2012

New Directions in Biology and Disease of Skeletal Muscle Conference

Fifth Biennial Conference

http://www.med.upenn.edu/muscle

2012 New Directions in Biology and Disease of Skeletal Muscle Conference

June 17-21, 2012
New Orleans, Louisiana

Objective

The New Directions in Biology and Disease of Skeletal Muscle Conference is returning to New Orleans, Louisiana in 2012. The fifth biennial meeting is being organized by H. Lee Sweeney and Elizabeth McNally. The conference will highlight current developments in muscle biology, disease, and therapy with presentations by leading international researchers.

The previous four meetings brought together muscle researchers who seldom attended the same specialty conferences. Many important scientific relationships and productive collaborations resulted from these interactions. As in the past, this meeting will provide a unique opportunity for collaborations to develop and thrive while exposing young scientists to a cross section of muscle and neuromuscular disease research.

The meeting format will be highly interactive and will be a combination of platform presentations and poster sessions. The Selection Committee was overwhelmed with the quality and research accomplishments from the abstracts submitted. The Poster Presentations will undoubtedly be a highlight of the 2012 NDBDSM. Over 125 posters will be on display at two Poster Sessions. Fourteen posters have been selected for Oral Presentations, during Sessions II thru Session VII

This year we are featuring two new additions to the program:

- MDA Translational Research Symposium on Sunday, June 17th, from 12 noon to 5:15 pm
  The goal of the MDA Translational Research Symposium is to give stakeholders an opportunity to review pathways by which neuromuscular disease therapies can be brought into clinical, registration-driven development and subsequently to the market. The symposium will highlight examples of therapeutics currently in development in both the academic and the corporate environments, and will include discussions of best practices for academic/industry relationships. Talks will include topics on intellectual property, funding mechanisms, available resources), and development considerations in the planning of clinical trials.
- PPMD Sponsored Workshop on Stem Cell Therapeutics on Thursday, June 21st from 8:00 am to 12 noon
  The aim of the stem cell workshop is to bring together leading experts in the field to define state-of-the-art stem cell therapy for neuromuscular diseases. Talks will define the obstacles which hinder efficient cell or cell and gene therapies for muscular dystrophy. The sessions also will explore exciting advances and the progress expected in the near future.

Conference Organizers

Elizabeth McNally, MD, PhD, The University of Chicago Medicine
H. Lee Sweeney, PhD, University of Pennsylvania

Program Committee

Elisabeth Barton, PhD, University of Pennsylvania
Alan Beggs, PhD, Children's Hospital Boston
Jill Rafael-Fortney, PhD, The Ohio State University
Laura Ranum, PhD, University of Florida
Melissa Spencer, PhD, University California Los Angeles
Volker Straub, MD, PhD, University of Newcastle, UK
Kathryn Wagner, MD, PhD, Kennedy Krieger Institute

Coordinator

Tharrie Daniels, The University of Chicago Medicine
Cheryl Fischer, University of Pennsylvania

2012 New Directions in Biology and Disease of Skeletal Muscle Conference

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KEYNOTE SPEAKER

Douglas C. Wallace, PhD

The Children's Hospital of Philadelphia
and University of Pennsylvania

A Mitochondrial Paradigm for Metabolic Degenerative Diseases

Sunday, June 17, 2012

6:00 PM **Opening Remarks and Introduction to Keynote Speaker**
Elizabeth McNally, MD, PhD
H. Lee Sweeney, PhD

6:15 PM **Keynote Speaker**
Douglas C. Wallace, Ph.D.

7:00 PM **Reception**

Douglas C. Wallace, PhD

Michael and Charles Barnett Endowed Chair in Pediatric Mitochondrial Medicine and Metabolic Disease
Director, Center for Mitochondrial and Epigenomic Medicine, Children's Hospital of Philadelphia Research Institute
Professor, Pathology and Laboratory Medicine, University of Pennsylvania

Dr. Wallace has been working on human and mammalian mitochondrial genetics for 40 years. He was the first to demonstrate that mammalian cells harbored cytoplasmically inherited genes by inventing the cybrid transfer technique in the early 1970s and using this system to demonstrate that mammalian chloramphenicol resistance could be transferred from cell to cell by fusing only a cytoplasmic fragment, a cytoplast, in the absence of a nucleus. He then proceeded during the 1970s to define the rules of mammalian mitochondrial genetics, culminating in his demonstration of the maternal inheritance of the human mitochondrial DNA (mtDNA) in 1980. From this foundation, his research followed two paths: the investigation of the nature and extent of human mtDNA variation in aboriginal populations and the quest for diseases resulting from mutations in the mtDNA. The population studies revealed that mtDNA variation was unique in that it correlated highly with the ethnic and geographic origins of indigenous peoples. This ultimately led to the realization that mtDNA variation was limited by natural selection and that mtDNA variation has been an important adaptive system for permitting people to survive and multiple in the range of different human environments. The quest for mtDNA diseases culminated in 1988 with the report by Wallace that Leber Hereditary Optic Neuropathy (LHON) was caused by a mtDNA missense mutation, making it the first maternally inherited mtDNA disease to be identified. Since that time, Wallace has not only shown that mtDNA mutations result in a wide range of clinical phenotypes but also that somatic mtDNA mutations are central to the aging process as well as for various age-related diseases such as Alzheimer and Huntington Disease. When the population specific mtDNA variation was compared to the frequency of common "complex" diseases, it was found that ancient mtDNA variation plays an important role in predisposition to many of these diseases. Thus, mtDNA variation has now been shown to be central to both rare and common multi-system diseases.

2012 New Directions in Biology and Disease of Skeletal Muscle Conference

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Sunday, June 17, 2012

Ballroom I & II, 12th Floor

10:00 AM - 6:00 PM **Registration**

Session I - MDA Translational Research Symposium

Chair: Cristina Csimma, PharmD, MHP

12:00 PM **WELCOME AND MEETING ANNOUNCEMENTS**
Elizabeth McNally, MD, PhD and **Lee Sweeney, PhD**

12:05 PM **INTRODUCTION**
Jane Larkindale, DPhil, Director of Translational Research, MDA

12:15 PM **MODELS OF TRANSLATION IN ACADEMIA**
Justin Fallon, PhD, *Bringing Biglycan to the Clinic*
Carrie Miceli, PhD and **Stan Nelson, MD**, *Development of Combination Therapy for Exon Skipping*
Kanneboyina Nagaraju, **DVM, PhD**, *Challenges of Setting up an Academic Startup Company*

1:00 PM **Peter J. Kelleher**
Working with your Technology Transfer Office - A Collaborative Process

1:40 PM BREAK

2:00 PM **Cristina Csimma, PharmD, MHP**
Translating Compounds into Therapeutics: Emerging Drug Development Model

2:40 PM **PANEL DISCUSSION: DRUG DEVELOPMENT RESOURCES**
MDA resources (MDA Venture Philanthropy) – **Jane Larkindale, DPhil**
NIH resources (TACT, TRND etc.) – **John Porter, PhD**
TREAT-NMD Advisory Committee for Therapeutics – **Volker Straub, MD, PhD**
Registries – **Sharon Hesterlee, PhD**
Pfizer drug database – **Carl Morris, PhD**

3:25 PM BREAK

3:40 PM **MODELS OF TRANSLATION AND FUNDING IN THE BIOPHARMACEUTICAL INDUSTRY**
Pfizer – **Carl Morris, PhD**
The burden of past failures on future drug development programs

Cytokinetics – **Fady Malik, MD, PhD**
Concept to Clinic: Skeletal Muscle Troponin Activators and their Application to Neuromuscular Diseases

PTC Therapeutics – **Stuart Peltz, PhD**
From concept to reality: the critical steps from initial drug descovery to clinical development

AVI Biopharma – **Ed Kaye, MD**
Phase 2b Results of Eteplirsen in Duchenne Muscular Dystrophy

Lawrence Charnas, MD, PhD
Shire HGT

Marc Blaustein
Halo Therapeutics' HT-100: A Patient-Partnered Drug Development Model

5:30 PM **SUMMARY OF SESSION, KEY MESSAGES AND LESSONS LEARNED**
John Porter, PhD

6:00 PM **OPENING REMARKS AND INTRODUCTION TO KEYNOTE SPEAKER**
Elizabeth McNally, MD, PhD and **Lee Sweeney, PhD**

5:45 PM BREAK (*15 minutes*)

6:15 PM **KEYNOTE SPEAKER**
Douglas C. Wallace, PhD, The Children's Hospital of Philadelphia and University of Pennsylvania
A Mitochondrial Paradigm for Metabolic Degenerative Diseases

7:00 PM **RECEPTION** – *The Plimsoll Club, 11th Floor*

2012 New Directions in Biology and Disease of Skeletal Muscle Conference

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New Orleans, Louisiana

Monday, June 18, 2012

Ballroom I & II, 12th Floor (except Poster Session)

Session II Inflammation and Fibrosis

Chair: Melissa Spencer, PhD

8:00 AM **Kanneboyina Nagaraju, PhD,** Children's National Medical Center
Inflammation in dysferlin deficiency

8:20 AM **Benedicte Chazaud, PhD,** Universite Paris Descartes
Inhibition and stimulation of myogenesis by differentially activated macrophages during human skeletal muscle regeneration

8:40 AM **Jeffery Molkentin, PhD,** Cincinnati Children's Hospital
p38 signaling defects in MD

9:00 AM **Pura Munoz Canoves, PhD,** Universitat Pompeu Fabra
Immune mediated mechanisms involved in development of fibrosis in mdx muscles

9:20 AM BREAK

10:00 AM **Elizabeth McNally, MD, PhD,** University of Chicago
Matrix regulation of muscle growth and fibrosis

10:20 AM **María Jose Acuna,** Pontificia Universidad Católica de Chile
Angiotensin 1-7, a novel agent that reduces fibrosis and improves strength in dystrophic skeletal muscle

10:40 AM **Kathryn Wagner, MD, PhD,** Kennedy Krieger Institute, John Hopkins University
Role of myostatin in fibrosis

11:00 AM LUNCH BREAK *on your own (2 hours)*

Session III Muscle Growth and Regeneration

Chair: Elisabeth Barton, PhD

1:00 PM **Grace Pavlath, PhD,** Emory University
Mechanisms of Muscle-Specificity in Oculopharyngeal Muscular Dystrophy

1:20 PM **Anton Bennett, PhD,** Yale University
Improvement in regenerative myogenesis and muscular dystrophy in mice lacking MKP-5

1:40 PM **Markus Ruegg, PhD,** Universitat Basel
Imbalance in mTORC1 signaling can cause severe myopathies

2:00 PM **Da-Zhi Wang, PhD,** Children's Hospital Boston
micro(RNA)management of muscle development and disease

2:20 PM BREAK

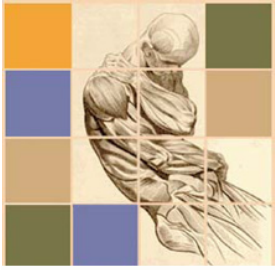
3:00 PM **Elizabeth Chen, PhD,** John Hopkins University
Cystoskeletal rearrangement in myogenesis

3:20 PM **Frank Naya, PhD,** Boston University
MEF2A regulates the Gtl2-Dio3 miRNA mega-cluster to modulate Wnt signaling in skeletal muscle regeneration

3:40 PM **Atsushi Asakura, PhD,** University of Minnesota
Genetic down-regulation or pharmacological inhibition of Flt-1 ameliorates the muscular dystrophy phenotype by increasing the vasculature in DMD model mice

4:00 PM BREAK *(1 hour)*

5:00 PM **POSTER SESSION I** *and Wine and Cheese Reception (3 hours)*
Azalea & Magnolia Ballrooms, 3rd floor



2012 New Directions in Biology and Disease of Skeletal Muscle Conference

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New Orleans, Louisiana

Tuesday, June 19, 2012

Ballroom I & II, 12th Floor (except Poster Session)

7:00 AM **POSTER SESSION II** *and Continental Breakfast*
Azalea & Magnolia Ballrooms, 3rd floor

Session IV Trafficking Defects in Muscle Disease

Chair: Alan Beggs, PhD

10:00 AM **Renzhi Han, PhD,** Loyola University
Mechanisms of muscle inflammation associated with defective membrane repair

10:20 AM **Jocelyn Laporte, PhD,** IGBMC
Defects in membrane trafficking and phosphoinositide pathways in centronuclear myopathies

10:40 AM **Ning Liu, PhD,** UT Southwestern
Role of muscle-specific microRNAs in skeletal muscle function and pathogenesis of muscular disorders

11:00 AM **Melissa Spencer, PhD,** UCLA
Impaired calcium calmodulin kinase signaling and muscle adaptation response in limb girdle dystrophy type 2A

11:20 AM **Alan Beggs, PhD,** Boston Children's Hospital, Harvard Medical School
Ultrastructural Pathology and Functional Deficits Can Be Reversed Following Protein-Replacement in a Murine Model of Myotubular Myopathy

11:40 AM **James Dowling, MD, PhD,** University of Michigan
Dominant mutation in CCDC78 in a novel centronuclear myopathy with cores

12:00 PM **Rita Barresi, PhD,** University of Newcastle
Secondary protein abnormalities in patients with anoctaminopathies

12:20 PM LUNCH BREAK *on your own (2 hours)*

Session V Nuclear Defects

Chair: Laura Ranum, PhD

2:30 PM **Maurice Swanson, PhD,** University of Florida
RNA regulation in muscle development and disease

2:50 PM **Stephen Tapscott, MD, PhD,** Fred Hutchinson Cancer Research Center, University of Washington
Inefficient epigenetic repression of DUX4 as a cause of facioscapulohumeral dystrophy (FSHD)

3:10 PM **Silvere van der Maarel PhD,** Leiden University Medical Center
Facioscapulohumeral muscular dystrophy: it takes two to tango

3:30 PM **Laura Ranum, PhD,** University of Florida
Repeat Associated Non-AUG (RAN) translation in myotonic dystrophy

3:50 PM BREAK

4:10 PM **Davide Gabellini, PhD,** Fondazione Centro San Raffaele del Monte Tabor
A chromatin-associated ncRNA regulates a Polycomb/Trithorax epigenetic switch in FSHD muscular dystrophy

4:30 PM **Isabelle Richard, PhD,** Genethon
The twists and turns of calpain 3 gene transfer

4:50 PM **Mani Mahadevan, MD,** University of Virginia
Proof of concept for a novel therapy for muscular dystrophy in myotonic dystrophy (DM1)

2012 New Directions in Biology and Disease of Skeletal Muscle Conference

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Wednesday, June 20, 2012

Ballroom I & II, 12th Floor

Session VI Signaling Defects and Therapies

Chair: Jill Rafael-Fortney, PhD

8:00 AM **Stefan Gehrig, PhD,** University of Melbourne
Turning up the heat on muscular dystrophy

8:20 AM **Dennis Guttridge, PhD,** Ohio State University
Targeting NF-KB as a potential therapy for Duchenne muscular dystrophy

8:40 AM **Ronald Victor, MD,** The Heat Institute, Cedars-Sinai Medical Center
Tadalafil alleviates functional muscle ischemia in patients with Becker muscular dystrophy

9:00 AM **Dean Burkin, PhD,** University of Nevada
Integrin signaling in muscular dystrophy

9:20 AM BREAK

10:00 AM **David Israeli, PhD,** Genethon
Distinctive serum miRNA profile in mouse models of striated muscular pathologies

10:20 AM **Basil Petrof, MD,** McGill University
CCR2 and TLR4 Inhibition Reveals the Therapeutic Potential for Targeted Modulation of Innate Immunity in DMD

10:40 AM **Rachelle Crosbie, PhD,** UCLA
Role of sarcospan and alpha7 integrin in laminin-binding, muscle force production, and amelioration of muscular dystrophy

11:00 AM LUNCH BREAK *on your own (2 hours)*

Session VII Gene Correction/Replacement and Novel Therapeutics

Chair: Kathryn Wagner, MD, PhD and Volker Straub, MD, PhD

1:00 PM **Anna Buj-Bello, PhD,** Genethon
Gene replacement therapy for myotubular myopathy

1:20 PM **Francesco Muntoni, MD,** UCLA
Exon skipping in Duchenne Muscular Dystrophy

1:40 PM **Louis Kunkel, PhD,** Children's Hospital Boston
Therapeutic development using zebrafish models of muscular dystrophy

2:00 PM **Thurman Wheeler, MD,** University of Rochester Medical Center
Myotonic dystrophy: identifying therapeutic candidates

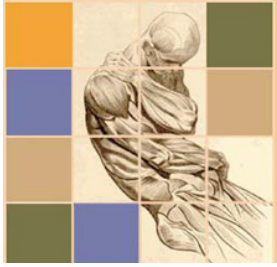
2:20 PM BREAK

3:00 PM **Barbara Smith, PT, PhD,** University of Florida
Phase I/II Trial of Diaphragm Gene Therapy for Pompe Disease: Initial Ventilatory

3:20 PM **Jackie McCourt,** University of Minnesota
Stability of human dystrophin constructs skipped around exon 45

3:40 PM **Guy Odom, PhD,** University of Washington
Development of an extracorporeal circuit for the regionalized delivery of rAAV

6:30 PM **RIVERBOAT CRUISE** *(4 hours)*



2012 New Directions in Biology and Disease of Skeletal Muscle Conference

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Thursday, June 21, 2012

Ballroom I & II, 12th Floor

Session VIII PPMD Sponsored Workshop on Stem Cell Therapeutics

Chair: Gillian Butler-Browne, PhD

9:00 AM **INTRODUCTION**

9:10 AM **Yvan Torrente, MD, PhD,** University of Milan
Preclinical experience and perspectives of a clinical trial using CD133 stem cells

9:30 AM **Francesco Saverio Tedesco, MD, PhD,** University College London
Mesoangioblast-based therapy for DMD: current clinical experimentation and novel pre-clinical strategies

9:50 AM **Yan Cherel, DVM, PhD,** College of Veterinary Medicine Oniris
and **Karl Rouger, PhD,** INRA UMR703
MuStem cells: a promising tool for DMD therapy

10:30 AM BREAK

10:45 AM **Gillian Butler-Browne, PhD,** Institut de Myologie UMRS 974
Autologous myoblast transplantation for OPMD

11:05 AM **Bruno Peault, PhD,** UCLA
A perivascular reserve of therapeutic stem cells for musculoskeletal regeneration

11:25 AM **Jean-Thomas Vilquin, PhD,** UPMC UM76, AIM INSERM U974
Aldehyde dehydrogenase activity identifies distinct cell populations within human muscle: new candidates for cell therapy

11:45 AM **CLOSING REMARKS** *(15 minutes)*

2012 New Directions in Biology and Disease of Skeletal Muscle Conference

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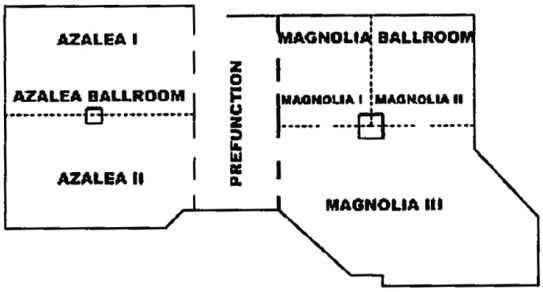
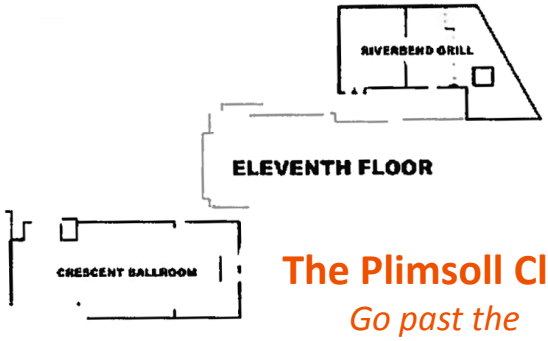
THE WESTIN
NEW ORLEANS
CANAL PLACE

Monday & Tuesday Poster Sessions

THIRD FLOOR



Reception Sunday 7:00 P.M.

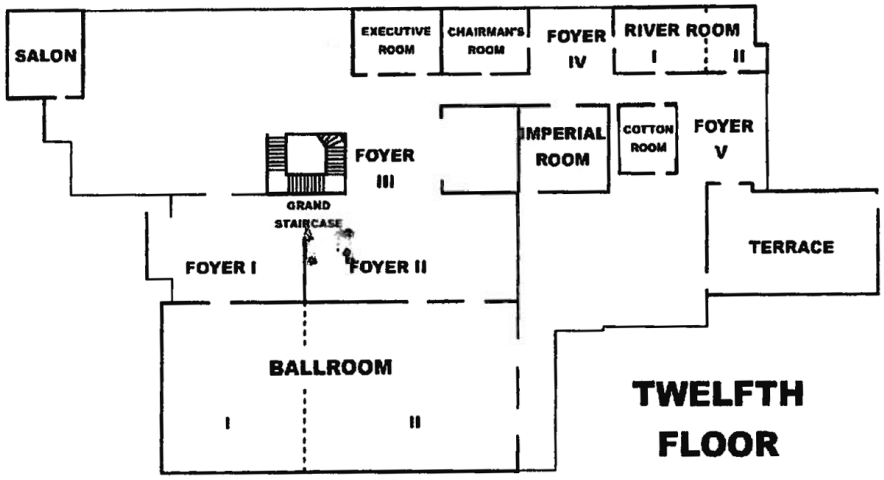


The Plimsoll Club

Go past the
River 127 Restaurant

*** Business Attire ***
NO jeans or sneakers
NO t-shirts or shorts

All Sessions



2012 New Directions in Biology and Disease of Skeletal Muscle Conference

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MAJOR FUNDING SUPPORT

MDA Muscular Dystrophy Association
Fighting Muscle Disease

Parent Project Muscular Dystrophy
LEADING THE FIGHT TO END DUCHENNE

AFMTÉLÉTHON
INNOVER POUR GUÉRIR

National Institute of Neurological Disorders and Stroke

National Institutes of Health

Office of Rare Diseases Research
National Institutes of Health

NIAMS

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ADDITIONAL FUNDING SUPPORT

Aurora Scientific Inc.

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JAIN FOUNDATION
ORCHESTRATING A CURE
LGMD2B DYSFERLINOPATHY MIYOSHI

Pfizer

Shire
Human Genetic Therapies

PTC THERAPEUTICS

COALITION TO CURE CALPAIN 3
OVERCOMING WEAKNESS WITH STRENGTH

AVIBioPharma

Riverboat Cruise with the New Orleans Steamboat Company

All Aboard !!!!

THE NATCHEZ STEAMBOAT

Wednesday, June 20, 2012

Boarding Starts at 6:30 pm

Ship Sails at 7:00 pm

Enjoy a Evening of Sailing on the Great Mississippi Riverat a

Cajun Food Festival

Live Jazz Band

Open Bar

Local Beers

Hors d”oeuvres

DIRECTIONS: Leave the Hotel and head to the River Front.

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68. Small heat shock protein B7 (HspB7) is essential for sarcomerogenesis in muscle development.
Yan, Yu-Ting, Academia Sinica

69. Injury-induced Pathological Ca2+ Entry in Dysferlinopathic Skeletal Muscle
Ziman, Andrew, University of Maryland, Baltimore

70. Transgenic mice expressing human LTBP4 display skeletal muscle fiber hypertrophy.
Ceco, Ermelinda, University of Chicago

71. The role of Smad3 in regulating weightlessness-induced muscle atrophy and its molecular mechanism
Chen, Xiaoping, China Astronaut Research and Training Center

72. Molecular mechanism of sarcolemmal nNOS localization in vivo
Duan, Dongsheng, University of Missouri

73. Neuronal nitric oxide synthase-deficient mice have slower phosphocreatine recovery kinetics and reduced microvascular response following muscle contractions
Forbes, Sean, University of Florida

74. Deficiency of the fast twitch muscle fiber protein alpha-actinin-3 alters muscle adaption in response to denervation and immobilisation
Garton, Fleur, The Children's Hospital at Westmead

75. Human cell interactions during muscle regeneration: consequences for cell therapy.
Mouly, Vincent, Myology Institute

76. A common human null polymorphism in the ACTN3 gene, leading to alpha-actinin-3 deficiency, enhances muscle endurance performance and response to training by increasing calcineurin activity
Quinlan, Kate, University of Sydney

77. A common human null polymorphism in the ACTN3 gene, leading to alpha-actinin-3 deficiency, enhances muscle endurance performance and response to training by increasing calcineurin activity
Quinlan, Kate, University of Sydney

78. Muscle regeneration in the MRL mouse is AMP-activated protein kinase dependent
Roberts, Nathan, University of Illinois at Chicago

79. Imbalance in mTORC1 signaling can cause severe myopathies
Ruegg, Markus, Biozentrum, University of Basel

80. neb: a zebrafish model of nemaline myopathy due to nebulin mutation
Telfer, William, University of Michigan

81. Longitudinal changes in MRI transverse relaxation time in boys with Duchenne muscular dystrophy
Willcocks, Rebecca, University of Florida

82. Improvement in regenerative myogenesis and muscular dystrophy in mice lacking MKP-5
Bennett, Anton, Yale University School of Medicine

83. Insulin-like Growth Factor-I E-peptides increase muscle mass via the IGF-IR but at the expense of satellite cell depletion.
Brisson, Becky, University of Pennsylvania

84. Integrin Signaling in Muscular Dystrophy
Burkin, Dean, University of Nevada School of Medicine

85. An in vitro Model for Load-Sensing in Muscle Cells
Catherine, Moorwood, University of Pennsylvania, School of Dental Medicine

86. Cytoskeletal Rearrangement in Myogenesis
Chen, Elizabeth, Johns Hopkins University School of Medicine

87. Utrophin is Regulated by Sarcospan-Dependent Akt Activation: Requirement for Muscle Repair
Crosbie-Watson, Rachelle, University of California, Los Angeles

88. Cell-specific regulation of myostatin signaling
Hoogaars, Willem, Leiden University Medical Center

89. Metabolic consequences of ACTN3 deficiency â€“ more than a structural muscle protein
Houweling, Peter, Children's Hospital Westmead

90. Mul1, a novel E3 Ligase, Induces Mitophagy in Response to Muscle Wasting Stimuli
Kambadur, Ravi, Nanayang Technological University

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91. p38-alpha MAPK relieves epigenetic gene silencing to endorse the skeletal muscle differentiation program
Mal, Asoke, Roswell Park Cancer Institute

92. Evaluation of calcium clearance between different mouse models for Duchenne muscular dystrophy
Mazala, Davi, University of Maryland

93. The role of contraction in skeletal muscle development: a study of focal adhesion kinase in vivo.
Mazelet, Lise, Queen Mary University of London

94. Decreased Mitochondrial Coupling and Functional Capacity in mdx Skeletal Muscle are Unaffected by Sildenafil But Contribute to a Bioenergetic Crisis In Vivo
Percival, Justin, University of Miami, Miller School of Medicine

95. Mohawk is a Novel Regulator of Inflammation in Response to Muscle Damage.
Rawls, Alan, Arizona State University

96. Cancer cachexia induces increased oxidant damage and mitochondrial dysfunction in the diaphragm
Smuder, Ashley, University of Florida

97. Impaired calcium calmodulin kinase signaling and muscle adaptation response in limb girdle dystrophy type 2A
Spencer, Melissa, UCLA

98. Myoblast-derived induced pluripotent stem cells for muscular dystrophy therapy
Asakura, Atsushi, University of Minnesota Medical School

99. Satellite cells engraft in host mouse muscles following intra-arterial delivery
Bengtsson, Niclas, University of Washington

100. Autologous myoblast transplantation for OPMD
Butler-Brown, Gillian, Institut de Myologie UMRS 974 - UPMC University

101. Rapid generation of induced pluripotent stem cells (iPSCs) from the urine of a patient with Duchenne muscular dystrophy
Childers, Martin, Wake Forest University

102. Human Induced Pluripotent Stem Cells as a Novel Model System to Study Dystrophic Cardiomyopathy
Jennifer, Strande, Medical College of Wisconsin

103. Homeobox proteins Barx2 and Pax7 are functional antagonists downstream of Wnt signaling in muscle
Makarenkova, Helen, The Neurosciences Institute

104. The effect of mesenchymal stem cells in a PEGylated fibrin gel seeded on an extracellular matrix on skeletal muscle recovery in a volumetric muscle loss model.
Merscham, Melissa, University of Texas-Austin

105. Immortalized human dystrophic cells: a tool to assess pathophysiology and therapeutic strategies.
Mouly, Vincent, Myology Institute

106. MEF2A regulates the Gtl2-Dio3 miRNA mega-cluster to modulate Wnt signaling in skeletal muscle regeneration
Naya, Frank, Boston University

107. Expanding donor muscle cell engraftment with modulating factors
Parker, Maura, Fred Hutchinson Cancer Research Center

108. Perivascular Multipotent Stem Cells for the Therapy of Skeletal Muscle and other Organs
Peault, Bruno, The University of Edinburgh, UK, UCLA

109. Preclinical experience and perspectives of a clinical trial using CD133 stem cells
Torrente, Yvan, University of Milan

110. Aldehyde dehydrogenase activity identifies distinct cell populations within human muscle: new candidates for cell therapy
Vilpin, Jean-Thomas, UPMC UM76, AIM, INSERM U974, CNRS UMR 7215, Groupe Hospitalier Pitie-Salpetriere, Paris, France

111. microRNAs in skeletal muscle satellite cells and muscle regeneration
Wang, Da-Zhi, Children's Hospital Boston and Harvard Medical School

112. Low levels of dystrophin improve survival of the dystrophin/utrophin double knockout mouse
Aartsma-Rus, Annemieke, Leiden University Medical Center

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113. Gene replacement therapy for myotubular myopathy
Buj Bello, Anna, Genethon

114. MicroRNA Inhibition to Facilitate Muscle Regeneration and Regulate Fiber Type
Cheng, Cindy, Duke University

115. Sphingolipids modulate the inflammatory gene signature in mdx mice
Doering, Jonathan, Virginia Polytechnic Institute and State Univeristy

116. Intramuscular AAV9 Administration Results in Motoneuron and Skeletal Muscle Expression: Implications for Pompe Disease
Falk, Darin, University of Florida

117. Muscle Stem-cell therapy is improved by reducing the fibrosis associated to muscular dystrophies
Gutierrez, Jaime, Catholic University of Chile

118. Targeting NF-KB as a potential therapy for Duchenne muscular dystrophy
Guttridge, Denis, Ohio State University

119. Growth factor delivery via PEGylated fibrin gel for the treatment of skeletal muscle injuries
Hammers, David, The University of Texas at Austin

120. Evaluation of fragment AAV for the treatment of dysferlinopathy
Hirsch, Matthew, University of North Carloina

121. Distinctive profile of serum miRNA in mouse models for muscular dystrophies
Israeli, David, Genethon

122. Targeting PAX3-FKHR as a strategy for therapeutic benefit of alveolar rhabdomyosarcoma
Jothi, Mathivanan, Roswell Park Cancer Institute

