The Ottawa Conference on New Directions in Biology & Disease of Skeletal Muscle

May 5-8, 2010 -- Ottawa, Canada

Keynote Speakers:

Dr. Eric Olson & Dr. Andrew Marks

Bringing together basic, clinical and translational researchers from distinct venues including academia and industry to address the challenges of understanding muscle disease including the muscular dystrophies and to form collaborations to meet these challenges.

Organizers:

Drs. Elizabeth McNally, Lee Sweeney, Rashmi Kothary, Lynn Megeney, & Robin Parks

THE OTTAWA CONFERENCE ON NEW DIRECTIONS IN BIOLOGY AND DISEASE OF SKELETAL MUSCLE

The Westin Hotel, Ottawa, CANADA

May 5th – 8th, 2010

Objective of the Conference

The goal of this meeting is to facilitate research progress with the goal of translation to therapy for muscular diseases. It brings together basic, clinical and translational researchers from distinct venues including academia and industry to address the challenges of understanding muscle disease including the muscular dystrophies and to form collaborations to meet these challenges.

The first "New Directions in Biology & Disease of Skeletal Muscle" meeting was held in 2004 in San Diego, the second was held in 2006 in Dallas, and the third was held in New Orleans in 2008. The current session is being held jointly with "The Ottawa Conference on Neuromuscular Disease", which is held every three years.

This year's conference will highlight current developments in muscle biology, disease, and therapy. It will provide opportunity for new collaborations and relationships to form and will allow young scientists to be exposed to muscle disease biology.

PROGRAM

WEDNESDAY, May 5, 2010

2 pm – 7 pm	REGISTRATION
3 pm – 4:20 pm	**Opening Session** – **Intro by Lee Sweeney** Industry Session PTC - Pfizer - Acceleron
4:45 pm – 6 pm	*"Translating basic research into therapy for muscular dystrophy"* John Porter, Ph.D., Muscular Dystrophy Coordinating Committee and National Institute of Neurological Disorders and Strokes Sharon Hesterlee, Ph.D., Parent Project Muscular Dystrophy Jane Larkindale, D. Phil., MDA Venture Philanthropy, Muscular Dystrophy Association
6 pm – 7:30 pm	**Reception at The Westin Hotel**
7:30 pm	**Keynote Speaker Eric Olson – Intro by Lynn Megeney**

THURSDAY, May 6, 2010

7:30 am – 8:30 am	Continental Breakfast + Poster setup
8:30 am – 10 am	**Session I - Muscle Biology – Chair: Lou Kunkel** Lou Kunkel, Children's Hospital Boston Stephen Tapscott, Fred Hutchinson Cancer Research Center Alfred Goldberg, Harvard Medical School
10 am – 10:20 am	Coffee
10:20 am – noon	**Session I** continued Emanuela Gussoni, Children's Hospital Boston Lynn Megeney, Ottawa Hospital Research Institute *Short Talk:* Elisabeth Barton, University of Pennsylvania *Short Talk:* Justin Percival, University of Washington
noon – 1:30 pm	Lunch
1:30 pm – 3 pm	**Session II – Therapy - Chair: Lee Sweeney** Jeff Chamberlain, Univ. of Washington School of Medicine Eric Hoffman, Children's National Medical Center, Washington Xaio Xaio, Univ. North Carolina Eshelman School of Pharmacy
3 pm – 3:20 pm	Coffee
3:20 pm – 4:50 pm	**Session II** continued Lee Sweeney, University of Pennsylvania School of Medicine Elizabeth McNally, University of Chicago *Short Talk:* Thurman Wheeler, University of Rochester *Short Talk:* Denis Furling, Institut de Myologie, France *Short Talk:* Jeff Molkentin, Howard Hughes Medical Institute
5 pm – 7 pm	**POSTERS (Governor General Room)**

FRIDAY, May 7, 2010

7:30 am – 8:30 am	Continental Breakfast, **Posters**
8:30 am – 10 am	**Session III - Disease Pathology I – Chair: Kevin Campbell** Eric Shoubridge, McGill University Kevin Campbell, Howard Hughes Medical Institute Maurice Swanson, University of Florida *Short Talk:* Ronald Cohn, Johns Hopkins University
10 am – 10:20 am	Coffee
10:20 am – noon	**Session III** continued Melissa Spencer, Univ. of California Los Angeles Health Sciences Kathryn Wagner, John Hopkins School of Medicine Carsten Bonnemann, The Pennsylvania Muscle Institute *Short Talk:* Seng Cheng, Genzyme Corporation
noon – 1:30 pm	Lunch
1:30 pm – 3 pm	**Session IV – Signaling – Chair: Bernard Jasmin** Bernard Jasmin, University of Ottawa Rachelle Crosbie, Univ. California Los Angeles DMD Research Center *Short Talk:* Jeff Dilworth, Ottawa Hospital Research Institute
3 pm – 3:20 pm	Coffee
3:20 pm – 4:50 pm	**Session IV** continued Gordon Lynch, University of Melbourne Robert Korneluk, CHEO Research Institute *Short Talk:* Emidio Pistilli, University of Pennsylvania
5 pm – 6 pm	**POSTERS**

6:30 pm	**BANQUET – MUSEUM OF CIVILIZATION**

SATURDAY, May 8, 2010

7:30 am – 8:30 am	Continental Breakfast, **Posters**
8:30 am – 9:30 am	**Keynote Speaker Andrew Marks Intro by Beth McNally**
9:30 am – 10:30 am	**Session V - Disease Pathology II – Chair: Rashmi Kothary**
	Charlotte Sumner, John Hopkins Medicine
	Brunhilde Wirth, Institute of Human Genetics at the University Hospital of Cologne
	Short Talk: Dean Burkin, University of Nevada
10:30 am – 10:50 am	Coffee
10:50 am – 12:30 pm	**Session V** continued
	Rashmi Kothary, Ottawa Hospital Research Institute
	Frederic Charbonnier, Laboratoire de Neurobiologie des Réseaux Sensorimoteurs
	Short Talk: Amanda Ward, Baylor College of Medicine
12:30 pm – 1:30 pm	Lunch
1:30 pm – 3 pm	**Session VI -Stem Cells – Chair: Michael Rudnicki**
	Amy Wagers, Harvard Medical School
	Brad Olwin, University of Colorado
	Swomitra Mohanty, University of California, Berkeley
3 pm – 3:20 pm	Coffee
3:20 pm – 4:30 pm	**Session VI** continued
	Pura Munoz-Canoves, Universitat Pompeu Fabra, Barcelona
	Michael Rudnicki, Ottawa Hospital Research Institute
	Short Talk: Frédéric Trensz, University de Sherbrooke

Dr. Eric Olson, Keynote Speaker

Professor
UT Southwestern Medical Center at Dallas, Department of Medical Biology

Endowed Title: Robert A. Welch Distinguished Chair in Science
Annie and Willie Nelson Professorship in Stem Cell Research
Pogue Distinguished Chair in Research on Cardiac Birth Defects

Dr. Olson's laboratory studies muscle cells as a model for understanding how embryonic cells adopt specific fates and how programs of cell differentiation and morphogenesis are controlled during development. There are three major muscle cell types: cardiac, skeletal and smooth, which express distinct sets of genes controlled by different combinations of transcription factors and extracellular signals.

They have focused on discovering novel transcription factors that control development of these muscle cell types and remodeling in response to cardiovascular and neuromuscular diseases. The processes involved in muscle development are evolutionarily ancient and conserved across diverse organisms. This conservation has enabled them to take a cross-species approach to dissect this problem by identifying myogenic regulatory genes in the fruit fly or in vertebrate embryos and to use these genes to perform gain and loss-of-function experiments in vivo and in vitro.

Their long-term goal is to delineate the complete genetic pathways for the formation and function of each muscle cell type and to use this information to devise pharmacologic and genetic therapies for inherited and acquired muscle diseases in humans.

Dr. Andrew Marks, Keynote Speaker

Chair, Dept. of Physiology & Cellular Biophysics
Founding Director, Clyde & Helen Wu Center for Molecular Cardiology

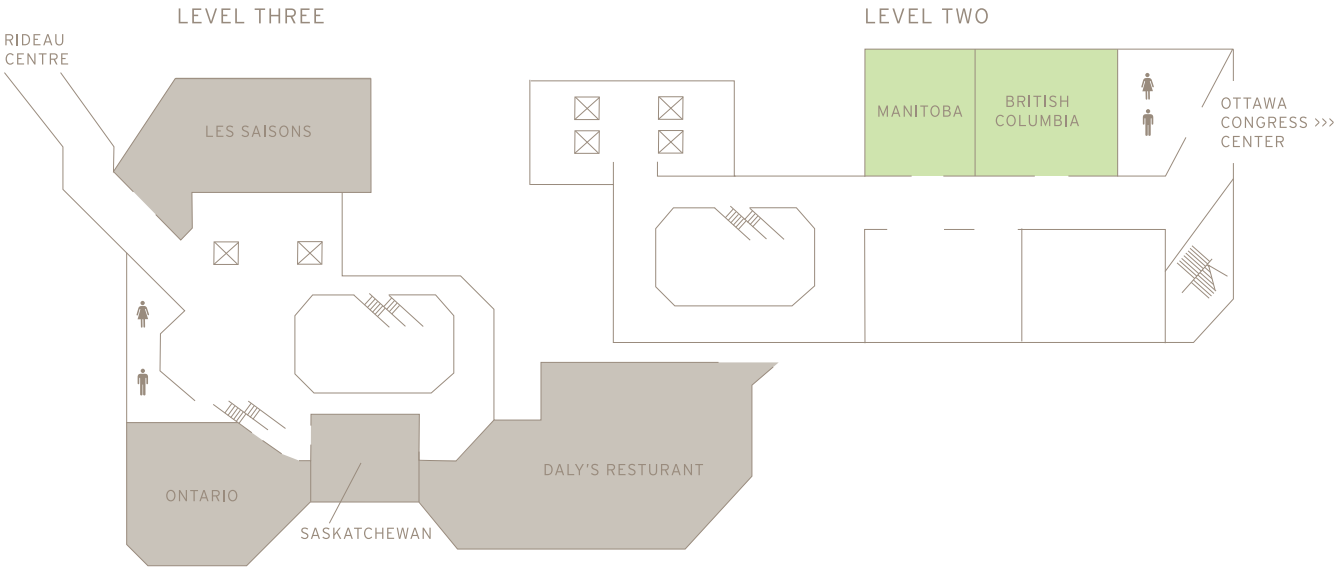
The Marks lab is devoted to improving basic understandings of mechanisms that regulate calcium dependent signals including muscle contraction and cell growth. In particular the lab focuses on the structure/function relationships of ion channel macromolecular complexes including the ryanodine receptor/ calcium release channel (RyR) and the IP3 receptor. The lab employs a wide range of techniques including molecular and cell biology, confocal calcium imaging, structural biology, muscle physiology, and generates genetically altered animal models of human diseases to test hypotheses.

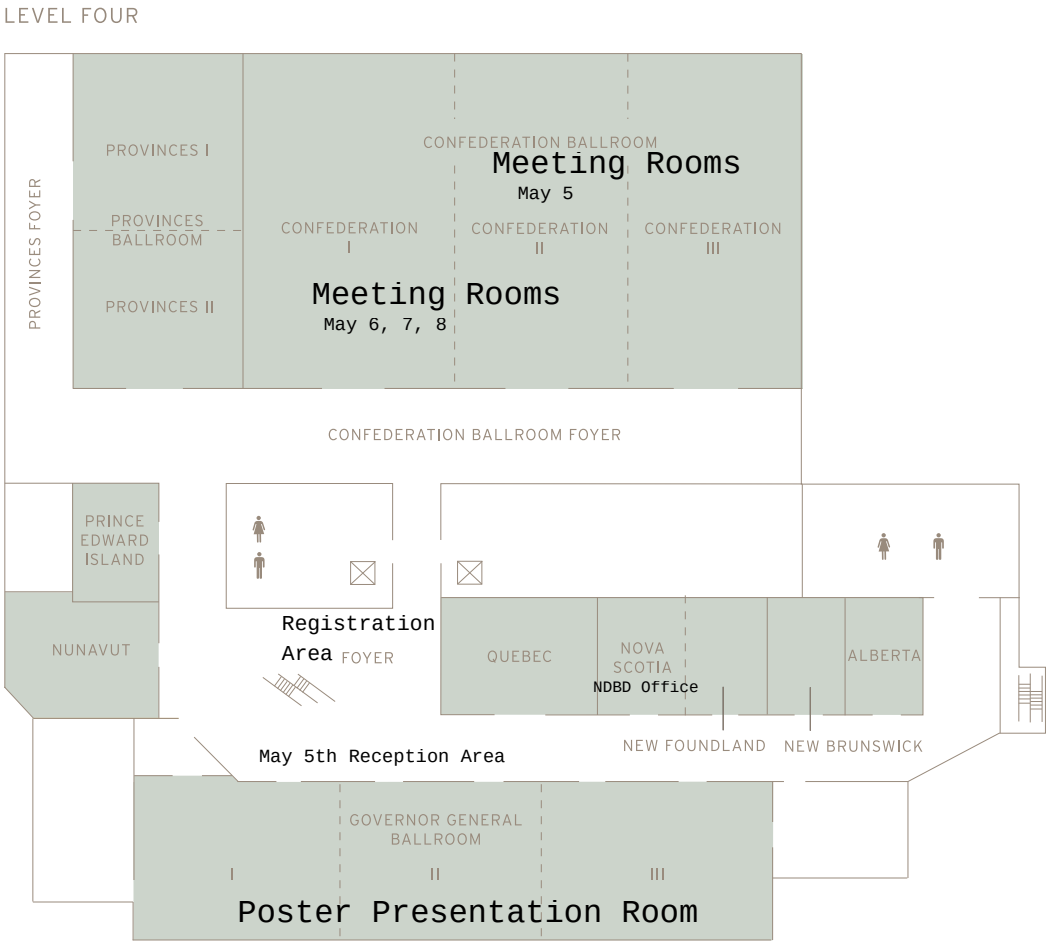
As a basic scientist and a physician, Andrew Marks, M.D., a cardiologist, chairman of the physiology department, and Founding Director of Wu Center for Molecular Cardiology, looks for ways to turn observations from the clinic or lab into new therapies for patients. This approach is responsible for the first drug eluting stent used to prevent coronary artery stent restenosis, and for a first-of-its-kind treatment for heart failure.

The research on heart failure and sudden cardiac death began with a clinical observation: the body's "fight or flight" nervous system is active at all times in heart failure patients - not just during times of stress - a condition that puts constant pressure on the heart. In the lab, Dr. Marks found that the constant stimulation of the heart alters the function of the calcium channel that controls the heart beat and strength of contraction of the heart. In heart failure, the channel starts leaking calcium, which can weaken the heart's pumping action and sets up the potentially fatal arrhythmias that are responsible for half of all deaths from heart failure.

Andrew Marks and his team found that a leak in RyR channel in the heart causes heart failure and cardiac arrhythmias - and have synthesized novel compounds called "calcium channel stabilizers" that fix the leak and prevent heart failure and arrhythmias in animal models. These novel therapeutics are being developed for testing in patients.

FLOOR PLANS





THE WESTIN
OTTAWA

The Ottawa Conference on New Directions in Biology & Disease of Skeletal Muscle

Museum of Civilization Dinner, May 7, 2010

6:00 pm – Buses will pick up for trip to Museum of Civilization (Westin Lobby)
6:30 pm – Cash Bar / Museum Viewing (Canada Hall, 3rd Floor)
8:00 pm – Dinner
9:15 pm – Guest Speaker Introductions
9:20 pm – Guest Speaker: **Danielle Campo**
10:00 pm – Buses will pick up for return to Westin Hotel

Menu:

Baby greens with cucumber slices, cherry tomatoes, and a balsamic vinaigrette

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Butternut squash soup

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Roasted chicken supreme with a wild mushroom sauce, root vegetables and Roasted new potatoes

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Maple mousse cake, served with maple syrup and a vanilla sauce

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Regular and decaffeinated coffee
Selection of tea and herbal teas

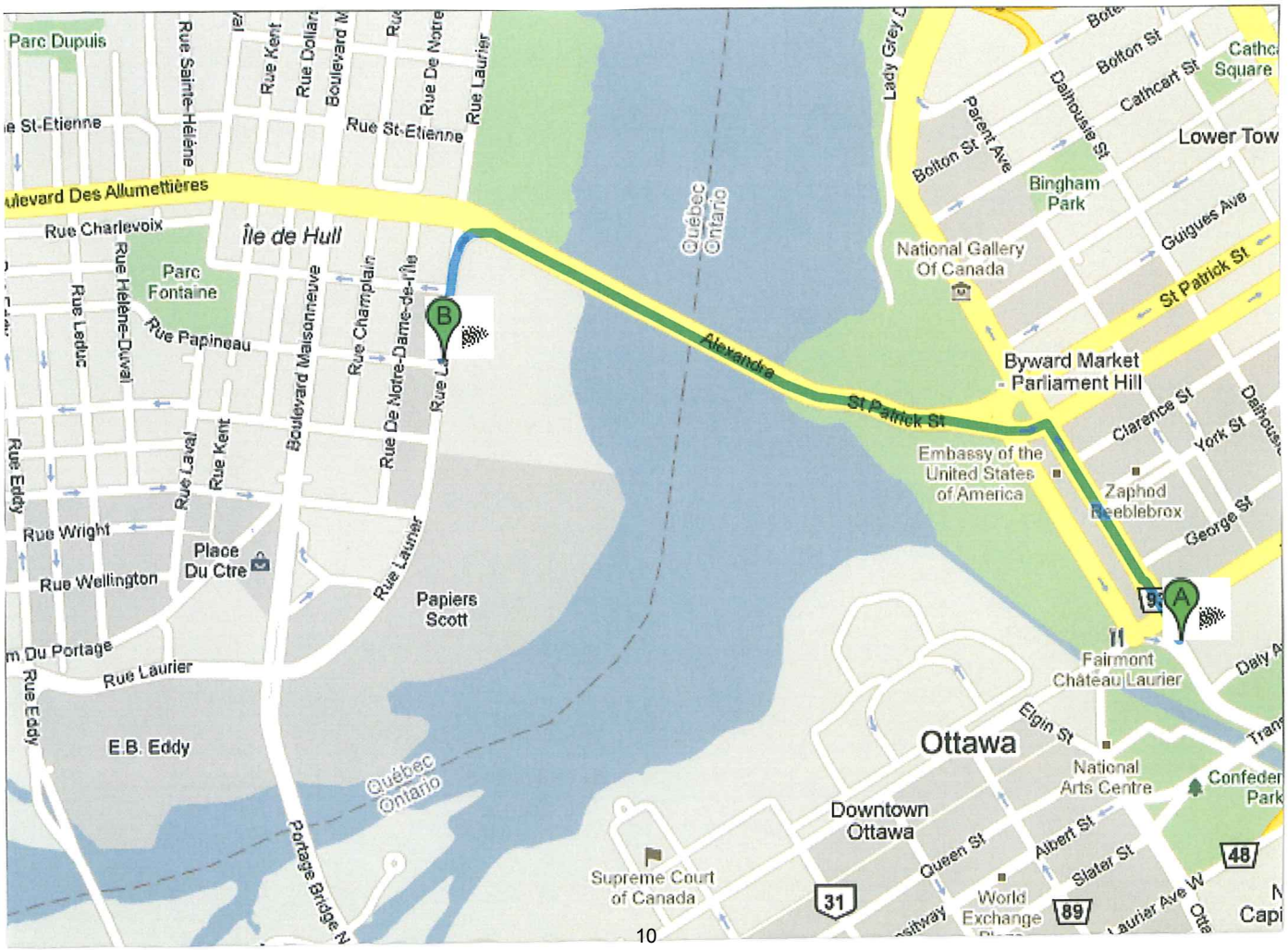
Walking directions to Canadian Museum of Civilization

1.8 km – about **22 mins**

A The Westin Ottawa
11 Colonel By Drive
Ottawa, ON K1N 9H4
(613) 560-7000

1. Head **northwest** on **Colonel By Dr** toward **Rideau St**	51 m
2. Continue onto **Sussex Dr**	400 m
3. Turn **left** at **Murray St**	72 m
4. Continue onto **St Patrick St**	400 m
5. Continue onto **Alexandra** Entering Quebec	450 m
6. Continue onto **Boulevard Des Allumettières**	150 m
7. Turn **left** at **Rue Laurier**	280 m

B Canadian Museum of Civilization
100 Laurier Street
Gatineau, K1A 0M8



Danielle Campo, Guest Speaker

Danielle Campo knows what it takes to achieve goals. Only in her early twenties, she has achieved more than most of us will accomplish in a lifetime. Danielle brings that hard work and dedication to Muscular Dystrophy Canada as an Ambassador.

At the 2000 Paralympic Games in Australia, Danielle won a stunning three gold medals and one silver in freestyle swimming. That year she was named Ontario's Junior Citizen of the Year. She was then all of fifteen years old.

In 2004 at the Athens games, Danielle won a silver and two bronze medals in relay and freestyle. Her athletic achievements defy the usual limits that come with congenital fibre type disproportion, the rare form of muscular dystrophy, which is Danielle's diagnosis.

A graduate from Ontario's Fanshawe College, Danielle has turned her attention to working life. At the Children's Aid Society in Windsor, Ontario, Danielle works for social change. "So many kids don't have enough encouragement, enough motivation. They need respect and support and stimulation to try something different, to believe that they can change and that they can have much fuller lives. Their parents need respect and support too - perhaps even more of it than the kids do - in order to be able to try new ways and discover hope."

Danielle continues to participate in Muscular Dystrophy Canada fundraising events and conferences lending her support and encouragement.

KEYNOTE SPEAKER ABSTRACTS

New Insights into the Genetic Networks of Skeletal Muscle Disease
Olson, Eric, Keynote Speaker

Department of Molecular Biology, UT Southwestern Medical Center, Dallas, TX

Adult skeletal muscle fibers respond to changes in activity, motor innervation, and hormonal signaling by modifying their phenotype to maintain a balance between physiological demand and functional capacity. This phenotypic plasticity is achieved through activation of intracellular signaling pathways that modulate myofiber gene expression. Skeletal muscle also exhibits remarkable regenerative potential following injury, through the activation of satellite cells, which promote muscle repair. We are seeking to define the gene regulatory mechanisms responsible for skeletal muscle remodeling and regeneration. Recently, we discovered that myogenin, in addition to its role as an essential regulator of skeletal muscle development, also regulates muscle atrophy by directly activating the expression of E3 ubiquitin ligase genes. We have also found that that myogenic coactivator MASTR cooperates with Pax7 to maintain adult muscle structure and function and to promote satellite cell-dependent regeneration. The signaling pathways and transcriptional networks that control skeletal muscle growth, regeneration and remodeling are intertwined with a collection of microRNAs that act as negative regulators of gene expression. We have identified signature expression patterns of microRNAs associated with diverse diseases of skeletal and cardiac muscle. Gain- and loss-of-function studies in mice have revealed striking functions for these microRNAs in numerous facets of muscle biology, including the control of sarcomerogenesis, myosin expression, fibrosis, hypoxia, angiogenesis, myocyte survival, and cytoskeletal dynamics. Especially intriguing is the discovery of a network of muscle-specific microRNAs embedded within myosin heavy chain genes, which control fast versus slow myofiber switching, muscle performance and thyroid hormone sensitivity of striated muscles. Disease-inducing microRNAs can be persistently silenced in vivo through systemic delivery of anti-miRs, allowing for the direct therapeutic modulation of disease mechanisms. Therapeutic opportunities for manipulating microRNA biology in the settings of muscle disease will be discussed.

Role of leaky ryanodine receptors in impaired exercise capacity
Marks, Andrew, Keynote Speaker

Columbia University

During exercise, the major Ca2+ release channel required for excitation-contraction coupling (ECC) in skeletal muscle, the ryanodine receptor (RyR1), is progressively PKA hyperphosphorylated, nitrosylated, and depleted of the phosphodiesterase PDE4D3 and the RyR1 stabilizing subunit calstabin1 (FKBP12), resulting in leaky channels that cause muscle fatigue in mice and humans. A novel small molecule (S107) that prevents depletion of calstabin1 from the RyR1 complex improved force generation and exercise capacity, reduced Ca2+-dependent neutral protease calpain activity and plasma creatine kinase levels. Thus, Ca2+ leak via calstabin1 depleted RyR1 channels leads to defective Ca2+ signaling, muscle damage and fatigue. Next, we identified a structural and functional defect in RyR1 in the mdx mouse model of muscular dystrophy. RyR1 isolated from mdx skeletal muscle exhibited an age-dependent increase in S-nitrosylation coincident with the dystrophic changes in the muscle. RyR1 S-nitrosylation depleted the channel complex of calstabin1 resulting in leaky channels. Preventing the depletion of calstabin1 from the RyR1 complex with S107 inhibited the SR Ca2+ leak, reduced biochemical and histologic evidence of muscle damage, and improved muscle function. Thus, SR Ca2+ leak via RyR1 likely contributes to muscle weakness in muscular dystrophy and preventing the RyR1-mediated SR Ca2+ leak may provide a novel therapeutic approach.

SPEAKER ABSTRACTS

Mechanistic and molecular insights into fatigue in muscular dystrophy

Campbell, Kevin, Chair, Speaker
Campbell, Kevin[1], Rader, Erik[1], Crawford, Robert[1], Faulkner, John[2], Weiss, Robert[1], Chamberlain, Jeffrey[3], Moore, Steven[1], Kobayashi, Yvonne[1]
[1]University of Iowa, Iowa City, IA 52242, [2]University of Michigan, Ann Arbor, MI 48109, [3]University of Washington, Seattle, WA 98195

Many neuromuscular conditions are characterized by excessive fatigue that is disproportionate to activity level; notably, some patients experience this fatigue without a somatic disease. To understand the molecular basis of this fatigue, we used an interdisciplinary approach, combining an in vivo activity assay, genetically defined mice, Laser Doppler and magnetic resonance imaging, and patient biopsy analysis. We show that loss of skeletal muscle sarcolemma nNOS exacerbated fatigue experienced after mild exercise and caused exercise-induced muscle edema in dystrophic muscle. This loss leads to deficiency in contraction-induced cGMP-dependent attenuation of local vasoconstriction, resulting in narrowing of the muscle vasculature post-exercise. We show that skeletal muscle sarcolemmal nNOS detection is decreased in a large number of distinct myopathies, suggesting a common fatigue mechanism. Finally, we show in mice that pharmacological enhancement of nitric oxide-cGMP signaling from active muscle relieved this fatigue, and in dystrophic mice, relieved exercise-induced muscle edema. These findings provide a novel therapeutic strategy to address fatigue symptoms in neuromuscular disease patients where sarcolemma nNOS is deficient.

In vivo NMDA-receptor activation accelerates motor-unit maturation, protects spinal motor-neurons and enhances SMN expression in severe Spinal-Muscular-Atrophy mice.

Charbonnier, Frederick, Speaker
Universita de Paris Descartes, UMR 8194 CNRS, Paris, France

Spinal Muscular Atrophy (SMA), a lethal neurodegenerative disease which occurs in childhood, is due to the misexpression of the SMN protein in motor neurons. It is still unclear whether activating motor-units in SMA could correct the delay in the post-natal maturation of the motor unit and could result in neuroprotection. In the present work, we demonstrate that an appropriate NMDA-receptor activation in SMA-like mice significantly accelerated motor-unit post-natal maturation, counteracted apoptosis in the spinal cord and induced a marked increase of SMN expression resulting from either a transcriptional or a post-transcriptional effect. The NMDA treatment strongly extended the lifespan in two different SMA mouse models. The analysis of the intracellular signaling cascade that lay downstream the activated NMDA receptor revealed an unexpected reactivation of a CaMKII/AKT/CREB pathway that could induce an activation of SMN2 gene expression in SMA spinal cord. Therefore, pharmacological activation of spinal NMDA receptors could constitute a useful strategy for both increasing SMN expression and limiting motor-neuron death in SMA spinal-cord.

Novel mechanisms to enhance utrophin expression and muscle cell function

Crosbie, Rachelle, Speaker
University of California, Los Angeles, CA 90095

Duchenne muscular dystrophy is caused by dystrophin mutations that lead to structural instability of the sarcolemma membrane, myofiber degeneration/regeneration, and progressive muscle wasting. We have discovered two novel mechanisms for upregulating components of the dystrophin-and utrophin-glycoprotein complexes. We have found that overexpression of the transmembrane protein sarcospan results in altered muscle cell glycosylation and increased expression of adhesion complexes that mediate extracellular matrix attachment. We also show that myogenic Akt signaling in mdx mice promotes increased expression of utrophin, which replaces the function of dystrophin thereby preventing sarcolemma damage and muscle wasting. In contrast to previous suggestions that increased Akt in dystrophy was a secondary consequence of pathology, our findings demonstrate a pivotal role for this signaling pathway such that modulation of Akt can significantly affect disease outcome by amplification of existing, physiological compensatory mechanisms. Our efforts have revealed two complementary approaches that promote membrane stability. Importantly, both approaches are specifically applicable to Duchenne muscular dystropphy and may also be effective in a wide range of congenital muscular dystrophies.

Goldberg, Alfred, Speaker
Goldberg, A[1], Zhao, J[1], Brault, J[1], Henrike, B[1], Cohen, O[1]
[1]Harvard Medical School

In recent years there has been dramatic progress in our understanding of the cellular mechanisms responsible for the atrophy of skeletal muscle that occurs with fasting, disuse, nerve injury, and many systemic diseases (e.g. cancer cachexia). In these diverse conditions, the rapid muscle wasting results largely from excessive protein degradation by the ubiquitin proteasome pathway. In these rapidly atrophying muscles, but not in aging-related muscle wasting (sarcopenia), there is a common program of transcriptional changes, in which a set of atrophy-related genes ("atrogenes") are induced or repressed coordinately. Among the most induced proteins are components of the ubiquitin-proteasome pathway, especially the muscle-specific ubiquitin ligases, atrogin-1 and MuRF1. MuRF1 is critical in the ordered disassembly and degradation of the myofibrillar apparatus, especially thick (myosin) filaments. However, a distinct E3 catalyzes loss of thin (actin) filaments whose identification will be discussed. These atrogenes are induced by the FoxO family of transcription factors, and overproduction of FoxO3 by itself causes dramatic muscle atrophy. In addition, FoxO3 stimulates the cell's other main proteolytic system, the autophagic/lysosomal pathway, and in atrophying muscles, mRNAs for many autophagy-related genes are induced by FoxO3. These two pathways are also coordinately regulated by mTOR kinase. Its inhibition with rapamycin causes a rapid activation not only of autophagy but also of the ubiquitin proteasome pathway in muscle and other cells. Thus, during fasting and other types of atrophy, the cell's two main proteolytic systems are activated coordinately to cause the breakdown of different cellular components; the loss of

contractile proteins via the ubiquitin-proteasome pathway and of organelles (e.g. mitochondria) via autophagy.

