIH RO1AR049881 (RWG).

106. LMP-420: A Transcriptional Inhibitor of TNF with Therapeutic Potential for Muscle Diseases

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The mdx mouse, a Duchenne Muscular Dystrophy model, shows susceptibility to muscle injury and high levels of serum creatine kinase (CK); a phenotype intensified by exercise. Muscular degeneration is exacerbated by a chronic inflammatory process in which tumor necrosis factor alpha (TNF) has a central role. TNF blockade, with either specific antibodies or soluble receptors, reduces necrosis in mdx mice. LMP-420, a purine nucleoside analog, is an orally-active inhibitor of TNF transcription that could be more easily administered than TNF antagonists requiring injection. The effect of 25 mg/kg/day of LMP-420 in 4 week-old mdx mice submitted to a treadmill running program for 5 weeks was evaluated using the following parameters: quantitative histopathological analyses of gastrocnemius and diaphragm and CK levels. LMP-420 treated mice showed significantly-decreased CK levels and amelioration of muscular degeneration, fewer inflammatory infiltrates, decreased degenerati on/regeneration ratios and Evans blue dye infiltrated myofibers. Our data suggest that the inhibition of TNF production by a small molecule transcriptional inhibitor is beneficial for retarding dystrophinopathy and may lead to an option for future clinical trials for DMD.

107. Reduced muscle necrosis and long-term benefits in dystrophic mdx mice after cV1q (blockade of TNF) treatment

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Tumour Necrosis Factor (TNF) is a potent inflammatory cytokine that appears to exacerbate damage of dystrophic myofibres in-vivo. The mono-clonal murine specific antibody cV1q (Centocor USA) that blockades TNF demonstrated significant anti-inflammatory effects in dystrophic mdx mice (an animal model for DMD). cV1q administration significantly protected dystrophic myofibres against necrosis in both young and in adult mdx mice subjected to 48 hours voluntary exercise. Long-term studies (up to 90 days) in voluntarily exercised mdx mice showed beneficial effects of cV1q treatment with reduced histological evidence of myofibre damage and a striking decrease in serum creatine kinase levels. A convincing measure of improved muscle function in cV1q treated mdx mice was that they ran significantly more than control mdx mice, further demonstrating protective effects of anti-TNF therapy on dystrophic muscle function. In the absence of exercise long-term studies with cV1q di d not reduce dystropathology in mdx mice. These long-term studies with cV1q in exercised (and unexercised) mdx mice (1) emphasise the importance of exercise for drug testing in this dystrophic mouse model and (2) confirm the benefits of anti-TNF drugs on dystrophic muscle. These data support an important role for inflammation in exacerbation of muscular dystrophy and suggest new drug interventions (much already in wide clinical use) to reduce the clinical severity of DMD.

108. siRNA Mediated Allele Specific Silencing of Dominant Negative COL6A3 Mutation Causing UCMD

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Congenital muscular dystrophy type Ullrich (UCMD) leads to severe weakness in early childhood. It is caused by mutations in collagen VI genes (COL6A1-3). Heterozygous exon skipping in N-terminal part of triple helical domains of COL6 genes are frequent cause of UCMD. In-frame skipping of exon 16 in the alpha3(VI) mRNA is the most frequent one in which the mutant chain interferes with the wildtype allele exerting a strong dominant negative effect on COL6 assembly. Haploinsufficiency for the alpha3(VI) chain is asymptomatic. We investigate RNAi mediated knockdown of the dominant negative transcript as a therapeutic option, ideally transforming the dominant negative situation to an asymptomatic haploinsufficiency state. We designed siRNA oligos targeted at exons 15/17 junction created by loss of exon 16 in the alpha3(VI) transcript. We co-transfected siRNA oligos with plasmids expressing GFP fused wild type or mutant COL6A3 triple helical domains into 293T cells. We show that one oligo (siRNA#1) is able to knock down the mutant COL6A3 target specifically. We then transfected siRNA oligos into dermal fibroblasts from UCMD patients with this mutation. We show by RT-PCR and immunostaining that siRNA#1 specifically silenced the mutant transcript with improved extracelluar COL6 deposition. Chemical chaperones have been used to ameliorate ER stress caused by protein misfolding such as occurs in the dominant COL6 mutations. Combining siRNA with chemical chaperones appeared to further improve extracellular Col6 deposition in patient cells. This study shows that siRNA mediated approach has the potential to specifically silence dominant negative mutations causing severe UCMD, thus establishing RNAi as a promising molecular approach for treatment of dominant negative UCMD. This work was supported by grants from MDA USA (MDA3896) and NIH/NIAMS (R01AR051999) to CGB and by NIH grant AR053251 to MLC.

109. Beyond the bench and towards the bedside: Resources designed to facilitate clinical studies of the burdens of muscle disease.

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As explored at the NIH sponsored Burden of Muscle Disease Workshop (January 26-27, 2005), muscular dystrophy symptoms impact the quality of life (QoL) and economics of patients and caregivers (www.niams.nih.gov/News_and_Events/Meetings_and_Events/). Large populations of patients, along with validated and more efficient measurement of QoL are needed. Our purpose is to describe current NIH resources designed to facilitate large scale assessments of QoL and recruitment of patients into clinical studies. The NIH sponsored Patient-Reported Outcomes Measurement Information System (PROMIS) is an initiative designed to revolutionize the way patient-reported outcome tools are selected and used in clinical research and practice evaluation. It aims to establish a national resource for accurate and efficient measurement of patient-reported symptoms and other health outcomes in clinical practice. The NIH sponsored Registry of Myotonic Dystrophy (DM) and Facioscapulohumeral Muscular Dystrophy (FSHD) Patients and Family Members is designed to facilitate research by making available to investigators anonymous data on Registry members and by helping investigators recruit subjects into their clinical studies. It also provides educational materials to patients and family members. Combining QoL tools with improved physical measurements will provide a firm basis for integrative approaches to the management of muscular dystrophy.

110. Moving to human trials- the TREAT-NMD approach

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TREAT-NMD is a European Union funded Network of Excellence aimed at accelerating trials of cutting edge therapies in rare inherited neuromuscular diseases. As such is seeks to harmonise practice in this area and integrate efforts so that there is greater collaboration and progress can be enhanced. The network addresses the pathway from the identification of promising molecules and how these are identified in a consistent way to the delivery of therapies to the patients. A crucial aspect of this is the "trial readiness" of the target population. The initial phases of the network have focussed on DMD and SMA, disseminating and developing standards of care and diagnosis and working on registries and trial networks. The approach is now broadening to other neuromuscular diseases. The network does not seek to be exclusive, and is working collaboratively with many groups worldwide. Funding is from the European Union Framework 6 programme.

111. Progression of physical limitations of myotonic dystrophy type 1 (DM1) patients enrolled in the NIH Registry and discussion of endpoint measures in clinical trials

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Many forms of muscular dystrophy exhibit significant symptoms beyond muscle degeneration and weakness. To develop effective treatments requires measuring the effect of the interventions in a way that allows comparisons with what is known about normal progression of disease. Our purpose is to analyze the progression of physical limitations in myotonic dystrophy type 1 (DM1) patients. The NIH sponsored Registry of DM and FSHD Patients and Family Members has enrolled 589 DM1 patients (average age 44.2 y ± 15.6; 50.3% female). Average CTG size is 531.3 ± 432.5 (n=271).The % of individuals experiencing a life changing event or developing a new requirement for adaptive aid is low in patients enrolled in the Registry greater than 1 yr and up to 6 yrs (n=505 individuals with 1,380 person years of follow up). Data indicated that 2.7% of patients died, 0.9% lost employment, 1.6% received a pacemaker, 1.7 % required use of a leg brace, and 2.6% required use of a wheelchair. Data indicates that physical milestones may not be an appropriate endpoint measure in clinical trials. Potential surrogate measures in DM1 trials may include measures of muscle strength, muscle mass, manual dexterity, and timed functional tests. Further studies are needed to develop a more comprehensive evaluation of disease progression and how these manifestations impact clinical care, quality of life, economic burden, and endpoint measures in clinical trials. Funding: NIH Contract NO1-AR-5-2274.

112. Optimization of therapeutic antisense-mediated exon skipping for Duchenne muscular dystrophy

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Antisense-mediated exon skipping is one of the most promising therapies for Duchenne muscular dystrophy. It employs antisense oligoribonucleotides (AONs) to induce exon skipping during pre-mRNA splicing of DMD transcripts, restoring the reading frame and generating partially functional Becker-like dystrophins. Proof of concept was obtained in cultured muscle cells from 15 patients. Recently, exon 51 skipping and local dystrophin restoration was shown in 4 patients treated with a single intramuscular AON injection (van Deutekom et al., this meeting). Currently, we are optimizing systemic delivery of AONs for subsequent clinical trials. In parallel, we are assessing the specificity and bio-distribution of different AON chemistries after local and systemic treatment. Notably, our hDMD model contains a complete copy of the human DMD gene stably integrated into the mouse genome and allows testing of human specific AONs in vivo. First results indicate that morpholino an d 2'-O-methyl phosphorothioate AONs are equally efficient for most human target sequences, but that morpholinos may be less sequence specific. Finally, we are furthering our work on multiexon skipping (AJHG 2004, 74:83-92) to broaden the mutational scope of this approach.

113. Preclinical Drug Studies In Mdx Mice: A Challenge To Speed Up Pharmacotherapy Of Duchenne Muscular Dystrophy

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Prompt-to-use drugs targeting disease-related pathways can lead to a safer than steroids pharmacotherapy of Duchenne Muscular Dystrophy. Moreover, drugs may help to avoid rejection and enhance efficiency of gene/cellular therapies in the future. Here comparative data of a large in vivo preclinical screening on the model of exercised mdx mice are presented. Outcome of drug treatment is evaluated on a large array of in vivo and ex vivo disease-related parameters by means of a multidisciplinary approach. Immunosuppressive/anti-citokine drugs (cyclosporine A, etanercept) contrast exercise-induced weakness, degeneration-related impairment of muscle chloride channels, high levels of creatine kinase and of pro-fibrotic TGF-beta1 as well as muscle degeneration. Similar results are observed after a partial restoration of dystrophin by a chronic gentamicin treatment, suggesting that the inflammation-dependent degeneration can be highly sensitive to dystrophin presence, and supporting the possible synergism of therapies able to control inflammation and to enhance dystrophin level. By contrast, the alteration of calcium homeostasis is unrelated to inflammatory cascade and is more strongly affected by the absence of dystrophin. This alteration requires more direct drug action on the pathways related to abnormal calcium entry, as obtained with either the phosphodiesterase inhibitor pentoxifylline or taurine (Telethon-Italy GGP05130).