123. Therapeutic development using zebrafish models of muscular dystrophy
Kunkel, Louis, Children's Hospital Boston/Harvard Medical School

124. Ultrastructural Pathology and Functional Deficits Can Be Reversed Following Protein-Replacement in a Murine Model of Myotubular Myopathy
Lawlor, Michael, Medical College of Wisconsin

125. Responses To ActRIIB-mFc Treatment Are Specific to Individual Muscles in the Mtm1p.69C Murine Model of Myotubularin Deficiency.
Lawlor, Michael, Medical College of Wisconsin

126. A MICRORNA REGULATED VECTOR PREVENTS CARDIAC TOXICITY OF CALPAIN 3 GENE TRANSFER
Le Roy, Florence, Genethon

127. RNAi therapy for Limb girdle muscular dystrophy type 1A
Liu, Jian, Nationwide Children's Hospital

128. Proof of concept for a novel therapy for muscular dystrophy in myotonic dystrophy (DM1)
Mahadevan, Mani, University of Virginia

129. Allogenic bone marrow transplantation (BMT) ameliorates muscle atrophy, degeneration, and weakness in DMRV mice
Malicdan, May Christine, NIH/NHGRI

130. A survey of pre-clinical studies of FDA-approved drugs in the mdx mouse model of Duchenne muscular dystrophy (DMD)
Malik, Vinod, Research Institute at Nationwide Children's Hospital

131. Stability of human dystrophin constructs skipped around exon 45.
McCourt, Jackie, University of Minnesota

132. Viral muscle gene delivery and targeting NOS in muscular dystrophy
McCullagh, Karl, National University of Ireland Galway

133. Exon skipping in Duchenne Muscular Dystrophy
Muntoni, Francesco, UCL Institute of Child Health

134. Metabolic changes in sialic acid synthesis pathway in GNE-myopathy model mice with long-term sialic acid treatment.
Noguchi, Satoru, National Institute of Neuroscience, NCNP

LIST OF ABSTRACTS

ABSTRACTS

Disease Biology

1. Angiotensin-(1-7), a novel agent that reduces fibrosis and improves muscular strength in dystrophyc skeletal muscle

Acuña, Maria[1], VÃ-o, Carlos[1], Santos, Robson[3], Cabello-Verrugio, Claudio[2], Brandan, Enrique[1]
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Duchenne muscular dystrophy (DMD) is a genetic myopathy that results in skeletal muscle fibrosis and loss of muscle strength. Several factors have been involved in DMD fibrotic progression such as Transforming Growth Factor type beta (TGFb) and Angiotensin II (AngII). It is known that Angiotensin 1-7 (Ang1-7) counteracts Ang II fibrotic effects in other tissues. We studied the effect of Ang1-7 on extracellular matrix (ECM) deposition, TGFb signaling and muscle strength. We found that Ang1-7 reduces TGFb induced Smad-dependent signaling and ECM deposition on treated myoblasts, being this effect mediated by the Ang1-7 transducer receptor Mas. Using the DMD murine model, the mdx mice, we found that systemic infusion or oral administration of Ang1-7 significantly reduced TGFb signaling and exercise-induced fibrosis resulting in an improvement of muscle strength. The effects observed were mediated by Mas receptor, since infusion of the Mas receptor antagonist (A-779) showed an augmented fibrosis, TGFb signaling and decreased muscle strength. Moreover, the mdx-Mas KO mice show an increased muscle damage, fibrosis and TGFb signaling compared to mdx mice, together with a decreased muscle strength evaluated by an exercise resistance test. In conclusion, Ang1-7 could be a novel therapeutic agent to treat muscle fibrosis associated to DMD. (Fondecyt 11080212, FONDAP 1398001, ConicytPFB12/2007, MDA89419, Conicyt AT-24100061, CARE)

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Disease Biology

2. Zebrafish and Mouse Models of Cofilin-2 Deficiency to Understand Human Muscle Disease Secondary to Cofilin-2 (CFL2) Mutations

Agrawal, Pankaj[1], Joshi, Mugdha[1], Gupta, Vandana[1], Fernandez-Gonzalez, Angeles[1], Kourembanas, Stella[1], Beggs, Alan[1]
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Cofilin-2 (CFL2) is an actin-binding protein that belongs to AC group of proteins (cofilin-1 (CFL1) and destrin (DSTN)), and its mutations cause nemaline myopathy with minicores. We have created mouse models of cofilin-2 deficiency, including Cfl2-conditional KO (Cofi) to obtain constitutive (Cfl2-/-) and tissue-specific cofilin-2 excision, and knock-in of the p.A35T-CFL2 human mutation. Cfl2-/- mice died by day (P) 8 with severe skeletal muscle weakness, and revealed sarcomeric disruptions, actin accumulations and nemaline bodies within muscles. Timing of the weakness coincided with cofilin-1 disappearance from within the myofibers. Mice with cardiac muscle-specific cofilin-2 excision died later (median age = 3.5 months), with reduced ejection fraction, atrial thrombosis and cardiac degeneration. Lung inflation studies on Cfl2-/- mice revealed large alveolar spaces with alveolar septation defects. p.A35T-Cfl2 knock-in mice also died by P8, and mimicked human disease. Cofilin-2 was knocked down in zebrafish (ZF) using morpholinos (MO), and rescue attempted by co-injecting cfl2-MO with WT-CFL2, p.A35T-CFL2, CFL1 and DSTN mRNAs. The cfl2-knockdown embryos had delayed hatching, decreased spontaneous tail coiling, poor activity with curved body, bent tail, and shortened length. Disruption of the sarcomeres and nemaline bodies were noted on EM. Co-injecting CFL2-WT mRNA with MO rescued a significant proportion of ZF embryos.

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ABSTRACTS

Disease Biology

3. Regulation of dystrophin-deficient muscle by an ankyrin-encoded microRNA

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In the context of Duchenne Muscular Dystrophy (DMD), the absence of a functional dystrophin protein results in cellular membrane tearing, abnormal calcium signaling, cardiac arrhythmias, and lethality in patients with DMD. Here we report the ankyrin-1.5 embedded microRNA, miR-486, is reduced in expression in DMD muscle due to the disruption of a novel dystrophin-ankyrin1.5 interaction. We demonstrate that this ankyrin1.5-dystrophin interaction is essential for maintaining normal myofiber structure, myogenic differentiation, and normal levels of miR-486 in muscle. Transgenic overexpression of miR-486 in mdx5cv (dystrophin-mutant) mice resulted in improved serum biochemistry, reduced apoptosis, and improved muscle regeneration following injury. Transient overexpression of miR-486 by minicircle DNA injections, also resulted in similar improved muscle physiology in the mdx5cv mouse. MicroRNA-486 overexpression in mdx5cv mice resulted in increased phosphorylated-AKT (activated) and modulation of levels of an mTor-dependent pathway, specifically Pgc1alpha. Additionally, the overexpression of Pgc1alpha is linked to miR-486 overexpression as a consequence of AKT activation. Together, these studies demonstrate that therapeutic overxpression of miR-486 or Pgc1alpha has significant therapeutic potential through its modulation of an AKT/mTor-dependent pathway in dystrophin-deficient muscle. (NIH/NINDS)

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Disease Biology

4. Post-transcriptional regulation of PABPN1 expression: Implications for Oculopharyngeal Muscular Dystrophy

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The nuclear poly(A) binding protein 1 (PABPN1) binds with high affinity to polyadenosine RNA and regulates polyadenylation as well as mRNA export. Mutations of the N-terminus of PABPN1 resulting in expansion of a 10 alanine stretch to 12â€“17 alanines lead to oculopharyngeal muscular dystrophy (OPMD). OPMD is a rare, late onset disease characterized by eyelid drooping, difficulty in swallowing, and weakness in the proximal limb muscles. Although PABPN1 is a critical regulator of polyadenylation and is ubiquitously expressed, how alanine expansions in PABPN1 lead to a muscle-specific disease is unknown. As PABPN1 plays an essential role in RNA metabolism, any impairment of its function should, in theory, affect numerous tissue types, but the intrinsic characteristics of muscle may make this tissue more vulnerable to the effects of mutant PABPN1. Indeed, our results reveal that muscle tissue contains significantly lower levels of PABPN1 mRNA and protein as compared to unaffected tissues. This lower amount of PABPN1 in muscle could sensitize this tissue to the deleterious effects of mutant PABPN1. To examine the mechanism underlying the decreased expression of PABPN1 in muscle as compared to other tissues, we examined PABPN1 transcript stability. Our results reveal that the half-life of the PABPN1 mRNA is decreased in muscle relative to other tissues, suggesting a post-transcriptional regulatory mechanism specific to muscle tissue. An in vitro RNA decay assay confirmed these results using tissue extracts prepared from muscle as compared to extracts prepared from kidney. We are currently testing

whether changes occur in the stability of the wild type PABPN1 transcript as compared to the mutant transcript encoding alanine-expanded PABPN1. We are also defining the cis/trans-factors that modulate PABPN1 transcript stability in muscle. Understanding the unique characteristics of PABPN1 regulation in muscle will lead to a greater understanding of the molecular mechanisms underlying OPMD and possible contribute to the development of new therapeutic interventions. Funding: Development Grant from the Muscular Dystrophy Association (MDA157523) and National Institute of Health grant NS059340.

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Disease Biology

5. Post-Translational Modification of PABPN1, The Protein Affected in Oculopharyngeal Muscular Dystrophy

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Oculopharyngeal muscular dystrophy (OPMD) is a late onset, autosomal dominant disease that presents with weakness of the muscles of the eyelids, pharynx and proximal limbs. The causative mutation is an alanine expansion in the gene encoding polyadenosine-binding protein nuclear 1 (PABPN1). PABPN1 is vital for the polyadenylation of nascent RNA transcripts. Despite the ubiquitous expression of PABPN1, OPMD symptoms present in a subset of muscles. Post-translational modification of PABPN1 could be one of the factors responsible for the tissue-specificity of OPMD. Our goal is to characterize the phosphorylation of both wildtype and mutant alanine expanded PABPN1 in muscle cells to gain insight into the tissue-specificity and pathogenesis of OPMD. Previous studies reveal that PABPN1 is essential for myogenesis in vitro, and that nuclear bulk poly (A) tail lengths decrease in murine muscle cells during differentiation, suggesting that polyadenylation is regulated during this process. Consistent with potential regulation of PABPN1, we find that both wildtype and the mutant alanine expanded forms of PABPN1 are phosphorylated during myogenic differentiation in human muscle cells in vitro. PABPN1 is also phosphorylated on serine and threonine residues in murine muscles in vivo, with the phosphorylation status changing during muscle regeneration. Together these data suggest that phosphorylation-mediated modulation of PABPN1 function could be vital for muscle homeostasis. Characterizing the regulation of PABPN1 will further our understanding of its function in muscles and potentially pave the way for the identification of novel molecular targets, which can be exploited for the rational development of therapies for OPMD.

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Disease Biology

6. Secondary protein abnormalities in patients with anoctaminopathies

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Recessive mutations in the Anoctamin 5 gene (ANO5) cause limb-girdle muscular dystrophy type 2L (LGMD2L) and type 3 Miyoshi myopathy (MMD3). These conditions have been called â€œanoctaminopathiesâ€ and they are clinically similar to dysferlinopathies, another group of muscular dystrophies which include LGMD2B and Miyoshi myopathy. Preliminary tests of commercial antibodies directed against ANO5 have not yielded encouraging results and new antibodies are not fully characterized yet. Therefore very little is known about cellular localisation and function of ANO5. To gain insights on the role of ANO5 and its potential functional interactions, we compared the expression of various proteins involved in muscle disease in biopsies from ANO5 patients and patients with other known diagnoses. Interestingly, a variable degree of sarcolemmal dysferlin deficiency was detected in a large number of LGMD2L biopsies, but cytoplasmic expression was maintained and a band of normal intensity on immunoblot was seen. Although this was not a specific finding of ANO5 patients and secondary Dysferlin reduction was also seen in other patientsâ€™ biopsies, this feature was significantly more frequent in ANO5 patients. Abnormal sarcolemmal expression of dysferlin was also observed in LGMD2A and 1C, caused by defects in the dysferlinâ€™ s binding partners CAPN3 and CAV3, respectively. These results suggest that some functional interaction between dysferlin and ANO5 may occur.

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Disease Biology

7. Characterization of novel ANO5 antibodies

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The ANO5 gene is mutated in LGMD2L and a non-dysferlin Miyoshi myopathy, MMD3. Recessive ANO5 mutations are associated with sarcolemmal lesions and defective membrane repair. In European patients the ANO5 mutation, c.191dupA, is present in both MMD3 and LGMD2L patients and is emerging to be a common cause of adult onset muscular dystrophy. ANO5 belongs to the anoctamin protein family, 10 human proteins (ANO 1-10) sharing a similar structure consisting of eight transmembrane domains, a re-entry loop domain and the novel DUF590 domain. Recent studies have shown that ANO1 and ANO2 function as calcium activated chloride channels (CaCCs), which are ion channels gated by increases in intracellular Ca2+ concentration. Heterologous expression of ANO5 and other anoctamins has shown that not all anoctamins are CaCCâ€™s. The function of ANO5 is not known as yet. Its biochemical characterization in muscle has been limited due to lack of specific and freely available antibodies. We have generated monoclonal antibodies to ANO5 using specific N-terminal and C-terminal peptides and characterized the hybridomas which detect overexpressed ANO5 fusion proteins in C2C12 cells. Once the validation tests complete successfully, the novel antibodies for ANO5 will provide an important resource for diagnostics and research.

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ABSTRACTS

Disease Biology

8. Differential Interaction of Dystrophin and Utrophin with Microtubules

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The dystrophin gene encodes a 427 kD cytoplasmic protein expressed in striated muscle which links the costameric actin cytoskeleton to the extracellular matrix via the dystrophin-glycoprotein complex. Our lab previously demonstrated that dystrophin directly binds microtubules with high affinity (Kd=0.43uM) and organizes them into a rectilinear lattice beneath the sarcolemma. Transgenic expression of nearly full-length dystrophin on the dystrophin-deficient mdx background restores microtubule organization, which is lost in mdx mice. Using in vitro cosedimentation assays, we conclude that microtubule binding activity maps to a region between hinge 3 and repeat 24. Conversely, utrophin binds microtubules in vitro with 10-fold lower affinity than dystrophin and transgenic expression of utrophin is not sufficient to rescue the disorganized microtubule network in mdx muscle. Our results suggest that any deficiency in microtubule function due to dystrophin loss may not be restored by utrophin upregulation. Therefore, it is important to determine if any pathologies can be attributed to the microtubule disorganization due to dystrophin loss. This work was supported by the NIH Training Program in Muscle Research AR007612 and NIH RO1 AR042423.

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Disease Biology

9. Mutations in the satellite cell gene MEGF10 cause a recessive congenital myopathy with minicores

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We describe a novel myopathy characterized by severe weakness, respiratory impairment, scoliosis, joint contractures, and both dystrophic and myopathic histology. Whole genome sequence from one patient was filtered using linkage data and variant databases. A single gene, MEGF10, contained nonsynonymous mutations that co-segregated with the phenotype. Affected subjects were compound heterozygous for missense mutations p.C326R and p.C774R. Screening MEGF10 in 190 patients with unexplained myopathies revealed a heterozygous mutation, p.R71W, in one subject. All three mutations were absent from at least 645 control subjects. MEGF10 contains 17 EGF-like domains, each of which contains eight cysteine residues that form disulfide bonds. Both the p.C326R and p.C774R mutations alter one of these residues, which are completely conserved in vertebrates. Murine Megf10 is known to preserve the regenerative potential of muscle satellite cells. Here, knockdown of megf10 in zebrafish resulted in impaired motility and disorganized muscle tissue, corroborating the pathogenicity of the human mutations. Our data establish the importance of MEGF10 in human skeletal muscle and suggest satellite cell dysfunction as a novel myopathic mechanism. (NIH K08 NS048180, Genise Goldenson Fund, Children's Hospital Boston Pilot Grant, Muscular Dystrophy Association Grant 186796, Gimbel Foundation, NIH P30 HD18655, NIH P50 NS40828)

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Disease Biology

10. The Na+/Ca2+ exchanger 1 (NCX1) exacerbates dystrophic pathology of the hindlimb while rescuing the diaphragm

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Recent evidence from mouse models suggests that increased sarcolemma Ca2+ influx contributes to dystrophic pathology in skeletal muscle. Thus, one potential therapeutic strategy would be to increase sarcolemmal Ca2+ efflux or clearance. Expression of NCX1 is increased in the mdx and Sgcd mouse models. We initially hypothesized that such upregulation in NCX1 would be compensatory by helping increase sarcolemmal Ca2+ efflux, hence reducing myofiber necrosis and pathology. To mechanistically evaluate this hypothesis we generated transgenic mice that overexpress the NCX1 under the control of the human skeletal a-actin promoter. Surprisingly, NCX1 transgenic mice developed progressive hindlimb pathology. When NCX1 transgenic mice were crossed with the mdx and Scgd-/- dystrophic models, hindlimb pathology was dramatically exacerbated. However, NCX1 overexpression rescued pathology in the diaphragm of both dystrophic models. This suggests that NCX1 overexpression increased reverse mode exchange activity and net Ca2+ influx in the hindlimb while increasing forward mode activity and Ca2+ clearance in the diaphragm. These results suggest that inhibition of reverse mode activity could represent a novel treatment approach. This work was supported by grants from the NIH, Howard Hughes Medical Institute, and the Jain Foundation.

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Disease Biology

11. Inhibition and stimulation of myogenesis by differentially activated macrophages during human skeletal muscle regeneration

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Skeletal muscle repair is associated with macrophages (MPs). Soon after injury, infiltrating MPs are pro-inflammatory and stimulate myogenic cell precursor proliferation in vitro. Then MPs switch their phenotype to anti-inflammatory M2 MPs that directly support myogenesis and myofiber growth. We investigated the effects of differentially activated MPs on the successive steps of myogenesis and muscle repair in human, both in vitro and in vivo. We showed in vitro that proinflammatory MPs inhibited myogenic cell fusion while anti-inflammatory MPs stimulated myogenic differentiation and increased the secondary fusion. We identified a series of cytokines and growth factors involved in the anti and pro-myogenic properties of pro- and anti-inflammatory MPs, respectively. In vivo, thanks to collaboration with M. Kjaer (Copenhagen), we analyzed regenerating human muscle sections. A dozen of myogenic and macrophagic markers were explored. We showed that regenerating myofibers containing no myogenin positive cell were associated with MPs that preferentially expressed pro-inflammatory markers (COX2, iNOS). Inversely, regenerating myofibers containing myogenin positive cells were associated with MPs that preferentially expressed anti-inflammatory markers (Arginase I, CD206, CD163). These results show that during human muscle regeneration, myogenesis is finely regulated by successive MPs subsets. (Funding: ANR, EU-FP7, EU)

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Disease Biology

12. AAV-MTM1 prolongs survival and rescues severe muscle weakness in mouse and canine models of X-linked myotubular myopathy

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Mutations in the myotubularin gene (MTM1) result in X-linked myotubular myopathy (XLMTM), a fatal pediatric disease of skeletal muscle characterized by small myofibers with frequent central nuclei and abnormal mitochondrial accumulations. Patients with XLMTM typically present with severe hypotonia, muscle weakness and respiratory failure. Previous local studies in Mtm1-mutant mice demonstrated potential efficacy of gene therapy to treat the disease. We now report the first results of intravenous delivery of an adeno-associated virus serotype 9 (AAV9) vector expressing myotubularin under the muscle-specific desmin promoter in mouse models of the disease. Myotubularin was rapidly and persistently expressed in muscles throughout the body, including the diaphragm, and this translated into robust improvement of skeletal muscle pathology and contractile force, with normal motor activity of treated mutant mice. The lifespan of both constitutive (KO) and muscle-restricted (mKO) Mtm1 deficient mice, which normally survive around 7-8 weeks, was prolonged up to the end of the 6 and 12 month-study, respectively. In addition, we also report results for the direct delivery of an AAV8 vector carrying the canine MTM1 gene (cMTM1) into the cranial tibialis (CT) muscle of the recently characterized canine model of the disease. We observed dramatic improvement in the strength of limb muscles treated with AAV8-cMTM1 to levels approaching that of age-matched normal littermates. This response was already detectable at 1 week post-injection, and improvement continued until a terminal measurement at 6 weeks post-injection. Concomitantly, the AAV-injected XLMTM muscles showed a substantial increase in mass and myofiber size, and decreases in pathological features. Our findings provide proof-of-principle that AAV-mediated myotubularin replacement is highly efficient in rescuing the muscular phenotype of both small and large animal models of myotubular myopathy.Funding: MDA, AFM, JFF, NIH

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Disease Biology

13. Modulation of Muscle Regeneration and Inflammation by TGFb1 and IL-1b.

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Inflammatory muscle pathologies such as Duchenne and Limb Girdle muscular dystrophy 2B are marked by inflammatory foci that consist of infiltrating macrophages and express pro-inflammatory cytokines. The aim of this study is to examine how these factors affect muscle regeneration directly by testing their effects on cellular proliferation and differentiation of myogenic cells. Microarray profiles of muscle cells derived from MDX and A/J mice show upregulated IL1b and TGFb pathway signaling. TGFb signaling is implicated in controlling the balance between regeneration, inflammation and fibrosis in dystrophic skeletal muscle. We proposed that canonical TGFb signaling mediates muscle regeneration and fibrosis whereas non-canonical signaling mediates inflammation synergistically with IL-1b. To test this, we investigated the effects of TGFb1 and IL-1b on satellite cell proliferation and differentiation. We further extended these studies to include macrophages, another critical component of inflammatory muscle disease. We used a co-culture system of myoblasts and macrophages to ascertain effects of classically activated (M1) and alternatively activated (M2) macrophages on myoblasts. The results of our studies should help to elucidate the mechanisms of the pathogenesis of dystrophin and dysferlin deficiencies and will direct us to a better-informed selection of anti-inflammatory therapies. Funded by Jain Foundation

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Disease Biology

14.

Role of sarcospan and alpha7 integrin in laminin-binding, muscle force production, and amelioration of muscular dystrophy

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Although sarcospan is a core component of the dystrophin- and utrophin-glycoprotein complexes, its role within muscle is assumed to be inconsequential based on the apparently normal phenotype of sarcospan-deficient mice. We performed a rigorous analysis of sarcospan-null mice and discovered that loss of sarcospan decreases levels of the dystrophin- and utrophin-glycoprotein complexes and impaired laminin-binding activity. Furthermore, sarcospan-deficient muscle is more susceptible to eccentric-contraction induced injury, despite the increased levels of alpha7beta1 integrin. This is the first demonstration of a muscle phenotype in sarcospan-deficient mice. To genetically test whether sarcospan affects integrin function and whether integrin compensates for loss of sarcospan, we generated mice lacking both sarcospan and alpha7 integrin (DKO). Muscle regeneration, sarcolemma integrity, and fibrotic accumulation were markedly exacerbated in DKO mice and were remarkable similar to DMD muscle. Expression of the dystrophin- and utrophin-glycoprotein complexes, laminin-binding, and Akt signaling were negatively impacted in DKO muscle resulting in severely diminished specific force properties. Our data demonstrate that sarcospan is the only common component of the three major laminin-binding complexes in muscle and that these interactions are important for extracellular matrix attachment and force development.(NIH)

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Disease Biology

15. EHD1 mediates vesicle trafficking required for normal muscle growth and development

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Mutations in dysferlin cause muscular dystrophy, and loss of myoferlin impairs normal myoblast fusion leading to smaller muscles in vivo. These ferlins are C2-domain containing proteins that display calcium-sensitive phospholipid binding, an interaction thought to be critical for vesicle fusion and trafficking and membrane repair in muscle. The EHD protein family has also been implicated in intracellular trafficking, especially endocytic recycling, where they may mediate cytoskeletal reorganization. We previously showed that the C2 domains in myoferlin, Fer1L5, and to a lesser extent, dysferlin bind directly to EHD proteins in vitro. We now characterized muscle from EHD1-null mice and found that loss of EHD1 leads to a decrease in the number of large muscle fibers as well as a substantial increase in serum creatine kinase levels, indicative of defective myoblast fusion and defective sarcolemmal function. Cultured EHD1-null myoblasts display defective fusion accompanied by mislocalization of caveolin-3 and Fer1L5. EHD1-null muscle also had malformed T-tubules. These data, taken together with the interaction with ferlin proteins, suggests that the EHD proteins coordinate important events in muscle growth and maintenance likely through their interaction with ferlin proteins and the ability to reorganize the cytoskeleton. Supported by NIH NS047726 and the Muscular Dystrophy Association

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Disease Biology

16. Dominant mutation in CCDC78 in a novel centronuclear myopathy with cores

Dowling, James[1], Davidson, Ann[1], Majczenko, Karen[1], Camelo-Piragua, Sandra[1], Li, Xingli[1,] Joshi, Sucheta[1], Li, Jun[1], Burmeister, Margit[1]

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Congenital myopathies are genetically heterogeneous disorders that typically present in childhood with hypotonia and weakness and are most commonly defined by characteristic muscle biopsy changes. Approximately 40% of congenital myopathies are currently genetically unresolved. We identified a family with a novel congenital myopathy characterized by distal weakness and biopsy changes that include cores and central nuclei. To identify the causative genetic abnormality in this family, we performed whole exome capture and next generation sequencing. A missense variant in the previously uncharacterized CCDC78 gene was detected. This variant alters RNA transcript processing and results in a 222 bp in-frame insertion. CCDC78 is expressed in skeletal muscle, enriched in the perinuclear region and the triad, and found in intracellular aggregates in patient muscle. Modeling of the CCDC78 mutation in zebrafish resulted in changes mirroring the human disease, including abnormal RNA processing, altered motor function and abnormal muscle ultrastructure. Using a combination of next generation sequencing and modeling in the zebrafish, we thus have identified a mutation in CCDC78 associated with a unique centronuclear myopathy with cores. Funding: NIH

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ABSTRACTS

Disease Biology

17. Phosphoinositide kinases in muscle development and disease

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Phosphoinositides (PIs) are low abundance phospholipids required for the regulation of diverse cellular processes including membrane trafficking. The 3-PIs (PI3P and PI3,5P2) appear especially important for skeletal muscle, as mutations in the 3-PI phosphatase Mtm1 results in myotubular myopathy (MTM), a devastating childhood myopathy. The function(s) of the kinases that generate these PIs have yet to be explored in skeletal muscle. The goal of this study is to determine the role of these enzymes in muscle development as well as to assess their relationship to MTM pathogenesis. To this end, we have developed and studied muscle specific knockout mice for Vps34 and Pik3c2b, 3-PI kinases expressed in muscle, and for Fig4, knockout of which disturbs PI3,5P2 kinase function. Knockout of Vps34 results in a severe dilated cardiomyopathy and a skeletal muscular dystrophy. Knockouts of Pik3c2b and Fig4, conversely, only minimally affect muscle structure and function. We also generated double mutant mice combining kinase knockouts with Mtm1 knockouts. Analyses of these mice are ongoing and are designed to test the impact of reduction of 3-PI levels on the severe phenotype associated with loss of Mtm1 function. Funding: NIH and MDA

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Disease Biology

18. Dystrophin deficiency compromises force production of the extensor carpi ulnaris muscle in the canine model of Duchenne muscular dystrophy

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Loss of muscle force is a salient feature of Duchenne muscular dystrophy (DMD), a fatal disease caused by dystrophin deficiency. Assessment of force production from a single intact muscle has been considered as the gold standard for studying physiological consequences in murine models of DMD. Unfortunately, equivalent assays have not been established in dystrophic dogs. To fill the gap, we developed a novel in situ protocol to measure force generated by the extensor carpi ulnaris (ECU) muscle of a dog. Muscle pathology and contractility were compared between normal and affected dogs. Absence of dystrophin resulted in marked histological damage in the ECU muscle of affected dogs. Muscle weight-normalized isometric tetanic force was significantly reduced in dystrophic dogs. Importantly, eccentric contraction induced a significantly greater force loss in affected dogs. To our knowledge, this is the first convincing demonstration of force deficit in a single intact muscle in the canine DMD model. The method described here will be of great value to study physiological outcomes following innovative gene and/or cell therapies. NIH/MDA/Jesseâ€™s Journey Foundation

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Disease Biology

19. Vesicle-associated protein secretion from dystrophin deficient myotubes

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Although dystrophin is suggested to link the intra and extracellular cytokeleton network, deficiency in this function is not enough to explain the onset or progression of Duchenne muscular dystrophy. The present study shows that mdx myotubes secrete twice as much protein as wild type. An aberrant vesicle trafficking in mdx myotubes was suggested by analysis of the secretome and proteome profiles. We confirmed that mdx myotubes contained LAMP-1 positive vesicles containing myosin light chain-1- a marker oversecreted in mdx myotubes- as well as secreted vesicles with aberrant densities. The secretome and proteome profiles were normalized when mdx myotubes were treated with morpholinos to rescue dystrophin expression. Altogether, these results suggest that increased protein secretion is due to a dysregulation of vesicle trafficking resulting from dystrophin deficiency. Furthermore, LAMP-1 accumulation under the plasma membrane in 16-day-old mdx muscles is consistent with the idea that an aberrant vesicle trafficking could occur in vivo as a pre-pathogenic process. We hypothesize that the export of proteins through vesicles occurs before the onset of the pathological cascade, continues thereafter, and contributes to the pathophysiogy of DMD. Funded by: NIH, Foundation to Eradicate Duchenne, Wellstone Center, European Communityâ€™s Seventh Framework Programme project MYOAGE, ANR Genopath , AFM

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Disease Biology

20. Early Disease Phenotypes in Dystroglycanopathy Mice

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Dystroglycanopathies are a family of congenital and limb-girdle muscular dystrophies in which the post-translational glycosylation of alpha-dystroglycan is impaired. Without proper glycosylation, alpha-dystroglycan binding to laminin and other extracellular matrix proteins is disrupted, leading to skeletal muscle dystrophy with variable heart, brain and eye involvement. Molecular mechanisms downstream of alpha-dystroglycan dysfunction must initiate disease pathology; however, the nature of such early disease pathways in congenital (severe) cases is unknown. In this study, we use a conditional fukutin knockout mouse, a model of severe dystroglycanopathy, to analyze skeletal muscle early in the disease process. We find evidence of dystrophic pathology in young knockout mice. Furthermore, histological, biochemical and genetic analyses suggest that specific signaling pathways are altered early in dystroglycanopathy disease. Understanding these signaling events early in the disease process is essential for developing therapeutic strategies to minimize the severity of congenital dystroglycanopathies. (Research supported by UGARF)

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ABSTRACTS

Disease Biology

21. A chromatin-associated ncRNA regulates a Polycomb/Trithorax epigenetic switch in FSHD muscular dystrophy.

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Repetitive sequences account for more than 50% of the human genome. Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common muscle disorders. It is an autosomal dominant disease associated to reduction in the copy number of the D4Z4 repetitive sequence mapping to 4q35. By an unknown mechanism, D4Z4 deletion causes an epigenetic switch leading to de-repression of 4q35 genes. We found that the Polycomb group of epigenetic repressors targets D4Z4 in healthy subjects and that D4Z4 deletion is associated to reduced Polycomb silencing in FSHD patients. We have identified DBE-T, a novel chromatin-associated non-coding RNA produced selectively in FSHD patients that coordinates de-repression of 4q35 genes. DBE-T is doing this by directly recruiting the Trithorax group protein Ash1L to the FSHD locus, driving histone H3 lysine 36 dimethylation, chromatin remodeling and 4q35 gene transcription. This study provides insights into the biological function of repetitive sequences in regulating gene expression and on how mutations of such elements can influence the progression of a human genetic disease. AFM, Dulbecco Telethon Institute, ERC, FSHD Global, FSH Society, Italian MoH, MDA.

