2014

New Directions in Biology and Disease of Skeletal Muscle Conference

Sixth Biennial Conference

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

2014 New Directions in Biology and Disease of Skeletal Muscle Conference

June 29 - July 2, 2014
Chicago, Illinois

Objectives

This is the text box.The New Directions in Biology and Disease of Skeletal Muscle is being held in Chicago June 29 – July 2, 2014. T his meeting brings together scientists working to understand mechanisms and develop new therapies for muscle disease, especially the muscular dystrophies. The "New Directions" meeting differs from other topically related meetings because of its focus on bringing together industry and academic attendees with focus evaluating laboratory based observations and assessing or testing suitability for therapy in the preclinical and clinical setting. This meeting was developed in response the to MD Care Act and the recognition that devising and testing therapy for rare neuromuscular disorders requires organization and coordinated efforts among all stakeholders. In addition to the focus on identifying and testing therapeutic pathways, the New Directions meeting places a high emphasis on inclusion of trainees and young investigators, as it is recognized that the challenges of these medical problems will require a diverse and prolonged effort to realize cures for these devastating disorders.

Objective 1: The presentation and sharing of unpublished data. This meeting emphasizes the presentation of unpublished work. Early access to information allows for new collaborations to form to move scientific discovery forward faster into translation.

Objective 2: Promotion of collaboration between industry and academic investigators. As targets are increasingly moving towards development, preclinical and clinical testing, the interaction and partnership between industry and academia is increasingly important. The first session of this meeting is designed to promote industry and advocacy group participation.

Objective 3: Clinical trial planning and outcome. We will devote a specific session to outcomes and endpoints for clinical trials for neuromuscular disease especially the muscular dystrophies and hope to contribute to improved consensus and understanding of appropriate expectations for clinical trials.

Objective 4: Identify both common and unique targets for each muscle disease. This meeting provides a format where multiple different mechanisms of muscle disease are covered providing a backdrop to identify common elements that can be manipulated therapeutically.

Objective 5: Provide trainees and young investigators a forum in which to present data and to encourage trainees to remain studying neuromuscular disease. Trainees are expected to present posters, and senior and junior investigators are engaged by evaluating these presentations.

Conference Organizers

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

Program Committee

James Dowling, Hospital for Sick Children
Renzhi Han, Loyola University Medical Center
Jan Lammerding, Cornell University
Kenneboyina Nagaraju, Children's National Health System
Isabelle Richard, Généthon, France
Charlotte Sumner, Johns Hopkins School of Medicine
Silvère van der Maarel, Leiden University Medical Center

Coordinator

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

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KEYNOTE SPEAKER

Fred Turek, PhD

Northwestern University

Genetics of circadian rhythms and sleep: Modern life battles ancient drives (and muscle function)

Sunday, June 29, 2014

5:00 PM **Opening Remarks and Introduction to Keynote Speaker**
Elizabeth McNally, MD, PhD
H. Lee Sweeney, PhD

5:10 PM **Keynote Speaker**
Fred Turek, Ph.D.

7:00 PM **Reception**

Fred Turek, PhD
Charles & Emma Morrison Professor of Biology
Director, Center for Sleep & Circadian Biology
PhD, Stanford

Research in the Turek laboratory is focused on the study of sleep and circadian rhythms, with special interest in identifying genes that regulate sleep and circadian rhythms. In addition to our work on rodents, we have established extensive collaborations with clinical researchers. Studies in humans are aimed at shifting the human clock in an attempt to alleviate mental and physical problems that are associated with disorders in circadian time-keeping, particularly in the elderly and in shift-workers.

Mice don't stay up late to party or watch a movie. The nocturnal creatures stick to their naturally evolved body clocks: they get shut-eye during the day and feed and play at night. Exhibiting similar clock function to humans at a molecular level, mice have allowed Fred W. Turek, PhD, Charles E. and Emma H. Morrison Professor of Biology in the Weinberg College of Arts and Sciences, and of neurology at the Feinberg School and CSCB director, to separate behavior from biology in the study of the circadian clock system.

"Humans are the only species that disobey their biological clocks on a regular basis," says Dr. Turek, who was a member of the pioneering Northwestern research team that identified the first clock gene in mammals. "However, we can turn our mice into shift workers, so to speak, by changing their light-dark cycles so we can better understand the biological processes of circadian disruption."

Forced to work the "graveyard" shift, the mice in his studies have mirrored ailments often seen in humans with similarly misaligned biological rhythms. Late night and early morning workers, for example, often fight the battle of the bulge. Dr. Turek and his colleagues found the same to be true of animals that ate when normally they would be sleeping. The midnight munchers packed on as much as 48 percent more weight over their baseline than mice fed the same food at natural waking hours. Female shift workers such as nurses and flight attendants with constant jet lag have been shown to experience fertility and menstrual problems. The Turek research group discovered that mice with severe circadian disruption had significantly poorer pregnancy outcomes.

In addition, we are using both pharmacological and non-pharmacological approaches to determine if we can reverse the effects of aging on the circadian clock system in both rodents and humans. Our sleep, circadian and metabolic studies are focused on how disruption in these interactions can lead to obesity, diabetes and CVD.

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Sunday, June 29, 2014

11:00 AM - 5:00 PM **REGISTRATION**

Industry Workshop: Therapeutics in the Clinic for Muscular Dystrophy

WELCOME AND MEETING ANNOUNCEMENTS
Elizabeth McNally, MD, PhD and **Lee Sweeney, PhD**

1:00 - 1:30 PM **HALO THERAPEUTICS**
Diana Escolar, Chief Medical Officer
Preliminary Saftey and Novel Biomarker data from a Phase 1b/2a Clinical Program of HT-100 in Duchenne Muscular Dystrophy

1:30 - 2:00 PM **SUMMIT CORPORATION plc**
Jon Tinsley, CSO
Development of small molecule Utrophin transcription modulators for DMD

2:00 - 3:00 PM **CATABASIS PHARMACEUTICALS, INC.**
Michael Jirousek, Founder and CSO
Blocking NFκB signaling in muscular dystrophy

3:00 - 3:30 PM **PFIZER, INC.**
Carl Morris, Sr. Director Muscle Biology & Protein Therapeutics
Rare Diseases Research Unit
Anti-myostatin therapy in muscular dystrophy

3:30 - 4:00 PM **PTC THERAPEUTICS**
Stuart Peltz, CEO
The road to an approved drug for DMD

4:00 - 4:15 PM **Theo Smart** *and* **Lee Sweeney**
Facilitating Future Clinical Trials in DMD
Drafting a Policy Guidance for the US Food and Drug Administration (FDA)

4:00 - 5:00 PM BREAK

5:00 - 6:00 PM **KEYNOTE SPEAKER**
Fred Turek, Northwestern University
Genetics of circadian rhythms and sleep: Modern life battles ancient drives (and muscle function)

6:00 - 7:00 PM BREAK

7:00 - 9:00 PM **WELCOME RECEPTION**

2014 New Directions in Biology and Disease of Skeletal Muscle Conference

June 29 - July 2, 2014 Chicago, Illinois

Monday, June 30, 2014

Session I Clinical Trial Update/Planning

Chair: Elizabeth McNally University of Chicago

8:00 - 9:20 AM

Alessandra Ferlini, Ferrara
Biomarkers in dystrophin restoration therapy

Thomas Voit, Institute de Myologie
Quantifiable measures of force and function in DMD

Charles Thornton, Rochester University
Evaluation of response to anti-CTG expansion treatment in DM1

Kathy Swoboda, Utah
Clinical outcome measures for SMA

9:20 - 10:00 AM BREAK

Session II SMA Animal Models, Preclinical

Chair: Charlotte Sumner, John Hopkins University

10:00 - 11:20 AM

Livio Pellizzoni, Columbia University
Dysregulationof RNA processing in spinal muscular atrophy

Adrian Krainer, Cold Spring Harbor
Antisense splicing modulcation for therapy and modeling of SMA

Charlotte Sumner, Johns Hopkins University
Animal models of SMA

Chien-Ping Ko, University of Southern California
Disruption for NMJ and muscle in SMA pathogenesis and effects of novel therapeutics

New and Notable

Chair: Lee Sweeney, University of Pennsylvania

11:20 AM - 12: 20 PM

Jocelyn Laporte*, IGBMC
Reducing dynamin 2 rescues myotubular myopathy in mice

Joel McDade*, University of Michigan
Rapid recruitment of sarcolemma-derived dysferlin is critical for membrane repair in skeletal muscle

Alexis Demonbreun*, University of Chicago
Annexin A6 modifies muscular dystrophy by mediating sarcolemmal repair

12:20 - 2:00 PM **LUNCH BREAK** (on your own)

2:00 - 4:30 PM **POSTER SESSION I** (Even-numbered posters)

Session III Congenital Muscular Dystrophies

Chair: James Dowling, Michigan/Toronto

4:30 - 6:10 PM

Carsten Bönnemann, NIH
Collagen VI and beyond: disorders of the myomatrix

Markus Ruegg, University of Basel
Therapy development for MDC1A

James Dowling, Hospital for Sick Children, Toronto, Ontario
Mutations in LMOD3 cause severe nemaline myopathy

Tobias Willer, The University of Iowa
Dystroglycanopathy Muscular Dystrophies

Rhonda Bassel Duby, UTSW
Discovery of genes involved in myopathy

** Presenter selected from abstract submissions*

6:10 PM **DINNER** (on your own)

6:15 - 8:30 PM **BREAKOUT SESSION – Manton Center for Orphan Disease Research & Joshua Frase Foundation**

Titin-related disorders *(Invitation Only)*

2014 New Directions in Biology and Disease of Skeletal Muscle Conference

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Tuesday, July 1, 2014

Session IV Inflammation in Muscle Injury and Disease

Chair: Renzhi Han, Loyola

8:00 - 10:00 AM

Fayyaz Sutterwala, Iowa
NLRP3 inflammasome in cell necrosis-induced sterile inflammatory response

James G. Tidball, UCLA
Myeloid cells in muscular dystrophy

Renzhi Han, Loyola University Medical Center
Innate immune system in muscular dystrophy

Eric P Hoffman, Children's National Medical Center
*Modified steroids for DM**D***

Catherine Moorwood*, University of Pennsylvania
Caspase-12 ablation preserves muscle function in the mdx mouse

Davy Vanhoutte*, CCHMC
Thrombospondin-4 functions in endoplasmic reticulum (ER) stress-based adaptation that protects skeletal muscle from disease

10:00 - 10:30 AM BREAK

Session V Nuclear Membrane Mechanisms of Muscle Disease

Chair: Jan Lammerding, Cornell University

10:30 - 11:50 AM

Jan Lammerding, Cornell University
Abnormal nuclear mechanics and mechanotransduction in laminopathies

Gisele Bonne, Insitute of Myology, Paris, France
New insights in parthophysiological mechanisms of laminopathies

Howard Worman, Columbia University
Signaling defects in laminopathies

Lori Wallrath, University of Iowa
Pathological changes in muscle gene expression caused by mutant lamins

11:50 - 2:00 PM LUNCH BREAK (on your own)

2:00 - 4:30 PM **POSTER SESSION II** (odd-numbered posters)

Session VI Genetics and Epigenetics of FSHD

Chair: Silvere van der Maarel, LUMC

4:30 - 6:10 PM

Silvere van der Maarel, LUMC
Genetics and Epigenetics of FSHD

Peter Jones, University of Massachusetts
Epigenetic variability and gene expression in facioscapulohumeral muscular dystrophy

Scott Harper, The Ohio State University
Mouse models for FSHD and preclinical testing

Rabi Tawil, University of Rochester
Clinical and molecular biomarkers, trial readiness in FSHD

Jeff Miller*, Boston University
FSHD pathogenesis: Expression of DUX4-FL inhibits protein turnover and induces nuclear aggregation of TDP-43

7:00 PM **BANQUET DINNER – THE SIGNATURE ROOM AT THE 95TH** John Hancock Building

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Wednesday, July 2, 2014

Session VII Limb Girdle Muscular Dystrophies

Chair: Isabelle Richard, Généthon, France

8:00 - 10:00 AM

Isabelle Richard, Généthon, France
Therapeutical strategies in limb-girdle muscular dystrophies

Ralph Knöll, Imperial College, London
DNAJB6 In LGMD1D

Bjarne Udd, Finland
Innate immunity system in muscular dystrophy

Sandra Cooper, Australia
Caplain: an overdrive switch for dysferlin membrane repair?

Louis Kunkel, Boston
Zebrafish models of muscular dystrophy

Jaiswal Jyoti*, Children's National Medical Center
Cellular mechanism for poor myofiber repair in LGMD2B and identification of a new drug target

10:00 - 10:30 AM BREAK

Session VIII Gene Correction and Other Therapy

Chair: Kenneboyina Nagaraju, CNMC

10:30 AM - 12:30 PM

Francesco Muntoni, UCL
Exon skipping for DMD

Luis Garcia, CNRS
Approaches for exon skipping

Kanneboyina Nagaraju, CNMC
Early inflammatory pathways in dystrophy

Rachelle Crosbie-Watson*, UCLA
Only mega-complexes of utrophin and integrin can functionally compensate for muscular dystrophy

Michael Lawlor*, Medical College Wisconsin
Treatment with ActRIIB-mFc improves lifespan, behavior and pathology in the Acta1 H40Y murine model of nemaline myopathy

Jennifer Strande, Medical College Wisconsin
Using IPSCs with dystrophin gene mutations to compare oxidative response between cardiac and skeletal muscle

12:30 PM **MEETING ADJOURNS**

2014 New Directions in Biology and Disease of Skeletal Muscle Conference

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MAJOR FUNDING SUPPORT

National Institute of Neurological Disorders and Stroke

Parent Project Muscular Dystrophy

LEADING THE FIGHT TO END DUCHENNE

Office of Rare Diseases Research

National Institutes of Health

National Institutes of Health

NIAMS

2014 New Directions in Biology and Disease of Skeletal Muscle Conference

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ADDITIONAL FUNDING SUPPORT

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ORCHESTRATING A CURE

LGMD2B DYSFERLINOPATHY MIYOSHI

HALO THERAPEUTICS

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ORCHESTRATING A CURE

LGMD2B DYSFERLINOPATHY MIYOSHI

Is this year's Proud Sponsor
of the
2014 New Directions in Biology and Disease of Skeltal Muscle Banquet Dinner

7:00 pm on Tuesday, July 1, 2014

Enjoy Fine Dining at The Signature Room at the 95th
in the John Hancock Building

Business Casual; no shorts, gym shoes, or athletic wear. Jacket and tie are optional

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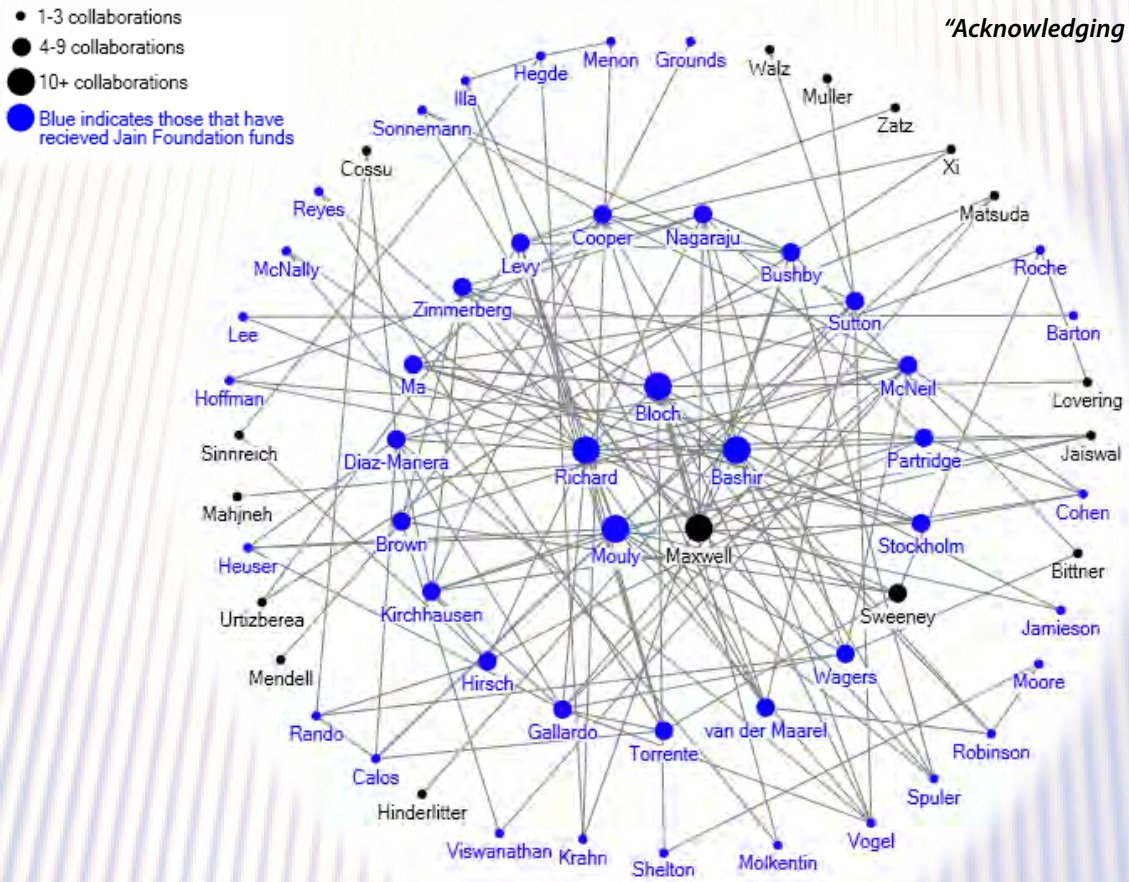
LGMD2B DYSFERLINOPATHY MIYOSHI

JAIN FOUNDATION is this year's Proud Sponsor of the 2014 New Directions in Biology and Disease of Skeletal Muscle Conference Banquet Dinner



Enjoy Fine Dining at The Signature Room at the 95th in the John Hancock Building

7:00 P.M. on Tuesday, July 1, 2014

Business Casual Dress: no shorts, gym shoes or athletic wear. Jacket and tie are optional.

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Disease Biology

1. **Acuña, Maria** - Catholic University of Chile
   ACE2 is augmented in dystrophic skeletal muscle and plays a role in decreasing associated fibrosis
2. **Agrawal, Pankaj** - Boston Children's Hospital and Harvard Medical School
   SPEG interacts with myotubularin (MTM1) and its deficiency causes centronuclear myopathy with dilated cardiomyopathy
3. **Albrecht, Douglas** - Jain Foundation
   MRI/MRS analysis of lower limb and hip muscles of dysferlin deficient mice reveal a specific defect in proximal leg muscles
4. **Alexander, Matthew** - Boston Children's Hospital/Harvard Medical School
   MicroRNA-486 overexpression ameliorates the disease pathology of dystrophin-deficient muscle
5. **Banks, Glen** - University of Washington
   Myofiber branching rather than myofiber hyperplasia contributes to muscle hypertrophy in mdx mice
6. **Batra, Abhinandan** - University of Florida
   Physiological changes in skeletal muscle of leg based on MRI and strength measures in children with Duchenne muscular dystrophy and Collagen VI myopathy
7. **Beedle, Aaron** - University of Georgia
   Magnetic Resonance versus Histological Imaging of Dystrophic and Acutely Injured Skeletal Muscle: Evaluation of MRI as an Outcome Measure for Therapeutic Trials in Muscular Dystrophy Mice
8. **Beedle, Aaron** - University of Georgia
   Rabbit monoclonal antibodies for the detection of alpha-dystroglycan core protein
9. **Belanto, Joseph** - University of Minnesota
   Microtubule Binding Distinguishes Dystrophin from Utrophin
10. **Chadwick, Jessica** - The Ohio State University
    Elucidating the Role of Mineralocorticoid Receptors in Skeletal Muscle as a Potential Therapeutic Target for Duchenne Muscular Dystrophy
11. **Chamberlain, Christopher** - University of Minnesota
    A comparative urine metabolomics approach to identify novel pathogenic mechanisms and therapeutic outcome measures for Duchenne muscular dystrophy
12. **Clarke, Nigel** - Children's Hospital at Westmead
    Mutations in LMOD3 cause severe nemaline myopathy by disrupting thin filament organisation in skeletal muscle
13. **Cohen, Tatiana** - Kennedy Krieger Institute
    Smad7 regulates skeletal muscle growth, fiber type specificity and regeneration
14. **Crosbie-Watson, Rachelle** - University of California, Los Angeles
    Only mega-complexes of utrophin and integrin can functionally compensate for muscular dystrophy
15. **de Greef, Jessica** - University of Iowa
    Dysferlin deficiency exacerbates skeletal muscle pathology in a mouse model for the collagen VI-related myopathies
16. **Demirtas, Berjan** - Istanbul University
    Giant fibres in fetal ovine muscle
17. **Demonbreun, Alexis** - The University of Chicago
    Annexin A6 modifies muscular dystrophy by mediating sarcolemmal repair
18. **Dowling, James** - Hospital for Sick Children *(No Poster)*
    Lysosomes and muscular dystrophy
19. **Evesson, Frances** - Boston Children's Hospital/Harvard Medical School
    Membrane repair in living muscle cells

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20. **Fitzgerald, Jamie** - Henry Ford Hospital
    A Mutation In The Alpha 6 Chain Of Type VI Collagen Disrupts E-C Coupling And Leads To A Lethal Muscular Dystrophy
21. **Gidaro, Teresa** - Institut de Myologie
    Cellular effectors of the exacerbated fibrosis in affected muscles of oculopharyngeal muscular dystrophy
23. **Gidaro, Teresa** - Institut de Myologie
    Excessive daytime sleepiness in Myotonic Dystrophy type 1 patients: an unsolved clinical target requiring translational research approach
24. **Girgenrath, Mahasweta** - Boston University
    Losartan treatment rescues the dysregulation of Matricellular proteins in LAMA2 deficient congenital muscular dystrophy
25. **Gokhin, David** - The Scripps Research Institute
    Control of thin filament lengths by sarcomeric tropomodulin isoforms: insights from mouse models
26. **González, David** - Pontificia Universidad Católica de Chile
    CTGF is up-regulated in transgenic SOD1G93A and denervated muscles
27. **Grounds, Miranda** - The University of Western Australia
    Lipid accumulation in dysferlin-deficient muscles
28. **Guadagnin, Eleonora** - NIH
    Investigating the molecular mechanisms underlying the progression of collagen VI-related muscular dystrophies
29. **Heydemann, Ahlke** - UIC, CCVR
    The MRL mouse strain is naturally resistant to high fat diet-induced hyperglycemia
30. **Heydemann, Ahlke** - UIC and CCVR
    Investigating the regenerative potential of the MRL mouse strain's mitochondrial genome.
31. **Himeda, Charis** - University of Massachusetts
    Myogenic enhancers regulate expression of the Facioscapulohumeral muscular dystrophy associated DUX4 gene
32. **Jaiswal, Jyoti** - Children's National Medical Center
    Cellular mechanism for poor myofiber repair in LGMD2B and identification of a new drug target
33. **Jones, Peter** - University of Massachusetts Medical School
    Epigenetic variability and gene expression in facioscapulohumeral muscular dystrophy
34. **Kerr, Jaclyn** - University of Maryland School of Medicine
    Nrf2 activity in dystrophy: a disease modifier and therapeutic target
35. **Kodippili, Kasun** - University of Missouri - Columbia
    Nitric oxide (no) dependent attenuation of sympathetic vasoconstriction is impaired in contracting skeletal muscles of duchenne muscular dystrophy dogs
36. **Kramer, Henning** - GlaxoSmithKline
    Age Modulates the Rate and Extent of Skeletal Muscle Recovery Following Eccentric Muscle Damage in Mdx Mice
37. **Lamar, Kay-Marie** - University of Chicago
    Amelioration of muscular dystrophy by transgenic expression of Ltbp4
38. **Lek, Angela** - Boston Children's Hospital
    A transgenic zebrafish model for FSHD
39. **Lek, Monkol** - Massachusetts General Hospital
    Systematic identification of causal mutations in severe muscle diseases using various genomic technologies
40. **Loehr, James** - Baylor College of Medicine
    Genetic inhibition of Nox2 activity improves contractile function and pathology in young and mature mdx muscle.
41. **Londhe, Priya** - The Ohio State University
    Microvesicles containing miRNAs promote muscle cell death in cancer cachexia via TLR7

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42. **Marcelino Nunes, Andreia** - University of Nevada, Reno
    Dynamic laminin isoform switching and basement membrane buildup during epaxial skeletal muscle development in the mouse embryo
43. **Marshall, Jamie** - Boston Children's Hospital
    Transgenic overexpression of human heme oxygenase 1 does not ameliorate muscular dystrophy in the mdx5CV mice
44. **McDade, Joel** - University of Michigan
    Rapid Recruitment of Sarcolemma-derived Dysferlin is Critical for Membrane Repair in Skeletal Muscle
45. **Metairon, Sabrina** - IPEN/CNEN-SP
    DETERMINATION OF INORGANIC ELEMENTS IN BIOLOGICAL TISSUES OF DYSTROPHIC MICE STRAINS USING NAA
46. **Mian, Luhe** - National Institutes of Health (NIH)
    Cardiac impairment in GNE myopathy
47. **Miller, Daniel** - University of Washington
    A DUX4 Target Reporter Demonstrates Momentary but Lasting Effects of DUX4 Expression in Human FSHD Myoblasts and Provides a useful Drug-screening Platform for FSHD Therapies.
48. **Miller, Gaynor** - University of Sheffield
    MacLow, a new inducible model for investigating the role of CD68 positive macrophages in muscular dystrophy.
49. **Miller, Jeffrey** - Boston University School of Medicine
    FSHD pathogenesis: Expression of DUX4-FL inhibits protein turnover and induces nuclear aggregation of TDP-43.
50. **Montanaro, Federica** - The Research Institute at Nationwide Children Hospital
    Dystrophin stabilizes the membrane repair complex in the diaphragm
51. **Moorwood, Catherine** - University of Pennsylvania School of Dental Medicine
    Caspase-12 Ablation Preserves Muscle Function in the mdx Mouse
52. **Partridge, Terence** - Children's National Medical Center
    Human muscle regeneration and aging: a new model in immuno-deficient mice.
53. **Partridge, Terence** - Children's National Medical Center
    Muscles of the mdx mouse are simultaneously dystrophic, hypertrophic, hyperplastic and atrophic
54. **Partridge, Terence** - Children's National Medical Center
    Nuclear membrane protein lamin A/C does not play by the rules.
55. **Parvatiyar, Michelle** - UCLA
    Beta-adrenergic stimulation reveals a role for sarcopsan in cardiac hypertrophy
57. **Patrinostro, Xiaobai** - University of Minnesota
    Quantitative Comparison of Utrophin in Dystrophin-Deficient Mouse and Human Skeletal Muscle
58. **PELLIZZONI, LIVIO** - Columbia University *(No Poster)*
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59. **Piers, Adam** - Murdoch Children's Research Institute
    The role of the extracellular matrix protease ADAMTS5 in normal and dystrophic skeletal muscle
60. **Pratt, Stephen** - University of Maryland School of Medicine
    Recovery of altered neuromuscular junction morphology and muscle function in mdx mice after injury
61. **Rosenberg, Paul** - Duke University School of Medicine
    STIM1 in skeletal muscle development, contractility and regeneration
62. **Ruegg, Markus** - University of Basel *(No Poster)*
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    The sepn1 knockout zebrafish: a novel genetic model of SEPN1-related myopathy
65. **Smith, Lucas** - University of Pennsylvania
    Collagen content does not alter the passive mechanical properties of fibrotic skeletal muscle in mdx mice
66. **Smith, Lucas** - University of Pennsylvania
    MMP13 is required for efficient skeletal muscle regeneration in mouse model of muscle injury
67. **Smith, Sarah** - University of Toronto
    Exploring secondary molecular pathways as therapeutic targets for merosin-deficient congenital muscular dystrophy
68. **Swanson, Lindsay** - Boston Children's Hospital & Harvard Medical School
    Clinical Features of X-linked Myotubular Myopathy Carriers
69. **Tjondrokoesoemo, Andoria** - Cincinnati Children Hospital Medical Center
    Genetic overexpression of Serpina3n rescues muscular dystrophy
70. **Udd, Bjarne** - University of Helsinki *(No Poster)*
    DNAJB6 in LGMD1D
71. **van der Maarel, Silvère** - Leiden University Medical Center *(No Poster)*
    Genetics and Epigenetics of FSHD
72. **Vanhoutte, Davy** - Cincinnati Children's Hospital Medical Center
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74. **Veeranki, Sudhakar** - University of Louisville
    Hyperhomocysteinemia (HHcy) inhibits satellite cell regenerative capacity through p38 MAPK signaling
75. **Vieira, Natassia** - Boston Children's Hospital
    A novel genetic modifier of Duchenne muscular dystrophy phenotype
76. **Villalta, S. Armando** - University of California San Francisco
    Regulatory T cells suppress muscle inflammation and injury in the mdx mouse model of Duchenne muscular dystrophy.
77. **Vo, Andy** - University of Chicago
    QTL analysis using the superhealing MRL strain reveals distinct genetic regions that modify muscular dystrophy and cardiomyopathy in mice
78. **Vohra, Ravneet** - University of Florida
    Age dependent changes in cardiac and skeletal muscle T2 in gamma-sarcoglycan deficient mice
79. **Wada, Eiji** - The University of Tokyo
    Dietary phosphorus overload exacerbates dystrophic phenotypes of the dystrophin-deficient mdx mouse
80. **Wallrath, Lori** - University of Iowa
    Pathological changes in muscle gene expression caused by mutant lamins
81. **Windish, Hillarie** - Jain Foundation
    Dysferlin deficiency in mdx mice does not exacerbate the behavioral phenotype
82. **Zou, Yaqun –** NINDS, NIH
    Inhibiting Myostatin signaling to Treat Collagen VI Deficient Myopathy
83. **Zou, Yaqun –** NINDS, NIH
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105. Spinazzola, Janelle - University of Pennsylvania
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106. Afzal, Muhammad - Medical College of Wisconsin
Using iPCs with Dystrophin Gene Mutations to Compare Differences in Oxidative Stress Responses between Cardiac and Skeletal Myocytes

107. Anson, Blake - Cellular Dynamics International
Human induced pluripotent stem cell derived skeletal myoblasts: A new human-based platform for basic research and drug discovery utilizing skeletal myotubes from healthy and clinical populations.