114. Myostatin blockade in a murine knockout model of limb girdle muscular dystrophy type 2G (LGMD2G)

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LGMD2G has been described in humans as a muscular pathology occurring due to absence of the sarcomeric protein, TCAP. Until now, research in LGMD2G has been limited by the lack of an available animal model. We propose to perform the first comprehensive assessment of function in TCAP-null mice, and to further characterize the phenotype by describing muscle function following tissue damage. In keeping with a clinical interest in treatment of muscle wasting, we will investigate whether myostatin blockade ameliorates dystrophic muscle pathology observed in TCAP (-/-) mice by crossing TCAP (-/-) mice with myostatin (-/-) mice. We anticipate that the resulting TCAP/myostatin (-/-) double knockout will improve muscle function observed in TCAP (-/-) mice, and establish a foundation for use of myostatin-blocking agents in patients. Here, we will describe prerequisite experiments: 1) confirmation of knockout (KO) status, and 2) preliminary rotor-rod performance data, w hich indicates a tendency towards a functional deficit in TCAP-null mice. KO was confirmed using standard western blotting techniques with both a custom N-terminus-specific TCAP antibody, and a commercially available TCAP antibody. Performance on the rotor-rod was assessed longitudinally and was poorer in TCAP KO mice vs controls (n=5-34 mice/group) at 2 months (62± 36 vs 77±20 sec, mean±SD), 3 months (65±33 vs 72±34 sec), and 4 months (56±27 vs 71±14 sec).

115. siRNA Mediated Allele Specific Selective Silencing of a Dominant Negative COL6A3 Mutation Causing UCMD

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Congenital muscular dystrophy type Ullrich (UCMD) leads to severe weakness in early childhood. It is caused by mutations in the three collagen VI genes (COL6A1-3). Heterozygous exon skipping mutations in the N-terminal part of the triple helical domains of COL6 genes are frequent causes of UCMD. In-frame skipping of exon 16 in the alpha 3(VI) collagen mRNA is the most frequent mutations in which the mutant chain interferes with the wild type allele exerting a strong dominant negative effect on COL6 assembly. Haploinsufficiency for the alpha 3(VI) chain is asymptomatic. We investigated RNAi mediated knockdown of the dominant negative transcript as a therapeutic option, ideally transforming the dominant negative situation to an asymptomatic haploinsufficiency state. We designed siRNA oligos targeted at the exon 15/17 junction created by the loss of exon 16 in the alpha 3(VI) transcript. We co-transfected siRNA oligos with plasmids expressing GFP fused wild type or mutant COL6A3 triple helical domains into 293T cells. We show that one oligo (siRNA#1) is able to knock down the mutant COL6A3 target specifically. We then transfected siRNA oligos into dermal fibroblasts from UCMD patients with this mutation. We show by RT-PCR and immunostaining that siRNA#1 specifically silenced the mutant transcript leading to improved extracellular COL6 deposition. Chemical chaperones have been used to ameliorate ER stress caused by protein misfolding such as occurs in the dominant COL6 mutations. Combining siRNA with chemical chaperones appeared to further improve extracellular COL6 deposition in patient cells. This study shows that siRNA mediated approach has the potential to specifically silence dominant negative mutations causing severe UCMD, thus estab lishing RNAi as a promising molecular approach for treatment of dominant negative UCMD. This work was supported by grants from MDA USA (MDA3896) and NIH/NIAMS (R01AR051999) to CGB.

116. Pre-clinical and clinical development of 2OMePS antisense oligonucleotides for Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) patients suffer from a progressive, severe muscle-wasting disease due to frame-disrupting mutations in the DMD gene and a complete loss of functional dystrophin. Antisense oligonucleotide compounds (AONs) have recently shown therapeutic promise for DMD patients. By inducing specific exon skipping during mRNA splicing AONs have been successful in repairing the open reading frame in cultured muscle cells from patients, as well as in the *mdx* mouse model. This resulted in novel expression of internally deleted, partially functional dystrophin proteins as found in the less severe Becker muscular dystrophy patients (BMD). We recently completed a successful proof-of-concept study, demonstrating safety, tolerability, and dystrophin restoring effect of a single, intramuscular dose of antisense oligonucleotide PRO051. Based on positive results with 2OMePS antisense compounds in non-clinical pharmacodynamic, pharmacokinetic and toxicity studies in mice, rats and monkeys, we are now preparing for phase I/II studies. An ascending-dose, open label study will be done in 5-16 year old DMD patients. Eligible patients will receive PRO051 via systemic injections. Several safety parameters and muscle function and strength will be assessed before, during and after treatment. Muscle biopsies will be taken to determine specific exon skipping and dystrophin protein expression.

117. Muscle function recovery in dystrophic dog after exon skipping gene therapy

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Duchenne Muscular Dystrophy (DMD) is an X-linked recessive disorder due to mutations in the gene that encodes dystrophin. Most of these mutations consist in large genomic deletions, although their extent is not directly correlated with the severity of the phenotype. Out-of-frame deletions lead to abortion of translation, dystrophin deficiency and severe DMD phenotypes, while internal deletions that produce in frame mRNAs leading to shorter proteins are responsible for a milder myopathy known as Becker Muscular Dystrophy (BMD) 1. About 80% of the out-of-frame mutations could be theoretically rescued after restoring the translational frame by using exon skipping strategies 2. Here we used gene transfer in a large animal model of DMD, the Golden Retriever Muscular Dystrophy (GRMD) dog 3, to achieve the precise skipping of multiple exons spaced over 125,000 bp of the dystrophin pre-mRNA and the re-expression of a functional protein. This led to sustained correction of the dystrophic phenotype in extended muscle areas and muscle strength recovery. Exon skipping was obtained with U7snRNAs (U7smOPT) 4 carrying antisense sequences designed to mask determinants of exon 6 and 8 definition. These U7smOPT were introduced into skeletal muscle fibres by using Adeno Associated Viral (AAV2/1) vectors. After two months, levels of dystrophin were almost normal in transduced fibres. Histological examination revealed that the dystrophin glycoprotein complex was restored and that spontaneous muscle damages were stopped. Muscle architecture was fully corrected and fibres displayed the hallmarks of mature and functional units. Muscle force as well as NMR imaging indices reflecting muscle structural and functional integrity were improved. Our study documents for the first time the recovery of dystrophin at the scale of entire limbs in a large animal and thus represents a critical milestone for the development of clinical trials in human patients.

NUCLEAR MEMBRANE

118. Deletion of lamin A/C lysine 32 is responsible for abnormal muscle maturation associated with proliferation and differentiation defects in mice

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Lamin A and C, encoded by LMNA gene, localize at the inner face of the nuclear membrane and interact with multiple proteins and DNA. Mutations reported all along the LMNA gene are responsible of multiple diseases including Emery-Dreifuss muscular dystrophy (EDMD). Among them, the deletion of the lysine 32 leads to a severe phenotype with the first clinical signs appearing before the age of 2 and a loss of ambulation before the age of 10. Homozygous knock-in mice reproducing this mutation show a pronounced reduction of lamin A/C levels. At birth, they are undistinguishable from their wild type littermates but they rapidly develop an overall growth retardation in weight and size and die around 15 days of life. Histological analysis of mutant muscles shows a reduction of fiber cross section area, an increased proportion of fibers with central nuclei and embryonic myosin heavy chains expression compared to wild type littermates resembling a maturation defect. Culture of mutant myoblasts shows a reduced proliferation rate and a delayed differentiation with major nuclear abnormalities: several nuclear proteins interacting with lamin A/C are mislocalized, the chromatin is abnormally distributed and the nuclear shape is severely altered.

119. Lmo7, an emerin-binding transcription activator, regulates C2C12 myoblast differentiation

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X-linked Emery-Dreifuss muscular dystrophy (EDMD) is inherited through mutations in EMD, which encodes the inner nuclear membrane protein emerin. EDMD is characterized by progressive skeletal muscle weakening, contractures of major tendons, heart muscle dysfunction and life-threatening irregular heart rhythms. We identified Lmo7 as a high-affinity (125 nM) emerin-binding protein that is specifically disrupted by the EDMD-causing P183H emerin mutation. Mice lacking Lmo7 have dystrophic muscles, suggesting that Lmo7 is important for muscle regeneration or function. Lmo7 regulates the expression of a number of muscle and cardiac genes, and interacts with four of these genes (CREBBP, NAP1L, LAP2 and EMD) by chromatin immunoprecipitation. Lmo7 was downregulated in C2C12 myoblasts to test if Lmo7 regulates myogenic differentiation. Importantly, downregulation of Lmo7 resulted in a 4.5-fold decrease in myotube formation after six days and overexpression of Lmo7 caused a 3.2-fold increase in myotube formation after five days. Lmo7 downregulation resulted in decreased myogenin and MyHC expression, but no change in MyoD or Myf-5 expression was detected. These results suggest a model whereby Lmo7 regulates genes important for myoblast commitment to differentiation or fusion, or both. We propose that misregulation of Lmo7-mediated gene expression and subsequent muscle regenerative dysfunction contributes to the X-EDMD phenotype.

120. Mice Expressing Mutant Nesprin-1 Display Emery Dreifuss Muscular Dystrophy.

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Mutations in the inner nuclear membrane proteins lamins A and C cause diseases affecting skeletal and heart muscle. Produced from a single gene, lamins A and C contribute to the LINC complex that LInks the Nucleoskeleton with the Cytoskeleton via the nesprin and SUN proteins. Nesprins 1 and 2 are giant spectrin-repeat containing proteins that have long and short isoforms. The nesprins contain a transmembrane anchor that tethers to the nuclear membrane followed by a domain that resides within the lumen between the inner and outer nuclear membrane. The luminal domain binds directly to SUN proteins. In order to investigate nesprin-1, we generated mice expressing carboxy-terminally truncated nesprin-1. This strategy produces nesprin-1 lacking its transmembrane and luminal domains that together are referred to as the KASH domain. Mice carrying this mutation are born in non-Mendelian ratios with approximately half dying at birth from respiratory failure. Mutant muscle displayed mislocalization of nuclei under the neuromuscular junction as well as a reduced number of neuromuscular junctions and hindlimb weakness. As in Emery Dreifuss muscular dystrophy, with age the mice develop contractures affecting the spine and producing kyphoscoliosis, as well as cardiac conduction defects. These data demonstrate the importance of the LINC complex for normal neuromuscular function. Supported by MDA.