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Disease Biology

22. A Role for Misregulated Myoblast Fusion in Rhabdomyosarcoma Pathogenesis

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Rhabdomyosarcoma (RMS) is a malignancy of skeletal muscle myoblasts that fail to exit the cell cycle and are irreversibly blocked from differentiating into syncytial muscle. The most aggressive form of RMS is caused by the PAX-FOXO1 fusion oncoprotein, but the underlying PAX-FOXO1 pathogenetic mechanisms that impede differentiation and promote neoplastic transformation remain unclear. Using a Drosophila PAX-FOXO1 model, we show that mutation in the myoblast fusion gene rolling pebbles (rols) suppresses PAX-FOXO1 lethality and that rols acts as PAX-FOXO1 target gene. In mammalian myoblasts, gene silencing of a mammalian rols ortholog, Tanc1, reveals that Tanc1 is essential for myoblast fusion, but dispensable for myocyte differentiation. Misexpression of PAX-FOXO1 in myoblasts upregulates Tanc1 and blocks differentiation, while reducing Tanc1 expression back to normal levels restores both fusion and differentiation. Furthermore, decreasing human TANC1 expression causes RMS cells to lose their neoplastic state, undergo fusion, and form syncytia. Taken together, these findings identify misregulated myoblast fusion caused by TANC1 misexpression as a RMS neoplasia mechanism and suggest fusion molecules as targets for RMS therapy.

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Disease Biology

23. Hsp72 induction reduces muscle deterioration and preserves muscle function in severely affected dystrophic mice

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Duchenne muscular dystrophy (DMD) is the most severe of the muscular dystrophies. Dystrophin-deficient muscle fibres are fragile and susceptible to influx of Ca2+ that activates inflammatory and muscle degenerative pathways. Repeated cycles of degeneration and increasingly inadequate regeneration results in loss of muscle fibers and fibrotic infiltration with significant functional impairments. We tested the hypothesis that increasing intramuscular heat shock protein 72 (Hsp72) expression would preserve muscle strength and ameliorate the dystrophic pathology in mouse models of DMD. Increasing Hsp72 expression improved muscle architecture, strength, and contractile function of severely affected diaphragm muscles in mdx dystrophic mice. We found the sarcoplasmic/endoplasmic reticulum Ca2+ ATPase (SERCA) to be dysfunctional in severely affected muscles of mdx and dko mice, and that Hsp72 interacts with SERCA to preserve its function under conditions of stress, ultimately contributing to the reduced muscle degeneration seen with Hsp72 upregulation. These data demonstrate the significant therapeutic potential of increasing Hsp72 expression for DMD and related disorders. This work was supported in part by the National Health and Medical Research Council (Australia, Project grant 1009114), the Association Francaise contre les Myopathies (France) and the Muscular Dystrophy Association (USA). SMG was supported by a National Heart Foundation Postgraduate Scholarship (Australia).

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Disease Biology

24. Myofibre growth and turnover in the mdx mouse

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Existing characterization of muscle growth in the mdx mouse is limited to gross measures of muscle mass or inferences from sectioned tissues. Here we present a systematic longitudinal survey of whole myofibre size and myonuclear addition from 1 week to 6 months of age. Single myofibres were isolated from WT and mdx mice and their size was determined by Phalloidin-based f-Actin Quantification (PhAct). Numbers of myonuclei and quiescent satellite cell (Pax7+ve) nuclei were counted for each myofibre. At 1 week old, average mdx myofibre size was similar to WT, but was low from 2 to 4 weeks, becoming similar at 6 weeks, then larger at 3 and 6 months. Myonuclear content, which paralleled WT up until 4 weeks, was increased in the older mdx as large numbers of myonuclei become sequestered into centralized linear arrays. Despite the increase in myonuclear number, the satellite cell pool remained constant as a proportion of total myonuclei. Abnormal mdx satellite cell adhesion was evident as a dearth of Pax7+ve nuclei on isolated myofibres but not in whole muscle sections. To evaluate muscle turnover after the 3-4 week phase of growth by myoblast proliferation, older mice were given BrdU and the fate of BrdU+ve cells was analyzed at different time points. This systematic analysis of post-natal whole myofibre growth clarifies several previously ill-understood aspects of the mdx pathology, and reveals unsuspected pre-myonecrotic features of newborn mdx mouse. (MDA)

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Disease Biology

25. Loss of Inactive Lipid Phosphatase Mtmr12 Results in Centronuclear Myopathy in Zebrafish

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X-linked myotubular myopathy (XLMTM) is an X-linked congenital disorder caused by mutations of myotubularin (MTM1). Males are born with severe generalized hypotonia and weakness with respiratory insufficiency. Myotubularin belongs to a large family of conserved lipid phosphatases that include both catalytically active and inactive members (i.e., MTMRs). Biochemically, inactive MTMRs have been shown to form heteroligomers with active members within the myotubularin family through protein-protein interactions. However, the functional significance of most of these interactions remains unknown. By in vitro as well as in vivo studies, we have identified that catalytically inactive MTMR12 binds to myotubularin in skeletal muscle. Knockdown of the mtmr12 gene in zebrafish resulted in skeletal muscle myopathy and impaired motor function. Analysis of mtmr12 morphant fish showed pathological changes with central nucleation, disorganized t-tubules, myofiber hypotrophy and whorled membrane structures similar to those seen in myotubularin deficiency in humans. Biochemical studies showed that deficiency of MTMR12 results in reduced stability of myotubularin protein in zebrafish. Loss of myotubularin also resulted in reduced levels of MTMR12 protein in C2C12 cells, mice and humans. Moreover, XLMTM mutations within the myotubularin interaction domain disrupt binding to MTMR12 in vitro. These studies in cells, zebrafish, mice and humans strongly support that interactions between myotubularin and MTMR12 are required for the stability of their functional protein complex in normal skeletal muscles. This work highlights an important physiological function of inactive phosphatases. These findings propose novel therapeutic approaches for myotubular myopathy by identification of drugs that could stabilize the MTM1-MTMR12 complex and hence may treat this disorder. Funding Source: NIH (NIAMS), Lee and Penny Anderson Family Foundation, William Randolph Hearst Fund, French and American Muscular Dystrophy Associations.

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Disease Biology

26. Mechanisms of muscle inflammation associated with defective membrane repair

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Dysferlin plays a role in the plasma membrane repair by promoting vesicle fusion with the plasma membrane. Dysferlin-deficient patients and mice show prominent muscle inflammation. Previously, we and other groups showed that the innate immune system including the complement pathway and the NLRP3 (Nod-like receptor family, pyrin domain containing 3) inflammasome are activated in the dysferlin-deficient muscle. However, the molecular mechanisms that initiate and perpetuate the immune activation are not well understood. It is conceivable that unrepaired membrane disruptions in the dysferlin-deficient muscle result in prolonged leakage of the intracellular vesicles. In particular, the cytosolic leaflet (containing the charged lipids) of the intracellular vesicles gets exposed to the immune system. Here we showed that the charged vesicles potently activate the NLRP3 inflammasome to induce the pro-inflammatory cytokine IL-1beta secretion from macrophages. We further identified that ROS-dependent

activation of the non-selective calcium channel TRPM2 by the charged vesicles plays an essential role in the NLRP3 inflammasome activation. These findings suggest that targeting the TRPM2-mediated activation of the NLRP3 inflammasome could be a novel therapeutic target for the diseases associated with defective membrane integrity.

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Disease Biology

27. Clinico-pathological findings of DNAJB6-myopathy

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Background: DNAJB6 is a chaperon-associated protein, which is known to have multiple functions including a role to suppress protein aggregation. Mutations in the DNAJB6 gene were recently identified in one LGMD1E and one distal myopathy family. Objective: To characterize clinical and pathological findings of patients with DNAJB6 mutation. Methods: Mutation screening of DNAJB6 was performed for a total of 192 patients with unclassified LGMD or myofibrillar myopathy. Detailed clinical and pathological analyses were performed. Results: We identified 4 Japanese families with 2 novel heterozygous missense mutations in DNAJB6. Both mutations cause an amino acid substitution at p.Phe93. All patients showed adult-onset slowly progressive proximal-dominant muscle weakness, with no brain and cardiac involvement. Muscle biopsy showed mild disorganization of myofibrils with few cytoplasmic inclusions and rimmed vacuoles. Conclusion: G/F domain of DNAJB6 may have an important role in skeletal muscle function. (Japan Society for the Promotion of Science, the Ministry of Health, Labor, and Welfare, Neurological and Psychiatric Disorders of NCNP)

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Disease Biology

28. Alpha-actinin-3: a novel genetic modifier of Duchenne muscular dystrophy (DMD)

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Homozygosity for a common null polymorphism (R577X) in the ACTN3 gene results in deficiency of the fast muscle fiber protein, alpha-actinin-3, in ~18% of the worldâ€™s population. ACTN3 genotype significantly influences muscle performance in elite athletes and in the general population. On this basis we hypothesised that ACTN3 genotype may contribute to the phenotypic variation in disease progression seen in DMD patients. Analysis of the CINRG natural history cohort reveals alpha-actinin-3 deficient DMD patients have significantly reduced 10m walk times, indicative of greater overall muscle function and slower disease progression. Consistent with this, we have also shown that Actn3 KO/mdx mice are protected against eccentric damage, have reduced fibre branching and enhanced recovery from fatigue, compared to WT/mdx mice. The protective effect of alpha-actinin-3 deficiency in both DMD patients and the mdx mouse confirms ACTN3 as a novel genetic modifier of DMD pathogenesis. Genetic modifiers have immediate importance in the stratification and analysis of results of therapeutic trials and have the potential to provide insight into the molecular pathogenesis of DMD. Our studies in Actn3 KO/mdx mice suggest that alpha-actinin-3 deficiency ameliorates the dystrophic phenotype through activation of the calcineurin pathway and the up-regulation of oxidative muscle metabolism pathways. (NHMRC)

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Disease Biology

30. Sarcospan amelioration of muscular dystrophy is dependent on integrin

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Many therapeutic approaches to the treatment of Duchenne muscular dystrophy (DMD) are centered around the attempt to replace the dystrophin-glycoprotein complex with the remaining adhesion glycoprotein complexes, which also provide stability to the sarcolemma by maintaining the connection between the extracellular matrix and the sarcolemma: the alpha7 beta1 integrin complex and the utrophin-glycoprotein complex. We have shown that sarcospan interacts with alpha7 beta1 integrin to maintain the levels of both the dystrophin- and utrophin-glycoprotein complexes and is a core component of the dystrophin- and utrophin-glycoprotein complexes. Furthermore, over-expression of sarcospan stabilizes the levels of both the utrophin-glycoprotein complex and the alpha7 beta1 integrin complex to the sarcolemma in the mdx mouse model of DMD. Thus, sarcospan is likely an important candidate therapeutic protein in the treatment of muscular dystrophy. To determine if alpha7 beta1 integrin association with the utrophin-glycoprotein complex is important for sarcospanâ€™s amelioration of mdx dystrophic pathology, we generated triple knockout (TKO) mice lacking dystrophin, alpha7 integrin, and sarcospan as well as sarcospan transgenic mice lacking dystrophin and alpha7 integrin. We discovered that sarcospan and alpha7 beta1 integrin stabilize alpha-dystroglycanâ€™s association with the utrophin-glycoprotein complex regulating the attachment of the extracellular matrix with the sarcolemma. Furthermore, we demonstrated that alpha7 beta1 integrin is important for maintaining increased levels of the utrophin-glycoprotein complex upon the over-expression of sarcospan. Genetic and chemical approaches to ameliorate muscular dystrophy by increasing utrophin at the sarcolemma rely on the assumption that utrophin alone is sufficient to restore muscle function. Our data reveal that integrin and sarcospan are necessary determinants of utrophin function. This work was supported by grants from the Genetic Mechanisms Pre-doctoral Training Fellowship USPHS National Research Service Award GM07104, the Edith Hyde Fellowship, and the Eureka Pre-doctoral Training Fellowship to J.L.M.; Undergraduate Research Fellows Program to E.C.; Tegger Foundation and Swedish Research Council (524-2009-619) to J.H.; NIH/NIAMS (R01 AR053697) to D.J.B; and NIH/NIAMS (R01 AR048179) to R.C-W.

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Disease Biology

31. Facioscapulohumeral muscular dystrophy family studies of DUX4 expression provide evidence for disease modifiers and a quantitative model of pathogenesis

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Facioscapulohumeral muscular dystrophy (FSHD) is the most prevalent myopathy afflicting both children and adults. FSHD pathology has been proposed to be caused by the misexpression of an

aberrantly spliced mRNA from the DUX4 (double homeobox 4) gene resulting in production of a pathogenic protein, DUX4-FL, whose expression has been exclusively linked to FSHD myogenic cells and muscle tissue. Here we report the analysis of DUX4 mRNA and protein expression in our large Wellstone Muscular Dystrophy Center collection of myogenic cells and muscle biopsies derived from FSHD affected subjects and their unaffected first-degree relatives. We confirmed that stable DUX4-fl mRNA and protein were expressed in myogenic cells and muscle tissues derived from clinically affected FSHD subjects. However, we also report DUX4-fl mRNA and protein expression in muscle biopsies and myogenic cells from genetically unaffected relatives of the FSHD subjects, although at a significantly lower level than in FSHD. Interestingly, several genetically diagnosed adult FSHD subjects yet to show clinical manifestations of the disease in the assayed muscles showed DUX4-fl expression. These results establish that DUX4-fl expression per se is not sufficient for FSHD muscle pathology and indicate that quantitative modifiers of DUX4-fl expression and/or function such as epigenetic status and family genetic background are determinants of FSHD muscle disease progression.

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Disease Biology

32. Delayed skeletal muscle development in spinal muscular atrophy mice

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The disruption of the survival motor neuron (SMN1) gene leads to the neurodegenerative disease spinal muscular atrophy (SMA). SMA has traditionally been considered to be strictly a motor neuron disease, however the contributions of intrinsic muscle defects to the SMA phenotype have not been thoroughly studied. Histological analyses of skeletal muscles from SMA mice revealed intact sarcomere organization albeit with an increased number of immature myofibers. This observation led us to investigate the expression of the myogenic regulatory factors (MRFs) in SMA muscle. The expression of myogenic proteins Pax7, MyoD, myogenin and MRF4 was delayed in muscles from severe Smn-/-;SMN2 and less severe Smn2B/-SMA mice. We detected aberrant expression of MRFs as early as post-natal day 2 in SMA mice, well before onset of phenotype. Misregulation of MRFs was observed in muscles independent of motor neuron denervation and degeneration, suggesting that this abnormality was intrinsic to muscle. In summary, we demonstrate delayed expression of the myogenic program and increased presence of immature myofibers in SMA mice suggesting that reduced Smn levels retard muscle development. CIHR, MDA USAThe disruption of the survival motor neuron (SMN1) gene leads to the neurodegenerative disease spinal muscular atrophy (SMA). SMA has traditionally been considered to be strictly a motor neuron disease, however the contributions of intrinsic muscle defects to the SMA phenotype have not been thoroughly studied. Histological analyses of skeletal muscles from SMA mice revealed intact sarcomere organization albeit with an increased number of immature myofibers. This observation led us to investigate the expression of the myogenic regulatory factors (MRFs) in SMA muscle. The expression of myogenic proteins Pax7, MyoD, myogenin and MRF4 was delayed in muscles from severe Smn-/-;SMN2 and less severe Smn2B/-SMA mice. We detected aberrant expression of MRFs as early as post-natal day 2 in SMA mice, well before onset of phenotype. Misregulation of MRFs was observed in muscles independent of motor neuron denervation and degeneration, suggesting that this abnormality was intrinsic to muscle. In summary, we demonstrate delayed expression of the myogenic program and increased presence of immature myofibers in SMA mice suggesting that reduced Smn levels retard muscle development. CIHR, MDA USA

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Disease Biology

33. Disruption of dystroglycan-pikachurin interaction underlies the molecular pathogenesis of eye abnormalities in dystroglycanopathy

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Dystroglycanopathy is a group of congenital and limb-girdle muscular dystrophies. Hypoglycosylation of dystroglycan is a hallmark of these disorders. Eye abnormalities are often associated with severe dystroglycanopathy; however the molecular basis of ophthalmologic phenotype is not completely understood. Pikachurin, the most recently identified ligand of dystroglycan, plays a crucial role in the formation of the photoreceptor ribbon synapse. Here we characterize their interaction and pathophysiological roles. Biochemical binding assays show that pikachurin-dystroglycan binding is calcium dependent and insensitive to heparin and high NaCl concentration. Using dystroglycan deletion constructs and tissue samples from dystroglycanopathy mouse models, we show that Large-dependent modification and the GlcNAc-beta1,2-branch on O-mannose of dystroglycan are necessary for pikachurin binding. Immunofluorescence analysis reveals a disruption of pikachurin localization in the photoreceptor ribbon synapse of these model animals. Together, our data demonstrate that post-translational modification on O-mannose, which is mediated by Large and POMGnT1, is essential for pikachurin binding and proper localization, and suggest that their disruption underlies the molecular pathogenesis of eye abnormalities in dystroglycanopathy. This work was supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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Disease Biology

34. Development of a Minimally-Invasive, Longitudinal Eccentric Muscle Damage Model in the Mdx Mouse

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Muscular dystrophy (MD) refers to a diverse cluster of inborn degenerative muscle disorders characterized by progressive muscle wasting, weakness, loss of mobility, and premature death. The dystrophin-deficient mdx mouse is commonly used preclinically to represent Duchenne muscular dystrophy, however the severity of pathology in unstressed mdx mice is comparatively mild after an initial 4-6 wk flurry of degeneration/regeneration cycles, making assessment of potential therapies challenging. We designed a novel non-terminal method for measurement of in situ limb force that can be used to measure recovery from limb damage. Under anesthesia, the limb was positioned securely at 90 degrees and anchored to a motorized foot plate and force transducer. Needle electrodes were inserted adjacent to the sciatic nerve in the thigh to induce a net plantarflexion response upon field stimulation. Mice then underwent a unilateral eccentric muscle contraction protocol in which the hind limb plantarflexor muscles were forcibly lengthened using the automated footplate while being concomitantly stimulated to contract. The resultant microtrauma induced a rapid and reproducible isometric force deficit that was monitored longitudinally until resolution of the damage. In WT mice, a bout of 60 eccentric contractions decreased twitch and tetanic force ~30% 24-hours after injury, and took 2-3 weeks to recover. In contrast, middle-aged (8-month) male mdx mice exhibited a severe, ~80% force reduction following the same loading parameters, with a protracted recovery timeline (6-8 weeks) and a new, inferior baseline at resolution (80% of initial force). The magnitude of the force deficit and the kinetics of recovery could be titrated by adjusting the number the contractions. Mdx mice that performed a bout of either 2 or 6 eccentric contractions exhibited a mitigated force deficit and serum creatine kinase response, as well as a more rapid and complete recovery time course following damage. In conclusion, we have developed a new in vivo

research model that measures functional recovery of skeletal muscle following physiological damage, and potentially enhances the translational value of mdx mice. *All studies were conducted after review by the Institutional Animal Care and Use Committee at GSK and in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals.

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Disease Biology

35. Absence of post-phosphoryl modification in dystroglycanopathy mouse models and wild-type tissues expressing non-laminin binding form of alpha-dystroglycan

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Aberrant glycosylation of alpha-DG with reduced laminin-binding activity is a biochemical hallmark of a group of muscular dystrophy commonly referred to as dystroglycanopathy. Among causative genes for dystroglycanopathy, it has been reported that fukutin and LARGE are involved in phosphodiester-linked modification of O-mannose on alpha-DG. Fukutin-related protein (FKRP) is a responsible gene of dystroglycanopathy, however its precise function is still unknown. In this study, we use several dystroglycanopathy mouse models to demonstrate that FKRP is also involved in the phosphodiester-linked modification. Furthermore, we have found that the glycosylation status of alpha-DG in lung and testis is minimally affected by defects in fukutin, LARGE or FKRP. Alpha-DG prepared from wild-type lung- or testis-derived cells lacks the post-phosphoryl moiety and shows little laminin-binding activity. These results suggest that post-phosphoryl modification not only plays critical roles in the pathogenesis of dystroglycanopathy but also is a key determinant of alpha-DG functional expression as a laminin receptor in normal tissues and cells. Ministry of Health, Labor, and Welfare of Japan Intramural Research Grant (23B-5) for Neurological and Mental Disorders and Grant-in-aid for Scientific Research (A)23249049 from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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Disease Biology

36. Role of laminin alpha 2 in myogenesis

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Congenital muscular dystrophy MDC1A is a common form of muscular dystrophy caused by mutations in the alpha 2 subunit of laminin 211(Lama2).Laminin211 is a heterotrimeric protein mainly expressed in the basement membrane of skeletal muscles, and plays a crucial role in the structural and functional integrity of the muscle fibers. The DyW mouse is an animal model for MDC1A that truly represents the manifestation and progression of the disease in humans. MDC1A is characterized by severe muscle wasting, progressive muscle weakness, and little to no regeneration. This could be the result of compromised proliferation and differentiation of myoblasts, which normally play a crucial role in muscle regeneration. To elucidate the role of Lama2 in myogenesis, its expression in the myoblast cell line C2C12 was knocked down using small interfering RNA. Lama2 silenced C2C12 cells show reduced proliferation by hemocytometric analysis and Ki67 staining as well as reduced expression of myogenin under differentiation conditions. These results indicate that Lama2 may play an important role in myoblast proliferation and differentiation. (Dr. Girgenrath's research is funded through MDA and Cure CMD)

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Disease Biology

37. Defects in membrane trafficking and phosphoinositide pathways in centronuclear myopathies

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Centronuclear myopathies are rare congenital myopathies defined by muscle weakness and abnormal organelle positioning. Several forms have been described : the most severe is the X-linked form, also called myotubular myopathy, and is due to mutations in the 3-phosphoinositide phosphatase myotubularin (MTM1); the membrane remodeling protein amphiphysin 2 (BIN1) is mutated in some autosomal recessive cases; and the large GTPase dynamin 2 (DNM2) is mutated in dominant centronuclear myopathy. All these proteins appear to regulate membrane remodeling and/or trafficking in cells, while the link with organelle mis-positioning has remained elusive. Data in different animal models as drosophila, zebrafish, mouse and dog point to defects in the structure of the triad, underlying the excitation-contraction coupling, and provide a rationale for the muscle weakness observed in patients. Abnormalities in triad markers and in BIN1 appear common to the different forms of centronuclear myopathy when muscle biopsies from patients with different mutations are analyzed. However, the role of myotubularin, amphiphysin and dynamin in skeletal muscle is not well characterized, and their functional link is not established. These points will be discussed in the presentation.

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Disease Biology

38. Satellite cell depletion and dysfunction correlates with disease progression in severe murine myotubularin deficiency

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X-linked myotubular myopathy (XLMTM) is a severe congenital myopathy caused by deficiency of the lipid phosphatase, myotubularin. While a number of structural and physiological abnormalities have been found in myotubularin deficiency, the phenotype of myogenic stem cells is unclear. In the present study, we evaluated the viability, proliferative capacity and in vivo engraftment potential of prospectively isolated myogenic cells obtained from the severely-symptomatic murine (Mtm1delta4) model of myotubularin deficiency. Mtm1delta4 muscle contains fewer myogenic cells than WT littermates, and the number of myogenic cells decreases with age. The behavior of Mtm1delta4 myoblasts is also abnormal, as they engraft poorly into C57Bl6 Rag1null mdx5cv mice and display decreased proliferation and increased apoptosis in comparison to wild type myoblasts in vitro. When evaluating the Pax7+ satellite cell population in vivo by quantitative PCR and immunohistochemistry, Mtm1delta4 animals showed significantly lower Pax7 expression and numbers of satellite cells in comparison to age-matched WT littermates. Additionally, the deficiency of satellite cells became more marked as disease progressed. These studies demonstrate specific abnormalities in myogenic cell number and behavior that may relate to the progression of disease in myotubularin deficiency, and may also be used to develop in vitro assays by which novel treatment strategies can be assessed. Funded by: NIH, MDA, Joshua Frase Foundation, Lee and Penny Anderson Family Foundation

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Disease Biology

39. Role of muscle-specific microRNAs in skeletal muscle function and pathogenesis of muscular disorders

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MicroRNAs (miRNAs) are a class of small non-coding RNAs that modulate cellular phenotypes by inhibiting expression of mRNA targets. miRNAs play key roles during skeletal muscle differentiation, and changes in miRNA expression are associated with various skeletal muscle disorders. We show that the muscle-specific miRNAs, miR-133a and miR-206, play pivotal roles in skeletal muscle function and disease. Mice with genetic deletions of miR-133a-1 and 133a-2 develop adult onset centronuclear myopathy in fast-twitch myofibers, accompanied by impaired mitochondrial function, fast-to-slow myofiber conversion, and disarray of muscle triads, the sites of excitation-contraction coupling. These abnormalities mimic human centronuclear myopathies and can be ascribed, at least in part, to dysregulation of the miR-133a target Dynamin 2, a GTPase implicated in centronuclear myopathy in humans. MiR-206 is enriched in activated satellite cells and is strongly up-regulated in during skeletal muscle injury and in mdx mice, a model of Duchenne muscular dystrophy. Mice lacking miR-206 showed delayed skeletal muscle regeneration in response to cardiotoxin injury. Loss of miR-206 also accelerates and exacerbates the dystrophic phenotype of mdx mice. MiR-206 promotes satellite cell differentiation and fusion by suppressing a collection of negative regulators of myogenesis. Our findings reveal that essential roles of miR-133 and miR-206 in muscle regeneration and pathogenesis of muscle diseases.

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Disease Biology

40. The Role MMP-13 in Skeletal Muscle Regeneration

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Skeletal muscle requires timely expression of genes for satellite cell based regeneration in coordination with extracellular matrix remodeling. We have identified a gene, MMP13, which breaks down fibrillar collagen in the resolution of muscle damage. To determine the timecourse of MMP expression and activity in regenerating muscle, cardiotoxin (CTX) injections were used to create reproducible muscle regeneration in adult mice. Muscles were harvested 1-14 days post CTX injection and processed for qRT-PCR, immunoblotting, and histological analysis. MMP9 was only elevated early in the repair process while MMP2 had transient elevation peaking 1 week post injury. In contrast, MMP13 expression was not increased until 1 week post injury and elevation persisted throughout the time course. To determine the necessity of MMP13 expression in regeneration we injected CTX into MMP13 ko mice and compared the resolution of damage to C57 mice. Parameters of muscle regeneration included embryonic myosin heavy chain and centrally nucleated fibers. We also investigated the fibrosis formation using trichrome staining. We crossed mdx mice with MMP13 koâ€™s to investigate the role in repeated injury. These data allow us to evaluate MMP13 in muscle regeneration after both acute injury and chronic injury. This work is supported by the NIH (AR057363), through the Wellstone Core (AR052646), and by the Pennsylvania Muscle Institute Training Fellowship (AR053461).

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Disease Biology

41. Dysferlin and affixin in sarcolemmal repair

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Background: Dysferlin is a sarcolemmal protein that is defective in Miyoshi myopathy and LGMD2B. In the presence of Ca2+, dysferlin accumulates around the damaged membrane site and is suggested to mediate sarcolemmal repair. We previously reported that affixin is a dysferlin-binding protein and co-localizes with dysferlin at the sarcolemma of normal human skeletal muscle. The immunoreactivity of affixin was reduced in sarcolemma of dysferlinopathy muscles. We also reported that affixin regulates reorganization of cytoskeletal actin. Objective: To examine the possibility that affixin is involved in sarcolemmal repair. Methods: We examined the calcium-dependency of dysferlin-affixin association by immunoprecipitation (IP) using mouse skeletal muscle and COS-7 transfectants. To clarify molecular behavior of affixin in sarcolemmal repair, GFP-tagged human affixin was expressed in FDB of mice (C57BL/6J and dysferlin-deficient A/J) by electroporation. Membrane wound-repair assay of single myofiber was performed using two-photon laser microscopy. Results: IP revealed that the association of dysferlin and affixin was abolished in the presence of calcium. However, the association of dysferlin and caveolin-3 was not affected in different calcium concentrations. GFP-tagged affixin accumulates at the wounded site in C57BL/6J mice, however accumulation of affixin was not observed in A/J mice. These results suggest affixin involvement in sarcolemmal repair. (Intramural Research Grant (23-5) for Neurological and Psychiatric Disorders of NCNP, Grant-in-Aid for Scientific Researchï¼ŒHealth and Labour Sciences Research Grants)

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Disease Biology

42. Overexpression of calpain inhibitor calpastatin results in reduction of myocardial damage in dystroglycan-deficient dystrophic cardiomyopathy

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We have shown that cardiac specific disruption of dystroglycan (cDGKO), or loss of dystroglycan function by aberrant glycosylation (LARGEmyd), leads to cardiomyopathy with focal patches of cardiac myocyte damage and fibrotic remodeling. This study tested the hypothesis that inhibition of calpains, activated as a consequence of sarcolemma damage, will slow disease progression in the dystrophic heart. Mice with calpastatin overexpressed in the heart (TgCpstn) were crossed to cDGKO mice. TgCpstn-cDGKO mice (50wks) have reduced focal deposition of collagen and reduction in expression of cardiomyopathic markers compared to cDGKO mice. To test whether calpains were exerting effects upstream or downstream of sarcolemma damage, we assessed myocellular uptake of immunoglobulin in vivo. Surprisingly, a small reduction in the number of patches with IgG uptake was observed in TgCpstn-cDGKO mice compared to cDGKO mice. Even more strikingly, the size of individual patches was markedly decreased from an average of >10 cells per patch in cross sections of cDGKO mice, to an average of ~1.2 cells per patch in TgCpstn-cDKO mice (similar to WT). This prevention of expansion of damage to neighboring cells was evident even in animals after isoproterenol injections. These data suggest that inhibition of calpains improves the ability of the dystrophic myocardium to self-contain an initial injury and prevents the expansion of cell damage to neighboring cells (NIH).