108. Lu, Aiping - University of Pittsburgh
Progression of muscular dystrophy in Dystrophin/utrophin-/- mice is associated with rapid muscle progenitor cell exhaustion

109. Myburgh, Kathy - Stellenbosch University
Skeletal muscle crush injury and neuromuscular juntion regeneration: painting a picture in immunofluorescence

Therapy

110. Arthur, Peter - UWA
l-2-oxothiazolidine-4-carboxylate (OTC) protects dystrophic muscles from damage in mdx mice

111. Banks, Glen - University of Washington
Muscle structure influences utrophin expression in mdx mice

112. Chamberlain, Joel - University of Washington
Systemic AAV-mediated RNA interference improves RNA toxicity in a mouse model of myotonic dystrophy

113. Chrzanowski, Stephen - University of Florida
Intramuscular Heterogeneity in Duchenne Muscular Dystrophy Determined By Magnetic Resonance Imaging

114. Doering, Jonathan - Virginia Tech
Dietary sphingolipids modulate muscle power and inflammation in mdx mice.

115. Dominov, Janice - University of Massachusetts Medical School
A Deep Intronic Mutation in Dysferlin Leads to Expression of a Pseudoexon in Miyoshi Myopathy Patient Cells: Use of Antisense Oligonucleotides to Promote Normal DYSF Expression

116. Duan, Dongsheng - University of Missouri
Safe and bodywide muscle gene transfer in young adult Duchenne muscular dystrophy dogs

117. Escolar, Diana - DART Therapeutics, Inc
Preliminary Clinical Safety and Biological Activity Data of HT-100, a Novel Therapy for Duchenne Muscular Dystrophy

118. Friesen, Westley - PTC Therapeutics
Next Generation Readthrough Drugs to Treat Nonsense Mediated Muscular Dystrophies

119. Gao, Quan - University of Chicago
Determining the minimal functional unit of gamma sarcoglycan

120. Garcia, Luis - University of Versailles
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121. Gibbs, Elizabeth - UCLA
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Therapy

ABSTRACTS

Disease Biology

1. ACE2 is augmented in dystrophic skeletal muscle and plays a role in decreasing associated fibrosis

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Duchenne muscular dystrophy (DMD) is characterized by absence of dystrophin, muscle wasting and fibrosis. We demonstrated that infusion of angiotensin-1-7 (Ang-1-7) normalized skeletal muscle architecture, decreased fibrosis, and improved muscle function in mdx mice. In this study, we investigated the presence, activity, and localization of ACE2, the enzyme responsible for Ang-1-7 production, in wild type (wt), mdx and in a model of induced chronic damage in wt mice. All dystrophic muscles studied showed higher ACE2 activity than wt. Immunostaining studies suggest that ACE2 was localized at the sarcolemma and, to a lesser extent, associated to interstitial cells. Similar results were observed in the model of induced chronic damage. Interestingly, Ang-1-7 infusion resulted in a reduction of ACE2 in dystrophic muscle. Furthermore, we evaluated the effect of ACE2 overexpression in mdx tibialis anterior (TA) muscle and showed that expression of ACE2 reduced the fibrosis associated with TA dystrophic muscles. Indeed, fewer inflammatory cells infiltrating the mdx muscle were observed. This evidence supports ACE2 as an important therapeutic target to improve dystrophic muscle phenotype. (Fondecyt1110426, Fondecyt3140323, CAREPFB12/2007)

2. SPEG interacts with myotubularin (MTM1) and its deficiency causes centronuclear myopathy with dilated cardiomyopathy

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Centronuclear myopathies (CNM) are characterized by muscle weakness and increased central nucleation within myofibers. X-linked myotubular myopathy, the most common severe form of CNM is caused by mutations affecting myotubularin (MTM1), a lipid phosphatase. To increase our understanding of myotubularin function, we conducted a yeast-2-hybrid screen to identify MTM1-interacting proteins. The protein product (SPEG) of the striated preferentially expressed gene (SPEG) was identified as an MTM1-interacting protein, confirmed by immunoprecipitation and immunofluorescence studies. SPEG knock out was previously associated with severe dilated cardiomyopathy in a mouse model. Using whole exome sequencing, we identified SPEG mutations in three CNM patients, including two with dilated cardiomyopathy, from three families. SPEG protein was markedly reduced in their muscles evaluated by immunofluorescence and western blots. Examination of muscle samples from Speg-knock out mice showed increased frequency of central nuclei. SPEG mutation leads to a phenotype with similarities to myotubular myopathy, likely because of its interaction with myotubularin, with dilated cardiomyopathy due to its strong expression and critical role in cardiac muscle.

Disease Biology

3. MRI/MRS analysis of lower limb and hip muscles of dysferlin deficient mice reveal a specific defect in proximal leg muscles

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of age using magnetic resonance imaging (MRI) to compare the volumes of 2 lower limb muscles (TA, gastrocnemius) and two hip muscles (psoas, gluteus maximus) with C57BL/6 control animals. The composition (fat/water content) and metabolite levels of the gastroc and gluteus was also monitored by magnetic resonance spectroscopy (MRS). Beginning at 9 months of age, Bla/J mice showed gross abnormalities in their hip muscles. Bla/J gluteus maximus and psoas muscles were reduced in volume, and areas of tissue atrophy and fat infiltration were detected. These changes correlate with increased lipid signals in 1H-MRS in gluteus maximus. In contrast, there was no decline in volume or change in composition of the lower limb muscles of the BlaJ animals. We conclude that hip muscles of dysferlin deficient mice are dramatically more affected than the commonly studied lower limb muscles, and that MRI and MRS can be used to reliably monitor disease progression and assess the potential of possible therapies in these muscles. Using this platform, we have begun evaluating the efficacy of rationally selected FDA-approved drugs.

4. MicroRNA-486 overexpression ameliorates the disease pathology of dystrophin-deficient muscle

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Duchenne muscular dystrophy (DMD) is caused by mutations in the dystrophin gene that result in the dysregulation of many signaling pathways that interact directly or indirectly with the dystrophin protein. Previously, we identified miR-486 as being strongly reduced in its expression levels in the dystrophin-deficient mouse and muscle biopsies of human DMD patients. Here we report that transgenic overexpression of the muscle-enriched microRNA, miR-486, in mdx5cv (dystrophin-mutant) mice resulted in improved serum biochemistry, reduced apoptosis, increased myofiber size, and improved muscle physiological force output. Using a bioinformatic approach, we identified DOCK3, dedicator-of-cytokinesis-3, as being a direct downstream target of miR-486 in skeletal muscle. Manipulation of DOCK3 expression in myoblast cell culture had strong effects on normal and DMD myoblast apoptosis, and on the RAC1/RHOA signaling pathway. Together, these studies demonstrate that stable overexpression of miR-486 ameliorates many of the signs of the disease pathology of dystrophin-deficient muscle.

Disease Biology

5. Myofiber branching rather than myofiber hyperplasia contributes to muscle hypertrophy in mdx mice

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Banks, Glen,

Muscle hypertrophy in the mdx mouse model of Duchenne muscular dystrophy (DMD) can partially compensate for the loss of dystrophin by maintaining peak force production. Histopathology examination of the hypertrophic muscles suggests the hypertrophy primarily results from the addition of myofibers, and is accompanied by motor axon branching. However, it is unclear whether an increased number of innervated myofibers (myofiber hyperplasia) contribute to muscle hypertrophy in the mdx mice. Here we directly compared myofiber number, myofiber branching, and innervation, from 3-20 weeks of age. We found that a 28% increase in the number of fibers in transverse sections of muscle correlated with a 33% increase in myofiber branching. The dystrophic pathology coincided with profound changes to innervation of the muscles that included temporary denervation of necrotic fibers, fragmentation of synapses, and ultraterminal axon sprouting. However, the total number of neuromuscular junctions remained constant. Thus, muscle hypertrophy in mdx mice results from myofiber branching rather than myofiber hyperplasia.

6. Physiological changes in skeletal muscle of leg based on MRI and strength measures in children with Duchenne muscular dystrophy and Collagen VI myopathy

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Duchenne muscular dystrophy (DMD) and Collagen VI myopathy (COL6) cause progressive muscle weakness and disability. Both diseases are caused by mutation of genes encoding for muscle structural protein and are characterized by infiltration of fatty and fibrotic tissue. Magnetic resonance imaging (MRI) and spectroscopy (MRS) show promise as sensitive, noninvasive biomarkers in DMD, but have not been extensively examined in COL6. We examined several MRI biomarkers and specific torque, in age-matched groups of DMD and COL6 subjects and unaffected controls

MRI and MRS were acquired in lower extremity muscles and were analyzed for cross sectional area (CSA), contractile area, T2 (MRS), and fat fraction (FF). Strength, normalized to contractile area, was measured for the Knee Extensors and Plantar flexors.

Fat fraction and T2 were elevated in both COL6 and DMD compared to controls. Thigh CSA and contractile area were lowest in COL6. In the lower leg, DMD had highest CSA and contractile area. Strength measures were most impaired in DMD.

COL6 subjects had widespread atrophy, contributing to weakness, while DMD subjects had force deficits even in muscle groups with increased contractile area. (Funding: NIH)

Disease Biology

7. Magnetic Resonance versus Histological Imaging of Dystrophic and Acutely Injured Skeletal Muscle: Evaluation of MRI as an Outcome Measure for Therapeutic Trials in Muscular Dystrophy Mice

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Abnormal glycosylation of alpha-dystroglycan impairs the structural link between the extracellular matrix and the cytoskeleton causing dystroglycanopathy-type muscular dystrophy. There are no therapies for dystroglycanopathies and variability in phenotypes complicates the assessment of therapeutic effect. Our aim is to assess the correlation between magnetic resonance (MR) imaging and histological measurements to determine the reliability and suitability of MR imaging for future therapeutic trials in animal models. Dystroglycanopathy knockout (KO) and littermate (LC) mice were scanned by T2 relaxation MRI for comparison with histological assays. T2 relaxation MRI of 7.5 week mice clearly correlated with disease. However, this measurement was not a reliable indicator of mouse genotype or disease severity in 13 week mice. We hypothesized that the degenerative/regenerative status in younger mice has a more distinct MRI profile, so we performed T2 relaxation MRI in wild-type mice with toxin-induced regeneration. Our data suggest that MRI may be useful for prescreening mice for drug trials, but that optimization is required for reliable detection of therapeutic outcomes. Funded by the University of Georgia College of Pharmacy.

8. Rabbit monoclonal antibodies for the detection of alpha-dystroglycan core protein

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Alpha-dystroglycan provides an essential structural link between the extracellular matrix and the dystrophin-glycoprotein complex. Binding of alpha-dystroglycan to extracellular matrix proteins requires a rare O-mannose glycan structure on its mucin domain. Functionally glycosylated alpha-dystroglycan can be well detected by mouse monoclonal antibodies IIH6 and VIA4-1. However, in dystroglycanopathy-type muscular dystrophies and other disease states, the O-mannose glycan is abnormal and detection by glycosylation-specific antibodies is lost. Here, we report the generation of rabbit monoclonal antibodies targeting the alpha-dystroglycan C-terminus. These antibodies successfully detect both normal and abnormally glycosylated alpha-dystroglycan from dystroglycanopathy mice using Western blot and immunofluorescence techniques. Furthermore, we demonstrate that these monoclonal antibodies are useful in other species and other types of muscular dystrophy. Overall, these rabbit monoclonal antibodies against the alpha-dystroglycan core protein offer a new tool for muscular dystrophy research. Funded by the University of Georgia College of Pharmacy.

Disease Biology

9. Microtubule Binding Distinguishes Dystrophin from Utrophin

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Dystrophin and utrophin are similar proteins that link actin filaments with a complex of sarcolemmal glycoproteins, yet localize to distinct subcellular domains. Transgenic utrophin overexpression improves many aspects of the mdx mouse and utrophin upregulation is under investigation as a potential therapy for DMD. Here, we compared the microtubule (MT) binding activity of dystrophin with utrophin and analyzed several transgenic mouse models to identify mdx phenotypes that remain despite transgenic utrophin overexpression. Our in vitro analyses revealed that dystrophin binds MTs with high affinity and pauses MT polymerization whereas utrophin does neither. Transgenic utrophin overexpression does not rescue MT lattice disorganization, loss of torque production after eccentric contractions, or physical inactivity after mild exercise. Finally, our data suggest that physical inactivity correlates with loss of sarcolemmal nNOS and loss of torque production correlates with MT lattice disorganization. Supported by the NIAMS grant RO1 AR042423 to JME. JJB was supported by the NIH Training Program in Muscle Research (AR007612) and a U of M Doctoral Dissertation Fellowship. Muscle functional assessments were supported by NIH P30 AR057220.

10. Elucidating the Role of Mineralocorticoid Receptors in Skeletal Muscle as a Potential Therapeutic Target for Duchenne Muscular Dystrophy

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Our lab identified a potential new treatment for Duchenne Muscular Dystrophy using the mineralocorticoid receptor (MR) antagonist spironolactone and ACE inhibitor lisinopril. Drug studies using dystrophic Het (utrn+/-;mdx) mice showed a dramatic improvement in both respiratory and limb muscle force and a reduction of ongoing muscle damage, in addition to preventing cardiomyopathy. We show MR is present in both skeletal muscle tissue and myogenic cultures, supporting a direct affect by MR antagonists on skeletal muscle. Global analysis of gene expression between treated and untreated dystrophic mice identified potential molecular targets to unravel the drugs' mode of action. Preliminary microarray data comparing quadriceps muscle from lisinopril and spironolactone treated het mice to untreated controls revealed changes in the expression of several gene targets with known roles in striated muscle, which are currently being confirmed using RT-PCR, immunofluorescence and western blot analysis. Myogenic cultures treated with MR agonists and antagonists are being used to test whether these potential downstream targets are affected in a cell autologous manner in skeletal muscle and represent bona fide MR gene targets. NIH funded.

Disease Biology

11. A comparative urine metabolomics approach to identify novel pathogenic mechanisms and therapeutic outcome measures for Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a lethal muscle wasting disorder caused by mutations in dystrophin. Because muscle is highly metabolic, we hypothesized that dystrophin loss would dramatically alter the metabolomic profile of DMD patients and mouse models. Urine is ideal for metabolomic analysis and biomarker discovery in DMD because it: 1) contains a diverse array of measurable metabolites; 2) is non-invasive; and 3) provides a general overview of metabolic status, but does not require certification for collection. Using a semi-untargeted metabolomic screen, we have shown that Krebs cycle metabolites are reduced in mdx urine. We have developed a multiple reaction monitoring mass spectrometric assay for urinary succinate and validated these results in an independent cohort of mdx mice. Also, we have evidence that expression of either recombinant dystrophin or utrophin increases succinate in mdx urine to normal levels. Finally, we have shown that succinate is reduced in a small cohort of DMD patients and appears to correlate with disease severity. These data provide evidence of a non-invasive biomarker with potential for use in future DMD clinical trials. CMC is funded by the NIH Training Program in Muscle Research (AR007612).

12. Mutations in LMOD3 cause severe nemaline myopathy by disrupting thin filament organisation in skeletal muscle

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Nemaline myopathy (NM) is a disorder of the skeletal muscle thin filament characterised by muscle dysfunction and electron-dense protein accumulations (nemaline bodies). Pathogenic mutations have been described in nine genes to date, but the genetic basis remains unknown in many cases. We used whole exome sequencing in two families with NM and gene sequencing in over 540 additional NM probands to identify and characterise a new genetic cause of NM. We identified homozygous or compound heterozygous variants in LMOD3, which encodes leiomodin 3 (Lmod3) in 21 patients from 14 families. Affected individuals had severe generalised weakness and hypotonia from birth, and most affected individuals died in the neonatal period. We demonstrated that Lmod3 is expressed from early muscle differentiation, localises to thin filaments with enrichment at the pointed ends, and has strong actin nucleating activity. Loss of Lmod3 in patient muscle results in shortening and disorganisation of thin filaments. Knockdown of lmod3 in the zebrafish replicates this phenotype. These findings define a new genetic subtype of NM and demonstrate an essential, previously unrecognised role for Lmod3 in the regulation of sarcomeric thin filaments in skeletal muscle.

Disease Biology

13. Smad7 regulates skeletal muscle growth, fiber type specificity and regeneration

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Identification of myostatin as a negative regulator of muscle growth, 17 years ago, has led to the development of multiple novel drugs for muscle degenerative and wasting diseases. The approaches aimed at inhibiting myostatin currently involve extracellular targets such as inhibiting the activation of myostatin or preventing the binding of myostatin to its receptor. Another potential mechanism of blocking myostatin is to target its natural intracellular antagonist, Smad7. To study how Smad7 regulates muscle, we examined muscle growth and regeneration in Smad7-/- mice. We report that loss of Smad7 results in deficits in muscle growth. Smad7-/- muscle is weaker than WT in in vitro force generation and in vivo gait analysis, due to hypoplasia, hypotrophy and fiber type switching from the glycolytic towards the oxidative state. Smad7-/- muscle also shows delays in regeneration in response to injury and defective proliferation and differentiation in vitro. We propose that the defects observed occur due to compensatory upregulation of the Smad2/3 pathway in the absence of Smad7. Thus, modulation of Smad7 may represent another approach to inhibiting myostatin for the treatment of muscle disorders.

14. Only mega-complexes of utrophin and integrin can functionally compensate for muscular dystrophy

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It is well-established that overexpression of utrophin and alpha7beta1 integrin ameliorate disease in the mdx mouse model of Duchenne muscular dystrophy (DMD), but the compensatory mechanism has remained elusive. We first establish that alpha7beta1 integrin and the utrophin-associated glycoprotein complex directly interact to form a large mega-macromolecular complex. Furthermore, we demonstrate that networks of sarcospan, a tetraspanin-like protein, nucleate alpha7beta1 integrin and the utrophin-associated glycoprotein complex into high-density, buoyant mega-complexes that activate Akt growth signals. Using double and triple knockout mice, we show that sarcospan-mediated amelioration of muscular dystrophy in DMD mice is dependent on the incorporation of utrophin and alpha7beta1 integrin into mega-complexes, even when they are individually expressed at therapeutic levels. (NIH NIAMS; MDA)

Disease Biology

15. Dysferlin deficiency exacerbates skeletal muscle pathology in a mouse model for the collagen VI-related myopathies

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Mutations in COL6A1, COL6A2, and COL6A3 underlie the collagen VI-related myopathies that are characterized by muscle weakness, joint hypermobility and contractures. Collagen VI is believed to function in anchoring the basement membrane to the underlying connective tissue, but the exact mechanism leading to muscle degeneration is still unknown. By mimicking a human missense COL6A2 mutation, we generated a mouse model for the collagen VI-related myopathies. Col6a2 mice present with few necrotic and centrally nucleated fibers at young age; they develop significant skeletal muscle pathology only at 9 months of age. We hypothesized that disrupting the muscle membrane repair mechanism in Col6a2 mice would exacerbate their skeletal muscle pathology. We therefore generated Col6a2 mice that lack dysferlin, a protein involved in the membrane repair of damaged sarcolemma. Col6a2/dysferlin-deficient mice develop more severe skeletal muscle pathology than Col6a2 or dysferlin-deficient mice. Most notably these mice present with a significant percentage of centrally nucleated fibers, even at young age. Our data show that the Col6a2/dysferlin-deficient mice provide a useful model to further characterize the role of collagen VI in skeletal muscle.

16. Giant fibres in fetal ovine muscle

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The study examined the occurrence of giant fibres in fetal muscle from undernourished and control ewes. Giant fibres were rounded in shape and larger than adjacent normal fibres. They showed generally strong ATPase –alkaline positive reaction. Based on ATPase staining the giant fibres are classified as type II or fast fibres. In EM, Transverse sections showed that regular hexagonal array of myofilaments was absent in the giant fibres. Longitudinal sections showed that sarcomer length was shorter than normal fibres. Giant fibres are clearly hypercontracted fibres. Undernutrition did not seem to have significant effect on the occurance of giant fibre. In the two restricted animals the high incidence of giant fibres was observed. It is suggested that giant fibres, since they are present prenatally, results from defects in the developing muscle fibres leading to structural and metabolic anomalies within the fibres. These defects such as inadequte amount of sarcoplasmic reticulum, leaky membranosus of sarcoplasmic reticulum or deficiency in the production of ATP by the mitochondria prevents relaxation of muscle and causes hypercontraction.

Disease Biology

17. Annexin A6 modifies muscular dystrophy by mediating sarcolemmal repair

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Many monogenic disorders, including the muscular dystrophies, display phenotypic variability despite the same disease-causing mutation. To identify genetic modifiers of muscular dystrophy, we used quantitative trait locus mapping and whole genome sequencing in a mouse model. This approach uncovered a modifier locus on chromosome 11 associated with increased membrane damage. Whole genome and RNA sequencing identified Anxa6, encoding annexin A6, as a modifier gene. ANXA6 belongs to family of calcium dependent phospholipid binding proteins that facilitate membrane repair, accumulating at sites of damage. A synonymous variant in exon 11 creates a cryptic splice donor resulting in a truncated annexin A6 protein, ANXA6N32. Live cell imaging of annexin A6 in myofibers showed that annexin A6 orchestrates a repair zone and cap at the site of damage. In contrast, expression of even low levels of ANXA6N32 disrupted the annexin A6-rich cap and repair zone and interferes with normal annexin A6 translocation resulting in visible leak of intracellular contents. In vivo, the presence of ANXA6N32 was associated with reduced membrane-associated annexin A6 in damaged myofibers. These data highlight Anxa6 as a modifier of muscle injury. (NIH)

18. Lysosomes and muscular dystrophy *(No Poster)*

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Muscular dystrophy is fundamentally considered a disease of impaired membrane integrity. Gene mutations that cause muscular dystrophy are most commonly identified in gene products that govern cell-extracellular matrix adhesion. However, it has become increasingly recognized that abnormalities in intracellular pathways, particularly those that govern protein and organelle degradation such as autophagy, the ubiquitin-proteosome system, and apoptosis, are present and meaningfully contribute to muscular dystrophy disease pathogenesis. My laboratory is generally interested in the role of the endolysosome in muscle homeostasis and disease. We (and others) have recently discovered that lysosomal dysfunction may serve not only as a "secondary" abnormality in muscular dystrophy, but also as a primary driver of disease. In this talk, I will present recent and evolving data that explores the interrelationship between lysosomal function/dysfunction and muscular dystrophy.

Disease Biology

19. Membrane repair in living muscle cells

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We aim to better understand the mechanism by which skeletal muscle repairs plasma membrane damage. We are interested in the contribution of the protein dysferlin to repair, since absence of dysferlin leads to a form of late onset muscular dystrophy. We present our optimized damage assay to study repair in live cells, with the goal to track the precise damage activated intracellular movements of dysferlin.