REPEATS AND CONTRACTIONS IN MUSCULAR DYSTROPHY

121. Functional studies of the mouse Dux4 homologue

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Facioscapulohumeral muscular dystrophy (FSHD) is caused by deletions within the D4Z4 tandem array on chromosome 4q35. Although the pathological mechanism underlying the disease is till unclear, one model proposes that the deletions disrupt or perturb expression of a putative homeobox gene (DUX4) encoded by the repeat. We have identified D4Z4 orthologues in Afrotheria (elephants and related species), the first time that any D4Z4 homologue was shown to exist in non-primate species. We also identified a homologues array in mice and rats. Comparison of the organization of mammalian D4Z4 loci identifies two very striking properties. First, there is conservation only of the DUX4 ORF. Second, all of the homologues are organized as multiple copies in a head to tail arrangement, where the repeat units within an array are almost identical within a species but differ between species. Expression of the mouse Dux array was confirmed by RT PCR, RNA fluorescence and tissue in si tu hybridization. We are currently examining the functions of the mouse Dux protein with the aim of developing in vivo and in vitro models of FSHD. Funded by the MDA, USA, the FSH Society and The Medical Research Council, UK.

122. CTCF couples insulation and perinuclear localization of the D4Z4 subtelomeric element in FSHD but not in control cells.

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Disorders of different levels of the epigenetic regulations have been observed in numerous human pathologies. Both genetic and epigenetic alterations contribute to the Facio-Scapulo-Humeral Dystrophy (FSHD) linked to the reduction of a number of D4Z4 repeated elements at the 4q35 locus. The consequence of this rearrangement remains enigmatic but deletion of this repeat to a threshold of 11 copies might epigenetically dysregulate the FSHD-gene(s) in patients through position effect variegation (PEV) and our goal was to test the function of D4Z4 on the regulation gene silencing. We generated several constructs where different number of D4Z4 were cloned downstream of a reporter gene and followed the expression of the gene over an extended time in culture. We showed that D4Z4 is a bona fide insulator element protecting from PEV and able to block enhancer-promoter communication. The multivalent CTCF protein binds to D4Z4 and participates in this insulation activity. Unlike other human telomeres, the 4q35 locus is localized at the periphery of the nucleus and might interact with A-type Lamins. We showed that D4Z4 displaces a telomere toward the nuclear periphery in the presence of CTCF. We further demonstrate that the perinuclear activities of D4Z4 are lost upon multimerization of the repeat, suggesting that the D4Z4 array at the 4q35 locus acts as a CTCF-dependent insulator in FSHD patients but not in normal individuals and might impact on the expression of the genes causing FSHD. Thus, our findings might elucidate the mechanism by which D4Z4 contraction might contribute to this dystrophy.

123. MBNL3 inhibits muscle differentiation by disrupting MEF2D beta-exon splicing

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Mammalian muscleblind-like (MBNL) proteins are proposed regulators of myogenesis and are implicated in myotonic dystrophy (DM). Disrupted regulation of alternative splicing has been linked to the myotonia, insulin resistance and cardiac conduction defects of DM. However the mechanism responsible for the associated muscle degeneration remains unclear. We have reported that mammalian MBNL3 functions as an inhibitor of myogenesis. Microarray analysis revealed that myocyte enhancer factor 2 (MEF2) target genes are down-regulated by MBNL3 overexpression in C2C12 mouse myoblasts. Inclusion of the alternatively spliced beta-exon to produce the more active isoform of MEF2D is hindered in these cells. Expression of the beta(+) but not the beta(-) MEF2D isoform is sufficient to overcome the inhibitory effects of MBNL3 on muscle gene expression and myotube formation. RNA immunoprecipitation studies suggest that MBNL3 interacts with the MEF2D message in vivo. We have establ ished a MEF2D minigene assay to map the cis-acting elements that regulate beta-exon splicing. We hypothesize that silencing of MEF2 beta-exon splicing by MBNL3 antagonizes the expression of MEF2 target genes necessary for muscle differentiation. A defect in beta-exon MEF2 splicing also is detected in C2C12-CTG200 cells, a tissue culture model of DM. Targeting MBNL3 activity may represent a potential therapeutic approach for combating the muscle wasting of DM.

124. Specific sequence variations in 4qter are associated with FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) progressively affects the muscles of the face, shoulders and upper-arms, often in an asymmetric fashion. FSHD is associated with contraction of the polymorphic macrosatellite repeat D4Z4 on chromosome 4q35. Several disease models have been proposed in which D4Z4 contraction affects gene expression by epigenetic mechanisms. We here demonstrate that D4Z4 contraction alone is insufficient to cause FSHD but that these contractions need to occur on a specific allelic background of 4qter. By studying sequence variations in the D4Z4 region by using a stable simple sequence length polymorphism proximal to D4Z4, a SNP within D4Z4 and the 4qA/4qB variation distal to D4Z4 in a large cohort of individuals, we demonstrate that the subtelomeric domain of chromosome 4q can be subdivided in at least nine haplotypes, each defined by specific sequence variations close to and within D4Z4. We also show that repeat contractions on at least two 4q haplotypes are not associated with FSHD. We propose that each of these haplotypes has a unique sequence signature and that specific SNPs in the disease haplotype are essential for the development of FSHD. By studying the worldwide distribution of these haplotypes, we demonstrate that all haplotypes arose before modern humans migrated out of Africa and that differences in a llele frequencies may have important consequences for the incidence of FSHD in different world populations.

125. Regulation of TNF mRNA stability by CUGBP1 in muscle cells and myotonic dystrophy

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Pathogenesis of myotonic dystrophy type I (DM1) has been linked with altered expression, phosphorylation and localization of the CUGBP1 RNA-binding protein. The roles of CUGBP1 in regulating splicing and translation of clinically relevant genes have been investigated in detail. However, we and others have also implicated CUGBP1 as a modulator of poly(A) shortening and mRNA decay.

Our results show that CUGBP1 binds with high affinity and specificity to the 3'UTR of the TNF mRNA and recruits the PARN deadenylase to mediate rapid poly(A) shortening in vitro. Importantly, shRNA-mediated knockdown of either CUGBP1 or PARN in C2C12 myoblasts results in stabilization of the TNF transcript. Similarly, expression of CUG repeat RNA in these cells also causes TNF mRNA stabilization. Finally, treatment of cells with phorbol ester, which has been shown to affect CUGBP1 phosphorylation and function, inhibits decay of TNF mRNA.

Our results have significant implications for DM1 patients as TNF levels are routinely elevated in this disease. We suggest that this may be mediated through altered function of CUGBP1. Experiments are planned to identify other targets of this mRNA decay pathway that may be affected in DM.

Funded by AHA, MDA and NIH.

126. Looking for the Elusive FSHD-Determining Sequence Proximal to D4Z4 Repeats at 4q35: High-Resolution DNaseI Hypersensitivity Profiling with Tiled Microarrays

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Almost all patients with facioscapulohumeral muscular dystrophy (FSHD), a dominant disease, have a short array (<11 units) of tandem 3.3-kb repeats (D4Z4) at 4q35. Nonpathogenic arrays have 11-100 units. There are almost identical arrays, at 10q26 but these are phenotypically neutral despite high homology 42 kb proximal to 4q and 10q D4Z4 arrays and in all distal sequences (15-25 kb). Therefore, there should be a disease-related sequence >42-kb proximal to 4q35 D4Z4, but standard microarray expression analyses failed to reveal its identity. With tiled microarrays, we have examined DNaseI hypersensitive sites at 4q35 in one FSHD and one control myoblast cell strain so far. We found that the terminal 1-MB region containing D4Z4 has far fewer DNaseI hypersensitive sites (DHS) and annotated genes at 4q than at 10q. FSHD and control myoblasts gave very similar DHS at FRG1 and none at FRG2 or TUBB4Q, all candidate FSHD genes at 4q35. DHS at four unexpected positions wer e observed in both samples from 15 kb to 1.1 MB proximal to D4Z4 on 4q35. Three of these overlapped heterologous short tandem repeats (STRs) with unit sizes of 32 - 52 bp. Some additional DHS seen only in the FSHD myoblasts were located 0.2 - 1.4 MB proximal to D4Z4. Several of these were also associated with an STR. Similar analyses are ongoing in other FSHD and control myoblast cell strains to determine if FSHD-specific DHS can be detected that are linked to undocumented genes or unusual chromatin structures. Supported in part by NIH grant R01 NS048859 and an FSH Society Grant.

127. In vivo study of alternative splicing factors using rAAV mediated gene transfer

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Over 70% of human genes encode alternatively spliced pre-mRNAs that are translated into proteins which can differ from one another enormously in their functions that affect many aspects of human development and physiology. Most of our knowledge for alternative splicing has been obtained from use of in vitro system and only a little has been studied in vivo. Muscleblind-like (MBNL) proteins are alternative splicing factors that promote exclusion of fetal exons during development. Also, their functional loss is known to result in myotonic dystrophy (DM), which is a multisystemic degenerative disease. In order to study in vivo function of MBNL1, we employed recombinant adeno-associated virus (rAAV) mediated gene transfer to modulate the expression of Mbnl1 in various mouse tissues either locally or systemically. Mbnl1 overexpression in a transgenic mouse model for DM simultaneously reversed DM associated myotonia and missplicing of target pre-mRNAs. This study demons trates that rAAV-mediated gene transfer is a powerful system to study alternative splicing factors in vivo.

128. Viral-mediated gene transfer to analyze RNA splicing during muscle development

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Over 70% of human genes encode alternatively spliced exons and this process is particularly prominent during skeletal muscle development. The muscleblind-like (MBNL) and CUGBP1/ETR3-like factors (CELF) proteins are antagonistic splicing factors that control postnatal splicing of alternative fetal and adult exons. Loss of MBNL, and/or enhancement of CELF, splicing function by poly(CUG) RNAs is believed to cause the neuromuscular disease myotonic dystrophy (DM). To test the hypothesis that MBNL1 coordinately regulates the splicing of specific sets of developmentally regulated exons in muscles, we have used a systemic injection approach and recombinant adeno-associated virus (rAAV)-mediated gene transfer to modulate the expression of several splicing regulators, including Mbnl1, Cugbp1 and hnRNP A1, in vivo. This study demonstrates that splicing mechanisms can be analyzed in vivo using rAAV-mediated gene transfer and provides a novel therapeutic approach to reverse D M-associated muscle pathology.