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Disease Biology

43. p38 signaling defects in MD

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http://www.cincinnatichildrens.org/research/divisions/m/mcb/labs/molkentin/default/

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Disease Biology

44. Critical Role of Connective Tissue Growth Factor in the Development of Fibrosis and Skeletal Muscle Strength in Duchenne Muscular Dystrophy

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Muscular dystrophies are characterized by a decrease of skeletal muscle mass and force and an increase in fibrosis. Connective tissue growth factor (CTGF) is overexpressed in muscular dystrophies and correlates with the severity of fibrosis in many diseases. However, the role of CTGF in Duchenne Muscular Dystrophy (DMD) and associated fibrosis remains unknown. We evaluated the effect of loss of CTGF expression in a genetically model (mdx-CTGF+/- mice) and a decreased CTGF activity model, mdx mice treated by two months with blocking CTGF antibodies (FG-3019, Fibrogen). Using both approaches we show a decrease of fibrotic protein levels together with a significant less muscle damage compared to mdx mice, as evidenced by cytological analyses and reduced levels of myogenic precursor markers like myogenin and embryonic myosin. Remarkably, the decrease or blocking of CTGF caused an improvement of muscle strength. On the other hand, overexpression of CTGF in wild type mice using an adenovirus, a significant and transient increase of fibrotic proteins together with a significant decrease of muscle strength was observed. These results show the importance of CTGF in the patho-physiology of muscular dystrophies and suggest that inhibition of fibrosis has important effect improving muscle strength. Therefore CTGF targeting might have significant potential in development of novel therapies for DMD and related diseases.

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Disease Biology

45. Histological effects of neuraminidase 1 deficiency on skeletal muscle regeneration

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Neuraminidase-1 (Neu1) regulates the catabolism of sialoglycoconjugates in lysosomes. Congenital Neu1 deficiency in children is the basis of sialidosis, a neurosomatic disorder whose symptoms include hypotonia, muscle weakness and osteskeletal deformities. Mice with Neu1 deficiency develop an atypical form of muscle degeneration characterized by abnormal fibroblast proliferation and expanded extra cellular matrix (ECM), with invasion of muscle fibers by ECM components, cytosolic fragmentation and muscle atrophy. Muscle regeneration was induced by intramuscular administration of cardiotoxin (CTX) in the right tibialis anterior (TA) muscles of Neu1-/- mice and normal controls. Muscle regeneration was analyzed 7, 14, 21 and 28 days after lesion. Muscle fiber maturation was not affected in Neu1 mice during regeneration. However, muscle fiber cross sectional area was reduced in Neu1-/- compared to Neu1+/+, and the endomisium space was enlarged 21 and 28 days after lesion in Neu1-/- muscle. The determination of Neu1 role on muscle physiology is important to understand the neuromuscular clinical manifestations reported in patients with Neu1 deficiency and the importance of lysosomes and the sialic acid metabolism on the physiopathogenesis of muscle diseases (supported by FAPESP 2009/02937-4 and 2011/03853-9).

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Disease Biology

46. Aberrant protein secretion as a biomarker of dystrophic muscle in vivo and in vitro

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The proteome and secretome of tissue cultured myotubes of mdx and WT mice were compared by SILAC. Mdx myotubes lost twice as much protein as WT, mainly intact proteins, in a 1.5 to 4 fold excess over WT. Most conspicuous of these were Myosin-light chains which were readily detectable by Western blot in the culture medium of mdx myotubes and in the serum of pre-pathological mdx mice. This loss is specific to particular proteins and is not attributable to cell damage, cell death or to generalized leakiness. Instead it is associated with production by the mdx cells of abnormally sized microvesicles, to abnormal lysosomal activity and with abnormalities of protein synthesis and retention of specific proteins both in tissue culture and in muscle fibres in vivo. We show that these abormalities can be largely reversed by restoration of dystrophin expression by exon-skipping induced by antisense morpholinos. We are using the excess secretion of myosin light chains in tissue culture as a screen of potential therapeutic agents. In addition, we are investigating the phenomenon of chronic excessive protein secretion and pre-pathological myofibre atrophy in vivo as important and previously unsuspected components of dystrophinopathies separate from the process of myonecrosis. Funding, DOD, FEDS,NIH

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Disease Biology

47. Mechanisms of Muscle-Specificity in Oculopharyngeal Muscular Dystrophy

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Oculopharyngeal muscular dystrophy (OPMD) is a autosomal dominant disease of late onset characterized primarily by eyelid drooping and difficulties in swallowing. Although mutations in the ubiquitously expressed PABPN1, an mRNA binding protein, cause OPMD, much is still unknown regarding the mechanism by which mutant PABPN1 leads to muscle-specific pathology. Due to the fact that PABPN1 appears to play an essential role in RNA metabolism, any impairment of its function should, in theory, affect numerous cell and tissue types, but the intrinsic characteristics of skeletal muscle may make this tissue more vulnerable to the effects of mutant PABPN1. One area of our focus is on the role of PABPN1 in skeletal muscle. We find that PABPN1 is essential for myoblast proliferation and differentiation in vitro. Muscle tissue shows significantly lower levels of PABPN1 protein as compared to unaffected tissues potentially due to the greater instability of PABPN1 mRNA we observe in skeletal muscle. We are currently examining the mechanisms that regulate PABPN1mRNA instability in muscle. Another area of research is focused on the biology of pharyngeal satellite cells, which are critical for muscle repair and growth. Compared to limb satellite cells, pharyngeal satellite cells in vivo are increased in number and demonstrate enhanced proliferation and fusion with myofibers under basal conditions. We are currently examining the effects of mutant PABPN1 on the proliferation, differentiation and fusion of pharyngeal satellite cells. Given the onset of OPMD in middle age, we are also analyzing these parameters in aged pharyngeal satellite cells. Understanding the muscle-specific roles of PABPN1 will provide mechanistic insights into the development of pathology in OPMD. (NIH)

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Disease Biology

48. Interstitial Stem Cells: At the Crossroads of Muscle Regeneration and Fibrosis

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Duchenne Muscular Dystrophy (DMD) is a severe muscular dystrophy with a high incidence of 1 in 3500 male births. It is characterized by progressive muscle degeneration leading to loss of ambulation in the early teens and death in the early twenties. Available corticosteroid treatments can prolong ambulation but do not change the outcome of the disease. Disease severity and outcome closely correlate with the progressive replacement of muscle tissue by fibrotic and adipose tissue. This process is accompanied by a progressive loss of muscle regenerative potential. Therefore, identification of the mechanisms that regulate the balance between fibrotic cells and myogenic cells is crucial for the development of treatment approaches for DMD that are expected to impact morbidity and mortality. We have identified a population of interstitial stem cells with the dual potential to generate muscle stem cells and fibroblast/adipocyte precursor cells. We found that these cells are susceptible to the dystrophic muscle environment and mirror disease pathology at a cellular level by generating large numbers of fibroblast and adipocyte precursors while shutting down de novo formation of muscle stem cells. Expression profiling identified a signaling pathway that is down-regulated in interstitial stem cells from mdx mice and more globally in dystrophic muscle from mice and DMD patients. Systemic in vivo activation of this pathway in mdx mice resulted in rapid restoration of myogenesis in interstitial stem cells and significantly improved histological parameters of disease pathology in skeletal muscles. These results suggest that these interstitial stem cells are at the crossroads of muscle and fibrosis/fat regulation and have proven useful in identifying a new pathway with potential for therapeutic development in DMD.

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Disease Biology

49. CCR2 and TLR4 Inhibition Reveals the Therapeutic Potential for Targeted Modulation of Innate Immunity in DMD

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In Duchenne muscular dystrophy (DMD), inflammation is an important driver of the disease process. Chemokines and Toll-like receptors (TLRs) are key initiators of innate immunity. The CCL2-CCR2 chemokine axis is critical in promoting monocyte/macrophage recruitment, while TLR4 recognizes not only microbial LPS but also endogenous ligands (so-called "danger signals") generated by tissue damage. In this study, we report that interference with either CCR2 or TLR4 interactions, by both genetic and pharmacologic means, has major benefits for the phenotype of mdx mice. Hence genetic ablation of CCR2 or TLR4 in mdx mice results in decreased central nucleation, reduced fibrosis, and improved force-generating capacity of the diaphragm. There is a broad downregulation of multiple proinflammatory genes and a dramatic reduction of macrophages in both CCR2- and TLR4-ablated mdx diaphragms. Furthermore, flow cytometry reveals a significant shift away from Ly6C+ ("inflammatory", or M1) macrophages. To evaluate the potential of pharmacotherapy, mdx mice also received either: 1) subcutaneous mesenchymal stromal cells engineered to systemically deliver a CCR2-inhibiting fusion protein consisting of GM-CSF with a truncated form of CCL2, or 2) oral glycyrrhizin, an inhibitor of the endogenous TLR4 ligand, HMGB1. In both cases, we observed significant benefits in treated mice similar to those found in mdx mice with genetic ablation of CCR2 and TLR4. These studies suggest the potential for targeted inhibition of innate immunity via CCR2 or TLR4 as an alternative to the current use of high-dose corticosteroids to suppress inflammation in DMD. Funding: CIHR, FRSQ

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Disease Biology

50. Alterations in Neuromuscular Junction Morphology Following Contraction-induced Injury

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Much attention has been dedicated to myofiber damage due to lengthening contractions (LC), however the neuromuscular junction (NMJ) may display changes that contribute to altered excitation contraction coupling. The purpose of this study was to compare NMJ morphology and function in healthy and injured muscles after LCs. We used a model that induces an injury to the quadriceps by LCs through a 60o arc of knee motion in mdx and wild type (WT) mice. The extent of NMJ transmission failure (NTF) and EMG changes were assessed one day after injury. Injured and uninjured muscles were then prepared for staining by perfusion-fixation and whole mount preparations where acetylcholine receptors were identified by alpha-bungarotoxin (BTX). We measured total stained area (TSA), total stained perimeter (TSP), total area (TA) and total perimeter (TP) of NMJs via staining with BTX. After injury, WT mice experienced a relatively mild loss in absolute force (39 ± 6 %) compared to mdx (76 ± 8 %). The injury resulted in a significantly altered morphology of motor endplate (ME) for mdx mice only. Specifically, MEs decreased in TSA (12.5 %) and increased in TSP (5.2 %), TA (25.8 %) and TP (10.5 %). EMG and NTF data showed corresponding changes in function for mdx. The results show that muscle strain injury causes morphological changes to the NMJs in dystrophic muscle and that changes in the NMJ can contribute toward force loss. Funded by NIH-NIAMS.

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Disease Biology

51. Pathological mechanisms of autosomal recessive centronuclear myopathy

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Centronuclear myopathies (CNM) are a group of congenital disorders characterized by hypotonia and typical skeletal muscle biopsies showing small rounded fibres with central nuclei. Several forms have been documented: a severe X-linked form with mutations in myotubularin MTM1 (XLMTM or XLCNM), a dominant form with mutations in dynamin 2 (ADCNM) and our group has identified mutations in amphiphysin 2 / BIN1 in patients with autosomal recessive centronuclear myopathy (ARCNM), including two mutations leading to a premature stop codon. We also showed that BIN1 mutations impair different functions of the protein. The aim of this research is to better understand the role of amphyphysin2 / BIN1 in healthy muscle and in the pathology of CNM. We are characterizing constitutive (CMV) and muscle-specific (HSA) knockout mice lines generated by targeted homologous recombination in ES cells, for exon 11 (muscle specific exon; encoding a phosphoinoside (PI) binding domain) and exon 20 (the last exon coding for the SH3 domain) of BIN1. The first deletion is expected to disrupt the regulation of BIN1 by PIs (and thus potentially the link with MTM1) while the deletion of exon 20 should disrupt the interaction with DNM2. Our results have confirmed that total (CMV) deletion of exon 11 doesnâ€™t alter the splicing of neighbouring exons. The life expectancy of these KO mice is not reduced compared to the littermates, and they have a very mild phenotype, with nuclear mislocalization (a CNM feature) significantly impaired. Both the CMV and HSA KO of exon 20 was perinatally lethal. This work should lead to the first characterization of an animal model for autosomal centronuclear myopathy and to a better comprehension of pathological mechanisms, which are still not known.

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Disease Biology

52. Repeat Associated Non-AUG (RAN) translation in myotonic dystrophy

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Our understanding of the molecular basis of diseases caused by microsatellite expansions, including myotonic dystrophy, has been built on studying the expected effects of the mutations based on their location within predicted coding and non-coding regions. Recently, however, it has become apparent that most of these mutations, including the DM1 CTG•CAG expansion are bidirectionally expressed. Additionally, we made the surprising and unexpected discovery that the canonical rules of translation do not apply for CTG•CAG repeat expansions and that CAG and CUG expansion transcripts can express homopolymeric expansion proteins in all three frames without an AUG start codon. This Repeat-Associated Non-AUG (RAN) translation is hairpin structure dependent and occurs without frameshifting or RNA editing. We have now performed additional cell-culture studies to characterize the RNAs capable of undergoing RAN translation and the cellular factors and conditions that favor this process. In the first series of experiments, we show RAN translation occurs in two or more frames across a variety of triplet and tetranucleotide repeat motifs including the DM2 CCTG•CAGG repeat expansion motif. Additionally, we show that RAN translation across human myotonic dystrophy type 1 (DM1) CAG-antisense expansion transcripts results in the accumulation of a DM1 polyGln expansion proteins in multiple organ systems including skeletal muscle and cardiomyocytes in affected human and DM1 mouse tissue. The nuclear co-localization of the apoptotic marker, caspase-8 and DM1 polyGln protein in infiltrating leukyocytes within thromboses of mouse cardiac tissue suggests a role for polyGln toxicity in DM1. Additional studies are currently underway to assess the role of RAN proteins in myotonic dystrophy.

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Disease Biology

53. nNOS modulates muscle force and fatigue with contrasting effects in wild type and mdx mice

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We have generated transgenic mice expressing either nNOSalpha or nNOSmu in skeletal muscle. These transgenics have been bred onto the mdx and C57Bl10 backgrounds where the effect on muscle performance was evaluated. Using in vitro tools to analyze diaphragm muscle physiology in the nNOSalpha transgenic mice, we found that young (8 week) Tg mice show higher specific force (Sp) and improved fatigue recovery of diaphragm when compared to the non Tg littermates. In contrast, when mdx mice were analyzed, young Tg mice show no difference from non Tg littermates. At older ages (16 weeks), wild type mice carrying the nNOSalpha transgene have a smaller increase in Sp compared to their non Tg littermates. The presence of the nNOSalpha transgene did not alter the Sp in 16 week old mdx mice, but they show more susceptibility to fatigue than the Tg negative littermates. In situ analysis of the TA muscle show that Sp is similar in 8 weeks old wild type mice with and without the nNOSalpha transgene. However Tg mice are less susceptible to fatigue in the TA muscle than the negative littermates, as we observed in diaphragm. Effects on mdx TA muscles are expected to be similar to those observed in diaphragm, increasing fatigue susceptibility. Analysis and comparison with nNOSmu Tg mice, carrying the skeletal muscle isoform of nNOS, will provide evidence about the role of the mu-insert and the assumed functional equivalence between the alpha and mu isoforms FUNDING: NIH RO1ARO56221, Pre-doctoral fellowship from CONICYT, Chile, to Daniela L. Rebolledo

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Disease Biology

54. Skeletal muscle atrophy caused by deficiency of Neuraminidase 1 in mice

Rizzato, Vanessa[1], Neves, Juliana[1], Fappi, Alan[1], Godoy, Tiago[1], van de Vlekkert, Diantha[2,] Chadi, Gerson[1], D'Azzo, Alessandra[2], Zanoteli, Edmar[1]

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Deficiency of the enzyme neuraminidase 1 (NEU1), responsible for the catabolism of sialic acid-containing glycoconjugates is associated with sialidosis. Children affected by the disease have systemic and neurological abnormalities. Neu1-/- mice are a close phenocopy of the condition. Histology of Neu1-/- muscle reveals expansion of the epimysial and perimysial spaces, proliferation of fibroblast-like cells and abnormal deposition of collagens. Muscle fibers located adjacent to the expanded connective tissue underwent invagination of their sarcolemma, which resulted in infiltration of the fibers by fibroblast-like cells and extracellular matrix and in their progressive cytosolic fragmentation. To understand the atrophy process in Neu1 deficiency, sciatic denervation was performed in Neu1-/- mice and littermates controls (2-3 mo). After 7, 14 and 21 days of denervation, gastrocnemius muscles (GA) were excised for analysis. The muscle fibers from transgenic animals presented decreased size, increased endomisial space and expanded connective tissue compared to the controls. An up-regulation of Neu1 RNAm in the atrophied muscle was observed. After denervation, muscle fibers from Neu1-/- mice had bigger size variability compared to wild type fibers. Overall these studies highlight a novel role of NEU1 in the processes that follow initiation of an atrophic phenotype in skeletal muscle (Supported by FAPESP 2009/02937-4 _ 2011/03857-4).

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Disease Biology

55. Initial characterization of a novel porcine model of Becker muscular dystrophy

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Duchenne muscular dystrophy (DMD) is caused by a mutation in the dystrophin gene which results in production of a non-functional protein. Becker muscular dystrophy (BMD) is caused by a mutation in the dystrophin gene resulting in a partially functional protein product or an insufficient amount of functional dystrophin. These diseases share many physiological similarities including muscle dysfunction, impaired mobility, and death; albeit generally disease progression is slower and more variable in BMD compared to DMD. Currently, there are three primary models for these diseases including two mouse models and a dog model. Although these models have contributed substantially to our current understanding of BMD and DMD, there are significant limitations to each that reduce their utility in translational research necessitating the search for alternative models. We have recently discovered a porcine model with an apparent mutation in the dystrophin gene such that at 8 weeks of age dystrophin protein expression is reduced 90% by Western blot compared to control and is supported by immunohistochemistry. Serum CK activity was increased 2-fold and muscle injury and fatty infiltration were apparent in muscle taken from 8 week old affected animals compared to healthy animals. This initial characterization strongly suggests the discovery of a novel mutation in dystrophin that is associated with a BMD-like phenotype in the pig. Supported by the USDA and NIH.

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Disease Biology

56. P2X7 receptor knockout alleviates the pathology in the mdx mouse model of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is the most common and lethal inherited muscle disease. Many secondary cellular and metabolic dysfunctions downstream of the primary dystrophin absence contribute to this pathology. We have previously demonstrated significant P2X7 purinergic receptor abnormalities in muscles of the mdx mouse model of DMD. Here, in two specific mdx/P2X7(-)double-knockout (dKO) mouse strains we have shown that P2X7 receptor removal or depletion significantly decreased dystrophic pathology. At 4 weeks post-natal, coinciding with a period of profound degeneration/regeneration in the mdx mouse, mdx/P2X7(-)dKO mice presented a reduced level of blood serum creatine kinase while molecular analyses indicated a shift from a pro-inflammatory to a pro-regeneration state. At both 4 weeks and 4 months, centrally nucleated fibres have a consistently greater Feretâ€™s diameter whilst isolated diaphragms generated greater specific force when subjected to tetanic field stimulation in mdx/P2X7(-) dKO animals. Our results suggest that the P2X7 receptor activation is an important contributing factor in this complex pathology and a potential therapeutic target for treatment of this disease. Supported by TC2N INTERREG (EU) and IBBS, University of Portsmouth.

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Disease Biology

57. Single amino acid changes in distinct domains of dystrophin can effect protein folding and cause disease, but not always

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Advancements in sequencing technology have revealed several Duchenne and Becker Muscular Dystrophy patients who encode only a single amino acid change in their DMD gene. Additionally, large amounts of single nucleotide variant (SNV) data are being generated from projects such as International HapMap and 1000 genomes. We compared the amino acid changes reported and identified many originally associated with disease but now listed as SNVs. There remain DMD mutations only associated with disease but they are differentially clustered in the protein. Both the N- and C-terminal domains have significantly greater proportions of disease-causing changes compared to the central rod domain, indicating they are less tolerant to primary sequence changes. Consistent with the concentration of disease-causing mutations in the dystrophin termini, we have previously shown that missense mutations in the N-terminal actin-binding domain cause thermal instability in vitro. We have now engineered amino acid changes located in the C-terminal or central rod region. Our biochemical analyses demonstrate that disease-causing changes significantly decrease the solubility and thermal stability of dystrophin while population changes do not. (NIH AR042423).

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Disease Biology

58. A chromosome 11 modifier of cardiopulmonary function in muscular dystrophy

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Mice lacking the dystrophin-associated protein Î³-sarcoglycan (Sgcg) develop cardiomyopathy and muscular dystrophy. Cardiac and muscle fibrosis in this model is enhanced in the DBA/2J background and is suppressed in 129T2/SvEmsJ background. We now conducted a genomewide screen for modifiers by characterizing 245 mice from an F3-F4 intercross using cardiac echocardiography and quantitative measures of Evans blue dye uptake and hydroxyproline content in multiple muscle groups. Mice were genotyped on two platforms using 2313 informative markers distributed across the genome. Phenotypes were associated with genetic markers using QTLRel, a program that accounts for relatedness. More than 30 quantitative trait loci (QTL) were identified. The abdominal muscles were the most severely affected muscle group with the greatest fibrosis and membrane leak. A region on chromosome 11 was significantly associated with membrane leak. This same chromosome 11 region was found to influence right ventricular mass, and pulmonary artery acceleration time, an indicator of pulmonary hypertension. The genomes of the parental strains were sequenced to identify genetic variants responsible for these QTLs. The chromosome 11 locus contains >40 genes, and we identified potentially important variants. These correlations reflect the importance of abdominal muscles for breathing and cardiac function and identify significant new modifiers for muscular dystrophy. NIH, Wellstone Muscular Dystrophy Cooperative Group

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Disease Biology

59. RNA Regulation in Muscle Development and Disease

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Skeletal muscle development requires the precise coordination of transcriptional and co/post-transcriptional events for the stage-specific expression of protein isoforms during embryonic and postnatal periods. In the neuromuscular disease myotonic dystrophy (DM), this coordination is disrupted by the expansion of C(C)TG microsatellites that are subsequently transcribed into pathogenic C(C)UG repeat expansion RNAs. These C(C)UGexp RNAs are toxic because they disrupt the normal regulation of alternative splicing factors, including the CELF and MBNL proteins, so that fetal isoforms are expressed in adult tissues. In previous studies, we have provided experimental support for this RNA-mediated disease model by generating Mbnl1 knockout (KO) mice that develop characteristic features of DM muscle disease, including myotonia, muscle pathology (central myonuclei, split fibers, fibrosis) and retention of fetal alternative splicing patterns in adults. Here, we report the generation and characterization of Mbnl2 and Mbnl3 KO mice and the analysis of Mbnl combinatorial KOs. Our studies reveal tissue-specific requirements for the Mbnl proteins and provide additional evidence in support of the MBNL loss-of-function model for DM. Research support provided by the NIH (AR046799, NS048843).

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ABSTRACTS

Disease Biology

60. Inefficient epigenetic repression of DUX4 as a cause of facioscapulohumeral dystrophy (FSHD)

Tapscott, Stephen[1], Geng, Linda[2], Yao, Zizhen[1], Snider, Lauren[1], Young, Janet[1,] van der Maarel, Silvere[3], Gentleman, Robert[4], Tawil, Rabi[5]

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Facioscapulohumeral dystrophy (FSHD) is one of the most common inherited muscular dystrophies. Recent studies indicate that the inefficient epigenetic suppression of a retrogene, DUX4, embedded in the D4Z4 repeats causes FSHD. DUX4 is normally expressed in the human germline and epigenetically suppressed in somatic tissues, possibly through an siRNA mediated repression of direct repeats. The inefficient repression in FSHD muscle results in occasional bursts of DUX4 expression. DUX4 is a double homeobox transcription factor. We show that DUX4 expression in skeletal muscle activates transcription of many genes normally expressed in the germline. The genes regulated by DUX4 are reliably detected in FSHD muscle but not in controls, providing direct support for the model that misexpression of DUX4 is a causal factor for FSHD. In addition, DUX4 binds and transcriptionally activates endogenous retrotransposons and modulates the innate immune response. These finding suggest several specific pathophysiologic mechanisms for FSHD, identify candidate biomarkers for disease diagnosis and progression, and provide a roadmap for therapeutic development.

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Disease Biology

61. Facioscapulohumeral muscular dystrophy: it takes two to tango

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Facioscapulohumeral muscular dystrophy is associated with a chromatin relaxation defined by DNA hypomethylation and changes in histone modifications of the D4Z4 repeat leading to a variegated expression pattern of the DUX4 retrogene encoded within each D4Z4 unit. In the common form of this disease, FSHD1, chromatin relaxation is accomplished by contraction of the D4Z4 repeat below a size of 11 units. In FSHD1, D4Z4 chromatin relaxation is mostly restricted to the shortened array. In the second form, FSHD2, D4Z4 chromatin relaxation is observed on both chromosomes 4 and on the homologous arrays on chromosome 10 independent of repeat size. In FSHD2 families, D4Z4 hypomethylation can segregate as a dominant trait suggesting that there is a genetic basis for FSHD2. D4Z4 chromatin relaxation needs to occur on a specific haplotype to cause FSHD. These FSHD-permissive haplotypes contain a polyadenylation (pA) signal for the DUX4 gene facilitating stable expression of DUX4 mRNA in FSHD skeletal muscle. FSHD non-permissive alleles do not contain this pA signal and therefore fail to produce stable DUX4 transcripts upon chromatin relaxation.We established mouse models for FSHD having key genetic, epigenetic and molecular features of FSHD. Mice with D4Z4 arrays of 12 units do not express DUX4 in somatic cells with D4Z4 being heterochromatic. Mice with D4Z4 arrays of 2 units show a variegated expression pattern of DUX4 in muscle cells having a less condensed chromatin structure. Like in humans, ectopic expression of DUX4 in mice activates germ line and early stem cell programs as well as specific classes retrotransposons. It also suppresses the innate immune system making these mice good models for future mechanistic and therapeutic studies. Funding: NIH, MDA, FSH Society, NWO, Stichting FSHD

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Disease Biology

62. LARGE glycosylates the alpha7beta1 integrin and regulates expression and laminin-binding in muscle

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The alpha7beta1 integrin is a major laminin-binding receptor in skeletal, cardiac and vascular smooth muscle. Loss of the alpha7 integrin causes congenital muscular dystrophy and has been shown to be a major modifier in various muscle diseases. Overexpression of the alpha7 integrin in muscle can rescue mouse models of Duchenne muscular dystrophy and merosin deficient congenital muscular dystrophy type 1A. The alpha7beta1 integrin is a glycoprotein, however the enzymes that glycosylate the alpha7beta1 integrin in muscle are unknown. LARGE is a protein glycosyltranferase which has been shown to glycosylate alpha-dystroglycan to promote laminin binding in muscle cells. In this study we tested the hypothesis that LARGE glycosylates the alpha7beta1 integrin in skeletal muscle. Our results show that overexpression of LARGE in C2C12 cells increased alpha7 integrin glycosylation and resulted in elevated levels of alpha7beta1 integrin and laminin-binding. These results indicate that the alpha7beta1 integrin is a targeted for LARGE-mediated glycosylation and drugs that target LARGE glycosylation may be therapeutic in the treatment of muscular dystrophy through elevated alpha7beta1 and alpha-dystroglycan. (Supported by NIH/NIAMS R01AR053697)

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Disease Biology

63. Mutation-directed studies on the function of the dystrophin ZZ domain

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DMD is typically associated with the loss of dystrophin (dys), which plays an important role in muscle fiber integrity via interactions with beta-dystroglycan (bDG) and other members of the transmembrane glycoprotein complex. The ZZ domain, a C-terminal cysteine-rich zinc-finger domain, has been implicated in forming a stable interaction between dys and bDG, but other potential binding partners have not been well investigated. In the ZZ domain, 9 of 11 reported missense mutations are associated with DMD (rather than the milder BMD) but the molecular pathogenesis of these is not well studied. Prior studies have only addressed effects on bDG binding, with discrepant results.

We are taking complementary approaches to characterizing ZZ domain function. First, we are testing the effect of three mutations on candidate binding partners; we have introduced mutations into dys constructs for in vitro transfection and in vivo (AAV) transduction, followed by co-immunoprecipitation of native or co-expressed candidates. Preliminary data suggest that although some mutations affect bDG binding, others result in more rapidly degraded protein (presumably due to instability). Second, we are using mass spectrometry to identify alterations in binding partners. Finally, we are analyzing the effects of missense mutations on the folding of the ZZ domain using both in silico modeling and circular dichroism studies. Here we present our work in progress.

DMD is typically associated with the loss of dystrophin (dys), which plays an important role in muscle fiber integrity via interactions with beta-dystroglycan (bDG) and other members of the transmembrane glycoprotein complex. The ZZ domain, a C-terminal cysteine-rich zinc-finger domain, has been implicated in forming a stable interaction between dys and bDG, but other potential binding partners have not been well investigated. In the ZZ domain, 9 of 11 reported missense mutations are associated with DMD (rather than the milder BMD) but the molecular pathogenesis of these is not well studied. Prior studies have only addressed effects on bDG binding, with discrepant results. We are taking complementary approaches to characterizing ZZ domain function. First, we are testing the effect of three mutations on candidate binding partners; we have introduced mutations into dys constructs for in vitro transfection and in vivo (AAV) transduction, followed by co-immunoprecipitation of native or co-expressed candidates. Preliminary data suggest that although some mutations affect bDG binding, others result in more rapidly degraded protein (presumably due to instability). Second, we are using mass spectrometry to identify alterations in binding partners. Finally, we are analyzing the effects of missense mutations on the folding of the ZZ domain using both in silico modeling and circular dichroism studies. Here we present our work in progress.