BMPR1a is necessary for proliferation of interstitial skeletal muscle progenitors

Gussoni, Emanuela, Speaker
Gussoni, Emanuela[1], Huang, Ping[1]
[1]Children's Hospital Boston and Harvard Medical School, Boston, MA 02115

Satellite cells, the known stem cells of skeletal muscle, reside between the basal lamina and the sarcolemma of myofibers. Other myogenic progenitors distinct from satellite cells have also been isolated. In human fetal muscle, we reported the existence of interstitial Myf5+ BMPR1a+ progenitors that proliferate upon stimulation with BMP4, expressed by muscle side population (SP) cells. To study if BMPR1a is important in myogenic cells, its expression was ablated in Myf5+ progenitors in vivo. Mutant mice are born runted and remain significantly smaller throughout life compared to wild-type littermates. Proliferation of myogenic cells derived from mutant mice is significantly decreased compared to control. The percentage of prospective myogenic progenitors (Sca-1- CD45-) is significantly lower in mutant mice compared to controls, while the presumed non-myogenic fraction (Sca-1+ CD45-) is higher in mutant mice compared to controls. Fractionated Sca-1- CD45- and Sca-1+ CD45- cells from control and mutant mice were expanded in vitro. Sca-1- CD45- cells from mutant mice did not show a proliferation defect, while Sca-1+ CD45- cells did. It was also found that the cultured Sca-1+ CD45- fraction from control mice contained myogenic progenitors, which were severely reduced in mutant mice. These studies indicate that expression of BMPR1a is necessary for the proliferation of interstitial myogenic progenitors distinct from satellite cells.

Systemic anti-sense in DMD: Progress, and hurdles facing clinical implementation of exon-skipping

Hoffman, Eric, Speaker
Eric P Hoffman, Toshifumi Yokota, Qi Lu, Terence Partridge, Shin'ichi Takeda,
Children's National Medical Center, Washington DC

A therapeutic approach for Duchenne muscular dystrophy is systemic modulation of dystrophin gene splicing using antisense oligonucleotides (AOs) to restore the reading frame of the dystrophin gene (exon-skipping). Currently, exon skipping is generally felt to be the most tangible molecular therapeutic for the majority of DMD patients. However, it is also a unique challenge for drug development for a number of reasons. First, there are many exonic targets. Multiple AO drugs must be developed in order to provide this therapy to the majority of Duchenne dystrophy patients. This is not a single drug development program, but rather many programs. Second, there is a poor understanding of BMD-like (internally deleted) dystrophin mRNA and protein function. Clinically mild or assymptomatic Becker muscular dystrophy patients with large in-frame deletions are often cited as evidence for predicted clinical efficacy of exon skipping. However, a range of clinical symptoms are seen for any specific deletion. We have recently shown that 10 patients sharing the same common exon-skipping target in-frame deletion (exon 45-47), determined by MLPA in both DNA and mRNA, show a wide range of dystrophin protein amounts (5%-80%), and a range in clinical phenotypes (Kesari et al. 2008). Third, there are difficulties in showing pre-clinical efficacy of human DMD drugs in animal models. The allelic heterogeneity in mutant dystrophin genes, in both humans and animals, leads to many mutations, but few shared mutations between humans and animals. Most pre-clinical studies in mouse have been done in the mdx model, using a mouse exon 23 drug that has limited direct relevance to human drugs targeting hot-spot deletions (exons 45-55). Fourth, experimental systems for determining optimal AO target sequence vary, with in vitro systems showing relatively poor correlation with in vivo tests. We recently showed that only a single AO was needed for efficient exon skipping of the CXMD dog mutation in vitro. In contrast, this same AO was ineffective in vivo (Yokota et al. 2009). We describe progress in exon-skipping and suggest that this approach has the potential to improve the quality of life of many if not most DMD patients. However, manufacturing, regulatory, and business model challenges face implementation of this approach in the clinic.

Promotion of the slow myogenic program as a therapeutic approach for DMD

Jasmin, Bernard, Chair, Speaker
University of Ottawa, Centre for Neuromuscular Disease, and Ottawa Hospital Research Institute, Ottawa, Canada

Over the last several years, a significant portion of our efforts has focused on identifying the basic mechanisms regulating expression of utrophin A in muscle at the transcriptional and post-transcriptional levels. Our recent findings showed the role of several signaling pathways important for regulating expression and localization of utrophin A in slow versus fast skeletal muscles. In particular, we were the first to show that utrophin A is regulated by calcineurin signalling as well as via the transcriptional co-activator PGC-1alpha. Together, these findings provide strong support to our original working hypothesis indicating that promotion of the slow myogenic program can attenuate progression of the dystrophic phenotype by stimulating expression of utrophin A in skeletal muscle. In this context, we recently embarked on a series of pre-clinical studies aimed at elucidating the potential role of PPARdelta activation on utrophin A expression. To achieve this, we have used the PPARdelta specific agonist, GW501516, and demonstrated that chronic pharmacological activation of PPARdelta indeed results in an increase in utrophin A expression as well as in robust structural and functional improvements in mdx mouse muscle. These findings may prove important and could pave the way for additional and novel therapeutic interventions for DMD.

Regulation of NF-kB activity in muscle by cIAP1 and cIAP2

Korneluk, Robert, Speaker
Emeka K. Enwere, Rim Lejmi Mrad, Kristen Timusk, Shawn Beug, Douglas Mahoney, Herman Cheung, Nathalie Earl, Martine St. Jean, John Lunde, Bernard Jasmin, Eric LaCasse and Robert Korneluk
Apoptosis Research Center, Children's Hospital of Eastern Ontario Research Institute, [2]Department of Cellular & Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada.

The two highly similar cellular Inhibitors of APoptosis, cIAP1 and cIAP2, have recently been shown to be critical and redundant regulators of classical and alternative NF-kB signaling, particularly in response to activation by cytokines such as TNFalpha or TWEAK. This is of special relevance in skeletal muscle, where chronic NF-kB activation has been shown to be involved in the pathogenesis of muscle diseases such as skeletal muscle atrophy and muscular dystrophy. Given that the roles of both cIAP1 and cIAP2 in NF-kappaB signaling are now well established, we sought to understand their function in myogenesis under normal and disease conditions. Remarkably, we found that the chemically-induced downregulation of cIAP1 in primary myoblasts led to the formation of hypertrophic myotubes. Similar results were seen both with siRNA knockdown of cIAP1 and upon differentiation of primary myoblasts isolated from cIAP1-null mice. The cIAP1deficient mice themselves had increased muscle mass, as well as increased number and area of individual muscle fibers. Interestingly, in response to denervation-induced muscle atrophy, leg muscle fiber size was preserved in both cIAP1 and cIAP2 knockout mice compared to wild-type mice. Finally, we generated a mouse line by crossing cIAP1-null mice with the Duchenne muscular dystrophy mouse model (mdx). These double-mutant progeny showed striking amelioration of the dystrophic phenotype. Collectively, these results reveal cIAP1 and cIAP2 as novel regulators of skeletal muscle size and regeneration, and suggest the potential for cIAP modulation in the treatment of skeletal muscle atrophy and dystrophy.

Defects in cytoskeletal dynamics in a mouse model for spinal muscular atrophy

Kothary, Rashmi, Organizer, Chair, Speaker
Regenerative Medicine Program, Ottawa Hospital Research Institute and University of Ottawa

The disruption of the survival motor neuron 1 gene (SMN1), either by deletion, rearrangement, or mutation leads to the neurodegenerative disease Spinal Muscular Atrophy (SMA). SMA is the leading genetic cause of infant deaths affecting 1 in 10,000 live births and is characterized by the degeneration of motor neurons and skeletal muscle atrophy. Although SMA is primarily a motor neuron disease, the involvement of muscle in its pathophysiology has not been entirely ruled out. To study neuronal and muscle contributions to pathogenesis in SMA, we have developed an intermediate mouse model for the disease. In the present work, we have characterized altered actin cytoskeleton dynamics in this mouse model. This has allowed us to assess therapeutic approaches to attenuate the disease process in this mouse model. Funded by CIHR and MDA.

Identifying novel roles for beta-adrenoceptor signaling in skeletal muscle: implications for muscle growth, adaptation, regeneration and plasticity

Lynch, Gordon, Speaker
Lynch, Gordon[1], Ryall, James[1], Church, Jarrod[1], Koopman, Rene[1], Gregorevic, Paul[2]
[1]*Basic and Clinical Myology Laboratory, Department of Physiology, The University of Melbourne, Victoria 3010, Australia,* [2]*Laboratory for Muscle Research & Therapeutics Development, Baker IDI Heart and Diabetes Institute, Melbourne, Victoria 3004, Australia*

While the importance of beta-adrenoceptor (beta-AR) signaling in the heart has been well documented and continues to receive significant attention, it is only more recently that we have begun to appreciate the importance of this pathway in skeletal muscle (Lynch & Ryall, 2008). Stimulating the beta-AR signaling pathway with beta-agonists has therapeutic potential for muscle wasting since administration can elicit anabolic responses. Thus, the beta-AR signaling pathway holds promise as a target for the muscle wasting associated with sarcopenia, cancer cachexia, disuse, unloading, sepsis, metabolic disorders, neuromuscular diseases, and for hastening muscle repair after injury. However, concerns regarding possible adverse cardiovascular effects have so far limited clinical use of beta-agonists for muscle-based indications. We propose that better understanding beta-AR signaling in skeletal muscle will advance development of effective interventions for muscle wasting. To this end, we are using beta-AR knockout mice to examine beta-AR signalling in skeletal muscle development, injury and regeneration, plasticity and adaptation. Supported by the NHMRC (Australia) and the AFM (France) Lynch GS & Ryall JG (2008). Physiol Rev 88:729-67.

Dissecting modifier genes for muscular dystrophy

McNally, Elizabeth, Organizer, Speaker
McNally, Elizabeth[1], Swaggart, Kayleigh[1], Ceco, Ermalinda[1], Lim, Jackie[1], Palmer, Abraham[1], Heydemann, Ahlke[1]
[1]*University of Chicago, Chicago, IL 60637*

Mutations in more than 50 different single genes can lead to cardiomyopathy and may frequently be associated with muscle disease. In some instances, the severity of disease can be predicted by the precise mutation. However, more often there is a range of disease associated even with the identical gene mutation, and this is best seen in families who carry the identical gene mutation. Understanding the gene(s) that modify outcome may be useful for identifying pathways for therapy and predicting outcome. Additionally, dissecting the networks that combine to produce phenotype offers an alternative approach to understanding complex inheritance. We employed an unbiased mapping approach to search for genes that modify muscular dystrophy in mice. In a genomewide scan, we found a single strong locus on chromosome 7 that influences pathological features of muscular dystrophy, muscle membrane permeability and muscle fibrosis. Within this genomic interval, an insertion/deletion polymorphism of 36 bp in the coding region of LTBP4 was found. LTBP4 encodes a latent transforming growth factor beta binding protein, a protein that sequesters TGFb and regulates it availability to bind the TGFb receptor. These data identify LTBP4 as a target to regulate TGFb signaling to modify outcome in muscle disease. Additionally genomic mapping studies reveal the complexity of modifier genes that contribute to inherited heart and muscle disease.

Caspase 3 Activity Inhibits Self Renewal of Muscle Satellite Cells.

Megeney, Lynn A., Organizer, Speaker
Sprott Centre for Stem Cell Research, Program in Regenerative Medicine, Ottawa Hospital Research Institute and the Departments of Medicine and Cellular & Molecular Medicine, University of Ottawa.

Caspase 3 directly promotes the differentiation program in a wide variety of cell lineages including skeletal muscle. This non-death role for caspase 3 is mediated in part through the activation of CAD (caspase activated DNase). Once activated CAD induces genome wide DNA strand breaks leading to altered gene expression, events that are essential for promoting myoblast differentiation. Here, in addition to directly promoting cell differentiation, we demonstrate that caspase 3 also impairs the self renewal process in muscle stem cells (satellite cells), by targeting and inhibiting the muscle pluripotency factor Pax7. Using ChIP sequencing methodology, we have identified Pax7 as a direct CAD genomic target. CAD targeting of the Pax7 gene is concurrent with down-regulation of Pax7 expression during early stages of myoblast differentiation. In addition, we demonstrate that caspase 3 directly targets and cleaves the Pax7 protein to ensure efficient blockade of satellite cell self renewal. Taken together, our results demonstrate that caspase 3 activity acts at both early and late stages in the muscle cell life cycle, limiting self renewal while enhancing differentiation.

Isolation and Analysis of Satellite Cells from the Muscle Niche Using Microfluidics

Mohanty, Swomitra, Speaker
Mohanty, Swomitra[1], Conboy, Michael[1], Sohn, Lydia[1], Conboy, Irina[1]
[1]*University of California*

Research by the Conboy lab at the University of California-Berkeley, has focused on using muscle to model stem-cell mediated regeneration, specifically the decline seen with aging and disease. Satellite cells are the regenerative stem cells for muscle repair, and it has been discovered that cells residing in aged tissue retain their intrinsic ability to regenerate. Furthermore, it has also been found that aged differentiated muscle inhibits the response of these endogenous stem cells, resulting in reduced regenerative potential. During the regenerative process, satellite cells activate and migrate to the site of injury where some cells differentiate and become myogenic, while others retain stem cell like properties. Currently, expression of surface markers (e.g. Sca-1, CXCR4, and integrins) on these cells is used as a predictor for myogenic cell fate. The majority of current research has focused on bulk analysis of satellite cells with little research devoted to analysis of the muscle fiber, which has been identified as an important regenerative niche. One hurdle to studying the muscle fiber niche is the fact that very few satellite cells are present on each fiber (~5-15 cells per fiber). Traditional methods (e.g. FACS or MACS) of surface-marker analysis are not feasible, as these methods require exogenous labels, high shear forces, and thousands of cells for analysis. However, the Sohn lab at the University of California-Berkeley has developed a novel microfluidic-based method for label-free surface-marker analysis of a small number of cells (10-500). The method uses resistive-pulse sensing (RPS) to electronically detect specific cell-surface markers. Cells are not damaged because they flow through the device under low shear forces; consequently, they can be collected after screening for further analysis or culturing. Thus, this method enables us to analyze satellite cells from a single fiber and to study stem-cell regulation in a manner not possible with traditional methods.

Osteopontin is a novel therapeutic target in DMD that promotes inflammation and fibrosis

Spencer, Melissa, Speaker
Kramerova, Irina[1], Kudryashova, Elena[1], Vetrone, Sylvia[1], Ermaolova, Natalia[1], Overman, Julia[1], Martinez, Leonel[1], Miceli, M. Carrie[1], Spencer, Melissa[1]
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Inflammation and fibrosis are two features of the disease Duchenne muscular dystrophy. Our previous studies identified a protein, osteopontin (OPN), as an immunomodulator that promotes inflammation and fibrosis in the mouse model of DMD, the mdx mouse. For these studies, double mutant mice (DMM) were generated lacking both OPN and dystrophin. DMM muscles showed reduced infiltration of NKT-like cells and neutrophils. Elevated OPN appeared to correlate with the dystrophic process as OPN was significantly elevated in mdx serum and mdx and DMD biopsies and ablation of OPN resulted in decreases in serum CK and increases in muscle strength. Targetting NKT cells with asialo GM1, an antibody against a glycolipid that is present on NK cells, was shown to effectively decrease NK and NKT cells, but this treatment did not improve the mdx phenotype. Diaphragms and hearts of DMM mice had less fibrosis than mdx counterparts, concomitant with reductions in intramuscular TGFb. We tested whether the reductions in diaphragm fibrosis resulted in a functional improvement in diaphragm function and found a significant increase in minute ventilation in 8 month old DMM compared to age matched mdx mice. These studies suggest that OPN is a promising therapeutic target for reducing inflammation and fibrosis in DMD.

Synaptic abnormalities and failure of motor unit development in SMA mice

Sumner, Charlotte, Speaker
Sumner, Charlotte[1], Kong, Lingling[2], Rich, Mark[3], Wang, Xueyong[4], Mentis, George[5]
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The autosomal recessive motor neuron disease spinal muscular atrophy (SMA) is caused by insufficient expression levels of the survival motor neuron (SMN) protein. "Delta 7" mice are an animal model of severe SMA that show early, severe motor behavioral abnormalities and death at two weeks. In this mice, electrophysiological studies demonstrate synaptic dysfunction at both neuromuscular junction (NMJ) synapses and central afferent synapses onto motor neuron cell bodies. Morphologically, presynaptic terminals contain a paucity of synaptic vesicles suggesting that the SMN protein may play a role directly or indirectly in synaptic vesicle organization. These synaptic abnormalities are associated with myofibers that are hypotrophic and express an immature pattern of myosin heavy chain genes. Treatment of SMA mice with the histone

deacetylase inhibitor, trichostatin A, increases SMN protein levels, improves synaptic function, and results in increased maturity of the motor unit. Together these results suggest that impaired activity of SMN-deficient central and peripheral synapses results in a failure of motor unit development and muscle weakness in severe SMA mice.

Premature Stop Codon Suppression as a Therapy for DMD
Sweeney, Lee, Organizer, Chair, Speaker
University of Pennsylvania

Subsets of patients with a large number of genetic diseases have disease due to a premature stop (nonsense) mutation in the coding sequence of a protein. Thirty years ago it was shown that certain classes of antibiotics (aminoglycosides) suppress nonsense mutations in eukaryotic cells in culture. In the late 1990s we demonstrated that this approach could be used in an animal model of a human disease, Duchenne muscular dystrophy (DMD). The need for a safer drug to promote suppression of nonsense mutations underlying a large number of human diseases led to the development of PTC124 (ataluren). Cultured myotubes from the patients from a Phase 2a clinical trial in DMD designed to evaluate ataluren exposure and safety showed dose-dependent dystrophin expression in 100% of cases. However, the dose-response curve was not sigmoidal, but displayed a peak read-through at 5-10µg/ml, with decreasing read-through at higher levels of drug. Recent results from a blinded, placebo-controlled, long-term (48 weeks) efficacy trial in DMD suggest that this effect occurs in vivo as well as in vitro. This has important implications as to the future use of ataluren in patients with DMD and with other genetic disorders.

Reversal of Muscle Fibrosis with a Soluble Activin IIB Receptor.
Wagner, Kathryn, Speaker
Li, Z[1], Zhang, J[4], Wagner, K[1,2,3]
[1]The Kennedy Krieger Institute, [2]Departments of Neurology-The Johns Hopkins School of Medicine, [3]Departments of Neuroscience-The Johns Hopkins School of Medicine, [4]Departments of Radiology-The Johns Hopkins School of Medicine

Progressive fibrosis of skeletal muscle is a defining feature of the vast majority of muscular dystrophies. Although the exact consequences of endomysial fibrosis are unknown, it has been implicated in inhibiting diffusion of nutrients, reducing contractility and compliance and impeding muscle regeneration. Therefore, treatment of fibrosis is currently considered an important therapeutic goal for muscular dystrophy. One potential modulator of muscle fibrosis is myostatin, a highly conserved member of the TGF-b superfamily that is expressed almost exclusively in skeletal muscle, inhibiting proliferation and differentiation of myoblasts while stimulating proliferation of muscle fibroblasts. In the genetic absence of myostatin, skeletal muscle fibrosis is reduced in mdx and d-sarcoglycan mouse models of muscular dystrophy compared to their littermates with normal myostatin. Mdx mice, genetic models of Duchenne muscular dystrophy, exhibit progressive fibrosis in skeletal muscle over time. Treatment of 2-year-old mdx mice with a soluble activin IIB receptor , the putative receptor for myostatin, resulted in reduction of limb muscle fibrosis index, hydroxyproline content, extracellular matrix proteins and enhancement on muscle MRI compared to baseline. To our knowledge, this is the first demonstration of pharmacological reversal of pre-existing skeletal muscle fibrosis.

Plastin 3 protects against spinal muscular atrophy
Wirth, Brunhilde, Speaker
Institute of Human Genetics, Institute for Genetics and Center for Molecular Medicine Cologne, Univ. of Cologne, 50931 Cologne, Germany

Homozygous deletion of SMN1 causes spinal muscular atrophy (SMA), the most frequent genetic cause of early childhood lethality. In rare instances, however, individuals are fully asymptomatic despite carrying the same SMN1 mutations and the same number of SMN2 copies as their affected siblings, thereby suggesting the influence of modifier genes. By transcriptome-wide differential expression analysis we identified plastin 3 (PLS3; Xq23) to be highly expressed in all unaffected but not in the affected counterparts. PLS3 expression in blood turned out to be a rare variant, occurring only in 5% of controls. We found that PLS3 is highly expressed in spinal cord, associates with SMN, and together are part of a large multiprotein complex in spinal cord and they show similar subcellular locations in primary motor neurons. PLS3, as an actin bundling protein, influenced the F-actin levels known to be involved in axonal outgrowth and guidance. PLS3 knock-down severely affected axonal growth, whereas its overexpression induced axonal growth. Most importantly, over-expression of PLS3 rescued the axonal growth defects caused by reduced SMN levels in neuronal differentiated PC12 cells, in primary motor neurons of SMA mouse embryos and in an in vivo zebrafish SMA-model. Our data strongly support the view that the involvement of SMN in axonal biology is the major pathogenic defect in SMA.

Abstracts from the following speakers were not available at the time of printing:

Carsten Bonnemann
Pura Munoz-Canoves
Jeff Chamberlain
Lou Kunkel
Brad Olwin
Michael Rudnicki
Eric Shoubridge
Maurice Swanson
Steven Tapscott
Amy Wagers
Xaio Xaio

SHORT TALKS ABSTRACTS

ER Chaperone GRP94 Depletion in Skeletal Muscle Affects Muscle Growth and Architecture.

Barton, Elisabeth, Short Talk
Barton, Elisabeth[1], Tian, Zuozhen[1]
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Insulin-like growth factor (IGF) is essential for muscle regeneration and repair. From in vitro studies, it has been shown that the relationship between glucose-regulated protein 94 (grp94) and IGF secretion of skeletal muscle is linear, such that loss of grp94 activity inhibits IGF secretion. To address this effect in vivo, a muscle-specific conditional knockout mouse was developed (MCK-Cre grp94 FL/FL). Upon grp94 deletion, reduced body size and muscle mass were apparent. In order to determine the cause of smaller skeletal muscle, the muscle size and fiber type were analyzed. Skeletal muscles were harvested 8 weeks after birth. The fiber cross-sectional area analysis displayed the shifted distribution toward smaller fibers in grp94-deleted muscles, and especially loss of the largest gauge fibers compared to control. Fiber type analysis revealed that there was conversion in fiber type from slow to fast. In grp94-deleted soleus, the proportion of fast fibers, MHCIIa, was greater than that of slow fiber, MHCI, and in the EDL, there was an increase proportion of MHCIIb compared to controls. These differences may be a consequence of muscle adaptation to the lack of grp94 and local IGF to aid muscle growth. Alternatively, these factors may regulate early fiber type specification. These possibilities will be addressed in the near future.

Laminin-111 protein therapy prevents muscle disease and increases viability in the dyW mouse model of merosin-deficient congenital muscular dystrophy

Burkin, Dean, Short Talk
Burkin, Dean[1], Rooney, Jachinta[1], Doe, Jinger[1], Wuebbles, Ryan[1]
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Merosin-deficient congenital muscular dystrophy type 1A (MDC1A) is a lethal muscle wasting disease for which there is no effective treatment or cure. MDC1A is caused by mutations in the LAMA2 gene resulting in the absence of the laminin-alpha2 protein. Laminin-alpha2 is required for the formation of laminin-211 and -221 (merosin) which provides structural and functional integrity to skeletal muscle. MDC1A patients and the dyW mouse model exhibit delayed motor milestones, muscle wasting, elevated serum creatine kinase and decreased life expectancies. We have recently demonstrated that laminin-111 protein therapy can prevent muscle disease progression in the mdx mouse model of Duchenne muscular dystrophy. To determine if this protein therapy may benefit MDC1A patients, dyW mice were treated with laminin-111. Intramuscular injections of laminin-111 prevented apoptosis and inflammation associated with muscle disease, while systemic delivery of laminin-111 protein increased life expectancy. Treatment of primary myogenic cells from MDC1A patients with human laminin-111 prevented apoptosis. Our data indicate laminin-111 may serve as an effective protein replacement therapy in the treatment of MDC1A. Supported by NIH, Cure CMD and SAM.

AAV-mediated augmentation of SMN levels for spinal muscular atrophy

Cheng, Seng, Short Talk
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Spinal muscular atrophy (SMA) is a neuromuscular disease caused by a deficiency of SMN due to mutations in the SMN1 gene. Loss of SMN activity results in motor neuron cell death in the spinal cord. In this study, an AAV vector expressing human SMN (AAV8-hSMN) was injected at birth into the CNS of a mouse model of SMA. These injections resulted in widespread expression of SMN throughout the spinal cord that translated to a robust improvement in skeletal muscle physiology. Treated SMA animals also displayed significant improvements on behavioral tests indicating that the neuromuscular junction was functional. Importantly, treatment with AAV8-hSMN increased the median lifespan of SMA mice to 50 days compared to 15 days for untreated controls.Moreover, SMA mice injected with a self-complementary AAV vector showed a greater extension in median survival to 157 days. These data indicate that CNS-directed, AAV-mediated SMN augmentation is highly efficacious in addressing both the neuronal and muscular pathologies of a mouse model of SMA

Molecular Analyses of Hibernation Suggest Novel Treatment Strategies to Preserve Muscle Mass

Cohn, Ronald, Short Talk
Cohn, Ronald[1], Burks, Tyesha[2], Soleimani, Arshia[2], Simmers, Jessica[2], Lin, Benjamin[2], Leinwand, Leslie[3], Vaughan, Dana[4], Andres-Mateos, Eva[4]
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Skeletal muscle is the largest organ in the human body, comprising ~50% of total body weight. Disuse (e.g. immobilization, denervation, and microgravity), inherited neuromuscular disorders, and aging all result in debilitating loss of skeletal muscle. According to the current paradigm, loss of muscle mass results from insufficient muscle regeneration and an imbalance of protein synthesis and degradation. In addition, recent evidence demonstrates that improvement of mitochondrial function also plays a role in preservation of muscle mass. In contrast, hibernating mammals have evolved molecular mechanisms to survive prolonged periods of immobilization without any significant loss of muscle mass. We therefore explored the molecular pathways involved in the maintenance of muscle mass in the hibernating 13-lined ground squirrel (Ictidomys tridecemlineatus). We find that active muscle regeneration does not contribute significantly to preservation of skeletal muscle. Instead, we show absence of proteolysis and autophagy accompanied by synchronization of resistance and endurance exercise pathways that have been previously assumed to be mutually exclusive. Furthermore, we demonstrate characterization of a novel protein which plays a

critical role in mediating skeletal muscle preservation during hibernation independent of Akt activation. This protein contributes to the hypertrophic and cell survival response in mouse and human muscle. Our results identify novel key regulatory molecules responsible for these pathway alterations that will open up new avenues for therapeutic interventions in patients with a variety of inherited and acquired forms of skeletal muscle atrophy and degeneration. Supported by the NIH Director's New Innovator Award DP2 OD004515, by NIH 5K08NS055879 award and the MDA #101938.

p38 MAP Kinase signaling is required for fusion of myoblasts during myogenesis

Dilworth, F. Jeffrey, Short talk
Liu, Qi-Cai[1], Brand, Marjorie[1], Iratxeta-Perez, Carol[1], Dilworth, F. Jeffrey[1]
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It is well-established that the p38 MAP Kinase signaling pathway plays a critical role in the in myoblast differentiation. While many phosphorylation targets of p38 MAP kinase have been identified in muscle cells, it is not clear how activation of these factors leads to the formation of multinucleated myotubes. Using multiple cell lines with myogenic potential (C2C12 myoblasts, 10T1/2 fibroblasts, and MyoD-/-_Myf5-/- fibroblasts), we determined that expression of the muscle specific bHLH protein myogenin was directly modulated by p38 signaling. Based on these findings, we hypothesized that the critical effects of p38 on myogenesis are likely to be mediated through the regulated expression of myogenin. To address this possibility, we generated a C2C12 cell line that stably maintained an exogenous myogenin cDNA under the control of a Tetracyclin inducible promoter. Combining this system with the use of a pharmacological inhibitor of p38 activity (SB203580), we determined that several known p38 dependent genes could be reactivated in absence of p38 signaling if myogenin was expressed exogenously. Microscopy demonstrated that while no differentiation was observed in cells treated with SB203580, the exogenous expression of myogenin in the absence of p38 signaling allowed for the alignment of myoblasts. However, fusion of the myoblasts to form myotubes could not be achieved by introducing myogenin expression into the SB203580 treated cells. Microarray analysis was performed on cells treated with SB203580 in the presence or absence of exogenous myogenin expression. These areas demonstrated that 332 genes down-regulated by p38 signaling. Amongst these genes, 149 returned to normal levels of expression when myogenin levels were restored in the cells. Gene set enrichment analysis (GSEA) of genes that are regulated by p38, but not myogenin, demonstrates that factors previously implicated in cell fusion are enriched in this subset of the myogenic transcriptome. While it has long been established that p38 MAPK signaling regulates skeletal myogenesis, our work suggests a major role for the p38 signaling pathway is to promote fusion of mononucleated cells to generate myotubes.

RNA-based gene therapy to inhibit expanded CUG-RNA toxicity in myotonic dystrophy

Furling, Denis, Short Talk
Furling, Denis[1], Francois, Virginie[1], Klein, Arnaud[1], Beley, Cyriaque[1], Jollet, Arnaud[1], Lemercier, Camille[1], Garcia, Luis[1]
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Myotonic Dystrophy type1 (DM1), the most common neuromuscular disorders in adults, is an autosomal dominant disease caused by expanded CTG repeats in the 3'UTR of the DMPK gene. DM1 is representative of group of RNA-mediated disorders due to the expression of transcripts containing pathogenic expanded repeats. The mutant DMPK-mRNAs containing expanded CUG repeats (CUGexp) are retained into the nuclei as aggregates and altered the functions of splicing factors such as MBNL1, which is sequestered in these foci, leading to misregulation of alternative splicing and DM1 neuromuscular degeneration. Several strategies are under development to reverse the toxic effects of these pathologic RNAs. In this study, we have investigated the ability of a new modified human hU7-snRNAs to target CUGexp-DMPK transcripts. Different antisense sequences were imbedded within hU7-snRNA-derived lentiviral vectors and their efficacy were evaluated in muscle cells isolated from DM1 patients. An efficient silencing of CUGexp-DMPK mRNAs was measured in hU7-snRNA transduced DM1 cells. The number of foci was also significantly decreased, MBNL1 proteins were released and several aberrant splicing were corrected in treated-DM1 cells. In addition, defective myogenic differentiation of DM1 myoblasts was restored. In conclusion, we propose modified hU7-snRNA as a new RNA-based gene therapy approach to inhibit CUGexp-RNA toxicity and correct DM1 abnormalities.