129. A DNA repeat linked to facioscapulohumeral muscular dystrophy has a high density of potential guanine quadruplexes, Non-B DNA structures

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Facioscapulohumeral muscular dystrophy (FSHD) is linked to a short array of 3.3-kb repeats at 4q35 (<11 repeat units). Long D4Z4 arrays (11-100 repeat units) at 4q35 are nonpathogenic. The higher-order structure of D4Z4 chromatin must play a central role in the near-threshold effect of 4q array size on disease status. D4Z4's very high G+C content (73%) suggest a special structure in the array. Accordingly, we found that D4Z4 has an unusually high density of potential G-quadruplexes (PQS), non-B DNA structures containing four equal-length runs of G residues. There is increasing evidence for the biological importance of PQS. Seven D4Z4 oligonucleotides match the PQS hairpin consensus sequence, G(3-5)N(1-7)G(3-5)N(1-7)G(3-5). All gave characteristic circular dichroism (CD) spectra for G-quadruplexes. In addition, CD provided evidence for the formation of many bimolecular-type PQS from D4Z4, such as from G(4)CG(4). Pairing of similar non-adjacent sequences in quadru plexes might help organize the structure of the array by hydrogen-bonding between different parts of the array, probably stabilized by protein binding. D4Z4 interactions with distant proximal and nearby distal 4q35 regions are likely to be involved in FSHD. We also found clustered PQS matching the above consensus sequence in these regions. PQS may be involved in interactions between the array and distant sequences in cis, as well as within the array, in a novel type of pathogenic looping of chromatin. Supported in part by NIH grant R01 NS048859.

130. Therapeutic application for a cell culture model of myotonic dystrophy

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Myotonic dystrophy is the second most common cause of muscular dystrophy and the most common cause of the adult-onset type. The major form, DM type 1 (DM1), is caused by a CTG expansion in the 3' untranslated region (UTR) of the DMPK gene, which is transcribed and forms nuclear RNA foci. Expression of expanded RNA (DMPK-CUG) in mice and in cell culture reproduces cellular and molecular aspects of DM1. The mutant RNA reverses a developmentally regulated alternative splicing transition, directly causing the myotonia and insulin resistance that patients experience. However, the muscle wasting, which is responsible for the most common cause of DM1 mortality and morbidity, has yet to be explained. Cultured myogenic satellite cells from patients exhibit a significant differentiation defect likely to be directly relevant to this symptom. We are in the process of establishing inducible mouse C2C12 myoblast cell lines that express DMPK-CUG RNA to investigate the mechanism by which mutant RNA inhibits differentiation. Additionally, we will use these cell lines to develop a novel approach to reverse these effects using antisense oligonucleotides (ASOs) and endogenous RNase H activity. This work is supported by NIH R01AR/GM45653-09.

131. Severe skeletal muscle wasting in a tissue-specific, inducible mouse model for myotonic dystrophy, type 1

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The mechanism by which skeletal muscle wasting occurs in myotonic dystrophy (DM) remains unknown. In order to study the onset and progressive nature of skeletal muscle pathology we have created transgenic mice expressing 960 CUG repeats within exon 15 of the DMPK gene (EpA960) in an inducible and skeletal muscle-specific manner. EpA960 mice express exon 15 of the DMPK gene with CUG repeats, when a floxed concatemer of three polyadenylation sites is removed by a Cre-mediated recombination event. EpA960 mice have been crossed with mice expressing a skeletal muscle-specific modified Cre protein (HSA-Cre), which becomes activated in the presence of tamoxifen. EpA960/HSA-Cre bitransgenic mice express large amounts of DMPK-CUG960 RNA from the recombined allele post tamoxifen administration specifically in skeletal muscle. Seventy percent of animals exhibit severe and progressive muscle wasting as assessed by muscle function tests, gross appearance and MRI. Histological abnormalities including increased central nuclei, variation in fiber size and atrophy of muscle fibers are seen in bitransgenic mice post tamoxifen. Additionally bitransgenic mice produce waxing and waning myotonic runs, intranuclear RNA foci which colocalize with Mbnl, splicing abnormalities and elevated CUGBP1 protein levels all characteristic of DM. With this model we are investigating pathogenic mechanisms involved in skeletal muscle wasting.

132. High similarity of global muscle splicing defects in two mouse models of DM1

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Myotonic dystrophy is caused by expansion of CTG triplet repeats (or CTGG repeats) within a transcribed region. CUG-repeat RNA accumulates with splicing factors in abnormal nuclear foci whose appearance correlates with complex defects in muscle, heart, and nervous system function. Some of these defects can be traced to incorrect splicing of pre-mRNAs that depend on the splicing regulator Muscleblind-like 1 (MBNL1), a protein known to bind CUG-repeat RNA in the nuclear foci. To test the hypothesis that the primary effect of CUG-repeat RNA is to create a loss of MBNL1 function by sequestration, we compared global splicing changes in skeletal muscle from two mouse models of DM1, one expressing a CUG-repeat RNA, and another lacking a functional MBNL1 gene. We find that for nearly every splicing event that is significantly altered in either model compared to wild type, the magnitude of the change in splicing for each event is highly similar between the two models ($r > 0.8$), indicating that loss of MBNL1 function quantitatively explains almost all of the splicing changes observed due to expression of CUG repeats in mouse skeletal muscle. Supported by NIH.

133. Nuclear organization of gene expression in relation to skeletal muscle development and disease.

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The cell nucleus is remarkably complex in both function and internal sub-structure, with genome organization in nuclei intimately linked to cell-type regulation. By “mapping” interphase nuclei, we find that specific loci are spatially organized with distinct nuclear compartments. Many active protein-coding genes position precisely at the edge of ~20 SC35 domains or “speckles”, enriched in mRNA metabolic factors. In contrast, many silent loci preferentially organize in the peripheral heterochromatic compartment adjacent to the nuclear envelope, as we showed for the D4Z4 locus involved in FSH Dystrophy. In contrast, certain muscle-specific genes become associated with SC35 domains as they transition from myoblast to skeletal myotube. Many active genes cluster around each SC35 domain, and this relates to clustered gene organization into chromosome bands. Contrary to the notion that SC35 speckles are inert storage sites, our findings indicate that these are in fact “hubs” of activity within the nucleus. Our lab has also examined in detail the structural organization of pre-mRNA metabolsim, by visualizing spliced and unspliced transcripts as they emanate from the gene. Evidence indicates that transcription and most splicing occurs at the edge of the SC35 domains, with more mature mRNAs from some genes entering into the domain. However, this is a locus-specific property and not the case for all genes; for instance, dystrophin pre-mRNA, with 76 introns, is never found within SC35 domains, whereas MyHC pre-mRNA is essentially always within domains. Studies of mRNAs mutated in specific genetic diseases suggest that passage through SC35 domains may be linked to export of specific mRNAs. In myotonic dystrophy type 1, triplet repeat expansion in the 3′ UTR of DMPK causes the nuclear retention of the mutant mRNA. In both normal and DM1 muscle cells, the DMPK locus positions precisely at the outer edge of a factor-rich SC-35 domain. While the normal mRNA consistently is observed within the domain, the DM1 mutant transcripts detach from the gene but accumulate in granules that abut but do not enter SC-35 domains, suggesting that RNA entry into the domain is blocked. Despite their exclusion from these compartments, mutant transcripts are spliced. MBNL1 becomes highly concentrated with mutant RNA foci, and RNAi knockdown of MBNL1 promotes the accumulation or entry of newly synthesized mutant transcripts in the SC-35 domain. Collectively, these and other findings indicate that transit into and out of an SC35 domain are distinct steps in the early (post-splicing) transport of some mRNAs, and that mutant mRNAs in some genetic diseases become blocked at this step. Finally, results linking a large non-coding RNA to paraspeckles that form at the periphery of SC35 domains will be discussed.

BASIC MUSCLE BIOLOGY

134. Structural basis of familial hypertrophic cardiomyopathy caused by myosin point mutation R403Q

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Mutations in virtually all of the proteins comprising the cardiac muscle sarcomere have been implicated in causing Familial Hypertrophic Cardiomyopathy (FHC). The disease-causing point mutation R403Q in beta-myosin heavy chain (MHC) is one of the most lethal mutations linked to FHC and is located at the actomyosin interface. Here we examine the structural consequences of the R403Q mutation in a recombinant smooth muscle myosin subfragment (S1), whose kinetic features have much in common with slow beta-MHC. We obtained three-dimensional reconstructions of wild-type and R403Q smooth muscle S1 bound to actin filaments in the presence (ADP) and absence (apo) of nucleotide by electron cryomicroscopy and image analysis[1]. We observed that the R403Q mutation caused a severe disruption of the actomyosin interaction at the interface. This is the first demonstration that cardiomyocyte disarray, the hallmark of FHC, is the direct result of a molecular level interaction, an d not a secondary effect of change in global cardiac function. If this result is substantiated by the cardiac isoforms, these studies should facilitate the design of new therapeutics or diagnostics to treat the disease.

135. Subtle skeletal muscle defects in mice that lack alpha7 integrin and utrophin

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Dystrophin, alpha7beta1 integrin and utrophin are the major laminin receptors in skeletal muscle. Loss of dystrophin causes Duchenne muscular dystrophy. Both utrophin and the alpha7beta1 integrin have been shown to be major genetic modifiers of disease progression in mouse models for muscular dystrophy. To investigate the relative contributions of the alpha7 integrin and utrophin as modifiers in diseased skeletal muscle, we generated mice lacking both complexes (alpha7/utr-/-). Mice deficient in dystrophin and the alpha7 integrin (alpha7/mdx-/-) or utrophin (mdx/utr-/-) exhibit severe muscle pathology and die prematurely, while alpha7/utr-/- mice are viable and fertile. alpha7/utr-/- mice exhibit partial embryonic lethality and histological defects similar to alpha7 integrin null mice. Initial studies suggest that loss of the alpha7 integrin and utrophin results in more severe neuromuscular defects. Together these results suggest that mice lacking the alpha7 integrin and utrophin exhibit a mild muscle phenotype and the dystrophin glycoprotein complex may compensate for the loss of both utrophin and the alpha7beta1 integrin.

136. Altered muscle function in Actn3 knockout mice: mechanistic insights into the association between alpha-actinin-3 deficiency and human athletic performance

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Homozygosity for a common nonsense variant in the human ACTN3 gene (R577X) results in complete deficiency of the fast muscle fibre protein, alpha-actinin-3, in more than one billion individuals worldwide. We have previously shown that the 577XX null genotype is under-represented among elite sprint athletes, over-represented among elite endurance athletes, and associated with poorer muscle strength and sprint performance in non-athlete cohorts. The 577X allele has been positively selected during human evolution, suggesting that alpha-actinin-3 deficiency confers an adaptive benefit. We have generated a knockout (KO) mouse model of alpha-actinin-3 deficiency. Actn3 KO mice have reduced grip strength and increased intrinsic endurance capacity, consistent with the phenotype of 577XX humans. The muscle of KO mice displays a marked metabolic shift towards the more efficient oxidative pathway, with increased mitochondrial density, a significant decrease in the activity of the anaerobic enzyme lactate dehydrogenase, and increased activity of multiple mitochondrial enzymes. We have also shown a reduction in fast muscle fibre diameter and enhanced force recovery following fatigue in alpha-actinin-3 deficient mice. We propose that the effects of alpha-actinin-3 deficiency on muscle metabolism underlie recent positive selection on the 577X allele in humans, as well as the association between ACTN3 genotype and human athletic performance.