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Disease Biology

64. A Mitochondrial Paradigm for Metabolic and Degenerative Diseases

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The most energetically demanding organs and tissues in the body are the brain, heart, muscle, kidney, and endocrine. Hence, these tissues are preferentially affected by systemic mitochondrial defects resulting from changes in any of the 1000-2000 nuclear DNA (nDNA) or mitochondrial DNA (mtDNA) genes of the mitochondrial genome. Introduction of a mtDNA COI missense mutations (T6589C, V421A) into mouse causes a 50% complex IV defect and mitochondrial myopathy and cardiomyopathy. Homozygous mutations in the nDNA adenine nucleotide translocator (ANT1) cause myopathy and cardiomyopathy in mice and humans. In mice, the severity of the Ant1 -/- cardiomyopathy is accentuated by the mtDNA COI

mutation and in humans by different mtDNA lineages (haplogroups). A human mtDNA ND1 missense mutation (T3394C, Y30H) can cause blindness (LHON) at low altitude on one mtDNA background yet is enriched in high altitude Tibetans on a different mtDNA background. Alzheimer and Parkinson Disease risk is influenced by the mtDNA background and the accumulation of brain somatic mtDNA mutations. Thus, mitochondrial dysfunction may underlie many common complex diseases. (NIH grants NS21328,NS41850, AG24373, and DK73691)

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Disease Biology

65. Alternate translational initiation and amelioration of phenotype in the DMD gene

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Nonsense mutations within exon 1 of DMD do not result in severe DMD but instead lead to very mild BMD, due to an alternative initiation of translation at AUG codons within in exon 6. This leads to translation of a nearly full-length but N-terminal truncated dystrophin lacking the first half of the canonical actin binding domain 1 (ABD1). We have identified the motif encoded in exon 5 that recruits ribosomes for alternate translational initiation within exon 6, and using a dual luciferase reporter system we have determined that this motif is selectively activated in muscle cell lines but not fibroblasts or HEK cells. Our preliminary data suggest that this motif is an IRES (internal ribosome entry site) as complementary experiments have ruled out any promoter activity or aberrant splicing. The exceedingly mild clinical features of patients with an exon 1 DMD founder allele (p.Trp3X) suggest that the product of this IRES initiation is a highly functional protein. We are therefore exploring different strategies to induce IRES utilisation for therapeutic purposes in patients with mutations in exons 1 through 4. Research funding: Institution Funding

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Disease Biology

66. The role of the IGF-binding proteins (IGFBPs) in skeletal muscle signalling.

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IGF-1 signalling plays a major role in muscle growth and regeneration. IGF binding proteins (IGFBPs) regulate this signalling by binding to IGF-1. Recent microarray data showed that IGFBP2 and IGFBP5 were significantly down-regulated in hypertrophied skeletal muscles. Expression of the IGFBPs was investigated further in notexin model of induced muscle damage and regeneration and in the mdx mouse model of muscular dystrophy. IGFBP5 was significantly down-regulated following notexin induced damage and in mdx mouse limb and diaphragm muscles following voluntary exercise. Conversely, IGFBP2 was significantly up-regulated during the inflammatory stage of notexin induced regeneration. IGFBP2 is up-regulated early during muscle regeneration and may be expressed by infiltrating inflammatory cells. IGFBP5 may be highly expressed from myogenic cells later in regeneration when myoblasts are proliferating forming myotubes. Antisense oligonucleotides will be used to inhibit IGBP5 and IGBP2 in the mdx mouse. Based on results seen in the muscle regeneration models we hypothesise that inhibition of these binding proteins will increase the bioavailability of IGF-1 and result in improved disease pathology. Funding Body: Muscular Dystrophy Australia

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Disease Biology

67. A dual therapeutic approach enhances amelioration of the pathology of MDC1A in DyW mice by improving regeneration and reducing inflammation

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MDC1A is a severe form of congenital muscular dystrophy characterized by hypotonia and premature death. MDC1A results from deficiency in the laminin alpha 2 chain of laminin-211, an extracellular matrix protein mainly found in skeletal muscle and Schwann cells. Defects in laminin-211 cause major disruption of structural stability and signal transduction that leads to apoptosis, failed regeneration, and chronic inflammation. Overexpressing mIGF-1 in DyW mice, a model for MDC1A, enhances regeneration and leads to an improved phenotype; however, inflammation remains a persisting feature. Chronic inflammation and NFkappaB activity is upregulated in DyW muscles. We used NEMO Binding Domain peptide to inhibit NFkappaB activation in mIGF-1 overexpressing DyW mice to determine if dual therapy would have an additive effect on the pathology of MDC1A. These mice show further improvement in overall growth and function. Additionally, tibialis anterior muscles show marked improvement in pathology with larger, more uniform myofiber size as well as improved regeneration and reduced fibrosis. Our results demonstrate that a combinatorial therapeutic approach has promising potential as an effective treatment for MDC1A. (MDA and BU Sargent College)

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Disease Biology

68. Small heat shock protein B7 (HspB7) is essential for sarcomerogenesis in muscle development.

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HSPB7 (also cardiovascular heat shock protein, cvHSP) is belonged to the small heat shock protein (sHSP) family containing a conserved alpha-crystallin domain. Despite of the significant up-regulation in the dystrophin-deficient MDX diaphragm and the aging skeletal muscle, HSPB7 is expressed restrictedly in the striated muscle cells including cardiomyocytes through cardiogenesis and skeletal muscles during myogenesis. Furthermore, the results of IHC and confocal fluorescence microscopic analysis showed HSPB7 is mainly expressed in slow twitch muscle fibers and regenerating muscles in mouse model. Though many sHSPs have been demonstrated containing molecular chaperone activity in vitro in cell-free conditions and the stress protection activity in animal disease models, the function of HSPB7 remains enigmatic. To explore the biological function of Hspb7 in mouse development, the Hspb7 mutant mouse lines were established by gene targeting approach with lacZ reporter gene knock in. Heterozygous mutant can propagate and show no significant difference as the wild type animal. However, loss of HSPB7 in mouse results in embryonic lethality at E11.5 with cardiac development defects. The myofibrils with disarray sarcomere structures are observed by transmission electron microscopy in Hspb7 deficient hearts. Mal-patterning of sarcomeric proteins and loss of I-band structure were observed in the mutant cardiomyocytes. Additionally, we identified filamin C as the interacting protein of HSPB7 in cardiomyocytes. Taken together, our findings highlight the novel role of sHSPs affecting the sarcomerogenesis of the striated muscle cells.

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ABSTRACTS

Disease Biology

69. Injury-induced Pathological Ca2+ Entry in Dysferlinopathic Skeletal Muscle

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Limb Girdle Muscular Dystrophy 2B and Miyoshi Myopathy are linked to mutations in the protein, dysferlin. We have explored the onset and localization of cellular damage in muscles from dysferlin-null A/J mice and determined that the t-tubules are highly susceptible to both in vivo and in vitro injury and the primary site of damage. T-tubule damage in vitro is dependent upon extracellular Ca2+ and leads to acute disruption of Ca2+ homeostasis and ec-coupling. Cellular damage and disruption of Ca2+ regulation and ec-coupling in isolated fibers are largely prevented by acute treatment with the Ca2+ channel blocker, diltiazem. Diltiazem also protects dysferlin-null muscle from damage by large strain lengthening contractions in vivo. We conclude that (1) dysferlin stabilizes t-tubules, Ca2+ homeostasis and ec-coupling in mammalian skeletal muscle, (2) extracellular Ca2+ is required for A/J muscle to sustain damage in vitro, (3) Ca2+ flux through the L-type Ca2+ channel, which is blocked by diltiazem, mediates the damage caused by extracellular Ca2+, and (4) diltiazem may prove efficacious as a preventative treatment for dysferlinopathies. Supported by the NIH (1F32AR057647 to APZ), the Jain Foundation (to RJB and JAR), and the Muscular Dystrophy Association (to RJB).

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Muscle Biology

70. Transgenic mice expressing human LTBP4 display skeletal muscle fiber hypertrophy.

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[1]*University of Chicago, Department of Medicine,* [2]*Committee on Cell and Molecular Physiology, University of Chicago*

Latent TGFbeta binding proteins (LTBPs) sequester TGFbeta family members in the extracellular matrix, where they regulate TGFbeta activity. We previously demonstrated that Ltbp4 is a modifier of muscular dystrophy. Specifically, an insertion/deletion polymorphism in the proline-rich region of murine Ltbp4 segregates with enhanced membrane damage, increased fibrosis and produces elevated SMAD activity in Sgcg mice, a model for Limb Girdle Muscular Dystrophy 2C. To test whether human LTBP4 modifies muscular dystrophy, we now generated a mouse model that expresses human LTBP4 (hLTBP4 BAC) by inserting a human bacterial artificial chromosome encompassing the human LTBP4 gene. We characterized multiple founder lines carrying the hLTBP4 BAC transgene. RT-PCR analysis verified hLTBP4 BAC mRNA expression in heart and skeletal muscle of the transgenic mice. hLTBP4 BAC transgenic mice have increased skeletal myofiber cross sectional area without an increase in total muscle fiber number, suggesting that LTBP4 exerts an effect on muscle growth. hLTBP4 BAC mice were bred to mdx mice and preliminary data suggest an increase in fibrosis in the hLTBP4/mdx mice. These data support a model where LTBP4, as a matrix-associated protein, may regulate multiple TGFbeta family members to mediate muscle growth.

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Muscle Biology

71. The role of Smad3 in regulating weightlessness-induced muscle atrophy and its molecular mechanism

Chen, xiaoping[1], zhang, peng[1], liu, hongju[1], li, li[1], li, wenjiong[1]
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During spaceflight,weightlessness will induce skeletal muscle atrophy, which seriously impairs astronautsâ€™ performance on orbit and health recovery after return. However, the mechanism underlying weightlessness-induced muscle atrophy is still unclear. In the present study, we explored the role of Smad3 in regulating weightless muscle atrophy induced by mouse hindlimb suspending for 28 days and its molecular mechanism. Our results revealed that the weight loss and the decrease in cross-sectional area of slow-twitch soleus muscle were significantly inhibited in Smad3 knockout mice compared to their wild type littermates. Meanwhile, we found that Smad3 knockout could significantly down-regulate the expression of atrophy-specific genes Atrogin-1 and MuRF-1, and up-regulate the expression of slow type myosin heavy chain (MHCâ€“I) but down-regulate fast type myosin heavy chain (MHC-II) by real time PCR and western blot analysis. Furthermore, Smad3 knockout significantly rescued the impaired plasticity of satellite cells with enhanced myogenesis and muscle regeneration by MyoD and myogenin induce. Finally, using in vivo electroporation with the different domain deletion mutants of Smad3, we showed that the C-terminal domain of Smad3 is essential for the selective atrophy of slow-twitch soleus muscle and the transition of MHC-I into MHC-II during hindlimb suspending, which is the characteristic changes of space muscle atrophy. Therefore, the study demonstrated the important role of Smad3 in regulating weightlessness-induced muscle atrophy and its molecular mechanism. Our data indicate that Smad3 may be used as a potential marker to evaluate the degree of space muscle atrophy and its intervention effects of drugs or other protective measures. This work was supported by grants from National Basic Research Program of China (2011CB711000),Natural Sciences Foundation of China (31171144) and State Key Laboratory Grant of Space Medicine Fundamentals and Application (SMFA10A01).

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Muscle Biology

72. Molecular mechanism of sarcolemmal nNOS localization in vivo

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Absence of sarcolemmal nNOS plays a critical role in the pathogenesis of muscular dystrophy and muscle fatigue. We recently demonstrated that dystrophin spectrin-like repeats 16 and 17 (R16/17) are required for sarcolemmal nNOS localization (Lai et al JCI 119:624, 2009). However, it is not clear how the specific binding between R16/17 and nNOS occurs. To address this issue, we generated more than 45 different adeno-associated virus (AAV) vectors to express various sequence changes that may potentially involved in this interaction. These include point mutations, substitutions, in-frame deletions and phase alterations. Highly purified recombinant AAV-6 was prepared through two rounds of isopycnic ultra-centrifugation. AAV was delivered to the tibialis anterior muscles of mdx and mdx4cv, dystrophin-utrophin double knockout and dystrophin transgenic mice. Expression was confirmed using a panel of epitope specific antibodies that recognize modified dystrophins and nNOS. nNOS was also validated by in situ activity assay. Collectively, our results revealed a novel protein-protein interaction model that encompasses both stringency and flexibility. A better understanding of the noncanonical dystrophin-nNOS interaction may shed new light on basic muscle biology. Our rationally engineered new dystrophin and utrophin constructs have significant implications for Duchenne muscular dystrophy therapy. NIH/MDA

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Muscle Biology

73. Neuronal nitric oxide synthase-deficient mice have slower phosphocreatine recovery kinetics and reduced microvascular response following muscle contractions

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Lack of neuronal nitric oxide synthase (nNOS) localized to the sarcolemma has been suggested to contribute to impaired local perfusion to skeletal muscle following exercise. We hypothesized that mice lacking nNOS (nNOS-/-) would have slower recovery of phosphocreatine (PCr) and reduced microvascular response following muscle contractions. 31P-MRS data were acquired in male wild-type (WT, n=22) and nNOS-/- (n=14) mice using an 11.1 T MR system. Measures were obtained from the posterior lower hindlimb during three bouts of muscle contractions at 0.75 Hz for 10 min with 10 min recovery (low-intensity) and 5 Hz for 2 min with 15 min recovery (high-intensity). In a subgroup, the peak blood oxygenation level dependent (BOLD) response was measured using a 4.7 T MR system following 5 tetani separated by 3 minutes. The time constant of PCr recovery was slower ($p<0.05$) following the contractions in nNOS-/- than WT after both low- (139Â±9vs.221Â±14s) and high-intensity (143Â±9vs.196Â±13s). Furthermore, the peak postcontractile BOLD was lower ($p<0.05$) in nNOS-/- (1.7Â±0.1%) than WT (3.0Â±0.4%). The slower PCr kinetics and reduced postcontractile BOLD effect may be due to a reduced delivery of oxygen to skeletal muscle as a result of an inability to blunt sympathetic vasoconstriction. Supported by MDA.

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Muscle Biology

74. Deficiency of the fast twitch muscle fiber protein alpha-actinin-3 alters muscle adaption in response to denervation and immobilisation

Garton, Fleur[1], Seto, Jane[2], Houweling, Peter[2], Quinlan, Kate[1], Yang, Nan[2], North, Kathryn[2]
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A common null polymorphism (R577X) in the ACTN3 gene results in the absence of fast fiber-specific alpha-actinin-3 in ~18% of humans worldwide. Alpha-actinin-3 deficiency is detrimental to elite sprint athletes and may benefit endurance performance. We have developed an Actn3 knockout (KO) mouse which mimics the human phenotype. KO mice show reduced grip strength, resistance to fatigue and a shift towards slow/oxidative metabolism in fast fibers, without a shift in fiber type. Altered levels of regulators of calcineurin (RCAN) in the KO suggest involvement of the calcineurin-dependent signalling pathway. We aimed to investigate adaptation to muscle disuse, hypothesizing that Actn3 genotype would have a local influence on muscle adaptation, irrespective of neural innervation. Separate cohorts of KO and wild-type (WT) mice underwent 4 weeks immobilisation and 2 and 8 weeks of denervation. Atrophy stress decreased muscle fiber size, however KO muscle fibers demonstrated a lower threshold to undergo fiber type switch (as defined by myosin heavy chain isoforms) towards a slower phenotype. This revealed a local effect of alpha-actinin-3 altering muscle fiber type in response to adaptation which we propose is mediated through the calcineurin signalling pathway. Our findings have important implications for understanding local muscle biology signalling and individual responses to muscle disuse/disease and training in the human population. (National Health and Medical Research Council of Australia and Australian Research Council)

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Muscle Biology

75. Human cell interactions during muscle regeneration: consequences for cell therapy.

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Muscle regeneration involves several partners, including myoblasts, but also inflammatory cells. We assessed the behaviour of human myoblasts in an in vivo context using cellular implantation into regenerating TA of immunodeficient mice. We observed that implanted human myoblasts proliferate in situ during an early phase, and that differentiation occurs between 3 and 5 days post-transplantation, thus limiting both proliferation and dispersion of the precursors. We assessed the influence of the pro- (M1) or anti-inflammatory (M2c) macrophages by co-injecting them with myoblasts, and show that pro-inflammatory macrophages delay the in situ differentiation of myoblasts, thus allowing a longer period for proliferation and dispersion, and an increased participation of human cells to hostâ€™s regeneration. These interactions probably involve secretion of specific molecules or vesicles. Interestingly, some of the injected pro-inflammatory macrophages become anti-inflammatory later, thus allowing the differentiation of the implanted human myoblasts. Since macrophages can be easily prepared from patients, this offers a possibility to optimize cell therapy using autologous myogenic precursors. A project supported by AFM, ANR In-A-Fib, and EU (MYORES and MYOAGE).

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Muscle Biology

76. A common human null polymorphism in the ACTN3 gene, leading to alpha-actinin-3 deficiency, enhances muscle endurance performance and response to training by increasing calcineurin activity

Quinlan, Kate[1], Seto, Jane[2], Lek, Monkol[2], Zheng, Fiona[2], Garton, Fleur[2,] Houweling, Peter[2], North, Kathryn[2]

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Approximately 1 billion people worldwide do not express alpha-actinin-3 (a-act-3) due to homozygosity for a common null polymorphism in the ACTN3 gene. a-Act-3 deficiency alters athletic performance and human muscle function - it is detrimental to sprint and power performance. Our Actn3 KO mouse has revealed that a-act-3 deficiency leads to a shift in fast muscle fibre contractile and metabolic properties towards those normally associated with slower muscle fibres. We have demonstrated that, in addition to improved endurance performance at baseline, Actn3 KO mice respond more readily to exercise training with greater increases in running performance and fast-to-slow fibre shifts. By microarray, we have found that downstream targets of the calcineurin signalling pathway are altered in a-act-3 deficient muscle. We have now shown that the activity of calcineurin, which plays a critical role promoting fast-to-slow twitch fibre transition, is increased in KO mouse muscle. We also demonstrate that alpha-actinin-2 (which is upregulated in a-act-3 deficiency) competes with the negative regulator of calcineurin activity, calsarcin-2, for binding to calcineurin, providing a molecular mechanistic explanation for the increased calcineurin activity in a-act-3 deficient muscle. This change in calcineurin activity explains the enhanced endurance performance at baseline and the augmented response to endurance training in a-act-3 deficient muscle. (National Health and Medical Research Council of Australia and Australian Research Council)

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Muscle Biology

77. A common human null polymorphism in the ACTN3 gene, leading to alpha-actinin-3 deficiency, enhances muscle endurance performance and response to training by increasing calcineurin activity

Quinlan, Kate[1], Seto, Jane[2], Lek, Monkol[2], Zheng, Fiona[2], Garton, Fleur[2,] Houweling, Peter[2], North, Kathryn[2]

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Approximately 1 billion people worldwide do not express alpha-actinin-3 (a-act-3) due to homozygosity for a common null polymorphism in the ACTN3 gene. a-Act-3 deficiency alters athletic performance and human muscle function - it is detrimental to sprint and power performance. Our Actn3 KO mouse has revealed that a-act-3 deficiency leads to a shift in fast muscle fibre contractile and metabolic properties towards those normally associated with slower muscle fibres. We have demonstrated that, in addition to improved endurance performance at baseline, Actn3 KO mice respond more readily to exercise training with greater increases in running performance and fast-to-slow fibre shifts. By microarray, we have found that downstream targets of the calcineurin signalling pathway are altered in a-act-3 deficient muscle. We have now shown that the activity of calcineurin, which plays a critical role promoting fast-to-slow twitch fibre transition, is increased in KO mouse muscle. We also demonstrate that alpha-actinin-2 (which is upregulated in a-act-3 deficiency) competes with the negative regulator of calcineurin activity, calsarcin-2, for binding to calcineurin, providing a molecular mechanistic explanation for the increased calcineurin activity in a-act-3 deficient muscle. This change in calcineurin activity explains the enhanced endurance performance at baseline and the augmented response to endurance training in a-act-3 deficient muscle. (National Health and Medical Research Council of Australia and Australian Research Council)

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Muscle Biology

78. Muscle regeneration in the MRL mouse is AMP-activated protein kinase dependent

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The MRL mice have long been investigated for their superior healing ability when subjected to various wound injury models. Despite this long history, the mechanisms behind their extraordinary healing ability remain undefined. We have recently demonstrated that their healing phenotype extends to gamma-sarcoglycan mediated muscular dystrophy. Gamma-sarcoglycan null mice with 50% MRL nuclear genome displayed reduced fibrosis. In addition, MRL maternal inheritance reduced the fibrosis even further, indicating the MRL mitochondrial genome as imparting a beneficial effect. We have now investigated skeletal muscles and cultured myoblast cells from the MRL wild-type mice and have evidence that their metabolism is significantly different from muscle in control animals. At baseline, the MRL muscles have increased AMP-activated protein kinase (AMPK) and activated AMPK. These signaling increases result in increased mitochondrial area, increased glycolysis, and reduced reactive oxygen species. Through the use of AMPK-inhibitors and AMPK-activators, we have shown that the AMPK mediated metabolic differences are required for the MRL myoblast super-healing characteristics. (NIH-R01)

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Muscle Biology

79. Imbalance in mTORC1 signaling can cause severe myopathies

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The mammalian target of rapamycin, mTOR, regulates protein synthesis in response to nutrients and growth factors. mTOR assembles into two distinct multiprotein complexes, called mTOR complex 1, mTORC1, and mTORC2. Conditional deletion of the mTORC1-essential component raptor in skeletal muscle affects the metabolic and structural properties of muscle fibers and causes a severe myopathy after a few months so that mice eventually die of respiratory failure. A very similar phenotype has been reported for mice deficient for mTOR, indicating that mTOR in skeletal muscle mainly acts via mTORC1 and not mTORC2. In a new study, we conditionally deleted the upstream inhibitor of mTORC1, TSC1, in skeletal muscle. Unexpectedly, all muscles except soleus become significantly atrophic. This differential response of muscles is based on distinct changes in the expression of atrogenes, known to be regulated by Akt/FoxO signaling. Importantly, TSC1-deficient mice develop a severe myopathy at about 9 months of age characterized by the accumulation of inclusions and vacuoles in the fibers and a strong reduction in muscle force. We show that this pathology correlates with a strong impairment of autophagy. Interestingly, injection of the mTORC1 inhibitor rapamycin normalizes autophagy and removes the force deficits. In conclusion, our data provide strong in vivo evidence that proper activation of mTORC1 is important to maintain muscle size and function. Our data also suggest that strategies aimed at shifting the balance of mTORC1 activation as an attempt to treat myopathies might be detrimental for muscle.This work was supported by the Swiss National Science Foundation, the University of Basel and the Foundation for Research on Muscle Diseases.

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Muscle Biology

80. neb: a zebrafish model of nemaline myopathy due to nebulin mutation

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Nemaline myopathy is one of the most common and severe non-dystrophic muscle diseases of childhood. Patients typically present in infancy with hypotonia, weakness, delayed motor development, and bulbar and respiratory difficulties. Mutations in six different genes are associated with nemaline myopathy, with nebulin mutations being the most common. No treatments or disease-modifying therapies have been identified for this disease. One of the major barriers to treatment development is the lack of models amenable to rapid and coordinated testing of potential therapeutic strategies. To overcome this barrier, we have characterized the first zebrafish model of nemaline myopathy. This model, termed neb, harbors a recessive mutation in the nebulin gene that results in decreased Nebulin protein levels, a severe motor phenotype and premature lethality. In addition to impaired motor function, neb zebrafish exhibit many of the features associated with human nemaline myopathy. These include impaired force generation, altered thin filament length and the presence of specific histopathological changes, including the formation of nemaline bodies. In summary, neb zebrafish mirror the genetic, clinical and pathological aspects of nemaline myopathy due to NEB mutation, and thus are an excellent model for future therapy development for this devastating disorder. Research was funded in part by the Amendt-Heller Award from the Department of Pediatrics at the University of Michigan; by Foundation Building Strength; and by the National Institutes of Health.

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ABSTRACTS

Muscle Biology

81. Longitudinal changes in MRI transverse relaxation time in boys with Duchenne muscular dystrophy

Willcocks, Rebecca[1], Arpan, Ishu[1], Forbes, Sean[1], Lott, Donovan[1], Senesac, Claudia[1,] Walter, Glenn[2], Vandenborne, Krista[1]

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Duchenne muscular dystrophy (DMD) leads to inflammation in muscle tissue and fiber replacement by fat. Both of these pathophysiological changes increase the MRI transverse relaxation time (T2). The purpose of this study was to quantify longitudinal changes in T2 in boys with DMD. T2 weighted images were collected annually over 2 years in 16 boys with DMD (5-13 years). The soleus muscle was outlined on T2 maps on three consecutive axial slices. A T2 histogram was plotted, and the mean T2, full width at half maximum, and percentage of elevated pixels (pixels greater than the 95th percentile of a histogram derived from controls) were measured. Boys also completed a timed 30 ft walk. Mean T2 and elevated pixels increased significantly over one year, and all T2 variables increased significantly over two years. The increase in 30 foot walk time over two years was strongly and significantly correlated with the increase in mean T2 ($r=0.87$, $p<0.01$) and the increase in elevated pixels ($r=0.72$, $p<0.01$). All T2 variables increased more in 9-13 year old boys than in 5-6 or 7-8 year old boys. Mean T2 and elevated pixels increase with time and correlate with disease progression, demonstrating their potential for use in clinical trials. (MDA, PPMD, NIH)

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Signaling

82. Improvement in regenerative myogenesis and muscular dystrophy in mice lacking MKP-5

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Duchenne muscular dystrophy (DMD) is a degenerative skeletal muscle disease caused by mutations in dystrophin. The degree of functional deterioration in muscle stem cells determines the severity of DMD. The mitogen-activated protein kinases (MAPKs), which are inactivated by MAPK phosphatases (MKPs), represent a central signaling node in the regulation of muscle stem cell function. Here we show that MKP-5 negatively regulates muscle stem cell function in mice. MKP-5 initially controls JNK to coordinate muscle stem cell proliferation and then p38 MAPK to control differentiation. Genetic loss of MKP-5 in mice improves regenerative myogenesis and dystrophin-deficient mice lacking MKP-5 exhibit an attenuated dystrophic muscle phenotype. Hence, enhanced pro-myogenic MAPK activity preserves muscle stem cell function even in the absence of dystrophin and ultimately curtails the pathogenesis associated with DMD. These results identify MKP-5 as an essential negative regulator of the pro-myogenic actions of the MAPKs and suggest that MKP-5 may serve as a target to promote muscle stem cell function in the treatment of degenerative skeletal muscle disease.

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Signaling

83. Insulin-like Growth Factor-I E-peptides increase muscle mass via the IGF-IR but at the expense of satellite cell depletion.

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Skeletal muscle produces and responds to Insulin-like growth factor-I (IGF-I). The Igf1 gene encodes mature IGF-I and a carboxy-terminal extension called the E-peptide. In rodents, alternative splicing produces two Igf1 isoforms, Igf1a and Igf1b, leading to identical mature IGF-I, and E-peptides EA or EB. A debate exists over the potential activity of the E-peptides. To evaluate the effects of the E-peptides in vivo, we expressed the E-peptides in mouse skeletal muscle utilizing AAV vectors. To focus on the effects of the E-peptides, we introduced a missense mutation within mature IGF-I at Valine 44 (V44M). IGF-I with this mutation has 90X less binding affinity to the IGF-I Receptor (IGF-IR), and cannot activate downstream signaling pathways. We compared EA and EB (V44M-IA and V44M-IB) by injecting the AAV vectors into mouse hindlimbs. One month after injection with V44M-IB, C57 mouse muscles and fiber areas were significantly bigger than controls. Both V44M-IA and V44M-IB caused a reduction in satellite cell numbers. In mice lacking functional IGF-IR in muscle, there was no hypertrophy. Thus, the E peptide effects were IGF-I receptor dependent. In chronically regenerating muscles of the mdx mouse, there was also no hypertrophic response, suggesting that an active satellite cell pool did not enhance the effects of the E-peptides. We propose that the E-peptides do not have independent activity in vivo, but instead serve as modulators of IGF-I signaling. (NIH)

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Signaling

84. Integrin Signaling in Muscular Dystrophy

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The alpha7beta1 integrin is a transmembrane laminin receptor in skeletal, cardiac and vascular smooth muscle and is a major modifier of disease progression in Duchenne and congenital muscular dystrophies. Mutations in the alpha7 integrin gene cause congenital myopathy in both humans and mice. We have shown that loss of the alpha7 integrin in mdx mice results in a more severe dystrophic phenotype and reduced viability. In contrast, transgenic overexpression of the alpha7 integrin in mdx/utr-/- mice improves muscle pathology and life expectancy. The mechanisms underlying integrin-mediated rescue of dystrophic muscle remain unclear but likely involve improvements in muscle-matrix interactions, muscle repair and activation of cell signaling pathways. We have used cell-based and transgenic mice to investigate alpha7beta1 integrin signaling in skeletal muscle. Our results show that the alpha7beta1 integrin regulates Akt signaling, myostatin and utrophin expression to promote muscle growth, repair and survival. Together these results support the idea that drug-based therapies that boost alpha7beta1 integrin in muscle could be used in the treatment of muscle disease. (Supported by NIH/NIAMS R01AR053697)

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Signaling

85. An in vitro Model for Load-Sensing in Muscle Cells

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Alpha-, beta-, gamma- and delta-sarcoglycan are transmembrane proteins that form a subcomplex of the dystrophin glycoprotein complex (DGC) in skeletal muscle. Mutations in any one sarcoglycan result in complete or partial loss of other sarcoglycans and cause Limb Girdle Muscular Dystrophies (LGMD), while mutations in dystrophin result in loss of the entire DGC and cause Duchenne / Becker muscular dystrophies (DMD/BMD). Recently, we discovered that gamma-sarcoglycan (GSG) is tyrosine phosphorylated in response to mechanical load in vivo, and that this phosphorylation event is necessary for normal ERK1/2 signalling in response to stretch. To further investigate GSG-mediated mechanotransduction, we developed an in vitro stretching assay, in which satellite cells from wild type and GSG-null flexor digitorum brevis fibres are cultured and differentiated into myotubes, on a flexible silicone membrane. The cultures are subjected to an optimised stretching protocol that produces a robust ERK1/2 signal in wild type muscle cells, using a custom-designed apparatus. We used this assay to elucidate further components of GSG-dependent load-responsive signalling pathways, including p70S6 kinase. This assay could be used to better understand the contribution of aberrant load-sensing to the pathology of LGMD and DMD/BMD, and to test the ability of pharmacological agents to correct abnormal load-responsive signalling in the absence of GSG. Research funding: NASA, NIH

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Signaling

86. Cytoskeletal Rearrangement in Myogenesis

Chen, Elizabeth[1], Sens, Kristin[1], Zhang, Shiliang[1], Jin, Peng[1], Duan, Rui[1], Luo, Fengbao[1], Li, Shuo[1]
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A critical step in satellite cell-mediated muscle regeneration is the fusion of mononucleate myoblasts to form multinucleate muscle fibers. Thus understanding the mechanisms underlying myoblast fusion may inform potential therapeutic strategies aimed at treating various genetic and acquired degenerative muscle diseases. Studies in the past decade revealed striking molecular conservation in myoblast fusion between the fruit fly Drosophila and mammals, making Drosophila an ideal model system to dissect myoblast fusion in vivo. Our recent work in Drosophila began to unravel a novel cellular mechanism underlying myoblast fusion. We have shown that myoblast fusion is mediated by an invasive podosome-like structure (PLS) and that the dynamic actin cytoskeletal rearrangement within the PLS is essential for PLS invasion, which ultimately leads to fusion pore formation (Sens et al., 2010; Jin et al., 2011). Our recent work on the p21-activated kinases has further demonstrated that regulation of the actin filament assembly within the PLS is critical for PLS invasion and fusion pore formation. Results from these studies will be discussed and put in the context of muscle regeneration.