Method: We are using a 532nm pulsed ablation laser to make small focused cellular lesions, coupled with real time spinning disk confocal fluorescence microscopy to study repair in differentiated human and mouse myotubes.
Results: As in previous studies, we demonstrate that calcium is required for efficient membrane repair of damaged myotubes. Interestingly, we show that while dysferlin deficient myotubes have an impaired repair outcome compared to wild type myotubes, they still successfully repair ~30% of damage events.

Conclusions: Our assay provides a controlled, adaptable system to model muscle repair in live cells, achieving high temporal and spatial resolution imaging at sites of membrane damage. We are now using this protocol to study the exact role dysferlin plays in muscle membrane repair.
(Jain Foundation)

20. A Mutation In The Alpha 6 Chain Of Type VI Collagen Disrupts E-C Coupling And Leads To A Lethal Muscular Dystrophy

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Mutations in the COL6A1, COL6A2 and COL6A3 genes result CMD. Three new collagen VI genes have been described recently; COL6A4, COL6A5 and COL6A6. Since the a6(VI) chain, encoded by COL6A6, is present human skeletal muscle we conducted a mutation screen CMD patients previously excluded for mutations in COL6A1, -2 and -3. A COL6A6 mutation was detected in a neo-natal lethal CMD. The patient was severely hypotonic and exhibited no spontaneous respiration. The variant is a homozygous 5-bp deletion leading to a premature stop codon. Immunostaining demonstrates that a6(VI) protein is virtually absent in the patient muscle while levels of a1(VI), a2(VI) and a3(VI) are unchanged. The a6(VI) chain is a component of the transverse-tubule network in close association with the t-tubule marker, ryanodine receptor (RyR), a component of excitation-contraction (E-C) coupling. In the patient, but not control sample, RyR and DHPR, are almost completely absent. We conclude that in the absence of a6(VI), the key E-C coupling molecules are absent explaining why the child was severely hypotonic. We speculate that a6(VI) is critical for the formation, stability or maintenance of the t-tubule system in skeletal muscle. Supported by the MDA and NIH.

Disease Biology

21. Cellular effectors of the exacerbated fibrosis in affected muscles of oculopharyngeal muscular dystrophy

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OPMD is an autosomal dominant inherited, late onset degenerative muscle disorder. Affected pharyngeal muscles show atypical features such as an exacerbated fibrosis together with fiber atrophy and an increased number of satellite cells. This peculiar phenotype lead us to evaluate the behaviour of primary cell cultures from pharyngeal muscles of control and OPMD patients compared to control limb muscles, in order to decipher the respective role of myoblasts and fibroblasts. We followed in vitro the proliferation rate of both CD56+ and CD56- cell fractions. In vivo studies were also performed using a xeno-transplantation model in immunodeficient mice to follow their respective behaviour during muscle regeneration. Our study demonstrated that the CD56-cell fraction of pharyngeal muscle is different from that of limb muscle with a strikingly high proliferative capacity, inducing a rapid loss of myogenicity of primary cultures. The full characterisation of the role of these cellular effectors will help understanding the molecular and cellular mechanisms underlying the relationship between fibrosis and muscle atrophy in this muscle, essential step to develop new therapeutic strategies.

23. Excessive daytime sleepiness in Myotonic Dystrophy type 1 patients: an unsolved clinical target requiring translational research approach

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Excessive Daytime Sleepiness (EDS) is recognised as the most frequent non-muscular symptom in patients affected by Myotonic Dystrophy type 1 (DM1). EDS is characterized by persistent sleepiness, unaffected by naps interfering with work, domestic responsibilities, social life and ability to undertake daily tasks or activities. These features are experienced by both patients and parents as a prominent and debilitating element of DM1 life. The mechanisms leading to EDS in DM1 are largely unknown. Given their role in awakening state and the effect of methylphenydate in daytime sleepiness scale score in DM1 patients, monoaminergig neuron constitutes a likely key element in DM1 sleepiness.We thus compared spontaneous discharge, excitability and drug responsiveness of monoaminergic neurons from DMSXL mice, the mouse model of DM1, and WT animals, using both extracellular and patch clamp experiments in acute brain slices. We demonstrated that monomaminergic neurons present a lower spontaneous discharge and a lower response to apamine, wich suggest SK channel primary or secondary dysfunction or sub expression.This pilot project will lead to further experiments to elucidate the potential sub-expression or dysfunction of SK channel in DMSXL.

Disease Biology

24. Losartan treatment rescues the dysregulation of Matricellular proteins in LAMA2 deficient congenital muscular dystrophy

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LAMA2-deficient muscular dystrophy is a devastating disease caused by defects in the extracellular matrix (ECM) protein Laminin-a2. Absence of this protein results in structural instability and severe signaling dysregulation across the sarcolemma in a wide range of signaling pathways. The patients with MDC1A present with muscle weakness and hypotonia either at or soon after birth and often die from respiratory complications or failure to thrive. There are no effective therapies available to patients afflicted with MDC1A. Although this disease rooted in the ECM, it remains to be elucidated about role of dysregulation of matricellular proteins in driving the disease progression. Using the LAMA2DyW mouse model, we show that Laminin-deficiency leads to a marked dysregulation of Integrins. Specifically, Integrins alpha5 and alphaV, receptors for various matricellular proteins typically associated with fibroblasts and myofibroblasts, are greatly overexpressed in Laminin-deficient muscles. Further, we found that treatment with Losartan, an Angiotensin type 1 receptor inhibitor that leads to marked rescues of muscle fibrosis seen in these mice, causes a complete recovery in the expression of these molecules in DyW muscle. MDA &CureCMD

25. Control of thin filament lengths by sarcomeric tropomodulin isoforms: insights from mouse models

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The uniform lengths of skeletal muscle thin filaments are controlled by actin subunit exchange at pointed ends (P-ends). Each P-end is capped by two sarcomeric tropomodulin (Tmod) molecules, with Tmod1 and Tmod4 isoforms present in a 1:9 ratio. In Tmod1-null sarcomeres, P-ends are capped by Tmod3 (normally a sarcoplasmic reticulum-associated Tmod isoform) and Tmod4, preserving normal thin filament lengths. In Tmod4-null sarcomeres, P-ends are capped solely by Tmod1, also preserving normal lengths. Thus, Tmod1-null and Tmod4-null muscles deploy compensatory mechanisms to specify correct thin filament lengths. To discern the effect of sarcomeric Tmod depletion from mature muscle, we measured thin filament lengths in the mdx and mdx/mTR mouse models of Duchenne muscular dystrophy, in which Tmods are calpain-proteolyzed in a muscle use-dependent manner. Proteolysis of Tmod1 and/or Tmod4 in mdx and mdx/mTR muscles results in 10-12% increases in thin filament lengths, due to addition of actin subunits onto P-ends. These results show that Tmod1 and Tmod4 are individually dispensable for initial specification of muscle-specific thin filament lengths, but important for maintenance of correct filament lengths in mature myofibrils.

Disease Biology

26. CTGF is up-regulated in transgenic SOD1G93A and denervated muscles

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by degeneration of upper and lower motor neurons (MNs), causing muscle atrophy followed by progressive paralysis. Neurodegeneration and denervation observed in SOD1-mutated ALS cases focused the effort to understand how a mutant SOD1 act within MNs. Little is known about the role of mutated-SOD1 in skeletal muscle. Connective Tissue Growth Factor (CTGF) is a pro-fibrotic factor up-regulated in varied fibrotic diseases such as Duchenne muscular dystrophy (DMD). The reduction of CTGF slows down dystrophy progression in mdx mice, a murine model of DMD. However, there is no evidence showing the role of CTGF in ALS muscles.

We found an increase of CTGF together with ECM molecules induction of CTGF in both denervated and ALS post-symptomatic mice. Moreover, we observed an increase of nuclear pSmad3 in ALS muscle.

Our data suggest that CTGF could be involved in the progression of ALS disease, possibly contributing in muscle weakness and fibrosis. Current experiments are evaluating the effect of CTGF reduction in tgSOD1G93A and denervated muscles to elucidate its role in muscle wasting and fibrosis.

CARE PFB12/2007, FONDECYT 3140357, Conicyt Fellow

27. Lipid accumulation in dysferlin-deficient muscles

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Dysferlin is a membrane associated protein involved in protein vesicle trafficking and fusion. Gene defects result in the human dysferlinopathies Limb Girdle Muscular Dystrophy type 2B and Miyoshi Myopathy, and the A/J and BLAJ dysferlin-deficient mouse models of these diseases where the psoas and quadriceps are the most severely affected muscles. The precise basis for this adult-onset muscle disease pathology is not understood. We have shown that lipid replaces myofibres in dysferlin-deficient mice and humans. Oil red O showed many lipid droplets within the psoas and quadriceps of dysferlin-deficient A/Jdys-/- mice aged 8 and 12 months, and lipid droplets were also conspicuous within human myofibers from patients with dysferlinopathy. Electron microscopy of A/Jdys-/- psoas confirmed lipid droplets within myofibers and the presence of many adipocytes. The relationship between the conspicuous intermyofibrillar lipid and progressive adipocyte replacement in dysferlin-deficient muscles is not clear. These observations present a new focus for investigating the mechanisms that result in the progressive decline in function of dysferlin-deficient muscles.

Disease Biology

28. Investigating the molecular mechanisms underlying the progression of collagen VI-related muscular dystrophies

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Ullrich congenital muscular dystrophy (UCMD) presents at birth or in infancy with progressive muscle weakness, joint contractures and respiratory failure, and is caused by mutations in the three genes coding for collagen VI. Dysfunction of the collagen VI microfibrills in the extracellular matrix perturbs the normal extracellular matrix/muscle interface, leading to yet incompletely characterized downstream effects. Review of muscle biopsies from UCMD patients show that the histological appearance changes with disease progression, suggesting changing disease-driving pathways throughout the progression of the disease. In preparation for a molecular investigation of such pathways, we analyzed 39 UCMD patient muscle biopsies with an automated image analysis algorithm using the degree of atrophy, fibrosis, and regeneration as criteria to group the biopsies. We performed microarray-based expression profiling on the grouped patient biopsies and age-matched controls. Expression profiling results correlated with staging obtained from the automated image analysis algorithm. Further studies will establish the involvement of specific molecules in different stages of UCMD to identify suitable targets for therapeutic intervention. (NIH)

29. The MRL mouse strain is naturally resistant to high fat diet-induced hyperglycemia

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The MRL mouse strain is known for its ability to regenerate ear wounds, cardiac injury and skeletal muscle disease. As part of previously identified metabolic differences, wild type MRL skeletal muscles have higher levels of active, phosphorylated AMPK than wild type B6 mice. Due to their naturally and chronically increased levels of skeletal muscle pAMPK we hypothesized that the MRL mice would be resistant to high fat diet (HFD)-induced metabolic changes. After 12 weeks of CD or HFD the mice were analyzed. Despite gaining weight and increasing their fat deposits the MRL mice were resistant to all indicators of HFD-induced metabolic alterations assayed. Only the HFD B6 mice displayed hyperglycemia, resistance to insulin, hyperinsulinemia and hypersensitivity to glucose challenge. The HFD MRL skeletal muscles contained heightened levels of pAMPK. The MRL mouse strain is the first naturally occurring mouse strain that we are aware of that is resistant to HFD-induced metabolic changes. We hypothesize that these metabolic differences and plasticity provide the basis for the MRL mouse strain's super healing characteristics. Furthermore, the increased pAMPK suggests a proximal mechanism for these beneficial metabolic differences. NIH

Disease Biology

30. Investigating the regenerative potential of the MRL mouse strain's mitochondrial genome.

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We have been pursuing the remarkable ability of the MRL mouse strain to resist muscular dystrophy-mediated fibrosis. For a number of scientific reasons we have focused upon the genetic polymorphisms found in the MRL mitochondria and their effects upon the metabolic and regeneration characteristics of this mouse strain. We initially identified that maternal inheritance from the MRL line correlated with reduced muscular dystrophy-mediated fibrosis. We have also identified a number of metabolic differences in the wildtype MRL mice including increased pAMPK, mitochondria and glycolysis and decreased reactive oxygen species. We have generated 4 backcrossed breeding lines which separate the MRL nuclear and mitochondrial genomes using the very fibrotic DBA2/J as the opposing strain (nucMRLmitoM, nucMmitoDBA2/J, nucDmitoM and nucDmitoD). We are now poised to investigate the regenerative molecular signaling cascade initiated by the MRL mitochondrial genome. In the sixth generation of the four backcrossed lines we are analyzing the; ear wound healing ability, muscular dystrophy-mediated fibrosis, and metabolic characterizations. Ultimately we will be able to quantify the mitochondrial genome-mediated healing and regenerative abilities. NIH

31. Myogenic enhancers regulate expression of the Facioscapulohumeral muscular dystrophy associated DUX4 gene

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Facioscapulohumeral muscular dystrophy (FSHD) is linked to epigenetic dysregulation of the chromosome 4q35 D4Z4 macrosatellite. However, this does not account for the tissue specificity of FSHD pathology, which requires stable expression of DUX4-fl mRNA from the D4Z4 array in skeletal muscle. Here we describe the identification of two enhancers, DUX4 Myogenic Enhancer 1 (DME1) and 2 (DME2) which activate DUX4-fl expression in skeletal myocytes, but not fibroblasts. Chromatin immunoprecipitation analysis revealed that DME1 and 2 are enriched for enhancer marks and RNA Polymerase II occupancy, and Chromosome conformation capture analysis confirmed association of DME1 and 2 with the DUX4 promoter in vivo. Nucleosome occupancy and methylome sequencing analysis indicated that in most FSHD myocytes, both enhancers are associated with nucleosomes, but have hypomethylated DNA, consistent with a poised transcriptional state and with the observed DUX4 expression in rare myonuclei. Our data support a model in which DME1 and 2 are in a permissive state in most cells; in rare FSHD myocytes, these enhancers associate with the epigenetically de-repressed DUX4 promoter to drive the pathological misexpression of DUX4-fl. (NIH/AFM/MDA)

Disease Biology

32. Cellular mechanism for poor myofiber repair in LGMD2B and identification of a new drug target

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Mutations in Dysferlin gene leads to Limb Girdle Muscular Dystrophy 2B (LGMD2B). Dysferlin deficient muscle fibers are characterized by poor ability to repair from injury, but the underlying cellular mechanism for this deficit has remained elusive. Using patient and dysferlinopathic mouse cells and myofibers we have investigated the cellular basis of this deficit. We find that dysferlinopathic muscle cells undergo normal differentiation, but exhibit compromised repair ability even prior to differentiation. This repair deficit correlates with reduced number of lysosomes present at the sarcolemma. This reduction in sarcolemmal pool of lysosomes delays and reduces the extent of injury-triggered lysosomal exocytosis. This decrease in lysosome exocytosis causes reduced secretion of lysosomal enzyme acid sphingomyelinase (ASM). Acute treatment of dysferlinopathic muscle cells and myofibers with sphingomyelinase restores their ability to repair from focal injury. Thus similar to other ferlins dysferlin also regulates vesicle fusion and regulates sarcolemmal repair through this mechanism. These results point to ASM (a drug already in human clinical trials) as a potential therapy for dysferlinopathy.

33. Epigenetic variability and gene expression in facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is associated with epigenetic changes at the 4q35 D4Z4 macrosatellite leading to the misexpression of the DUX4 gene encoded within the repeat array. We have investigated if subtle differences in the epigenetic status of the 4q35 region could account for the variation in DUX4 expression among affected FSHD1 subjects and potentially relate to the lack of apparent muscle weakness in asymptomatic FSHD1 subjects. Family cohorts of myogenic cells were analyzed for DNA methylation status and tested for their sensitivity to the small molecules that can alter the chromatin state. We find that myogenic cells from FSHD1 affected subjects are epigenetically poised to express DUX4 compared to unaffected subjects; however, FSHD1 subjects show individual differences in their capacity to express DUX4 in response to altered epigenetic status. The epigenetic analysis of asymptomatic FSHD1 subjects closely resembled that of unaffected controls. Therefore, the epigenetic status of the 4q35 region more closely correlates with clinical FSHD than the genetic status, and individual epigenetic differences may impact variability in progression of disease pathology, age of onset and disease severity.

Disease Biology

34. Nrf2 activity in dystrophy: a disease modifier and therapeutic target

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We recently discovered X-ROS, a pathway by which stretch activates NADPH Oxidase 2 (NoX2) dependent ROS generation. In dystrophic (mdx) skeletal muscle, excessive X-ROS contributes to muscle injury and the dystrophic process. X-ROS pathway transcripts in both human DMD and mdx revealed a widespread alteration in redox homeostasis genes. The transcription factor Nrf2 is a master regulator Nrf2 of redox homeostasis. We hypothesized that reductions in Nrf2 enhance X-ROS signaling by reducing redox buffering capacity. We genetically silenced Nrf2 in a model of dysferlinopathy. With Nrf2 silencing, adult A/J mice had significant muscle-specific functional deficits and altered histopathology and X-ROS was dramatically enhanced. To directly address the effect of Nrf2 activity, we treated adult mdx mice with sulforaphane, a Nrf2 activator. With treatment, the sentinel antioxidant enzymes were significantly increased in the muscle and the enhanced X-ROS was ameliorated. We conclude that Nrf2 activity is a disease modifier in muscular dystrophy and that strategies that target its activation may be effective in slowing disease progression.

35. Nitric oxide (no) dependent attenuation of sympathetic vasoconstriction is impaired in contracting skeletal muscles of duchenne muscular dystrophy dogs

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The absence of sub-sarcolemmal protein dystrophin in Duchenne muscular dystrophy (DMD) leads to the delocalization of neuronal nitric oxide synthase (nNOS) from the cytosolic surface of the sarcolemma. Sarcolemmal nNOS is essential for sympatholysis, a process of attenuating alpha-adrenergic receptor mediated vasoconstriction in contracting muscles that results in a large increase of blood flow to the working muscles. nNOS delocalization compromises sympatholysis and hence causes functional ischemia and muscle damage in DMD patients. Here we hypothesize that the loss of sarcolemmal nNOS also disrupts protective sympatholysis in dystrophic dog muscle. To test this hypothesis, we compared hemodynamic responses to intra-arterial forelimb infusion of norepinephrine (an alpha-adrenergic vasoconstrictor) in normal and dystrophic dogs both at rest and during forelimb contraction. To verify our observations, we also applied nNOS inhibitors. We found that alpha-adrenergic vasoconstriction was attenuated in normal contracting dog muscle. This sympatholytic effect was also partially blocked by nNOS inhibitors. Importantly, consistent with what has been shown in human patients, sympatholysis was impaired in dystrophic dogs.

Disease Biology

36. Age Modulates the Rate and Extent of Skeletal Muscle Recovery Following Eccentric Muscle Damage in Mdx Mice

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The dystrophin-deficient mdx mouse is commonly used as a preclinical model of Duchenne muscular dystrophy, however the severity of pathology in unstressed mdx mice is comparatively mild, making assessment of potential therapies difficult. We hypothesized that an aged background might enhance the translational relevance of the mdx mouse model by evincing a more "human-like" environment, particularly when combined with a physiologic stressor. Therefore, we challenged young (8-wks) and older (8-months) mdx mice to an exercise bout consisting of 60 eccentric repetitions of the limb plantarflexor muscles and longitudinally evaluated the kinetics of force recovery. Both mdx age groups exhibited a massive and reproducible force deficit at day 1 post-damage (only 20% of starting force). Young mdx animals recovered rapidly to 90+% of initial undamaged force in as little as 13 days before plateauing at a new inferior steady-state level of limb function. In contrast, older mdx mice took nearly 4 times longer to recover (40-60 days), and the extent of recovery was also poorer (<80% of initial undamaged force). Taken together, we have identified an age-dependent delay in the rate and quality of skeletal muscle repair in mdx mice.

37. Amelioration of muscular dystrophy by transgenic expression of Ltbp4

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Genetic modifiers can strongly influence outcome in single gene disorders and in complex genetic traits. Our laboratory used a genomewide scan in mice to identify Ltbp4 as a modifier of muscular dystrophy (Heydemann et al., J Clin Invest., 2009). We subsequently found LTBP4 to be a modifier of human muscular dystrophy (Flanigan et al., Ann Neurol., 2013). LTBP4, latent TGF-beta binding protein, regulates the extracellular bioavailability of TGF-beta by matrix sequestration.

To further address how LTBP4 exerts its effects as a modifier, we hypothesized that LTBP4 may regulate myostatin, a negative regulator of muscle differentiation and growth. We performed in vitro co-immunoprecipitation experiments and found that LTBP4 complexes with myostatin.

To better understand the mechanism in which LTBP4 functions in muscular dystrophy pathogenesis, we generated transgenic mice expressing Ltbp4 in skeletal muscle. Transgenic positive mice have increased forelimb strength and have larger muscles compared to wild type mice. Transgenic mice were then bred with mdx mice, a mouse model of muscular dystrophy. Preliminary data suggests that the transgene improves membrane leak, fibrosis, and grip strength in mice with muscular dystrophy. (NIH)

Disease Biology

38. A transgenic zebrafish model for FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common forms of muscular dystrophy, characterized by asymmetric weakness of the facial, shoulder and upper arm muscles, accompanied by hearing loss and retinal vasculopathy. Although the causative gene for FSHD remains controversial, the primate specific retrogene, DUX4, is a leading candidate. There is currently no mammalian model that recapitulates the human FSHD pathology. Previously, our lab has shown that misexpression of very low levels of human DUX4 in zebrafish development recapitulates the phenotypes seen in FSHD patients. It was demonstrated that microinjection of human DUX4 mRNA into zebrafish eggs caused asymmetric abnormalities of the eyes and ears, and disorganization of fin and trunk muscles. Using a tamoxifen-controlled CreERt2-loxP system, we have now generated a transgenic DUX4 line that successfully reproduces the mosaic, low-level expression of DUX4. We show that activating DUX4 expression during development results in a degenerative muscle phenotype by day 7 post-fertilization. This stable line will enable us to control when, where and how much DUX4 is expressed to best model FSHD pathogenesis in zebrafish, allowing for better functional studies.

39. Systematic identification of causal mutations in severe muscle diseases using various genomic technologies

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Exome sequencing is a powerful and cost-effective approach for the identification of causal mutations in patients suffering from Mendelian diseases. However, exome analysis identifies a causal mutation in only 30-50% of sequenced families, indicating much work remains to be done to integrate various complementary genomic technologies to identify causual muations that may have been missed by exome sequencing.
We describe the development of an integrated pipeline for the identification of causal variants from exome, RNA sequencing and whole genome sequencing data and its application to a cohort of muscle disease patients. Our online application called xBrowse enables the intuitive analysis of family-based exome data, permitting researchers and clinicians to rapidly explore the effects of altering inheritance modes on the identification of potential causal mutations. Through xBrowse, our collaborators have access to gene-based RNA expression data across various human tissues, and a large reference panel of over 50,000 exomes.
We have applied this integrated approach to exome data from over 250 individuals consisting of families and probands affected by a range of neuromuscular diseases.

Disease Biology

40. Genetic inhibition of Nox2 activity improves contractile function and pathology in young and mature mdx muscle.

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Duchenne Muscular Dystrophy (DMD) is an X-linked progressive degenerative disease caused by a mutation in the gene encoding dystrophin. Dystrophic muscle is characterized by increased reactive oxygen species (ROS) production and Ca2+ influx, decreased contractile function and morphological alterations. In mdx mice, a model of DMD, nicotinamide adenine dinucleotide phosphatase (NADPH oxidase or Nox2) ROS production is elevated early in the disease progression, resulting in increased Ca2+ influx and decreased muscle function. We hypothesized that inhibiting Nox2 ROS would protect against the pathophysiological changes associated with dystrophy, which would be maintained as the mice aged. Genetic inhibition of Nox2 activity resulted in decreased ROS production and partial recovery of force in young (5-7 wks) mice. The increased force was maintained with age (15-18 wks) while mdx force declined. We also demonstrate that decreasing Nox2 ROS reduced Ca2+ influx and central nuclei while maintaining CSA and fiber type distribution in young and mature mice. Our data support our hypothesis that Nox2 inhibition improves the pathophysiology of mdx muscle and identify Nox2 as a potential therapeutic target to preserve muscle function in DMD.

41. Microvesicles containing miRNAs promote muscle cell death in cancer cachexia via TLR7

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MicroRNAs (miRNAs) are small, noncoding RNAs that regulate gene expression and, in cancers, are often packaged within secreted microvesicles. The cachexia syndrome is a debilitating state of cancer
that predominantly results from the loss of skeletal muscle mass, which is in part associated with apoptosis. How tumors promote apoptosis in distally located skeletal muscles has not been explored.
Using both tumor cell lines and patient samples, we show that tumor derived microvesicles induce apoptosis of skeletal muscle cells. This proapoptotic activity is mediated by a microRNA cargo, miR-21, which signals through the Toll-like 7 receptor (TLR7) on murine myoblasts to promote cell death. Furthermore, tumor microvesicles and miR-21 require c-Jun N-terminal kinase activity to regulate this apoptotic response. Together, these results describe a unique pathway by which tumor cells promote muscle loss, which might provide a great insight into elucidating the causes and treatment options of cancer cachexia.
This work was supported by National Institutes of Health Grants CA180057 (to Denis Guttridge) and U01 CA152758 (to Carlo Croce).

Disease Biology

42. Dynamic laminin isoform switching and basement membrane buildup during epaxial skeletal muscle development in the mouse embryo

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Skeletal muscle development starts early in embryogenesis, initiating a progressive build-up of muscle mass that proceeds well after birth. Laminins 111/511 are present during early stages of myogenesis, whereas laminins 211/221 are expressed during the later stages of muscle development. In MDC1A, the alpha2 laminin chain is absent and crippling muscle weakness is evident from birth. However it is unclear exactly when MDC1A starts manifesting itself. We undertook a detailed analysis of the expression and deposition of laminin isoforms during epaxial myogenesis in normal mouse embryos. We find that laminin alpha2 deposition correlates with (1) myotome development and (2) the onset of secondary myogenesis, while most of primary myogenesis occurs in the absence of assembled laminins. Surprisingly, 3D reconstruction of laminin immunolabeling at fetal stages reveals a complex pattern of matrix deposition. We are presently using this normal developmental pattern of laminin deposition to compare with the pattern observed in dyW-/- embryos, a mouse model for MDC1A. This comparative analysis will increase our understanding of the onset of muscle disease in MDC1A. PTDC/SAU-BID/120130/2010

43. Transgenic overexpression of human heme oxygenase 1 does not ameliorate muscular dystrophy in the mdx5CV mice

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Duchenne muscular dystrophy (DMD) is a progressive muscle wasting disorder caused by mutations in dystrophin and loss of the predominant adhesion complex at the muscle membrane, the dystrophin-glycoprotein complex (DGC). Upregulation of heme oxygenase 1 (HMOX1) ameliorates muscle pathology and improves lifespan in the zebrafish model of DMD. Using inducible transgenic models, we tested the ability of HMOX1 to ameliorate the mdx5CV murine model of DMD. Mice were crossed to produce dystrophin deficient mice overexpressing inducible HMOX1 under the control of the human skeletal actin (HSA) and ubiquitous (ROSA) promoters. The transgene was activated from 3 to 9 weeks of age with the administration of 2mg/ml doxycycline in the drinking water. Under these conditions, we did not observe a reduction in serum creatine kinase, amelioration of central nucleation, alleviation of exercise-induced fatigue, or improvement in specific force production in mdx5CV mice overexpressing systemic or skeletal muscle specific HMOX1. This work was supported by the Gimbel Foundation to L.M.K. and the Muscular Dystrophy Association (MDA) Development Grant MDA255059 to M.S.A.