137. Deregulated PKA activity and localization in muscular dystrophy

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Alterations in signaling pathway activity have been implicated in the pathogenesis of Duchenne Muscular Dystrophy (DMD), a degenerative muscle disease caused by a deficiency in the costameric protein dystrophin. Accordingly, the notion of the dystrophin-glycoprotein complex (DGC), and by extension the costamere, as harboring signaling components has received increased attention in recent years. The localization of most, if not all, signaling enzymes to this subcellular region relies on interactions with scaffolding proteins directly or indirectly associated with the DGC. One of these costameric scaffolds is myospryn, a large, muscle-specific protein kinase A (PKA) anchoring protein or AKAP. Previous studies have demonstrated a dysregulation of myospryn expression in human DMD suggesting a connection to the pathophysiology of the disorder. Here we report that dystrophic muscle exhibits reduced PKA activity resulting, in part, from severely mislocalized myospryn and the RIIalpha regulatory subunit of PKA. Furthermore, we show that myospryn and dystrophin coimmunoprecipate in native muscle extracts and directly interact in vitro. Finally, we reveal that the myospryn gene is a downstream target of PKA-CREB (cAMP response element binding protein) signaling and that numerous dysregulated genes in dystrophin deficient muscle are candidate cAMP-inducible genes and harbor putative CREB binding sites. Our findings reveal for the first time abnormalities in the PKA signal transduction pathway in muscular dystrophy. This work was supported by the NIH/NHLBI and the Muscular Dystrophy Association (MDA).

138. Ozz-E3 ubiquitin ligase targets sarcomeric thick filaments to regulate the embryonic isoform of myosin heavy chain during skeletal muscle development.

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Sarcomeric proteins are developmentally expressed as a series of isoforms appropriate for slow or fast contraction, and aerobic or anaerobic metabolism. Mechanisms must exist to enable isoform exchange and replacement while maintaining the highly ordered fine structure of sarcomeres. Ozz-E3 is a muscle-specific, developmentally regulated ubiquitin ligase, shown to target beta-catenin. Here, we show that Ozz also targets an epitope on the rod portion of embryonic myosin heavy chain (MyHemb), which forms the core of thick filaments, promoting its polyubiquitination and degradation by the proteasome. MyHemb expression persists during differentiation of Ozz–/– muscle cells, allowing us to ask whether myofilament isoform composition is regulated by dynamic exchange between soluble monomers and myofilaments, as seen in vitro, or if MyHemb is targeted in myofibrils and actively removed. Using a size exclusion column we found Ozz co-segregates with high molecular wei ght polymers in the insoluble sarcomeric fraction, consisting of MyHemb dimers, myosin light chains, and components of the Ozz ligase complex. Ozz, apparently, can access its binding sites within polymeric MyH. These observations lead to the novel idea that the ubiquitin-proteasome system, in addition to removing misfolded or damaged proteins, also can actively access structural proteins within large polymeric assemblies as part of the regulation of tissue development and growth. This research was funded by NIH grant AR049867, Cancer Center Core Grant CA021765, and the American Lebanese Syrian Associated Charities (ALSAC)

139. Extraocular muscles buffer calcium better than limb muscle: implications for preferential sparing in Duchenne's Muscular Dystrophy (DMD)

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DMD is the most common fatal, NMD in males and associated with widespread muscle wasting. Enigmatically, extraocular muscles (EOMs) are spared in DMD. Dysregulation of calcium homeostasis has been suggested to contribute to DMD muscle damage. We propose that EOM sparing is facilitated by differential calcium buffering properties of EOMs compared to limb (tibialis anterior-TA) muscle. We investigated the role of candidate proteins as well as compared calcium handling properties of cultured myotubes from EOMs and TA. We calculated from electron microscopy (EM) micrographs that large pale global fibers of EOMs have a 1.7 fold larger SR content than TA fibers. qPCR revealed higher mRNAs levels in EOMs for several calcium and SR related proteins including SERCA2. EOM myotubes show better buffering of elevated intracellular calcium, with 2.3-fold larger peaks and 1.7-times faster decay times. Taken together, a larger SR and differential expression of calcium buffering p roteins may contribute to the improved ability of EOMs to handle elevated intracellular calcium levels. This in turn would be predicted to protect EOMs from the calcium-mediated damage noted in DMD limb muscles. Funding: NIH EY015537 & EY013862

140. Structural and functional evaluation of branched myofibers in young and old mdx mice

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Skeletal muscle function is dependent on the regular organization of skeletal muscle structure. In dystrophic (dy/dy) muscle, malformed myofibers have been shown to be functionally weak and prone to injury when stimulated (Head et al, J Physiol, 1990). This concept was further strengthened by recent findings which show that, in the extensor digitorum longus muscle (EDL) of dystrophic (mdx) mice, susceptibility of whole muscle to injury from lengthening contractions is directly linked to the extent of myofiber branching within the muscle (Chan et. al., Am J Physiol-Cell, 2007). We examined the morphology of enzymatically-isolated muscle fibers of the EDL and flexor digitorum brevis (FDB) from young (2-3 months) and old (8-9 months) mdx and control mice (C57BL10). In young mdx EDL, 6% of the myofibers has visible malformations (i.e., inter-fiber splitting, branched ends, mid-fiber appendages). In contrast, 70% of myofibers in old mdx mice contained visible malformat ions. In the mdx FDB, malformation occurred in only 4% of the young myofibers, but 52% of the old myofibers. Age-matched controls did not display the altered morphology of the mdx muscles (0% in EDL and FDB). The membrane-associated (e.g., beta-spectrin) and cytoplasmic (e.g., desmin) cytoskeletal structures appear normal in the malformed mdx myofibers, and despite multiple branches, all had a single neuromuscular junction (TRITC-BTX). In mdx FDB's with significantly branched ends, an assessment of global, electrically evoked Ca2+ signals (Indo1PE-AM) revealed a 23.3 ± 4.8% decrease in amplitude and a 14.2 ± 6.2% increase in the decay time constant in myofibers with significant branching. No alteration in the basal myoplasmic [Ca2+] (i.e., Indo Ratio) was seen in malformed vs. normal mdx myofibers. In summary, aging mdx myofibers develop morphological malformations that are likely associated with alterations in myofiber Ca2+ signaling.

141. Characterization of the mammalian ferlins in myogenesis

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The ferlin family represents a class of C2 domain-containing proteins. Dysferlin, identified as the defective gene in limb-girdle muscular dystrophy type 2B, was the first mammalian ferlin characterized. Mice with mutations in dysferlin present a reduction in the ability to repair damaged muscle membranes. Otoferlin mutations have been linked to deafness. Highly homologous to dysferlin, myoferlin also contains six C2 domains, is also highly expressed in muscle, and required for proper myoblast fusion. Three novel members of the mammalian ferlin family have yet to be characterized: fer-1-like-4, fer-1-like-5, and fer-1-like-6. Fer1l4, fer1l5, and fer1l6 are widely expressed in the adult mouse and contain five, four, and six C2 domains, respectively. We generated ferlin specific antibodies and found that each protein is expressed during myogenesis in culture and exhibits unique localization patterns within developing myotubes. Based on these patterns, we hypo thesize that the ferlins play important roles in muscle development and damage repair. The characterization and investigation of three additional ferlins will help determine the molecular mechanisms in which the ferlins participate in muscle development and maintenance. Supported by NIH NS047726 and the Jain Foundation.

142. Characterization And Expression Of Alpha-Actinin Family Members During Zebrafish Development

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The alpha-actinins are an ancient protein family sharing a common evolutionary origin with other members of the spectrin-gene superfamily of actin-binding proteins. In skeletal muscle, alpha-actinins are thought to participate in maintaining sarcomeric integrity by anchoring the thin filaments. Alpha-actinin homologues have been identified in several different vertebrate as well as invertebrate species, yet detailed information on spatial and temporal expression of the alpha-actinin family in early embryonic development is lacking. In this work, we have utilized zebrafish as a model system to analyze potentially evolutionary conserved roles of alpha-actinins in muscle function. Although most vertebrates have four alpha-actinin genes, we find that the zebrafish genome includes five, each of which is highly homologous to other vertebrate orthologues. Three appear to encode sarcomeric isoforms, while two may be cytoskeletal. We identified two putative transcripts for zebrafish alpha-actinin-3 that positioned to different genomic loci whereas all other alpha-actinins had single transcript orthologues each. We further show the detailed expression pattern of these alpha-actinin genes in zebrafish from one cell stage to seven days post fertilization using whole mount in-situ hybridization. Future studies utilizing zebrafish model system will help a better understanding of alpha-actinins involving pathways in muscle function.

143. miRNA-mediated translational regulation of utrophin-A (UtrnA) mRNA by elements in its 5' and 3'-UTRs

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Utrn is the autosomal homolog of dystrophin, the product of the Duchenne's muscular dystrophy (DMD) locus and UtrnA regulation is of therapeutic interest for DMD. In addition to the miRNA target reported by Rosenberg et al. 2006, we find the Utrn 3'-UTR contains 4 miRNA target sites and the 5'-UTR contains an IRES element. Expression of Utrn-luc-3'UTR constructs in C2C12 cells is repressed when co-transfected with these miRNAs, conversely miRNA-inhibitors suppress the inhibitory effect. Ribosomal profiling and qPCR analysis show that the elements in the 5'- and 3'-UTRs of the UtrnA repress expression of the reporter luciferase mRNA predominantly at the translational level in C2C12 myotubes. The inhibition of reporter luciferase mRNA translation is unusually high (> 98%) in presence of both the 5' and 3'-UTRs. Further analysis using bicistronic luciferase reporter assay suggest that the 3'-UTR exhibits its inhibitory effect both on the IRES-mediated a nd cap-dependent translation, consistent with the model proposed by Petersen et al. Mol Cell. 2006. We suggest that translational repression by the 5' and 3'-UTRs of utrophin-A occurs at the post-initiation stage.

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144. Studies into the contribution of the FKHR (FOX01a) DNA binding domain to Pax3-FKHR DNA binding.