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Signaling

87. Utrophin is Regulated by Sarcospan-Dependent Akt Activation: Requirement for Muscle Repair

Crosbie-Watson, Rachelle[1], Marshall, Jamie[1], Holmberg, Johan[1], Chou, Eric[1], Ocampo, Amber[1], Oh, Jennifer[1], Peter, Angela[1], Martin, Paul[2]

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Utrophin is normally confined to the neuromuscular junction (NMJ) in adult muscle and partially compensates for the loss of dystrophin in mdx mice. By creating several transgenic and knockout mice, we demonstrate that sarcospan regulates Akt signaling to control utrophin expression. Furthermore, sarcospan determines glycosylation of alpha-dystroglycan by affecting levels of the NMJ-specific glycosyltransferase, Galgt2. In response to cardiotoxin injury, regenerating myofibers express utrophin and Galgt2 modified Î±-dystroglycan around the sarcolemma. Sarcospan-null mice display delayed differentiation after cardiotoxin injury due to loss of utrophin protein and diminished Akt signaling. Treatment of sarcospan-null mice with constitutively active Akt increases utrophin and restores muscle repair after injury, revealing an important role for the sarcospan-Akt-utrophin signaling axis in regeneration. Sarcospan improves cell surface expression of utrophin by increasing transportation of utrophin and dystroglycan, which accumulate in ER/golgi membranes of mdx mice. Our studies reveal new pathways that regulate utrophin expression at the cell surface that are required for muscle repair. (NIH)

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Signaling

88. Cell-specific regulation of myostatin signaling

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Myostatin (Mstn), TGF-beta and activin are members of the TGF-beta superfamily that inhibit skeletal muscle differentiation and muscle growth. In contrast to TGF-beta and activin, Mstn signaling acts largely muscle specific. We hypothesized that the muscle specific action of Mstn may in part be mediated by specific utilization of receptors and co-receptors. Interestingly, RNAi-mediated knockdown of type I receptors ALK4 and ALK5 showed that Mstn signaling in C2C12 cells is ALK4 dependent, whereas in non-myogenic cells (C3H10T1/2 and 3T3-L1) Mstn signaling required ALK5. In contrast, TGF-beta signaling was ALK5 dependent and activin signaling was ALK4 dependent in all cell types tested. Importantly, we showed that co-receptor Cripto was specifically required for Mstn signaling in C2C12 cells and primary mouse myoblasts and that Cripto protein is present in primary myoblasts but not in primary muscle fibroblasts. Moreover, Cripto enhanced Mstn signaling but inhibited activin signaling in 293T cells. Immunofluorescent staining showed that Cripto protein was present in low levels in quiescent satellite cells and was induced in activated satellite cells. On a functional level, Cripto, ALK4 or ALK5 knockdown accelerated myogenic differentiation of C2C12 cells. Together, these experiments identify a novel mechanism that regulates Mstn signaling in myoblasts, which distinguishes it from TGF-beta/activin signaling. (Duchenne Parent Project)

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Signaling

89. Metabolic consequences of ACTN3 deficiency - more than a structural muscle protein

Houweling, Peter[1], Berman, Yemima[2], Turner, Nigel[3], Quinlan, Kate[2], Yang, Nan[2], Cooney, Greg[3], North, Kathryn[2]

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Homozygosity for a common null polymorphism (R577X) in the gene ACTN3 results in absence of the fast muscle fiber protein alpha-actinin-3 in ~18% of the general population. ACTN3 genotype has been shown to influence elite and general athletic performance, muscle bulk and strength. Alpha-actinin-3 deficient fast muscle fibers show a shift towards a slow oxidative phenotype, resulting in reduced muscle strength and improved endurance performance. Using our unique Actn3 knockout (KO) mouse model we have determined that the absence of alpha-actinin-3 also influences how muscle stores and uses energy. Specifically KO mice show altered glucose metabolism, with improved glucose clearance at baseline and altered weight gain following a high fat diet (HFD) with KO mice being resistance to weight gain. These findings have been replicated in a number of human cohorts. Furthermore we have begun to identify the molecular pathways responsible for these changes highlighting alpha-actinin-3 as an important regulator of key metabolic pathways such as AMPK and calcineurin signaling. Alpha-actinin-3 is commonly considered to be a structural muscle protein, predominantly effecting skeletal muscle function and performance. The existence of a common genetic variant that affects the metabolic function of human muscle has important implications for health in particular regarding weight gain, obesity, type-2 diabetes and inherited metabolic disease. National Health and Medical Research Council of Australia (NHMRC)

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Signaling

90. Mul1, a novel E3 Ligase, Induces Mitophagy in Response to Muscle Wasting Stimuli

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Recent research shows that the dysfunction and subsequent loss of mitochondria (mitophagy) is a potent inducer of skeletal muscle wasting. However, the molecular mechanisms that govern the deregulation of mitochondrial function and number during muscle wasting are not fully understood. In this report, we show that different muscle wasting stimuli up-regulated a novel E3 ubiquitin ligase, mitochondrial E3 ubiquitin protein ligase 1 (Mul1), which facilitated mitophagy. Mul1 up-regulation was mediated by FoxO1/3 transcription factors. Overexpression of Mul1 in skeletal muscles and myoblast cultures was sufficient for the induction of mitophagy. Consistently, Mul1 suppression not only protected from the mitophagy, but also partially rescued muscle wasting despite the presence of muscle wasting stimuli. Up-regulation of Mul1 while increased mitochondrial fission, resulted in ubiquitination and degradation of mitochondrial fusion protein, Mfn2. Thus this novel mechanism, for the first time, explains the molecular basis for the loss of mitochondrial number during muscle wasting. Research funding by MOE is acknowledged

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Signaling

91. p38-alpha MAPK relieves epigenetic gene silencing to endorse the skeletal muscle differentiation program

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The p38 MAPK signaling pathway plays important roles in numerous biological processes including skeletal muscle differentiation. The latter process is manifested through the activation of previously repressed/silenced muscle differentiation-specific genes by the MyoD family of muscle regulatory factors. In this context, p38 MAPK has received intense scrutiny as being crucial for MyoD to mediate muscle-specific gene expression program. Previously, we demonstrated that histone methyltransferase KMT1A association with MyoD establishes an epigenetic gene silencing event in myoblast cells to restrict one of the first muscle-specific gene myogenin, whose expression is essential to execute the differentiation program. Recent findings of others, however, highlight the distinct role for different p38 MAPK family members in making decision to either restrict or induce muscle differentiation program. In particular, while p38-alpha promotes MyoD-mediated muscle-specific gene expression, p38-gamma facilitates KMT1A association with MyoD via phosphorylation to repress myogenin expression. Here, we will provide evidence that p38-alpha directed phosphorylation of KMT1A disables its association with MyoD, thereby endowing the suppression of KMT1A-impeded myogenin expression and differentiation. This work is supported by grant from NIH/NIAMS.

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Signaling

92. Evaluation of calcium clearance between different mouse models for Duchenne muscular dystrophy

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Duchenne muscular dystrophy is a progressive and devastating neuromuscular disease. Some of the mechanisms thought to contribute to disease severity include elevations in intracellular calcium (Ca2+), increased activation of calpains, and increased mitochondrial Ca2+ overload. Purpose: to evaluate differences in intracellular Ca2+ clearance between three dystrophic mouse models. Methods: control (CON; C57BL/10ScSn; n=3), mdx (n=5), mdx/Utr+/- (n=6), and mdx/Utr-/- (n=4) mice were used in the present study. Single muscle fibres from the flexor digitorum brevis were obtained by collagenase digestion and loaded with Fura-2 AM. Fibres were electrically stimulated at 50 and 100Hz and the time for Fura-2 ratio to return 75% back to resting ratio was used as an index of Ca2+ clearance (T75). Results: There were no differences between CON and dystrophic genotypes in T75 at 50 and 100Hz. However, there was a main effect for all dystrophic fibres (mdx, mdx/Utr+/- and mdx/Utr-/- combined) vs. CON with an increase in T75 at both frequencies (50Hz: 0.10 Â± 0.01s vs. 0.08 Â± 0.01s; 100Hz: 0.12 Â± 0.01s vs. 0.09 Â± 0.01s for dystrophic vs. CON, respectively; $p<0.05$). Conclusion: Our results suggest that Ca2+ clearance is prolonged in dystrophic muscle compared to control but is similar between different dystrophic mouse models. These data suggest that differences in Ca2+ clearance mechanisms may not be responsible for differences in disease severity between dystrophic models.

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Signaling

93. The role of contraction in skeletal muscle development: a study of focal adhesion kinase in vivo.

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Embryonic movements are important for establishing muscle structure and function. In zebrafish, paralysis causes myofibril disruption and conversely contraction leads to myofibril organisation in developing skeletal muscle. We are identifying the signalling pathway that regulates contraction-driven myofibril organisation. Understanding this aspect of development in vivo will facilitate research addressing skeletal muscle disease, such as dystrophy, in humans. Focal Adhesion Complex (FAC) links the extracellular matrix to the cytosol and plays a role in mechanotransduction. Focal adhesion kinase (Fak) is a key component of FAC. Therefore Fak is a potential candidate in the contraction-driven myofibril organisation pathway. Zebrafish have two fak genes, fak1b is ubiquitously expressed from gastrulation, whereas fak1a is expressed later in developing skeletal muscle. The impact of paralysis on fak expression was assessed using an immotile zebrafish mutant (cacnb1ts25). Levels of fak1a and fak1b expression in wild-type versus mutant embryos are comparable using qRTPCR. Treatment of wild type embryos with Fak inhibitors did not affect movement or muscle development. Taken together, our data suggests that Fak is not involved in myofibril organisation during development. We propose that the contraction-driven myofibril organisation is regulated via a novel signalling pathway and are testing this theory using a microarray approach. QMUL College studentship.

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Signaling

94. Decreased Mitochondrial Coupling and Functional Capacity in mdx Skeletal Muscle are Unaffected by Sildenafil But Contribute to a Bioenergetic Crisis In Vivo

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In Duchenne muscular dystrophy (DMD), dystrophin-deficiency disrupts nNOSμ - signaling and inhibits nitric oxide (NO)-stimulated cGMP synthesis. Increasing cGMP with sildenafil (Viagra®) partially circumvents defective NO signaling and reduces dystrophic pathology. Exactly how sildenafil ameliorates dystrophic pathogenesis is unknown. NO-cGMP pathways regulate mitochondrial content and function; thus impaired NO signaling may disrupt mitochondria in dystrophic muscle. Using 31P NMR and optical spectroscopy, we determine in vivo mitochondrial function in the mdx mouse model of DMD and test whether sildenafil's anti-dystrophic effects result from improved mitochondrial function. We find that dystrophin-deficiency reduces subsarcolemmal mitochondria, but increases intermyofibrillar mitochondria, leaving total mitochondrial content unchanged. Mdx mitochondria are markedly inefficient and exhibit substantial uncoupling of oxidative phosphorylation as well as lower maximal ATP synthesis rates. These defects decreased cellular ATP. Surprisingly, sildenafil had no effect on mdx mitochondria, suggesting that the benefits of sildenafil in mdx mice are independent of skeletal muscle mitochondrial function. More importantly, decreased mitochondrial coupling and capacity represent crucial pathogenic mechanisms in dystrophic muscle that contribute to a bioenergetic crisis, the hallmark of a degenerative phenotype. Funding, NIH, MDA.

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Signaling

95. Mohawk is a Novel Regulator of Inflammation in Response to Muscle Damage.

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The innate immune response plays an essential role in hypertrophic muscle growth in associated with non-injurious mechanical loading as well as regeneration of injured muscle. In addition to controlled clearance of necrotic muscle fibers, neutrophils and macrophage serve as a source of Th1 cytokines and Th2 cytokines necessary for activation and proliferation of satellite cells follow by their differentiation into new myofibers. Recruitment of the immune cells and satellite cells to the site of injury is dependent on the expression of chemokines belonging to the C-C and C-X-C ligand families. Elevated levels of chemokines contribute to chronic inflammation and pathologies associated with muscular dystrophies and idiopathic inflammatory myopathies. However, chemokine regulation in satellite cells and skeletal muscle remains poorly understood. Recently we observed that mice deficient for the transcription factor, Mkx, exhibit a muscle repair deficit consistent with a failure to stimulate a robust inflammation response, including persistent necrotic fibers, reduced muscle fiber number and caliber and reduced infiltration of macrophage. Satellite cells isolated from Mkx-/- muscle have reduced expression of several chemokines and Th1 cytokines associated with muscle repair. A similar reduction of transcription was observed in the injured Mkx-/- muscle for CCL2, a critical chemokine for recruiting phagocytic macrophage. We hypothesize that Mkx, is a key regulator of chemokine and cytokine transcription in response to muscle injury. Research supported by Muscular Dystrophy Association.

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Signaling

96. Cancer cachexia induces increased oxidant damage and mitochondrial dysfunction in the diaphragm

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Ventilatory insufficiency due to decreased diaphragm function is the leading cause of morbidity and mortality in many conditions (e.g. muscular dystrophy). In this regard, cancer cachexia is characterized by severe muscle wasting that significantly affects patientsâ€™ prognosis. Therefore, understanding the cellular mechanisms required for cachexia-induced skeletal muscle wasting is important. We tested the hypothesis that cancer cachexia-induced diaphragm dysfunction is associated with increased oxidative damage and mitochondrial dysfunction. CD2F1 mice were randomly assigned to either a control (CON) or tumor-bearing (TB) group. At the completion of the experimental period, animals in the TB group exhibited a ~24% decrease in body weight compared to CON animals. Additionally, there was a significant increase in diaphragm levels of both lipid peroxidation and protein carbonylation confirming that cancer cachexia results in an increase in oxidant damage. Furthermore, using permeabilized diaphragm muscle fiber bundles, our results reveal a significant decrease in the mitochondrial respiratory control ratio in TB animals, which is due to a reduction in state 3 respiration. Collectively, these results indicate that oxidative stress and mitochondrial damage is increased in diaphragm muscle as a result of cancer cachexia. Therefore, mitochondria may play an important signaling role in cancer cachexia-induced diaphragm dysfunction. This work was supported by NIH RO1 AR060209 and Bankhead-Coley Cancer Research Program Grant 09BN-09 awarded to ARJ and NIH RO1 HL087839 awarded to SKP.

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Signaling

97. Impaired calcium calmodulin kinase signaling and muscle adaptation response in limb girdle dystrophy type 2A

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Mutations in the non-lysosomal, cysteine protease calpain 3 (CAPN3) result in the disease limb girdle muscular dystrophy type 2A (LGMD2A). CAPN3 is localized to several subcellular compartments, including triads where it plays a structural, rather than a proteolytic, role. In the absence of CAPN3, several triad components are reduced, including the major Ca2+ release channel, ryanodine receptor (RyR). Furthermore, Ca2+ release upon excitation is impaired in the absence of CAPN3. In the present study we show that Ca-calmodulin protein kinase II (CaMKII) signaling is compromised in CAPN3 knockout (C3KO) mice. The CaMK pathway has been previously implicated in promoting the slow skeletal muscle phenotype. As expected, the decrease in CaMKII signaling that was observed in the absence of CAPN3 is associated with a reduction in the slow vs fast muscle fiber phenotype. We show that muscles of WT mice subjected to exercise training activate the CaMKII signaling pathway and increase expression of the slow form of myosin; however, muscles of C3KO mice do not exhibit these adaptive changes to exercise. These data strongly suggest that skeletal muscleâ€™s adaptive response to functional demand is compromised in the absence of CAPN3. In agreement with our mouse studies, RyR levels were also decreased in biopsies from LGMD2A patients. Moreover, we observed a preferential pathological involvement of slow fibers in LGMD2A biopsies. Thus, impaired CaMKII signaling and, as a result, a weakened muscle adaptation response identifies a novel mechanism that may underlie LGMD2A. This pathway is a target that should be explored for pharmacological intervention.

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Stem Cells

98. Myoblast-derived induced pluripotent stem cells for muscular dystrophy therapy

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Induced pluripotent stem cells (iPSCs) have been generated from a variety of somatic cells through the use of reprogramming factors. iPSCs hold tremendous therapeutic promise due to their capacity for unlimited expansion, ability to differentiate into any cell type, and immunological compatibility. Therefore, iPSCs constitute an ideal resource for cellular based DMD therapies. However, directed myogenic differentiation of iPSCs remains inefficient. Recent studies suggest that iPSCs retain a propensity to differentiate into their tissue of origin due to epigenetic memory. In light of these developments, we have recently established myoblast-derived iPSCs (MB-iPSCs) capable of unlimited expansion in vitro (Watanabe et al., Stem Cells, 2011). MB-iPSCs show a bias towards myogenic differentiation in embryoid body cultures and teratomas compared to fibroblast-derived iPSCs (FB-iPSCs). In addition, MB-iPSCs, but not FB-iPSCs, display low level expression of myogenic genes, including MyoD, indicating that myogenic genes are not fully silenced in MB-iPSCs during the reprogramming process. Therefore, MB-derived iPSCs can differentiate into myogenic cells more readily than FB-derived iPSCs, potentially forming the basis for a novel DMD therapy. MB-iPSCs also offer a valuable means of investigating the molecular mechanisms in the reprogramming process due to the well-defined nature of the muscle development process. Supported by MDA.

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Stem Cells

99. Satellite cells engraft in host mouse muscles following intra-arterial delivery

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Cell based therapies for Duchenne muscular dystrophy (DMD) and other muscle wasting disorders aim to introduce myogenic progenitor/stem cells to patients in order to restore muscle mass, regenerative capacity and performance. As multiple muscles are impacted in DMD, systemic treatments targeting all affected muscles are highly desirable. Intramuscular (IM) and intravenous cell injections are largely inefficient, due to limited cell migration and trapping of donor cells in filter organs. Intra-arterial (IA) cell delivery on the other hand presents an attractive avenue. To date, IA delivery of satellite cells has mostly been overlooked due to a belief that they are incapable of reaching muscles via circulation. However, clear evidence supporting this apparent limitation is scarce. In fact, our initial results indicate that freshly isolated satellite cells engraft in downstream muscles of host mdx mice following IA injection, based on the expression of donor specific myogenic reporter genes. Interestingly, we have determined that satellite cells of extraocular muscles (EOM) harbor superior proliferative capacity compared to those of limb muscles (both in vitro and in vivo following IM injection), possibly making EOM satellite cells ideally suited for transplantation studies. Based on these promising results we propose that IA delivery of satellite cells and EOM progenitors should be strongly considered for cell-based therapy of DMD. MDA, NIH

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Stem Cells

100. Autologous myoblast transplantation for OPMD

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Oculopharyngeal muscular dystrophy (OPMD) is an autosomal dominant inherited, slow progressing, late onset degenerative muscle disorder, characterized by progressive eyelid drooping (ptosis) and difficulties with swallowing (dysphagia). The pharyngeal and cricopharyngeal muscles (CPM) are among the specific targets in OPMD. The genetic mutation is an abnormal expansion of a (GCG)n repeat in the coding region of the ubiquitously expressed poly(A) binding protein nuclear 1 (PABPN1) gene, leading to an expanded polyalanine tract at the N-terminal of the protein.

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Stem Cells

101. Rapid generation of induced pluripotent stem cells (iPSCs) from the urine of a patient with Duchenne muscular dystrophy

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Mature human somatic cells can be reprogrammed to a primitive stage, resembling human Embryonic Stem cells (hES) isolated from embryos. These iPSCs acquire the same infinite self-renewal ability and pluripotent differentiation potential as ES cells. Because of these extraordinary properties, iPSCs can provide virtually infinite numbers of multiple types of somatic cells. Thus, iPSCs hold great potential for disease modeling, drug screening and regenerative medicine. New emerging technologies, such as microRNA delivery, have vastly improved the efficiency of the reprogramming process. However, iPSCs generation is still time-consuming (usually weeks) with relatively low efficiency. Moreover, most iPSCs derived from skin fibroblasts or peripheral blood cells, require invasive collection procedures. Here we show, for the first time, that iPSCs can be generated from the urine of a patient with Duchenne muscular dystrophy (DMD). Urine derived cells (UC) were expanded by culturing DMD patient urine sediment in tissue-culture treated plates. Isolated UCs were fast-proliferating. Moreover, they intrinsically expressed high levels of c-myc and klf4, two factors in the reprogramming cocktail. For comparison, DMD patient UCs and normal human fibroblasts were seeded on Matrigel-coated plates and transduced with a polycistronic lentiviral vector expressing human oct4, sox2, klf4 and c-myc. Virus-infected cells were maintained in original medium for 3 days before switching to hES medium mTeSR. ES-like colonies were observed around 7 days from UCs . Those colonies were alkaline phosphate positive, expressing the pluripotent surface markers ssea4 and Tra-1-81. Exogenous transgenes, as determined by red fluorescence reporter, started to silence around day 7 and complete transgene silenced colonies were noted around day 10 to 14. Isolated large colonies could be manually picked and passaged by day 12. In contrast, fibroblast derived iPSC colonies generally require 3 weeks. RT-PCR array and immunostaining confirmed the expression of pluripotent markers in several UC iPSC lines. An in vivo teratoma formation assay further confirmed the differentiation ability of iPSC to form three germ layers. Together, these data demonstrate the feasibility of rapid iPSC generation from the urine of a DMD patient.Funding: MDA, NIH

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Stem Cells

102. Human Induced Pluripotent Stem Cells as a Novel Model System to Study Dystrophic Cardiomyopathy

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Cardiomyopathy is a poorly understood consequence of Becker and Duchenne muscular dystrophy (BMD and DMD); in part due to the shortcoming of animal models. We determined whether induced pluripotent stem cells (iPSCs) derived from BMD and DMD patients could be differentiated into cardiomyocytes and retain a specific disease phenotype. We directed BMD- and DMD-iPSCs along with non-diseased (N)-iPSCs into cardiomyocytes using defined factors. We confirmed cardiomyocytes by observing contracting regions and immunolabeling for cardiac markers: sarcomeric alpha-actinin and cardiac troponin T. For further experiments, we identified cardiomyocytes by their expression of green fluorescent protein which was under the transcriptional control of a cardiac-specific promoter. We measured mitochondrial permeability transition pore (mPTP) opening using confocal microscopy; pore opening is a seminal event in cell death. mPTP opening occurred significantly earlier in BMD- and DMD-iPSC-derived cardiomyocytes (121Â±12 sec and 62Â±14 sec) when compared to N-iPSC-derived cardiomyocytes (163Â±13 sec). We have established the first human model of dystrophic cardiomyopathy based on directed differentiation of cardiomyocytes from iPSCs containing the genetic background of BMD and DMD. This model system can be used to probe the molecular defects that underlie the pathogenesis of cardiomyopathy that produce heterogeneous disease manifestations. (NIH R01HL034708 to ZJB)

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Stem Cells

103. Homeobox proteins Barx2 and Pax7 are functional antagonists downstream of Wnt signaling in muscle

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Homeobox transcription factors are key intrinsic regulators of myogenesis. We have shown that the homeobox factor Barx2 is a novel marker for muscle progenitor cells where it is co-expressed with Pax7 and muscle regulatory factors (MRFs). Analysis of Barx2 null mice has demonstrated critical roles for Barx2 in postnatal myogenesis including regeneration of acute and chronic muscle injury, and muscle maintenance during aging. Barx2 influences cell proliferation, migration, and fusion via targets such as cyclins, cytoskeletal proteins, adhesion molecules, and cell matrix-remodeling factors. Barx2 also interacts with MRFs such as MyoD and influences their binding to target genes. Moreover, Barx2 exists in a functional loop with MRFs, whereby MRFs directly regulate Barx2 expression and Barx2 directly or indirectly regulates MRFs. Recently we found that Barx2 also interacts with core transcriptional effectors of the Wnt pathway, beta-catenin and TCF/LEF, and regulates Wnt target genes. Intriguingly, MyoD synergizes with Barx2 in activation of a Wnt reporter gene, while Pax7 antagonizes the activity of Barx2 in this system. Several Wnt target genes as well as genes encoding components of the Wnt signalling pathway are misregulated in Barx2-/- satellite cells. Moreover, Barx2 is differentially regulated by canonical and non-canonical Wnts, indicating a functional loop between Barx2 and Wnts. Overall, our data suggest that Barx2 is an intermediate in Wnt/beta-catein signalling during myogenesis and that it integrates the activities of other intrinsic regulators such as MRFs and Pax7 with extrinsic Wnt signals. Misregulation of Wnt targets is likely to contribute to impaired growth and regeneration in Barx2 null mice. Acknowledgements: NIH NIAMS; Association Francaise contre les Myopathies (A.F.M)

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Stem Cells

104. The effect of mesenchymal stem cells in a PEGylated fibrin gel seeded on an extracellular matrix on skeletal muscle recovery in a volumetric muscle loss model.

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This study investigated the effect of bone marrow derived mesenchymal stem cells (MSCs) in a PEGylated fibrin gel (PEG) seeded into a decellularized extracellular matrix (ECM) on recovery of skeletal muscle following a volumetric muscle loss (VML) injury. Six to nine month old male Sprague-Dawley rats were used in this study. Approximately one-third of the skeletal muscle mass of the lateral gastrocnemius (LGAS) was removed from the LGAS, which was immediately replaced with an acellular ECM of the same dimensions. Seven days after injury, animals were injected with one of four solutions: saline (SAL), MSCs (MSC), PEGylated fibrin hydrogel (PEG), or MSCs in PEG (PEG+MSC). Maximal isometric tetanic tension (Po) of the LGAS was assessed fifty-six days after VML injury, followed by histological and immunohistological evaluation. Tetanic tension of the PEG+MSC treated group was significantly higher compared to all other treatment groups, although specific tension (N/cm2) in the PEG+MSC group was only significantly higher compared to SAL and PEG treated groups. However, LGAS mass was significantly higher in the PEG+MSC group compared to all other groups. These findings suggest the combination of the PEG+MSC lead to an increase in tissue deposition, resulting in greater maximal force, as well as total mass. Gross morphology of the repaired gastrocnemius was indistinguishable from the contralateral control. (Funding: DoD, NSF)

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Stem Cells

105. Immortalized human dystrophic cells: a tool to assess pathophysiology and therapeutic strategies.

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We generated immortalized human myoblast cell lines from healthy donors and patients, by neutralizing the mitotic clock by transduction with human telomerase (hTERT), and the stress dependant p16 pathway by transduction with cyclin-dependent kinase 4 (CDK-4). Pathological cell lines include DMD, FSHD, LGMD1C, LGMD2B, OPMD, Myasthenia Gravis, SMA, RYR deficiency or nesprin deficiency (some of them with various mutations including exon-skippable mutations). Their capacity to proliferate and differentiate in vitro was assessed as well as for some of these cell lines their capacity to participate to in vivo regeneration, by injecting them into regenerating TA muscles of immunodeficient mice.We have also set up a strategy to create myogenic cell lines from skin fibroblasts when a muscle biopsy is not available by transduction of both hTERT for immortalization, and inducible MyoD for myogenic conversion. These cell lines represent tools to assess pathophysiology and general or tailored therapeutic strategies, depending on the targeted mutation. All these cell lines can be shared with the scientific community on a collaborative basis. A project supported by AFM, Jain Foundation, Parents project NL, and EU (MYORES and MYOAGE).

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Stem Cells

106. MEF2A regulates the Gtl2-Dio3 miRNA mega-cluster to modulate Wnt signaling in skeletal muscle regeneration

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Understanding the molecular mechanisms of skeletal muscle regeneration is critical to exploit this pathway for use in tissue repair. Our data demonstrate that the MEF2A transcription factor plays an essential role in skeletal muscle regeneration in adult mice. Injured Mef2a knockout mice display impaired myofiber formation associated with a significant decrease in Pax7+ nuclei. We also document the existence of rare cells in regenerating muscle that co-express MEF2A and Pax7. MEF2A controls this process through its direct regulation of the largest known mammalian microRNA (miRNA) cluster, the Gtl2-Dio3 locus. A subset of the Gtl2-Dio3 miRNAs represses secreted Frizzled-related proteins (sFRPs), inhibitors of Wnt signaling. Consistent with these data, Wnt signaling is inhibited in regenerating Mef2a knockout muscle. Furthermore, overexpression of miR-410 and miR-433, two miRNAs in the mega-cluster that repress sFRP2, rescues myotube formation in Mef2a-deficient myoblasts. Thus, miRNA-mediated modulation of Wnt signaling by MEF2A is a requisite step for proper muscle regeneration, and represents an attractive pathway for enhancing regeneration of diseased muscle. This work was funded by the NIH.

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Stem Cells

107. Expanding donor muscle cell engraftment with modulating factors

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Transplantation of myogenic stem cells possesses great potential for long-term repair of dystrophic muscle. We developed a model of canine-to-murine xenotransplantation and a canine-to-canine model of allogeneic transplantation to re-approach cell transplantation and determine if it is a viable therapeutic option for muscular dystrophy. We used the xenotransplant model to show that CXCR4 is important for donor muscle cell engraftment, yet FACS sorted CXCR4-positive cells display decreased engraftment efficiency. Diprotin A, a positive modulator of CXCR4/SDF-1 binding, significantly enhanced engraftment and stimulated sustained proliferation of freshly isolated donor muscle derived cells in vivo. These results were recapitulated in cxmd canines using canine-to-canine allogeneic transplantation, highlighting the pre-clinical utility of the xenotransplantation model in assessing the relative efficacy of muscle stem cell populations.

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Stem Cells

108. Perivascular Multipotent Stem Cells for the Therapy of Skeletal Muscle and other Organs

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Adult multipotent progenitor cells, the archetype of which is the mesenchymal stem cell (MSC) can be recovered in culture from multiple tissues. In spite of its recognized therapeutic potential, the MSC has long been of unknown native identity, tissue distribution and frequency, and its extraction has relied entirely on extended culture. Hypothesizing that blood vessels naturally harbor primary MSC, we have identified and sorted to homogeneity perivascular cells from multiple human organs. Perivascular cells, which include pericytes in microvessels and adventitial cells around larger ones, express MSC markers and are natively multipotent. Pericytes, regardless of tissue origin, are identical to MSCs in terms of growth in vitro, developmental potential and ability to migrate, inhibit T-cell proliferation and support hematopoiesis. These results indicate a perivascular origin for mesenchymal stem cells. Accordingly, human pericytes regenerate bone and promote functional recovery in the diseased heart, lung and kidney. We have explored in detail the ability of perivascular cells to generate human skeletal muscle, both in culture and in vivo. Importantly, perivascular MSC progenitors can be purified in sufficient numbers to be used in cell therapy protocols without preliminary in vitro "expansion". These cells therefore exhibit all advantages of conventional MSCs in terms of tissue repair, but their efficacy is not compromised by the adverse effects commonly associated with in vitro culture.