Disease Biology

44. Rapid Recruitment of Sarcolemma-derived Dysferlin is Critical for Membrane Repair in Skeletal Muscle

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Dysferlin is hypothesized to facilitate vesicle fusion during sarcolemma repair, but how dysferlin-containing membranes contribute to membrane repair in muscle is unknown. We generated a novel muscle-specific reporter mouse expressing dysferlin-pHluorin GFP (dysf-pHGFP) and used the lumenal/extracellular pH-sensitive GFP to examine the dynamic behavior of dysferlin containing membranes following membrane wounding in live adult muscle fibers. We show that dysferlin is highly enriched in the sarcolemma and t-tubules in resting fibers. Dysf-pHGFP is rapidly recruited to laser-induced sarcolemma wounds (<10s), resulting in the formation of a stable dysferlin-rich domain at the lesion. Photobleaching specific pHGFP populations revealed that the majority of dysferlin recruited to lesions is derived from the adjacent sarcolemma. Disruption of the subcortical actin-cytoskeleton impairs dysferlin recruitment to wounds and also impairs membrane resealing in wild-type fibers. Our data support a novel model where cytoskeleton dependent recruitment of sarcolemma-derived dysferlin generates an “active zone” of high lipid binding capacity at wounds to facilitate muscle membrane repair. (AHA Predoctoral Fellowship 12PRE12050130)

45. Determination of inorganic elements in biological tissues of dystrophic mice strains using naa

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In this work the determination of inorganic elements in biological tissues (whole blood, bones and organs) of dystrophic mice, used as animal model of Duchenne Muscular Dystrophy (DMD), was performed using analytical nuclear technique. We investigated blood of dystrophic mice (spontaneous mutation) such as: A/J, Dmdmdx/J and SJL/J. The aim of this work was to determine reference values of elements of clinical and nutritional relevance (such as, Br, Ca, Cl, K, Mg, Na, S, Fe) in whole blood, tibia, quadriceps and hearts from A/J, SJL/J and Dmdmdx/J, dystrophic mice using Neutron Activation Analysis technique (NAA). To show in more details the alterations that this disease may cause in these biological tissues, correlations matrixes of DMD dystrophic mouse strain were generated and compared with C57BL/6J mouse control group. Furthermore, comparative analysis of blood between C57BL/6J, Dmdmdx/J, A/J and SJL/J species showed a decrease in Ca blood levels emphasizing the need of its evaluation in other animal models with muscular dystrophy. The alteration in some concentration among the elements in the health and diseased status indicates a connection between these elements in whole blood, tibia, quadriceps and heart.

Disease Biology

46. Cardiac impairment in GNE myopathy

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GNE myopathy is an adult-onset myopathy caused by mutations in the GNE gene, encoding a bifunctional enzyme in sialic acid biosynthesis. It is characterized by progressive skeletal muscle weakness and atrophy, with accumulation of rimmed vacuoles. Although GNE myopathy is known to preferential affect skeletal muscles, there had been reports indicating cardiac involvement. Our Natural History Study showed that cardiac involvement in patients may have a higher occurrence than expected.

In this study we established a systematic evaluation of the cardiac involvement in GNE myopathy by analyzing the mouse model Gne-/-hGNED176VTg. Histopathology showed rimmed vacuoles and disorganization of cardiac myofibrils. Lectin staining array corroborated the hyposialylation of O-linked glycoproteins. Echocardiogram revealed a decrease of cardiac function, also confirmed by MRI.

Our findings provide evidence that cardiac muscles are involved in GNE myopathy. We propose that hyposialylation of cardiac muscles can lead to impaired cardiac muscle contractility, and may be improved with sialylation-increasing therapies. It is important for clinicians to be aware of the possible occurrence of cardiac disease in GNE myopathy for proper treatment.

47. A DUX4 Target Reporter Demonstrates Momentary but Lasting Effects of DUX4 Expression in Human FSHD Myoblasts and Provides a useful Drug-screening Platform for FSHD Therapies.

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Facioscapulohumeral Muscular Dystrophy (FSHD) is a dominantly inherited adult-onset myopathy causing irreversible and untreatable asymmetric muscle wasting. FSHD is caused by epigenetic derepression of the D4Z4 macrosatellite array resulting in aberrant expression of DUX4, a transcription factor that is normally repressed. Activation of DUX4 targets results in an apoptotic cascade and subsequent cell death in culture. The expression profile of DUX4 has been difficult to investigate as the protein has not been detected directly in FSHD muscle biopsies and expression levels are very low in cultured cells. We constructed a reporter for DUX4 target activation to examine DUX4's pattern of expression and consequence of exposure in cultured myoblasts. We found that DUX4 expression is momentary in FSHD cultures but downstream effects are lasting. Also, activation of the DUX4 target reporter in live muscle cells was necessary for apoptosis. We have used this system as basis for a high-throughput siRNA screening and drug development platform in order to elucidate the epigenetic and transcriptional networks governing DUX4 production and to identify potential treatment options for FSHD patients. (NIH, Friends of FSH Research)

Disease Biology

48. MacLow, a new inducible model for investigating the role of CD68 positive macrophages in muscular dystrophy.

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Macrophages are versatile cells of the myeloid lineage which play an essential role in the immune response. Proinflammatory macrophages are also prevalent in diseases such as Duchenne muscular dystrophy (DMD) where they are thought to exacerbate muscle damage. Therefore, targeting specific macrophages in DMD patients may reduce muscle damage whilst avoiding some of the negative side effects observed with the use of corticosteroids. Previously, we generated the MacLow mouse model which allows the inducible depletion of CD68 positive (CD68+) macrophages. In this study the MacLow line was crossed with the mdx mouse model of DMD to generate MacLow-MD animals. Macrophage depletion was induced for two weeks and resulted in a 50% reduction in the number of CD68+ cells in the tibialis anterior of six week old MacLow-MD mice ($P<0.05$). Furthermore, the number of regenerating i.e. centrally nucleated, muscle fibres, in treated animals was significantly reduced by ~33% (P value <0.05). The present study describes a new model for studying the role of CD68+ macrophages in muscle disease and highlights the potential of these cells as novel targets for DMD therapy.

49. FSHD pathogenesis: Expression of DUX4-FL inhibits protein turnover and induces nuclear aggregation of TDP-43.

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Expression of the FSHD-associated protein DUX4-FL is known to alter gene expression and to be cytotoxic, but cell responses to DUX4-FL are not fully understood. Here we provide evidence that expression of DUX4-FL in human myotubes inhibits protein turnover and promotes abnormal aggregation of TDP-43, a protein associated with ALS and Inclusion Body Myositis. We found that expression of DUX4-FL from its endogenous promoter in human FSHD myotubes was accompanied both by an increase in ubiquitinated proteins and by aggregation of TDP43 in nuclei. As previously, DUX4-FL expression was detectable by immunostaining only in ~0.1% of differentiated, myosin-expressing cells. Exogenous expression of DUX4-FL, but not DUX4-S, with a BacMam vector similarly caused altered intracellular distribution of ubiquitinated proteins and nuclear aggregation of TDP43, as well as inhibition of protein turnover in a model system. MG132 inhibition of the ubiquitin-proteasome system produced changes similar to DUX4-FL. Our results identify DUX4-FL-induced inhibition of protein turnover and aggregation of TDP-43 as potential pathological mechanisms in FSHD that, unexpectedly, are shared with other neuromuscular diseases. Support by NIH, MDA, AFM.

Disease Biology

50. Dystrophin stabilizes the membrane repair complex in the diaphragm

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In Duchenne muscular dystrophy expression of the dystrophin protein is disrupted in all striated muscles leading to myofiber degeneration. However, some muscles are more severely affected than others, while some appear spared. The reasons behind these differences are currently not understood but may be relevant to treatment development.
In mdx mice, the diaphragm is the most severely affected muscle. Guided by a novel proteomics-based approach we developed to profile the interactome of dystrophin, we discovered that dystrophin interacts with key proteins of the dysferlin membrane repair complex in the diaphragm but not limb muscles. We further show that in the mdx diaphragm loss of dystrophin disrupts the dysferlin complex. Based on these results, we propose a new model where loss of dystrophin in the diaphragm leads to more severe pathology by increasing membrane fragility and concomitantly impairing membrane repair.
Funding: PPMD, AHA, Nationwide Children Hospital, Ohio State University, Muscle Group.

51. Caspase-12 Ablation Preserves Muscle Function in the mdx Mouse

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Duchenne muscular dystrophy (DMD) is a devastating muscle wasting disease caused by mutations in dystrophin. Several consequences of dystrophin deficiency are triggers of endoplasmic reticulum (ER) stress, e.g. loss of calcium homeostasis, hypoxia and oxidative stress. During ER stress, misfolded proteins accumulate and the unfolded protein response (UPR) is triggered, leading to adaptation or apoptosis. We hypothesised that ER stress is heightened in dystrophic muscles and contributes to the pathology of DMD. We observed increases in UPR signalling in DMD patient biopsies and muscles of the mdx mouse model of DMD. We crossed mdx mice with caspase-12-null mice, which are resistant to ER stress. Caspase-12 ablation preserved mdx muscle function, resulting in a 75% recovery of specific force generation and resistance to eccentric contractions. Compensatory hypertrophy was normalised, due to a decrease in fibre size. Fibre central nucleation was not significantly altered, but muscle fibre degeneration was substantially reduced. In conclusion, we have identified heightened ER stress as a novel contributor to the dystrophic phenotype. Caspase-4, the human equivalent of murine caspase-12, is a potential therapeutic target for DMD.

Disease Biology

52. Human muscle regeneration and aging: a new model in immuno-deficient mice.

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Our understanding of human muscle regeneration comes mainly from tissue culture investigations, which do not reproduce regenerative conditions found in vivo. We have developed a model for transplanting strips of human muscle into the anterior tibial compartment of an immunodeficient mouse and tying them into the tendons of the peroneal muscles.
Muscle biopsies were obtained from FSHD volunteers and unaffected relatives. Host mice were euthanized, at intervals, the grafted muscles were removed and examined histologically and for gene expression patterns.
The grafted muscle degenerates over a period of a few days and is progressively replaced by newly regenerated fibres, predominantly of human origin. The graft becomes revascularized by blood vessels derived from both graft and host cells and innervated by motor neurons of the host mouse and the muscle fibres express mature fast and slow myosins.
Importantly, muscle samples from cadavers also give rise to successful grafts and regenerate well, even from aged donors. This, ease of access to donor material opens new pathways to the study, in vivo, of aging of human muscle and for analysis of patterns of human muscle gene and protein expression in response to various stimuli.

53. Muscles of the mdx mouse are simultaneously dystrophic, hypertrophic, hyperplastic and atrophic

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An intensive study of the mdx mouse, the most-used animal model of muscular dystrophy reveals a number of previously unsuspected abnormalities. First, postnatal muscle growth shows retarded myonuclear increase and abnormal satellite cell function together with smaller myonuclear domain and excessive lysosomal content. Second, onset of myonecrosis at 3 weeks (dystrophy), triggers doubling of myonuclear number per fibre compared with WT but with reduced sarcoplasm per nucleus. Thus, mdx mouse muscles are larger than WT (hypertrophy) but contain only half the amount of contractile material per myonucleus (atrophy). Both the total muscle volume and the number of nuclei per volume are greater than WT; this, in addition to replacement of myonuclei lost by muscle degeneration, involves extensive proliferation of myogenic cells (hyperplasia).
We conclude that the mdx dystrophy is not a simple, degeneration/regeneration model but involves a gross disturbance of relationships within the muscle fibres. For most muscles, it provides a good model for analysis of the necrotic but not of the regenerative pathways and is a poor preclinical model for therapies based on replacement or augmentation of myogenic cell functions.

Disease Biology

54. Nuclear membrane protein lamin A/C does not play by the rules.

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Emerin and Lamin A/C are two nuclear proteins, defects in which underlie Emery-Dreifuss muscular dystrophy. Conventionally pre-lamin is thought to be translated in the cytoplasm and to be transported into the nucleus by virtue of a nuclear localizing signal which, in the syncytial muscle fibre should be shared, by diffusion, between neighbouring myonuclei. Such sharing is seen for Emorin but does not occur with lamin A/C. In human/mouse heterokaryon myofibres, mouse myonuclei do not acquire human lamin A/C in tissue culture. Nor does it occur in vivo, even when rare mouse myoblasts fuse with regenerating human fibres in muscle grafts. This lack of sharing persists for 20 weeks despite a lamin half-life of ~2 weeks. In tissue culture, lamin does not transfer from lamin competent mouse myonuclei to lamin-null myonuclei in myotubes although, Emerin does transfer from the lamin-null myonucei to emerin-null myonuclei within the same myotubes.
So far, lamin A/C is the only protein demonstrated to exhibit such stringent stay-at-home properties; it implies that both the transcript and the protein are tightly bound to the membrane of the nucleus that encodes the gene, making it a poor prospect for myoblast transplantation therapies.

56. Beta-adrenergic stimulation reveals a role for sarcopsan in cardiac hypertrophy

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Loss of dystrophin and sarcoglycans causes distinct muscular dystrophies, frequently resulting in development of dilated cardiomyopathy. In skeletal muscle, loss of sarcospan (SSPN) reduces abundance of the dystrophin-glycoprotein complex (DGC) at the sarcolemma causing deficits in force properties. In the current study, we demonstrate that genetic ablation of SSPN negatively impacts expression of the DGC in cardiomyocytes, while 1D integrin levels were increased as a compensatory mechanism. SSPN-null mice at 1-year of age displayed an exacerbated cardiac hypertrophic response after isoproterenol treatment compared to experimental controls. In addition, SSPN-null hearts were functionally compromised, had reduced cardiac output (ejection fraction) and impaired cardiac contractility (fractional shortening). Further analysis revealed increased fibrosis and elevated expression of cardiac stress markers in SSPN-null hearts relative to wild-type. Our findings support an important role for SSPN in regulating cardiac remodeling and demonstrate that loss of SSPN exacerbates the hypertrophic response to -adrenergic agonism. (NIH NIAMS, MDA USA)

Disease Biology

57. Quantitative Comparison of Utrophin in Dystrophin-Deficient Mouse and Human Skeletal Muscle

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Duchenne muscular dystrophy (DMD) is a debilitating genetic disorder with no cure that results from a mutation in the gene coding dystrophin. The protein utrophin is structurally homologous to dystrophin and binds many of the same proteins. While increased utrophin levels have been shown to correlate with improved prognosis in a small cohort of DMD patients, and transgenic overexpression of utrophin rescues many of the dystrophic phenotypes of the mdx mouse, the levels of utrophin in dystrophic mouse and human muscle have never been directly compared. We performed quantitative Western blot analysis of utrophin levels in muscles from WT and mdx mice, as well as in muscle biopsies obtained from non-dystrophic control and DMD patients, using full-length recombinant mouse or human utrophin, respectively, as standards. Our results demonstrate that utrophin levels in human muscle biopsies agree well with those measured in analogous mouse models and suggest that mice can serve as accurate models for development of utrophin-based therapies. Supported by the Lillehei Heart Institute Summer Research Scholars Program, the MDA, and NIH grant AR042423.

58. Dysregulation of RNA processing in Spinal Muscular Atrophy *(No Poster)*

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At the post-transcriptional level, expression of protein-coding genes is controlled by a series of RNA regulatory events including nuclear processing of primary transcripts, transport of mature mRNAs to specific cellular compartments, translation and ultimately, turnover. These processes are orchestrated through the dynamic association of mRNAs with RNA binding proteins and ribonucleoprotein (RNP) complexes. Accurate formation of RNPs in vivo is fundamentally important to cellular development and function, and its impairment often leads to human disease. The survival motor neuron (SMN) protein is key to this biological paradigm: SMN is essential for the biogenesis of various RNPs that function in mRNA processing, and genetic mutations leading to ubiquitous SMN deficiency cause the neurodegenerative disease spinal muscular atrophy (SMA). I will discuss the expanding role of SMN in the regulation of gene expression through its multiple functions in RNP assembly and advances in our understanding of how disruption of SMN-dependent RNA processing pathways can cause motor system dysfunction in SMA.

Disease Biology

59. The role of the extracellular matrix protease ADAMTS5 in normal and dystrophic skeletal muscle

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ADAMTS5 is an extracellular matrix remodelling protease that cleaves proteoglycans, such as versican, to help facilitate myoblast fusion. To investigate the role in muscle development and regeneration, we characterised post-natal growth in the tibialis anterior (TA) and extensor digitorum longus (EDL). C57/BL6:Adamts5-/- muscle fibres were smaller, and following three weeks of voluntary endurance exercise, failed to display the oxidative fibre-type shift typical of C57/BL6:Adamts5+/+ mice. The expression of ADAMTS5 is also elevated in mdx mice, the most commonly used animal model for studying Duchenne Muscular Dystrophy. We hypothesised that genetically removing ADAMTS5 from mdx muscle would reduce inflammation and muscle necrosis, thus ameliorating the pathology. Preliminary data shows that mdx:Adamts5-/- mice have reduced numbers of centralised nuclei, a marker of regeneration from muscle damage. TA specific force in mdx:Adamts5-/- mice was not different from mdx:Adamts5+/+ mice, but these mice did show improved resistance to fatigue. These results demonstrate that ADAMTS5 is involved in postnatal development and remodelling in normal muscle, and highlights a potential role in mdx muscle regeneration.

60. Recovery of altered neuromuscular junction morphology and muscle function in mdx mice after injury

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Considerable attention has been dedicated to studying myofiber damage and muscle plasticity, but less attention to damage after injury at or near the nerve terminal. The neuromuscular junction (NMJ) in mdx mice has altered morphology compared to wild-type (WT). The NMJ is further perturbed 24 hrs following injury, but it is not known if these changes occur immediately after injury or resolve during muscle recovery. We induced eccentric injury to the quadriceps of WT and mdx mice and followed the animals until full functional recovery. NMJ morphology and neuromuscular transmission failure (NTF) rates were assessed at 5 time points after injury: Days 0, 1, 7, 14 and 21. Injury resulted in a significant loss of maximal torque in WT (39±6%) and mdx (76±8%) quadriceps, with recovery by Day 7 (WT) or Day 21 (mdx). Significant changes in NMJ morphology and NTF were found only in mdx following injury and only at Days 0 and 1. The data indicate that contraction-induced injury caused immediate changes at the NMJ, disrupting both morphology and NMJ function. Such early changes at the NMJ after injury are consistent with mechanical disruption and could contribute to the increased susceptibility to injury in mdx mice.(NIH1R01AR059179 to RML)

Disease Biology

61. STIM1 in skeletal muscle development, contractility and regeneration

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Stromal interaction molecule 1 (STIM1) is an ER membrane protein that activates the Orai family of store operated Ca2+ channels. Recently our group showed that STIM1 plays a critical role in skeletal muscle development and contractility and has offered important insight into how Ca2+ entry pathways functions in muscle. In fact, mutations in the human STIM1 gene have been associated with a range of muscle phenotypes including loss of function mutations (hypotonia) and gain of function mutations (tubular aggregate myopathy). We have characterized several mouse models of both the GOF and LOF mutations to show how STIM1 and SOCE are important in neonatal muscle growth and metabolism. Because SOCE has been reported to be upregulated in muscle of mdx mice, we investigated the function of SOCE in the pathogenesis and regenerative capacity of dystropathology by crossing the STIM1 null mice with mdx mice. Our results indicate a crucial role for SOCE in the regenerative capacity of mdx muscle as the double mutant (mdx:STIM1 null) mice exhibit more severe pathology, greater membrane damage and reduced exercise capacity. Together these studies establish the importance of STIM1 and SOCE as a cause and as a modifier of skeletal myopathies.

62. Therapy development for MDC1A *(No Poster)*

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The myomatrix and its binding to the sarcolemma is important for the structural stability of muscle. The myomatrix consists of laminin-211 (Lm-211), collagen IV and several associated components. Assembly of the myomatrix is initiated by the binding of Lm-211 to its cell surface receptors and its self-assembly. Mutations in Lm-211 result in a severe form of congenital muscular dystrophy, called MDC1A. Loss of Lm-211 causes increased expression of Lm-411. However, Lm-411 binds only weakly to the sarcolemma and it cannot self-assemble. Previous proof-of-principle studies in MDC1A mouse models have shown that transgenic expression of mini-agrin, which binds to Lm-411 and to α-dystroglycan, strongly ameliorates the dystrophic phenotype. I will now show that introduction of a second transgene that allows self-polymerization of Lm-411 acts in synergy with mini-agrin. The improvements are seen on all levels, such as behavior, weight gain, histology of skeletal muscles and muscle force. In summary, our data provide formal proof that MDC1A is caused by defects in myomatrix assembly and its connection to the sarcolemma. Our data may significantly affect future attempts to develop new treatment strategies for MDC1A.

Disease Biology

63. Dystrophin insufficiency causes locomotor dysfunction in a swine model of dystrophinopathy

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Dystrophin deficiency or insufficiency results in Duchenne and Becker muscular dystrophies (BMD), respectively. We recently described a pig model with spontaneously occurring dystrophin insufficiency resulting in muscle injury. The purpose of this investigation was to determine the extent to which dystrophin insufficiency impairs locomotion in a BMD pig model. Three month old male BMD pigs (n=5) and healthy male littermates (n=7) walked on a 5 m mat with imbedded force transducers at a self-selected velocity so that gait parameters could be objectively evaluated. Stride length and stride velocity were decreased by 8% ($p<0.05$) and 20% ($p<0.05$), respectively, in affected pigs compared to control. In affected pigs, the duration of the stance phase was increased 5% ($p<0.05$) and the duration spent in the swing phase was decreased 8% ($p<0.05$). The distance the center of pressure traveled during the single stance phase decreased by 44% ($p<0.05$) and the variance increased 42% ($p<0.05$) in affected pigs compared to control. These objective data show dystrophin insufficiency is associated with changes in gait and that the BMD pig is an appropriate model of dystrophinopathy. Supported by R21 NS079603

64. The sepn1 knockout zebrafish: a novel genetic model of SEPN1-related myopathy

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SEPN1-related myopathies (SEPN1-RM) are a heterogeneous group of early onset muscular disorders characterized by hypotonia, respiratory insufficiency, and spinal rigidity. SEPN1-RM is caused by mutations in the human SEPN1 gene, coding for selenoprotein N (SelN), however, SelN function and the mechanisms behind these pathologies remain poorly understood. To understand the role of SelN in skeletal muscle development, we used TALE nucleases to generate germ line mutations in the zebrafish sepn1 gene. Sepn1 knockout embryos exhibit morphological abnormalities and impaired early motor function, evidenced by slow touch-evoked swimming and weak contractions in tail and trunk muscles. Electron micrographs of sepn1 mutant skeletal muscle show amorphous cores lacking intact contractile apparatuses, enlarged vesicles of the sarcoplasmic reticulum, and malformed mitochondria found in wide gaps between myofibers. These ultrastructural defects correlate with reduced contractile forces generated by sepn1 mutants following wave pulse stimulations. Mechanistic studies are ongoing; we have strong evidence to suggest that reactive oxygen species are dysregulated in the sepn1 phenotype. Funded by NIH R01AR044345, F31NS081928, MDA201302, and CureCMD.