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Alveolar rhabdomyosarcoma (ARMS) is an aggressive muscle sarcoma of children that is characterized by a (2;13)(p35;q14) chromosomal translocation that produces the oncogenic fusion protein Pax3-FKHR. Despite the fact that the binding affinity and sequence requirements for Pax3-FKHR are not as stringent as for wild-type Pax3, it is commonly held that Pax3 DNA binding domains are solely responsible for Pax3-FKHR DNA binding. In the absence of direct studies into the contribution of FKHR, the general consensus has been that the bisected FKHR DNA binding domain is unimportant for Pax3-FKHR DNA binding. However, the amino acid sequence of Pax3-FKHR reveals that most FKHR amino acids that make direct DNA contacts are present in the fusion protein, suggesting that FKHR DNA sequence elements may be important. We have determined that the FKHR DNA recognition sequence is more extensive than previously believed and that flanking elements are important for wild-type FKHR bind ing. Consistent with this data, Pax3 and the extended FKHR recognition sequence are found in close proximity in promoter elements regulated by Pax3-FKHR. Therefore, these lines of evidence indicate that the FKHR domain may contribute to the Pax3-FKHR DNA binding. In this work, we perform a systematic determination of Pax3-FKHR binding sequences and examine known targets of Pax3, Pax3-FKHR, and FKHR to determine what role the FKHR binding domain plays in Pax3-FKHR gene activation.

145. Novel flanking DNA sequences enhance FKHR (FOX01a) binding affinity but do not alter DNA bending

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FKHR (FOXO1a), a member of the forkhead winged-helix family of transcription factors, has positive and negative regulatory effects in myogenesis by controlling the rate of myotube fusion and impairing glycemic control in developed skeletal muscles. FKHR was first identified through its fusion to Pax3 as a result of the t(2;13)(q35;q14) translocation in Alveolar Rhabdomyosarcoma, a solid muscle tumor of adolescents. FKHR is insulin responsive and binds to the insulin response element (IRE). However, multiple forkhead family members with diverse biological functions also bind to the IRE. Therefore, additional DNA sequence elements may be required to provide increased binding affinity and specificity for FKHR. We have used the Systematic Evaluation of Ligands by EXponential enrichment (SELEX) to systematically identify additional DNA sequences important for FKHR binding. We demonstrate for the first time that in addition to the IRE, two novel sequence elements are important for maximal FKHR binding: (1) the reverse complement (5'-GT(A/C)AACA-3') and, (2) the flanking sequence (5'-ACAACA-3'). Although these additional elements do not contribute to FKHR-induced DNA bending, the presence of these elements do increase the affinity of FKHR DNA binding through a one-to-one binding stoichiometry and subsequently the ability of FKHR to activate transcription from a luciferase reporter construct and in biologically relevant systems.

146. Sarcoplasmic Reticulum Calcium Uptake And Speed Of Relaxation Are Depressed In Nebulin-Free Skeletal Muscle

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Recently, we have generated a novel nebulin KO mouse model. The underlying mechanisms for depressed contractility in nebulin KO mice involve reduced thin filament lengths. Altered Ca2+ homeostasis might also be involved, as gene expression analysis revealed that sarcolipin (SLN, a potent SERCA-inhibitor) is upregulated in nebulin KO mice. Therefore, we investigated SLN protein expression in nebulin KO mice, as well as SR Ca2+ uptake, myofiber Ca2+ transients, and muscle contractile performance. Western blotting was performed on soleus, EDL, and quadriceps. Ca2+ transport activity of quadriceps SR vesicles was assayed, and Vmax was determined. FDB fibers were enzymatically dissociated, and Ca2+ transients during field stimulation were determined using Fura2FF. Finally, we determined intact soleus contractility, and Ca2+-activated maximum force in skinned soleus fibers. In KO mice, SLN protein expression was highly upregulated in soleus, EDL and quadriceps, SERCA w as slightly downregulated, and phospholamban and calsequestrin were comparable between both groups. Ca2+ uptake of isolated SR vesicles was reduced in nebulin KO mice across a range of Ca2+ levels; Vmax was reduced by >3 fold. Ca2+ release upon field stimulation was comparable between FDB fibers from KO and wt mice. However, rate of Ca2+ reuptake was significantly reduced in KO fibers, as indicated by increased values for tau (i.e. the time course of transient decay). Twitch and tetanic force generation were reduced in KO mice (60 and 53% respectively), and tetanic half relaxation time was increased in nebulin KO mice. Finally, maximum Ca2+-activated force was reduced by ~36% in nebulin KO mice (note the significantly larger tetanic force reduction in KO). These data demonstrate that altered Ca2+ homeostasis plays an important role in muscle dysfunction of nebulin KO-induced skeletal. In addition, the present study suggests a functional relation between nebulin and SLN.

147. Titin Kinase (Tk) Region Differentially Modulates Excitation-Contraction Coupling In Skeletal Vs Cardiac Muscle

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It has been previously reported that in cardiac muscle the TK region regulates contractile function through effects on calcium handling. The role of the TK region in skeletal muscle contraction is unknown and here we investigated the effect of deletion of the TK region on skeletal muscle contractility. We used a conditional TK knock-out mouse model that has been described before. We determined titin isoform expression in LV, soleus, EDL, and FDB muscle and measured calcium transients in isolated LV myocytes using Fura2-AM, and in enzymatically dissociated FDB fibers using Fura2FF-AM. In skinned soleus fibers calcium sensitivity of force generation was determined as well as maximum Ca2+-activated force. We found 40-50% truncated (kinase deficient) titin in LV, soleus, and EDL and 10-20% in FDB. Cardiomyocytes had a reduced rate of calcium reuptake as has been shown before in a cardiac-specific TK KO model. Surprisingly, in FDB fibers from KO mice calcium reuptake w as faster compared to wt mice (tau: 7.8 vs 11.2 ms, KO vs wt respectively). Calcium sensitivity of force generation was reduced in skinned soleus muscle from KO (pCa50: 5.97 vs 6.11, KO vs wt respectively). Finally, maximum calcium-activated force was not significantly different between KO and wt soleus muscle (105 vs 96 mN/mm2, KO vs wt respectively). This work further establishes that the TK region modulates calcium kinetics. Interestingly we found that the effect is differential in cardiac and skeletal muscle (reduced rate of uptake in cardiac muscle and increased rate in skeletal muscle). Furthermore, the TK regulates calcium sensitivity of force generation in skeletal muscle. The TK-binding protein MURF-1 might be involved, as MURF-1 is known to degrade troponins and decreased binding capacity in the KO muscle might increase its cytosolic concentration and proteolytic activity.

148. Identification of a novel Z-band associated protein complex

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The KY protein underlies a form of muscular dystrophy in the mouse but its role in muscle remains elusive. KY interactions previously identified included fragments of filamin C and of a novel protein termed KIP. cDNA isolation and specific antibodies showed that at least three proteins are produced from the KIP locus: KIP1, KIP2 and KIP2a. Interaction assays within the Y2H system supported that KIP1, KIP2, KY and FLNC are part of a protein complex. Immunofluorescence analysis of striated muscles as well as transductions of cardiomyocytes revealed co-localization of KIP1, KIP2, KY and FLNC at the Z-band. KIP2 also locates at intercalated discs in heart sections. Endogenous KIP1 also shows nuclear localization and in the absence of the KY protein, there is a significant reduction of nuclear KIP1 in all adult muscles, a feature particularly exacerbated in dystrophic soleus muscle. Constitutive RNAi mediated down-regulation of KIP2 in C2C12 myoblasts does not affect p roliferation rates. However, once initiated to differentiate these cells fail to fuse and detach from the plate, suggesting that KIP2 has a crucial role at an early stage of the muscle differentiation pathway.

MUSCLE SYSTEMS BIOLOGY

149. Rescue of mechanical function, morphology and signaling by desmin plasmid transfection into muscles from desmin knockout mice

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Desminopathies result from lack of this intermediate filament protein. However, the basis for the pathology is not fully understood. We probed desmin's function by transfecting adult desmin knockout (DKO) muscle with plasmid DNA encoding desmin and measuring resulting structural and functional changes. Mice were transfected by electroporation and, after 7 days, we measured the regularity of the myofibrillar lattice and several responses to mechanical stretch including the deformability of nuclei, stress production, injury and c-Jun-kinase (JNK) phosphorylation. Desmin transfection reversed essentially all of the knockout phenotype in a quantitative manner—Z-disks were re-aligned, myonuclear deformability was restored, and stress production and the resulting injury response was also returned to near-wildtype levels in a dose-dependent, although nonlinear manner. Furthermore, the stretch-induced activation of JNK phosphorylation, not detectable in the DKO muscl es, was restored. These results demonstrate that the desmin intermediate filament is uniquely responsible for the spatial alignment of Z-disks and plays a central role in transducing mechanical stress: (1) from the force-generating cytoskeletal network to the surrounding tissue and (2) to the intracellular signaling apparatus.

150. Expression of the muscle glycogen phosphorylase gene in patients with McArdle disease: the role of nonsense-mediated mRNA decay

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Nearly 35% of all mutations identified in the muscle glycogen phosphorylase gene (PYGM) in patients with McArdle disease result in premature termination codons (PTCs), particularly the p.R50X, found in more than 50% of the mutated alleles in most Caucasian populations. Mutations resulting in PTC could trigger the degradation of mRNA through a mechanism known as nonsense mediated decay (NMD). To investigate if NMD affects the levels of transcripts containing PYGM mutations, 28 patients with McArdle disease, harbouring 17 different mutations with PTCs in 77% of their alleles, were studied. We assessed that 92% of patients showed NMD. The most frequent mutation (p.R50X) elicited decay in all the genotypes tested. Other PTC-producing mutations causing decay were: p.L5VfsX22, p.Q73HfsX7, p.E125X, p.N134KfsX161, p.W388SfsX34, p.R491AfsX7 and p.D534VfsX5. Missense mutations, and p.E797VfsX19, located in the last exon, were not affected by NMD. Exceptions to the rules gov erning NMD were found in the mutations p.A704V and p.K754NfsX49. The high number of mutations undergoing decay and their wide distribution along the PYGM gene in McArdle disease patients, strongly suggest that NMD could play an important molecular role in this disease.

151. Altered gene expression profiles in spastic muscle from wrist flexors and extensors in children with cerebral palsy determined from genechip analysis

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Gene expression profiling from patients with cerebral palsy (CP, n=6) were compared to control patients (CON, n=2) with no history of neuromuscular disease. Biopsies were taken from a wrist flexor (FCU, n=8) and extensor (ECRB, n=8). Patients were selected with two major degrees of CP clinical severity. Data were analyzed with a 2-way Welch ANOVA grouping by muscle (FCU vs. ECRB) and disease (CP vs. CON). Genes with a significant change in expression ($p<0.05$) with CP were viewed as altered and subjected to further analysis (n=184). Most effects of muscle type and severity were not statistically significant. A select list of 10 genes was subjected to quantitative PCR analysis to confirm the chip results. There was a highly significant correlation between the chip and QPCR results in the effect of CP on the genes above $p<0.0001$. The list of 184 altered genes was examined to determine which Gene Ontology (GO) categories were significantly altered. There were 18 altered categories many of which were muscle specific. Other categories of interest were extracellular matrix, regulation of cell adhesion, and proton transport. These genes and categories are being investigated to determine their role in CP and potential as targets for therapy.