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Stem Cells

109. Preclinical experience and perspectives of a clinical trial using CD133 stem cells

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Cell therapy is one promising approach to correct genetic diseases by contributing to tissue regeneration; stem cells can be isolated from a healthy donor or dystrophic patient. In the first case cells will be transplanted under a regime of immune suppression; in the second case, cells will have to be genetically corrected before transplantation in the same patient. The recent identification of different types of multi-potent stem cells, some suitable for protocols of cell therapy, has disclosed new perspectives in the treatment of genetic diseases. Our previous work indicated that CD133+ cells, a population of progenitor cells, produce functional improvement upon intra-arterial injection in a dystrophic mouse. Transplantation of engineered dystrophic canine muscle-derived CD133+ cells gave promising results in GRMD dogs, the most reliable animal model that shows a clinical phenotype very similar to DMD patients. Because of these results, we plan a pilot clinical trial, based on intra-muscular and intra-arterial transplantation of autologous engineered CD133+ cells. Efficacy and possible adverse effects will be evaluated to test whether this approach may represent a first step towards a therapy for muscular dystrophy.

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Stem Cells

110. Aldehyde dehydrogenase activity identifies distinct cell populations within human muscle: new candidates for cell therapy

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Aldehyde dehydrogenase (ALDH) activity is one hallmark of human primitive progenitors presenting broad regeneration capacities in vivo. We investigated for the presence of ALDH+ cell populations in striated muscles. Using the fluorescent substrate Aldefluor®, we described well-defined sub-populations of SSClo/ALDHbr cells extracted from dissociated biopsies. Two main sub-populations were discriminated according to CD34 expression. They expressed variable levels of associated markers (CD90, CD105, CD140b) but not endothelial (CD31) nor hematopoietic (CD45) markers. Upon sorting, ALDH+/34- cells from skeletal muscle developed in vitro as a population of CD56+ myoblasts able to form myotubes and participated efficiently to muscle regeneration in vivo in SCID mice, while ALDH+/CD34+ cells did not. Several isoforms of ALDH were expressed by muscle cells, sometimes overlapping. Immunohistofluorescence studies highlighted rare cells in endomysial position or in contact with vessels depending on isoforms expressed. ALDH+ cell populations may become new actors of muscle homeostasis or repair in cell therapy perspectives. Research funding sources: PPMD, AIM, DIM-Stempole Region Ile de France, INSERM, CNRS, UPMC.

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Stem Cells

111. microRNAs in skeletal muscle satellite cells and muscle regeneration

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Most of the time, adult skeletal muscle stem cells, or satellite cells, do little except slowly divide. After an injury, however, they are activated and help build new muscle fibers. The transcription factor Pax7 has two, seemingly contradictory jobs in satellite cells. Pax7 is required for satellite cell maintenance under physiological conditions. Upon satellite cell activation, Pax7 switches on the genes necessary for early differentiation. Intriguingly, Pax7 also stops the cells from terminal differentiating. The question is what shuts down Pax7 to allow myoblast differentiation program properly proceed. Using the in vitro differentiation of satellite cells as a model system, we identified two miRNAs, miR-1 and miR-206, which could play essential roles during satellite cell differentiation. Boosting the amounts of miR-1 and miR-206 in satellite cells slowed their division and speed up their differentiation. We showed that the two microRNAs target Pax7. Because a single miRNA can simultaneously repress the expression of multiple target genes, we predict that miR-1 and miR-206 provide the robustness for the efficient differentiation of satellite cells by repressing multiple stem cell maintenance genes including Pax7. Research in our lab was supported by the March of Dimes Birth Defect Foundation and National Institutes of Health DZ Wang is an Established Investigator of the American Heart Association.

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Therapy

112. Low levels of dystrophin improve survival of the dystrophin/utrophin double knockout mouse

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Duchenne muscular dystrophy is caused by the lack of functional dystrophin. There is no cure, but several clinical trials examine the therapeutic potential of compounds aiming to restore dystrophin synthesis. It is not known how much dystrophin is needed to slow down or prevent (parts of) the disease. To elucidate this, we have generated a mouse model with low dystrophin levels in a utrophin negative background based on skewed X-inactivation. Dystrophin expression was observed in a mosaic pattern and expression in the quadriceps muscle varied between 3 and 47% of wild-type levels, as assessed by Western blot. In a utrophin/dystrophin negative background, dystrophin levels <10% already significantly extended median survival from 70 to 182 days, while survival was further improved for levels >10%. Furthermore, motor function and histopathology and mRNA and serum biomarkers were normalized in a dystrophin level dependent manner. These results suggest that even low levels of dystrophin are already beneficial.

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Therapy

113. Gene replacement therapy for myotubular myopathy

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We have demonstrated that cell cultures isolated from non-affected OPMD muscles have a normal proliferation and differentiation phenotype, whereas cultures isolated from affected OPMD muscles have a reduced myogenicity as compared to control cells. Since OPMD is selectively expressed in a defined group of small muscles and satellite cells can be isolated and amplified from non-affected muscles, we conducted a phase I/II clinical trial in OPMD patients, consisting of the grafting of autologous non affected myoblasts into the pharyngeal muscle during a myotomy.

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Therapy

114. MicroRNA Inhibition to Facilitate Muscle Regeneration and Regulate Fiber Type

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Sarcopenia is muscle wasting that occurs in aged individuals and is characterized by loss of muscle mass in type II fibers. The regenerative potential of myofibers relies on satellite cells (SCs) found in the niche between the myofiber and basal lamina. Upon injury, cues from myofibers signal SCs to leave the niche and move outside the basal lamina. SCs then become myoblasts that proliferate and differentiate to regenerate muscle. Aged individuals experience delayed repair due to slowed reaction of SCs, which may affect sarcopenia and increase the risk for sarcopenic obesity. We hypothesize that microRNAâ€™s (miRâ€™s) have therapeutic applications to enhance, accelerate, and direct the regeneration process in aged individuals. Inhibiting proliferation-promoting miR-133a promoted mouse myoblast force production and increased differentiation of primary human myoblast (hSKM) 4.5-fold after 6 days, compared to transfection with scrambled control. Inhibition of senescence-promoting miR-34a will be used to enhance proliferative/functional hSKM. The metabolic coactivator PGC-1alpha leads to type I fiber development, which may protect against obesity. MiR-696, a repressor of PGC-1alpha, will be inhibited to increase type I fiber content.(NIH)

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Therapy

115. Sphingolipids modulate the inflammatory gene signature in mdx mice

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Duchenne Muscular Dystrophy (DMD) is a severe muscle wasting disease characterized by progressive muscle degeneration, increased deposition of connective and fat tissues and a clear inflammatory response. Sphingolipid (SL) metabolites are associated with the generation or perpetuation of low-grade chronic inflammation critical in atherosclerosis, obesity and cancer. Dietary SL, however, were shown to suppress intestinal inflammation. In this study, we test the hypothesis that dietary SL can modulate specific inflammatory gene markers associated with DMD. C57BL6 (WT) and MDX mice were fed an AIN 76A diet with or without 0.1% sphingomyelin for 3 weeks starting at age 5 weeks. Tibialis Anterior muscles from 3 mice of each treatment group (WT, WT + S, MDX, and MDX + S) were harvested and the extracted RNA was pooled for Affymetrix gene chip analysis, followed by RT-PCR confirmation of changes in individual mice. Fifty-three inflammatory gene markers were either up- or down-regulated in MDX compared to WT. Of these, sixteen with the greatest fold change were related to cytokines and the complement system. Both gene chip and RT-PCR analyses revealed dietary SL treatment favorably reversed the expression levels of up- and down-regulated genes in MDX mice. These data show that dietary SL modulate the inflammatory gene signature in the MDX mouse. Research Funding: VBI/Fralin Life Science Institute Core Resources Exploratory Grant program, Virginia Tech

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Therapy

116. Intramuscular AAV9 Administration Results in Motoneuron and Skeletal Muscle Expression: Implications for Pompe Disease

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Pompe disease is an autosomal recessive metabolic disease caused by the deficiency of the lysosomal enzyme acid alpha-glucosidase (GAA) and results in a progressive accumulation of lysosomal glycogen. This has a profound impact on cellular architecture and function, which leads to progressive cardiomyopathy, respiratory insufficiency and motoneuron dysfunction. Currently, the only treatment for Pompe disease is an enzyme replacement therapy (ERT) delivering recombinant human GAA protein. ERT must be administered every other week and while it has extended lifespan, a new natural history has evolved and patients eventually require ventilatory support. We have focused on an alternative strategy to produce GAA through the natural intracellular pathway as opposed to the extracellular, receptor-mediated uptake of ERT. We have optimized this strategy using adeno-associated virus (AAV) vectors to express long-term therapeutic levels of human GAA in cardiac, skeletal muscle, and the CNS resulting in improved cardiac and respiratory function. However, an incomplete restoration of skeletal muscle function or activation still remains. Therefore, skeletal muscle weakness may be attributed to a loss of neuromuscular junction integrity or axonal pathology and may have a profound impact on AAV9 retrograde transduction efficiency. We tested the hypothesis that an AAV9 vector encoding hGAA would result in retrograde transport and restore neuromuscular junction integrity in Pompe disease. One year old Pompe mice (Gaa-/-) were randomized to the following groups: untreated (Gaa-/-), AAV9-CMV-hGAA, or AAV9-DES-hGAA. AAV9 treated animals received a single injection of 1x1011vg in the right tibialis anterior muscle. One month post injection, the tibialis anterior muscle and lumbar spinal cord were analyzed for vector genome copy number and GAA activity. Significant levels of vector genomes were detected in the tibialis anterior (AAV9-DES-hGAA 1.5x105 Â± 3.1x104 vg/ug DNA; AAV9-CMV-hGAA 8.4x104Â± 1.7x104 vg/ug DNA) and lumbar spinal cord (AAV9-DES-hGAA 1.5x103 Â± 1.7x102 vg/ug DNA; AAV9-CMV-hGAA 2.5x103Â± 1.3x103 vg/ug DNA) suggesting efficient transduction of skeletal muscle and retrograde transport of AAV9. Activity of GAA in tibialis anterior lysates was 2396% and 1770% above wild-type in AAV9-DES-hGAA and AAV9-CMV-hGAA animals, respectively ($p<0.05$). Immunohistochemical assessment of neuromuscular junctions in the tibialis anterior revealed a restoration of integrity in AAV9-treated animals. In conclusion, our data suggest that Pompe disease results in a loss of neuromuscular junction integrity. Moreover, a single injection of AAV9-hGAA results in robust levels of vector copy number at both the site of injection and in associated motoneurons. NIH 5F32HL095282-03 and MDA 216676 (DJ Falk), TL1RR029889(MS Soustek), and PO1 HL59412 (BJ Byrne)

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Therapy

117. Muscle Stem-cell therapy is improved by reducing the fibrosis associated to muscular dystrophies

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The Duchenne muscular dystrophy (DMD) is a genetic disorder characterized by the absence of the protein dystrophin, causing myofiber degeneration, necrosis and subsequently accumulation of extracellular matrix (ECM) proteins in a process called fibrosis. The therapy with progenitor muscle cells is a promissory treatment for the DMD. Grafted cells must migrate and fuse with the regenerating fibers, restoring the expression of dystrophin. Previous attempts have shown low efficiency, but the exact causes remain unknown. Our aim was to study whether the success of the cell therapy is enhanced if the muscle fibrosis is diminished. Satellite cells obtained from freshly isolated skeletal muscle fibers from WT mice were transplanted in the Tibialis anterior (TA) muscles of mdx mice, a mouse model for DMD. We use genetic and pharmacological approaches that have shown to diminish fibrosis, mdx mice heterozygous for CTGF, a potent pro-fibrotic factor, or mdx mice treated with an anti-inflammatory drug (D07I1051). One month after cell transplantation, the number of dystrophin positive myofibers compared to control mdx mice was significantly increased in both models of diminished fibrosis. These results indicate that the muscle cell therapy can be improved under conditions where fibrosis is diminished, opening new approaches for the successful treatment of individuals with DMD. CONICYT-79090027, FONDECYT-11110010, CARE-PFB-12/2007, MDA-89419

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Therapy

118. Targeting NF-KB as a potential therapy for Duchenne muscular dystrophy

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It has been nearly ten years since the initial observations were made associating NF-ÎºB activity with dystrophic muscles in both the mdx mouse model and DMD patients. Based on results from genetic models, NF-ÎºB is thought to contribute to the pathology of DMD by functioning in multiple cellular compartments to promote inflammation and muscle damage, while also limiting the regenerative capacity of muscle progenitors. Since inflammation and failed regeneration are two hallmark features of DMD, NF-ÎºB has been considered a viable therapeutic target, and numerous strategies are currently being designed to impinge on this signaling pathway with the hopes of eventually improving DMD disease. Attempts from our laboratory have relied on a small peptide inhibitor of NF-ÎºB called NBD that functions by blocking the activity of the upstream IKK complex, directly responsible for mediating NF-ÎºB activation. Past studies using mdx and dko mice have shown that NBD is efficacious at not only improving the histopathology of skeletal muscle, but also in significantly rescuing diaphragm and cardiac function. In moving forward to develop NBD as a potential therapeutic for DMD, we performed a pre-clinical trial in the Golden Retriever model (GRMD). Dogs starting at 2 months of age were dosed for a four month period and both skeletal muscle histopathology and function were used as outcome measures. Results showed that NBD dosing was efficacious at reducing inflammation and necrosis from multiple limb muscles as well as improving pelvic limb muscle isometric force, which also correlated with diminishing joint contractures and greater ambulation. Overall, results support the benefits of NBD in dystrophic dogs and suggest that targeting the NF-ÎºB signaling pathway remains a viable option for the treatment of DMD. Funding Source: NIH/NINDS

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Therapy

119. Growth factor delivery via PEGylated fibrin gel for the treatment of skeletal muscle injuries

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Acute skeletal muscle injuries often result in prolonged functional impairments of affected muscles. In the current study, the controlled release of IGF-I from a biodegradable polyethylene glycol (PEG)ylated fibrin gel matrix was evaluated as a treatment for ischemia/reperfusion (I/R) injury of skeletal muscle. Two-hour tourniquet (TK)-induced I/R was induced in the hind limbs of male Sprague-Dawley rats. Intramuscular saline, bolus IGF-I (bIGF), PEGylated fibrin gel (PEG-Fib), or IGF-I conjugated PEG-Fib (PEG-Fib-IGF) treatments were administered after 24 hours of reperfusion. Functional and signaling data were collected at 14 and 4-day reperfusion groups, respectively, while histological assessments were performed on both time points. PEG-Fib-IGF treatment resulted in significant functional improvements and displayed improved histological characteristics over all other treatment groups after 14 days of reperfusion. At 4 days of reperfusion, PEG-Fib-IGF treated muscles showed enhanced activation of the PI3K/Akt pathway compared to PEG-Fib treatment, suggesting involvement of this pathway as a mediator of improved functional recovery. Increased myoblast activity was not evident as a result of PEG-Fib-IGF treatment. These results indicate controlled delivery of IGF-I from the PEG-Fib platform is a viable therapeutic treatment for skeletal muscle injuries. This study was funded by NSF to LJS.

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Therapy

120. Evaluation of fragment AAV for the treatment of dysferlinopathy

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Despite the application of adeno-associated viral (AAV) vectors in over 70 clinical trials, several technological deficiencies are apparent, including the relatively limited single-strand DNA packaging capacity of the AAV capsid (4.7kb). Regardless of this physical limitation, recent reports demonstrated large gene transduction following AAV-mediated delivery of gene â€œfragmentsâ€ that are presumably reconstructed to larger cassettes by host DNA repair processes. In the present work, we performed mechanistic analyses demonstrating that fragment AAV (fAAV) transduction is independent of non-homologous end joining and relies on a unique homologous recombination pathway present in all tissues tested. From a therapeutic standpoint, fAAV was enhanced for large gene transduction in skeletal muscle compared to the leading strategy that relies on the co-transduction of distinct AAV genomes followed by a particular concatemerization event. This enhanced large gene transduction by fAAV was especially notable at lower multiplicities of infection, which is consistent with the preferred clinical trend towards lower administered doses. Next, fAAV was produced using a 7.5kb therapeutic cassette consisting of codon-optimized dysferlin cDNA packaged in a chimeric muscle specific capsid. Characterization of the packaged genomes via Southern blotting demonstrated three distinct fragmented DNA species less than 5kb. However, despite the inability to package the entire 7.5kb cassette, fAAV-Opt-dysferlin transduction resulted in reconstructed dysferlin transcripts and Dysferlin protein production in cell culture. Following these in vitro experiments, the ability of fAAV to restore Dysferlin to the skeletal muscle of a deficient mouse model (BlaJ) was demonstrated. Currently, the therapeutic efficacy of fAAV-Opt-dysferlin transduction is under evaluation in the BlaJ mouse model using an established panel of enzymatic, behavioral, and histological disease phenotypes. In addition to these therapeutic parameters, we developed a versatile muscle cell labeling technique that quantitates muscle turnover in live animals. Collectively, this work demonstrates that fAAV transduction relies on a unique mechanism of DNA repair to mediate efficient large gene transduction in vitro and in vivo. In addition to the dysferlin gene therapy approach evaluated herein, these experiments have broad implications for the success of fAAV vectors as treatments for other genetic diseases requiring large gene delivery including cystic fibrosis, hemophilia A, and other types of muscular dystrophies. (Jain Foundation)

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Therapy

121. Distinctive profile of serum miRNA in mouse models for muscular dystrophies

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Biomarkers (BM) are critically important in diseases diagnosis and monitoring. Classical monitoring methods in muscular dystrophies (MD) are based on histological and molecular analyses of muscle biopsies. Such methods are time consuming, expensive, not quantitative, and require biopsies. The serum BM mCK is not specific to particular muscle pathology and badly correlate with severity. Expression profile of miRNA molecules is specific to tissue of origin and pathophysiological state. In the context of MD, specificity of muscle-miRNA profile to muscle pathology was recently demonstrated. Moreover, it was recently discovered that intracellular miRNA molecules are released into body fluids, stably expressed, and might be used for BM discovery. The present study aimed at identification of circulating miRNA BM in five mouse MD models. To obtain high quality data we elaborated a two-steps screening strategy, employing two independent and complementary miRNA quantification technologies. We have identified a set of serum miRNA commonly deregulated in a number of MD, and other miRNA which are pathology-specific, providing together pathology-specific circulating miRNA profile for every studied model. This study demonstrated clearly the utility of circulating miRNA for diagnosis and monitoring in muscular dystrophies.

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Therapy

122. Targeting PAX3-FKHR as a strategy for therapeutic benefit of alveolar rhabdomyosarcoma

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The chimeric PAX3-FKHR transcription factor, resulting from t(2;13) translocation, is present in a majority of alveolar rhabdomyosarcoma (ARMS), a pediatric skeletal muscle cancer. PAX3-FKHR-positive ARMS patients is associated with increased treatment failure and mortality rate. Prior studies have suggested that transcriptional activity of PAX3-FKHR contributes in part for ARMS tumorigenesis by altering cell growth, differentiation and apoptosis. Taken advantage of PAX3-FKHR as a transcription factor, we inspired to develop new agents able to inhibit its transcription activation function that could be useful for the treatment of ARMS. To achieve this goal, we have screened a small molecule chemical library consist of bioactive molecules using PAX3-FKHR-responsive cell-based readout ARMS system. This approach has identified a short list of potential compounds inhibiting PAX3-FKHR-dependent reporter gene transcription with no significant toxicity to the readout cells. We validated one such inhibitor of PAX3-FKHR (PFI-6) using molecular and cellular approaches in ARMS cells. The results showed that PFI-6 inhibits cell proliferation, anchorage independent growth and invasiveness of ARMS cells. Results further indicated that PFI-6 induced AKT activity abrogates PAX3-FKHR function via phosphorylation. Together, it appears that PFI-6 could be a potential candidate for development of anti-ARMS pharmaceuticals. This work is supported by grants from NIH/NIAMS and Roswell Park Alliance Foundation.

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Therapy

123. Therapeutic development using zebrafish models of muscular dystrophy

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The Muscular dystrophies are a group of degenerative muscle diseases in which the muscle forms normally at first, but starts to degenerate faster then it can be repaired. Any therapeutic approach that decreases the rate at which muscle degenerates will have a major impact on disease course. Inhibitors of PDE5 have been shown to change disease course in mouse models of the most common form, Duchenne dystrophy without restoration of dystrophin expression. Zebrafish models of dystrophin deficiency have been used to screen three libraries of human use approved and known mode of action compounds. In addition to PDE inhibitors, 6 other compounds impacting on the same pathway have been identified. These compounds not only prevent the onset of muscle phenotype in development, some of them also reverse an already present muscle phenotype. Combinations of compounds can result in dystrophin deficient fish surviving at near normal numbers. The 6 compounds and the PDE5 inhibitors influence disease progression and knowledge of their mode of action has lead to a key pathway, which merges, on Heme oxgenase 1. Similar screens of small molecules are in progress for other fish models of human muscular dystrophy. (NIH/NINDS, MDA)

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Therapy

124. Ultrastructural Pathology and Functional Deficits Can Be Reversed Following Protein-Replacement in a Murine Model of Myotubular Myopathy

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X-linked myotubular myopathy (XLMTM) is a congenital muscle disorder that causes severe perinatal weakness and is caused by deficiency of the lipid phosphatase, myotubularin. Mtm1delta4 and Mtm1p.R69C mice model severely- and moderately-symptomatic myotubularin deficiency, respectively, due to differences in the degree of myotubularin deficiency in these animals. Functional testing of the EDL and soleus muscles of Mtm1delta4 mice, which produce no myotubularin, revealed markedly impaired contractile function. Similar testing on Mtm1p.R69C mice, which produce small amounts of myotubularin, showed impaired contractile function only of the EDL muscle, with intact function of the soleus muscle. We then evaluated the consequences of injecting a prototypical targeted protein replacement agent (3E10Fv-MTM1) into the tibialis anterior muscle of Mtm1delta4 mice. Injection of low doses of 3E10Fv-MTM1 over 2 weeks produced a significant increase in contractile function and an improvement in ultrastructural sarcotubular organization. These findings suggest that even a low dose of myotubularin can produce significant improvement in myotubularin deficiency, and that targeted protein replacement with 3E10Fv-MTM1 could effectively treat the pathology and weakness observed in this disease. Funded by: NIH, MDA, Joshua Frase Foundation, Lee and Penny Anderson Family Foundation

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Therapy

125. Responses To ActRIIB-mFc Treatment Are Specific to Individual Muscles in the Mtm1p.69C Murine Model of Myotubularin Deficiency.

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X-linked myotubular myopathy (XLMTM) is a congenital muscle disorder that causes severe perinatal weakness and is caused by deficiency of the lipid phosphatase, myotubularin. A major pathological finding in XLMTM is myofiber smallness, and we proposed that therapeutically increasing myofiber size would cause symptomatic improvement in myotubularin deficiency. Recent studies have elucidated an important role for the activin receptor type IIB (ActRIIB) in the regulation of muscle growth, and have shown that ActRIIB inhibition results in significant muscle hypertrophy. We recently reported that treatment with the extracellular domain of the activin type IIB receptor fused to a murine Fc region (ActRIIB-mFc) produced a 17% extension of lifespan in severely symptomatic (Mtm1delta4) myotubularin-deficient mice, with transient increases in body mass, forelimb grip strength, and type 2b myofiber size. To evaluate the efficacy of ActRIIB-mFc in a less severe model of myotubularin deficiency, we conducted a similar trial in the moderately symptomatic (Mtm1p.R69C) myotubularin-deficient mouse model. In contrast to what was seen using Mtm1delta4 mice, treatment of Mtm1p.R69C mice produces minimal increases in body mass and no increase in forelimb grip strength. Treatment-induced myofiber hypertrophy in Mtm1p.R69C mice is restricted to the gastrocnemius muscle, in comparison to the hypertrophy in all muscles that was seen in treated Mtm1delta4 mice. Additionally, while treatment with ActRIIB-mFc produced proliferation of satellite cells in both the quadriceps and gastrocnemius muscles of treated WT mice, this satellite cell proliferation was restricted to the gastrocnemius muscle in treated Mtm1p.R69C mice. These findings illustrate the importance of microenvironmental factors in myotubularin deficiency, and suggest fundamental differences in the mechanism of muscle weakness in Mtm1delta4 and Mtm1p.R69C mice.

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Therapy

126. A microrna regulated vector prevents cardiac toxicity of calpain 3 gene transfer

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Genetic defects in calpain 3 protease leads to Limb-Girdle Muscular Dystrophy type 2A (LGMD2A), a disease of the skeletal muscle that affects predominantly the proximal limb muscles. There is no treatment for this disease to date. We previously demonstrated the efficient recovery of calpain 3 (CAPN3) proteolytic activity and muscle pathology following intramuscular injection of recombinant adeno-associated virus (rAAV) vectors expressing CAPN3 under the control of the desmin promoter in a murine model for LGMD2A (C3KO mouse). However, systemic administration of the same vector led to cardiac toxicity that we related to an unregulated proteolytic activity of calpain 3 in the heart. Following these results, we set up a strategy based on microRNA regulated vector to prevent CAPN3 transgene expression in the heart. We cloned the target sequence of the cardiac specific microRNA-208a downstream of CAPN3 cDNA and demonstrated in cellulo its capacity to down-regulate the expression of the carrier messenger in presence of miR-208a. New muscle specific promoters were also cloned and validated in cellulo. Several rAAV vectors carrying CAPN3 transgene and these regulatory elements were designed and validated in vivo. Our results indicate that the presence of the miR-208a target sequence in combination with the different promoters tested permit CAPN3 transgene expression in skeletal muscles without showing any cardiac toxicity in C3KO mice.

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Therapy

127. RNAi therapy for Limb girdle muscular dystrophy type 1A

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Limb-girdle muscular dystrophy refers to a group of 23 disorders characterized by progressive wasting and weakness of shoulder and hip girdle muscles. The onset and progression of LGMD varies among individuals and genetic subtypes. Preclinical gene therapy studies have shown promising results for some forms of LGMD, and importantly, one of these strategies (for LGMD2D) has been recently and successfully translated to human clinical trials. Nevertheless, current LGMD-targeted gene therapies involved gene replacement strategies for recessive forms, while treatments for dominant LGMDs (LGMD type 1; LGMD1) have been largely unexplored. This lack of focus on gene therapy for dominant LGMDs arose primarily because these diseases require disease gene knockdown, and the molecular tools to feasibly accomplish this did not exist until recently, with the emergence of RNAi. We hypothesized that patients with dominantly inherited LGMD would benefit from RNAi-mediated reduction of the pathogenic alleles underlying their disease. In this study, we developed the first RNAi-based, pre-clinical treatment for LGMD1A, caused by dominant mutation in one allele of the myotilin (MYOT) gene. Our strategy involved engineering MYOT-targeted artificial microRNA (miMYOT) vectors to knockdown mutant MYOT in muscles of an LGMD1A mouse model. Three months after treatment, we found miMYOT vectors, but not controls, significantly reduced soluble mutant MYOT protein to undetectable levels, and the protein aggregates, one of the characteristics of LGMD1A, were either absent or very small in treated muscles. Our miMYOT vectors also significantly improved muscle mass and whole muscle strength in LGMD1A mice. We are currently quantifying histopathological improvements, and determining whether our miMYOT vectors will similarly improve body wide functions using treadmill assays. These studies represent important first steps toward translating targeted RNAi gene therapy approaches for LGMD1A, and our RNAi strategies could be adapted to impact a large class of dominant muscle disorders.