Transgenic overexpression of SERCA1 mitigates muscular dystrophy in Mdx, sarcoglycan null, and TRPC3 transgenic mice

Molkentin, Jeffery, Short Talk
Goonasekera, Sanjeewa, Molkentin, Jeffery
Cincinnati Children's Hospital, Howard Hughes Medical Institute, Molecular Cardiovascular Biology

Muscular dystrophy refers to a clinically and genetically heterogeneous group of degenerative muscle disorders characterized by progressive muscle weakness and degeneration. Although the primary defect likely results from a loss of sarcolemmal integrity, the secondary molecular mechanisms leading to myofiber necrosis is debated. One hypothesis suggests that elevated cytosolic Ca^{2+} is an initiating event for many downstream sequelae resulting in myofiber necrosis. In this study we demonstrate the dystrophic phenotype observed in Mdx and delta-sarcoglycan null (DSG) mice is dramatically improved by skeletal muscle-specific overexpression of sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA1) using a transgenic approach. Moreover, dystrophic disease due to TRPC3 overexpression, and its associated Ca^{2+} influx mechanism for myofiber necrosis, was also improved with the SERCA1 transgene. Skeletal muscle from SERCA1 transgenic mice showed significantly elevated SERCA1 protein levels and 2-4-fold greater Ca^{2+}-ATPase activity, which enhanced Ca^{2+} clearance and the amplitude of the transient. Histologically, quadriceps, diaphragm and soleus muscles were analyzed for percent central nucleation at 3 months of age, revealing up to a

75% reduction in the three diseased models when the SERCA1 transgene was present. A similar decrease in fibrosis was also observed in the same muscle groups in the diseased models with the SERCA1 transgene. In addition, serum creatine kinase levels were also significantly reduced in DSG/SERCA1 TG and Mdx/SERCA1 TG mice compared to DSG or Mdx alone. Mechanistically, we show that SERCA1 overexpression corrects a deficit in Ca^{2+} re-uptake after twitch stimulation in diseased DSG myofibers, and it reduces mitochondrial swelling observed in DSG myofibers. Thus, our results demonstrate that enhanced SR Ca^{2+} ATPase activity improves both biochemical and histological evidence of muscle damage observed in three separate mouse models of MD by presumably increasing the rate of Ca^{2+} clearance from the cytosol, coupled with more efficient sequestration of Ca^{2+} in the SR and less of a "leak" phenomena.

Golgi and sarcolemmal nNOS control skeletal muscle bioenergetics by differentially regulating contraction-induced fatigue and vasoconstriction during exercise
Percival, Justin, Short Talk
Percival, Justin[1], Bible, Ken[1], Seigel, Michael[1], Marcinek, David[1], Froehner, Stanley[1]
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Neuronal nitric oxide synthase mu (nNOSmu) synthesizes the versatile messenger nitric oxide (NO). During exercise, loss of contraction-induced signaling by sarcolemmal nNOSmu to adjacent blood vessels has been proposed to cause exaggerated cage inactivity in nNOS knockout mice. We provide evidence against this proposal by showing that loss of sarcolemmal nNOSmu-mediated signaling does not cause skeletal muscle fatigue or post-exercise muscle weakness. Furthermore, nNOSmu is not the only source of NO in skeletal muscle. We demonstrate the existence of a novel nNOSbeta splice variant signaling pathway at the Golgi complex that regulates both fatigue resistance and post-exercise strength. NO is a well-known regulator of mitochondrial function; thus we investigated whether the fatigue from nNOSbeta-deficiency could result from abnormalities in mitochondrial integrity and function. Loss of nNOSbeta caused swelling and mislocalization of mitochondria, and decreased mitochondrial ATP production. Together these data suggest that during exercise, sarcolemmal nNOSmu regulates blood supply, (by opposing sympathetic vasoconstriction) while Golgi nNOSbeta simultaneously opposes skeletal muscle fatigue (by enhancing mitochondrial ATP synthesis). We conclude that nNOSmu and nNOSbeta signaling pathways play complementary roles in regulating muscle bioenergetics, that are critical for peak skeletal muscle performance.

Function in the mdx Mouse Model of Duchenne Muscular Dystrophy (DMD)
Pistilli, Emidio, Short Talk
Pistilli, Emidio[1], Bogdanovich, Sasha[1], Lachey, Jennifer[2], Seehra, Jasbir[2], Khurana, Tejvir[1]
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The activin receptor type IIB (ActRIIB) is a trans-membrane receptor for transforming growth factor-beta (TGFbeta) superfamily members, including but not limited to myostatin, that are involved in the negative regulation of muscle mass. We tested the translational hypothesis that blocking ligand binding to ActRIIB for 12 weeks would stimulate skeletal muscle growth and improve muscle function in the mdx mouse. Inhibition of ActRIIB signaling was achieved using a novel protein therapeutic composed of the extracellular portion of the ActRIIB fused to the Fc portion of murine IgG (sActRIIB), at doses of 1.0 and 10.0 mg.kg-1 body weight. After 12 weeks of treatment, the 10.0 mg.kg-1 dose caused a 27% increase in body weight with a concomitant 33% increase in lean tissue mass. Absolute tetanic force production of the extensor digitorum longus (EDL) muscle ex vivo was 31% and 39% greater in response to the 1.0 And 10.0 mg.kg-1 dose of sActRIIB, respectively. Circulating creatine kinase levels were lower in mice treated with the 10.0 mg.kg-1 dose of sActRIIB, compared to control. These data demonstrate that targeting the ActRIIB improves skeletal muscle mass and strength in the mdx mouse model of DMD, providing a therapeutic rationale for this molecule in treating skeletal myopathies. FUNDING: World Anti-Doping Agency (T.S. Khurana); AR053461 (E. Pistilli)

The Wnt Canonical Pathway Promotes Fibrosis in Skeletal Muscle through Sca1-Derived Muscle Resident Stromal Cells
Trensz, Frédéric, Short Talk
Trensz, Frédéric[1], Hanoun, Sonia[1], Cloutier, Alexandre[1], Richter, Martin[1], Grenier, Guillaume[1]
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Previous work reported a role for the Wnt canonical pathway in fibrosis formation of aged skeletal muscle. In this work, we scrutinized the dystrophic Mdx mouse model that displays important skeletal muscle fibrosis. Our results indicated that dystrophic muscles possessed an augmented muscle resident stromal cell (mrSC) population as compared to age-matched wild type mice. Stimulation of cultured mrSCs by Wnt3a promoted their proliferation and collagen expression, but it induced growth arrest and no collagen expression in cultured myoblasts. Moreover, muscle fibers cultured in the presence of Wnt3a showed no myogenic Pax7pos cell proliferation, instead promoting the increase in mrSCs. Intramuscular injection of Wnt3A in adult wildtype mice significantly enhanced the mrSC population and collagen deposition when compared to contralateral muscle. In addition, injection of the Wnt antagonist, DKK1, into Mdx mice, significantly reduced collagen deposition. Taken together, these results suggest that the Wnt canonical pathway acts on the mrSC population to increase the production of collagen that is observed during aging and other myopathies.

CUGBP1 overexpression in mouse skeletal muscle reproduces features of myotonic dystrophy type 1
Ward, Amanda, Short Talk
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The neuromuscular disease myotonic dystrophy type I (DM1) is the most common form of adult onset muscular dystrophy. The severity and type of symptoms affecting DM1 patients is highly variable, but they typically have severe problems associated with the skeletal muscle system including

myotonia, muscle atrophy, and progressive muscle weakness/wasting. Indeed, dysphagia and respiratory failure resulting from muscle wasting are major causes of death in individuals with DM1. The causative mutation in DM1, a CTG repeat expansion in the 3\'82\'c4\'f4 untranslated region of the DM protein kinase (DMPK) gene, leads to nuclear depletion of Muscleblind-like 1 (MBNL1) and increased steady state levels of CUG binding protein 1 (CUGBP1). Both MBNL1 and CUGBP1 function as regulators of alternative splicing during normal heart and skeletal muscle development, and their aberrant expression in DM1 results in widespread misregulation of alternative splicing. The pathogenic effects of MBNL1 depletion have previously been tested by the generation of MBNL1 knockout mice, but the consequence of CUGBP1 overexpression in muscle is not yet known. In a DM1 mouse model expressing DMPK-CUG960 repeat RNA in skeletal muscle, CUGBP1 up-regulation is temporally correlated with severe muscle wasting suggesting CUGBP1 may play a critical role in this process. To test this hypothesis, we generated transgenic mice with doxycycline-inducible and skeletal muscle-specific expression of CUGBP1. When fed a doxycycline-containing diet, MDAFrtTA/TRECUGBP1 mice had an 8-fold up-regulation of CUGBP1 in all skeletal muscles tested including the gastrocnemius, quadriceps, triceps, and soleus. Induced MDAFrtTA/TRECUGBP1 mice had reduced gastrocnemius muscle weight and impaired muscle function suggesting that aberrant up-regulation of CUGBP1 is detrimental to normal muscle processes. Histological examination of skeletal muscle from induced MDAFrtTA/TRECUGBP1 mice showed myofiber atrophy and degeneration, myofiber size variability, and an abundance of myofibers with centrally located nuclei. Excessive central nucleation is one of the most characteristic changes observed in DM1 muscle histology indicating CUGBP1 overexpression alone is capable of reproducing DM1-like features on histopathology. Lastly, a subset of misregulated alternative splicing events documented in DM1 patients were reproduced in induced MDAFrtTA/TRECUGBP1 mice. This study has shown that mouse muscle overexpressing CUGBP1 reproduces the molecular and physiological defects of DM1 tissue and establishes CUGBP1 as a major player in DM1 skeletal muscle pathogenesis. This work was supported by the NIH predoctoral NRSA fellowship F31NS067740 (A.J.W.) and NIH grants R01AR45653 and R01GM076493 (T.A.C.).

Intravenous delivery of antisense morpholinos normalizes RNA splicing and reduces myotonia in a transgenic mouse model of myotonic dystrophy type 1

Wheeler, Thurman, Short Talk
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Objective: To test whether morpholino oligos modified for systemic delivery can reverse symptoms in a transgenic mouse model of myotonic dystrophy type 1 (DM1). Background: DM1, the most common muscular dystrophy in adults, is caused by expression of an expanded CUG (CUGexp) repeat in the DM protein kinase mRNA. Clinical features of DM1 result directly from trans-dominant effects of this mutant RNA. For example, CUGexp RNA sequesters RNA binding proteins in the muscleblind-like (MBNL) family, leading to abnormal alternative splicing of select genes. Mis-splicing of the muscle chloride channel, ClC-1, causes myotonia in DM1. Intramuscular injection of a CAG repeat morpholino targeting the CUGexp RNA can displace Mbnl1 protein and eliminate myotonia in a transgenic mouse model of DM1 (Wheeler, 2009). To enhance tissue uptake after systemic delivery, morpholinos have been covalently linked to an octa-guanidine dendrimer. Design/methods: Two dendrimer-modified morpholinos were tested by tail vein injection of HSALR mice: 1) a CAG repeat and 2) a ClC-1 antisense that previously demonstrated selective skipping of ClC-1 exon 7a in mice (Wheeler, 2007). Control mice were injected with saline. Treatment assignments were randomized. Injections and electromyography were blinded. Results: Treatment with the CAG repeat oligo improved RNA mis-splicing and reduced or eliminated myotonia in all muscles tested. In mice treated with ClC-1 antisense, ClC-1 splicing was completely reversed and myotonia eliminated in all muscles tested. ClC-1 protein was restored to the muscle surface membrane in both groups of morpholino-treated mice. Identical dosing for one month produced a partial effect. Intravenous injection of saline had no effect. Conclusions: Dendrimer-modified morpholinos achieve whole-body correction of RNA mis-splicing and reduction of myotonia in a transgenic mouse model of DM1, supporting the feasibility and effectiveness of systemic delivery of antisense oligos as treatment for DM1. Study supported by: University of Rochester Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center (NIH/NS48843) and NIH (AR46806)

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POSTER ABSTRACTS

Disease Biology

1. Hypernitrosylated ryanodine receptor/calcium release channels are leaky in dystrophic muscle

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Duchenne muscular dystrophy (DMD) is characterized by progressive muscle weakness and early death resulting from dystrophin deficiency. Loss of dystrophin results in disruption of a large dystrophin glycoprotein complex (DGC) leading to pathologic calcium (Ca2+)-dependent signals that damage muscle cells. We have identified a structural and functional defect in the sarcoplasmic reticulum (SR) Ca2+ release channel/type 1 ryanodine receptor (RyR1) in the mdx mouse model of muscular dystrophy that may contribute to altered Ca2+ homeostasis in dystrophic muscles. RyR1 isolated from mdx skeletal muscle exhibited an age-dependent increase in S-nitrosylation coincident with dystrophic changes in the muscle. RyR1 S-nitrosylation depleted the channel complex of FKBP12 (or calstabin1 for calcium channel stabilizing binding protein) resulting in leaky channels. Preventing the depletion of calstabin1 from the RyR1 complex with an RyR-specific compound, S107, that binds to the RyR1 channel and enhances the binding affinity of calstabin1 to the nitrosylated channel, inhibited the SR Ca2+ leak, reduced biochemical and histologic evidence of muscle damage, improved muscle function and increased exercise performance in mdx mice. Thus, SR Ca2+ leak via RyR1 due to progressive S-nitrosylation of the channel and calstabin1 depletion likely contributes to muscle weakness in muscular dystrophy and preventing the RyR1-mediated SR Ca2+ leak may provide a novel therapeutic approach.

Disease Biology

2. Renin Angiotensin system and skeletal muscle fibrosis: new strategies to modulate induction of fibrotic response to Connective Tissue Growth Factor (CTGF/CCN-2)

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Duchenne muscular dystrophy (DMD) is characterized by development of fibrosis. Transforming Growth Factor beta (TGFb), Connective Tissue Growth Factor (CTGF) and Renin Angiotensin System, whose fibrotic exponent is Angiotensin II (AngII), are involved in fibrotic progression. Using mdx mice, we observed that exercise-induced fibrosis, determined by an increase in collagen, fibronectin and CTGF levels, was prevented by treatment with losartan, an AngII receptor type1 (AT1) blocker. This reduction in fibrosis was accompanied by an improvement of muscle function. Using a model of muscular fibrosis induced by CTGF, we observed an increase of fibrosis, which was reduced by treatment with losartan. In skeletal muscle cells, AngII increases collagen and CTGF levels. Interestingly, the rise of CTGF induced by AngII was reduced by treatment with an inhibitor of TGFb signaling, suggesting a crosstalk between AngII and TGFb signaling pathways in the induction of CTGF. The results suggest that AngII induces CTGF by a mechanism involving TGFb signaling and the fibrotic effect mediated by CTGF can be inhibited by blocking AT1 and could be a good target for treatment of muscular fibrosis. Fondecyt11080212, FONDAP1398001, ConicytPFB12/2007, MDA89419.

Disease Biology

3. Cofilin-2 deficiency causes severe myopathy with actin accumulations in a knockout mouse model

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Background: Congenital myopathies (CMs) are rare skeletal muscle disorders of genetic origin. They usually present with early-onset skeletal muscle weakness of varying severity causing delayed motor milestones, respiratory difficulties and often death in early life. We recently identified a family with two siblings diagnosed with CM, and having nemaline bodies and minicores in their muscles, to carry a homozygous missense mutation A35T in the cofilin-2 gene (CFL2). Cofilin-2 is an actin binding and depolymerizing protein expressed primarily in skeletal muscles and heart. It belongs to AC group of proteins that include cofilin-1 and destrin proteins. Objective: Create a mouse model of cofilin-2 knockout (KO) and evaluate the phenotype to understand the molecular function of cofilin-2 in muscle development and/or maintenance. Design/Methods: We created a conditional knockout mouse model for Cfl2. These mice were bred with various Cre recombinase-expressing mice to obtain complete and muscle-specific Cfl2 knockouts. These included mice with Cre expressed in zona pellucida (Zp3-cre) for complete KO, both cardiac and skeletal muscles (ACTA1-cre), skeletal muscle only (Mef2C) and post-natal cre expression in skeletal/cardiac muscles (CK-MM-cre). Results: Lack of cofilin-2 was associated with severe myopathy in the KO mice. Their average life span ranged from 7 days for complete KO to 28 days for conditional KO mice bred with those expressing cre using CK-MM promoter. KO animals were significantly smaller in both length and weight compared to their siblings with intact cofilin-2. Their muscles showed extensive areas of muscle degeneration on H&E and oxidative staining. Electron microscopy showed fibers with minicores, absent Z lines, disrupted sarcomeres and actin accumulations. On immunofluorescence, diffuse actin and alpha-actinin-2 staining was present in several fibers. On Western blot analysis, increased amounts of actin and alpha-actinin-2 were present in the KO compared to control. No obvious cardiac involvement was present on light and electron microscopy or on performing echocardiograms on KO and sibling control mice. Further, the skeletal muscle did not show any defects in the KO fetuses (E15.5) suggesting an important role for cofilin-2 plays in muscle maintenance, but not in development. Conclusions: Cofilin-2 deficiency causes severe skeletal myopathy associated with muscle degeneration and actin accumulations in a mouse model. Cofilin-2 plays a critical role in muscle maintenance.

Disease Biology

4. Loss of nuclear poly(A)-binding protein 1 (PABPN1) causes defects in myogenesis and mRNA biogenesis

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The nuclear poly(A) binding protein 1 (PABPN1) is a ubiquitously expressed protein that plays a critical role in polyadenylation. Short expansions of the polyalanine tract in the N-terminus of PABPN1 lead to oculopharyngeal muscular dystrophy (OPMD), which is an adult onset disease characterized by eyelid drooping, difficulty in swallowing, and weakness in the proximal limb muscles. Although significant data from in vitro biochemical assays define the function of PABPN1 in control of poly(A) tail length, little is known about the role of PABPN1 in mammalian cells or specifically, muscle cells. To assess the function of PABPN1, we examined the effects of PABPN1 depletion using siRNA in primary mouse myoblasts from extraocular, pharyngeal and limb muscles. PABPN1 knockdown significantly decreased myoblast proliferation and differentiation during myogenesis in vitro. At the molecular level, depletion of PABPN1 depletion led to a shortening of mRNA poly(A) tails, demonstrating the cellular function of PABPN1 in polyadenylation control in a mammalian cell. In addition, PABPN1 depletion caused nuclear accumulation of poly(A) RNA, revealing that PABPN1 is required for proper poly(A) RNA export from the nucleus. Together, these experiments demonstrate that PABPN1 plays an essential role in myoblast proliferation and differentiation, suggesting it is required for muscle regeneration and maintenance in vivo.

Disease Biology

5. Exon-level expression profiling in FSHD

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Facioscapulohumeral Muscular Dystrophy (FSHD) is a progressive muscle disorder that has been associated with a contraction of 3.3-kb repeats termed D4Z4 on chromosome 4q35. FSHD is typically characterized initially by facial muscle weakness followed by scapular fixator, humeral, truncal, and lower-extremity muscles weaknesses. It is also observed in FSHD a wide clinical inter- and intrafamilial variability, ranging from wheelchair-bound patients to asymptomatic carriers. Despite several studies have been focused on trying to find the gene responsible for this disease, no genes have been definitively linked to FSHD yet. Multiple lines of evidence indicate that FSHD is caused by a complex and uncommon mechanism. One of the proposed models to explain the molecular basis of FSHD is that D4Z4 deletion might induce abnormal transcriptional activity in cis and in trans. To get at whether there is abnormal transcriptional activity in FSHD we are using the Gene Exon 1.0 ST Arrays (Affymetrix), which have about 4 probes per exon, enabling two complementary levels of analysis â€“ gene expression and alternative splicing, and we selected muscle tissue samples from related affected, asymptomatic carrier and healthy control from 3 different families. Our preliminary results show that at the gene level our data corroborate with our previous results comparing the gene expression profiling using the HGU133.2 platform, and at the exon level the misspliced genes seem to be mostly associated with cell signaling, cell death and protein trafficking in FSHD, but these results still need to be validated. This study of the exon-level expression profiling from asymptomatic carriers and related affected patients has allowed a comprehensive analysis of the expression profiling in FSHD and is a novel approach to try to enhance our understanding of this disease.

Disease Biology

6. Lateral force transmission through desmin can initiate dystrophy in mdx mice

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Duchenne muscular dystrophy (DMD) is a fatal muscle wasting disease caused by mutations in dystrophin, but the pathogenesis remains unclear. Desmin is an intermediate filament protein that mechanically connects the contractile apparatus of striated muscles to the sarcolemma and organelles. Desmin expression is increased in the mdx mouse model of DMD. Here we examined the role of desmin in the pathogenesis of DMD by generating mdx:desmin double knockout mice (dko mice). We found that half of the dko mice died suddenly by approximately 3 months of age from a dilated cardiomyopathy. In contrast, the dystrophic pathology of skeletal muscles was clearly improved in dko mice, resembling a mild dystrophy similar to desmin knockout controls. There was an increased level of utrophin on the sarcolemma, which did not connect to the contractile apparatus because there were no costameres. The muscles were highly susceptible to contraction-induced injury upon lengthening contractions, but the sarcolemma of most fibers (99%) remained impermeable to Evans blue dye infiltration. We conclude that desmin transmits lateral forces to the sarcolemma that can tear the membrane and initiate the dystrophic process in DMD.

Disease Biology

7. A genetic approach to phenocopy dystroglycanopathy disease severity in the mouse

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Disorders of alpha-dystroglycan glycosylation, secondary dystroglycanopathies, present in patients as congenital or limb girdle muscular dystrophy with variable severity and organ involvement. Mechanisms underlying the variable patient phenotypes in dystroglycanopathy patients are unclear, and as yet, no animal models exist that can fully account for both severe and moderate/mild disease phenotypes. To better understand the disparity in patient outcomes and develop therapeutic strategies, we generated mouse models for conditional disruption of alpha-dystroglycan glycosylation. Using different muscle specific promoters for gene deletion, we successfully bred mice with severe or milder muscular dystrophy skeletal muscle phenotypes. Severely affected mice were underweight, exhibited marked forelimb weakness, and were more prone to early mortality. Histologically, muscle degeneration/regeneration, fibrosis and fatty replacement were evident. In contrast, we also successfully generated mildly affected mice with significant histological changes and creatine kinase elevation consistent with muscular dystrophy, but without discernable forelimb weakness. These two mouse models bridge the phenotypic severity of dystroglycanopathy patients and offer new ideas as to the role of functional dystroglycan in muscle development and regeneration. Funded by: HHMI, NIH Wellstone MDCRC, MDA and AHFMR.

Disease Biology

8. Recessive mutations in the putative calcium-activated chloride channel Anoctamin 5 cause proximal LGMD2L and distal MMD3 muscular dystrophies

Bolduc, Véronique[1], Marlow, Gareth[2], Boycott, Kym[3], Baas, Frank[4], de Visser, Marianne[5], Mahjneh, Ibrahim[6], Bashir, Rumaisa[2], Brais, Bernard[2]

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The recently described human Anoctamin (ANO) protein family comprises 10 members, many of which correspond to calcium-activated chloride channels. We have identified the first recessive mutations in an ANO gene, ANO5, that result in a proximal limb-girdle muscular dystrophy (LGMD2L) in three French-Canadian families and in a distal non-dysferlin Miyoshi myopathy (MMD3) in Dutch and Finnish families. These mutations consist of an aberrant splice site, an one-base pair duplication and two missenses. The splice site and the duplication mutations introduce premature stop codons and consequently trigger nonsense-mediated mRNA decay. The LGMD2L phenotype is characterized by proximal weakness with asymmetrical quadriceps femoris atrophy. The MMD3 phenotype is associated with distal weakness in particular of calf muscles, and with defective membrane repair. Though the function of ANO5 protein is unknown, its putative calcium-activated chloride channel function may lead to important insights into the role of deficient skeletal membrane repair in muscular dystrophies. This study was supported by the Muscular Dystrophy Association (USA), the Muscular Dystrophy Campaign (UK), and the Jain Foundation.

Disease Biology

9. Characterization of skeletal muscle in Spinal Muscular Atrophy mice

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The disruption of the survival motor neuron 1 gene (SMN1), either by deletion, rearrangement, or mutation leads to the neurodegenerative disease Spinal Muscular Atrophy (SMA). SMA is the leading genetic cause of infant deaths affecting 1 in 10,000 live births and is characterized by the degeneration of motor neurons and skeletal muscle atrophy. Although SMA is primarily a motor neuron disease, the involvement of muscle in its pathophysiology has not been entirely ruled out. Therefore, the purpose of this study is to elucidate intrinsic and motor neuron associated muscle defects in SMA mouse models. We have performed electron microscopy studies on longitudinal and cross-sections of pre- and post-phenotype tibialis anterior muscles from severe SMA mice. Our analysis did not reveal any overt defects in skeletal muscle from severe SMA compared to wild-type counterparts. Moreover, we did not observe any evidence of increased skeletal muscle regeneration in SMA mice as assessed by hematoxylin and eosin staining. In addition, the fiber type distribution in SMA tibialis anterior muscles is similar to wild-type mice. Future studies will focus on the functional capacity of SMA skeletal muscle. At the moment, our results would indicate that SMA mice do not display overt skeletal muscle perturbations. The characterization of muscle defects in SMA mice will further our understanding of the contribution of this tissue in the SMA phenotype. Funded by CIHR and MDA.

Disease Biology

10. LITF: an A-type Lamin interacting transcription factor involved in myogenesis

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Mutations in the A-type Lamin gene (LMNA) are associated with age-related degenerative disorders of mesenchymal tissues, including Emery-Dreyfuss muscular dystrophy (EDMD) and Limb-Girdle Muscular Dystrophy. The molecular mechanisms that relate mutations in LMNA with different human diseases are poorly understood. Here we report the identification of an A-type Lamin-interacting Transcription Factor, LITF, a unique single copy vertebrate gene. Chromatin immunoprecipitation and gel mobility shift assays showed that LITF binds to DNA within close proximity of genes encoding transcription factors that control tissue-specific differentiation. Blocking LITF expression in C2C12 myoblasts down-regulates myogenic regulatory factors (Mef2C, MyoD, Myf6 & MyoG) and subsequently inhibits myogenic differentiation. Significant reductions in LITF expression was also observed in muscle biopsies of Duchenne muscular dystrophy patients ($p<0.0001$, NCBI GEO profile GDS610) and hearts of the LMNA H222P mouse model of EDMD ($p<0.01$, NCBI GEO profile GDS2746). These observations coupled with the molecular uniqueness of LITF suggest that it lies in a prominent position within myogenesis. Our discovery of LITF may provide the first direct molecular link between Lamin A/C and cellular differentiation. This connection alone may account for the role that Lamin A/C plays in cellular differentiation, aging and disease.

Disease Biology

11. Mouse Models of Hypokalemic Periodic Paralysis

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Hypokalemic periodic paralysis (HypoPP) is an inherited disorder of skeletal muscle excitability characterized by recurrent attacks of depolarization-induced weakness lasting hours to days. Missense mutations in the L-type Ca channel (CaV1.1) and the voltage-gated Na channel (NaV1.4) have been identified in HypoPP families. Curiously, all 14 mutations are at positively charged residues (arginines) in voltage-sensor domains. We have generated knock-in mutant mice harboring the CaV1.1/R538H and NaV1.4/R669H mutations. In vivo hypokalemic challenge by glucose + insulin infusion elicited a loss of muscle excitability (CMAP decrement and reduced force). In vitro contraction testing established a dose-response relation for K-induced weakness and established unequivocally that NaV1.4/R669H produces a HypoPP phenotype. In contrast, most NaV1.4 mutations give rise to HyperPP. Intermediate levels of K challenge (2-3 mM), produced a slow oscillation in

maximal tetanic force. This shift in muscle excitability likely underlies the process of recovery from an attack of periodic paralysis, and was shown to be oubain-sensitive. These new mouse models provide a unique opportunity for gaining new insights on the pathophysiology of HypoPP.

Disease Biology

12. Differential proteolysis of LTBP4 regulates TGFbeta release in muscular dystrophy

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Work from others has shown that attenuating TGFb activity ameliorates muscular dystrophy in mice. Latent TGFb binding proteins (LTBPs) sequester TGFb proteins in the extracellular matrix and regulate TGFb activity. Through a genomewide scan for modifiers of muscular dystrophy, we identified an insertion/deletion polymorphism in LTBP4 that segregates with muscular dystrophy severity. Using the Sgcg null model, we found that deletion of 36 bp encoding the proline rich region of LTBP4 associated with more severe muscular dystrophy. We expressed this region of LTBP4 in vitro and found that insertion of 12 amino acids into the proline rich region provided resistance to proteolysis. Muscle derived fibroblasts expressing the insertion Ltbp4 allele produced less SMAD signaling after TGFb exposure. This also correlated with reduced SMAD signaling in Sgcg null mice with this allele. Notably, human LTBP4 has an even larger deletion in the proline rich region. We found that the human LTBP4 proline rich region was highly susceptible to proteoylsis, offering an explanation for the high degree of fibrosis in human muscular dystrophy. Our data suggest a mechanistic role for LTBP4 in controlling TGFb bioavailability and muscle disease outcome.

Disease Biology

13. Gene transfer-driven inhibition of Cardiac Ankyrin Repeat Protein as a potential therapy for the correction of muscular dystrophies

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Muscular dystrophies (MD) are a group of genetic disorders characterised by progressive muscle degeneration and weakness, with a broad range of genetic and phenotypic variations. Up until now, the treatments, which are only palliative, aim at alleviating the symptoms. The research of new treatments, mainly orientated towards gene therapy, is largely impeded by the fact that each therapy has to be tailored for the targeted disease. In order to find a therapeutic target eligible for the correction of the largest possible number of MD, we previously screened the expression of pivotal proteins in several major MD and demonstrated that Cardiac Ankyrin Repeat Protein (CARP), a transcription regulator, is constitutively up-regulated in every muscular dystrophy considered, including the most common form Duchenne Muscular Dystrophy. Whether this up-regulation is beneficial or detrimental for the muscle tissue remains unknown, although the supposed anti-proliferative function of CARP is consistent with an aggravation of the phenotype. In the present work, we assess the down-regulation of CARP as a potential therapy in the alpha-sarcoglycan deficient mouse model. Two different CARP-specific Sh-RNA sequences vectorized into rAAV-[2/1] were injected directly in the TA muscles of 3-weeks old animals and the treatment efficiency was assessed after 1 or 3 months of expression. Although CARP mRNA level was efficiently reduced by our approach, it was never down-regulated to the level observed in control C57BL6 mice and was not reflected by a significant reduction of the protein expression. Accordingly, no histological improvement was detected. We also demonstrate that CARP over-expression occurs in both muscle fibres and mononucleated cells in alpha-sarcoglycan deficient muscle. Indeed, CARP staining is especially intense in small fibres organised in foci and in pax7-positive mononucleated cells. We are therefore assessing the histological effects of lentiviral-driven CARP down-regulation in mononucleated cells. Altogether, the analyses of these protocols will be useful to ascertain whether CARP is a suitable therapeutic target for the treatment of MD.

Disease Biology

14. Calpain 3 is a self-limiting orchestrator of rapid, local changes in cyto-architecture

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Limb-Girdle Muscular Dystrophy 2A (LGMD2A) is caused by mutations in Calpain 3 (CAPN3). Three hypotheses prevail for LGMD2A pathogenesis: 1) deregulated (in)activation of CAPN3, 2) protease mislocalisation, and 3) deregulation of substrates critically important for muscle fiber homeostasis. However, due the rapid autolysis of CAPN3 little is known of its substrates, and consequently the pathomechanism of LGMD2A remains elusive. By combining bio-informatics with biochemistry and molecular biology we identified a primary amino acid sequence motif underlying CAPN3 substrate cleavage. This motif is common to all 11 known CAPN3 substrates, can transform a non-substrate into a substrate, and accurately identifies 325 new substrates. Among the new CAPN3 target proteins we identified the Protein Inhibitors of Activated Stats (PIAS) family of E3 SUMO ligases as substrates for CAPN3. CAPN3 can negatively regulate PIAS3 activity in vivo and subsequent analysis of LGMD2A patient tissue showed SUMO2 to be deregulated. Further bioinformatic analysis of our predicted substrates revealed CAPN3's involvement in apoptosis and calcium signaling and suggests that CAPN3 functions as a self-limiting orchestrator of rapid, local changes in cyto-architecture.