Disease Biology

65. Collagen content does not alter the passive mechanical properties of fibrotic skeletal muscle in mdx mice

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Many skeletal muscle diseases are associated with progressive collagen accumulation leading to fibrosis and impaired muscle function. Increased collagen content and altered organization are thought to drive increased muscle stiffness. However, little is known about collagen organization in fibrotic muscle, and its functional consequences. To investigate the relationship between collagen content and organization with muscle mechanical properties, we studied mdx mice, a model for Duchenne muscular dystrophy that undergoes muscle fibrosis. Collagen content increased in the mdx soleus and diaphragm muscles. Collagen packing density, a parameter of collagen organization, was determined using circularly polarized light microscopy of picosirius red stained sections. EDL and soleus muscle had proportionally less dense collagen in mdx, while the overall proportion of loose collagen was increased in all mdx muscles. The mdx muscles were weaker, yet only the EDL had increased stiffness. Unexpectedly, passive stiffness did not correlate with collagen content and only weakly with collagen organization. We conclude that fibrosis does not lead to increased passive stiffness, and that collagen content is not predictive of muscle stiffness. (NIH)

66. MMP13 is required for efficient skeletal muscle regeneration in mouse model of muscle injury

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Skeletal muscle requires timely expression of genes for satellite cell-based regeneration in coordination with extracellular matrix remodeling. Matrix Metalloproteinases (MMPs) are a family of enzymes responsible for breakdown of extracellular matrix components. We have identified MMP13, which breaks down fibrillar collagen during the resolution of muscle damage, as a contributor to muscle regeneration. Unchallenged MMP13 null mice muscles have no significant difference in histology or mechanical properties compared to wild type. To determine the necessity of MMP13 expression in regeneration we injected cardiotoxin into MMP13 null mice and compared the resolution of damage to wild-type mice. Results show reduced muscle fiber size and vascularity of MMP null mice following injury. MMP13 null mice have similar collagen area within muscle, but that collagen is in a looser state. In order to determine the effect on satellite cells we conducted studies using primary cultures from MMP13 null and wild-type mice to show that MMP13 null cells have reduced migration. These data demonstrate that mice lacking MMP13 have decreased muscle regenerative capacity and indicate MMP13 as a therapeutic target to enhance muscle regeneration. (NIH)

Disease Biology

67. Exploring secondary molecular pathways as therapeutic targets for merosin-deficient congenital muscular dystrophy

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Merosin-deficient congenital muscular dystrophy (MDC1A) is caused by recessive loss-of function mutations in the laminin-α2 gene. Laminin 211, the major laminin isoform in skeletal muscle, binds sarcolemmal components to anchor the muscle fiber to the extracellular matrix. Analysis of the candyfloss (caf) zebrafish model of MDC1A has shown that muscle fiber degeneration is a result of contraction-induced muscle cell injury due to improper anchorage of the muscle fiber to the basement membrane. Additionally, mouse models of the disease have shed light on abnormalities in secondary molecular pathways, including increased expression of ubiquitin-proteasome-related and autophagy-related proteins. As there are currently no curative therapies for MDC1A, our lab utilizes the caf zebrafish model of the disease to uncover irregularities in underlying secondary molecular pathways in an effort to find new therapeutic targets to ameliorate disease pathology. We will suggest a new phenotypic characterization in homozygous caf mutant embryos, as well as provide insight into secondary pathways as potential therapeutic targets. (SickKids)

68. Clinical Features of X-linked Myotubular Myopathy Carriers

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X-linked myotubular myopathy, a severe congenital myopathy caused by mutations in the MTM1 gene, is characterized by profound generalized skeletal muscle weakness in affected males. To improve our understanding of whether female MTM1 carriers manifest symptoms, we developed a questionnaire to assess muscle weakness or other organ dysfunction in this population. Here we present data on a pilot group of 8 carriers. All carriers reported that they fatigue more easily than peers, and 50% have difficulty climbing stairs. 75% participated in sports as young adults, but 50% of that group recalled having endurance and strength concerns when compared to their peers. 37.5% of carriers were diagnosed with eye muscle weakness with 25% reporting ptosis. Other concerns described include scoliosis, joint laxity, skeletal asymmetry, and neck weakness. 50% of carriers also subjectively report having larger head sizes. 75% of carriers reported having issues maintaining low weight even with proper exercise and diet. 37.5% report recurrent difficulties with chewing and swallowing. Our preliminary data suggest that features of muscle weakness are present in this population, prompting further study of a larger group of MTM1 carrier females. (NIH)

Disease Biology

69. Genetic overexpression of Serpina3n rescues muscular dystrophy

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Destabilization of dystrophin- sarcoglycan complex is a cause of muscular dystrophies (MD) characterized by increased membrane fragility, myofiber necrosis and an inflammatory response that can also be pro-fibrotic. Serpina3n belongs to a serine protease inhibitor family with known functions in accelerating the wound healing process by modulating extracellular matrix remodeling. Elevated Serpina3n expression is observed in different mouse models of MD. Additionally, while Serpina3n is normally secreted and localized to an ECM like area of skeletal muscle fibers, it is also noticeably present within the cytoplasm of dystrophic myofibers, prompting us to create a transgenic with skeletal muscle-specific overexpression of Serpina3n (Serpina3n Tg). We show that dystrophic pathology observed in delta sarcoglycan null mice (sgcd-/-) and dystrophin mutant mice (mdx) are rescued by Serpina3n Tg. Myofiber necrosis is dramatically reduced as shown by decreased extent of central nucleation and fibrosis. Mechanistically, Serpina3n may work through stabilization of sarcolemma membrane and extracellular matrix, as evidenced by decreases in Evan's blue dye uptake and CK leak, which leads to restoration of running capacity in these dystrophy

70. DNAJB6 in LGMD1D *(No Poster)*

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The gene causing LGMD1D is a ubiquitously expressed co-chaperone DNAJB6 and not a muscle specific gene. The dominant mutations decrease the anti-aggregational effect of DNAJB6 and impair the functions of chaperonal complexes in which DNAJB6 is involved, leading to protein aggregations and secondary autophagic abnormality with rimmed vacuolar pathology. DNAJB6 is als involved in the chaperone-assisted selective autophagy (CASA) pathway, a major mechanism in protein re-cycling turnover for the maintenance of Z-disc sarcomeric integrity. CASA components HSPB8, BAG3 and STUB1 target proteins to macroautophagy. DNAJB6 interacts with several members of the complex and since autophagy can be targeted with drugs therapeutic options are investigated.

71. Genetics and Epigenetics of FSHD *(No Poster)*

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Fascioscapulohumeral dystrophy (FSHD) is caused by partial chromatin decondensation of the D4Z4 repeat array on chromosome 4 and transcriptional derepression of the D4Z4-encoded DUX4 retrogene in skeletal muscle. In FSHD1, D4Z4 chromatin relaxation and DUX4 expression is caused by contraction of D4Z4 repeat array to 1-10 units on a DUX4 polyadenylation signal containing allele. In FSHD2, D4Z4 chromatin decondensation of normal-sized D4Z4 repeat arrays is mostly caused by mutations in the structural maintenance of chromosomes flexible hinge domain containing 1 (SMCHD1) gene on chromosome 18. The chromatin modifier SMCHD1 binds to D4Z4 to maintain a repressed D4Z4 chromatin structure in somatic cells. Individuals with FSHD2 have reduced SMCHD1 activity at D4Z4 causing D4Z4 chromatin decondensation and DUX4 expression. SMCHD1 mutations can also modify disease severity in FSHD1.
In 52/61 FSHD2 families we identified heterozygous SMCHD1 mutations. Mutations were found throughout the locus but the damaging potential of a mutation depends on several factors, providing a molecular basis for the clinical variability in disease onset and progression.

Disease Biology

72. Thrombospondin-4 functions in endoplasmic reticulum (ER) stress-based adaptation that protects skeletal muscle from disease.

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Thrombospondin-4 (Thbs4) was recently shown to function inside cardiomyocytes by inducing a protective ER-stress response through a mechanism involving activating transcription factor 6alpha (ATF6a). We now extend this paradigm to myofibers and show that membrane instability, dystrophic pathology and loss of exercise capacity in both Sgcd-/- and mdx mice are dramatically rescued by skeletal muscle specific overexpression of Thbs4 (Thbs4Tg). In addition, Thbs4Tg and Sgcd-/-Thbs4Tg mice are protected against in situ lengthening-contraction induced injury. Conversely, targeted deletion of Thbs4 blunts the ER-stress response and worsens dystrophic pathology in Sgcd-/- and mdx mice. Mechanistically, we show that Thbs4 stabilizes the membrane via several interconnected mechanisms. First, Thbs4 accelerates ER-to-golgi and post-golgi protein trafficking in an ATF6a dependent manner. Next, sarcolemmal localization of DGC and integrins, as well as their associated proteins is enhanced in Thbs4Tg and Sgcd–/–Thbs4Tg muscles. Finally, Sgcd–/–Thbs4Tg myofibers display accelerated membrane resealing upon laser injury. Hence, our data reveal a Thbs4-dependent ER-stress based adaptation that protects skeletal muscle from disease.(NIH, HHMI)

74. Hyperhomocysteinemia (HHcy) inhibits satellite cell regenerative capacity through p38 MAPK signaling

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Recent evidence suggests that p38 MAPK signaling is upregulated with concomitant decline in Pax7 expression and ability of the satellite cells to contribute to the stem cell reservoir and long-term regenerative potential, causing elderly susceptibility to frailty and injury. HHcy has been shown to cause elderly frailty, compromised muscle function and susceptibility to injury. However, the mechanisms of HHcy induced skeletal muscle dysfunction remains unknown. Here, we show that there is increase in the p38 MAPK signaling, reduced Pax7 expression in the satellite cells of HHcy mice (CBS -/+) when compared to cells from WT. Moreover, there was reduced overall force generation and recovery after injury in CBS -/+ mice when compared to age matched WT mice. Furthermore, we show that treatment of isolated satellite cells briefly in vitro with Hcy resulted in dose dependent enhancement in p38 MAPK signaling and reduction of Pax7 expression. In addition pharmacological inhibition of p38 MAPK signaling enhanced stem cell population (Pax7 +ve), force generation and recovery after injury. Together these results suggest that HHcy is causing skeletal muscle frailty through satellite cell dysfunction involving p38 MAPK upregulation. NIH.

Disease Biology

75. A novel genetic modifier of Duchenne muscular dystrophy phenotype

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Duchenne muscular dystrophy (DMD) is an X-linked skeletal myopathy caused by mutations in the dystrophin gene. The secondary signaling pathways involved in the pathogenesis of DMD remain poorly characterized. Differently from humans, animal models of dystrophin-deficiency show great phenotypic variability. Through the study of clinical exceptions among the golden retriever muscular dystrophy (GRMD) dog we aim to identify signaling pathways that can modulate the disease pathogenesis. We performed gene expression profiling and found 114 genes differentially expressed between severely affected and mildly affected dog muscle. One gene had the same expression level in mildly affected and normal dogs. Phosphatidylinositol transfer protein (Pitpna) expression was reduced in mildly when compared to severely affected GRMD dogs. Modulation of pitpna gene expression in the dystrophin deficient zebrafish model leads to functional improvement. These findings could explain the phenotype variability in GRMD dogs and more importantly, these candidate genes open new possibilities for therapeutic approaches. This work was supported by FAPESP, the Duchenne Research Fund and the Gimbel Foundation.

76. Regulatory T cells suppress muscle inflammation and injury in the mdx mouse model of Duchenne muscular dystrophy.

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We tested the hypothesis that regulatory T cells modulate muscle injury and inflammation in Duchenne/Becker muscular dystrophy and the mdx mouse model of DMD. Although Tregs were largely absent in wild type mouse and normal human muscle, they were elevated in dystrophic muscle, displayed an activated phenotype and expressed interleukin-10 (IL-10). Depletion of Tregs exacerbated muscle injury and inflammation, which was characterized by an enhanced interferon-gamma response that primed M1 activation of muscle macrophages. To examine the therapeutic value of targeting Tregs during muscular dystrophy, we treated mdx mice with IL-2/anti-IL-2 complexes (IL-2c), and found that Tregs were increased in muscle, resulting in reduced expression of cyclooygenase-2 and myofiber injury in mdx mice. The amelioration of dystrophinopathy by IL-2c treatment was associated with an increased expression of IL-10 in dystrophic muscle. These findings reveal that Tregs modulate the progression of muscular dystrophy by suppressing type 1 muscle inflammation associated with muscle fiber injury, and highlight a novel application of Treg-modulating agents as as potential therapeutics for DMD.
This research was funded by NIH grants to SAV and JAB.

Disease Biology

77. QTL analysis using the superhealing MRL strain reveals distinct genetic regions that modify muscular dystrophy and cardiomyopathy in mice

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Muscular dystrophy with the same disease mutation yields clinical phenotypes that are variable suggesting the presence of genetic modifiers. The super-healing MRL background modifies disease phenotypes when introduced into a gamma-sarcoglycan (Sgcg) model of limb girdle muscular dystrophy. The MRL strain has been associated with improved corneal wound and cardiac repair. Quantitative trait locus (QTL) mapping was applied between these differentially affected Sgcg MRL/MpJ and 129T2/SvEmsJ mouse strains with muscular dystrophy. Animals were phenotyped using measures of muscle pathology and cardiac function. Muscles were harvested to measure sarcolemmal damage using Evans blue dye uptake. Fibrosis was measured using hydroxyproline determination in multiple muscle groups. Echocardiography, body mass, muscle mass, and heart mass were also determined. QTL mapping identified 9 significant loci associated with these measurements in chromosomes 7, 9, and 15. Additionally, whole-genome sequencing and RNA-seq was performed to prioritize candidate modifier genes. Together, these data support the presence of genetic modifiers for muscular dystrophy and cardiomyopathy from the superhealing MRL strain. NIH

78. Age dependent changes in cardiac and skeletal muscle T2 in gamma-sarcoglycan deficient mice

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The purpose of this study was to monitor cardiac and skeletal muscle T2 in a mouse model of limb girdle muscular dystrophy (LGMD-2C) at different ages. Cardiac and hindlimb muscles of gsg-/- mice were longitudinally imaged using MRI and spectroscopy at 4.7T Oxford magnet with a Varian/Agilent spectrometer. Cardiac and respiratory gated T2-weighted single spin-echo images of the heart were acquired (TR = 750 ms; TE = 12.5 and 30 ms; FOV = 25X25 mm2, slice thickness = 1.0 mm, averages = 8) using a custom-built quadrature volume coil. T2 values were derived using average signal intensity from the mid papillary region of the left ventricle at each TE. Single voxel STEAM 1H-MRS of the posterior compartment in the right lower hindlimb was acquired (32 TE's non-linearly spaced between 5-300 ms, TR 9000 ms), and analyzed using complex PCA with non-linear curve fitting. An age dependent reduction in myocardial T2 (4-5 months, 24.9±4.1 ms; 12 months, 19.0±1.3 ms) and hindlimb muscle T2 (4-5 months, 25.0±0.5 ms; 12 months, 22.8±0.9 ms) was observed. The findings of this study indicate that there is an age dependent reduction in muscle T2 in gsg-/- mice, possibly due to increasing tissue fibrosis with age. Wellstone U54AR05264

Disease Biology

79. Dietary phosphorus overload exacerbates dystrophic phenotypes of the dystrophin-deficient mdx mouse

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Duchenne muscular dystrophy (DMD) is a lethal X-linked disease with no effective treatment. Because dietary phosphorus (P)/phosphate (Pi) consumption is increasing and adverse effects of phosphate overloading have been reported in several disease conditions, we examined the effects of dietary P intake in a murine model of DMD, mdx mouse. Upon weaning, control and mdx mice were fed diets containing 0.7 g (low-P), 1.0 g (normal-P), or 2.0 g (high-P) P/100 g until they were 90 days old. Dietary P overload dramatically exacerbated the dystrophic phenotypes of mdx mice by increasing inflammation associated with infiltration of M1 macrophages. In contrast, minimal muscle necrosis and inflammation were observed in sedentary and even in exercised mdx mice fed a low-P diet, suggesting potential beneficial therapeutic effects of lowering dietary P intake on disease progression. Dietary P overloading promoted dystrophic disease progression in mdx mice whereas restricting dietary P intake improved muscle pathology and function.
Funding: Health and Labour Sciences Research Grant; Neurological and Psychiatric Disorder of National Center of Neurology and Psychiatry; ministry of Education, Culture, Sports, Science and Technology-Japan

80. Pathological changes in muscle gene expression caused by mutant lamins

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Mutations in the human LMNA gene encoding A-type lamins cause laminopathies, a collection of diseases that includes muscular dystrophy. We generated a Drosophila model of laminopathies in which human disease mutations are modeled in Drosophila lamin for functional analyses. Muscle-specific expression of mutant lamins causes cytoplasmic aggregation of NE proteins, induction of genes regulated by the redox-sensing transcription factor Nrf2, and muscle dysfunction. Nrf2 is normally sequestered in the cytoplasm by Keap-1. Oxidative stress causes Nrf2 to dissociate from Keap-1, translocate into the nucleus, and activate target genes. Paradoxically, muscles expressing mutant lamins were discovered to be under reductive stress, rather than oxidative stress. We propose that cytoplasmic aggregates of NE proteins stabilize the adaptor protein p62/SQSTM1, which binds Keap-1, allowing Nrf2 to translocate into the nucleus and activate gene expression. Our findings provide a novel mechanism for gene regulation by mutant lamins and suggest classification of laminopathies as protein aggregation disorders. (NIH)

Disease Biology

81. Dysferlin deficiency in mdx mice does not exacerbate the behavioral phenotype

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Literature suggests that loss of both dysferlin and dystrophin in the same animal has a compounding effect on muscle pathology. Conceptually, defective membrane repair due to the loss of dysferlin in an animal prone to muscle membrane lesions due to the loss of dystrophin, should exacerbate the muscle phenotype. We hypothesized that dysferlin/dystrophin double deficient mice might be a more robust model for evaluating genetic correction or replacement of dysferlin, as dysferlin deficiency alone in mice is so mild it is difficult to detect in the first 6 months. In this study, dysferlin deficient BLAJ mice were crossed with mdx mice, and compared to mdx mice in a variety of behavioral tests: open field activity, grip strength, rotarod performance, downhill treadmill running, and serum creatine kinase levels. Comparisons were made at 4, 12 and 26 weeks of age, along with histological evaluation of the muscles at necropsy. Surprisingly, we found little difference between the dysferlin/dystrophin double deficient mice and the dystrophin deficient mdx mice, suggesting that the absence of dysferlin in the mdx background does not alter behavioral or histological measures of muscle pathology during the first 6 months of life.

82. Inhibiting Myostatin signaling to Treat Collagen VI Deficient Myopathy

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Collagen VI related dystrophies(COL6-RD) are common early onset muscle disorders, caused by mutations in COL6A1-3, leading to an abnormal muscle extracellular matrix. Myostatin, a member of TGF-beta superfamily, is a negative regulator of muscle mass acting mostly via activin type IIB receptor and smad2/3 signaling. In muscle biopsies from patients with COL6-RD we found active myostatin levels to be higher compared to controls, while full length myostatin was unchanged. This suggests that myostatin signaling may be overactive, consistent with our hypothesis that the abnormal extracellular matrix caused by dysfunction of collagen VI may lead to more available active myostatin and thereby to exaggerated muscle atrophy. Therefore, blocking myostatin signaling as a therapeutic strategy may be helpful to rescue the muscle atrophy phenotype. To test this strategy, we crossed Col6a3 mutant mice with muscle specific dominant negative Acvr2B (M-DN) mice, the latter blocking myostatin signaling in the muscle due to the mutant receptor trapping active myostatin. We are evaluating these crosses for muscle size and strength as well as for histological improvement.

Disease Biology

83. Recessive and dominant mutations in COL12A1 cause a novel EDS/myopathy overlap syndrome in human and mice

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Collagen VI related myopathies are disorders of connective tissue with an overlap phenotype combining clinical involvement from the muscle and from the connective tissue. Not all patients displaying related overlap phenotypes between muscle and connective tissue have mutations in collagen VI. Here we report a homozygous recessive loss of function mutation and a de-novo dominant mutation in collagen XII (COL12A1) as underlying a novel overlap syndrome involving muscle and connective tissue. Two siblings homozygous for a loss of function mutation showed joint hyperlaxity combined with weakness precluding independent ambulation, while the patient with de novo missense mutation was more mildly affected, showing improvement including the acquisition of walking. A mouse model with inactivation of the Col12a1 showed decreased grip strength, delay in fiber type transition and a deficiency in passive force generation while the muscle seems more resistant to eccentric contraction induced force drop, indicating a role for a matrix based passive force-transducing elastic element in generation of weakness. This new muscle connective tissue overlap syndrome expands on the emerging importance of the muscle ECM in pathogenesis of muscle disease.

Muscle Systems Biology

84. The role of IL-15 receptor alpha antibody in reserving skeletal muscle following denervated Injury

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IL-15 is up-regulated within skeletal muscle after exercise. It's cognate receptor IL-15Ra is also highly expressed in skeletal muscle and forms a complex with IL-15 to stabilize IL-15 and modulate IL-15 bioavailability. The purpose of the current study is to explore the effect of IL-15Ra antibody on denervated skeletal muscle. In this study we performed a unilateral sciatic nerve crush to establish rapid muscle atrophy which was repaired over time. We show that after 2 weeks of treatment with IL-15Ra antibody soleus wet weight was increased in the denervated limb. IL-15Ra antibody treatment also altered the body composition in these mice by reducing fat mass. It has been shown that denervated muscle has a rapid increase in TNF-a signaling. When the IL-15Ra antibody was applied to C2C12 and/or Rhabdomyosarcoma cells, treated with TNF-a, it inhibited the induction of IL-6 and MuRf1 expression by TNF-a after 24 and 48 hours. Collectively these studies hypothesize that the mechanism of IL-15Ra antibody's protective effect on denervated muscle may be via directly regulating the catabolic signals induced by TNF-α signaling and indirectly via reduction in whole body fat mass which is reported to reduce local TNF-α levels.

85. Predicted miRNAs in murine dystrophin gene

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Duchenne muscular dystrophy (DMD) is a common X-linked disease characterized by mutations in the dystrophin gene. In order to identify new molecular networks of gene regulation specific for DMD, we predicted new microRNAs (miRNAs) within the dystrophin gene. We analyzed the gene sequence with three microRNA prediction algorithms (mirEval, mirFinder and MiR-abela) which examine the secondary structure and free-folding energy of their precursors, conservation of miRNA sequence or similarity with other miRNAs and clusterization with already known miRNA. We predicted 28 pre-miRNAs within the dystrophin gene. Fourteen of the predicted pre-miRNA resulted common to all three algorithms. Five putative pre-miRNA showed similarity with known miRNAs allowing to suppose that the dystrophin gene contains new transcription sites for annotated miRNAs. The other 23 predictions could represent new miRNA molecules. Validation of these predicted miRNAs within dystrophin gene will add new intrinsic molecular networks to the characterization of DMD pathogenesis and could explain the variability of the DMD clinical phenotypes. These findings will offer the opportunity to intervene at the miRNA level regulating specific pathways of this muscle disease.

Muscle Systems Biology

86. Differential response to Myogenic Regulatory Factors (MRFs) in fast and slow muscles of rats after atrophic stimulus following aerobic exercise.

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MRFs have been studied to improve the understanding of cellular and molecular mechanisms that underlie the process of atrophy, including MyoD and Myogenin. In this study, we investigated Soleus (Sol-oxidative and slow) and Plantaris (Pl-glycolytic and fast) muscles of rats with atrophic stimulus followed by aerobic training. Male Wistar rats (3 months) were divided into 5 groups (n = 8); C: control; I: immobilized; C7: control 7 days; R7: immobilized recovery for 7 days; E7: immobilized submitted to exercise for 7 days. I, R7 and E7 groups were submitted to a hind limb immobilization (7 days). Muscle atrophy was confirmed by cross-sectional area analysis. The E7 group was submitted to swimming exercise for 20 min with 3% of weight as overload. Sol and Pl muscles were submitted to morphological and gene expression analyses. The immobilization caused Sol and Pl muscles atrophy and aerobic training promoted Sol muscle area recovery, but not in the Pl muscle. There was significant increase in MyoD and Myogenin gene expression only in Sol muscle after aerobic training. Our data suggest that aerobic training is a good intervention for muscle atrophy recovery in slow muscle, but did not promote the recovery in fast muscle (FAPESP).

87. Thrombospondin-1 increases proliferation of muscle progenitor cells

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Chronic destruction of muscle fibers in muscular dystrophies leads to persistent inflammation, activation of satellite cells, and upregulation of thrombospondin-1 (TSP-1). A chronic increase in TSP-1 may not be favorable for repair since it is anti-angiogenic, inhibits an inflammatory environment and promotes fibrosis. We examined the effects of TSP-1 on muscle cells to determine whether it causes potentially negative effects that would impair repair. We found that mRNA levels of TSP-1 increased significantly in two models of muscle injury (laceration >575 fold increase, and cardiotoxin injection >25 fold increase) and that protein levels were ~40% larger in the γ-sarcoglycan deficient mouse. Conditioned media obtained from M1-, M2a- and M2c-like macrophages caused a significant increase in proliferation of C2C12 cells and contained a significantly higher TSP-1 concentration compared to media from non-activated macrophages. Treatment of isolated satellite cells with 25 nM TSP-1 also caused a significant increase in proliferation compared to untreated cells. Thus, local macrophage-released TSP-1 in chronic muscle damage may promote proliferation of satellite cells and contribute to pool exhaustion in muscular dystrophies. (NIH)

Muscle Systems Biology

88. Cardiopulmonary deficits in muscular dystrophy associate with abdominal muscle pathology

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In muscular dystrophy, there is variable involvement of distinct muscle groups. To compare pathology in multiple muscle groups and correlate pathology with cardiovascular outcomes, we conducted a detailed characterization of Sgcg mice from a genetically diverse background (DBA/2J and 129T2/SvEmsJ). Mice were examined at 8 weeks by echocardiography (n=160). Following sacrifice, individual muscle groups were studied using Evans blue dye to monitor muscle injury. Fibrosis was determined using hydroxyproline measurement. Of multiple muscle groups sampled, the abdominal muscles were found to have the greatest amount of Evans blue dye uptake, an indicator of muscle membrane leak and damage. The abdominal muscles were also found to have more fibrosis than other muscles, including the diaphragm muscle. The amount of diaphragm fibrosis correlated positively with fibrosis in the left ventricle, and abdominal muscle fibrosis correlated with impaired left ventricular function. Interestingly, diaphragm and abdominal muscle fibrosis were not correlated. Together these data reflect the recruitment of abdominal muscles as respiratory muscles in muscular dystrophy, a finding consistent with data from human patients.