152. Distinctive patterns of microRNA expression in extraocular muscles (EOMs)

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EOMs are a unique group of muscles that are anatomically and physiologically distinct from other skeletal muscles. EOMs show differential susceptibility to disease, exemplified by sparing in DMD. Here we investigated the expression pattern of microRNAs(miRNAs), as they may play a role in generating the unique EOM allotype. We used microRNA microarray chips (LCSciences) covering miRBase 10.0 to define the microRNAome of EOM and limb muscle (TA-tibialis anterior). 33 miRNAs were found to be differentially regulated based (19 up-regulated & 14 down-regulated) at $p < 0.01$. Bioinformatic tools and databases were used to integrate the results with our previous transcriptomic and proteomic EOM profiling data. Interestingly, miR-155 which we found to be down-regulated in EOM, has recently been shown to be up-regulated in many primary muscular disorders, including DMD (Eisenberg et al. PNAS 2007). In conclusion, the definition of the microRNAome of EOMs complements existing data about the molecular make-up of EOMs, which will help to explain their differential sensitivity to disease and may assist in development of therapeutic strategies.

Funding: NIH EY 013862.

153. Identification of the neuromuscular junction (NMJ) transcriptome of extraocular muscles (EOM) by laser capture microdissection (LCM).

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EOMs are a anatomically and physiologically distinct group of muscles. EOMs show differential susceptibility to disease, exemplified by sparing in DMD and involvement in myasthenia gravis. To better understand the unique EOM allotype, we examined the transcriptome at the neuromuscular junctions (NMJs) and extra-synaptic regions of EOMs. Approximately 6000 NMJs and equal amount of NMJ-free fiber regions were identified by ACh-esterase staining and captured using LCM. Profiles were generated for genes differentially expressed at synaptic and non-synaptic regions, using a FDR of 1 % and validated by qPCR and immunohistochemistry. 580 transcripts (504 up-regulated) were found to be differentially expressed at these regions. These included well-known, evolutionarily conserved, synaptic markers (e.g. nicotinic Acetylcholine receptor (ACHR) alpha, delta and epsilon subunits, utrophin and nestin) as well as a large number of novel genes. Our definition of the NMJ (synapti c) transcriptome provides insight into the mechanism of formation and functioning of the unique synapses and their differential involvement in diseases noted in the EOM allotype. Funding EY013862.

154. Consequences of dysferlin deficiency in human muscle tissue

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Background: Dysferlin deficiency causes several forms of muscle disease, most prominently limb-girdle muscular dystrophy type 2B and Miyoshi myopathy. Dysferlin is a sarcolemmal protein has been demonstrated to play a role in the calcium-dependent process of membrane resealing. Methods: To explore the molecular consequences of dysferlin deficiency in humans, we analyzed gene expression patterns in muscle samples obtained from 7 Miyoshi myopathy patients at biopsy and compared them to 4 controls using the Affymetrix U133Plus2.0 GeneChip system. We compared these to existing data on human dystrophin deficiency (Duchenne muscular dystrophy, DMD) and mouse dysferlin deficiency. Using principal component analysis (PCA), linear correlation coefficients, rank coefficients, Euclidean distances, and geometric fold change analysis, we compared data both within species and between species. Results: (1) PCA clearly separated the disease from control samples. (2) There were significant similarities between the human and mouse comparisons. (3) Multiple analyses suggested that dysferlin-deficient mouse quadriceps at 8 months had the greatest similarity with human disease. (4) A comparison of our dataset with an existing one on DMD demonstrated that PCA clearly separated both disease groups from controls. (5) Geometric fold change analysis demonstrated that a limited number of genes are discordantly regulated between the dysferlin and dystrophin-deficient datasets. Discussion: This study takes advantage of the vast repositories of gene expression now available to put our data in context. We draw two broad conclusions. (1) Age and muscle type are significant determinants of gene expression in muscle tissue, and different muscle types bear differing degrees of molecular similarities to muscles from other species. (2) Gene expression patterns in dysferlin deficiency and dystrophin deficiency are similar, with only a small number of genes discordantly regulated between them. Dystrophin and dysferlin localize differently and play different roles in the muscle fiber, suggesting that this close similarity between the disease states is a result of downstream changes common to the histology of both disorders, including inflammation and connective tissue infiltration. Further biochemical studies on protein products of genes of interest are ongoing.

155. Duration of untreated juvenile dermatomyositis (JDM) at diagnosis: Impact on muscle biopsy (MBx) gene expression profiles, comparisons with polymyositis (PM)

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Background: JDM with prolonged untreated disease often have: 1) less weakness, 2) normal muscle enzymes 3) persistent rash 4) pathologic calcifications 5) decreased nailfold capillaries and bone density. MBx show upregulation of IFN-alpha induced genes, also seen in PM. Purpose: To compare untreated JDM MBx gene expression profiles: a) for impact of chronic inflammation; b) with matched JPM MBx. Methods: Microarray data from 16 girls with JDM symptoms (Sx) 2 mo or more (long) were compared with 3 girls, Sx less than 2 mo (short). qRT-PCT confirmed array data on additional MBx (n=5/group). JPM (Sx less than 2 mo) were compared with matched JDM (n=4). Results: In MBx (long vs short): 79 genes were differentially expressed; 9 genes showed significant positive correlation with length of untreated disease. Upregulation of immune responses and vasculature remodeling genes, HLA-DQA1, smooth muscle myosin heavy chain, clusterin, plexin D1, tenomodulin were confirmed by qRT-PCR and increased DC-LAMP+ mature dendritic cells by immunohistochemistry in the long duration group. Comparing JPM profiles: 48 genes were increased over JDM; 31 genes were below JDM. IRF-2 was increased in JDM; TRADD and dystonin were increased in JPM. Conclusions: Duration of untreated JDM Sx impacts on vascular remodeling, immune response genes and DC LAMP+ cells. Differential expression is seen when JPM is compared with JDM, controlling for disease chronicity. Support: R01 AR48289.

156. Coupled Bistable Switches Control Cellular Memory and Developmental Plasticity in Human Skeletal Muscle Progenitors

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Understanding cell fate determination in muscle progenitors will facilitate the development of cellular therapy for muscle diseases. We identified a human marrow stromal cell subclone, WB15-M, which has developed spontaneously into skeletal muscle progenitors. WB15-M cells expressed alpha7-integrin, MyoD and Myf5, and were capable of differentiating into mature myofibers. Interestingly, when treated with inhibitors of Erk or p38 MAPK signaling, WB15-M cells exhibited enhanced expression of Pax7, Pax3 and Msx1, and acquired the ability to express alkaline phosphatase upon BMP2 stimulation, several properties seen in satellite cells. A dual differentiation assay revealed that MAPK inhibition induced a cell fate switch between myogenic or osteogenic differentiation. Clonal analysis showed that WB15-M cells responded to myogenic or osteogenic signals in an all-or-none fashion and exhibited cellular memory, hallmarks of bistable genetic switches. The cellular memory effect was contingent on the accumulation of lineage-determination factors and could be erased by brief cycloheximide exposure. We propose a model, in which coupled bistable switches control the inducible production of lineage-determination factors. A dynamical balance between these factors determines cell fate. This model explains the developmental stability and plasticity of human myogenic progenitors and provides a conceptual framework for further study. (Funded by the Illinois Regeneration Medicine Institute.)

157. Gene Expression Profiling of Muscle Samples from Patients Carrying SEPN1 Mutations

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Mutations of SEPN1 (encoding selenoprotein N) cause a group of related congenital myopathies including multiminicore disease. Selenoprotein N function is unknown, although data from our lab suggests a role in regulating redox state and calcium release from the endoplasmic reticulum. To further understand the downstream effects of SEPN1 mutations in skeletal muscle, we conducted gene expression analysis on muscle biopsies of 3 patients with SEPN1 mutations and 3 age-matched controls. Data were filtered for >2 fold change and 3 of 6 present calls, followed by one-way ANOVA with p-value of <0.105. Software by Ingenuity Systems was used to identify important biological mechanisms and pathways. Expression of 506 probe set IDs was significantly altered based on the above criteria. Interesting findings in the calcium regulation pathway included a two-fold decrease in CACNG1 expression, that encodes the voltage-dependent DHPR gamma-1 subunit. Reduction in this protein may lead to increased calcium influx through L-type Ca channels. In addition, CAMK2D, which encodes calcium/calmodulin-dependent kinase (upregulated 2 fold) is also linked to this pathway. Genes involved in oxidation-reduction pathways that were significantly upregulated included GPX3 and DIO2. Upregulation of CASP8 (2.2 fold) suggests calcium-dependent, cysteine-specific proteolysis that may lead to increased cell death.

158. The fallacy of foci: A heterodox view of mutant RNA in myotonic dystrophy

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Dystrophia myotonia type 1 (DM1; myotonic dystrophy) is an autosomal dominant disorder due to a large CTG expansion in the 3' untranslated region (UTR) of the DM protein kinase (DMPK) gene. Transcription of this gene yields a long CUGn-containing mutant (mut) RNA, in which clinical disease is associated with repeats of n=100-5000. Phenomenologically, the expression of mut RNA is correlated with the morphologic observation of ribonucleoprotein (RNP) precipitates ("foci") in the nuclei of DMPK-expressing cells. There has been an abiding but unsupported concept that the identification of proteins in these foci is essential for a conclusion of protein-mut RNA interactions. In this presentation, I contend that this is an unwarranted inference. Revision of this view is essential to restoring confidence in diverse data on mut RNA binding to proteins not in foci that have been discounted because of non-conformity with this premise, data that are essential to a scientifica lly sound understanding of the underlying pathobiology of this disease. A new model of mut RNA-protein interactions is proposed with distinct binding properties for soluble and insoluble (foci) mut RNA that accommodates these data without exclusions. I acknowledge present or past support from the Association Francaise contre les Myopathies (AFM) and the Muscular Dystrophy Association (MDA).