Disease Biology

15. Zebrafish MTMR14 is required for excitation-contraction coupling, developmental motor function, and the regulation of autophagy

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Myotubularins are lipid phosphatases that act to modify phosphoinositides and regulate membrane traffic. Mutations in several myotubularins are associated with human disease. Sequence changes in MTM1 and MTMR14 are associated with centronuclear myopathy. We previously demonstrated that morpholino mediated knockdown of zebrafish MTM1 results in significantly impaired motor function, abnormal excitation contraction coupling and severe histopathologic changes that are characteristic of human centronuclear myopathy. In this study, we examined the function of MTMR14 using a similar approach. As with MTM1, Knockdown of MTMR14 results in morphologic abnormalities, a developmental motor phenotype characterized by diminished spontaneous contractions and abnormal escape response, and impaired excitation-contraction coupling. In contrast to MTM1 knockdown, however, muscle structure is normal and not consistent with centronuclear myopathy. Double knockdown of MTM1 and MTMR14 dramatically impaired motor function and skeletal muscle ultrastructure. The combined effect of reducing levels of both MTMR14 and MTM1 was significantly more severe than either knockdown alone, an effect which is likely mediated, at least in part, by increased autophagy. In all, our results suggest that MTMR14 is required for motor function and, in combination with MTM1, is required for myocyte homeostasis and normal embryonic development.

Disease Biology

16. Gender influences cardiac function in the mdx model of Duchenne cardiomyopathy

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Cardiomyopathy is a leading cause of death in Duchenne muscular dystrophy (DMD). A valid mouse model will be extremely useful for studying disease pathogenesis and developing novel therapies. We recently demonstrated that aged mdx mice exhibit cardiomyopathy nearly identical to that seen in human patients (Bostick et al Circulation Research 102:121-30, 2008). To further characterize this model, we examined the influence of gender on the cardiac phenotype of aged mdx mice. We compared the left ventricular hemodynamics, heart weight to body weight ratio (HW/BW) and cardiac hydroxyproline content in 22-month-old male and female mdx mice. Surprisingly, only females showed the characteristic rightward (cardiac dilation) and downward (pressure reduction) shift of the pressure-volume loop. Additionally, maximum pressure and contractility were significantly worse in females. HW/BW ratios were significantly higher in female mdx mice. However, there was no difference in hydroxyproline content suggesting equivalent levels of cardiac fibrosis in both genders. Taken together, we found significant gender disparity in the cardiac function of aged mdx mice. Aged female mdx mice may represent a better model for studying Duchenne cardiomyopathy.

Disease Biology

17. nNOS mislocation reduces muscle force via RyR1 hypernitrosylation in a mouse model of Duchenne muscular dystrophy

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The loss of muscle force is a major clinical manifestation of Duchenne muscular dystrophy (DMD). However, molecular mechanisms underlying muscle weakening are not completely clear. DMD is due to dystrophin deficiency. Besides maintaining sarcolemmal integrity, dystrophin also recruits neuronal nitric oxide synthase (nNOS) to the sarcolemma. In DMD, nNOS delocalizes to the cytosol. While the loss of sarcolemmal nNOS leads to ischemic muscle injury in DMD, it remains controversial whether mislocalized nNOS contributes to muscle disease. Aberrant nNOS activation in the cytosol may lead to nitrosative damage. Surprisingly, studies have failed to show histopathology improvement in dystrophin/nNOS double knock mice. To resolve this discrepancy, here we tested the hypothesis that elevated cytosolic nNOS activity reduces muscle force by nitrosative modification of ryanodine receptor 1 (RyR1). Eliminating nNOS significantly enhanced specific forces of the EDL muscle in nNOS-null mdx4cv mice (n-dko). Further, eccentric contraction injury was tempered. Importantly, nitrosative stress was dramatically reduced and RyR1 hypernitrosylation was mitigated in n-dko muscle. Together, our results suggest that S-nitrosylation of RyR1 by cytosolic nNOS compromises force generation in dystrophin-null muscle. Reducing nitrosative stress may represent a promising strategy to enhance muscle strength in DMD patients.

Disease Biology

18. Defective myogenesis and 4q35 gene dysregulation in FSHD cell models

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FSHD is a dominant progressive myopathy that characteristically affects facioscapulohumeral musculature. While the disease is linked to contraction of the 4q35 D4Z4 repeat region, the mechanism by which FSHD arises is poorly understood. We are establishing a unique repository of tissue and primary cell samples from persons with FSHD and control relatives to develop cell models for the purpose of identifying and validating FSHD biomarkers and studying the FSHD disease mechanism. Real-time PCR assays using the Fluidigm Biomark System were performed to evaluate growth and differentiation conditions for FSHD and control cell cultures. FSHD cells exhibited lower levels of myoblast markers in proliferating cultures, and smaller inductions of differentiation genes over a 7-day differentiation timecourse. Differential expression of putative FSHD candidate biomarkers was also detected in FSHD cells. In remortalized FSHD cell clones with hTERT-mediated elongated telomeres, some rescue of myogenic defects was observed, although FSHD candidate genes

continued to be misregulated. Our findings provide evidence that FSHD pathology in muscle differentiation is manifest in cultured FSHD cells, and identify FSHD disease biomarkers. Funding: NICHD.

Disease Biology

19. Prolactin increases SMN gene expression via activation of STAT5 signalling pathway

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Spinal muscle atrophy (SMA) is an autosomal recessive neurodegenerative disease which is characterized by the loss of \'8c\'b1 motor neurons resulting in progressive muscle atrophy. Loss of functional Survival motor neuron (SMN) protein due to mutations or deletion in the SMN1 gene is the cause of SMA. A potential treatment strategy for SMA is to upregulate levels SMN protein originating from the SMN2 gene compensating in part for the absence of functional SMN1 gene. Sodium valproate, trichostatin A and aclarubicin, all agents which effectively enhance SMN2 expression, have been recently been shown to activate STAT5 in SMA-like mouse embryonic fibroblasts and human SMN2-transfected NSC34 cells. Given that Prolactin is also known to activate the STAT5 signaling pathway, we elected to assess its impact on SMN levels. In this manner we have demonstrated a significant induction in SMN mRNA and protein levels in NT2 and MN-1 cells upon treatment with Prolactin. We have demonstrated that activation of STAT5 pathway by Prolactin is necessary for this transcriptional upregulation of SMN gene. We have also found that Prolactin treatment induces SMN expression in brain and spinal cord samples of non-transgenic cd-1 mice in vivo. Impact of Prolactin on SMA mouse is currently under investigation. Presently there is no cure of SMA. This study provides a good mechanistic insight of how SMN protein is regulated through Prolactin via STAT5 pathway and its effect on the phenotype of the disease. This will help in the identification and characterization of STAT5 pathway activators as potential therapeutic compounds for the treatment of SMA.

Disease Biology

20. Motor neuron abnormalities in the ventral horn of L1 spinal cord from dystonia musculorum mice

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Dystonia musculorum (dt) is an inherited sensory neuropathy in mice that leads to sensory ataxia. While the primary pathology lies in the sensory neurons of dt mice, the overt movement disorder affecting the use of limbs suggests motor neurons may also be affected. We used a combination of techniques to determine the viability and role of motor neurons in the P15 dt mouse pathology. We found that dt mice have eccentric nuclei in alpha-motor neuron cell bodies in the ventral horn of the L1 spinal cord. Furthermore, there is a loss of alpha motor neuron cell bodies in dt spinal cords. A dramatic reduction in the total number of motor axons in the ventral root of dt mice was also evident. Among the motor neuron defects we observed were abnormal accumulation of phosphorylated neurofilaments (NF) in the proximal region (perikaryon) and bulb-like NF accumulation at the distal (presynaptic terminals) region of motor neurons. As well, neuromuscular junction staining of dt extensor digitorum longus (EDL) and tibialis anterior (TA) muscle fibers showed immature endplates and a significant decrease in axon branching compared to wild type littermates. Ultrastructure analysis revealed the presence of amyelinated alpha motor axons in the ventral root of the spinal nerve, suggesting a possible defect in oligodendrocyte function. Finally, motor behaviour tests revealed that motor performance is affected in dt mice. Overall, our work shows that motor neurons are not completely spared in dt mice and likely contributes to the dt phenotype. Funded by a grant from the CIHR.

Disease Biology

21. Distinct roles for laminin alpha1 globular domains in laminin alpha1 chain mediated rescue of laminin alpha2 chain deficiency

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Laminin (LM) alpha2 chain mutations cause congenital muscular dystrophy with dysmyelination neuropathy (MDC1A). One reason behind the obstacles in designing efficient therapy is that the pathology of MDC1A has not been fully understood. Previously, we demonstrated that LM alpha1 chain functionally replaces LM alpha2 chain in the neuromuscular system. Dystroglycan and integrins are major LM receptors. Unlike LM alpha2 chain, alpha1 chain binds the receptors by separate domains; globular (LG) domains 4 and LG1-3, respectively. Thus, the overexpression LM alpha1 chain that lacks LG4-5 dystroglycan binding domains in LM alpha2 chain deficient mice gives an opportunity to describe the molecular mechanisms underlying LM alpha1 chain mediated rescue of MDC1A. This overexpression resulted in prolonged lifespan and improved health of dystrophic mice. Most importantly, different muscle groups were spared to different degree - only the diaphragm and heart muscle were fully corrected - indicating an important variation in their requirements for LM-dystroglycan interaction. Furthermore, the regenerative capacity of the skeletal muscle did not depend on LM alpha1 LG4-5. However, this domain was crucial for preventing apoptosis in limb muscles and essential for myelination and myelin maintenance in peripheral nerve. These results show that alpha1 LG domains and consequently their receptors have disparate functions in the neuromuscular system.

Disease Biology

22. Transgenic HSP72 over-expression ameliorates the dystrophic pathology and improves muscle function in mdx dystrophic mice

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Duchenne muscular dystrophy (DMD) is characterized by the absence of dystrophin and results in muscle fiber fragility, disrupted Ca2+ handling, and activation of pathways responsible for muscle degeneration. Repeated cycles of degeneration and increasingly inadequate regeneration results in fibrotic infiltration within skeletal muscles and significant

functional impairments. Heat shock protein 72 (HSP72) has been shown to protect contractile function and improve Ca2+ handling in stressed cardiac myocytes. We tested the hypothesis that muscle-specific overexpression of HSP72 would ameliorate the dystrophic pathology and improve contractile function in mdx dystrophic mice. Female mdx mice were crossed with male mice overexpressing a muscle-specific rat inducible HSP72 transgene to yield mdx:HSP72 and mdx littermate controls. HSP72 overexpression improved specific force of diaphragm muscle strips and reduced collagen infiltration within the diaphragm. Whole body muscle breakdown, as determined from serum creatine kinase levels, was significantly less in mdx:HSP72 compared with mdx control mice. The improved dystrophic pathology with muscle-specific overexpression of HSP72 highlights therapeutic potential for DMD.

Disease Biology

23. A Zebrafish Model of Dynamin 2 (DNM2) Related Centronuclear Myopathy

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Mutations in dynamin-2 (DNM2) cause a dominant form of centronuclear myopathy (CNM) with a wide spectrum of clinical severity, ranging from severe congenital to mild late-onset forms. To examine the role of DNM2 in muscle development and disease, we identified the zebrafish homologue of human DNM2 and determined early expression patterns. Morpolinos were designed against DNM2 to knock down expression in developing embryos. DNM2 knockdown caused global developmental defects, including a shortened body structure, small muscle compartments and underdeveloped head and eyes. Behavioral analysis revealed severe motor defects in DNM2 morphant embryos. Immunohistochemistry revealed general disorganization and hypotrophy of individual muscle fibers. Notably, ultrastructural analysis showed significant disorganization of both the sarcoplasmic reticulum and T-tubules. We hypothesize that DNM2 plays a critical role in the formation and maintenance of the tubuloreticular network in muscle cells, and that disruption of this function leads to the phenotype seen in patients with CNM. In order to further examine the role of DNM2 in CNM, we have generated transgenic zebrafish expressing human DNM2-S619L, a mutation associated with severe congenital CNM. The study of DNM2 and associated proteins in zebrafish muscle offers a promising model for the study of CNM, and will lead to a greater understanding of the cellular dysfunction underlying this disease.

Disease Biology

24. TGFbeta signaling drives progression in muscular dystrophy

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Muscular dystrophy is a genetic disease caused by mutations in dystrophin and associated proteins. We previously generated deletions of the Drosophila melanogaster gene delta-sarcoglycan (Sgcd) that result in skeletal and cardiac muscle degeneration as a model of muscular dystrophy. Transforming Growth Factor beta (TGFb) signaling has been shown in human and mouse muscular dystrophy. Additionally, TGFb-neutralizing antibodies alleviate aspects of the muscle phenotype. We investigated whether TGFb signaling is pathogenic in our Drosophila model. Using the dad-lacZ reporter, we found TGFb activity in cells near the site of muscle tears. We found that exercise increased muscle injury and thus TGFb signaling in the Sgcd mutant. We decreased TGFb activity in flies by introducing heterozygous mutations of the co-SMAD Medea (homologous to SMAD4), or one of the two r-SMADs, MAD (SMAD1/5/8) or Smox (SMAD2/3). Mutations in Medea, Mad, or Smox restore skeletal muscle function in Sgcd mutants. Mutations in Medea and Smox, but not Mad, restore cardiac function suggesting some differences in the response of heart and muscle to SMAD signaling. We report the first genetic evidence that TGFb signaling is pathogenic in muscle degeneration.

Disease Biology

25. Dystrophin-deficient canine hindlimb flexor muscles display abnormally low stiffness

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Dogs with golden retriever muscular dystrophy (GRMD) model human Duchenne muscular dystrophy, with signs of progressive muscle weakness, contractures and gait abnormalities. Muscle stiffness (delta force/delta muscle length) has not been evaluated in GRMD dogs. We tested the hypothesis that GRMD muscles have greater-than-normal stiffness, similar to that reported in DMD patients. Stiffness was determined in hindlimb tibiotarsal flexor muscles of normal (n=5), carrier (n=7), and affected GRMD (n=10) dogs aged ~4 to 118 months with an eccentric protocol. Each dog was anesthetized, positioned with one hind paw affixed to a servomotor lever arm, and muscle contractions induced in tibiotarsal flexors by 1 sec percutaneous peroneal nerve stimulation at 50hz. Muscles contracted isometrically for 800 ms, and then the servomotor rotated the lever arm 29 degrees in extension at ~0.7 Lo/sec for 200 ms. Flexor stiffness for GRMD dogs (0.03 +/- 0.01 N-m/deg; mean +/- SD; $p<0.05$)) was ~67% less than normal (0.09 +/- 0.03) and ~57% less than carrier (0.07 +/- 0.02), while normal and carrier values were similar. These novel findings should be interpreted with respect to experimental conditions and state of pathology: first, dogs were anesthetized, which could have dampened normal neural drive; second, there is variable pathology among GRMD muscles with flexor muscles relatively spared. Thus, similar studies should be expanded to other muscle groups. Funding: National Center for Canine Models of DMD (NCDMD) (1U24NS059696-01A1; NINDS/NIAMS; Kornegay JN); MDA Infrastructure Grant, Natural History and Immunological Parameters in the German Shorthaired Pointer Muscular Dystrophy [GSHPMD] Dog (Kornegay JN).

Disease Biology

26. Lifting the nebula: novel insights in the molecular basis of muscle weakness in nemaline myopathy

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Nemaline myopathy (NM) is a common non-dystrophic congenital myopathy, with the most important clinical feature being unexplained muscle weakness. We studied the muscular phenotype of NM patients with a well-defined nebulin mutation (NEM2), using a multidisciplinary approach to study thin filament length regulation and muscle contractile performance. Since SDS-PAGE and Western blotting revealed greatly reduced nebulin levels in skeletal muscle of NM-NEB patients, we also carried out comparative studies with a nebulin knockout (NEB KO) mouse model. Mechanical studies revealed that muscle fibers from both NM-NEB and NEB KO muscle had increased tension cost, and reductions in ktr, calcium sensitivity of force generation, and cooperativity of activation. Studies also showed that both NM-NEB and NEB KO muscle had reduced thin filament length and that this lowers force on the descending limb of the force-sarcomere length relation. These findings indicate that in skeletal muscle, nebulin increases force through (1) increasing thin-filament length, and (2) increasing the crossbridge fraction that generates force via increasing thin filament activation and altering crossbridge cycling kinetics. These novel roles of nebulin in regulating muscle force add a new level of understanding to skeletal muscle function, and provide a mechanism for the severe muscle weakness in patients with nebulin-based nemaline myopathy.

Disease Biology

27. Functions of selenoprotein N and the ryanodine receptor intracellular calcium release channel in muscle differentiation and cell signaling

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Mutations that cause complete loss of Selenoprotein N (SepN) and mutations that affect the function of the RyR1 protein, the core protein component of the skeletal muscle Ryanodine Receptor intracellular Calcium Release Channel (RyR-CRC), result in congenital myopathies with a similar spectrum of cellular defects. To account for the similar phenotypes, we proposed that SepN functions as modifier of RyR-mediated calcium mobilization in vivo and that defects in calcium release account for the cellular aberrations observed in the congenital myopathies. This hypothesis is supported by our work (Jurynec et al. (2008). PNAS 105: 12485-90.) indicating: i) SepN interacts directly with the RyR-CRC, ii) SepN is required for normal calcium mobilization in the embryo, and iii) SepN is necessary for normal RyR-CRC function measured in vitro. Further, our data indicate SepN is an essential component of the RyR-CRC redox sensor. An alternative model has been set forth in which the primary function of SepN is to maintain the overall redox state of the cell and only indirectly affects muscle function (Arbogast et al. (2009). Ann Neurol 65: 677-686.). To understand the origins of the cell defects observed in SepN-related and RyR-related congenital myopathies, we analyzed the cell differentiation defects that occur in the zebrafish embryo upon loss of either SepN or RyR-CRCs. We find SepN and RyR-CRC function are required for precisely the same cell differentiation events and intercellular signaling events, supporting the model that both proteins function in a common molecular pathway. Specifically we find that Selenoprotein and RyR functions are required for carrying out Hedgehog growth factor signaling in a number of contexts. The effect of loss of SepN- or RyR-mediated Hedgehog signaling is a deficit in the induction of slow muscle fibers, a cell type-specific defect often observed in RyR- and SepN-related myopathies. We propose that defects in muscle development, specifically in slow muscle cell induction, contribute to the pathology of RyR- and SepN-related disease.

Disease Biology

28. The Zebrafish dag1 Mutant: A Novel Gene Model For Dystrophy

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In a forward genetic approach to identify genes responsible for congenital muscle diseases, we identified a zebrafish mutant that exhibits degenerating muscle fibers with impaired motility behavior and lethality by 7 dpf. Genetic mapping identified a genomic locus containing the human orthologue of DAG1 encoding dystroglycan, a glycoprotein and integral membrane component of the dystrophin-glycoprotein complex (DGC). Mutations of enzymes involved in glycosylation of dystroglycan result in several forms of muscular dystrophy. The dag1 gene in mutant fish contains three point mutations near the splice acceptor site of intron 1, resulting in unstable transcripts and complete absence of dystroglycan protein. Ultrastructural studies of dag1 muscles revealed structural abnormalities in triads at 2 dpf, preceding sarcolemmal damage, which becomes evident only at 4-5 dpf. Small and irregular shaped t-tubules, as well as highly disorganized terminal and longitudinal cisternae of sarcoplasmic reticulum, were seen in myofibers of dag1 fish. Immunofluorescence of WT fish confirmed expression of detectable dystroglycan at t-tubules, and co-localization with DHPR, suggesting that dystroglycan is required for t-tubule biogenesis. This zebrafish model thus provides unique opportunities in the understanding of biological functions of dystroglycan as disruption of this gene in higher vertebrates results in early embryonic lethality.

Disease Biology

29. Disease-causing missense mutations in actin binding domain 1 of dystrophin induce thermodynamic instability and protein aggregation

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Mutations in the dystrophin gene cause Duchenne muscular dystrophy (DMD) most commonly through loss of protein expression. In a small sub-population of patients,

missense mutations can cause DMD, Becker muscular dystrophy (BMD) or X-linked cardiomyopathy (XLCM). Nearly one-half of disease-causing missense mutations are located in actin-binding domain 1 (ABD1) of dystrophin. To test the hypothesis that ABD1 mutations cause disease by impairing actin binding activity, we engineered the K18N, L54R, D165V, A168D, L172H and Y231N mutations into the full-length dystrophin cDNA and characterized the biochemical properties of each mutant protein. The K18N and L54R mutations result in the most severe diseases in humans and each caused a small but significant 4-fold decrease in actin binding affinity, while the affinities of the other mutant proteins were not significantly different from WT dystrophin. More interestingly, WT dystrophin was observed to unfold in a single-step, highly cooperative manner. In contrast, all six mutant proteins were significantly more prone to thermal denaturation and aggregation. Our results suggest that missense mutations in ABD1 may all cause loss of function via protein instability and aggregation rather than loss of ligand binding. However, more severe disease states may be due to the combinatorial effects of mutations on both protein aggregation and impaired actin binding.

Disease Biology

30. The MRL genome inhibits late stages of muscular dystrophy

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The "superhealing" MRL mouse strain has been studied for its accelerated wound repair in many tissues. We hypothesized that the MRL genome also contained alleles that will aid chronic myopathic diseases such as muscular dystrophy and cardiomyopathy. To test this hypothesis we introduced the sarcoglycan-null allele (Sgcg), a model of Limb Girdle Muscular Dystrophy and cardiomyopathy, into the MRL mouse background strain. We analyzed disease progression in the Sgcg null F2 generation (MRL/Sgcg) comparing two different pathological traits, Evans Blue Dye uptake as a measure of membrane permeability and hydroxyproline content as an indication of collagen deposition and fibrosis. When disease progression was compared between the MRL/Sgcg mice and the founder Sgcg null mice, we found that membrane permeability of the dystrophic muscle was not modulated by the MRL background. In contrast, the fibrotic replacement of muscle was greatly attenuated in MRL/Sgcg mice to the point where hydroxyproline deposition was comparable to normal muscle. The lack of fibrosis was noted in the skeletal, diaphragm and cardiac muscles. These data illustrate that membrane permeability and fibrosis can be altered by different genetic modifiers and that abrogation of fibrosis alone is beneficial in muscular dystrophy. The future identification of the muscular dystrophy modifier genes from the MRL strain will identify pathways that may be useful to treat muscular dystrophy and predict outcome in patients.

Disease Biology

31. RNA-binding proteins HuR and AUF1 mediate skeletal muscle atrophy through the regulation of atrogenes MAFbx and MuRF1

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Skeletal muscle atrophy is a debilitating condition largely caused by muscle disuse but is also a major consequence of space flight, aging, and many diseases. To date, most studies dealing with the molecular events involved in atrophy have focused on transcriptional regulation. In contrast, the contribution of post-transcriptional mechanisms have been largely ignored. Here, we investigate the role of the RNA-binding proteins HuR and AUF1 during skeletal muscle atrophy. Analysis of whole cell and subcellular fractions from denervated mouse muscles and DEX-treated C2C12 cells revealed significant increases in HuR and AUF1. shRNA-mediated knockdown of HuR and AUF1 led to a clear reduction in DEX-induced atrophy, as evidenced by increased myotube diameter and decreased atrogene expression. RNA-IP of HuR and AUF1 revealed distinct binding to MAFbx and MuRF1 transcripts. Luciferase activity of both MAFbx and MuRF1 3â€™UTR reporter constructs increased upon DEX treatment, which was abolished upon knockdown of HuR and AUF1. These results indicate that HuR and AUF1 mediate skeletal muscle atrophy via stabilization of MAFbx and MuRF1. Thus, manipulating their expression during skeletal muscle atrophy may provide new therapeutic avenues. Funded by the Canadian Space Agency and CIHR

Disease Biology

32. DNAJB2 expression in normal and diseased human and mouse skeletal muscle

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DNAJB2, a co-chaperone regulator of Hsp70 that is expressed principally in the nervous system, has been recently reported to be upregulated in human skeletal muscle during its recovery from damage. Here we studied skeletal muscles of the dystrophic mdx mouse and patients with Duchenne muscular dystrophy and indeed identified DNAJB2 expression in regenerating fibers. Surprisingly, in both dystrophic and control mice and patients, DNAJB2 was also expressed in non-regenerating fibers, at the postsynaptic side of the neuromuscular junction. DNAJB2 functions as an adaptor molecule for the evacuation and degradation of proteins through the ubiquitin-proteasome system, and overexpression of DNAJB2 in models of the neurodegenerative disease spinobulbar muscular atrophy was shown to result in the reduction of protein inclusions. We therefore studied the possible relation of DNAJB2 expression to protein inclusion formation in skeletal muscle, in biopsies of several muscle pathologies associated with protein aggregation, and found in all of them a strong immunoreactivity with anti-DNAJB2 in aggregates and in vacuoles. We conclude that DNAJB2 is expressed in mouse and human skeletal muscle: at the neuromuscular junction of normal fibers, in the cytoplasm of regenerating fibers, and in protein aggregates and vacuoles in protein aggregate myopathies. We propose a role for DNAJB2 in protein turnover processes in skeletal muscle.

Disease Biology

33. Transgenic over-expression of gamma-cytoplasmic actin protects against eccentric contraction induced injury in mdx mice

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We previously reported that levels of gamma-cytoplasmic actin are elevated in dystrophin-deficient mdx mouse skeletal muscle. To investigate how elevated levels of gamma-cytoplasmic actin may influence the dystrophic phenotype, we transgenically over-expresssed gamma-cytoplasmic actin specifically in mdx skeletal muscle (mdx-TG mice). Levels of gamma-cytoplasmic actin in mdx-TG skeletal muscle were elevated 200-fold compared to mdx skeletal muscle and showed incorporation into thin filaments. Gross over-expression of gamma-cytoplasmic actin did not improve or exacerbate mdx muscle pathology. However, mdx-TG skeletal muscle had significantly improved specific force during eccentric contractions. Moreover, gamma-cytoplasmic actin over-expression was able to protect against force loss after the initial eccentric contractions. Further investigation into gamma-cytoplasmic actin's ability to protect mdx muscle during lengthening contractions may lead to insight into the mechanism of eccentric contraction-induced injury in dystrophin-deficient muscle.

Disease Biology

34. Residual laminin-binding activity and enhanced dystroglycan glycosylation by LARGE in novel model mice to dystroglycanopathy

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Hypoglycosylation and reduced laminin-binding of alpha-dystroglycan (alpha-DG) are common characteristics of a group of muscular dystrophy (dystroglycanopathy). Almost all cases of Fukuyama-type congenital muscular dystrophy, a severe form of dystroglycanopathy, are caused by a retrotransposal insertion in the fukutin gene. Here, we generated knock-in mice carrying the retrotransposal insertion in the mouse fukutin ortholog. Knock-in mice exhibited no signs of muscular dystrophy. Biochemical analyses detected minor levels of intact alpha-DG, and residual laminin-binding levels (~50% of normal) in knock-in mice. In contrast, intact alpha-DG is undetectable in the dystrophic Large(myd) mouse, and laminin-binding is markedly reduced. These data indicate that a small amount of intact alpha-DG is sufficient to maintain muscle integrity in knock-in mice. Transfer of the LARGE gene increased glycosylation and laminin-binding of alpha-DG in both knock-in mice and the POMGnT1 mutant mouse, which is another model of dystroglycanopathy. These data suggest that even partial restoration of alpha-DG glycosylation by replacing or augmenting glycosylation-related genes might deter disease progression and thus provide therapeutic benefits.

Disease Biology

35. Zebrafish Model for FKRP muscular dystrophies

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Mutations in Fukutin-related protein (FKRP) have been linked to a variety of phenotypes including Walker-Warburg syndrome (WWS), Limb Girdle Muscular Dystrophy (LGMD) 2I and congenital muscular dystrophy 1C (MDC1C). To examine the FKRP functions and to reveal the mechanism of muscle diseases, zebrafish were used as an animal model. Downregulating FKRP expression in zebrafish by two different morpholinos resulted in embryos with developmental defects similar to those observed in human muscular dystrophies associated with mutations in FKRP. The FKRP morphants showed phenotypes involving alterations in somitic structure and muscle fiber organization, as well as defects in developing eye morphology. Additionally, they were found to have a reduction in alpha-dystroglycan glycosylation and a shortened myofiber length. Moreover, co-injection of fish or human FKRP mRNA along with the morpholino restored normal development, alpha-dystroglycan glycosylation and laminin binding activity of alpha-dystroglycan in the morphants. Co-injection of the human FKRP mRNA containing causative mutations found in human patients of WWS, MDC1C and LGMD2I, could not restore their phenotypes significantly. Interestingly, these morphant fish having human FKRP mutations showed a wide phenotypic range similar to that seen in humans. These zebrafish may be good models for studies of pathomechanism of FKRP muscular dystrophies.

Disease Biology

36. NADPH oxidase is a primary source of oxidative stress in dystrophic muscle

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Recent studies have shown that Reactive Oxygen Species (ROS) contribute to the pathogenesis of muscle disease in mdx (dystrophic) mice. Here, we have investigated the role of NADPH oxidase as a primary source of the oxidative stress in these mice. The NADPH oxidase proteins gp91phox, p67phox and rac 1 were increased 2 - 3 fold in tibialis anterior muscles from mdx mice compared to wild type. Importantly, this increase occurred in 19 day old mice, before the onset of muscle necrosis. This suggests that NADPH oxidase is a primary cause of oxidative stress in dystrophin-deficient muscle rather than a secondary response to damage and inflammation. Studies on young adult mdx mice also showed increased NADPH oxidase protein expression and ROS production, using a chemiluminescent ROS assay. In addition, stretched contractions increased resting intracellular calcium in single mdx muscle fibers, which was significantly reduced by an NADPH oxidase inhibitor. Taken together, these results suggest that NADPH oxidase is a primary source of the increased basal and stretch-induced ROS production, which contributes to the loss of calcium homeostasis in mdx muscle. Support from the

NH&MRC, Australia.

Disease Biology

37. Dysferlin plays an evolutionarily conserved role in cholinergic synaptic signaling in C. elegans and mammals

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Dysferlin gene mutations cause LGMD2B, but the pathogenesis mechanisms are not fully understood. To better understand Dysferlin function, we studied the founding member of the Dysferlin gene family, C. elegans fer-1. Previously, we have shown that fer-1 is expressed in C. elegans muscle. Here, we show that fer-1 mutants exhibit specific defects in the clustering of post-synaptic acetylcholine receptors (AChRs) and are resistant to drugs that activate cholinergic signaling. Quantitative biomechanical studies of C. elegans motility reveal that fer-1 mutants swim with reduced efficiency, as compared to wild type animals. Acute administration of a cholinesterase inhibitor rescued the excitability and biomechanical properties of fer-1 muscle. To determine whether the synaptic functions of fer-1 are evolutionarily conserved, we examined cholinergic synaptic signaling in the A/J mouse model of Dysferlin deficiency. Using an in vivo platform for simultaneous measurements of muscle force and electrical activity, we observed defects in muscle force generation and electromyograms upon repetitive neuronal stimulation. Preliminary studies suggest that the rep-stim defects can be reversed through administration of an FDA-approved acetylcholinesterase inhibitor. These data suggest that Dysferlin plays an evolutionarily conserved role in cholinergic synaptic signaling and reveal a myasthenic-like component to LGMD2B disease pathogenesis.