89. Differential regulation of N-terminal region of the slow, fast and cardiac myosin binding protein-C

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Myosin binding protein-C (MyBP-C) is a thick filament protein with structural and regulatory functions in striated muscles. MyBP-C has three isoforms: fast-skeletal (fMyBP-C), slow-skeletal (sMyBP-C) and cardiac (cMyBP-C). Do structural variations in the N-terminal regions of MyBP-C isoforms confer different modulatory capacities? To answer this question, we used in vitro actin-binding and motility assays to characterize recombinant proteins encoding N' regions of sMyBP-C, fMyBP-C and cMyBP-C. Electron microscopy suggests that MyBP-C isoforms have similar interactions with the thin filament. Motility assays showed that while cMyBP-C and fMyBP-C N' fragments inhibited thin filament motion when fully activated, the sMyBP-C N-terminal fragment was significantly less inhibitory. Additionally, studies in intact cells overexpressing full-length MyBP-C isoforms were used to examine how molecular changes translated to the cellular level. Our studies demonstrated faster relaxation kinetics in sMyBP-C and fMyBP-C relative to cMyBP-C. Together, we demonstrate that, despite similar actin binding, differences in the N' structure of MyBP-C isoforms alter regulation of sarcomere functional properties. (NIH)

Muscle Systems Biology

90. Interleukin 15 receptor alpha (IL15Ra)-deficient mice are resistant to obesity

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IL15Ra is the widely expressed primary binding partner for IL15. We previously reported that, in fast twitch muscle, lack of IL15Ra promotes an oxidative/slow twitch phenotype, with increased mitochondrial biogenesis and fatigue resistance. IL15Ra SNPs have been linked to high endurance capacity, obesity and metabolic syndrome, but the effects of IL15Ra on metabolism are unknown. To address this, control B6129 and IL15Ra knockout (IL15Ra-/-) mice were fed normal chow (NC) or 45% fat diet (HFD) for 16 weeks. IL15Ra-/- mice were resistant to diet-induced obesity (DIO), gaining 18% in weight compared to 38% in controls. HFD IL15Ra-/- mice had less body and liver fat accumulation than controls. The leaner phenotype of IL15Ra-/- mice was associated with increased energy expenditure and diet induced thermogenesis, a strong induction of ucp1 in brown adipose tissue, and enhanced muscle fatty acid oxidation by muscle mitochondria. However, these changes did not improve glucose tolerance in IL15Ra-/- mice. These findings identify novel roles for IL15Ra in metabolism and obesity.
(Institute for Translational Medicine and Therapeutics' (ITMAT) Transdisciplinary Program in Translational Medicine and Therapeutics, UPenn)

91. Full-Length Dysferlin Expression Driven by Engineered Human Dystrophic Blood-Derived CD133+ Stem Cells

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The protein dysferlin is abundantly expressed in skeletal and cardiac muscles, where its main function is membrane repair. Mutations in the dysferlin gene are involved in two autosomal recessive muscular dystrophies: Miyoshi myopathy and limb-girdle muscular dystrophy type 2B. Development of effective therapies remains a great challenge. Strategies to repair the dysferlin gene by skipping mutated exons may be suitable only for a subset of mutations, while cell and gene therapy can be extended to all mutations. Herein, we show for the first time the in vitro production of full-length dysferlin mediated by a lentiviral vector in blood-derived CD133+ stem cells isolated from patients with Miyoshi myopathy. Transplantation of engineered blood-derived CD133+ stem cells into scid/blAJ mice resulted in sufficient dysferlin expression to correct functional deficits in skeletal muscle membrane repair. Multi-exon skipping of blood-derived CD133+ stem cells isolated from the same patients led to partial dysferlin reconstitution in vitro, but failed to ameliorate the dystrophic phenotype in vivo. Our data suggest that lentivirus-mediated delivery of full-length dysferlin in stem cells isolated from Miyoshi myopathy patients is a feasible stra

Muscle Systems Biology

92. Evolutionarily acquired functions of BetaM as a muscle-specific regulator of metabolic gene expression

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Co-option of orthologous ATP1B4 genes in placental mammals transformed Na,K-ATPase BetaM subunit into muscle-specific BetaM protein of the inner nuclear membrane. Eutherian BetaM temporarily and strongly expressed during perinatal muscle development, then sharply down-regulated, and again transiently up-regulated during muscle regeneration. We show that BetaM is a component of MyoD transcriptional complex in neonatal skeletal muscle and up-regulates MyoD expression in cultured muscle cells by altering chromatin structure and recruiting SWI/SNF to the MyoD promoter. Atp1b4 ablation in mice results in lower body weight, severe growth retardation and high mortality of knockout neonates. mRNA sequencing of skeletal muscle from neonatal WT and KO male littermates revealed strong down-regulation of fast-twitch and up-regulation of slow-twitch muscle genes, and broad changes in lipid metabolic genes expression. Notably, KO mice exhibit enhanced metabolic rate and insulin sensitivity, and are resistant to high-fat diet-induced obesity. These data indicate that BetaM has an essential role in regulating skeletal muscle gene expression during development, and loss of its developmental expression alters muscle metabolic programming.

93. A Role for Cytoplasmic Actins in Mitochondrial Stability

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Actin is made up of six different isoforms; four muscle and two non-muscle cytoplasmic actins (CY-actins). CY-actins have been implicated in many cellular functions but recently a role has been shown for CY-actin in mitochondrial dynamics. Previously, we showed that knockout of either of the two CY-actins in skeletal muscle results in progressive myofiber death. Using transmission electron microscopy, we identified structural abnormalities in the mitochondria and the sarcoplasmic reticulum (SR/ER) of aged muscle lacking CY-actin. Additionally, we observed enrichment of CY-actin isoforms in purified mitochondria associated membrane (MAM) fractions, believed to represent the functional interface between the ER and mitochondria. Further experiments suggest that structural abnormalities at the MAM may lead to a loss of mitochondrial function, which is supported by increased actin filament association with mitochondria after exposure to cellular stressors. Together these data support a potential role for the CY-actins in stabilizing mitochondria at the site of interaction with the SR/ER. Supported by NIAMS grants RO1 AR042423 and P30 AR057220.

Muscle Systems Biology

94. Identification of transcriptional targets of human DUX4 during zebrafish development

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Facioscapulohumeral muscular dystrophy (FSHD) is a progressive muscle wasting disorder caused by contractions of repeated units within the macrosatellite D4Z4 on chromosome 4q35 and derepression of the toxic transcription factor double homeobox 4 (DUX4) in skeletal muscle. There is currently no animal model for FSHD. We recently demonstrated that expression of very low levels of the toxic full-length isoform of human DUX4 in zebrafish embryos leads to gross abnormalities, including some of the phenotypes observes in FSHD patients. In order to identify and characterize developmental target genes and pathways of DUX4 in the zebrafish we conducted two complementary, high-throughput genomic analyses. Using the ChIP-seq approach we seek to identify genome-wide DUX4 binding sites and with the RNA-seq method we identify a large number of genes whose expression is dramatically changed as a result of DUX4 expression during the first 12 hours post fertilization in the zebrafish.

95. The new kid on the block? Microtubules and Duchenne muscular dystrophy (DMD)

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Association with dystrophin and implication in DMD pathology has put skeletal muscle microtubules in the spotlight. Microtubules play essential structural and transport roles linked to their protean nature: they are the most rigid of cytoskeletal elements, yet are dynamic, constantly growing and shrinking. Muscle microtubules form a 3-dimensional network that differs considerably from the "classic" radial network of proliferating cells. To decipher the organization of muscle microtubules we have expressed GFP-tagged markers in the flexor digitorum brevis (FDB) muscle of the mouse and visualized them in live fibers, in vivo and ex vivo. While individual muscle microtubules are highly dynamic, their bundling creates a durable lattice-like network. Adult muscle fibers have no centrosomes but instead nucleate microtubules from static Golgi elements and nuclear membranes. Growing from these centers, microtubules seem to be reoriented by dystrophin, suggesting how the microtubule network becomes disordered in the mdx mouse. We are now directly testing this model on mdx muscle fibers and hope the results will bring light on the role of microtubules in DMD and other muscle diseases.

Muscle Systems Biology

96. Dynamic Proteomics: a platform for proteome-wide interrogation of anabolic response and non-invasive biomarker discovery in skeletal muscle

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Development of effective therapies for muscle disorders is limited by a paucity of translational biomarkers. We have developed a novel platform, Dynamic Proteomics, using in vivo heavy water labeling combined with tandem mass spectrometry, which can quantify the fractional synthesis rates (FSR) of 100's of proteins simultaneously from <10 mg of tissue. Moreover, we have developed a plasma-based non-invasive assessment of skeletal muscle protein synthesis, by measuring FSR of plasma Creatine Kinase M-type (CK-M). We will present data validating the approach in rodent models and humans, highlighting the impact of anabolic stimulation on the FSR of 100's of muscle proteins across the proteome, including structural, cytosolic and mitochondrial proteins. Validity of CK-M as a "virtual biopsy" of skeletal muscle protein synthesis was demonstrated by the high correlations between the FSR of plasma CK-M with FSR of muscle CK-M and other muscle proteins in rodents and humans. We conclude that proteome dynamics reveal the detailed muscle proteostatic responses to anabolic interventions, and that FSR of plasma CK-M provides a minimally-invasive translational biomarker of skeletal muscle protein synthesis. (Office of Naval Research)

97. Neutrophil and macrophage content of injured muscles from young and old mice

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Aging is associated with delayed and possibly incomplete repair after skeletal muscle injury, but the underlying mechanisms are not entirely understood. We hypothesized that age-related deficiency in muscle repair is associated with an inappropriate leukocyte response, specifically a persistence of neutrophils and pro-inflammatory macrophages and a delay or reduction in the accumulation of anti-inflammatory (M2) macrophages. To test our hypothesis, we injured muscles of young and old mice with lengthening contractions in situ and analyzed the muscles 2 or 5 d later. Regardless of age, injury increased neutrophil (Ly6G+), total macrophage (CD68+) and M2 macrophage (CD163+) content by 2 d. By 5 d, neutrophils declined and total macrophages increased dramatically while M2 macrophages increased to a lesser extent. We found no evidence of persisting neutrophils or reduced accumulation of M2 macrophages in old muscles. Instead, we generally found more neutrophils and macrophages (total and M2) in muscles of old mice at both timepoints. Future studies will examine whether increased leukocyte content contributes to deficient repair with age or reflects a compensatory effect of an age-related decrease in leukocyte function. (NIA)

Muscle Systems Biology

98. Evaluating Contraction-Induced Impairment in Fiber Excitability Using Magnetic Stimulation

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Muscle fibers lacking dystrophin can have damaged plasmalemma following contractions which may impact the transmission of action potentials. Impairment of fiber excitability has been shown to be a mechanism of strength loss following in vivo contractions by mdx mice. No such determination of transient strength loss has been reported in boys with DMD. This pilot study developed a technique to non-invasively assess fatigue-induced changes in strength and fiber excitability of wrist extensors using magnetic stimulation along with EMG. Fatigue protocol: isometric contraction at 60-70% of maximum voluntary contraction (MVC) maintained as long as possible; followed by 15s rest and repeated until 60% of MVC could not be maintained >5s. In healthy adults, fatigue decreased voluntary and stimulated contraction force suggesting a peripheral component to fatigue. Voluntary activation % was unchanged suggesting no central component. Compound muscle action potential amplitude was unchanged indicating no impairment of fibers to conduct action potentials. Developing this technique will enable us to determine the extent to which contractions affect fiber excitability and transient strength loss in muscles of boys with DMD. (CTSI)

Signaling

99. NRIP regulates skeletal muscle contraction via CaN-NFATc1 and CaMKII autophosphorylation pathways

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We previously found a gene named nuclear receptor interaction protein (NRIP) (JBC 2005, NAR 2008). NRIP is a calcium-dependent calmodulin binding protein (Ca+2/CaM) (JV 2011). CaM contains four Ca+2-binding sites and regulates muscle contraction. Hence, in this study, we purposely investigated the function of NRIP in muscle contraction. We firstly generated conventional NRIP knock out mice (NRIP-/-). Here, we demonstrated that NRIP-/- mice lost muscle strength from the assays of in vitro muscle contraction test with neuromuscular blocker to rule out the nerve effect on muscle contraction; plus NRIP-/- mice showed the susceptibility to fatigue during repetitive contraction. Additionally, the exercise performance using rotarod and treadmill tests, NRIP-/- mice showed weaker than wild type mice. Taken together, NRIP plays a role in muscle strength and endurance. Due to Ca+2/CaM can activate calcineurin phosphatase (CaN) and calmodulin kinase II (CaMKII) for muscle contraction, we then investigated the mechanisms of NRIP-involved in muscle strength. Due to CaN can dephosphate NFATc1 that then translocates to nucleus to induce slow myosin gene expression. The results showed that NRIP-/- mice exhibited lower dephosphate NFATc1 and lower

100. IFN-gamma resets muscle cell fate though a multistep mechanism employing the PRC2 complex

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The inflammatory cytokine, IFN-gamma orchestrates a diverse array of fundamental physiological processes. We have recently identified the essential roles that IFN-gamma and CIITA, the MHC class II transactivator, play in the inflammatory response of muscle. We have discovered that IFN-gamma and CIITA inhibit myogenesis by modifying gene regulation in a muscle cell subject to inflammation. We show that CIITA first interacts with JARID2, a non catalytic subunit of PRC2 complex, which induces a paused RNAPII phosphorylated at serine 5. Subsequently, the additional subunits of the PRC2 complex, including the catalytic subunit EZH2, are recruited in a JARID2 dependent manner. This recruitment is concurrent with the loss of RNAPII and the observed methylation of H3K27. Our data show that both CIITA and IFN-gamma block myogenesis by the induction and recruitment of the PRC2 complex, which is normally silenced in a differentiating cell. We show that the presence of high levels of IFN-gamma in vivo, resulting from muscle damage or diseases such as muscular dystrophy, results in a up regulation of the PRC2 complex, suggesting that IFN-gamma employs the PRC2 complex to inhibit muscle differentiation in vivo.

Signaling

101. Reduced Hedgehog Signaling in Muscular Dystrophy Impairs Muscle Regeneration and Function

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In Duchenne muscular dystrophy (DMD) progressive failure of muscle regeneration and fibrosis contribute to disease progression. It is therefore important to define pathways that regulate these two processes. We have reported that the dystrophic muscle environment blocks myogenesis in interstitial muscle stem cells and instead promotes their fibro-adipogenic differentiation (Penton et al., PLoS ONE, 2013). A screen for differentially activated signalling pathways revealed a major role for Hedgehog (Hh) signalling in regulating myogenesis in interstitial stem cells. We report here for the first time that in dystrophic mdx mice, a model of DMD, Hh signalling is decreased in skeletal muscles, interstitial stem cells, satellite cells and fibroblasts. Mdx mice treated in vivo with a specific Hh agonist to re-activate Hh signalling show decreased inflammation, enhanced regeneration and decreased fibrosis in the diaphragm along with increased resistance to exercise-induced fatigue. Our results identify Hh as a new regulator of muscle regeneration, inflammation and fibrosis that is impaired in muscular dystrophy. Importantly, this pathway can be targeted by small molecules with beneficial effects. (NCH/OSU, PPMD, MDA)

102. Abnormal intracellular signaling in Fktn-deficient dystroglycanopathy muscle

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Secondary dystroglycanopathies are a subset of muscular dystrophies caused by aberrant glycosylation of dystroglycan (DG). Hypoglycosylation of alpha-DG prevents it from binding to laminin and other extracellular proteins, disrupting a link between the extracellular matrix and the intracellular cytoskeleton and rendering the sarcolemma susceptible to damage. FKTN encodes fukutin, a gene causative for dystroglycanopathy. Skeletal muscle from conditional Fktn KO mice shows a progressive dystrophic phenotype and mirrors the spectrum of phenotypic severity observed in human patients. Analysis of muscle growth and survival pathways in skeletal muscle of Fktn KO mice revealed abnormal expression of key signaling proteins and downstream targets. Further, chronic pharmacological inhibition of selected signaling processes improved histopathology of treated KO mice. Whether aberrant intracellular signaling is an intrinsic defect of dystroglycanopathy muscle fibers or if it is indicative of global events occurring in muscle throughout disease progression must be resolved to assess the contribution of abnormal signaling events to disease pathology and to evaluate the suitability of signaling pathway targets for therapeutic intervention.

Signaling

103. Biomechanical Strain Vehicles for Fibroblast-Directed Skeletal Myoblast Differentiation and Myotube Functionality in a Novel Coculture

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Fibroblasts embedded within muscle respond to strain by secreting cytokines that induce myoblast differentiation and we hypothesize regulate myotube function. A coculture was established to allow crosstalk between fibroblasts in Bioflex wells and myoblasts situated on non-deformable coverslips. CSDS modeling repetitive strain/injury and ALDS modeling manipulative therapy were applied to fibroblasts. Non-strained myoblasts in uniculture and coculture served as controls. After fibroblasts had induced myoblast differentiation, myotube contraction was assessed by ACh perfusion. CSDS-fibroblasts increased myotube contractile sensitivity vs. uniculture ($P<0.05$). As contraction is dependent upon ACh binding, expression and clustering of AChRs were measured. CSDS-fibroblasts increased AChR expression ($P<0.05$). Agrin-treated myotubes were then used to design a computer algorithm to identify αBGT-stained AChR clusters. ALDS-fibroblasts increased AChR clustering ($P<0.05$); while CSDS-fibroblasts disrupted clusters from forming. Strain-activated fibroblasts mediate myotube differentiation with multiple functional phenotypes. Cellular strategies aimed at improving muscle functionality may serve as novel targets in neuromuscular disorders. AOA

104. Developing a Resistance Running Wheel System for Mice

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Running wheels are extensively used in rodent endurance but not in resistance training studies. Recent studies in mice show that voluntary wheel running against resistance can promote resistance-like training effects. We designed a computer-controlled system to provide graded resistance to running wheels and performed a 16-wk pilot study. Initially, 5 C57Bl/6 mice were sedentary (SED) and 9 were given free access to the resistance wheels. After system troubleshooting, the runners' wheels were set to one of two resistances (set as % body mass): low resistance (LR; ~2%; n=3) or moderate resistance (MR; ~16%; n= 6). After 6 wk at constant load, the MR group increased work/day by 496% ($p= 0.0155$), while the LR group did not change. During the 6-wk training period, maximal hindlimb in-vivo plantar flexor torque (PFT) did not change for SED or either running group. Absence of increased PFT despite the increased work output for MR was surprising. In a follow-up study, we will increase wheel resistance, increase training duration, and explore both endurance and strength adaptive signaling pathways. Our ultimate aim for the system is to study the effects of resistance-like training in mouse disease models.

Signaling

105. Archvillin: A New Player in Skeletal Muscle Mechanical Signal Transduction

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Deficiency in the gamma-sarcoglycan subunit (gSG) of the DGC is a feature of muscular dystrophies, and is sufficient to induce muscle degeneration and signaling defects in adaptation to mechanical load. Mechanisms of gSG mediated signaling functions and the proteins involved are poorly understood. We identified the muscle-specific protein archvillin (AV) as a gSG interacting protein. AV expression and localization were analyzed by IB, qRT-PCR and IHC in hindlimb muscles of C57Bl/6 (WT), gSG-null (gsg-/-), and mdx mice. AV protein and mRNA expression is significantly upregulated in gsg-/- muscle, but is similar to WT levels in mdx muscle. In cross-sections, AV is upregulated at the sarcolemma in gsg-/- muscle, but absent in mdx muscle. Mechanical perturbation of TA muscles with an in situ eccentric contraction protocol revealed that AV associates with ERK in a stimulus-dependent manner, but this interaction is lost in gsg-/- and mdx muscle. Reintroduction of gSG in gsg-/- muscle by rAAV injection restores AV levels towards that of WT. These results suggest that AV expression is dependent on the SG complex and that AV may impact ERK signaling and contribute to the aberrant ERK signaling observed in dystrophic muscle. NIH T32AR053461

Stem Cells

106. Using iPCs with Dystrophin Gene Mutations to Compare Differences in Oxidative Stress Responses between Cardiac and Skeletal Myocytes

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Mechanisms of oxidative stress contributing to muscular dystrophy are not well understood. We studied two patient-specific iPC lines with Dys gene mutations (D-iPCs) to study the mechanism of stress induced injury in derived cardiomyocytes (iCM) and skeletal myocytes (iSkM). D1 (exon 3-6 deletion), D2 (exon 45-53 deletion) and wild type (N)-iPC lines were differentiated into iCM or iSkM. mRNA expression for SOD1 and SOD2 was reduced in D1/D2-iCM as compared to N-iCM. Stress conditioning significantly elevated reactive oxygen species (ROS) in D1/D2 vs. N-iCM, induced loss of mitochondrial membrane potentials($\Delta\psi m$) and cell injury. To check ROS source, iCM were pretreated with inhibitors against NOS, NOX, XO and mitochondria. The allopurinol (XO-blocker) pretreatment on iCM normalized ROS levels, $\Delta\psi m$ and reduced cell injury after stress; but had no effect on elevated ROS in D1/D2 iSkM. Our results suggest elevated ROS in iCM after stress is due in part to decreased expression of anti-oxidants and increased ROS derived from XO. XO does not contribute to increased ROS levels in iSkM. Allopurinol may be a treatment of choice for dystrophic cardiomyopathy but is unlikely to have therapeutic benefit for skeletal myopathy. NIH/CTSI-MCW

107. Human induced pluripotent stem cell derived skeletal myoblasts: A new human-based platform for basic research and drug discovery utilizing skeletal myotubes from healthy and clinical populations.

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Availability of relevant cell types that accurately represent the human condition for use in basic research, disease modeling, drug screening, and therapeutic applications is severely lacking. The generation of induced pluripotent stem cells (iPSCs) from somatic cells of an individual is a first step in the development of such models. However, the potential use of iPSC derived cells types requires a robust, scalable, and consistent differentiation methodology. Here we describe the derivation, characterization and in vitro differentiation potential of human iPSC-derived Skeletal Myoblasts. When directed to differentiate these cells express markers consistent with development of paraxial mesoderm and skeletal myoblasts. After cryopreservation, thaw, and replating they robustly express skeletal muscle structural proteins including Tropoinin T and Myosin Heavy Chain (MHC). After continued differentiation in vitro, these cells are also highly enriched for skeletal muscle transcription factors, including Myogenin. Consistent with expression of Myogenin and MHC, these cells can readily form myotubes, as measured by fusion index quantitation. In addition, these cultures are competent to phosphorylate AKT in response to IGF-1 signaling, wh

Stem Cells

108. Progression of muscular dystrophy in Dystrophin/utrophin-/- mice is associated with rapid muscle progenitor cell exhaustion

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Duchenne muscular dystrophy (DMD) is a deadly genetic disease characterized by a lack of dystrophin expression and progressive muscle weakening and wasting. Researchers have observed that despite the lack of dystrophin at birth, the histopathological signs of muscle weakness do not become apparent until 3-5 years of age. This happens to coincide with the exhaustion of the muscle progenitor cell (MPC) pool. In this study we isolated MPCs from the skeletal muscle of young (2 weeks) and old (6 weeks) dKO (dystrophin/utrophin double knock out) mice, which have a maximum lifespan of 6 to 8 weeks and is the mouse model closely resembles the disease progression observed in DMD patients. We found that MPCs isolated from the old dKO mice have a reduced ability to proliferate and differentiate compared to MPCs isolated from young dKO mice. In addition the result of muscle progenitor cell marker Pax7 staining indicated that MPCs significantly decreased during disease progression. These observations suggest that blocking the exhaustion of the MPC pool could be a new approach to improve muscle weakness in DMD patients. This work was supported in part by grants from the National Institute of Health (NIH1P01AG043376-01A1).

109. Skeletal muscle crush injury and neuromuscular juntion regeneration: painting a picture in immunofluorescence

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The neuromuscular junction (NMJ) endplate is composed of a coral-like arrangement of acetylcholine receptors (AChRs). AChRs scatter from their sub-synaptic position after injury. New AChRs are synthesized to contribute to rebuilding the NMJ structure or existing AChRs re-aggregate to their original location. We hypothesised that satellite cells (SCs) contribute to de novo AChR resynthesis.

BALB/C mice were subjected to lower hindlimb crush injury. Gastrocnemius and plantaris muscles were harvested on day 1, 3, 5, 7, 10, 14 and 28 post-injury. Bromodeoxyuridine (BrdU) was administered via mini-osmotic pumps for 7 days post injury. Longitudinal sections were assessed using super resolution confocal microscopy (Carl Zeiss). We used fluorochrome conjugated α-bungarotoxin to visualise the NMJ and Hoechst to ascertain localisation of nuclei. SCs were identified using CD56. Z-stack images were used to create 3-D images of the NMJ/nuclei relationship. Snap-frozen samples were analyzed for AChR subunit mRNA.

We observed accumulation of BrdU+ SCs at the site of injury in proximity of the NMJs. This localisation alludes to a specialised role for SC's in the events surrounding neuromuscular regeneration.
South African MRC and NRF.

Therapy

110. l-2-oxothiazolidine-4-carboxylate (OTC) protects dystrophic muscles from damage in mdx mice

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Oxidative stress, caused by reactive oxygen species (ROS), have been hypothesized to exacerbate disease pathology in muscle. One mechanism whereby ROS can cause cellular dysfunction is by oxidising protein thiol groups to disrupt protein function. We developed a fluorescent labelling technique to measure protein thiol oxidation. Using the mdx mouse model of dystrophy, we found protein thiol oxidation was elevated in dystrophic muscles, which included contractile proteins as well as proteins involved in calcium handling and glycolysis. Protein thiol oxidation in mdx muscle was high in pre-necrotic areas, suggesting a role in pathology. Therefore we have tested the effectiveness of OTC (l-2-oxothiazolidine-4-carboxylate), a cysteine based drug that targets protein thiol oxidation, to decrease protein thiol oxidation in dystrophic mdx muscles. Six weeks of OTC treatment significantly decreased protein thiol oxidation in 12 week old mdx muscles. OTC also improved muscle grip strength, decreased plasma creatine kinase (myofiber damage), decreased myeloperoxidase activity (inflammation) and restored muscle taurine to normal levels. OTC has potential as a drug intervention to decrease disease severity in Duchenne Muscular Dystrophy.

111. Muscle structure influences utrophin expression in mdx mice

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Duchenne muscular dystrophy (DMD) is a severe muscle wasting disorder caused by mutations in the dystrophin gene. To examine the influence of muscle structure on the pathogenesis of DMD we generated mdx4cv:desmin double knockout (dko) mice. The dko male mice died of apparent cardiorespiratory failure at a median age of 76 days compared to 609 days for the desmin-/- mice. An ~2.5 fold increase in utrophin expression in the dko skeletal muscles prevented necrosis in ~91% of 1a, 2a and 2d/x fiber-types. In contrast, utrophin expression was reduced in the extrasynaptic sarcolemma of the dko fast 2b fibers leading to increased membrane fragility and dystrophic pathology. Utrophin could form costameric striations with alpha-sarcomeric actin in the dko to maintain the integrity of the membrane, but the lack of restoration of the NODS complex and desmin coincided with profound changes to the sarcomere alignment in the diaphragm, deposition of collagen between the myofibers, and impaired diaphragm function. We conclude that the dko mice may provide new insights into the structural mechanisms that influence endogenous utrophin expression that are pertinent for developing a therapy for DMD.

Therapy

112. Systemic AAV-mediated RNA interference improves RNA toxicity in a mouse model of myotonic dystrophy

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RNA interference (RNAi) offers a promising approach for dominant genetic disorders that involve gain-of-function mechanisms. One candidate disease for RNAi therapy is myotonic dystrophy type 1 (DM1). DM1 is caused by expansion of a CTG repeat in the 3' UTR of the DMPK gene. The expression of DMPK mRNA containing an expanded CUG repeat (CUGexp) leads to defects in RNA processing. We designed miRNA-based RNAi hairpins to target the CUGexp in the human alpha-skeletal actin long-repeat (HSALR) mouse model of DM1. RNAi expression cassettes were delivered to HSALR mice using adeno-associated virus (AAV) shuttles injected intravenously. Vector delivery reduced disease pathology in muscles of the HSALR mice, including a reduction in the CUGexp mRNA, a reduction in myotonic discharges, improved patterns of pre-mRNA splicing, reduced myofiber hypertrophy, and a decrease in myonuclear foci containing the CUGexp mRNA. Improvements in hallmarks of DM1 in the AAV RNAi-treated HSALR mice indicate that myonuclear defects characteristic of DM1 can be improved with a systemic RNAi approach. Efficient AAV-mediated delivery of RNAi has the potential to provide a long-term therapy for DM1 and other dominant muscular dystrophies. (NIH, MDA)

113. Intramuscular Heterogeneity in Duchenne Muscular Dystrophy Determined By Magnetic Resonance Imaging

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Duchenne muscular dystrophy (DMD) is characterized by progressive muscle degeneration, loss of functional abilities, and early mortality. Differences between muscles affected in DMD are established, but less is known about alterations within individual muscle groups. Thus far, Magnetic Resonance (MR) imaging has been used to assess muscle pathology at the mid-belly region of muscles only. This study investigated intramuscular heterogeneity along the entire length of several muscle groups in DMD and control subjects.