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Therapy

128. Proof of concept for a novel therapy for muscular dystrophy in myotonic dystrophy (DM1)

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Using an inducible/reversible mouse model of myotonic dystrophy (DM1) due to DMPK 3â€™UTR mRNA toxicity and expression arrays, we identified a membrane receptor that was highly induced in heart and skeletal muscles and reverted with reversal of phenotypes. This was confirmed by various molecular analyses and correlated with levels of toxic RNA and muscle pathology. Importantly, we found this receptor also induced in DM1 skeletal muscles. We obtained mice that were null for the receptor and its ligand from a major pharmaceutical company. We bred these mice with our RNA toxicity mice. EMG, ECG, treadmill running, grip strength, RT-PCR, westerns and histologic analyses demonstrated significant benefit in lifespan, muscle function, histopathology, and regeneration in RNA toxicity mice lacking either the receptor or ligand. Using systemic delivery of a novel therapy that binds the ligand, we found significant benefit in muscle histopathology, run distance and grip strength. No therapies exist for DM1. Our results are proof of concept for this therapy in skeletal muscle function and histopathology in RNA toxicity. It is already in clinical trials and may be 'the first in human' compound for treating muscular dystrophy in DM1. (NIH)

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Therapy

129. Allogenic bone marrow transplantation (BMT) ameliorates muscle atrophy, degeneration, and weakness in DMRV mice

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GNE myopathy, previously known as distal myopathy with rimmed vacuoles (DMRV), or hereditary inclusion body myopathy (hIBM), is an autosomal recessive myopathy characterized clinically by progressive weakness and atrophy involving the distal muscles, and pathologically by the presence of myofiber vacuolation and degeneration. GNE myopathy is secondary to mutations in the GNE, a gene which encodes for the bifunctional enzyme which catalyzes the crucial steps in sialic acid biosynthesis. Despite the identification of the causative gene, no therapy has been approved for this debilitating disorder, as the pathomechanism has remained elusive. We and others have shown that hyposialylation is one of the key players in disease pathomechanism. This notion is supported by our recent publications showing that the myopathic phenotype in the existing DMRV/hIBM mouse was prevented by giving exogenous oral sialic acid metabolites modified ManNAc conjugates. Despite these promising results, several issues remain in designing an oral therapeutic regimen for this disabling myopathy, one of which is the extremely rapid excretion of orally administered sialic acids. In this study, we aim to establish autologous bone marrow transplantation as another justified choice for considering therapy, and as a continuous source of sialic acid-producing cells, thus maintaining an acceptable level in the circulation. After whole bone marrow cells obtained from eGFP mice were given to irradiated donor DMRV/hIBM mice, we saw that the lifespan, motor performance, and muscle force production remarkably improved. In addition, analysis of muscle pathology showed diminished number of characteristic trimmed vacuoles and intracellular accumulation of amyloid. The improvement in phenotype correlated well with the sialic acid in tissues and plasma. Interestingly, the level of cell surface sialylation of isolated leukocytes from bone marrow translpanted DMRV/hIBM mice increased through time, supporting the idea the hematopoetic cells can be a good source of sialic acid produciton. the Bone marrow transplantation by itself is not a new technique and thus could easily be adapted in DMRV/hIBM treatment. We envision that with this strategy, GNE myopathy patients can have a lasting supply of sialic acid that may benefit them towards disease recovery.â€ƒFunding: NIN/NCNP, AFM, NDF

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Therapy

130. A survey of pre-clinical studies of FDA-approved drugs in the mdx mouse model of Duchenne muscular dystrophy (DMD)

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Oral corticosteroids are the only therapy shown to alter the clinical course of DMD, but with significant side effects. Many drugs approved by the FDA for other diseases have been evaluated in mdx mice, the most widely used model of DMD. However, the assays used in pre-clinical studies are highly variable and comparison between studies is cumbersome. In an attempt to identify promising candidates for further pre-clinical or clinical studies, we analyzed all pre-clinical studies of FDA-approved drugs in mdx mice published since 1995. Parameters were collected in five categories (whole animal physiology; skeletal muscle pathology; skeletal muscle physiology; cardiac pathology; cardiac physiology) for analysis. Among 187 studies identified, 58% contained physiological and pathological data; 36% scored pathology alone; 11% assessed both skeletal and cardiac muscle; and only 3% studied both skeletal and cardiac muscle by both pathology and physiology. Pathology included limb skeletal muscle alone (38%); limb muscle and diaphragm (30%); limb muscle, diaphragm, and cardiac muscle (9%); diaphragm (8%) and cardiac muscle (6%). 43% of studies only analyzed one muscle. Our review highlights the importance of uniform study parameters, and the need to meaningfully compare studies. We are developing a web-based tool that will allow comparative analysis of the mdx literature to be made available as a resource for the scientific community. Funded by PPMD

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Therapy

131. Stability of human dystrophin constructs skipped around exon 45.

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Exon-skipping therapeutics are under investigation to delete disease-causing mutations from dystrophin transcripts. We showed that internal sequence deletions can result in dystrophin instability and aggregation (Henderson et al 2011), raising question of whether exon skipping affects dystrophin stability. To address this question, we compared the biophysical properties of purified full-length human dystrophin with proteins deleted for exons 44-45 (hDys-ex44/45), or exons 45-46 (hDys-ex45/46). By circular dichroism spectroscopy and differential scanning fluorimetry, hDys-ex44/45 and hDys-ex45/46 both showed thermal stabilities equivalent to full-length dystrophin. In contrast, a dystrophin deleted for exons 43-44 (hDys-ex43/44) was too unstable to be expressed and purified for further characterization. While our results suggest that exon 45 skipped dystrophins are stable, a remaining caveat is that each of the dystrophins analyzed were contaminated by a large N-terminal fragment that may have influenced the biophysical analyses. Therefore, we are currently optimizing a dual affinity purification strategy employing distinct N- and C-terminal epitope tags. Supported by Ryanâ€™s Quest, the MDA and NIH grants AR042423 and AR007612.

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Therapy

132. Viral muscle gene delivery and targeting NOS in muscular dystrophy

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Duchenne Muscular dystrophy (DMD) occurs due to the loss of the sarcolemmal localised protein dystrophin. Dystrophin is linked to several proteins including neuronal nitric oxide synthase (nNOS), which is involved in intracellular signaling. Disruption of nNOS is thought to contribute to the dystrophic pathology. The aim of the project is to conduct AAV delivery of nNOS and/or an associated signaling molecule to the mdx mouse model of DMD. This work entails the development of an AAV2/9 viral vector, initially with the green fluorescent protein (eGFP) gene driven by the muscle specific Spc512 promoter. Specificity of the vector was demonstrated in the C2C12 muscle cell line. The vector was locally delivered to the tibialis anterior (TA) muscle as well as systemic delivery via the tail vein. Muscles were analyzed for fluorescence and expression confirmed with qRT-PCR and widespread GFP antibody cross-reaction. Successful transduction of the TA muscle was observed 7 days post-exposure to the AAV2/9Spc512eGFP vector and was optimal in younger (3-4 week old) mdx mice. Delivery of the nNOS transgene using the AAV2/9 vector was unsuccessful likely due to the large transgene size (4.3 Kb). We turned our attention to nNOS associated signaling proteins of a size more feasible for packaging into the AAV. This work establishes for the first time, the efficacy of using muscle specific viral-transgene expression to modulate nNOS in the preclinical animal model of DMD. This research was funded by grant HRA/2009/79 from Health Research Board (HRB) Ireland to KMC.

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Therapy

133. Exon skipping in Duchenne Muscular Dystrophy

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The improved understanding of the regulation of dystrophin pre-mRNA splicing, and development in antisense oligonucleotides (AOs), have very rapidly moved in the last decade from in vitro experiments to in-vivo studies in animal models to phase I; IIa; IIb and now III clinical trials in DMD. The pace of the development of this novel approach is one of the fastest in recent drug development programs. It took less than 10 years from the publication of the first description of the use of AO to modify the splicing of the dystrophin gene in cultured mdx muscle cells (2008); to the publication of the results of the first intramuscular proof of concept study in DMD boys (2007, followed by a second one in 2009). In 2011 two separate repeated systemic dosing studies using 2 different AOs backbones (2â€™Omethyl and Morpolino, 2Ome and PMO respectively) have been published; in early 2012 the outcome of a phase IIb study using PMO AO was announced, and currently two large international randomised placebo controlled studies using the 2Ome AOs are underway. All these studies target exon 51; a phase I study targeting exon 44 was completed in 2011; and additional studies targeting other dystrophin exons are at advanced planning stages.

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Therapy

134. Metabolic changes in sialic acid synthesis pathway in GNE-myopathy model mice with long-term sialic acid treatment.

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GNE-myopathy is an autosomal recessive disorder characterized by muscle atrophy, weakness that initially involves the distal muscles, and presence of accumulated proteins and rimmed vacuoles in myofibers. This disease is secondary to mutations in the GNE gene, which encodes an essential enzyme in sialic acid biosynthesis. We recently showed that muscle atrophy and weakness were completely prevented in the GNE-myopathy model mouse after treatment with sialic acid (NeuAc and sialyllactose) and its precursor (ManNAc). In this study, we aimed to clarify whether the long-term treatment with sialic acid of GNE-myopathy model will influence sialic acid biosynthetic pathway. Recent report showed the endogeneous sialic acid synthesis in colon is inactivated or activated dependent on milk sialic acid level in neonatal suckling rats. In this study, at first we examined the expression of genes encoding the enzymes and transporter in sialic acid biosynthesis pathway in various organs in non-treated wild mouse by quantitative PCR. The genes involved in synthesis are highly expressed in liver, while those involved in degradation are highly in kidney, suggesting liver is an anabolic organ and kidney is a catabolic organ for sialic acid synthesis. We examined the expression of genes in skeletal muscle, liver and kidney of GNE-myopathy model mouse after long-term treatment with NeuAc and sialyllactose or ManNAc for more than 300 days. The genes for catabolic enzymes were further up-regulated in kidney, and the genes for anabolic enzymes were down-regulated in skeletal muscle in the treated mice, however the change in gene expression was smaller in disease model compared to that in control littermates. These results suggest that the long-term sialic acid administration may cause the decreasing effects of treatment and the effect on the expression of sialic acid-metabolic genes are variable with compounds administered. Research funding source: NIN/NCNP, Japan

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Therapy

135. Evaluation of an extracorporeal system developed for tissue metabolic exchange, oxygenation, and regionalized delivery of rAAV

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Duchenne muscular dystrophy, the most common inherited muscle disorder of children, is caused by mutations within the dystrophin gene and results in progressive muscle wasting. Several genetic intervention strategies are under investigation, and progress towards clinical gene therapy with adeno-associated viral (AAV) vector delivered transgenes remains promising. However, given the challenge of transducing the vast musculature in patients, we have designed a vector delivery system which incorporates dialysis equipment. Dialysis provides for ultrafiltration of vector, diffusive delivery of bicarbonate, electrolytes and oxygen into the circuit and subsequently, the tissues. Here we have evaluated our circuit design utilizing rAAV6 expressing the reporter gene human placental alkaline phosphatase. A number of pre-circulating conditions were evaluated including Ringers, (+) non-ionic surfactant poloxamer P188, (+) albumin, or whole blood, and varying pre-circulation time. Retention of rAAV6 was evaluated by qPCR/Souhern blot of vector genomes and cellular transduction assays. Over a circulation time course of one hour while assaying various aliquot time points we obtained a range of results across the multiple parameters ranging from approximately 0 to 90% of viable rAAV6 retention. In summary, this circuit has the potential for prolonged and enhanced delivery of vector while minimizing potential known side effects to the patient.

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Therapy

136. Developing NBD as a therapeutic for DMD: Results from a GRMD trial

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Duchenne muscular dystrophy (DMD) is a lethal muscle disease with no effective treatment. Genetic, biologic, and pharmacological studies indicate that inhibiting the NF-kB pathway improves pathology and function in dystrophic mice. To continue our work to determine if a peptide called NBD, which inhibits the IKK activating complex of NF-kB, can be developed to treat DMD, we initiated studies in a large animal model, golden retriever muscular dystrophy (GRMD) dogs. 2-month-old unaffected (n=3) and GRMD (n=6) dogs were given 10 mg/kg NBD 3x/wk IV for 4-months. All dogs survived the 4-month study, demonstrating for the first time that NBD can be administered systemically to a non-rodent species. Encouragingly, NBD treated GRMD dogs gained significantly more weight compared to a natural history GRMD cohort. Current studies are underway to complete safety and efficacy parameters, including blood cell counts and chemistries, joint angle, muscle function, hind limb MRI, histological and biochemical analysis on 4 hind limb muscles, and necropsy. These results will provide a better indication of the potential of NBD to be developed as a therapeutic for DMD. Work was supported by an NIH U01 grant to DCG and JNK and an F32 postdoctoral fellowship to JMP.

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Therapy

137. AAV-mediated genes transfer for restore the functional dysferlin.

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Deficiencies in dysferlin are the cause of limb-girdle muscular dystrophy type 2B and Miyoshi myopathy. Dysferlin is a transmembrane protein shown to play a role in the repair of the plasma membrane by vesicle fusion, providing a possible hypothesis for the pathophysiology of these diseases. No treatment is available for dysferlinopathies and gene therapy is complicated by the fact that the dysferlin cDNA is large. To correct dysferlin-deficiency, two therapeutic strategies were developed in dysferlin-deficient mice (B6.A/J-Dysfprmd): transfer of minidysferlin and a strategy relying on the concatemerization property of the Adeno-Associated Viral (AAV) vector to transfer the full-length dysferlin gene by means of two AAV vectors. Both strategies led to correction of the membrane repair deficit. However, only the concatemerization strategy improved muscle histology. In confirm this difference; we studied muscles from animals subjected to our experimental therapeutic strategies for their ability to recover from eccentric exercise using the large-strain injury (LSI). LSI consists in 15 repetitive lengthening contractions and generates an injury from which normal muscle recovers without the need of neomyogenesis. This test showed that the minidysferlin do not prevent myofiber degeneration in dysferlin-deficient muscle and the concatemerization strategy is efficient to protect the muscle from physical stress.

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Therapy

138. The twist and turns of calpain 3 gene transfer

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Genetic defects in Calpain 3 leads to Limb-Girdle Muscular Dystrophy type 2A, a disease of the skeletal muscle that affects predominantly the proximal limb muscles. There is no treatment for this disease to date. In a attempt to define a therapeutic strategy, we evaluated the potential of recombinant adeno-associated virus (rAAV) vectors for gene therapy in a murine model for LGMD2A. Efficient and stable transgene expression was obtained in the skeletal muscle after intramuscular and loco-regional administration. Moreover, its presence resulted in improvement of the histological features and in therapeutic efficacy at functional level. However, a cardiac toxicity that we related to an unregulated activity of calpain 3 was subsequently observed when the vectors were transferred using systemic administration. Following these results, we developed new AAV vectors with skeletal muscle restricted expression. The observations made after intravenous injection of this new generation of vectors indicated that we improved the safety of calpain 3 gene transfer since no sign of cardiac toxicity was noticed even with high doses of vector.

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Therapy

139. MuStem cell: a promising tool for DMD therapy

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Duchenne Muscular Dystrophy (DMD) is a genetic muscle disease resulting from dystrophin lack and without effective treatment. Somatic stem cell identification has given new impetus to neuromuscular disease therapy. We isolated resident muscle stem cells based on adhesion properties in healthy dog, characterized them and investigated their systemic delivery in the clinically relevant DMD animal model, the Golden Retriever Muscular Dystrophy (GRMD) dog. Delayed adherent cells, named MuStem cells, displayed high proliferation rate and multi-lineage differentiation potential even though they appeared to be committed to the myogenic lineage. They were negative for haematopoietic, endothelial and blood lineage cell markers. After intra-muscular injection in immunosuppressed GRMD dog, MuStem cells contributed to myofiber regeneration, satellite cell replenishment and dystrophin expression. Importantly, when intra-arterially delivered, they contributed to a striking and persistent clinical improvement, long-term dystrophin expression and muscle damage correction, Overall, with regard to their in vivo efficacy MuStem cells may provide a source of adult stem cells for future clinical trial of DMD patients.

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Therapy

140. Arbekacin as a therapeutic readthrough inducer for treatment of nonsense mutation-mediated Duchenne muscular dystrophy

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Translational readthrough of a premature termination codon is a promising therapeutic method in more than 2,400 distinctly inherited human diseases. We previously reported that negamycin, a dipeptide antibiotic, that binds to the ribosomal decoding site and alters translational accuracy, successfully restored dystrophin expression with less toxicity than gentamicin in mdx mouse, which carries a premature termination codon in the dystrophin gene. In order to measure translational readthrough activity with quantitative accuracy, we established a novel transgenic mouse strain, named READ (Readthrough Evaluation and Assessment by Dual reporter). We found that arbekacin induced the in vivo nonsense suppression in READ mice dose-dependently, and promotes the accumulation of dystrophin, reduction of serum creatine kinase activity and improvement of contractile function in mdx mice. Moreover, arbekacin exhibits restoration of dystrophin expression on muscle cell obtained by biopsies from Duchenne muscular dystrophy patients caused by nonsense mutations. We have validated the efficacy of arbekacin on dystrophin-deficient muscle that we ultimately wish to treat. Arbekacin is a brekthrough readthrough-inducing drug for muscular dystrophy patients harboring nonsense mutations. This work was supported in part by The Ichiro Kanehara Foundation; Fugaku Foundation; Sasakawa Grants for Science Follows (to MS); a grant from the New Energy and Industrial Technology Development Organization; a Grant-in-Aid for Scientific Research (B); a Grant-in-Aid for Exploratory Research from the Japan Society for the Promotion of Science; a Health and Labour Sciences Research Grant for Research on Psychiatric and Neurological Diseases and Mental Health; a research grant for Nervous and Mental Disorders from the Ministry of Health, Labour, and Welfare, Japan (to MM); Health and Labour Sciences Research Grant for Comprehensive Research on Disability Health and Welfare (H22-016); Intramural Research Grant (23-5) for Neurological and Psychiatric Disorder of NCNP to RM.

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Therapy

141. Phase I/II Trial of Diaphragm Gene Therapy for Pompe Disease: Initial Ventilatory Outcomes.

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Pompe disease is a disease caused by a deficiency of acid alpha-glucosidase (GAA) enzyme, and the infantile form leads to severe cardiac hypertrophy and respiratory failure. The currently approved treatment, enzyme replacement therapy (ERT), reduces cardiomyopathy and increases survival. However, many surviving patients experience progressive ventilatory insufficiency and eventually require mechanical ventilation (MV). We report an open-label, Phase I/II clinical study of diaphragm gene therapy with the goal to restore function. To date, 3 MV-dependent children on chronic ERT have completed enrollment, received intramuscular rAAV1-CMV-GAA vector delivery into the diaphragm, and underwent follow-up ventilatory testing for up to 180 days. No serious adverse events occurred and no clinically significant changes in safety labs were noted. Most recent assessments showed improvements in maximum tidal volume and maximum voluntary ventilation for all subjects. Furthermore, all subjects also were able to generate improved spontaneous breathing without MV assistance. In conclusion, the initial results support the safety and feasibility of AAV-mediated gene therapy for ventilatory failure in early-onset Pompe disease. (NIH)

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Therapy

142. Mature-IGF-I excels in muscle recovery following disuse atrophy compared to pro-IGF-IA

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Prolonged disuse of skeletal muscles results in atrophy, which are prone to injury once physical activity is resumed. This project aims to enhance muscle function upon the reintroduction of physical activity after atrophy with insulin-like growth factor-I (IGF-I), which is known to activate both proliferation and differentiation signaling pathways and to result in muscle hypertrophy. We tested the effects of mature-IGF-I and pro-IGF-IA on muscle rehabilitation in vivo using hindlimb suspension to induce muscle atrophy. Wild type mice were injected in one hindlimb with viral vector to increase the expression of pro-igf1a or mature-igf1, and the mice were suspended for 7 days. They were then returned to normal activity for 3, 7, and 14 days. The IGF-I injected muscles hypertrophied in non-suspended mice, but only excess mature-IGF-I succeeded in maintaining relative muscle mass and strength during suspension. Fiber size analysis revealed that mature-IGF-I injected muscles recover faster during the reloading process. While both excess pro-IGF-IA and mature-IGF-I were successful in inducing hypertrophy in skeletal muscles, pro-IGF-IA failed to prevent the suspension-induced atrophy. We conclude that mature-IGF-I provides more efficient recovery of muscle following disuse or atrophy. (NIH P01 HD059751)

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Therapy

143. Mesoangioblast-based therapy for DMD: current clinical experimentation and novel pre-clinical strategies

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Gene and cell therapy of Duchenne Muscular Dystrophy (DMD) is complex because of the large size of dystrophin gene (2.4Mb) and cDNA. In recent years, intra-arterial mesoangioblast (MAB: vessel-associated stem cells) transplantation caused amelioration of DMD animal models: this was mainly due to their ability to cross the vessel-wall. Cells similar to MABs were isolated from human skeletal muscle and are currently under clinical experimentation for a Phase I/II trial based upon four consecutive intra-arterial infusions of HLA-matched donor-derived MABs (EudraCT no. 2011-000176-33). While safety is the primary objective of the study, a possible modification in patientsâ€™ muscle function is also being measured. Autologous transfer of genetically corrected cells would be desirable, since it would not require immune-suppression. To this end, we reported recently the amelioration of a model of DMD by a novel strategy that combines Human Artificial Chromosome (HAC)-mediated dystrophin gene-replacement with mouse MAB transplantation. Indeed, at variance with conventional gene therapy vectors, HACs can carry large genetic regions and remain episomal. We are currently extending this approach to human MABs, engineering next generation HACs for future clinical translation of this autologous strategy for DMD. Research funding: European Community, European Research Council, Medical Research Council UK, Telethon, Duchenne Parent Project and Italian Ministries of Research and Health.

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Therapy

144. Optimizing systemic antisense delivery regimens in dystrophic mouse models

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Currently antisense oligonucleotides (AON) are investigated as a therapeutic approach for Duchenne muscular dystrophy (DMD), which is caused by the absence of dystrophin. AONs aim to restore the dystrophin reading frame by skipping targeted exon(s), thereby allowing the production of slightly shorter, but largely functional proteins. Proof of concept has been shown in cultured muscle cells, the mdx mouse model and recently in clinical trials by GSK/Prosensa (GSK2402968) and AVI Biopharma (AVI-4658). Efficient exon skipping for subcutaneously injected 2â€™-O-methyl phosphorothioate (2OMePS) AONs (used by GSK/Prosensa) has been obtained in dystrophic mouse models and patients. To further optimize dosing regimens, mice were injected with 200 mg/kg/week divided over weekly, biweekly or daily injections. For skeletal muscles no differences in skipping levels were found, but daily dosing resulted in higher skipping and dystrophin levels for heart and diaphragm. Furthermore we tested the tolerability of long-term treatment in mouse models with different disease-severity. No signs of toxicity were observed after 6 months treatment and, interestingly, the therapeutic effect was larger in more severely effected mice. (Dutch Duchenne Parent Project)

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Therapy

145. Genetic down-regulation or pharmacological inhibition of Flt-1 ameliorates the muscular dystrophy phenotype by increasing the vasculature in DMD model mice

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Recent works have shown the importance of the vasculature in the pathogenesis of Duchenne Muscular Dystrophy (DMD). Previously, we demonstrated (Verma, Hum. Mol. Genet., 2010) that capillary density and muscle perfusion can be increased in the mdx (DMD model) mice by heterozygous mutation in Flt-1 gene (mdx:Flt-1+/- mice). Interestingly, the mdx:Flt-1+/- mice show improved histological and functional muscle parameters compared with the mdx mice. Consequently, the mdx:utrophin-/-:Flt-1+/- mice display significantly higher survival rates compared with the mdx:utrophin+/+ mice. In this study, we show that these improvements are mediated due to a pro-regenerative mechanism from an increase in satellite cells rather than protection from contraction induced injury. In vivo hemodynamics studies reveled no cardiac insufficiency in mdx:Flt-1+/- mice compared to mdx mice. Lastly, pharmacological treatment of mdx mice with anti-Flt-1 peptide showed increased capillary density, increased tissue perfusion and more importantly, improved muscle histology. These data further validate inhibition of Flt-1 as a therapeutic target for the treatment of DMD. The work was funded by the MDA and the Gregory Marzolf Jr. Foundation.

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Therapy

146. Tadalafil alleviates functional muscle ischemia in patients with Becker muscular dystrophy

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BECKER muscular dystrophy (BMD) is a progressive and ultimately fatal X-linked muscle wasting disease for which there is no treatment. Allelic with Duchenne muscular dystrophy (DMD), BMD is caused by mutations in the gene encoding dystrophinâ€"a structural cytoskeletal protein that also targets other proteins to the sarcolemma. Among these is neuronal nitric oxide synthase (nNOSÂµ), which binds to spectrin-like repeats in dystrophinâ€™s rod domain. With exercise of healthy skeletal muscle, sarcolemmal nNOSÂµ-derived nitric oxide (NO) attenuates local Î±-adrenergic vasoconstriction thereby optimizing perfusion. We found previously that this protective mechanism is defectiveâ€"causing functional muscle ischemiaâ€"in dystrophin-deficient muscles of the mdx mouse and of children with DMD, in whom nNOSÂµ is misplaced from the sarcolemma to the cytosol. Here we report that this protective mechanism also is defective in men with BMD caused mainly by dystrophin mutations that delete exons encoding the nNOSÂµ binding site, because the vasoconstrictor response (measured as a decrease in muscle oxygenation) to reflex sympathetic activation was not appropriately attenuated during exercise of the dystrophic muscles. Moreover, in a randomized placebo-controlled cross-over trial, we show that functional muscle ischemia is alleviated and normal blood flow regulation fully restored by boosting NO-cGMP signaling with a single dose of the phosphodiesterase (PDE5A) inhibitor tadalafil. These translational human studies further support an essential role for sarcolemmal nNOSÂµ in the normal modulation of sympathetic vasoconstriction in exercising human skeletal muscle and implicate the NO-cGMP pathway as a putative new drug target for BMD.

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Therapy

147. Role of Myostatin Fibrosis

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Skeletal muscle fibrosis is a defining feature of the muscular dystrophies in which contractile myofibers are replaced by fibroblasts, adipocytes and extracellular matrix. This maladaptive response of muscle to repetitive injury is progressive, self-perpetuating and thus far, has been considered irreversible. We have previously shown that myostatin, a known endogenous modulator of muscle growth, stimulates normal muscle fibroblasts to proliferate. We now demonstrate that myostatin also regulates the proliferation of dystrophic muscle fibroblasts, and increases resistance of fibroblasts to apoptosis through Smad and MAPK signaling. Inhibiting myostatin signaling pathways with a soluble activin IIB receptor (ActRIIB.Fc), reduces resistance of muscle fibroblasts to apoptosis in vitro. Systemic administration of ActRIIB.Fc in senescent mdx mice, a model of muscular dystrophy, significantly increases the number of muscle fibroblasts undergoing apoptosis. This leads to the reversal of pre-existed muscle fibrosis as determined by histological, biochemical and radiographical criteria. These results demonstrate that skeletal muscle fibrosis can be pharmacologically reversed through induction of fibroblast apoptosis. This work was supported by the Muscular Dystrophy Association

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Therapy

148. Follistatin improves skeletal muscle regeneration following various forms of muscle injury

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Skeletal muscle recovery following injury is dependent upon precise control of activating myogenesis from satellite or mesenchymal stem cells, while limiting fibrosis; therefore, injured muscle could benefit significantly from therapies that both stimulate muscle regeneration and inhibit fibrosis. Myostatin is a member of the transforming growth factor-Î² superfamily (TGF- Î²) and a negative regulator of muscle regeneration. Activin A is also a member of the TGF- Î² family and has been demonstrated to be an integral component of the inflammatory response. Follistatin (Fst), which can bind to and neutralize both myostatin and Activin A, could potentially increase muscle mass, improve strength and attenuate inflammation in the context of skeletal muscle injuries. The aim of current study was to evaluate if local delivery of Fst would attenuate muscle damage and accelerate myofiber regeneration following various forms of muscle injury. Fst was injected delivered into gastrocnemius daily. Muscle mass, fiber necrosis, and fiber regeneration were assessed following injury 1, 3, and 7 days. qRT-PCR analysis were conducted to evaluate the genes associated with myogenesis, inflammation (Myf5 and Myogenin) and contractile elements (desmin and Î±-actin). Following the various forms of chemical injury, muscle wet weight was elevated at each day that is representative of the degenerative and regenerative phases. Local administration of Fst treatment didnâ€™t change the muscle wet weight at day1, increased it at day 3 and day 7 after treatment. Inflammation, fibrosis and muscle function have also been assessed in each model, delineating a role of Follistatin in its ability to augment muscle repair and function. In this comprehensive analysis Fst in muscle injury, Fst was shown to improve skeletal muscle recovery through accelerating muscle regeneration and preventing fibrosis.

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Therapy

149. Myotonic dystrophy: identifying therapeutic candidates

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Myotonic dystrophy type 1 (dystrophia myotonica; DM1) is the most common muscular dystrophy in adults. DM1 is caused by an expanded CTG repeat in the 3â€² untranslated region of the DM protein kinase (DMPK) gene. DMPK transcripts contain an expanded CUG repeat that is toxic to cells through an RNA gain-of-function mechanism. Therapeutic agents designed to reduce toxicity of the CUG repeat RNA hold promise for improving clinical features of DM1. Recent studies have identified antisense oligonucleotides (ASOs) that reverse the DM1 phenotype in a transgenic mouse model by inducing body-wide degradation of the pathogenic RNA. Strategies to identify therapeutic ASOs in pre-clinical models and translate this approach for clinical trials will be discussed. Funding: NINDS (U01NS072323; K08NS064293)

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Therapy

150. Long-Term Rescue of Skeletal and Cardiac Muscles in Dystrophic Mdx Mice by Peptide-Conjugated Morpholino

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Exon skipping has been demonstrated with capability to correct frame-shift and nonsense mutations of Duchenne Muscular Dystrophy (DMD). Peptide-conjugated PMO (PPMO) offers significantly higher efficiency than PMO with the ability to induce near normal levels of dystrophin and restore functions in both skeletal and cardiac muscles. Here, we examined the long-term (one year) efficacy and toxicity of systemic administration of PPMO targeting exon 23 of dystrophin gene in mdx mice. Half life of the dystrophin expression was about 2 months in skeletal muscles but shorter in cardiac muscle. Biweekly injection of 1.5 mg/kg PPMO induced less than 5% dystrophin expression in skeletal muscles with limited muscle function improvements and no dystrophin in the cardiac muscle. Monthly injections of 30mg/kg PPMO restored dystrophin to more than 50% normal levels in all muscles including cardiac muscle with reduced serum CK levels, significantly decreased CNF, no inflammatory cell accumulation and highly uniform muscle fiber population. Significant improvement in skeletal muscle function was clearly observed by grip force measurement. Our result demonstrated for the first time that long-term repeated administration of PPMO could be safely applied to achieve significant therapeutic effect for long-term treatment of DMD with tolerable toxicity.

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Therapy

151. AAV9 mediated FKRP gene therapy restores the expression of functional glycosylation of Î±-DG and improves muscle function

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Mutations in FKRP gene are associated with a wide range of muscular dystrophies from mild limb-girdle muscular dystrophy (LGMD) 2I to severe Walker-Warburg syndrome (WWS) and muscle-eye-brain disease (MEB). The characteristic biochemical feature of these diseases is the hypoglycosylation of α dystroglycan (αDG) in muscles. Currently there is no effective treatment available. Recombinant adeno-associated viral (rAAV) vector-mediated gene replacement represents a promising therapeutic strategy for the treatment of muscular dystrophy. In this study we examined the systemic therapeutic effects of AAV9-CB-FKRP gene therapy in the FKRP mutant mouse model with P448L missense mutation. Our results showed that systemic delivery of AAV9-CB-FKRP vector resulted in expression of FKRP in all skeletal muscles and with the highest level in cardiac muscle and Diaphragm. Consistent with our previous observation, FKRP protein localized at Golgi apparatus in muscle fibers. Expression of FKRP consequently restored functional glycosylation of αDG in all skeletal and cardiac muscles. Significant improvement in muscle pathology and functions was observed during 15 week treatment. This was supported by serum tests showing significant reduction in the level of serum creatine kinase. Our results also showed that levels of FKRP expression was correlated to the levels of functionally glycosylated αDG. AAV9-CB promoter mediated limited FKRP expression in kidney and liver with no detectable toxicity, indicating the applicability of the vector system to FKRP-related muscular dystrophy.

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Cytokinetics	http://www.cytokinetics.com/
Jain Foundation	http://www.jain-foundation.org/
Muscular Dystrophy Association	http://www.mdausa.org/
National Institute of Arthritis and Musculoskeletal and Skin Diseases	http://www.niams.nih.gov/
National Institute of Neurological Disease and Stroke - NINDS	http://www.ninds.nih.gov/
National Institutes of Health - NIH	http://www.nih.gov/
Office of Rare Diseases Research	http://rarediseases.info.nih.gov/Default.aspx
Parent Project Muscular Dystrophy	http://www.parentprojectmd.org/site/PageServer?pagename=nws_index
Pfizer	http://www.pfizer.com/research/
PTC Therapeutics	http://www.ptcbio.com/
Shire Human Genetic Therapies	http://www.shire.com/shireplc/financialreports/ar2005/pages/review/hgt.html

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NIAMS

MDA Muscular Dystrophy Association
Fighting Muscle Disease

National Institutes of Health

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Parent Project Muscular Dystrophy
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