Disease Biology

38. Amphiphysin 2 (BIN1) and triad defects in several forms of centronuclear myopathies

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Centronuclear myopathies (CNM) are congenital myopathies characterized by generalized muscle weakness and mislocalization of muscle fiber nuclei. Genetically distinct forms exist and mutations in amphiphysin 2 (BIN1) were recently identified in autosomal recessive cases. BIN1 has been implicated in membrane remodeling in brain and skeletal muscle. In this study, we show that the two BIN1 isoforms expressed in skeletal muscle possess the phosphoinositide-binding domain and are specifically targeted to the triads, close to the DHPR-RYR1 complex. Cardiac isoforms neither contain this domain nor localize to the triads, suggesting that splicing of BIN1 regulates its specific function in skeletal muscle. Immunohistofluorescence and electron microscopy analysis of muscles from patients with BIN1 mutations reveal aberrations of BIN1 localization and triad organization. These defects are observed in the three human forms of CNM and two different mammalian CNM models. In addition to a previously reported implication of BIN1 in cancer as a tumor suppressor, these findings sustain an important role for BIN1 skeletal muscle isoforms in membrane remodeling and organization of the excitation-contraction machinery. We propose that aberrant BIN1 localization and defects in triad structure are part of a common pathological mechanism shared between the three forms of centronuclear myopathies.

Disease Biology

39. Human muscle-derived progenitor cells express neuronal and glial markers in vitro and promote peripheral nerve repair

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Our group has recently isolated populations of human muscle-derived progenitor cells (hMDPCs) from human skeletal muscle using a modified preplate technique. We aimed to examine the in vitro expression and differentiation potential of hMDPCs toward neuronal and glial lineages, and determine whether the transplantation of hMDPCs into a critical sciatic nerve defect in adult SCID (immuno-deficient) mice could improve regeneration and functional recovery following injury. Our study demonstrated that the hMDPCs expressed both neuronal and glial markers and could undergo myogenic and neurogenic differentiation in vitro. Furthermore, these cells greatly enhanced the rate of nerve repair via the induction of nerve fiber regeneration and myelin-producing Schwann cells. Functional recovery after sciatic nerve injury was evident by improved walking patterns indicating reinnervation. In addition, gastrocnemuis muscle weight and area of the muscle fibers in the hMDPCs transplanted group were comparable to the contra-lateral uninjured control group and significantly greater than the PBS-treated mice at 72 weeks post-implantation. Here we identified human muscle as a source of progenitor/stem cells capable of to adopting a neuronal and/or Schwann cell phenotype, which could ultimately be used in cell-mediated therapies and tissue engineering applications for the treatment of peripheral nerve injuries.

Disease Biology

40. Impaired contractile properties in myotubularin deficient mice and dogs

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X-linked myotubular myopathy (XLMTM) causes severe perinatal weakness and often results in respiratory failure. XLMTM is caused by a congenital deficiency of myotubularin, implicated in T tubule organization and impaired excitation-contraction coupling. To evaluate the role of myotubularin in muscle contractile performance, we studied muscles from both murine and canine XLMTM models. Ex vivo field stimulation of soleus muscles from mildly weak, myotubularin deficient (Mtm1-/-) mice (n=5) resulted in markedly lower-than-normal

isometric force in comparison to wild type controls at 25-28 days of age. In contrast, stimulated EDL muscles from Mtm1-/- animals (n=7) produced only slightly lower-than-normal isometric force. Compared to wild type controls, Mtm1-/- animals demonstrated a right-shifted force-frequency relationship. Repeated in vivo force testing in Labrador retrievers (6 normal, 5 carriers, 1 affected) revealed progressive impairment of isometric contraction in the affected dog. Similar to Mtm1-/- mice, a right shift in the force-frequency relationship was also observed in the affected animal. These novel findings support the idea of a primary excitation-contraction coupling defect in myotubularin deficiency.

Disease Biology

41. Genetic ablation of CC class chemokine receptor 2 (CCR2) improves myofiber structure and function in the dystrophic (mdx) diaphragm

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Background: In the mdx mouse model of Duchenne Muscular Dystrophy (DMD), the diaphragm most closely resembles the human phenotype. The infiltration of inflammatory cells into dystrophic muscles propagates the pathology of DMD. We have previously reported that CC class chemokines, which attract macrophages and other inflammatory cell types found within dystrophic muscles, are highly upregulated in the mdx diaphragm. Objectives: We hypothesized that genetic ablation of CCR2, a CC class chemokine receptor which is important for monocyte/macrophage trafficking, would attenuate pathological changes within the mdx diaphragm. Methods and Results: Mdx/CCR2-/- mice were generated and evaluated at 2 ages (6 weeks and 3 months). Histological analysis revealed that mdx/CCR2-/- diaphragmatic myofibers were significantly larger and had fewer central nuclei than their mdx counterparts. In mdx/CCR2-/- mice, multiple proinflammatory genes were downregulated by quantitative RT-PCR, and immunostaining revealed a significant reduction in the number of macrophages contained within the diaphragm. In addition, maximal force production and the ability to maintain force following eccentric contractions were both significantly improved in mdx/CCR2-/- diaphragms compared to mdx mice. Conclusions: In the severely affected mdx diaphragm, which has a high level of inflammation and resembles human DMD, the loss of CCR2 function has beneficial effects upon the structure and function of dystrophic muscle fibers. Therefore, these findings suggest that CCR2 and/or its ligands could be useful therapeutic targets in DMD.

Disease Biology

42. Fiber type switching and centronuclear myopathy in mice lacking miR-133a

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Skeletal muscle is composed of heterogeneous myofibers with distinctive contractile and metabolic properties. Slow twitch (type I) myofibers display an oxidative metabolism and resistance to fatigue, whereas fast twitch (type II) fibers are primarily glycolytic and suited for rapid bursts of activity. Adult myofibers are highly plastic and can switch between slow and fast phenotypes in response to work load, hormonal stimuli, or disease. We show that the muscle-specific microRNA, miR-133a, suppresses accumulation of slow-twitch myofibers. Mice with genetic deletions of miR-133a-1 and miR-133a-2 (dKO) display increased numbers of slow fibers in soleus and GP muscle. Interestingly, miR-133a does not affect embryonic development of slow muscle fibers. Suppression of slow myofiber gene expression by miR-133a can be attributed, at least in part, to inhibition of calmodulin-1, which is necessary for maintaining the slow fiber gene program. Adult miR-133a dKO mice also develop centronuclear myopathy with a marked increase in centrally located nuclei in type II myofibers, without apparent signs of inflammation, fibrosis or myocyte death. miR-133a directly targets dynamin 2, a GTPase known to be important for the development of centronuclear myopathy in humans. Together, our studies demonstrate an essential role for miR-133a in the maintenance of myofiber identity, growth and homeostasis of skeletal muscle.

Disease Biology

43. Genetic Analysis of Potential Modifiers of Muscular Dystrophy in DM1

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Myotonic muscular dystrophy (DM1), the most common neuromuscular disorder in adults is the first example of a disorder caused by RNA toxicity. DM1 is caused by a (CTG) expansion in the DMPK gene resulting in a mutant RNA that dominantly causes disease by affecting specific RNA binding proteins (sequestration of muscleblind (MBNL1) and increased CUG-binding protein (CUGBP1) through hyperphosphorylation of CUGBP1 by protein kinase C isoforms alpha and beta). Recently, we developed the first inducible mouse model of RNA toxicity with which we showed that effects of RNA toxicity are reversible in skeletal and cardiac muscle. This was correlated with increased CUGBP1 in skeletal muscle. We also identified a novel mechanism of RNA toxicity, namely induction of a cardiac specific transcription factor, NKX2-5 in skeletal muscle. To study the role of these factors in DM1, we have generated mice expressing the toxic DMPK 3'UTR RNA in the absence of CUGBP1, PKC-alpha and PKC-beta, and transgenic mice that overexpress NKX2-5. These mice are currently being studied by EMG, ECG, treadmill, grip strength, histopathology and molecular means. Results from ongoing experiments will be presented.

Disease Biology

44. C/EBPbeta persistent expression in satellite cells inhibits skeletal muscle regeneration in cancer cachexia

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Cancer cachexia is a paraneoplastic syndrome characterized by an important skeletal muscle and adipose tissue atrophy, leading to fatigue and weakness, and causing significant death. Although the weight loss seen in patients is often concomitant with anorexia, increase food administration is

unable to reverse the effects, suggesting that poor nutritional intake is not the only factor involved in cachexia progression. Most studies on cachexia investigated catabolism pathways to explain the aforementioned effects, although only limited publications looked at the anabolism response. Using both in culture and in vivo models of cachexia, we assessed the contribution of skeletal muscle regeneration to the development of muscle wasting. We report that the muscle anabolic response is inhibited by cachectic stimuli and that these effects are mediated, at least in part, by the persistent expression of transcription factor CCAAT/Enhancer Binding Protein beta, in muscle satellite cells.

Disease Biology

45. Ectopic Calcification is caused by elevated serum inorganic phosphate in mdx mice

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Ectopic calcification was found in skeletal muscle of mdx mouse, an animal model of Duchenne muscular dystrophy. The calcium deposits were composed of hydroxyapatite, a crystallized form of calcium phosphate, and the serum inorganic phosphate (Pi) level in the mdx mice was approximately 1.4 times higher than that in the normal B10 mice, suggesting that Pi plays a critical role in the generation of ectopic calcification. When C2C12 mouse myoblasts were cultured under high-Pi concentration equivalent to mdx serum, expressions of osteogenic markers such as osteocalcin and Runx2 were up-regulated. At higher Pi concentration, myogenin expression and myogenic differentiation were retarded while osteogenic differentiation and generation of calcium deposition were obvious in culture. Moreover, ectopic calcification decreased significantly in mdx mice fed with Pi-reduced diet. We therefore conclude that the Pi-induced osteogenesis of muscle cells is responsible for ectopic calcification in mdx mouse skeletal muscle.

Disease Biology

46. CTCF binds to D4Z4 and regulates transcription of DUX4

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Facioscapulohumeral muscular dystrophy (FSHD) is caused by contraction of the D4Z4 repeat on chromosome 4q, which is associated with regional loss of repressive chromatin marks. The underlying disease mechanism remains speculative but these epigenetic changes might result in pathologic expression of DUX4. Recent data revealed that D4Z4 can act as an insulator protecting genes from position effect. We demonstrate a role for the chromatin insulator protein CTCF in vivo. While in fibroblasts and myoblasts from control individuals D4Z4 is not occupied by CTCF, in FSHD there is discrete, yet significant binding of CTCF to at least three sequences within the D4Z4 unit. Consistent with the role of CTCF in protecting genes from epigenetic silencing, we observed a significant loss of both histon H3K9 trimethylation and DNA methylation at CTCF binding sites at D4Z4 in FSHD alleles. The identified CTCF binding sites are located on each side of the transcription start site of DUX4. Down regulation of CTCF resulted in significantly reduced DUX4 transcripts levels in FSHD myoblasts. Thus aberrant CTCF binding at D4Z4 in FSHD may interfere with the setting of repressive epigenetic marks and results in inappropriate DUX4 transcription.

Disease Biology

47. Function and dysfunction of muscle side population cells: a potential factor in muscular dystrophy pathogenesis

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Duchenne Muscular Dystrophy (DMD) is characterized by muscle fiber necrosis and chronic inflammation surrounding degenerating fibers. Secondary effects that further accelerate disease progression include a gradual reduction in muscle regenerative potential and the replacement of muscle tissue with adipose and fibrotic tissue. There is currently no clear mechanism to explain either of these outcomes but it has been suggested that alterations in the activity of muscle resident stem cells could account for reduced regeneration and constitute a source of fibrotic cells in dystrophic muscle. We are interested in a specific population of muscle stem cells known as side population (SP) cells identified by their exclusion of the Hoechst 33342 dye. Characterization of SP cells has thus far been limited to in vivo transplantation studies due to a lack of an in vitro system capable of sustaining SP cell proliferation and muscle differentiation. We have developed an in vitro culture system to characterize muscle SP cells in isolation from normal as well as damaged tissue. In agreement with in vivo studies, we show that under our culture conditions, muscle SP cells isolated from intact wild type muscle give rise to PAX7+ satellite cells, differentiate into myotubes, and also give rise to fibroblasts. However, muscle SP cells derived from dystrophic mdx5cv muscle or cardiotoxin-injured muscle do not differentiate towards a myogenic lineage. Instead, SP cells isolated from a damaged muscle environment show an increased proliferation rate, exhibit enhanced fibroblast differentiation, and gain the ability to differentiate into adipocytes. Our data indicate that proliferation as well as cell fate choices of muscle SP cells are altered in damaged muscle suggesting that muscle SP cells could play a role in the deregulation of muscle regeneration in muscular dystrophy. Our in vitro system should prove a useful tool to complement in vivo studies and dissect the molecular mechanisms that regulate SP cell differentiation and proliferation. An increased understanding of the regulation of muscle SP cells may provide mechanisms for disease progression in DMD as well as generate new avenues for therapeutic interventions focusing on alterations of muscle resident stem cell activity.

Disease Biology

48. Bidirectional transcription enhances instability of expanded CTG repeats

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Myotonic dystrophy type 1 (DM1) is caused by expansion of a CTG repeat in DMPK. The DM1 expansion is unstable in somatic cells, leading to marked heterogeneity of repeat length in different tissues. Progression of DM1 may result from growth of the repeat in somatic cells over time. Our present understanding of the mechanisms that underlie repeat instability is incomplete. Previous work in bacteria and in human fibrosarcoma cells has suggested that instability of expanded CTG:CAG repeats is enhanced by transcription across the repeat tract. These studies, however, focused on contractions of the repeat, rather than expansions that predominant in human DM1. To study the effects of transcription in one or both directions, we derived normal human fibroblasts that have single-copy genomic integrations of highly-expanded CTG repeats. We observed instability of 800 CTG repeats in quiescent (contact-inhibited) and proliferating cells. Instability of the repeat was enhanced by transcription in the forward or reverse direction. Transcription of the repeat in both directions, which is reported to occur at the human DM1 locus, resulted in marked instability, which was greater than the sum of instabilities with transcription in the forward and reverse directions. Bidirectional transcription was frequently associated with expansions or contractions of more than 100 repeats. A similar phenomenon was observed for 250 CTG repeats, though it was less pronounced. Our results suggest that bidirectional transcription is important factor in generating the remarkable genetic instability of expanded CTG repeats in DM1. This cell model may provide a useful tool for studying interventions that stabilize the repeat, which potentially could delay the onset or slow the progression of DM1. This work comes from the Wellstone Muscular Dystrophy Cooperative Research Center at the University of Rochester (NIH U54NS48843) with support from the NIH (AR046806, AR48143) and the Muscular Dystrophy Association.

Disease Biology

49. Effects of muscle-specific inhibition of the NF-kappaB pathway on diaphragmatic dysfunction during sepsis

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Background: Acute respiratory failure is common in severe infections (sepsis) and often accompanied by diaphragmatic weakness. The transcription factor NF-kappaB plays a central role in the upregulation of several proinflammatory mediators implicated in this process. However, the specific role and influence of NF-kappaB activation within the muscle fibers themselves during sepsis is not known. Objective: To determine whether muscle-specific inhibition of NF-kappaB activation protects against diaphragmatic dysfunction during sepsis. Methods and Results: Transgenic mice with skeletal muscle-specific inhibition of NF-kappaB activation (termed MISR), achieved by muscle creatine kinase (MCK) promoter-driven expression of the IkappaB-alpha superrepressor, were compared to their non-transgenic littermates. Sepsis was induced by systemic injection of E. coli endotoxin (LPS), and mice were evaluated 24 hours later. Western blot confirmed expression of the transgene within the diaphragm. In transgenic mice, phosphorylation of IkappB-alpha and the p65 subunit of NF-kappaB were inhibited following exposure to LPS. The levels of several proinflammatory mediators (IL-1, IL-6, MCP-1) tended to be lower in the diaphragms as well as the serum of transgenic mice. In addition, MISR transgenic diaphragms showed significantly greater tetanic force production than non-transgenic mice after LPS injection. Conclusions: Activation of NF-kB within muscle fibers plays a major role in the loss of diaphragmatic force generation associated with sepsis. Funding Source: Canadian Institutes of Health Research, Fonds de la recherche en sante du Quebec, Quebec Respiratory Health Network, McGill University Health Centre

Disease Biology

50. Aberrant splicing of the annexin VII gene in myotonic dystrophy type 1 muscle satellite cells is independent of Mbnl1 and CUGBP1 activities and impairs their fusion into myotubes in vitro

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Myotonic dystrophy type 1 (DM1) and type II (DM2) are dominantly inherited multisystemic disorders. DM1 is triggered by the pathological expansion of a (CTG)n triplet repeat in the DMPK gene, whereas a (CCTG)n tetranucleotide repeat expansion in the ZNF9 gene causes DM2. Several DM pathologies, such as myotonia and diabetes, are caused by defects in alternative RNA splicing or spliceopathy. We report here a novel splicing anomaly in DM1. We show that the pre-mRNA for annexin VII (ANXA7) is aberrantly spliced during muscle satellite cell differentiation in vitro, leading to the expression of a shorter, non-muscle isoform. The larger transcript, bearing an additional exon at the 5' end, is normally expressed in the brain, heart, and skeletal muscle. We determined that fetal DM1 myotubes express almost exclusively the shorter isoform, whereas both isoforms were present in unaffected muscle cells. In contrast, annexin VII splicing was normal in adult DM2 muscle cells. When restored, the expression of the muscle-specific isoform improved the fusion of fetal DM1 satellite cells by increasing their myonuclear number and their overall fusion. Overexpression of muscleblind (Mbnl1), a protein involved in the DM spliceopathy, did not restore normal ANXA7 splicing in DM1 cells. Similarly, expression of CUGBP1 in normal myoblasts did not reproduce the aberrant splicing observed in DM1 cells. These results suggest that the missplicing of annexin VII mRNA underlies the fusion defect of fetal DM1 satellite cells and possibly contributes to the delay in muscle maturation in the patients. This is the first demonstration of a splicing defect that is specific for DM1 only. Our data also suggest that annexin VII is a novel player in the process of muscle satellite cell fusion.

Disease Biology

51. Inhibition of the p97/VCP/Cdc48 ATPase promotes muscle growth and retards atrophy by inhibiting protein degradation via the ubiquitin-proteasome and the autophagic/lysosomal pathways

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p97/VCP/Cdc48 ATPase binds ubiquitinated proteins and is essential for their degradation by the ubiquitin proteasome pathway. It also functions in the assembly of myofibrils, and p97 mutations cause Inclusion-Body Myopathy with ubiquitinated protein inclusions and sarcomeric disorganization. We studied the possible role of p97 in the excessive proteolysis causing muscle atrophy. During denervation atrophy of mouse Gastrocnemius, content of p97 and its cofactors, Ufd1 and p47, increase 2-3 fold when degradation of myofibrillar proteins is accelerated. Electroporation in mouse muscles of a dominant negative (DN) ATPaseless p97 (but not p97WT) attenuated atrophy induced by denervation and food deprivation. Surprisingly, in normal muscle, p97DN expression or depletion of endogenous p97 markedly increased weight and fiber diameter within 1 week. In myotubes, expression of p97DN (but not p97WT) did not alter rates of protein synthesis but inhibited proteasomal protein degradation, causing accumulation of ubiquitinated proteins and, surprisingly, also reduced lysosomal protein degradation, thereby raising the total protein content. Various types of muscle atrophy involve activation of FoxO-transcription factors and p97DN completely blocked the proteolysis promoted by caFoxO3. Thus, p97 is essential in the degradation of muscle proteins by both proteasomal and lysosomal pathways and in the accelerated proteolysis leading to atrophy. Funded by MDA and NIH (NIA).

Disease Biology

52. The Mtm1C205T knock-in mouse: a new model of myotubular myopathy with a mild phenotype

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MTM1 mutations cause myotubular myopathy (MTM) an X-linked centronuclear myopathy. MTM1 encodes the lipid phosphatase myotubularin. The short life span (<6 weeks) of the Mtm1 knockout mouse limits the scope of pre-clinical trials that can be attempted so a model with a milder phenotype is needed. Using data from human genotype-phenotype correlation studies we chose to model the recurrent C205T mutation, which leads to the R69C missense change in a catalytically inert domain of myotubularin. We predicted that a hypofunctional protein would result. Knockin mice appear unaffected at birth but by 2 months they have less body mass (15.8+/-11.3 gm) than wild type (21.2+/-12.7 gm) ($p<0.001$) and it remains about half that of wild type mice between 3 and 12 months. Knockin mice generate less grip strength force (0.07+/-0.008 kg) than wild type (0.1+/-0.02 kg) ($p<0.001$) at 2 months and it remains 60% of wild type between 3 and 12 months. Fifty percent of affected mice live 50 weeks. Histopathology reveals small myofibers with centrally placed nuclei. Unexpectedly, C205T disrupts splicing by inappropriately excluding exon 4 in most mRNAs, and myotubularin is not detected in immunoblots. However, some full length mRNA containing the C205T mutation is present, which may provide enough residual functional myotubularin to account for the relatively mild phenotype. The mild MTM phenotype of the Mtm1C205T mouse makes it an ideal complement to the knockout mouse in pre-clinical trials. KO8NS49095; MDA155638

Disease Biology

53. Role of the RNA-binding protein Staufen1 in Myotonic Dystrophy type 1

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Myotonic Dystrophy type 1 (DM1) is a disorder caused by the abnormal expansion of a CUG repeat in the 3'UTR of the DMPK mRNA. The RNA becomes toxic to the cell by the sequestration and misregulation of RNA-binding proteins and splicing factors, inducing aberrant splicing of several RNA. We have initiated a series of experiments aimed to determining whether the RNA-binding protein Staufen1 participates in the DM1 pathology. We show that Staufen1 interacts with the 3'UTR of the DMPK mRNA both in vitro and in myogenic cells. We also show that Staufen1 is involved in pre-mRNA splicing regulation, especially of the insulin receptor, a known aberrantly spliced gene in DM1. We demonstrate that Staufen1 can regulate the expression of the mutant RNA, and therefore be an important regulator in the disease. In conclusion, we propose that Staufen1 is a RNA-binding protein regulated by the toxic RNA. It is hoped that this study will lead to a deeper understanding of the DM1 disease, and in turn uncover new therapeutic targets. This work is supported by the CIHR, AFM and Rachel Fund.

Disease Biology

54. Delayed aggregation progression of PABPN1 in OPMD

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Oculopharyngeal muscular dystrophy (OPMD) is an autosomal dominant late onset myopathy caused by an alanine expansion in the poly(A) binding protein nuclear 1 (expPABPN1). Affected muscles show intranuclear inclusions (INI) containing aggregated expPABPN1. INI are used as a pathological marker for OPMD, however, INI of wild type (WT) PABPN1 are also naturally form. Only expPABPN1 aggregates are cytotoxic, suggesting that toxicity is caused by intermediate aggregation species of expPABPN1. Using high-resolution microscopy we show that PABPN1 aggregation is a multi-step process, where transitional pre-INI structures are formed from existing soluble molecules, while subsequent INI formation depends on protein accumulation. Different set of molecular partners and PABPN1 mobility distinguish pre-INI of WT-PABPN1 from those formed by expPABPN1 while INI structures are indistinguishable. In contrast to the WT PABPN1, expPABPN1 has a higher mobility in pre-INI leading to slower integration into pre-INI foci, indicating that expPABP1 aggregation is delayed in the pre-INI step. As we show that an affinity binder for PABPN1 specifically reverses pre-INI

structures, we suggest that pre-INI can be specifically targeted for disaggregation.

Disease Biology

55. Mono and di-allelic titinopathies in a mouse model carrying the c-terminal FINmaj titin mutation

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Tibial Muscular Dystrophy (TMD) and Limb Girdle Muscular Dystrophy 2J (LGMD2J) are allelic disorders caused by mutations at the end of the giant titin. Both clinical presentations have been initially identified in a large Finnish family and linked to a mutation (commonly referred to as FINmaj) in the last exon of titin. When this mutation is present on only one allele, it leads to the late onset mild distal TMD and on both alleles to the early onset severe LGMD2J. To study the pathophysiology of these two diseases, a mouse model carrying the FINmaj mutation was created. Although heterozygous (HE) and homozygous (HO) animals have a normal life span, abnormalities in the transmission ratio suggest a partial embryonic lethality. In HE mice, dystrophic myopathology appear late at 9 months of age in few distal muscles including the Tibialis Anterior. In HO mice, the first signs appear in the Soleus at 1 month of age and extend to most muscles at 6 months of age. Interestingly, the cardiac muscle is also severely affected in HO mice. As seen in LGMD2J, the mutation leads at molecular level to a loss of the very end of titin C-terminus and to secondary protein instability of calpain 3, a known partner of titin. Overall, this model shows a myopathology with high similarity to the human situation. It is currently utilized as a tool to decipher, at molecular level, the pathophysiological mechanisms underlying the two clinical presentations.

Disease Biology

56. The role of STIM1 in skeletal muscle metabolism

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It has long been recognized in skeletal muscle that release of Ca+2 stores following membrane depolarization, or excitation contraction coupling (ECC), is a critical determinant of muscle contractility and performance. In contrast, Ca+2 entry pathways have long been dismissed as irrelevant to muscle physiology. But recent evidence, from our lab and others, indicates that SOCE plays an important role in SR Ca+2 store repletion, fatigue limitation, and regulation of muscle specific gene expression. Stromal interaction molecule 1 (STIM1), the ER/SR Ca+2 sensor that activates SOC channels (Orai1) and is enriched in oxidative muscle fibers. Mice in which STIM1 was specifically deleted in skeletal muscle display defects in skeletal muscle growth and compromised perinatal survival. Metabolic profiling, biochemical enzymatic assays and gene expression studies were used to study muscles of STIM1 null mice and show marked impairment in oxidative metabolism. To explain how STIM1 influences oxidative metabolism we measured mitochondrial Ca+2 and found a marked reduction in basal and evoked [Ca+2]m in STIM1 null fibers. Thus, our studies support a model in which STIM1-mediated SOCE plays a critical role in muscle signaling and metabolism.

Disease Biology

57. THE ROLE OF SMN IN CO-TRANSCRIPTIONAL ALTERNATIVE SPLICING

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Spinal muscular atrophy (SMA) is amongst the leading genetic cause of infants death and it is characterized by specific degeneration and loss of spinal cord motoneurons. It is caused by disruption of the survival of motor neuron gene (Smn1), which plays an essential role in the cytoplasmic assembly of core spliceosomal snRNP. Besides its well-characterized role in snRNP assembly, it was suggested that the nuclear pool of SMN might play a more direct role in pre-mRNA splicing and/or transcription, although the molecular mechanism remained unknown. In this context, we have previously uncovered a methyl-dependent interaction between the SMN Tudor domain and CA150, a protein thought to be involved in bridging transcription and splicing. Indeed, it was found that the CA150-SMN Tudor interaction is required for the regulation of alternative splicing by CARM1, a transcriptional coactivator-associated methyltransferase (Cheng et al., Mol. Cell 2007). Moreover by using a large scale analysis, a recent study from the lab of Dr Dreyfuss (Zhang et al., Cell 2008) suggested for the first time that SMA is in fact a general splicing disease. Based on these results, we hypothesize that SMN plays a direct role in the regulation of alternative splicing. We will be presenting our recent progess in assessing the roles of SMN and CARM1 in regulating several aspects of post-transcriptional RNA processing. (GS is supported by a grant of AFM, JC is supported by CIHR).

Disease Biology

58. Passive mechanical properties of muscle fibers in hamstring contractures of children with spastic cerebral palsy

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Children with cerebral palsy (CP) often develop hamstring contractures and concomitant knee joint stiffness. The elements responsible for muscle contracture stiffness are unknown. Our study investigates the mechanics of these elements using single muscle fibers and fiber bundles. Biopsies were obtained from gracilis (GR) and semitendinosus (ST) during hamstring lengthening surgeries for patients with CP and from hamstring autografts in ACL reconstruction for typically developing (TD) patients. Fibers were dissected and attached to a force transducer and a motor arm with sarcomere length measured via laser diffraction. The fiber was stretched in 0.25 sarcomere increments with stress-relaxation measured over a 2 minute interval for 10 stretches. Bundles were measured similarly. The tangent modulus of stress-relaxed tension in fibers was equal in contractured and control fibers for both GR (CP 18.0\'ac\'b11.7 kPa/\'ac\'b5m; TD 21.5\'ac\'b12.3 kPa/\'ac\'b5m) and ST (CP 22.8\'ac\'b12.6 kPa/\'ac\'b5m; TD 22.5\'ac\'b12.3 kPa/\'ac\'b5m). When scaled to fiber bundles contractured muscle was significantly stiffer than control, GR (CP 72.8\'ac\'b118.2 kPa/\'ac\'b5m; TD 37.5\'ac\'b14.4 kPa/\'ac\'b5m) ST (CP 41.5\'ac\'b17.5 kPa/\'ac\'b5m; TD 25.9\'ac\'b13.0 kPa/\'ac\'b5m). These results demonstrate that

hamstring contracture observed in CP is not due to altered muscle cell mechanics but to the ECM connecting fibers. This implicates a major role of ECM in increased passive stiffness in joint contracture and may provide a basis for future contracture therapies.

Disease Biology

59. Role of Survival Motor Neuron (SMN) protein nuclear trafficking in the assembly and function of neuronal RNA Granules

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Spinal muscular atrophy (SMA) is a common, autosomal recessive neurodegenerative disease whose pathological hallmark is the degeneration and loss of spinal cord alpha-motor neurons resulting in paralysis and muscular atrophy. The survival of motor neurons gene (SMN) is the SMA causative gene. The mechanisms involved in the release of SMN from nuclear CBs (Cajal Bodies) to perform its suggested role in pre-mRNA splicing, and how it is then re-exported to the cytoplasm remain largely unknown. Since SMN nuclear import is tightly coupled to its involvement in assembly of snRNPs (small nuclear ribonucleoproteins) in the cytoplasm, we hypothesize that SMN release from Cajal bodies and subsequent nuclear export could also be coupled to its role in the formation of neuronal RNP granules complexes. We are currently examining the role of the CRM1-dependent pathway and the mRNA export pathway in SMN nuclear trafficking and export.

Disease Biology

60. Clinical characterization and mapping of the locus of a new form of childhood-onset limb-girdle muscular dystrophy

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Limb girdle muscular dystrophies are a heterogeneous group of pathologies characterized by weakness and wasting of the limb girdle muscles, with typical sparing of the face. We have recruited a group of five living and reviewed the records of six deceased distantly related French-Canadians of Acadian descent affected by a childhood-onset form of recessive LGMD. All cases originate from the small archipelago of the Magdalen Islands isolated in the Gulf of St-Lawrence. All cases present with limb girdle weakness on average at the age of seven years but they lose walking at a wide range of ages. Children have normal motor milestones and intelligence. With time, they develop macroglossia, decreased pulmonary function, hyperlordosis, large calves and mild to moderate contractures. Creatine kinase levels are elevated (663-10,000 U/L) in the first decades, but are back to normal at later stages. Muscle pathology showed non-specific dystrophic changes without any specific histological findings. Homozygosity mapping was used for analysis based on the likely sharing of the same founder mutation. A chromosomal region of not previously associated with a muscular dystrophy on chromosome 17q21.31 was uncovered. This study presents the description of a new recessive childhood-onset limb-girdle muscular dystrophy and the mapping of its original chromosomal locus.