MR images were acquired on a 3T whole body scanner. A grading scale was used to assess involvement throughout the length of the lower legs. Our techniques confirmed that some muscles are selectively affected, while others are relatively spared across subjects. Most importantly, it was determined that greater involvement occurred preferentially at the tendinous insertion, particularly in the earlier stages of disease.
Myogenic enhancers regulate expression of Therefore, these findings emphasize the importance of evaluating the entire length of muscle to determine the full extent of DMD, particularly in the early stages of the illness or when effects of interventions are monitored. Funding was provided by NIAMS, MDA, and PPMD.

Therapy

114. Dietary sphingolipids modulate muscle power and inflammation in mdx mice.

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Duchenne Muscular Dystrophy (DMD) is characterized by progressive muscle degeneration and inflammation. Evidence suggests sphingolipid metabolites can modulate the inflammatory response in cancer, but may also modulate skeletal muscle Ca2+ handling. We tested these possibilities in mdx mice. C57BL10 (WT) and mdx mice were fed AIN 76A diet ±0.1% Sphingomyelin (S) for 7 wks starting at age 4 wks (WT±S, mdx±S). At age 11 wks, in mdx+S vs WT mice, inflammatory markers were unchanged (IL-6) or up-regulated (Acp5, 63-fold and C1qa, 45-fold; $p<0.05$). WT+S in vitro EDL stress output was greater than all groups at 50-150 Hz. In mdx+S vs mdx mice, max power occurred at a lower activation frequency (19 vs 37 Hz). Preliminary data suggest SERCA1 levels were unchanged by S, whereas DHPRα1 levels were reduced in both WT+S (~91% vs WT) and mdx+S (~59% vs mdx; both $p < 0.05$), but were 4.6-fold higher in mdx+S vs WT+S ($p <0.05$). These data suggest that in mdx mice, 0.1%S modulates the inflammatory gene signature, and reduces the activation frequency for max muscle power. The mechanism for increased power in mdx mice treated with 0.1%S is presently unclear. We suggest dietary sphingolipids could potentially improve muscle power in DMD boys.

115. A Deep Intronic Mutation in Dysferlin Leads to Expression of a Pseudoexon in Miyoshi Myopathy Patient Cells: Use of Antisense Oligonucleotides to Promote Normal DYSF Expression

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Abnormal dysferlin expression leads to Miyoshi myopathy and limb-girdle muscular dystrophy type 2B. Using myogenic cells from Miyoshi myopathy patients, we recently identified a new deep intronic mutation in dysferlin intron 44i that alters the splicing of mRNA and leads to the inclusion of a pseudoexon between exons 44 and 45 (DYSF PE44.1). DYSF PE44.1 maintains the reading frame, adding 59 amino acids within the conserved C2F domain, likely disrupting function. We used an exon-skipping strategy and antisense oligonucleotides (AONs) to induce altered splicing in this PE44.1 mutant region of DYSF mRNA. This mutation provides a unique opportunity to evaluate AON-mediated exon-skipping because AONs designed to prevent DYSF PE44.1 splicing will restore the normal mRNA splicing pattern and thus normal protein (as opposed to a modified or truncated version currently possible with other exon targets). We show that AONs targeting DYSF PE44.1 reduce expression of the mutant mRNA splice form and restore higher levels of the normal form of mRNA and greater protein levels in vitro. Understanding the efficiency and biological effects of this process will advance the use of AONs toward clinical application for dysferlinopathies.

Therapy

116. Safe and bodywide muscle gene transfer in young adult Duchenne muscular dystrophy dogs

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The ultimate goal of Duchenne muscular dystrophy (DMD) gene therapy is to treat all muscles in the body. Global gene transfer has been demonstrated in rodents using adeno-associated virus (AAV). However, translation to large mammals has encountered formidable challenges. Over the last few years, we have developed safe and bodywide AAV transduction in newborn normal dogs. However, severe side-reactions were observed when AAV was delivered systemically to affected puppies. Investigators had to euthanize the dogs because of marked inflammation, growth delay, muscle atrophy and contractures. Here we report the first successfully whole body AAV delivery in a young adult DMD dog. A total of 7E14 particles of Y731F AAV-9 were injected intravenously to a 2-m-old affected dog. The dog tolerated injection well. General condition and body weight of the dog were stable throughout the study. Hematology and blood biochemistry were unremarkable. One-month muscle biopsy showed robust gene transfer. To examine bodywide transduction efficiency, the dog was euthanized at 14 weeks after injection. We observed widespread transgene expression in every muscle. On average, the transduction efficiency reached 30% (range, 5 to 80%).

117. Preliminary Clinical Safety and Biological Activity Data of HT-100, a Novel Therapy for Duchenne Muscular Dystrophy

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In DMD, a chronic inflammatory response leads to abnormal fibrosis. The resulting endomysial fibrosis directly impairs force transmission and muscle regeneration, and decreases muscle perfusion and is the only myopathologic parameter correlated with poor motor and cardiorespiratory outcome. HT-100 is an orally-delivered anti-fibrotic, anti-inflammatory agent with effects on molecular, pathology, and functional phenotypes of dystrophic mice, making it a promising candidate for DMD.

Studies HALO-DMD-01 and HALO-DMD-02 are open-label, single and multiple ascending dose and Extension studies to evaluate the safety, tolerability, PK, and early PD signals of HT-100 in steroid-treated DMD boys. Total treatment duration is approximately 7 months. Multiple safety, PK and exploratory PD endpoints, including serum biomarkers of fibrosis and inflammation are being assessed.

To date, 17 boys have been enrolled in 3 Cohorts. The boys were exposed to HT-100 at doses ranging from 0.005 to 0.02 mg/kg/d for up to 92 days. There have been no safety signals to date. Preliminary biomarker and safety data will be presented.

Therapy

118. Next Generation Readthrough Drugs to Treat Nonsense Mediated Muscular Dystrophies

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Nonsense mutations result in premature termination of protein translation and are the cause of at least 5 to 15% of the individual cases of most inherited diseases. PTC Therapeutics has developed ataluren (PTC124) an orally delivered, small molecule that is currently being tested in a pivotal trial for DMD. PTC-408 is structurally distinct from ataluren and promotes readthrough by a novel mechanism different from that of aminoglycosides or ataluren. Full length readthrough protein can be detected in cell models of readthrough at concentrations of PTC-408 as low as 10 nM. In MEFS derived from mice with a nonsense-mutation causing mucopolysaccharidosis type I-Hurler (MPS I-H), PTC-408 produced functional, full length α-L-iduronidase. Preclinical testing in the mdx mouse, a model of Duchenne muscular dystrophy (DMD), demonstrated that PTC-408 induces production of full length dystrophin protein. Our goal is to identify and develop a next generation readthrough drug with greater activity and potency than ataluren to treat DMD and other diseases caused by nonsense mutations.

119. Determining the minimal functional unit of gamma sarcoglycan

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Mutations in the gene encoding sarcoglycan, gamma (SGCG) lead to muscular dystrophy. The SGCG gene is composed of 8 exons. The most common mutation in SGCG is a deletion of thymidine 525 in exon 6, resulting in a frame shift and loss of protein. To restore the reading frame, skipping of exons 4-7 would be required, resulting in an internally truncated protein, referred to as “mini-gamma”. The functionality of mini-gamma was tested using heterologous expression in cells, or in separate experiments transgenesis in sarcoglycan null flies or mice. Expression of mini-gamma in flies demonstrated correct intracellular localization, similar to full-length sarcoglycan. Additionally, mini-gamma restored the heart function and improved the skeletal muscle function of sarcoglycan null flies. In transgenic mice, mini-gamma was enriched in membrane-bound fraction of muscle lysates and correctly localized at the muscle plasma membrane. Co-immunoprecipitation demonstrated that mini-gamma interacted with other sarcoglycans. These data provide evidence that mini-gamma is a stable protein that assumes the molecular role of full-length gamma sarcoglycan. Therefore, exon skipping is a viable strategy to correct LGMD 2C.

Therapy

120. Tricyclo-DNA a new chemistry for splice-switching approaches *(No Poster)*

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Antisense oligonucleotide technologies are promising therapies for numerous disorders including the classical neuromuscular disease Duchenne muscular dystrophy causing progressive deterioration of muscle function, cognitive impairment, cardiomyopathy, respiratory failure and premature death. However, although dystrophin restoration using naked oligonucleotide compounds has already been published in mouse and in clinical trials, these studies have failed to show marked clinical benefit.

Here, we demonstrate the therapeutic potential of a new class of DNA analogs (tcDNA) that fulfills most important prerequisites for splice-switching strategies. This novel chemistry represents a major advance because of the unprecedented efficiency of dystrophin rescue in all muscles, heart and brain, after intravenous administration in DMD mouse models. We show that whole-body rescue of dystrophin was correlated with improvement of critical parameters such as the respiratory function, which is the main cause of death in DMD patients, as well as cognitive enhancement.

The very properties of tcDNA-AONs make them particularly attractive for life-changing therapies in many neuromuscular diseases including neurologic disorders.

121. Overexpression of murine sarcospan ameliorates muscle pathology in the mdx mouse model

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Duchenne muscular dystrophy (DMD) is a degenerative muscle disorder caused by mutations in the dystrophin gene and characterized by progressive muscle wasting. Dystrophin is a component of the dystrophin-glycoprotein complex (DGC), which provides structural stability during muscle contraction. One of the core components of the DGC is the transmembrane protein sarcospan (SSPN). We have previously shown that moderate overexpression of human SSPN (3-fold) ameliorates dystrophy in mdx mice. However, mice expressing high levels of hSSPN transgene (10-fold and greater) exhibit a severe muscle phenotype and early lethality. To determine whether this pathology is specific to overexpression of human SSPN, we generated new transgenic lines overexpressing murine SSPN. Mice expressing high levels of mSSPN (approximately 20-fold) appear healthy and exhibit no muscle phenotype, demonstrating that high levels of SSPN are not inherently toxic. We show that overexpression of mSSPN ameliorates dystrophic pathology in mdx skeletal muscle, and we additionally evaluate improvement in muscle strength. These results support the use of SSPN as a therapeutic target in DMD and bring us closer to developing an applicable therapy (NIH NIAMS, MDA USA).

Therapy

122. Long-term phosphodiesterase 5 inhibition with Tadalafil preserves heart function in GRMD dogs

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Cardiomyopathy is a leading cause of death among Duchenne muscular dystrophy (DMD) patients. Inhibition of the cGMP-selective phosphodiesterase (PDE), PDE5, has received much interest in the last decade as a treatment for heart failure, and more recently for both skeletal muscle and cardiac components of DMD. The purpose of this study was to evaluate the efficacy of long-term PDE5 inhibition in the prevention of dystrophic cardiomyopathy. Nine-month old golden retriever muscular dystrophy (GRMD) dogs were treated with the PDE5 inhibitor Tadalafil for 16 months, and were examined by echocardiogram after 9, 12, and 16 months of treatment. Treated dogs demonstrated highly preserved left ventricular systolic and diastolic function, most notably ejection fraction and fractional shortening were increased, while relaxation time was reduced compared to control dogs during the course of this study. Histological evaluations revealed substantially reduced signs of pathology and fibrosis with Tadalafil treatment. Importantly, no adverse effects of long-term treatment were observed. These preclinical data provide strong support for the use of PDE5 inhibition for the treatment of DMD cardiomyopathy.

123. Substantial improvement of dystrophin-deficient skeletal muscle phenotype in a preclinical evaluation of CAT1041 compound

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Inhibitors of the nuclear factor kappa B (NFkB) pathway have demonstrated promising efficacy in reducing the skeletal muscle deterioration and fibrotic replacement associated with dystrophinopathies. In this preclinical study, CAT1041, an orally-delivered NFkB inhibitor, was evaluated as a potential treatment option for Duchenne muscular dystrophy (DMD). Using mdx mice that were fed CAT1041 in chow, short-term treatment showed reductions in muscle damage and increases beneficial signaling markers compared to control-diet fed animals, while long-term treatment muscles revealed greater resistance to mechanical damage. In mdx mice allowed ad libitum access to running wheels for 6 months to worsen dystrophic phenotype, CAT1041 treatment resulted in increased running and greater muscle hypertrophy than control diet mice. CAT1041 treatment also greatly improved limb muscle and diaphragm function, while reducing incidence of fibrosis in this cohort of mice. Furthermore, 9 months of CAT1041 treatment in the GRMD dog model of DMD yielded improved ventilatory function and less fibrosis than untreated GRMD dogs. These data suggest that CAT1041 has strong potential as a treatment option for DMD patients.

Therapy

124. Modified steroids for DMD *(No Poster)*

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Glucocorticoids (prednisone and deflazacort) are considered standard of care for Duchenne muscular dystrophy, with an acute (3-6 months) increase in strength and mobility, delayed loss of ambulation, and less scoliosis. However, extensive side effects profiles from chronic steroid use are a concern, including weight gain, osteopenia, mood changes (behavioral outbursts), growth stunting, delayed pubertal onset, and adrenal suppression. A common side effect seen in other indications of glucocorticoids is muscle wasting via the AKT1/FOXO pathway – this pathway is also stimulated in dystrophin-deficient muscle and may mitigate some of the strengths gains. We chemically dissected three subactivities of glucocorticoids (VBP15). This drug shows ~100-fold reduction of the transactivation subproperty (GRE-mediated transcriptional response), retained transrepression activity (NFkB inhibition), and improves physicochemical membrane properties of VBP15 were improved relative to prednisone, enabling greater membrane stability in dystrophin-deficient myofibers. VBP15 is entering clinical development, and the proposed clinical program is discussed.

125. Impaired viability of muscle precursor cells in muscular dystrophy with glycosylation defects and amelioration of its severe phenotype by limited gene expression

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Dystroglycanopathy (DGpathy) is caused by abnormal glycosylation of dystroglycan (DG). To establish effective treatment, we generated 2 distinct conditional knock-out (cKO) mice for fukutin, one of the causative genes for DGpathy. Myofiber-selective fukutin-cKO mice showed mild muscular dystrophy whereas muscle precursor cell (MPC)-selective cKO mice exhibited severe phenotypes. MPC-selective cKO mice showed impaired MPC proliferation, differentiation, and muscle regeneration, suggesting that these abnormalities contribute to the severe pathology. Since our data suggested that frequent cycles of degeneration/regeneration accelerate substantial and/or functional loss of MPC, we expected that protection from myofiber degeneration provides therapeutic effects even in mouse models with MPC defects; therefore, we restored fukutin expression in myofibers. Adeno-associated virus (AAV)-mediated rescue of fukutin that was limited in myofibers ameliorated the severe pathology even after disease progression. Considerably low AAV titers were associated with therapeutic effects. Our findings indicated that fukutin-deficient DGpathy is a regeneration-defective disorder, and gene therapy is a feasible treatment for the wide range of DGpathy.

Therapy

126. Reducing dynamin 2 rescues myotubular myopathy in mice

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Centronuclear/myotubular myopathies (CNM) are associated with muscle weakness and nuclei misposition in skeletal muscle. Currently, no effective treatments exist. Loss of MTM1 (myotubularin) or overexpression of wildtype DNM2 (dynamin 2) in skeletal muscle in mice cause a CNM-like phenotype, suggesting MTM1 and DNM2 function in a common pathway, where either MTM1 loss-of-function or DNM2 gain-of-function lead to the CNM phenotype.
To test this hypothesis, we reduced the expression of DNM2 in Mtm1-/y mice that reproduce a CNM phenotype with a progressive myopathy leading to death by 6-12 weeks. Mtm1-/yDnm2+/- mice survived up to 2 years, classical CNM histology including fiber atrophy and nuclei mispositioning were prevented or strongly delayed and reduced, and muscle strength was increased. Downregulation of Dnm2 selectively in skeletal muscle in young Mtm1-/y mice showed the rescue is cell autonomous and can potentially revert the progression of the phenotype. We identified MTM1 and DNM2 are in a common pathway regulating muscle organization and force. We introduce the original concept of 'cross-therapy' where one form of the disease (MTM1) can be rescued by decreasing expression of another gene mutated in CNM (DNM2).

127. Treatment with ActRIIB-mFc improves lifespan, behavior and pathology in the Acta1 H40Y murine model of nemaline myopathy

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Nemaline myopathy (NM) is one of the three main types of congenital myopathy, and it is associated with mutations in nine genes. NM is currently diagnosed on muscle biopsy by the presence of cytoplasmic rod-like structures (nemaline rods), although these structures may not be major contributors to the weakness seen in this disease. Myofiber smallness is also found in many cases of NM and may represent a cause of weakness that can be counteracted by treatment. In this study, we used ActRIIB-mFc (an inhibitor of myostatin signaling) to induce myofiber hypertrophy in the Acta1 H40Y mouse model of nemaline myopathy to evaluate this treatment strategy. Acta1 H40Y mice develop weakness and nemaline rod formation, and half of untreated male hemizygote mice die between 1-3 months of life. ActRIIB-mFC treatment of Acta1 H40Y mice has thus far shown dramatic improvements in lifespan, animal mass, and open field activity and more modest trends toward increased forelimb grip strength. Early pathological studies also indicate increased muscle mass (across all major muscles) and myofiber size in ActRIIB-mFc treated mice. These preliminary studies demonstrate the substantial potential of ActRIIB-mFc in the treatment of NM.

Therapy

128. TWEAK/Fn14, a novel pathway and therapeutic target in myotonic dystrophy

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Myotonic dystrophy type 1 (DM1), the most prevalent muscular dystrophy in adults, is characterized by progressive muscle wasting and multi-systemic complications. DM1 is the prototype for disorders caused by RNA toxicity. Currently, no therapies exist. Here, we identify that Fn14, a member of the TNF receptor super-family, is induced in mouse models of RNA toxicity and in tissues from DM1 patients, and that its expression correlates with severity of muscle pathology. This is correlated with increased signaling through the NFKB pathways in both mouse and human tissues. In mice with RNA toxicity, genetic deletion of either Fn14 or its ligand, Tweak, results in increased survival, reduced muscle pathology and better function. This is associated with reduced NFKB activation and evidence of increased muscle regeneration and increased PGC-alpha expression. Importantly, blocking TWEAK/Fn14 signaling with an anti-TWEAK antibody likewise increases survival, improves muscle histopathology and functional outcomes in affected mice. These results reveal new avenues for therapeutic development and provide proof of concept for the first clinically available therapy to treat muscular dystrophy in DM1.

129. Suppression of nonsense mutation with Arbekacin, an authorized aminoglycoside antibiotic in mouse models and human cells of Duchenne muscular dystrophy

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Translational readthrough of premature termination codons is a promising therapeutic approach for genetic diseases caused by nonsense mutations. Here, to identify a potent readthrough-inducing drug with lower toxicity than gentamicin, we have screened a library of kanamycin-related antibiotics. Arbekacin was identified as a potent inducer of readthrough activity in the READ mouse, a transgenic strain that was developed for the detection of readthrough activity. Subcutaneously administered arbekacin to mdx mice promoted the accumulation of dystrophin, reduction of serum creatine kinase activity, and improved contractile function. Moreover, arbekacin treatment helped restore dystrophin expression in human muscle cells isolated from Duchenne muscular dystrophy (DMD) patients with nonsense mutations in the dystrophin gene. Together, these results suggest that arbekacin represents an important chemical entity for the potential treatment of DMD and other genetic disorders caused by nonsense mutations. Arbekacin is now on clinical trial for DMD patients with nonsense mutation in Japan.

Therapy

130. Skeletal muscle damage and contraction-induced torque loss are attenuated in mdx/Utr-/- mice by SERCA1 overexpression

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Elevated intracellular Ca2+ has been implicated in DMD disease progression. SERCA1 overexpression was previously shown to ameliorate the dystrophic phenotype in the mdx model. Our goal was to assess the effects of SERCA1 overexpression in the more severe mdx/Utr-/- mouse model of DMD. Methods: WT, mdx/Utr-/- and mdx/Utr-/-/+SERCA1 mice were used at ~12 wks of age. Results: Body and muscle mass were reduced in mdx/Utr-/- vs. WT but in mdx/Utr-/-/+SERCA1 there was no difference from WT. Maximal isometric torque was reduced by 76% in mdx/Utr-/- vs. WT but attenuated to 43% by overexpression of SERCA1. Markers of muscle damage (% central nucleated fibres, necrotic area, and serum creatine kinase) were higher in mdx/Utr-/- vs. WT and all were attenuated in mdx/Utr-/-/+SERCA1. Torque deficit after eccentric injury was 100±1.0% in mdx/Utr-/- vs. 37±6.5% in WT mice and attenuated to 56±2.5% in mdx/Utr-/-/+SERCA1 mice. Conclusion: These data indicate that SERCA1 overexpression ameliorates functional impairments and cellular markers of damage in the more severe mdx/Utr-/- mouse model. Further, these findings support targeting intracellular Ca2+ control as a therapeutic approach to DMD.

131. In vitro stability of gene therapy and exon-skipped dystrophins

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Despite its large size, dystrophin is a highly stable protein, demonstrating cooperative unfolding during thermal denaturation as determined by circular dichroism spectroscopy. In contrast, internal deletions have been associated with loss of cooperative unfolding and protein aggregation. Several emerging therapy options for DMD utilize internally deleted micro-dystrophins and multi-exon skipped dystrophins that produce partially functional proteins but the stability of such internally-truncated proteins has not been investigated. In this study, we analyzed the in vitro stability of dystrophin gene therapy constructs as well as dystrophins skipped around exon 45. Our results reveal that not all gene therapy constructs display stability consistent with full-length human dystrophin. However, dystrophins skipped in-frame around exon 45 all show stability profiles congruent with intact human dystrophin. Our results suggest that the in vitro stability of human dystrophin is less sensitive to deletions at natural exonic boundaries than larger deletions present in some gene therapy constructs. Supported by Ryan's Quest, the MDA (218545) and the NIH (RO1 AR042423 and P30 AR057220).

Therapy

132. Macrophages modulate skeletal muscle restoration in a volumetric muscle loss model

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Despite the robust capacity of skeletal muscle to regenerate, in volumetric muscle loss (VML) injuries, muscle's intrinsic repair mechanisms are inadequate to replace functional tissue. The use of a decellularized ECM scaffold as a therapeutic approach for functional skeletal muscle reconstruction has been used in various animal models with disparate outcomes, and there is currently a search for therapies to improve scaffold integration and muscle regeneration. The success of biological scaffold incorporation is primarily dependent on the type of cell infiltrate and duration of the inflammatory response post implantation. We have shown that inflammatory infiltrate of the ECM is heavily dominated by CD45+/CD11b+ cells up to 21 days post implantation. Interestingly, only 30% of CD11b+ cells were identified as macrophages (Mp) at 14 days. Mp play an important role in the degradation and remodeling process of ECM scaffolds and promote skeletal muscle repair. We believe increasing Mp numbers in the ECM will hasten the muscle repair process, and to further enhance the regenerative response, Mp were coinjected with mesenchymal stem cells. Our data suggest Mp increase functional restoration in our VML injury model. (NIH to LJS)

133. Repurposed cancer therapeutics as treatments for DMD

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We have identified tyrosine phosphorylation of dystroglycan, the key transmembrane laminin receptor, as central to the loss of the entire DGC from the sarcolemma in Duchenne muscular dystrophy (DMD). Preventing phosphorylation of dystroglycan in mdx mice by mutation of a key tyrosine residue ameliorates the dystrophic phenotype. Studies in mouse myoblasts also demonstrate that treatment with proteasome or tyrosine kinase inhibitors increases levels of non-phosphorylated dystroglycan. Furthermore, by inhibiting tyrosine phosphorylation, ubiquitination or proteasomal degradation in sapje zebrafish, a fish model of DMD, dystroglycan phosphorylation was reduced and the dystrophic phenotype rescued. A significant improvement in sapje swimming ability was observed using tyrosine kinase or proteasome inhibitors already FDA approved as cancer therapeutics.
These studies demonstrate the potential of inhibiting dystroglycan tyrosine phosphorylation as a therapeutic strategy for DMD. Several of the effective compounds are already in clinical use so obtaining orphan drug status for their repurpose could be a rapid and effective route to DMD therapy in their own right or as adjuncts to other therapies currently in clinical trials.

Therapy

134. Utrophin (Utrn) upregulation via AAV let7 miRNA sponge in vivo

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Utrn, the autosomal homologue of dystrophin is of great therapeutic interest since its over-expression can compensate for dystrophin's absence in animal models of DMD. Utrn is subject to considerable let7 miRNA-mediated repression and blocking the interaction is predicted to "repress the repression" and upregulate Utrn expression. To enable this strategy we generated a miRNA sponge containing 8 repeats of the let-7 binding region of the Utrn 3'UTR (pZac-let7). Transfection into C2C12 cells led to c. 1.3 fold Utrn protein upregulation ($p<0.05$); levels comparable to those achieved using 2OMePS oligos to block let7 interaction with 3'UTR of Utrn. In preclinical studies, 4 wk old mdx mouse tibialis anterior (TA) muscles were injected with AAV2/9-pZac-let7 sponge and controls injected with AAV2/9-pZac in vivo. After one month AAV-pZac-let7 sponge treated mdx muscles showed biochemical, morphological and physiological signs of improvement. Treated mice had c. 2-fold upregulation of Utrn protein, 13% less CNF's and c. 15% reduction in force drop at 1 and 5 min after in situ lengthening contractions. Together, these studies support for miRNA repression-mediated utrophin upregulation as a promising therapeutic development for DMD. (MDA)

135. Role of dystrophin quantification in DMD trials *(No Poster)*

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Duchenne muscular dystrophy (DMD) is caused by mutations in the dystrophin gene, which prevent the full translation of dystrophin. Experimental therapies aimed at restoring dystrophin expression are progressing in clinical trials and dystrophin quantification appears a logical study outcome. However a number of issues require attention. Firstly, it is necessary to use standardised biochemical outcome measures to reliable quantify dystrophin. This is not trivial due to the scarcity of this protein; the lack of a protein standard for quantification; and different properties of mutant dystrophin. Secondly, while it is clear that dystrophin can be used as a pharmacodynamics biomarker, it is less clear if it could also be used as a surrogate endpoint. This implies that there should be a correlation between dystrophin expression and clinical benefit. Several variables play a role, ranging from the age and residual muscle mass of treated patients, the type of dystrophin restored (i.e. different deleted proteins might have different properties) and the pattern of protein expression (i.e. patchy versus homogenous). My presentation will be focused on these issues

Therapy

136. Dietary sodium nitrate alleviates functional muscle ischemia in Becker muscular dystrophy

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Mutations in the gene encoding the cytoskeletal protein dystrophin lead to skeletal muscle nitric oxide (NO) deficiency and functional muscle ischemia. The NO-cGMP pathway therefore constitutes a putative new drug target for dystrophinopathy. Indeed, we and others have shown that acute treatment with a phosphodiesterase 5 (PDE5) inhibitor alleviates functional muscle ischemia in dystrophic skeletal muscle. However, PDE5 inhibition does not rescue skeletal muscle production of NO, and thus may not fully recapitulate the cytoprotective effects of endogenous NO production. We therefore asked whether acute treatment with sodium nitrate—a natural NO-donor—would also alleviate functional muscle ischemia in dystrophinopathy patients. First, we show that the vasoconstrictor response during exercise—measured as a decrease in muscle oxygenation to reflex sympathetic activation— is not appropriately attenuated. Then, we show that acute oral treatment with sodium nitrate restores blood flow regulation and alleviates functional muscle ischemia. These data suggest that sodium nitrate can recapitulate the normal regulation of blood flow in exercising dystrophic skeletal muscle, which is normally achieved by sarcolemmal nNOS.