159. Distinctive patterns of miRNA expression in human muscular disorders

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The muscular disorders are a heterogeneous group of over thirty different inherited diseases characterized by muscle wasting and progressive weakness of variable distribution and severity, manifesting at any age from birth to middle years, and resulting in significant morbidity and disability. Although considerable progress has been made in our understanding of the overall complexity of the pathogenesis of the various muscular disorders, the underlying molecular pathways remain poorly understood and the decisive secondary factors responsible for the variability in the clinical phenotypes are still mainly unknown. In light of their involvement in modulating cellular phenotypes we hypothesized that miRNAs might be involved in the regulation of the pathological pathways leading to muscle dysfunction. We describe a comprehensive miRNA expression profile aiming to identify new and modifying elements involved in the regulatory networks of muscle and the signature patte rn of 185 miRNAs associated with ten common myopathological conditions in human. While five miRNAs (146b, 221, 155, 214, 222) were found to be consistently dysregulated in all samples analyzed in the study suggesting that these miRNAs are involved in a common underlying regulatory pathway among all diseases other miRNAs were identified to be dysregulated only in one given disease and not in any of the others. miRs- 486, 485-5p, 331, 30e-5p, 30d, 30a-5p, 26a, 22, 193b, 101, 95, Ambi-miR-7075 and Ambi-miR-13156 all in muscle biopsies taken from Duchenne patients; miR- 517* in FSHD, Ambi-miR-10617 in LGMD2A, miR- 301 in LGMD2B and miR- 302c* in Miyoshi myopathy. In nemaline myopathy a much larger set of 36 different miRNAs were uniquely dysregulated. Hence, in contrast to a common underlying miRNA-regulatory pathway suggested with some of the diseases analyzed, this finding might point to a unique regulatory mechanism. The subsequent identification of potential target genes and the unraveling of biological signaling pathways involved in this regulatory level in these disorders, point to an additional dimension of regulation of muscle function mediated by miRNAs. Together with the tight post transcriptional regulation at the mRNA level identified in Duchenne and Miyoshi myopathy and specific mRNA:miRNA predicted interactions, some of which are directly involved in compensatory secondary response functions and others in muscle regeneration, these findings suggest an important role of miRNAs in the pathology of muscular dystrophy.

160. A model for efficacy of methylprednisone: re-synchronization of the tissue repair process

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We used a systems biology and data integration approach to define molecular networks associated with progressive weakness and wasting in muscle disease, and the effect of corticosteroids (methylprednisone) on these networks. We studied muscle biopsies of 11 types of neuromuscular disease relative to normal controls (117 biopsies, 234 microarrays). We identified molecular networks associated with progressive wasting and weakness. These networks were then studied in normal muscle regeneration (mouse; 27 time points), where we found that different nodes of the pathology-associated network were expressed at distinct times during muscle regeneration. This led to the model that dystrophic myofibers in different stages of regeneration show inappropriate cross-talk, resulting in induction of fibrosis, failed regeneration, and muscle wasting. Study of these same networks in a methylprednisone treated muscle model (rat; 17 time points) showed that key members of the pa thology/regeneration-associated network were regulated by corticosteroids in a 24-hour cycle. This suggests a model for efficacy of steroids, where corticosteroids functions by re-synchronizing the regenerative process, thereby decreasing inappropriate network cross-talk, and permitting resolution of the tissue damage (effective repair). Funded by: Department of Defense W81XWH-05-0334, NIH (3R01 NS29525-13,5R24HD050846-02, 1U54HD053177).

161. Microarray expression profiling reveals gene expression associated with the dystrophin-glycoprotein and alpha7beta1 integrin complexes

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Muscular dystrophies are frequently caused by compromised transmembrane linkage between the cytoskeleton and extracellular matrix. Two major linkage complexes in skeletal muscle, the dystrophin-glycoprotein complex and alpha7beta1 integrin complex, both mediate such connections. Mutations of components of these complexes result in different muscular dystrophies. We previously demonstrated that enhancing alpha7beta1 integrin levels can alleviate development of severe muscular dystrophy in mice lacking both dystrophin and utrophin (mdx/utrn-/-). Moreover, whereas mice deficient in either dystrophin or alpha integrin develop mild muscular dystrophies, mice lacking both proteins manifest a severe muscle disease. To identify genes associated with each linkage system, as well as possible biomarkers and therapeutic targets, global gene expression profiles in skeletal muscle from 5 week old wild type, mdx, mdx/utrn-/-, a7-/-, and mdx/a7-/- mice were examined using Affy metrix microarrays. Expression of distinct sets of genes associated with each complex was identified. Genes associated with the severity of the different dystrophies and potential candidate genes for diagnosis and therapeutic interventions were also identified. These results provide new insights into how the dystrophin-glycoprotein and the alpha beta integrin complexes may affect muscle gene expression. Supported by the NIH and MDA.

162. Computational Modeling of Regulatory Networks in Muscle Differentiation

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Inactivity, muscular dystrophies and cachexia are hallmarked by muscle wasting, essentially a consequence of an imbalance between muscle degeneration and regeneration. To identify the key players and to infer the integrated effects of inhibitory and activating signals is important for therapies aimed at enhanced muscle regeneration. In vitro differentiation of myoblasts is a well-known model system for in vivo regeneration. Myoblast differentiation is tightly controlled at the transcriptional level. The aim of our research is to derive computational models that describe the transcriptional regulation of muscle differentiation in vitro and to evaluate their relevance for in vivo regeneration. For this, we employ Bayesian Networks that can capture causal relationships between a regulator and its target. We learn Bayesian Networks from public as well as own expression profiling and chromatin immunoprecipitation data, obtained with microarray or massively parallel seq uencing methodology. Confidence in the learned networks is enhanced by integration with different computationally and experimentally-derived meta-data, like predicted transcription factor binding sites, transcription factor activities, mRNA stability, and literature associations. Our approach as well as some examples and results will be demonstrated during the talk. P.A.C. 't H. is funded by NWO VENI grant no. 2005/03808/ALW.

163. Alternative splicing and miRNA regulation during myogenesis

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Many studies describe how developmental changes in gene expression define stem cell identity, few address the role of alternative splicing (AS) regulation. Using splicing-sensitive microarrays, we are examining AS regulation during differentiation of mouse C2C12 myoblasts. We have identified hundreds of changes in AS that occur during myogenic differentiation but not when the same cells undergo osteogenic differentiation. A number of these altered splicing events appear to represent potential nodes in interconnected regulatory pathways. For example, numerous mRNAs are alternatively processed such that their 3'-UTRs are switched, thus altering their putative microRNA (miRNA) binding sites. Additionally, other genes with changes in expression encode intronic miRNAs, suggesting coordinate regulation of these miRNAs with their host gene. By determining how AS and miRNA regulation are coordinated with respect to each other, in particular at 3'-UTRs, we will g ain insight into their contribution to proper myogenesis, and how their misregulation leads to altered muscle development in diseases like myotonic dystrophy.

164. miRNAs in diseased muscle

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MicroRNA (miRNA)-mediated control of protein expression is likely a widely used mechanism for posttranscriptional regulation of important cellular pathways, including muscle development and disease. To determine if changes in miRNA populations might be linked to muscular dystrophies, we have carried out a comparative miRNA expression profiling of muscle samples obtained from patients with 10 different groups of muscle diseases. Among 428 human miRNAs surveyed, a subset of 185 human miRNAs were found to be differentially expressed at a significant level ($p < 0.05$, false discovery rate < 0.05) in at least one of the 10 muscle conditions compared with the control panel. Among this set of differentially expressed miRNAs, the expression profile in human tissues has been previously established for 145. Of these, 60% are known to be expressed in adult muscle (as well as in other tissues). Most of the differentially expressed miRNAs were up-regulated in the different dise ases. Five miRNAs (146b, 221, 155, 214, 222) were found to be consistently dysregulated in all samples analyzed in the study across the various diseases thus suggesting the possibility a common underlying regulatory pathway among these diseases. Using a meta prediction tool of human miRNA targets that integrates all of the leading prediction methods into an improved predictor of human miRNA targets, we were able to compare predicted targets to actual mRNA array results from published studies. There was a high degree of correlation of the observed mRNA changes with miRNA predicted targets. Our current studies are to validate these miRNA results in cell culture.

165. Essential role for microRNAs during skeletal muscle development

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microRNAs (miRNAs) regulate gene expression post-transcriptionally by targeting mRNAs for degradation or by inhibiting translation. miRNAs are important for organogenesis of a variety of tissues including heart, brain, limb, lung and skin. To gain insight into the roles of miRNAs in mammalian skeletal muscle development, we eliminated miRNA function specifically in the myogenic compartment during embryogenesis. Mutant mice with reduced skeletal muscle miRNAs die perinatally and display decreased skeletal muscle mass accompanied by muscle cell death and myofiber degeneration. Interestingly, the miRNA mutant muscle phenotype is strikingly similar to that observed during aging. Our findings demonstrate that miRNAs are critical components required for embryonic myogenesis and suggest that alterations in miRNA-mediated gene regulation may contribute to age related sarcopenia. Current studies focus on elucidating the role of miRNAs in apoptotic pathways.

166. The transcriptional and post-transcriptional regulation of skeletal muscle gene expression

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The expression of MyoD is sufficient to convert a fibroblast to a skeletal muscle cell. A combination of expression analysis and chromatin immunoprecipitation studies show that MyoD directly activates genes expressed both early and late during the program of cell differentiation. Temporal patterning of gene expression is achieved, at least in part, through a feed-forward mechanism: MyoD is sufficient to activate early genes, however, the activation of late genes requires both MyoD and additional transcription factors induced by MyoD. In the absence of MyoD, the other participating transcription factors are not sufficient to efficiently initiate expression of the target genes. This is due, at least in part, because these factors cannot bind to the promoters in the absence of MyoD, either because they cannot locate the promoters in chromatin or because they cannot recruit necessary chromatin remodeling proteins, or both. At a subset of promoters, the homeobox protei n Pbx is necessary for MyoD to localize a gene within chromatin and initiate transcription. This demonstrates a specific mechanism of targeting MyoD to loci in inactive chromatin and reveals a critical role of homeodomain proteins in marking specific genes for activation in the muscle lineage. Gene suppression by Myod-induced myogenesis occurs largely through induction of micro-RNAs. Supported by NIAMS.

167. Elucidating alternative splicing misregulation in myotonic dystrophy type 1 (DM1)

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When MBNL1 function is depleted by CUG-repeat RNA in DM1, many alternative splicing events are misregulated. For some, MBNL1 is directly required. For others, MBNL1 may be indirectly required through its splicing of other splicing factors. To better understand how the DM1 phenotype arises, we need to understand these relations. I am approaching this through estimates of "mutual information", a statistical measure of dependence. Given several alternative splicing events attributed to MBNL1 loss, I estimated their mutual information with MBNL1 expression levels over a large, diverse expression dataset. I divided the splicing events into two subsets according to the level of mutual information with MBNL1. For each subset, I searched the proximal introns for enriched motifs. The subset with greater MBNL1 mutual information showed a motif similar to the sequence targeted by MBNL1; the subset with less was enriched for the FOX1 splicing factor binding motif. T he splicing of the FOX1 mRNA is altered when MBNL1 function is lost, such that the predicted FOX1 protein would lack its RNA-binding domain. I plan to extend this approach to tease direct from indirect effects of MBNL1 loss of function in DM1.

NOTES

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