Disease Biology

61. D4Z4 repeat contraction-independent FSHD2 is clinically identical to FSHD1

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Approximately 5% of patients with facioscapulohumeral muscular dystrophy (FSHD) have no contraction of the D4Z4 repeat. These patients, known as FSHD2 patients, however show similar chromatin changes in D4Z4 as patients with D4Z4 contractions (FSHD1). This suggests that a change in chromatin structure of the D4Z4 repeat unifies FSHD1 and FSHD2. In order to establish whether FSHD2 patients are phenotypically identical to FSHD1, we critically evaluated the clinical, genetic and epigenetic features in 33 FSHD2 patients, the largest cohort of FSHD2 patients known to date. The pattern of clinical involvement in FSHD2 is identical to FSHD1. In contrast to FSHD1, however, most FSHD2 patients are sporadic (67%). We did no observe gender differences in disease severity in FSHD2, but, overall, average disease severity in FSHD2 was identical to that reported in FSHD1. No correlation was found either between D4Z4 repeat size and disease severity or between degree of hypomethylation and disease severity. All FSHD2 patients carry at least one D4Z4 repeat on the permissive 4qA161 haplotype and in most patients, this repeat is <80 kb in size. In conclusion, a hypomethylated and medium-sized D4Z4 repeat on the permissive haplotype 4qA161 is observed in all patients with FSHD2. Clinically, FSHD2 patients are indistinguishable from patients with FSHD1, providing further evidence that FSHD2 represents a true FSHD subgroup. Supported by the Fields Center, Prinses Beatrix Fonds and the Netherlands Organization for Scientific Research

Disease Biology

62. Altered Muscle MRI Characters After Downhill And Horizontal Running Exercise In mdx Mice

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BACKGROUND: Duchenne muscular dystrophy (DMD), the most common form of muscular dystrophy, is caused by absence of protein dystrophin. The lack of dystrophin leads to a greater susceptibility to contraction-induced muscle damage in the mdx mouse, a DMD homologue. AIM: To monitor the effects of acute downhill running on muscle transverse relaxation time (T2) from magnetic resonance imaging (MRI). Mice underwent one of two running protocols on a motorized treadmill: downhill running at 14 degrees decline (n =11 mdx, n = 6 controls) or for 45 minutes at 8-10 m/min. MRI was conducted before the exercise bout, immediately afterwards, 24 and 48 hours following exercise. RESULTS: Higher muscle T2 of lower hindlimb muscles was observed in mdx compared to controls both pre-exercise and at each time point following downhill running. Furthermore, the medial compartment of the lower hindlimb muscles appeared to be most susceptible to an increase in muscle T2 following downhill

running. However, after a bout of horizontal running mdx mice did not experience an increase in muscle T2 at 24 and 48 hours. Muscle T2 was strongly correlated with the histological evidence of muscle damage from Evan's Blue dye ($R2 = 0.77$, $p < 0.01$). CONCLUSION: Downhill running induces an acute elevation in muscle T2 indicative of muscle damage in mdx mice. MRI is valuable, non-invasive approach for examining muscle damage and recovery in multiple muscle groups simultaneously.

Disease Biology

63. Dysfunctional autophagy underlies the pathogenesis of VCP associated inclusion body myopathy

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Inclusion body myopathies (IBM) are a family of muscle degenerative disorders with unclear pathogenesis. Pathologic features include ubiquitinated aggregates, tubulofilamentous inclusions and rimmed vacuoles (RVs). RVs are accumulations of membranous and proteinaceous debris presumed to be autophagic in origin. However to date no clear evidence has supported impaired autophagy in IBM pathogenesis. Mutations in valosin-containing protein (VCP) cause the multisystem degenerative disorder, IBMPFD or IBM associated with paget's disease and fronto-temporal dementia. We found that VCP was essential for autophagy and specifically autophagosome maturation to an autolysosome. Moreover, IBMPFD mutations in VCP disrupted this function resulting in the accumulation of non-degradative autophagosomes. These autophagosomes failed to acidify and fuse with lysosomes in cell culture and a transgenic animal model of IBMPFD myopathy. Consequently, IBMPFD myopathy mouse muscle accumulates autophagosome substrates such as ubiquitin, p62 and LC3II which localize to RVs. Additionally, IBMPFD myopathy mouse muscle fails to degrade an aggregated protein in response to an autophagic stimulus. We suggest that IBMPFD is a disorder of dysfunctional autophagy and may explain the pathogenesis of other IBMs with similar pathology.

Muscle Biology

64. Satellite and non-satellite cell origins for cell-based therapy of muscle wasting disorders

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One of our main research goals is to identify progenitor cells that can be delivered systemically and enhance myofiber repair in muscle wasting disorders. Specifically, we focus on bona fide satellite cells and pericytes (contractile cells engulfing the microvasculature endothelium) from limb, diaphragm and extraocular muscles. Whereas limb and diaphragm muscles are somite-derived and deteriorate in DMD and other muscular dystrophies, extraocular muscles (EOMs) originate from head mesenchyme and are not affected by dystrophin-dystroglycan impairments. For isolating and tracing satellite cells and pericytes we use lineage specific reporter mice and antigen-based cell sorting. Our studies show that EOM satellite cells and pericytes outperform their limb and diaphragm counterparts in their proliferation and reserve cell renewal in culture. Additionally, EOM myogenic progenitors contain a subpopulation that displays historical expression of smooth muscle myosin heavy chain based on Cre-Lox mouse studies. We have begun analyzing the behavior of the different populations upon their delivery to host mdx mice. We further aim to elucidate the molecular basis for the performance advantage of the progenitors from EOMs. Support: NIH, MDA

Muscle Biology

65. p38 MAP Kinase signaling is required for fusion of myoblasts during myogenesis

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It is well-established that the p38 MAP Kinase signaling pathway plays a critical role in the in myoblast differentiation. While many phosphorylation targets of p38 MAP kinase have been identified in muscle cells, it is not clear how activation of these factors leads to the formation of multinucleated myotubes. Using multiple cell lines with myogenic potential (C2C12 myoblasts, 10T1/2 fibroblasts, and MyoD-/-_Myf5-/- fibroblasts), we determined that expression of the muscle specific bHLH protein myogenin was directly modulated by p38 signaling. Based on these findings, we hypothesized that the critical effects of p38 on myogenesis are likely to be mediated through the regulated expression of myogenin. To address this possibility, we generated a C2C12 cell line that stably maintained an exogenous myogenin cDNA under the control of a Tetracyclin inducible promoter. Combining this system with the use of a pharmacological inhibitor of p38 activity (SB203580), we determined that several known p38 dependent genes could be reactivated in absence of p38 signaling if myogenin was expressed exogenously. Microscopy demonstrated that while no differentiation was observed in cells treated with SB203580, the exogenous expression of myogenin in the absence of p38 signaling allowed for the alignment of myoblasts. However, fusion of the myoblasts to form myotubes could not be achieved by introducing myogenin expression into the SB203580 treated cells. Microarray analysis was performed on cells treated with SB203580 in the presence or absence of exogenous myogenin expression. These areas demonstrated that 332 genes down-regulated by p38 signaling. Amongst these genes, 149 returned to normal levels of expression when myogenin levels were restored in the cells. Gene set enrichment analysis (GSEA) of genes that are regulated by p38, but not myogenin, demonstrates that factors previously implicated in cell fusion are enriched in this subset of the myogenic transcriptome. While it has long been established that p38 MAPK signaling regulates skeletal myogenesis, our work suggests a major role for the p38 signaling pathway is to promote fusion of mononucleated cells to generate myotubes.

Muscle Biology

66. The double-stranded RNA-binding protein, Staufen1, is involved in myogenic differentiation

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Staufen is a double-stranded RNA-binding protein first discovered in Drosophila, where it was shown to be involved in mRNA transport and localization during oogenesis

and the development of the central nervous system. In mammals, two Staufen homologues, Staufen1 and Staufen2, have been identified. They have also been shown to play a role in mRNA transport and localization in neurons through the formation of Ribonuclear protein complexes (RNPs). Staufen1, in particular, has been associated with other functions including two types of post transcriptional regulation: mRNA decay (Staufen-Mediated Decay) and increased protein translation. The goal of the present study is to determine the role of Staufen1 in skeletal muscle, specifically during myogenic differentiation. Our strategy was to caracterize the effects of Staufen1 over-expression on C2C12. Our results show that that over-expression of Staufen1 in C2C12 myoblasts delays the differentiation process and affects proper myotube formation. Immunofluorescence experiments reveal that Staufen1 over-expression causes a decrease in the fusion and differentiation indexes and leads to the formation of myotubes with significantly fewer nuclei. We show, by western blot, that the protein expression of the early and late markers of differentiation, myogenin and Myosin Heavy Chain, is delayed following induction of differentiation. Using quantitative RT-PCR (qRT-PCR) we show that myogenin mRNA expression levels are also delayed. This delay coincides with a significant increase in c-myc protein expression during proliferation and up to 24 hours after induction, after which time the levels returned to control levels. Since c-myc has been shown to inhibit C2C12 differentiation by repressing p21 and MyoD promoter activity, we looked at p21 and MyoD mRNA levels by qRT-PCR and found that they were both decreased after induction. In conclusion, our results indicate that Staufen1 negatively regulates skeletal muscle differentiation by affecting c-myc protein expression levels.

Muscle Biology

67. The role of XRCC1 in the repair of transient DNA strand breaks during skeletal muscle differentiation

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Caspase 3 has increasingly demonstrated a cell autonomous non-apoptotic function in several developmental programs. The proteolytic activity of Caspase 3 has an established requirement in skeletal muscle differentiation however how this activity is harnessed to promote differentiation has not been fully elucidated. Under apoptotic conditions Caspase 3 has a known function in inducing DNA fragmentation through Caspase Activated DNAse (CAD) and our laboratory has revealed a highly controlled action of Caspase 3 activated CAD in inducing transient and specific DNA strand breaks during skeletal muscle differentiation. The transient nature of these DNA strand breaks suggests an active DNA repair program is initiated to maintain genomic integrity. This study aims to delineate the DNA repair mechanism employed to repair these transient DNA strand breaks. The results currently obtained implicate the involvement of XRCC1 in the repair of the transient Caspase3/CAD induced DNA strand breaks during C2C12 myoblast differentiation. The observed involvement of XRCC1 but not ATM or DNA Ligase IV suggests the transient DNA strand breaks may be repaired through a process referred to as the back-up non-homologous DNA repair pathway. Further studies using an XRCC1 null mouse model will be required to understand the precise role of XRCC1 in the repair of CAD induced DNA damage during skeletal muscle differentiation.

Muscle Biology

68. Modeling susceptibility of human muscle fibers to high mechanical strain

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Many muscle diseases are characterized by a heightened susceptibility to injury by contractile activity. Evidence points to mechanical strain as an initiating event in the injury process, but it is unclear whether all muscle cells have similar sensitivity to strain. Vastus lateralis (VL) biopsies were obtained from 10 healthy volunteers (25 Â± 2 yrs). Peak Ca2+-activated force of 128 skinned fiber segments was assessed before (pre-force) and after (post-force) a single eccentric contraction (25% strain). Fiber type was based on fiber myosin heavy chain (MHC) isoform content. Multiple linear regression was used to model post-force. The best model, accounting for > 90% of the variability in post-force, included the predictors pre-force and fiber type: type I 0.85(pre-force) + 10.62 kN/m2 type IIa 0.85(pre-force) + 10.64 kN/m2 type IIa/IIx 0.85(pre-force) - 4.44 kN/m2 Our model reveals a strain-sensitive, but fiber type-independent, component to the injury process (pre-force). When this factor was held constant, type I and IIa fibers showed similar susceptibility to injury ($p = 0.988$) while fibers co-expressing type IIa and IIx MHC showed significantly greater force deficits ($p < 0.001$). This fiber type dependent component of the injury process may have clinical significance as the size of the type IIa/IIx population, which comprises ~20% of VL fibers in healthy individuals, is highly responsive to physiological and environmental factors.

Muscle Biology

69. Skeletal muscle-derived BDNF promotes expression of myosin heavy chain IIB and regulates muscle regeneration

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In adult skeletal muscle, brain-derived neurotrophic factor (BDNF) expression is restricted to satellite cells. Studies in our laboratory have shown that siRNA-mediated depletion of BDNF results in precocious differentiation of myoblasts in culture, suggesting a role in regulation of myogenic differentiation (J. Neuroscience, 2006). To expand on these findings and determine whether BDNF plays a similar role in vivo, we employed a Cre/Lox approach to generate a mouse in which BDNF is specifically depleted from skeletal muscle cells. In the absence of muscle-BDNF, expression of myosin heavy chain (MyHC) IIB was decreased at the transcript and protein levels. In addition, we found decreased expression of the satellite cell marker Pax7 compared to control littermates. Because satellite cells are responsible for postnatal growth and repair of skeletal muscle, we next examined whether regenerative capacity was compromised in the absence of muscle-BDNF. Indeed, BDNF-depleted muscle showed delayed

expression of several markers of regeneration (Pax7, MyoD, Myogenin, embryonic MyHC) 2-5 days following injury. Furthermore, we observed delayed appearance of newly regenerated fibers in the absence of muscle-BDNF. Together these findings suggest that muscle-derived BDNF plays at least two functions in skeletal muscle compartment; in promoting expression of fast MyHC IIB and in regulating myogenic differentiation during injury/repair. Funding by CIHR and MDA.

Muscle Biology

70. The utrophin A 5'-UTR drives cap-independent translation exclusively in skeletal muscles of transgenic mice and interacts with eEF1A2

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Duchenne Muscular Dystrophy (DMD) is a severe degenerative muscle disease caused by the lack of functional dystrophin in muscle tissues of young boys. A potential therapeutic strategy for DMD is to increase endogenous levels of utrophin, an autosomal homologue of dystrophin. One of the molecular mechanism in which utrophin A expression was shown to be regulated via the 5'UTR, where it was shown to drive cap-independent internal ribosome entry site (IRES)-mediated translation. Transgenic mice harbouring either control or the utrophin A 5'UTR bicistronic reporter transgene were generated. Utrophin A IRES activity was found exclusively in skeletal muscles and not in other tissues examined. In addition, the eukaryotic elongation factor 1A2 (eEF1A2), a muscle specific trans-factor, was found to bind to the 5'-UTR of utrophin A. Regions of the utrophin A 5'-UTR that bound eEF1A2 also mediated cap-independent translation in C2C12 muscle cells. When comparing with cells overexpressing eEF1A2, cultured cells lacking eEF1A2 had reduced IRES activity. Together, these results suggest an important role for eEF1A2 in driving cap-independent translation of utrophin A in skeletal muscle.

Muscle Biology

71. Consequences of the difference splice variants of LITF

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LITF is a novel A-type Lamin Interacting Transcription Factor that targets several developmentally important mesoderm-associated transcription factors (Six1, Six4, Mef2c, MyoG, Jun & Oct4). Our lab has previously shown that LITF is required for normal myogenesis in C2C12 cells. LITF specific immunoblotting analysis revealed the expression of seven different isoforms of LITF, differentially expressed in different tissues including heart, skeletal muscle, brain, lung and liver. The objective of this study is to determine the biological consequence of the different LITF splice variants in mesodermal determination. Differentiation of mouse satellite cells revealed the appearance of an additional LITF splice form in myotubes. Expression of this LITF splice form in C3H-10T1/2 cells, a mesenchymal stem cell line, did not promote differentiation of these multipotent cells into myotubes. Bioinformatics analysis has revealed the presence of a highly conserved sumoylation consensus sequence within LITF. Current experiments are investigating the biological role that sumoylation plays in LITF function.

Muscle Biology

72. Dissecting the cellular motif for dystrophin-mediated sarcolemmal nNOS localization

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Duchenne muscular dystrophy is a severe inherited muscle disease caused by dystrophin deficiency. An important function of dystrophin is to anchor nNOS to the sarcolemma. We recently found that dystrophin repeats 16 and 17 (R16/17) are crucial for nNOS localization. However, the underlying mechanism is not clear. Each repeat contains 3 alpha-helices. Here we tested the hypothesis that R16/17-mediated nNOS anchoring depends on correct alpha-helix phasing and composition. To study alpha-helix phasing, we sequentially deleted one of the 6 alpha-helices of R16/17 in a R16/17 containing microgene. AAV was used to introduce these microgenes to mdx mice. The original microgene efficiently recruited nNOS to the membrane. However, none of the modified microgenes restored sarcolemmal nNOS. To study alpha-helix composition, we swapped each alpha-helix of R16/17 with the corresponding alpha-helix of R18 and performed a yeast-2-hybrid assay. Except for the first alpha-helix of R17, replacing other alpha-helices of R16/17 did not compromise nNOS interaction. To confirm the in vitro finding, we engineered these chimeric repeats into the microgene and performed AAV gene transfer. In contrast to in vitro result, in vivo study suggests that the second, third alpha-helix of R16 and the first alpha-helix of R17 are all required for anchoring nNOS. In summary, our results suggest that correct phasing and composition is critical for dystrophin-mediated sarcolemmal nNOS localization.

Muscle Biology

73. Full-length utrophin cannot anchor nNOS to the sarcolemma

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Duchenne muscular dystrophy (DMD) is a lethal muscle disease caused by dystrophin deficiency. In normal muscle, dystrophin help maintain sarcolemmal stability by linking the extracellular matrix and the cytoskeleton. Further, dystrophin recruits neuronal nitric oxide synthase (nNOS) to the sarcolemma. Reduced sarcolemmal integrity has been considered as a major pathogenic mechanism in DMD. Interestingly, recent studies suggest that failure to anchor nNOS to the membrane also contributes to muscle fatigue. Over the last two decades, a great variety of therapeutic modalities have been explored to treat DMD. A particularly attractive approach is to increase utrophin expression. Utrophin shares considerable sequence homology, structural similarity and functional

properties with dystrophin. Here, we test the hypothesis that utrophin also brings nNOS to the sarcolemma. The full-length utrophin cDNA was expressed in dystrophin-deficient mdx mice by gutted adenovirus or via transgenic over-expression. Subcellular nNOS localization was determined by immunofluorescence staining, in situ nNOS activity staining and microsomal preparation western blot. Despite supra-physiological utrophin expression, we did not detect nNOS at the sarcolemma. Our results suggest that full-length utrophin does not anchor nNOS to the sarcolemma. This finding may have important implications in developing utrophin-based DMD therapies.

Muscle Biology

74. Metformin treatment of insulin resistance alleviates dystrophic muscle pathology

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Duchenne muscular dystrophy (DMD) is a fatal muscle disease that originates from mutations in the dystrophin gene leading to mechanical instability and damage of the affected myofiber. Progression of the dystrophic pathology is strongly influenced by alterations in signaling proteins and pathways. We have previously shown that the stress activated kinase JNK1 is elevated in dystrophic skeletal muscle and inhibition of this kinase can diminish myofiber damage. Interestingly, JNK1 activation is known to induce skeletal muscle insulin resistance by targeted serine phosphorylation of IRS-1. Here we show that mdx skeletal muscle is associated with defective insulin mediated signaling events involving upregulation of IRS-1 serine phosphorylation, down regulation of IRS-1 dependent signaling components (pAkt, pGSK3-beta), altered localization of the insulin sensitive glucose transporter protein GLUT-4 and loss of myofiber glycogen content. Interestingly, use of the insulin agonist metformin significantly reduced the dystrophic muscle pathology and restored metabolic disturbances, in contrast to insulin treatment. Following metformin treatment the observed reduction in membrane fragility paralleled an improvement in exercise tolerance compared to control mice. In conclusion, these data suggest that use of insulin agonists/mimetics may limit the disease progression in dystrophic muscle associated with impaired insulin signaling.

Muscle Biology

75. Differential recovery kinetics of alpha-actinin isoforms following FRAP studies of myofiber Z-lines

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The alpha-actinin proteins act as actin crosslinkers and mediate interactions between a host of additional cytoskeletal and sarcomeric proteins: Actn1 and Actn4 crosslink actin filaments in the cytoskeleton, while Actn2 and Actn3 serve a crucial role in anchoring actin filaments to the sarcomeric Z-line. In an effort to better understand the protein-protein interactions of each alpha-actinin isoform, as well as further structure/function analysis of the alpha-actinin family, we are investigating their association dynamics at the Z-line. To determine differences in alpha-actinin Z-line dynamics, we are using primary mouse FDB (flexor digitorum brevis) cultures, which we have determined contain predominantly (>95%) type II fibers. We are expressing GFP tagged Actn1, Actn2, Actn3, and Actn4 in these cultures and utilizing FRAP (Fluorescence Recovery After Photobleaching) to photobleach and quantitate their recoveries. Our progress thus far on Actn1, 2, and 4 indicates that different isoforms recover to different extents: with Actn2 recovering the least, Actn1 recovering to an intermediate degree, and Actn4 recovering most fully. This suggests that different alpha-actinin proteins have unique association kinetics at the Z-line, and that we can use this methodology to further investigate specific sequences of each actinin that lead to these functional differences.

Muscle Biology

76. Arginine Methylation of RNA Binding Proteins by CARM1 Regulates Motor Neuron Differentiation

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Spinal Muscular Atrophy results from loss of the survival of motor neuron (SMN) gene, whose protein product is a key component in the assembly of pre-mRNA splicing machinery. It is unclear how a deficiency in SMN and loss of its function results in a disorder leading to selective degeneration of motor neurons. In motor neuron-like cells, we have found that coactivator-associated arginine methyltransferase 1 (CARM1) is involved in a signaling pathway that regulates the switch from proliferation to differentiation by modifying the properties of a specific RNA binding substrate. We have identified an mRNA whose interaction with the RNA-binding protein is regulated by CARM1 methylation, and are currently investigating additional targets. Since SMN can serve as an adaptor module for arginine methylated proteins, we propose that SMN functions in the MN-1 differentiation pathway as an adaptor protein, through methylation-dependent interactions with specific RNA binding proteins. These findings may help to elucidate the specific role of arginine methylation and SMN in motor neurons, and in turn, provide crucial insights into the cell-specific pathophysiology of spinal muscular atrophy.

Muscle Biology

77. Elucidation of the Regulatory Role of microRNAs in Cardiac Gene Expression during Remodeling of the Growing Heart

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microRNAs are abundant in most tissues and are implicated in many physiological and pathological cellular processes. microRNAs are essential to the post-transcriptional regulation of ~30% of protein-coding genes. Cardiomyocyte terminal differentiation occurs shortly after birth in most mammals and is characterized by a transition from hyperplastic to hypertrophic growth. Key molecular mechanisms and pathways regulating this perinatal transition have yet to be elucidated. We hypothesize that microRNAs are critical for regulating the normal transition from a fetal to an adult heart. We aim to identify the genes involved in the transitional period and to determine the role of microRNAs within the perinatal

cardiogenomic programs. Total RNA was isolated from mice whole hearts at embryonic day 19, postnatal day 1, 3, 5, 7, 10, as well as from adult hearts. Both mRNA and microRNA expression was quantified by microarrays. Temporal patterning and ontological analysis of mRNA and microRNA expression patterns revealed an inflection point between 5 and 7 days post-birth representing an undescribed perinatal cardiogenomic program. The molecular relationship between microRNAs and mRNAs that define the perinatal program will be discussed.

Muscle Biology

78. Knock down of two mtmr1 paralogs cause broad developmental defects in zebrafish

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The myotubularin related gene family (MTMRs) encodes over a dozen related active and inactive lipid phosphatases that act on a variety of phosphatidylinositol-3-phosphates. Mutations of the prototypic member, MTM1, cause X-linked myotubular myopathy. MTMR1 lies only 20kb distal to MTM1, arose from an intrachromosomal duplication and shares 75% similarity at protein level. We have generated a novel antibody against MTMR1 and found that Mtmr1 is ubiquitously expressed during mouse development, the protein level being higher in the central nervous system than in skeletal muscle. In order to better understand the function of MTMR1 in early development, we have used zebrafish as a model organism. We describe the spatial and temporal expression patterns for two MTMR1 orthologues in zebrafish (68-69% aa similarity), including a newly identified mtmr1b paralog with 72% similarity to mtmr1a. Both orthologues are ubiquitously expressed, since the early stages of embryonic development, including within rudiments of brain, eye, heart and skeletal muscles throughout somitogenesis. Morpholino mediated knock down of both transcripts causes broad morphological abnormalities including reduced brain compartmentalization. Morphants for both genes are paralytic but only mtmr1b morphants exhibit significantly impaired myogenesis. These results suggest that MTMR1 plays a role in the development of various tissues, including brain and muscle.

Muscle Biology

79. Genomic characterization of Six1 function during skeletal muscle differentiation

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The Six family of transcription factors counts six members in vertebrates (from Six1 to Six6). Six1 and Six4 appear to be important for skeletal muscle development: Six1-/- mouse neonates die at birth due to thoracic skeletal defects and severe muscle hypoplasia affecting most of the body muscles. The molecular targets and mode of action of Six1 are poorly characterized. The Myogenic Regulatory Factors (MRFs) are transcription factors which are also critical for the determination and terminal differentiation of skeletal muscle. MRFs include MyoD, Myf5, myogenin and MRF4. Based on earlier data, we propose that Six1 has a genome-wide function and it cooperates with MRFs by co-regulating the expression of certain genes. We are using functional genomic approach (ChIP-on-Chip) to identify genes directly regulated by Six1. Using the C2C12 mouse myoblast cell line as a model of muscle differentiation, we have found that Six1 expression declines during differentiation, which suggests a putative mode of regulation of Six1 activity. We then performed chromatin immunoprecipitation (ChIP) with extracts from myoblast or cells undergoing differentiation. We found that Six1 significangly binds the regulatory region of genes involved in cell cycle, transcription and development significantly through Mef3 motif. Furthermore, we confirmed that Six1 and Myogenin share some targets by ChIP and co-transfection of Six1 and Myod has a synergistic effect in reporter assay, indicating that these two families could take part in combinatorial regulation of gene expression.

Muscle Biology

80. Diffusion tensor MRI to assess damage in healthy and dystrophic skeletal muscle after lengthening contractions

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Diagnosis of acute muscle strains is still typically made based on physical exam and patient history, but muscle injuries can be detected with magnetic resonance imaging (MRI). Conventionally, muscle strains are revealed best by T2-weighted MRI, which optimizes contrast between edema and muscular tissue. The purpose of this study was to determine if variables calculated from diffusion tensor imaging (DTI) would serve as an earlier and more sensitive marker of damage after a muscle strain injury in dystrophic (mdx) and control mice. We hypothesize that DTI biomarkers will provide a more informative assessment of muscle injury, and these measures will further elucidate the increased susceptibility to injury of the mdx model. Unilateral injury to the tibialis anterior muscle (TA) was induced by 15 maximal lengthening contractions in mdx mice (n=3) and control mice (n=3). High resolution T1 and T2-weighted structural MRI (100µm x 100µm x 750 µm) including T2 mapping and spin echo DTI (150µm x 150µm x 750 µm x 12 directions) were acquired on a 7T MRI system. T2 and diffusion tensor parameters (ie apparent diffusion coefficient [ADC], fractional anisotropy [FA], axial and radial diffusivity [AD and RD]) were calculated at each pixel location. TA imaging was compared to functional changes and HE staining and immunofluorescent labeling of TA cross-sections. Injury was confirmed by a significant loss of isometric torque (85% in mdx vs. 42% in controls). Parameter values were calculated as the percent increase in relation to the contralateral, uninjured TA of each animal. Greater increase in ADC, AD, and RD of the injured muscle was present in the mdx mice vs. controls when compared at proximal, medial, and distal locations of the muscle. These changes were paralleled by decreases in FA. On average, changes in DTI parameters were 1.8x greater in the mdx mice vs. controls. In comparison, changes in T2 did not indicate clear differences between mdx and controls. These data suggest that DTI may be a better indicator of muscle injury, even at early time points where the MR signal changes are dominated by local edema.Supported by grants from the Muscular Dystrophy association (MDA, grant 4278) and NIH-NIAMS (K01AR053235) to RML

Muscle Biology

81. Force Response During Stretch of Skeletal Muscle Fibers: Effects of Ca2+ and Blebbistatin

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During slow stretch of an activated muscle, force increases in two phases. The force attained at this transition, referred to as critical force (Pc), occurs in a critical sarcomere length extension (Lc). Pc has been attributed either to the number of attached crossbridges before stretch or to crossbridges in a pre-power stroke state. We investigated the mechanisms of stretch forces by altering either the number of crossbridges attached to actin or the crossbridge state. We performed experiments in which (a) activated fibres were stretched by 3% Lo (speeds of 1.0, 2.0 and 3.0 Lo/s-1) in pCa2 of 4.5, 5.0, 5.5 and 6.0, and (b) activated fibers were stretched by 3% Lo, at 2 Lo/s-1 in solution containing 5microM of the myosin inhibitor blebbistatin. When fibers were activated at a pCa2+ of 4.5, Pc was 2.47 \'ac\'b1 0.11 Po, and increased at high Ca2+ concentrations. Lc did not change with different Ca2+ concentrations (Lc = 14.34 \'ac\'b1 0.34 nm\'82\'c4\'a2HS-1). The velocity of stretch did not influence Pc and Lc. Fibers activated in blebbistatin showed a higher Pc (2.94 \'ac\'b1 0.17 Po) and Lc (16.30 \'ac\'b1 0.38 nm\'82\'c4\'a2HS-1) than control fibers (Pc 2.31 \'ac\'b1 0.08 Po; Lc 14.05 \'ac\'b1 0.63 nm\'82\'c4\'a2hs-1). Our results suggest that force generated during stretch is caused by both the number crossbridges attached to actin and crossbridges in a pre power-stroke state, which can be stretched by large amplitudes before detaching from actin.

Muscle Biology

82. Validation of primary human skeletal muscle cells for target identification screens and for screening emerging therapies that target muscle atrophy

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Muscle weakness leading to frailty is a major public health problem that is predicted to escalate in the future with the increasing number and proportion of older adults. There is an unmet need for therapeutic strategies that can slow the effects of ageing on muscle function and restore muscle size and strength in the frail elderly so as to maintain or improve their quality of life. Identifying targets and testing emerging drugs for age-related muscle weakness requires a cell-based system that reliably predicts in vivo effects in both pre-clinical rodent models and humans. The use of rodent cell lines such as mouse C2C12 or rat L6 myoblasts has been reported widely. However, there have been only limited reports of human skeletal muscle cells being used as screening tools. Therefore, we examined several commercially available sources of primary human skeletal muscle cells for their capacity to reproducibly differentiate into multinucleated, myosin heavy chain (MHC)-positive myotubes and respond to well-characterized inducers of atrophy (such as Dexamethasone and Myostatin) and hypertrophy (Insulin-like Growth Factor I). In addition we tested their capacity to be transfected or infected using a variety of delivery methods to introduce a Green Fluorescent Protein (GFP) reporter gene into both myoblasts and differentiated myotubes. These included lipid-based delivery, nucleofection (using Amaxa technology) and adenoviral infection. Human skeletal muscle myoblasts (HSMMs) from Lonza Walkersville Inc. and Human Skeletal Muscle Derived Cells (SkMDCs) from Cook Myosite Inc. demonstrated reliable and timely differentiation into multinucleated MHC-positive myotubes. They also responded to atrophy and hypertrophy stimulators. In addition, our results show that well-characterized inhibitors of myostatin blocked myostatin-induced atrophy in both HSMMs and SkMDCs. Furthermore, both cell lots were transfected and infected with varying efficiencies. Finally, our ongoing work is characterizing inhibition of gene expression in these cells, via siRNA and shRNA knockdown approaches. In summary, our findings validate two commercially available primary human skeletal muscle cell sources for use in screening assays aimed at identifying compounds that regulate skeletal muscle cell atrophy and hypertrophy and potentially in identifying new targets and signaling pathways that regulate skeletal muscle cells via gene knockdown screens.