137. Zinc is a promoting factor of proliferation and activation on myogenic cells through insulin pathway

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Zinc is one of the essential elements in the human body that has multiple roles, such as cell growth and DNA synthesis. However, the effects of zinc on myogenic cells are not fully investigated. In this study, we focused on the effects of zinc on myogenic cells. We first examined the effect of zinc on proliferation of C2C12 by adding EdU to proliferating C2C12 cells. EdU-incorporated cells were increased with ZnCl2. We also used reserve cell of C2C12 as a model of quiescent MSCs in vitro to investigate the role of zinc on activation of myogenic cells. The percentage of BrdU positive cells was increased with ZnCl2 in a dose dependent manner. Furthermore, we focused on insulin signal pathways. We found that both Akt and ERK were phosphorylated after ZnCl2 treatment, and these proteins involved in activation of reserve cells. Moreover, we assessed combinatorial effects of zinc and insulin. The combination dramatically increased the percentage of BrdU positive cells compared with cells treated with each agent individually, and enhanced phosphorylation of Akt. We concluded that zinc is a promoting factor for proliferation and activation of myogenic cells. (supported by MEXT)

Therapy

138. Adeno-Associated Virus-8 and 9 Yields Unique Systemic Transduction in Newborn Dog Striated Muscle

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Adeno-associated virus (rAAV) is a highly promising gene therapy vector for treating inherited diseases such as Duchenne muscular dystrophy (DMD). Numerous studies have shown efficient systemic gene transfer with AAV-8 and 9 in rodents. However, few studies have examined these AAV vectors in large animals. Here we compared AAV-8 and AAV-9 in neonatal dogs using an alkaline phosphatase (AP) gene vector. The highly purified AAV vector was delivery via the jugular vein to newborn puppies in the absence of pharmacological intervention or immune suppression. Two different doses were tested including a low dose at ~2e14 vg/kg body weight and a high dose at ~8e14 vg/kg body weight. AP expression was examined at the age of 2.5 or 12 months by whole body necropsy. Both AAV-8 and AAV-9 resulted in robust transduction in skeletal muscles in a dose-independent manner. Minimal expression was found in internal organs. Heart transduction showed dose-dependence but AAV-8 consistently yielded significantly higher transduction than AAV-9 at the same dose. Our results suggest that AAV-8 may represent a better vector for body -wide muscle gene transfer in dogs.

139. Antisense oligonucleotide-mediated knockdown of TGF-β/myostatin type I receptors as a potential therapy for Duchenne muscular dystrophy.

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Duchenne Muscular Dystrophy (DMD) is characterized by skeletal muscle fibrosis and impaired muscle regeneration. TGF-β/myostatin signaling is shown to be directly involved in the progression of muscular dystrophy. We therefore have developed a method to selectively inhibit the function of type I myostatin/TGF-β receptors Acvr1b (ALK4) and Tgfbr1 (ALK5) with antisense oligonucleotide (AON)-mediated exon skipping in vitro and in DMD mouse models (mdx and mdx/utrophin-/-).

Our results show efficient downregulation of both ALK4 and ALK5 after AON-mediated exon skipping and ALK5 downregulation showed increased myogenic differentiation in vitro using C2C12 cells and primary myoblasts from different DMD mouse models. In addition, efficient AON-mediated ALK4 and ALK5 knockdown was achieved in vivo after intramuscular injection in mdx mice. Similar to in vitro experiments, short-term ALK4 and ALK5 AON treatment resulted in significant increase in myogenic gene expression.

To summarize, our experiments suggest AON-mediated targeting of myostatin/TGF-β receptors may provide a potential therapy to selectively inhibit myostatin and TGF-β signaling and improve muscle quality and function.

Therapy

140. Intramuscular sex steroid hormones are associated with the regulation of muscle force and power in women

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Estrogen (E2) responsive tissues, such as skeletal muscle, may suffer from hormone deficiency after menopause leading to decrements in function. However, recent reports have shown 1) E2 to be synthesized in muscle and 2) systemic and intramuscular hormone levels to be unequal. We investigated the association between intramuscular (IM) sex steroids and muscle force and power in premenopausal women and postmenopausal monozygotic twin sister pairs discordant for the use of E2-based hormone replacement. As enzymatic conversion of biologically inactive DHEA to testosterone (T) and further to E2 or dihydrotestosterone (DHT) is sequential, separate linear regression models were used for each hormone. IM E2, T, DHT and DHEA proved to be significant, independent predictors of force and power (models adjusted for age and systemic E2). We also show that, muscle cells take up and synthesize hormones parallel to transcriptional activation of their receptors. The results show intracrine actions of sex steroids to take place and to be associated with force and power regulation in skeletal muscle. Therefore, manipulating IM hormone synthesis as a means to combat muscle weakness warrants further investigation. (Funding: EU-FP7, Finnish Academy)

141. Safety and Efficacy of Dysferlin Gene Replacement Utilizing Homologous Overlap Vector Delivery

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There is no cure for dysferlinopathies, a group of related progressive muscle wasting disorders caused by absent or mutant dysferlin (DYSF). DYSF is too large to be accommodated with canonical adeno-associated virus (AAV) packaging, and thus we constructed a dual vector system using AAV to deliver and restore DYSF in muscle. Packaged in the rh.74 serotype, this two vector system (AAV.DYSF.DV) is defined by a shared1 kb region of homology. We compared the efficiency and safety of DYSF.DV delivery in DYSF deficient mice and non-human primates (NHPs) in preparation for clinical trials. Dysf-/- mice were treated with AAV.DYSF.DV by intramuscular injection, isolated limb perfusion, and systemic injection. DYSF.DV expressed efficiently when delivered through arterial limb perfusion or systemically via the tail vein and restored membrane repair capacity in a dose-dependent manner. A single systemic dose of 1012vg resulted in widespread gene expression and restored functional deficits in the diaphragm. In NHPs, DYSF.DV delivery was analyzed for gene expression and toxicity using a FLAG tag. Findings showed clear evidence that functional dysferlin was translated without toxicity laying the foundation for clinical trial.

Therapy

142. Adoptive transfer of ischemia/reperfusion-conditioned macrophages enhances functional recovery of skeletal muscle after tourniquet-induced ischemia/reperfusion injury

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Tourniquet-induced ischemia reperfusion (TK-I/R) is severe skeletal muscle injury with extensive pathophysiology. Detrimental consequences of ischemia-reperfusion (I/R) injury to skeletal muscle involve prolonged post-reperfusion recovery periods, functional impairments and loss of muscle function. Routine use of tourniquets in clinic and in the military makes TK-induced muscle injury a serious clinical problem. The etiology of I/R injury in skeletal muscle is complex and is mediated by the global tissue energy depletion, overproduction of tissue damaging reactive oxygen species, excessive inflammation, vascular damage and tissue necrosis.
We have shown that adoptive macrophage transfer of I/R-conditioned macrophages significantly enhances skeletal muscle regeneration 14 days after TK-I/R injury (as evidenced by 15% increase in functional recovery of maximal force over saline injection). At the time of transfer, macrophages exhibit several features of M2 macrophages characterized by reduced expression of inflammatory and up-regulated expression of pro-regenerative/anti-inflammatory genes. The significant upregulation of IGF-I and transient up-regulation of IL-10 gene expression may mediate beneficial effects on muscle repair.

143. TXA127 Increases Wheel-Running and Reduces Fibrosis and Oxidative Stress in Mice with Established Limb Girdle Muscular Dystrophy-2F

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Mutations in sarcoglycan delta (Sgcd) cause limb girdle muscular dystrophy-2F (LGMD-2F) in animals and humans. Angiotensin-[1-7] (Ang1-7), a peptide of renin-angiotensin system, possesses antifibrotic, vasodilator and sympathoinhibitory properties. We have reported that administration of Ang1-7 for 8-9 wks, beginning soon after weaning, prevents autonomic and skeletal muscle dysfunction in Sgcd-/- mice [Hypertension, 2012]. In this study, our goal was to determine if TXA127 (Ang1-7 developed by Tarix) would increase locomotor activity and reduce dystrophic pathology in Sgcd-/- mice after LGMD-2F is established. Sgcd-/- mice, 28-35 wks of age, were infused with saline vehicle (n=8) or TXA127 (500 mcg/kg/day, n=8) via minipumps for 8 wks. Mice were housed individually in cages containing a running-wheel to quantify activity. Quadriceps muscles were harvested for histological and oxidative stress analyses. Sgcd-/- mice treated with TXA127 exhibited a seven-fold increase in wheel-running, 55% decrease in fibrosis and 50% decrease in oxidative stress ($P<0.05$ vs. vehicle-treated). Conclusion: Our results demonstrate that administration of TXA127 provides an effective therapeutic approach in muscular dystrophy. (Tarix Pharmaceuticals)

Therapy

144. Identification, characterization and utilization of an integrin enhancing compound, IEC-9, in the treatment of Duchenne Muscular Dystrophy.

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Duchenne Muscular Dystrophy (DMD) is a progressive, muscle wasting X-linked disease that affects 1 in every 3500 males. DMD is caused by mutations within the gene encoding dystrophin that result in the absence of the protein. Recent studies have shown that the integrin alpha7beta1 is a major modifier of disease progression in mouse models of DMD and drugs that promote alpha7beta1 integrin levels in muscle may be therapeutic in the treatment of DMD. Utilizing high-throughput drug discovery technology, we identified a potent small molecule compound currently annotated as integrin enhancing compound-9 (IEC-9) that enhanced the Beta-galactosidase activity in alpha7+/lacZ mouse myogenic cells. IEC-9 increases alpha7B integrin protein expression in mouse C2C12 and telomerized human DMD myoblasts and myotubes. Initial studies also show that the kinase MAP4k4 is a potential molecular target of IEC-9, the inhibition of which could lead to increased integrin expression. Preliminary data shows mdx mice treated with a 1mg/kg/day dose of IEC-9 for two weeks had improved strength and an increased level of alpha7 integrin protein in muscle, indicating in vivo on target efficacy. Hence IEC-9 could serve as a potential therapeutic for DMD.

145. Delivery of a rAAV9 U7snRNA vector targeting exon 2 results in widespread dystrophin expression in the Dup2 DMD mouse model

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Accounting for around 6% of all cases of Duchenne muscular dystrophy, exon duplications cases provide an excellent avenue for new exon skipping therapies. We sought to test the efficacy of virally-mediated duplication skipping in the novel Dup2 mouse, modeling the most common single-exon duplication (exon 2) seen in DMD patients. A targeting construct was created containing 4 copies of a modified U7snRNA, targeting both the splice donor or acceptor sites of exon 2 (U7sn RNA-ACCA). Both TA IM injections and tail vein IV injections were done in 8 week mice and then analyzed 4 weeks later. RT-PCR reveals widespread exon 2 skipping, with the simultaneous presence of all 3 predicted transcripts – duplicated exon 2 (Dup2), wild-type, and deleted exon 2 (Del2) – in variable proportions. Dystrophin expression and location was confirmed by immunoblot as well as by immunofluorescence. These results demonstrate the utility of the Dup2 mouse model as a tool for testing potential duplication exon-skipping strategies. They confirm that delivery of rAAV9.U7snRNA is able to induce exon 2 skipping and to drive the production of a functional dystrophin protein, suggesting a promising strategy for future clinical development.

Therapy

146. A personalized, therapeutic approach for Duchenne muscular dystrophy caused by missense mutations

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Duchenne and Becker muscular dystrophies are caused by a wide variety of mutations in the gene encoding dystrophin. Nonsense mutations as modeled by the mdx mouse are specifically targeted by stop codon read-through approaches, while out-of-frame deletions can be treated by exon-skipping technologies. Patients with missense mutations are a small, orphaned population, which could also potentially benefit from personalized approaches. Here we have generated and characterized stable muscle cell lines modeling disease-causing missense mutations in full-length dystrophin. Our data suggest that missense mutant dystrophin is rapidly degraded by the proteasome, which can be countered by small molecule therapeutics. Additionally, we have generated two mouse models encoding unique, disease-causing missense mutation in dystrophin. Our initial results indicate dystrophin protein abundance correlates with the severity of disease seen in the modeled patients. Increasing mutant protein levels by proteasome inhibition may be a promising avenue of personalized therapy for muscular dystrophy patients with missense mutations. Supported by NIH grant RO1 AR042423 and AHA fellowship 12PRE12040402.

147. Sulforaphane improves muscle function and protects muscle from oxidative damage in mdx mice via NF-E2-related factor 2 signaling pathway

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Background: Sulforaphane (SFN), one of the most important isothiocyanates in the human diet, is known to have chemopreventive and antioxidant activities in different tissues via activation of NF-E2-related factor 2 (Nrf2) signaling pathway. But the effect of SFN in Duchenne muscular dystrophy (DMD) is unknown. This work was undertaken to evaluate if SFN can improve muscular dystrophy in mdx mice.
Method: 4-weeks-old male mdx mice were treated with SFN (2 mg/kg) for 8 weeks. Then the forelimb grip was checked and running distance was tested by an acute exhaustive exercise protocol in a treadmill. Then the muscles were collected for oxidative damage and antioxidant enzyme assay.
Result: SFN treatment significantly increased the muscle force and running distance of mdx mice, and the activities of plasma lactate dehydrogenase(LDH) and plasma creatine phosphokinase (CK), as well as muscle MDA levels were decreased. Further, the muscle phase II enzymes NQO1, HO-1 expression and activity were increased by SFN treatment.
Conclusion: Collectively, these results show that SFN can improve muscle function and protect dystrophic muscle from oxidative damage in mdx mice.
Key words: Sulforaphane; Nrf2; DMD; oxidative damage

Therapy

148. Clinical and Molerucla Biomarkers; trail readiness in FSHD *(No Poster)*

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The recent description of a unified model for Facioscapulohumeral muscular dystrophy (FSHD) means that there are now defined, FSHD-specific therapeutic targets. With the prospects of therapeutic trials in the near future, clinical trial preparedness become of utmost importance. The current state and future prospects for clinical outcome measures as well as molecular and imaging biomarkers will be discussed.

149. Galectin-1 protein therapy for the treatment of Duchenne Muscular Dystrophy

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Duchene Muscular Dystrophy (DMD) is a fatal neuromuscular disease that affects nearly 20,000 children. DMD is caused by mutations in Dystrophin the central component of the sarcolemmal Dystrophin Glycoprotein Complex (DGC). The Utrophin Glycoprotein Complex (UGC) and Alpha7Beta1 Integrin complex are known to stabilize skeletal muscle sarcolemma in the absence of Dystrophin. Previous studies show elevated levels of these compensatory complexes can ameliorate disease pathology in mouse models of DMD. Galectin-1 is normally found in skeletal muscle where it plays a role in muscle repair. Galectin-1 binding to critical skeletal muscle proteins led us to hypothesize that increasing Galectin-1 might serve to stabilize the sarcolemma and serve as a therapeutic for DMD. To test this hypothesis, recombinant mouse Galectin-1 (rMs Gal-1) was produced and used to treat myogenic cells. Our results show rMs Gal-1 increased protein levels of several members of both UGC and α7β1 Integrin in myogenic cells. Treated mdx mice exhibited improved functional and pathological assessment of key sarcolemmal proteins. Together our results demonstrate for the first time that Galectin-1 is an exciting new protein therapeutic for the treatment of DMD.

Therapy

150. AAV-Mediated Gene Therapy to the Rescue

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While significant progress has been made to characterize dystroglycanopathies, muscular dystrophies associated with aberrant glycosylation of alpha-dystroglycan (alpha-DG) that are linked to progressive pathological changes in skeletal muscle and exhibit a spectrum of clinical severity, few efforts have been made in developing effective treatment options. A viable therapeutic approach arises in gene therapy, one we have investigated using adeno-associated virus (AAV) mediated LARGE or FKRP expression in a mouse model with an FKRP mutation. This study provides proof-of-concept that both LARGE and FKRP expression can ameliorate the dystrophic phenotypes caused by FKRP mutation and successfully generate functional glycosylation of alpha-DG. However, because LARGE overexpression also produces hyperglycosylated alpha-DG which may be detrimental to muscle function, determining optimal levels of LARGE expression could be crucial for achieving glycosylation of alpha-DG with normal function and justify its use as a one-for-all gene therapy. In contrast, FKRP expression produces functionally glycosylated alpha-DG comparable to that in normal muscle, suggesting that FKRP gene therapy may be more suitable for FKRP-related dystroglycanopathies

151. Phosphodiesterase 5 inhibition rescues functional sympatholysis in Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is a progressive X-linked muscle wasting disease caused by mutation of the gene encoding the cytoskeletal protein dystrophin, leading to defective vasomodulation of exercising skeletal muscle and exercise-induced vasospasm. Phosphodiesterase (PDE)5 inhibition corrects each of these vascular phenotypes— at least in the mdx mouse. We sought to translate these promising preclinical findings to actual patients with DMD, by assessing exercise-induced attenuation of reflex sympathetic vasoconstriction (i.e. sympatholysis)— a protective mechanism that matches oxygen delivery to metabolic demand. Our results demonstrate: 1) that sympatholysis is impaired in contemporary DMD, despite routine treatment with medications known to rescue sympatholysis in commonly acquired adult cardiovascular diseases; 2) that acute PDE5 inhibition with tadalafil rescues sympatholysis in a dose-dependent manner; and 3) that PDE5 inhibition is the mechanism of action – as our results were confirmed with a second PDE5 inhibitor (sildenafil). Together, these data advance the vascular hypothesis of DMD, and implicate PDE5 inhibition as a novel therapeutic treatment option for patients suffering from dystrophinopathy.

Therapy

152. AAV-SERCA2a gene therapy ameliorated dystrophic symptoms in aged-mdx mice

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Duchenne muscular dystrophy (DMD) is the most common X-linked muscle disease caused by dystrophin deficiency. In dystrophic myocytes, the cytosolic calcium level is significantly elevated. The supra-physiological calcium activates calcium-sensitive protease and causes muscle degeneration and necrosis. Cytosolic Ca2+ recycling is mainly performed by the sarco/endoplasmic reticulum calcium ATPase (SERCA) pump. Here we hypothesize that adeno-associated virus (AAV) mediated SERCA2a gene transfer can improve calcium recycling and mitigate muscle disease. Dystrophin-deficient mice are mildly affected until they are very old. To test our hypothesis, we delivered the human SERCA2a gene to 22-m-old mdx mice via the tail vein at the dose of 6E12 viral genome particles/mouse. Two months after gene therapy, we observed robust bodywide SERCA2a expression by immunofluorescence staining and western blot. Forelimb grip force and several ECG parameters were significantly improved compared to that of age/sex-matched untreated mdx mice. Nevertheless, there were nominal changes in muscle histology and heart hemodynamics. Our study suggests that AAV-mediated SERCA2a gene therapy may represent a highly promising modality to treat DMD.

153. Successful use of out-of-frame exon 2 skipping induces in vivo IRES-driven expression of a highly functional N-truncated dystrophin isoform: promising approach for treating other 5' dystrophin mutations

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Most mutations that truncate the reading frame of the DMD gene result in loss of dystrophin expression and lead to Duchenne muscular dystrophy. However, amelioration of disease severity can result from alternate translation initiation beginning in DMD exon 6 that results in the expression of a highly functional N-truncated dystrophin. This novel isoform results from usage of an internal ribosome entry site (IRES) within exon 5 that is glucocorticoid-inducible. IRES activity is confirmed in patient muscle by both peptide sequencing and ribosomal profiling. Generation by exon skipping of a truncated reading frame upstream of the IRES leads to synthesis of a functional N-truncated isoform in both patient-derived cell lines and in a new DMD mouse model, where expression protects muscle from contraction-induced injury and corrects muscle force to the same level as control mice. This treatment that corrects muscle force to the same level as control mice, support that this novel therapeutic approach will be beneficial for 5% of patients with mutations within the 5' exons of DMD.

Therapy

154. Modulation of membrane repair by recombinant MG53 protein as a therapeutic approach to muscular dystrophy

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Duchenne Muscular Dystrophy (DMD) and other dystrophies arising from mutations in the dystrophin/dystroglycan complex produce pathology, at least in part, due to sarcolemmal membrane fragility. Several other forms of muscular dystrophy, including limb girdle muscular dystrophies, have been linked to defective membrane repair machinery. Mice deficient in dystrophin and utrophin (dko) exhibit early onset of muscular dystrophy, muscle weakness, and death around 10-12 weeks of age. In this study, this dko model of DMD was used to mimic the human DMD phenotype and allow the therapeutic effects of recombinant human MG53 (rhMG53) to be measured. MG53 is a tripartite motif (TRIM)-family protein shown to be an essential component of the acute membrane repair machinery. Further studies have shown that rhMG53 can increase the membrane repair capacity of cell membranes and act as a possible therapeutic agent for treatment of muscular dystrophy in the mdx mouse. Since these initial studies were conducted with a short course of treatment here we were interested to determine if chronic application of rhMG53 can cause long term improvement of dystrophic symptoms including force generation, structural defects and membrane integrity.

155. Imaging therapeutic efficacy in live mice with myotonic dystrophy

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Mis-regulated alternative splicing is a hallmark of cell dysfunction in myotonic dystrophy type 1 (DM1). Aberrant splicing outcomes in muscle are reversible in DM1 mice and biomarkers of weakness in DM1 patients. To speed drug discovery, we developed a novel mouse model for non-invasive in vivo detection of alternative splicing outcomes. This “therapy reporter” model features a DsRED/GFP bi-chromatic construct. Expression of DsRED or GFP is determined by splicing of an upstream minigene, Serca1 exon 22. Inclusion of exon 22 results in DsRED expression (high in wild-type), while exclusion of exon 22 shifts the reading frame and results in GFP expression (high in DM1). Fluorescence detection by in vivo spectroscopy using a transdermal fiber optic probe (30 - 120 seconds) and imaging (1 - 30 seconds) is rapid. Quantification of the DsRED/GFP fluorescence ratio enables precise determination of splicing outcomes that is independent of expression levels. Therapeutic ASOs increase the DsRED/GFP ratio similar to wild-type. Serial imaging identified a clear therapeutic effect within 3 doses (8 days) and confirmed a several-week duration of action while limiting the number of mice needed. Support: NIH.

Therapy

156. Magnetic resonance measures of muscle size and quality are altered in the upper extremity of 7-16 year old boys with DMD

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Duchenne muscular dystrophy (DMD) causes progressive weakness and disability, including the loss of upper extremity function. Magnetic resonance imaging (MRI) and spectroscopy (MRS) provide insight into the progression of this disease in the legs and show promise as quantitative biomarkers, but these methods have not been used to examine the upper arm or shoulder. In this preliminary study, we performed MRI and MRS examinations as well as strength and function testing of the arms of 18 boys with DMD (11 ± 2 yrs, 16 ambulatory) and 5 unaffected controls (12 ± 3 yrs). Boys with DMD displayed both atrophy and fatty infiltration in the upper arm and shoulder muscles. As well, quantitative measures of muscle quality (fat fraction measured by 1H-MRS and 3 point Dixon MRI and transverse relaxation time (T2) measured by 1H-MRS and T2 weighted MRI) were significantly altered in DMD, even in boys as young as 7. Quantitative measures of muscle fat content were significantly correlated with total Performance of Upper Limb test score. These preliminary data suggest that MR measures of muscle quality in the arms have potential to be used as biomarkers in both ambulatory and nonambulatory boys with DMD.

157. Efficacy of Glucocorticoid steroid in the FKRP mutant mice model.

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Fukutin-related protein-muscular dystrophy (FKRP-MD) is one of the most common single gene muscular dystrophies, and associated with defects in glycosylation of alpha-dystroglycan. Currently, there is no effective therapy available and no experimental therapies have been reported. Glucocorticoid steroids (steroid) have become the standard treatment for Duchenne muscular dystrophy and other muscular dystrophies, but its applicability has not been evaluated for the treatment of FKRP-MD either in an animal model or in a clinical setting. In this study, we used the FKRP P448L mutant mouse strain representing moderate LGMD2I to assess steroid and currently available non-steroid anti-inflammatory drugs for their therapeutic efficacy in vivo. Predinisolone treatment for 2 months with twice a week and daily oral administration of 5mg/kg improves muscle pathology with significant reduction in muscle degeneration. As expected, steroid treatments did not significantly improve the muscle strength. No significant changes in histology and functions of the liver and kidney were observed in steroid treated mice when compared to the saline-treated mutant mice. The results suggest a limited benefit of the steroid to the dystroglycanopathies.

Therapy

158. Identifying Alpha7 Integrin Enhancing Small Molecules for the Treatment of Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is a lethal muscle disorder caused by mutations in the gene encoding dystrophin that results in loss of the dystrophin protein. Dystrophin nucleates a transmembrane complex that links laminin in the extracellular matrix to actin in the cytoskeleton and provides structural integrity to the sarcolemma. The alpha7beta1 integrin is a heterodimeric receptor in skeletal muscle that also links laminin to the actin cytoskeleton. Studies have demonstrated that increased expression of the alpha7 integrin alleviates disease progression and improves survival in mouse models of DMD, while loss of the alpha7 integrin in dystrophin-deficient mdx mice results in more severe muscle disease. We have initiated a high throughput (403,000 compounds) drug discovery program to identify small molecules that increase alpha7 integrin in skeletal muscle. The top “hits” from this screen consists of four compound families. The on-target activity of these compounds to increase alpha7 integrin has been verified by western blotting using human DMD myotubes. Compounds identified in this screen may be used to further elucidate regulation of alpha7beta1 integrin expression and may serve as promising therapeutics for DMD.

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