Muscle Biology

83. Myogenin and class II histone deacetylases control skeletal muscle atrophy by inducing E3 ubiquitin ligases

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Neurogenic atrophy is caused by discontinuation of the nerve supply to muscle and results in a reduction of muscle mass and eventual paralysis. Here we show that myogenin, an essential regulator of muscle development, controls muscle atrophy following denervation. Myogenin is up-regulated in skeletal muscle in response to denervation and directly activates the expression of skeletal muscle E3 ubiquitin ligases MuRF1 and atrogin-1, which promote muscle proteolysis and atrophy. Thus, adult mice lacking myogenin show preservation of muscle mass with diminished expression of MuRF1 and atrogin-1 following denervation. Similarly, mice lacking class II histone deacetylases HDAC4 and 5 in skeletal muscle fail to up-regulate myogenin following denervation and are resistant to muscle atrophy. Conversely, forced expression of myogenin in adult skeletal muscle of mice lacking HDAC4 and 5 is sufficient to induce muscle atrophy following denervation. These findings reveal a previously unrecognized role for myogenin in regulating neurogenic muscle atrophy and suggest new therapeutic targets to ameliorate muscle atrophy caused by denervation. This work was supported by NIH grant.

Muscle Biology

84. Novel O-mannosyl phosphorylation of alpha-dystroglycan is required for laminin binding: implications for congenital muscular dystrophy

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Alpha-dystroglycan (alpha-DG) is a ubiquitously expressed cell-surface glycoprotein that serves as a high affinity laminin receptor. Here we show that alpha-DG receptor function requires phosphorylation of O-mannosyl glycan, and that laminin-binding is dependent on the formation of a

phosphodiester linkage. Based on mass spectrometry- and NMR-based structural analyses, we identified a novel phosphorylated O-glycan (GalNAc-beta-1,3-GlcNAc-beta-1,4-[PO4-6-Man]) on the mucin-like domain of recombinant alpha-DG. We also demonstrated that patients with muscle-eye-brain disease and Fukuyama congenital muscular dystrophy, as well as mice with myodystrophy (Largemyd), are defective in a post-phosphoryl modification of this phosphorylated O-linked mannose, and that this modification is mediated by the like-acetylglucosaminyltransferase (LARGE) protein. These convergent mechanisms to pathology offer an explanation for how the forced expression of LARGE can circumvent defects in alpha-DG modification in cells from patients with these congenital muscular dystrophies, as reported previously (Barresi R. et al., Nat Med. 10:696-703, 2004). These findings expand our understanding of the mechanisms that underlie congenital muscular dystrophy.

Muscle Biology

85. At Diagnosis, Untreated Juvenile Polymyositis (JPM) and Juvenile Dermatomyositis (JDM) Muscle Biopsies (MBx) Share A Type I Interferon (IFN) Induced Cascade But Differ In Pathophysiology

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To compare gene expression profiles from untreated JPM, JDM and control MBx, and subsets with <2mos of disease symptoms. Methods: Three JPM and 12 JDM MBx profiles (Affymetrix [HG-U133A and MAS]) were normalized to 3 control MBx (mean age [yrs]: 12.39+7.72; 6.15+3.93; 8.96+1.66; respectively) with Genespring. Analysis of probe sets (<2 MAS 5.0 present calls) used Benjamini and Hochberg false discovery rate. In JDM and JPM with symptoms <2mos, profiles and serum IFN-alpha activity were compared. Antibodies to DC-LAMP, BDCA1 and BDCA2 identified maturity of dendritic cells (myeloid [mDC] or plasmacytoid [pDC]) (Student's t-test). Results: Principal component analysis of all samples showed separation of the 3 groups. Although JDM and JPM profiles had upregulation of genes induced by Type I IFN (OAS, IFIT, IRF, ISG) and protein folding stress response (PPIA, PDIA, PFDN, HSP), they differed in magnitude (JDM>>JPM) and in the expression of genes such as HBA, 18srRNA, and OSTF. Also, JDM trended toward higher serum IFN-alpha activity than children with JPM ($p=0.067$); only 1 JPM had detectable IFN-alpha. pDCs were increased in MBx from JDM > JPM ($p=0.014$), and they were immature. Conclusions: Although JPM and JDM share Type I IFN induced and stress response genes, they differ in magnitude and in mobilization of the immune response. We speculate that after disease initiation, the pathophysiology of JDM and JPM diverges. Support: NIAMS R01 AR48289

Muscle Biology

86. Measuring The Components Of Muscle Atrophy And Hypertrophy In Growth, And In Dystrophy

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Signaling pathways behind the control of muscle growth, atrophy and strength are partly known but we lack rigorous quantitative analysis of the accompanying cellular activities; notably, the roles of 3 main independent variables: myonuclear number, nuclear domain and amount of contractile apparatus. This limits our ability to interpret the mechanisms of action of agents that combat atrophy or muscle weakness. Therefore we have developed simple methods for measuring myonuclear number and myonuclear domain in terms both of total volume and amount of contractile apparatus. Turnover of cells in these processes is assessed by measuring loss of BrdU-labelled myonuclear cohorts. These methods permit us to compare postnatal muscle growth in wild-type and myopathic mice. In the wild-type EDL muscle almost all of the cell proliferation has occurred by 3 weeks of age but a further 5-fold growth occurs by accumulation of contractile apparatus and some increase in non-contractile sarcoplasm, unaccompanied by any significant myonuclear turnover. In mdx mice, initial growth follows the wild-type pattern but the disease process provokes mounting increase in myonuclear number and fibre size that is not fully paralleled by increase in contractile apparatus, together with rapid and complete turnover of muscle nuclei. These data provide reliable measures of the cellular components of loss or gain of muscle size and strength in disease and under therapy.

Muscle Biology

87. Rates of force development in MgADP-activated myofibrils isolated from skeletal muscles

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Myosin cross-bridges bound to actin play a significant role in the regulation of thin-filament activation. However, myosin-based regulation is a complex process that influences, and is influenced by, the Ca2+ regulatory pathway. We investigated the sole contribution of myosin regulation of isometric force (Po), rate of force development (Kact) and redevelopment (Ktr), by comparing myofibrils activated by either Ca2+ (pCa2+ = 4.5) or MgADP (10 mM); the latter induces strong binding of cross-bridge to actin independently of Ca2+. Rabbit psoas myofibrils were attached to an atomic force cantilever in a newly developed system with a time resolution of 4 microseconds. The myofibrils were set at a sarcomere length of 2.5 microns before activation, induced by rapid solution exchange. Maximal Po/cross-sectional area was 112.48 +/- 10.77 nN and 129.92 +/- 24.12 nN for Ca2+- and MgADP-activated myofibrils, respectively. After force development, myofibrils underwent a rapid shortening-stretch protocol and Ktr was measured during force recovery. Ca2+-activated myofibrils presented a faster Kact (4.83 +/- 2.00/sec) and Ktr (13.68 +/- 2.37/sec) than MgADP-activated myofibrils (Kact 0.28 +/- 0.03/sec; Ktr 1.58 +/- 1.36/sec). The results suggest that myosin-based activation plays an important role in force regulation that is independent of Ca2+, through a process that is much slower than that associated with the Ca2+ regulatory mechanism.

Muscle Biology

88. Loss of the IL15Ralpha Gene Increases Endurance and Alters Metabolic Characteristics of Muscle

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The purpose of these experiments was to determine the role of interleukin-15 receptor alpha (IL15Ralpha) and IL15 on skeletal muscle function and morphology in vivo using IL15Ralpha knockout (KO) mice, IL15KO mice, and IL15 transgenic (TG) mice. IL15RalphaKO mice ran 4-fold greater distances in cage running wheels compared to IL15KO, B6129 control and BL/6NTAc control mice, displaying an increased endurance capacity. The fast extensor digitorum longus (EDL) muscles from IL15RalphaKO mice showed several characteristics consistent with a transition to a slow, more oxidative phenotype, including: lower isometric forces, increased resistance to fatigue, longer relaxation time, lower twitch:tetanus ratio, and a leftward shift of the single fiber area histogram toward smaller fiber sizes. These EDL muscles also had an increase in positive staining for succinate dehydrogenase. The molecular signature of muscles from IL15RalphaKO mice included: upregulation of the transcription factor PPARdelta, and PGC-1alpha (genes regulating mitochondrial biogenesis); altered expression of calcium-related SERCA2 and calsequestrin; and greater protein content of cytochrome-c oxidase subunit Va. These phenotypes were not observed in IL15KO or IL15TG mice, demonstrating a novel role of IL15Ralpha in controlling the phenotype of fast skeletal muscles in vivo. FUNDING: NIH (EY013862) and World Anti-Doping Agency (T.S. Khurana); AR053461 (E. Pistilli)

Muscle Biology

89. Elucidation the functional role of LITF in myogenesis

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Our lab has identified LITF, a novel regulatory factor that binds to DNA within close proximity of key mesoderm transcription factors, Six1, Six4, Mef2c, MyoG, Jun and Oct4. These are essential in muscle development. Downregulated LITF expression in C2C12 myoblasts downregulates myogenic regulatory factors (MyoD and MyoG) and subsequently delays myogenic differentiation. We hypothesize that LITF may be involved in regulating the expression of myogenic transcription factors. Gene expression profiling of the C2C12 myoblasts compared to LITF stably knocked down in C2C12 cells revealed that 131 transcription factors and regulators are differentially expressed (Mef2c, Pax7, Hes6, and Myf6) with 92 significantly ($p<0.0001$) downregulated; some of which were previously identified in the LITF ChIP-CHIP: FOXC1, MEF2C, PITX2 and RXRA. This global downregulation of transcription factors and regulators is further emphasized by the significant ($p<0.0001$) downregulation of 492 (70%) of the 754 genes differentially expressed upon downregulation of LITF in C2C12 myoblasts. Current studies are exploring the direct molecular link between LITF functions and signaling pathways during muscle differentiation.

Muscle Biology

90. Bcl-2 associated factor 1 (Bclaf1): role in skeletal muscle differentiation

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Bclaf1 (also known as Bcl-2 transcription factor or Btf) was first identified in a screen for proteins that interact with anti-apoptotic members of the Bcl-2 family of proteins. We have recently uncovered a critical role for Bclaf1 in lung development. Lungs of mice deficient in Bclaf1 fail to form alveoli and accumulate excess smooth muscle cells, suggesting a role for this protein in cell lineage maintenance or differentiation. As earlier studies demonstrated, Bclaf1 is highly expressed in skeletal muscle compared to other tissues, and therefore we queried a role for this protein in skeletal muscle differentiation. We analyzed the expression of Bclaf1 protein and mRNA following induction of the skeletal muscle differentiation program in C2C12 cells. A dramatic decrease in nuclear Bclaf1 steady-state protein but not mRNA was observed during differentiation. In contrast, two smaller Bclaf1 protein species were observed to increase in the cytoplasm during differentiation. Interestingly, the differentiation-induced decrease in nuclear Bclaf1 and increase in cytoplasmic species were reversed in the presence of the caspase inhibitor ZVAD. Differentiating C2C12 cells showed increased myocyte fusion and myofiber dimensions following siRNA-targeted reduction of Bclaf1. Our findings indicate a role for Bclaf1 in the skeletal muscle differentiation program.

Muscle Biology

91. p300 represses FOXO signaling in skeletal muscle through its acetyltransferase activity

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Forkhead Box O (FOXO) signaling is increased in response to various catabolic conditions and is both sufficient and required for the normal atrophy phenotype. Although FOXO regulation by Akt is well characterized in skeletal muscle, additional levels of FOXO control have been identified in a variety of cell types which have yet to be fully explored in skeletal muscle. The current study provides evidence to support the acetyltransferase (HAT) activity of p300 in regulating FOXO signaling in skeletal muscle. Specifically, transfection of a dominant negative (d.n.) form of p300, which lacks HAT activity, is sufficient to activate a FOXO-responsive reporter in weight bearing muscle and further increases FOXO activation during muscle disuse. In contrast, transfection of WT p300 represses FOXO reporter activation during muscle disuse. Subsequent experiments demonstrate p300 to repress FOXO1 and FOXO4 gene expression and increase the protein turnover of FOXO1 and FOXO3a, both of which required its HAT activity. Co-transfection of WT or d.n.p300 with FOXOs 1, 3 or 4 further demonstrate that the HAT activity of p300 represses the transcriptional capacity of FOXO3a, since co-transfection of d.n.p300 with FOXO3a synergistically increased reporter activation. In summary, our findings suggest that p300 HAT activity exerts multiple levels of control on FOXO signaling in skeletal muscle that includes differential regulation of the FOXO homologues.

Muscle Biology

92. Unique transcriptional profiles in wrist muscles from patients with cerebral palsy

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Spastic muscle in cerebral palsy (CP) undergoes alterations secondary to upper motor neuron lesions (UMNL). Muscle alterations represent a critical disability of patients with CP and often result in contracture requiring surgery. Our objective is to define transcriptional changes associated with CP muscle. Two muscle biopsies were extracted from each patient, a wrist flexor and extensor. Biopsies were obtained during tendon transfer surgery or wrist facture surgery for controls. Among present transcripts CP biopsies clustered separately from controls. 205 transcripts were significantly altered in CP. Transcripts from various physiological pathways were altered in CP. Altered excitation-contraction coupling would be expected based on changes in transcripts associated with Ca2+ release during excitation and Ca2+ binding during relaxation. Myosin heavy chain transcripts were altered toward faster isoforms. Many extracellular matrix transcripts were increased with basal lamina components undergoing the most dramatic shifts. Myogenic potential showed confounding alterations of increases in both IGF1, a hypertrophy signal, and myostatin, a hypertrophy inhibitor. These adaptations were not characteristic of those observed in the transcriptome of other muscle disease states. These results will direct further research required to understand the unique mechanism of muscle adaptation to UMNL and development of innovative therapies.

Muscle Biology

93. Interferon Responsive Factor 2 Binding Protein 2 is a hypoxia-inducible coactivator of Vascular Endothelial Growth Factor-A expression in skeletal muscle

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Expression of VEGFA can be upregulated by the transcription factor TEAD4 in hypoxic endothelial cells. Here, we found TEAD4 alone or with the cofactor vestigial-like 4 (VGLL4) only modestly activated VEGFA expression. In a yeast two-hybrid cDNA library screen we identified IRF2BP2 as a specific cofactor of VGLL4. Coexpression of IRF2BP2 with TEAD4/VGLL4 or TEAD1 alone activated a VEGFA promoter 7-8 fold and increased endogenous VEGFA expression in C2C12 myoblasts. IRF2BP2 mRNA expression is high in human cardiac and skeletal muscles and increases during muscle differentiation of C2C12 cells. In mouse embryos, IRF2BP2 protein was ubiquitous but became restricted to the lung, heart and skeletal muscle late in foetal development. IRF2BP2 protein levels increased throughout myogenic differentiation, and IRF2BP2 was detected in adult human heart, but surprisingly only low levels were detected in adult human and rat skeletal muscles. However, ischemic rat hindlimb muscles or ischemic mouse myocardium have markedly elevated levels of IRF2BP2, indicating that the protein is hypoxia-inducible. Thus, we identify IRF2BP2 as a novel hypoxia-inducible coactivator of VEGFA expression in hypoxic cardiac and skeletal muscles.

Muscle Biology

94. A novel role for caspases during cardiac hypertrophy

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Cardiac hypertrophy is an adaptive response in which the heart grows in size to normalize output during cardiac stress. As heart cells are considered terminally differentiated, organ growth results from an increase in the size of individual cardiomyocytes as opposed to their division. Caspases are well characterized proteins, known primarily for their role in apoptosis leading to cell death. Many apoptotic characteristics are also seen during cellular processes including muscle cell differentiation and immune cell development. Recently it was shown that inhibition of caspase-3 abolished the ability of the heart to undergo pathological hypertrophy in vivo. Research also suggests that caspase inhibition may prevent left ventricular remodeling following myocardial infarction. Results shown here indicate that inhibition of caspase-3 and -8 minimizes hypertrophic growth in cardiomyocytes. Phenylephrine induced an approximate 80% increase in cell size after 24 hours however this growth was attenuated with the addition of caspase inhibitors. Cell staining confirmed the effects were observed in cardiomyocytes. These data suggest that caspase-3 and/or caspase-8 may be involved in hypertrophic growth. Preliminary results suggest that caspase activity may not be directly responsible for the observed effect. Further investigation into the localization of caspases during hypertrophy may explain how these proteins are acting to mediate growth.

Muscle Biology

95. Expression profiling and improved muscle-specific transcript annotation with complementary next generation sequencing technologies

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The annotation of transcripts in genome databases is incomplete. Many transcript isoforms specific to certain tissues remain to be discovered. We used next generation sequencing technologies to better annotate muscle-specific first exons and 3'-untranslated regions (3'-UTR) and discovered new genomic regions important for the regulation of muscle-specific gene expression. To this end, we performed a qualitative and quantitative analysis of the starts and ends of mRNA molecules in proliferating and differentiated C2C12 myoblasts with Cap Analysis of Gene Expression (CAGE) and Serial Analysis of Gene Expression (SAGE). We identified 4,304 and 3,846 genes differentially expressed between proliferating and differentiated cells by CAGE and SAGE respectively, with an overlap of 2,144. We found good concordance with previous microarray results, but also many myogenesis-related genes that were not present or detectable on the microarrays. We identified 196 novel regulatory regions specific for the two differentiation stages. These regions appeared to contain conserved myogenic transcription factor binding sites and are most likely involved in

the regulation of the myogenic gene transcription program. We conclude that next-generation sequencing of CAGE and SAGE libraries provide highly consistent expression level measurements and enrich current genome annotations with tissue-specific promoters and alternative polyadenylation sites.

Muscle Biology

96. The role of cIAP2 in skeletal muscle atrophy

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Skeletal muscle atrophy occurs as a secondary result from a number of conditions such as cancer cachexia, prolonged bed rest, and AIDS. Unfortunately, the cellular and molecular mechanisms behind atrophy are still poorly understood. It was recently found that the cellular inhibitors of apoptosis (cIAP1 and cIAP2) proteins play vital, yet redundant roles in the regulation of the NF-kappaB pathway, which is one of the most significant signaling pathways correlated with the loss of skeletal muscle mass in a number of conditions. We asked whether cIAP2 plays a role in skeletal muscle atrophy, using a denervation model which results in a consistent and rapid loss of muscle mass over a matter of days. cIAP2-/- and wild-type mice were denervated by removing a small portion of the sciatic nerve in the mid-thigh region of one limb, while the opposite limb was used as control. Fourteen days following sciatic nerve denervation, muscle tissue was harvested and the fiber cross-sectional area measured. We found that in the cIAP2-/- mice, fiber size was spared when compared with their wild-type counterparts. These data suggest that cIAP2 is a key regulator of skeletal muscle atrophy.

Muscle Biology

97. The Role of SMN in the assembly of Axonal RNA transport Granules

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Spinal Muscular atrophy (SMA) is a genetic neuromuscular disorder that is caused by the loss of the survival motor neuron gene, mainly affecting the population of motoneuron in the lower spinal cord. Despite the large body of work trying to clarify the function of SMN in all cells, the molecular defects leading to motoneuron-specific pathologies and development of SMA remains unresolved. Interestingly, motoneurons from SMA mice show a normal survival in cell cultures, but a significant reduction in axon growth associated with a mislocalization of beta-actin mRNA in the distal axons. It has been shown that SMN co-localizes in RNA-containing foci in axons, although the precise nature of these granular bodies as well as the axonal function of SMN remains to be determined. SMN has a TUDOR domain, which serves as a sensor of arginine methylation in cellular proteins. Mutations occurring in the Tudor domain lead to loss of this methyl-sensing capacity and are found in human patients who present severe cases of SMA, underscoring the functional relevance of this domain in the etiology of the disease. Our group has identified novel SMN Tudor domain interacting proteins from spinal cord tissues, many of which are known components of neuronal RNA granules. These proteins are involved in the transport and localized expression of mRNA in axons of neuronal cells. Based on these observations we hypothesize that SMN participates in the assembly and function of neuronal RNA granules. We will present our most recent progress in verifying this working model.

Signaling

98. The continuing enigma that is Leukaemia inhibitory factor

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Since the first description of a role for Leukemia inhibitory factor (LIF) in myoblast proliferation the early nineties the role that LIF plays in skeletal muscle regeneration has remained an enigma. Early studies purported a mitogenic role and suggested that enhanced regeneration was the direct result of increased precursor cell proliferation. We show that the effect of LIF is more likely related to cell death. In fact in C2C12 cells LIF treatment did not significantly increase the rate of DNA synthesis. LIF treatment does however significantly reduce staurosporine-induced cell death. Consistent with previous reports we show that LIF delays mytoube formation but contrary to early reports we show that the LIF receptor is expressed in myotubes with the protein being localized to the nucleus. Importantly, our more recent data suggests that there is a role for LIF in regeneration but it is more likely related to LIFs role as an inflammatory mediator, regulating macrophage activity, or as a stress signal to the hypothalamic-pituitary-adrenal axis. We are therefore pursuing the role of LIF in skeletal muscle not only as a myogenic agent but as cytokine which aids in regulating other non-myogenic cells during the regenerative response.

Signaling

99. Identification of endogenous phosphorylation sites in murine dystrophin by mass spectrometry

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Dystrophin is a 427 kDa protein containing an NH2-terminal actin-binding domain, a rod domain, a cysteine-rich domain, and a C-terminal domain. It forms a sarcomeric glycoprotein complex referred to as the dystrophin-glycoprotein complex (DGC), which may have both mechanical and signaling functions at the sarcolemma. Post-translational modification, such as phosphorylation, is known to regulate signaling events and previous studies have shown that the dystrophin protein can be phosphorylated in vitro. However, whether dystrophin is phosphorylated in vivo and how this affects protein function remains to be shown. To explore this, we used mass spectrometry to identify phosphorylated amino acids in endogenous murine dystrophin. Dystrophin was immunoprecipitated from mouse muscle or C2C12 cell lysate and subjected to SDS-PAGE. Dystrophin bands were excised from the gel and subjected to in-gel proteolytic digest, after which the resultant peptides were analysed by LC-MS/MS. Approximately 50-60% of the dystrophin sequence has been analyzed by mass spectrometry, resulting in the identification of 2 phosphorylated threonine residues within the rod domain, as well as 3 phosphorylated serine residues within the C-terminal domain. This is the first demonstration that dystrophin is phosphorylated on multiple amino acids in vivo. We are

continuing to investigate how phosphorylation may affect the function and stability of dystrophin.

Signaling

100. The Insulin-like growth factor-I E peptides drive myoblast proliferation but oppose muscle growth: a new anti-growth factor?

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Insulin-like growth factor-I (IGF-I) regulates and increases muscle growth. IGF-I is produced as a propeptide, and its C-terminal portion, the E peptide, is cleaved from the mature IGF-I protein. Due to alternative splicing, rodent IGF-I mRNA has two isoforms: A and B. The IGF-I proteins from igf1a and igf1b are identical, whereas E peptides A and B (EA and EB) differ in size, sequence, and possibly activity. The EB peptide has been shown to stimulate proliferation in a number of cell types, but the activity of EA has not been addressed, nor has the mechanism of action of either E peptide been established. To determine if EA or EB possess bioactivity, C2C12 cells, a mouse myoblast cell line, were exposed to synthetic peptides. Both E peptides increased myoblast proliferation and caused a dose-dependent increase in ERK1/2 phosphorylation. Pharmacological inhibition of MEK, preventing ERK1/2 phosphorylation, reduced the proliferation response. To determine if the E peptides had activity in vivo, viral delivery of EA or EB was performed into hind limb muscles of young (2-3 week old) wildtype mice. Increased expression of both EA and EB caused increased nuclear content and decreased muscle mass and force, which counters the normal pro-growth actions of IGF-I. These results demonstrate that both EA and EB have similar proliferative activity, and that they may drive cell division at the expense of muscle growth.

Signaling

101. Proteasome inhibition improves muscle phenotype of laminin alpha2 chain deficient mice

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Severe muscle atrophy is a prevalent feature of congenital muscular dystrophy with laminin alpha2 chain deficiency (MDC1A). Muscle atrophy occurs by a change in the normal balance between protein synthesis and protein degradation and molecules associated with these pathways could become new targets for drug therapy against MDC1A. Protein degradation in skeletal muscle is mainly mediated by the activity of the ubiquitin-proteasome pathway and the autophagy-lysosome pathway, respectively. Using the dy3K/dy3K mouse model of MDC1A, we here show that members of the ubiquitin-proteasome system are upregulated and that the global ubiquitination of the protein is raised in dystrophic limb muscles. Also, autophagy related genes are increased in dy3K/dy3K muscles. Negative regulation of Akt has been shown to enhance proteasome protein breakdown, autophagy and apoptosis and accordingly, we demonstrate that phosphorylation of Akt is diminished in diseased muscles. Specifically, we show that treatment with MG-132 reduces fibrosis, increases muscle fiber diameters, enhances locomotive activity and reduces apoptosis. Moreover, we demonstrate that transient inhibition of proteasome by MG-132 significantly improves the dystrophic dy3K/dy3K phenotype. In summary, these studies promote better understanding of the disease process and could lead to a drug therapy in patients suffering from MDC1A.

Signaling

102. Identification of the anti-apoptotic protein cIAP1 as a regulator of skeletal muscle mass and regeneration

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The factors controlling muscle regeneration are still poorly understood, and are of clinical interest in a wide variety of conditions ranging from cancer cachexia to muscular dystrophy. We recently determined that the cellular inhibitor of apoptosis 1 (cIAP1) protein is a positive regulator of the NF-kappaB signaling pathway, which is a known inhibitor of myoblast differentiation. However the role of cIAP1 in myogenesis is unknown. Surprisingly, we found that downregulation of cIAP1 in primary myoblasts, using a specific small-molecule antagonist (SMAC mimetic), led upon differentiation to formation of hypertrophic myotubes with considerably more nuclei than controls. This effect occurred in spite of a distinct delay in cell cycle exit, and in expression of the myogenic markers myogenin and myosin heavy chain. Similar results were seen both with siRNA knockdown of cIAP1 and upon differentiation of primary myoblasts isolated from cIAP1-/- mice. The cIAP1-/- mice themselves had increased muscle mass, as well as increased number and area of individual muscle fibers. Finally, targeted inactivation of the non-canonical NF-kappaB signaling pathway was sufficient to restore normal myotube formation to cIAP1-/- myoblasts. Together these results suggest that cIAP1 is a key regulator of skeletal myogenesis.

Signaling

103. The propeptide form of insulin-like growth factor I provides stable source of protein for driving muscle growth

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Insulin-like growth factor-I (IGF-I) is a mitogenic and myogenic peptide that plays a critical role in skeletal muscle development and growth. It is produced as a precursor polypeptide and undergoes post-translational cleavage generating mature IGF-I peptide. The mature peptide has been recognized as the bioactive molecule of IGF-I produced after the endoproteolytic removal of the carboxy-terminal E peptides; however there is increasing evidence that E peptides may possess autonomous and/or complementary action to mature IGF-I in skeletal muscle. The aim of this study was to identify the optimal forms of IGF-I for promoting muscle growth. Viral delivery was used to express the different IGF-I propeptides (IGF-IA and IGF-IB) as well as mature IGF-I in mouse skeletal muscle, and Ea-, Eb- and mature peptide-specific antibodies were utilized to detect the processing products derived from each IGF-I isoform. It was found that the predominant IGF-I species detected in muscles were propeptides: non-glycosylated IGF-IEa and IGF-IEb, and glycosylated IGF-IEa. The protease furin converted all propeptides to mature IGF-I. Confirmation of

mature IGF-I activity was achieved through exposure of myoblasts to pro- and mature IGF-I. These results support that IGF-I propeptides comprise storage and/or stable forms of IGF-I in skeletal muscle, regulating the bioavailability and activity of mature IGF-I.

Signaling

104. New signal transduction pathways for regulating muscle gene expression

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Quantitative proteomics has identified transcription factors with no previously known function in muscle gene regulation; e.g. MAZ & KLF3. MAZ expression increases >4-fold and KLF3 expression is initiated during muscle differentiation, suggesting that both play roles during this transition. MAZ binds sequences that differ from the database binding site; & control sites with divergent sequences were found in many muscle gene promoters: e.g., Myogenin, MEF2C, Six4, Skel alpha-actin, Desmin, & their occupancy was verified by ChIP. Similar studies identified multiple KLF3 binding sites in MCK & other muscle promoters, & 2 KLF3 isoforms were found in muscle nuclear extracts. Since KLF3 lacks an activation domain, its transcriptional role would require interaction with proteins that contain activation domains. A search for KLF3 binding partners disclosed strong interaction with SRF, & a specific KLF3-SRF synergism was demonstrated in COS cell transactivation studies with reporter constructs that contain KLF3, but no SRF binding motifs. This suggests that signaling pathways impinging on SRF can regulate genes lacking SRF binding sites via SRF-KLF3 interactions. Since KLF3 binding motifs are present in many muscle genes & since SRF is present prior to myogenesis, KLF3 expression during terminal differentiation could activate a previously unknown SRF-KLF3 pathway that provides many additional mechanisms for muscle gene regulation. (NIH)

Signaling

105. Phosphorylation of Pax7 regulates satellite cell proliferation through Pax7 protein stability

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Pax7, a paired and homeobox transcription factor, plays predominant roles in the survival and self-renewal of satellite cells and controls the myogenic determination factors (MRFs), Myf5 and MyoD expression. However, the molecular mechanisms of transcriptional regulation for Pax7 remain poorly understood. To investigate the possibility that Pax7 is regulated by phosphorylation, we analyzed Pax7 protein which purified from FLAG tagged Pax7 overexpressing primary myoblasts by tandem mass spectrometry, and found two phospho-serine sites in Pax7. Mutation analysis of phosphorylation sites revealed that mutants do not affect transcription activity of Pax7, by instead cause protein instability. To identify the kinase for Pax7 phosphorylation, we conducted a screening by using the multiplexed in vitro kinase assay, and identified casein kinase 2 (CK2) and GSK3 which are involved in non-canonical and canonical Wnt-signaling. To verify the phosphorylation of Pax7 by CK2 and GSK3, we performed in vitro kinase assays using purified protein and confirmed phosphorylation of Pax7. Moreover, specific inhibition of GSK3 by inhibitors including Wnt3a and siRNA induces Pax7 degradation in primary myoblasts and decreases the satellite cell proliferation on muscle fiber. Therefore, we conclude that phosphorylation of Pax7 by GSK3 plays an important role in proliferation of satellite cells through Pax7 protein stability.

Signaling

106. Alpha-Syntrophin modulates myogenin expression

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alpha-Syntrophin (Snta), a scaffolding protein that links signaling proteins to the dystrophin complex, is expressed in the early stages of differentiation but its role in myogenesis has not previously been investigated. In this study, we examined the relationship between Snta expression and the expression of myogenin, a key muscle regulatory factor. In the absence of Snta, myogenin expression is delayed in cultured primary skeletal muscle cells and in regenerating muscle compared to wild-type models. To confirm the relationship between Snta and myogenin expression, we compared the differentiation pattern of C2 cells with that of Sol8 cells which express Snta at much lower levels. Myogenin expression in Sol8 cells began 12 h later than in C2 cells under the same culture conditions. Myogenin expression was greatly reduced when C2 cells were transfected with Snta-specific siRNA, whereas myogenin was expressed approximately 12 h earlier in Sol8 cells transfected with a vector over-expressing Snta. Immunoprecipitation experiments showed that Snta associates with the protein Mixed Lineage Leukemia 5, a regulator of myogenin expression. From these results, we conclude that Snta plays an important role in regulating myogenesis by modulating myogenin expression. This study was supported by the Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) (KRF-2008-531-E00004) and by NIH (NS33145).

Signaling

107. Exploring the cytoplasmic role of the p38 mitogen-activated protein kinase in differentiating myocytes

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The p38 family of mitogen-activated protein kinases were first identified as mediators of the cellular stress response but have also been shown to be essential for differentiation, particular in the case of myocytes. p38 is known to play a critical role in myogenic gene regulation, aiding in the initiation of myocyte differentiation through the activation of specific genes. However, the possible involvement of p38 in other cellular processes during myogenesis, particularly outside of the nucleus, is not well studied. To gain a better perspective as to p38's involvement in myogenesis we have begun developing a

whole-cell lysate kinase assay that will allow for the identification of substrates for any kinase in any cell type. The initial application of such an approach to p38 has led to the identification of several new potential substrates with a wide range of functions, for example cytoskeletal organization, vesicle formation and function, and protein quality control. Currently we are evaluating the biological relevance of these targets to myogenesis, as well as attempting to improve the ability and ease of use of our substrate finding protocol. This type of approach should prove as an efficient means for generating a top-down view of p38's involvement in myocyte differentiation, and create a greater understanding of p38's global cellular function.

Signaling

108. Activation of the extrinsic and intrinsic cell death pathways promote myoblast differentiation via the coordinated activation of Caspase 3

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Recent studies have shown that caspase 3 is required for differentiation in a variety of cell lineages, including skeletal muscle, neural & ES cells, across a diversity of species, from Drosophila to human. However it remains unclear whether there is an equally conserved mechanism for up-regulating this protease in non-death cellular responses. Does the non-apoptotic induction of caspase 3 utilize a pathway that is unique to this cell fate or is it dependent on the intrinsic (int.) & extrinsic (ext.) cell death pathways? Here, we show that early initiation of skeletal muscle differentiation relies not only on the activity of caspase 3 but also upon the stimulation of both the ext. & int. pathways. Inhibition of caspase 8 & 9 using shRNA severely attenuates myoblast fusion (50-60%) & myotube formation (53-61%) in vitro, thus supporting the idea that both play an important role in differentiation. Using IP, a protein-protein interaction was identified between active caspase 8 & c-FLIP reinforcing the involvement of the ext. pathway. Moreover, we provide novel evidence that the int. pathway is involved by demonstrating a change in mitochondrial membrane potential. Differentiating cells show a transient color change which suggests an increase in permeability & interestingly coincides with the release of cytochrome C. These results supplement current data that caspases have pleiotropic functions, including an indispensable abililty to regulate cell fate.

Signaling

109. TWEAK/Fn14 System is a Critical Regulator of Denervation-Induced Skeletal Muscle Atrophy in Mice

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Skeletal muscle atrophy occurs in a variety of clinical settings, including cachexia, disuse and denervation. Inflammatory cytokines have been shown to be mediators of cancer cachexia; however, the role of cytokines in denervation and immobilization induced skeletal muscle loss remains unknown. Here we demonstrate that a single cytokine, TWEAK, mediates skeletal muscle atrophy which occurs under denervation conditions. Transgenic expression of TWEAK induces atrophy, fibrosis, fiber-type switching, and the degradation of muscle proteins. Importantly, genetic ablation of TWEAK decreases the loss of muscle proteins and spared fiber cross-sectional area, muscle mass, and strength after denervation. Expression of the TWEAK receptor Fn14, and not the cytokine, is significantly increased in muscle upon denervation, demonstrating an unexpected inside-out signaling pathway; the receptor up-regulation allows for TWEAK activation of NF-kB, causing an increase in the expression of the E3 ubiquitin ligase MuRF1. This study reveals a novel mediator of skeletal muscle atrophy, and indicates that the TWEAK/Fn14 system is an important target for preventing skeletal muscle wasting.

Signaling

110. Caspase3/ caspase activated DNase induced DNA strand breaks regulate gene expression during skeletal muscle differentiation.

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Caspase 3 activity is required for the differentiation of skeletal muscle but how this signal is utilized remains to be clearly elucidated. Recently we have demonstrated that caspase 3 activates the caspase activated DNase (CAD) to induce specific DNA strand breaks that regulate the expression of the cyclin-dependent kinase inhibitor, p21 (Larsen et al. PNAS 2010). The extent of DNA strand breaks observed during differentiation suggests that a large number of genomic loci may be regulated in this manner. Here we demonstrate caspase3/CAD targets a number of additional critical regulatory genes involved in skeletal muscle differentiation.

Signaling

111. AMP kinase activation stimulates expression of the slow myogenic program and improves muscle morphology in mdx mice

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The AMP kinase activator AICAR has been labeled as an exercise mimetic compound for its ability to recapitulate the skeletal muscle plasticity indicative of the beneficial adaptive response to habitual physical activity, including the induction of slow, oxidative myofibers. Thus, the purpose of this study was to evaluate the effects of AICAR treatment on muscle gene expression, as well as the dystrophic pathology in mdx animals. Acute AICAR exposure in C2C12 cells enhanced AMPK and ACC phosphorylation, and elicited significant increases in the mRNA expression of utrophin A, PGC-1 alpha, as well as cytochrome c oxidase subunits. Chronic AICAR administration to adult (6 weeks) mdx and wild-type mice for 4-5 weeks evoked the slow, oxidative myogenic program at both the mRNA and protein levels, including increases in utrophin A and PGC-1 alpha concomitant with a reduction in the transcriptional

co-repressor RIP140. AICAR-induced molecular adaptations were accompanied by morphological improvements in muscles rich in slow, oxidative myofibers. Our data suggest that AICAR-evoked muscle plasticity results in beneficial adaptations in mdx mice and represents a novel therapeutic avenue for further pursuit. Funds from MDA and CIHR.

Signaling

112. Modulation of cell spreading and cell substrate adhesion dynamics by dystroglycan

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Dystroglycan (DG) is a ubiquitously expressed cell adhesion protein. Much of the previous research into DG function has focused on mature muscle, where DG plays an important role in costameric adhesion complexes. Here we have investigated the role of DG in myoblasts to try and elucidate more fundamental roles in cell adhesion dynamics. We show that altering DG expression affects adhesion on different substrates and can modulate the number and size of cell adhesions. DG-knockdown cells exhibit increased numbers of large (fibrillar) adhesions and consistent with this we see slow but persistent migration. However, DG-overexpressing cells show a decrease in fibrillar adhesions, combined with more motile but less persistent migration. Through an SH3 domain phage display screen we have identified the vinculin-binding protein vinexin as a binding partner for DG. By mapping and mutating the binding site we show that this interaction plays a role in DG-mediated cell adhesion and spreading. Finally, by immunostaining we demonstrate conclusively the presence of beta-DG in focal adhesion structures. Together, our data support a role for DG in affecting cell spreading, substrate adhesion and migration by modulating cell adhesions dynamics.

Signaling

113. ER Chaperone GRP94 Depletion in Skeletal Muscle Affects Muscle Growth and Architecture

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Insulin-like growth factor (IGF) is essential for muscle regeneration and repair. From in vitro studies, it has been shown that the relationship between glucose-regulated protein 94 (grp94) and IGF secretion of skeletal muscle is linear, such that loss of grp94 activity inhibits IGF secretion. To address this effect in vivo, a muscle-specific conditional knockout mouse was developed (MCK-Cre grp94 FL/FL). Upon grp94 deletion, reduced body size and muscle mass were apparent. In order to determine the cause of smaller skeletal muscle, the muscle size and fiber type were analyzed. Skeletal muscles were harvested 8 weeks after birth. The fiber cross-sectional area analysis displayed the shifted distribution toward smaller fibers in grp94-deleted muscles, and especially loss of the largest gauge fibers compared to control. Fiber type analysis revealed that there was conversion in fiber type from slow to fast. In grp94-deleted soleus, the proportion of fast fibers, MHCIIa, was greater than that of slow fiber, MHCI, and in the EDL, there was an increase proportion of MHCIIb compared to controls. These differences may be a consequence of muscle adaptation to the lack of grp94 and local IGF to aid muscle growth. Alternatively, these factors may regulate early fiber type specification. These possibilities will be addressed in the near future.

Signaling

114. Skeletal muscle atrophy rescue in chronic diseases and disuse conditions by muscle-specific deletion of Traf6

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The TNF receptor associated factor (TRAF) family of proteins is a group of seven adapter proteins (TRAF1-TRAF7), that link a wide variety of cell surface receptors to the intracellular signaling proteins. TRAF proteins are defined by a conserved C-terminal domain, TRAF domain, which is responsible for homo- and hetero-dimerization of TRAF proteins as well as with other cognate surface receptors. The TRAF domain in all TRAFs (except TRAF1), contains N-terminal RING-finger domain. The deletion of N-terminal RING domain of TRAF proteins leads to generation of dominant negative TRAF mutants, suggesting its critical role in downstream effector functions. We also found that TRAF6 protein levels are dramatically reduced during differentiation suggesting its important role in myogenesis. Accumulating evidence strongly suggests that TRAF6 auto-ubiquitination activates kinase complexes such as TAK1 and IKK, which is dependent on RING-domain. As TRAF6 RING-finger domain is important for the activation of important pathways such as NFkB and Akt/PKB. In the present study, we have investigated the role of TRAF6 RING-finger domain in skeletal muscle atrophy and regeneration. Recent experiments in our laboratory have shown that TRAF6 expression levels have significantly increased in denervation, cachexia and injury. To investigate the role of TRAF6 RING-finger domain in atrophy and regeneration, we have generated mice with muscle specific deletion of TRAF6 exon 7. Our data suggests that skeletal muscle specific deletion of TRAF6 significant reduced the expression of several atrophy markers such as Murf-1, Atrogin-1 and Dystrophin and rescued the expression of muscle structural proteins such as myosin heavy chain, laminin and sarcomeric Î±-actin. In addition, we also found that TRAF6 deletion significantly reduced the expression of autophagy markers such as beclin, LC3B-I and LC3B-II. Finally, our data also indicates that there is a significant attenuation of cardiotoxin induced-injury in TRAF6 deleted mice.

Signaling

115. Gene networks regulated by p38alpha in skeletal myogenesis

Perdiguero, Eusebio[1], Sousa-Victor, Pedro[1], Ballestar, Esteban[2], Kumar, Roshan[3], Young, Richard[3], Munoz-Canoves, Pura[1]
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Findings published over the past years have established a key role for the p38 MAP kinase signaling pathway in the control of muscle gene expression and myotube formation. We have recently demonstrated that myoblasts lacking p38alpha were unable to differentiate and form multinucleated myotubes. We found that p38alpha not only promotes muscle differentiation and fusion, but it is a critical regulator of myoblast cell cycle exit, a necessary step prior to

commencing the muscle differentiation gene program. To elucidate the global gene expression program regulated by p38alpha during myogenesis, we undertook two approaches: microarray expression and ChIP-on-chip analyses using cells with or without activated p38alpha. We have found different gene networks regulated by p38alpha: for example, expression of muscle specific genes is blunted in cells lacking activated p38alpha, while proliferation-specific genes and cell cycle regulators are up-regulated. Interestingly, new chromatin- and transcription- associated genes appear to be modulated by p38alpha, suggesting a potential new role in myogenesis. On the other hand, and consistent with the occupancy of osmo-responsive genes by Hog1/p38 MAPK in yeast, ChIP-on-chip analysis for p38alpha in myoblasts revealed its association with specific networks of gene promoters, including transcription factors, developmental regulators, and muscle-related genes.

Signaling

116. Myostatin induces the degradation of sarcomeric proteins via Smad3 signalling during cachexia

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Myostatin, a transforming growth factor-beta (TGF-beta) super-family member, has been well characterized as a negative regulator of muscle growth and development. Myostatin has been implicated in several forms of muscle wasting including cachexia. Using molecular and cellular approaches, here we have characterized the signaling pathway and downstream target proteins degraded during myostatin induced cachexia. Treatment with Myostatin resulted in activation of muscle specific E3 ligases, Atrogin-1 and MuRF-1 followed by ubiquitination of sarcomeric proteins. Molecular analysis also indicated that Smad3 signaling is required for myostatin mediated up-regulation of FoxO1 and Atrogin-1 expression. However, Smad3 signaling was found to be dispensable for FoxO3 and MuRF1 induction by myostatin. In order to identify the downstream target proteins degraded by myostatin during cachexia co-immunoprecipitation technique was used. Tandem Affinity Purification of both N and C terminally tagged Atrogin-1 identified that the sarcomeric proteins (myosin heavy chain and myosin light chain), factors involved in protein synthesis, mitochondrial proteins, chaperones and microtubule binding proteins were ubiquitinated and degraded by myostatin treatment. PGC-1alpha is one of the metabolic regulators and down regulation of PGC-1alpha causes the skeletal muscle atrophy through FoxO3 activation. We found that myostatin treatment causes significant down regulation of PGC-1alpha in Smad3 dependent manner. Based on this data we concluded that myostatin induces the skeletal muscle wasting by degrading sarcomeric and other cytosolic proteins by up regulating the Atrogin-1, FoxO1 and down regulating PGC-1alpha via Smad3 signaling mechanism.

Signaling

117. Wnt7a and regulation of regenerative myogenesis

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Wnt signaling plays an important role in regulating developmental programs through embryonic development and in regulation of stem cell function in adult tissues. Wnt7a, a member of class II Wnt proteins involved in activation of noncanonical Wnt signaling, stimulates the symmetric expansion of satellite stem cells. Remarkably, Wnt7a induces hypertrophy via Frizzled7. We investigated the effects of Wnt7a overexpression and Wnt7a application using C2C12 cells as well as primary myoblasts on induction of hypertrophy as well as signaling pathways involved in Wnt7a induced hypertrophy. We generated C2C12 cells which overexpress Wnt7a-HA. After differentiation of these cells muscular hypertrophy became obvious. The fiber diameter was significantly increased compared to control cells after five days of differentiation whereas the fiber length was not altered. Another significant change we observed was the increased fusion index of C2C12 cells expressing Wnt7a-HA. Similar results were obtained applying recombinant Wnt7a protein to C2C12 cells as well as primary myoblasts prior to or during differentiation. Since Wnt7a is a member of class II Wnt proteins we investigated the application of different recombinant Wnt proteins (Wnt3a, Wnt5a, Wnt7a) and their effects on hypertrophy. Remarkably, both Wnt3a and Wnt5a did not have any effect on hypertrophy in C2C12 cells giving rise to the notion that Wnt7a has unique characteristics leading to hypertrophy.

Signaling

118. TrkB signaling represents a novel therapeutic target for neuromuscular diseases

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Brain-derived Neurotrophic Factor activation of the tyrosine kinase receptor trkB.FL modulates neuromuscular synapse maintenance and function, however it is unclear what role the alternative splice variant, truncated trkB (trkB.T1), may have in the peripheral neuromuscular axis. In mice null for trkB.T1 we demonstrate a significant gain in neuromuscular performance both in vivo and in situ. In vitro assays demonstrated that the gain-in-function in trkB.T1 null animals resulted specifically from increased muscle contractility, a mild myofiber hypertrophy and increased electrically evoked calcium release. In the trkB.T1 null muscle we identified a trkB.FL specific increase in Akt activation in resting muscle as well as a significant increase in trkB.FL, Akt and p70 activation in response to contractile activity. Further assays determined that trkB.T1 null mice are resistant to eccentric injury, likely via an Akt-p70 dependent increase in the expression of mRNA and proteins involved in cytoskeletal stability and membrane repair. We conclude that the trkB signaling pathway represents a novel target for intervention across diseases characterized by deficits in muscle contractility or increased susceptibility to muscle

injury. Funding: NIH-RC2 NR011968 to CWW and SGD

Stem Cells

119. CCAAT/Enhancer Binding Protein beta: a novel regulator of myogenesis and satellite cell marker

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In response to muscle injury the differentiation of muscle satellite cells is induced. Activated satellite cells enter the cell cycle and produce committed myogenic precursor daughter cells which begin expression of the myogenic bHLH transcription factor MyoD. Despite an important role for MyoD in adult myogenesis, relatively little is known about the regulation of its expression in satellite cell-derived cells. We have identified CCAAT/Enhancer Binding protein beta (C/EBPbeta), a bzip transcription factor, as a novel marker of muscle satellite cells, and inhibitor of MyoD expression in satellite cells, mesenchymal stem cells and C2C12 myoblasts. The failure to upregulate MyoD expression in cells ectopically expressing C/EBPbeta correlates with a decrease in myogenic factor expression, myosin heavy chain expression and fusogenic activity and recapitulates the phenotype of MyoD-/- skeletal muscle precursor cells. In activated satellite cells, C/EBPbeta expression is lost suggesting that C/EBPbeta may act in quiescent satellite cells to prevent myogenesis in the absence of muscle injury.

Stem Cells

120. Muscle mononuclear cells and their role in adult zebrafish myogenesis

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The current study of muscular dystrophy revolves around mouse, dog, and human patient samples in vivo models and primary myoblast cell cultures. Recently, zebrafish models of muscular dystrophy have emerged as a cost-effective animal model for study. Despite their attractiveness, no current methods exist to effectively isolate, culture, and characterize isolate zebrafish myoblasts from adult dorsal muscle. To better investigate the usefulness of zebrafish muscle stem cells, we have compared the histology of muscle from that of mammals and zebrafish and functionally characterized a mononuclear muscle stem cell population residing in the adult zebrafish dorsal muscle. Early mononuclear cells can be isolated from zebrafish and express many of the muscle markers that have been reported in mammalian cells. Transcriptome analysis of these zebrafish skeletal muscle mononuclear cells in culture as they underwent myogenic differentiation revealed a downregulation of proliferative genes, and an upregulation of myogenic structural genes. Together, these studies highlight an evolutionary conservation of myogenic differentiation between zebrafish and mammals and further enhance the use of zebrafish myoblasts for cell culture experiments.

Stem Cells

121. Skeletal Mosin Light Chain Kinase Regulates Muscle Lineage Decisions by the Phosphorylation of MEF2C

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MEF2 transcription factors play a regulatory role in several lineages. In skeletal myogenesis, MEF2 factors amplify and synergize with the MRFs, thus establishing commitment into cells with a more limited regenerative capacity. While phosphorylation is known to mediate MEF2 transcription regulation, lineage-specific regulation is unknown. Here, we provide evidence that the phosphorylation of a conserved threonine residue at the MEF2 domain of MEF2C by skeletal MLCK, specifically enhances skeletal and not cardiac myogenesis. In P19 embryonic pluripotent cells, skeletal development was specifically impaired upon overexpression of non-phosphorylation mimic mutant. Phosphorylation at T80 mediates the interaction of MEF2C with histone deacetylases, p300 and PCAF, and their differential recruitments into skeletal, but not cardiac, muscle promoters. Gain- and loss-of-function studies revealed that skMLCK is sufficient and necessary for skeletal myogenesis, indicating a novel function for skMLCK in controlling the crucial transition of skeletal muscle progenitors into skeletal myoblasts, and going beyond its known role in phosphorylating myosin to regulate muscle contraction. Further, we provide evidence that phosphorylation is crucial for the recruitment of histone deacetylases to specific promoters in a lineage-dependent fashion.

Stem Cells

122. The effect of muscle contractile activity on the regenerative potential of mouse and human skeletal muscle

Ambrosio, Fabrisia[1], Plassmeyer, Joshua[1], Brown, Elke[1], Fitzgerald, G. Kelley[1], Deasy, Bridget[1], Boninger, Michael[1], Rando, Thomas[2], Huard, Johnny[2]

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Aging typically results in dramatic declines in muscle regeneration. Fortunately, rejuvenation of the muscle microenvironment powerfully restores functionality. Here, we investigated the anti-aging effect of muscle contractile activity on skeletal muscle regenerative potential. Old (24 months) mice were divided into Injury (n=9) and Electrical stimulation (E-stim) (n=8) groups. All animals received a contusion to the tibialis anterior (TA). One day after injury, E-stim animals began daily TA stimulation protocol. E-stim dramatically enhanced myofiber regeneration, and resulted in increased strength 9 days after injury, compared to controls (p=0.00). E-stim also enhanced serum Klotho levels, an age-related regulator of stem cell regenerative potential (p=0.04). Next, we investigated the effect of contractile activity on regenerative potential in aged humans. There exists a subpopulation of human muscle precursor cells, myoendothelial (Myo-Endo) cells, that co-express myogenic and endothelial markers. These

cells exhibit an increased regenerative potential when compared to cells expressing only endothelial or myogenic markers. In elderly individuals, we found that completion of a 12-week muscle activity protocol resulted in a marked increase in the %Myo-Endo cells (Pre: 3.6±5.7% (n=6); post: 13.2±9.2% (n=4)). These results suggest that contractile activity may enhance the regenerative potential of aged mouse and human muscle.

Stem Cells

123. Smad3 Functions to Maintain Quiescent Satellite Cell Pool of Skeletal Muscle Through Pax7 Up-Regulation

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Smad3 has been shown to mediate differential inhibition of myoblasts by TGFbeta family. However, the role of Smad3 in the genetical commitment of muscle satellite cells remains unclear. Here, we found that Smad3 expressed selectively in vivo quiescent satellite cells and in vitro reserve cells. Smad3 deficiency led to an increase in the proportion of activated satellite cells with Pax7-/MyoD+ as well as earlier and hypertrophic either neo-myofibers during muscle regeneration in vivo or myotubes during differentiation in vitro, indicating enhanced myogenesis of satellite cells. Importantly, reconstituting Smad3 expression could rescue Smad3 deficiency-enhanced myogenesis by increasing the proportion of quiescent satellite cells with Pax7+/MyoD-, which was restored by PD98059 treatment and Pax7 knockdown. Furthermore, ERK1/2 activation determined the binding of Smad3 to the promoter of Pax7. This is the first study demonstrating that Smad3 can act genetically upstream of Pax7 to regulate myogenic commitment of quiescent satellite cells via ERK1/2 activation-dependent Pax7 transcriptional activation.

Stem Cells

124. Ex Vivo Expanded Human Muscle Stem Cells show increased levels of reactive oxygen species and increased sensitivity to oxidative stress

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Muscle stem cell therapy is a promising approach for the treatment of skeletal muscle disorders such as Duchenne muscular dystrophy (DMD), sports injuries or trauma. Effective cell therapy for muscle diseases, like DMD, will need to transplant large numbers of cells to reach more than 600 muscles or to replace muscle loss due to traumatic injury. Yet, challenges in obtaining clinically relevant cell doses arise from both the insufficient number of stem cells harvested from the source and also limited ex vivo proliferative capacity of human cells. Hence, the use of human muscle stem cells requires strategies that permit their ex vivo expansion yet maintain their health, phenotype and function. Here, we examined the effect of growth factor expansion methods and the use of catalytic antioxidants on the ex vivo aging of human muscle stem cells. We observed a significant increase in reactive oxygen species in populations treated with basic fibroblast growth factor, FGF2, as compared to unstimulated controls (measured by dihydroxyrhodamine 123). We also found that in the presence of oxidative stress, a condition likely to be present following in vivo cell transplantation, muscle stem cells exposed to extensive ex vivo FGF2 stimulation showed a significant increase in ROS production after H2O2 induced oxidative stress. Our results show that the catalytic antioxidant FBC-007 does not affect short-term cell growth rate, and it prevents myogenic differentiation and maintains cells in an undifferentiated state. Ongoing studies will examine the effect of expansion and the use of the catalytic antioxidant on the in vivo muscle regeneration potential of the human muscle cells.

Stem Cells

125. Improved muscle repair through transplantation of VEGF-expressing muscle stem cells in mdx skeletal muscle

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We have isolated a population of skeletal muscle stem cells (skMSC) that, when compared with myoblasts, display an improved regeneration capacity, exhibit better cell survival, and improve myogenesis and angiogenesis. We and others have observed that the origin of some muscle stem cells may reside within the blood vessel walls. Indeed we observe that a subset of preplate-isolated skMSC express both myogenic and endothelial markers; and transplantation of a purified population of myoendothelial cells gives rise to increased levels of skeletal muscle regeneration as compared to transplantation of purified myogenic cells. Here, we investigated the role of vascular endothelial growth factor (VEGF)-mediated angiogenesis in muscle stem cell transplantation-based skeletal muscle regeneration in mdx mice, a model of muscular dystrophy. We studied skMSC and skMSC transduced to overexpress VEGF. No differences were observed in vitro, however, after in vivo transplantation, we observe an increase in angiogenesis and endogenous muscle regeneration as well as a reduction in muscle fibrosis in muscles transplanted with VEGF-expressing cells when compared to control cells. In contrast, we observe a significant decrease in vascularity and an increase in fibrosis in the muscles transplanted with skMSCs expressing sFlt1 (VEGF-specific antagonist) when compared to control skMSCs. These results show that VEGF-expressing cells do not increase the number of dystrophin-positive fibers in the injected mdx muscle, when compared to the control skMSCs. Together the results suggest that the transplantation of VEGF-expressing skMSCs improved skeletal muscle repair through modulation of angiogenesis, regeneration and fibrosis in the injected mdx skeletal muscle.

Stem Cells

126. Caspase 3 Promotes Skeletal Muscle Differentiation by Limiting Satellite Cell Self Renewal

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Satellite stem cells are indispensable for the growth and regeneration of skeletal muscle. Nevertheless, the mechanisms that determine whether activated satellite cells self renew or differentiate into committed muscle progenitors

remains unknown. Caspase proteases are traditionally viewed as conserved cell death proteins, yet recent observations have demonstrated that caspase activity is required for muscle stem cell differentiation. Here, we sought to identify whether caspase 3 influenced cell fate by altering the balance between self renewal and differentiation. Using the isolated single fiber model, we have observed that caspase 3 activity is elevated during early stages of satellite cell derived myogenesis. Specifically, we have noted that caspase activity is restricted to Pax7(+) satellite cells and not the mature myonuclei. Peptide inhibition of caspase 3 activity resulted in increased numbers of the self-renewing Pax7(+)/MyoD(-) satellite cells with a corresponding decrease in the number of proliferating Pax7(+)/MyoD(+) cells. These results suggest that caspase 3 is a key determinant in cell fate decisions and promotes differentiation in part by limiting satellite cell self renewal.

Stem Cells

127. Canonical Wnt signaling and retinoic acid regulate Sox7 expression

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The in vitro muscle development pathway for the embryonic carcinoma cell line, P19, parallels that of the embryo, with the same transcription factors regulating gene expression. During the process of vertebrate skeletal myogenesis, signaling molecules are secreted from tissues surrounding the somite. These extracellular cues activate a cascade of transcription factors that leads to the expression of the myogenic regulatory factors, which control the end-stages of myogenesis. A family of transcription factors that has been characterized for their involvement in muscle development is the Sox family. Data analysis in P19 cells has revealed that Sox7 can initiate the entire pathway leading to skeletal muscle formation. Recent studies investigated the contribution of soluble signaling molecules to the regulation of sox7 expression. Treatment with retinoic acid (RA) accelerated Sox7 and Wnt3a expression, and activation of the Wnt pathway led to upregulation of Sox7, most likely by direct interaction of beta-catenin with the promoter, as demonstrated by ChIP. It is likely that RA acts upstream of the Wnt signaling pathway, since it was unable to overcome the repression of Sox7 expression by a non-functional beta-catenin. Therefore Sox7 can be placed downstream of both the Wnt pathway and RA signaling during myogenesis in P19 cells. Preliminary results show that stable Sox7-expression in the presence of low levels of RA causes a redirection of cell lineage from skeletal muscle to neurons. These studies have provided a clearer understanding of the pathways that regulate the expression of genes involved in the regulation of muscle precursor cell fate.

Stem Cells

128. The Functional Characterization of Wdr68 Regulation of Pax7 Activity in Myogenesis

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In response to tissue trauma, quiescent satellite cells in adult skeletal muscle undergo proliferative activation and migrate to the site of injury where they differentiate and fuse to mend and replace damaged myofibers. The tightly synchronized expression of myogenic regulatory factors plays a critical role in orchestrating the regenerative process. In recent studies, we identified the transcription factor Pax7 as able to recruit the Wdr5-Ash2L-MLL2 histone methyltransferase complex to the -57.5kb Myf5 enhancer and to activate Myf5 transcription. We also identified Wdr68 as a putative Pax7 binding protein. Wdr68 is a scaffold protein thought to integrate Shh signaling but about which very little is known. Therefore, we set out to characterize a role for Wdr68 with Pax7 in early myogenesis. The interaction of endogenous Pax7 and Wdr68 was validated by reciprocal co-immunoprecipitation and western blot analysis. Manipulation of Wdr68 levels by siRNA knockdown in primary myoblasts followed by real-time PCR analysis of Pax7 target genes suggests that Wdr68 acts as a negative regulator of Pax7. Since preliminary ChIP experiments indicate that Wdr68 does not bind chromatin at known Pax7 binding sites, we hypothesize Wdr68 functions to regulate Pax7 nuclear localization. Together, these experiments provide new insights into the molecular control of Pax7 function during regenerative myogenesis. This work is supported by funding from NSERC, NIH, HHMI and the University of Ottawa.

Stem Cells

129. The role of Pax7 isoform expression during myogenesis

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The post-natal regenerative capacity of skeletal muscle is attributed to a distinct population of myogenic satellite cells which function as lineage-committed precursors to replace damaged terminally differentiated muscle cells. The development and maintenance of this satellite cell lineage is dependant on the activity Pax7. Alternative splicing events of the highly conserved paired-box domain of Pax7 generates four functional isoforms due to the inclusion or exclusion of a glutamine (Q+/-) residue and/or a glycine-leucine (GL+/-) dipeptide at the exon 2/3 or 3/4 junctions, respectively. These various Pax7 isoforms exhibit distinct tertiary structures, possess differential DNA binding affinities and specificities, and therefore, may evoke the potential to differentially activate target genes. However, a specific function for the Pax7 alternative splicing events has not been described. Our findings suggest that the GL dipeptide change modifies downstream target gene activation. Preliminary data shows that while all isoforms can induce the proliferative myogenic regulatory factor Myf5 in myogenic cells, only two Pax7 isoforms (Q-GL-and Q+GL-) can initiate Myf5 expression in non-myogenic cell types, suggesting a functional importance for this GL+/- splicing event. Elucidation of the molecular mechanisms regulating Pax7 isoform expression, and further understanding of the isoform-specific target gene regulation, will provide a greater understanding of the molecular regulation present during myogenesis.

Stem Cells

130. Post-natal muscle-derived stem cells rescue progeria stem cell dysfunction and promote angiogenesis

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Severely reduced expression of the DNA repair endonuclease ERCC1-XPF leads to a dramatic progeriod syndrome (disease of accelerated aging) in humans. Genetic deletions of the excision repair cross-complementation group 1 (Ercc1) gene in mice causes reduced lifespan with premature onset of severe aging that mimics human progeria. Muscle-derived stem cells (MDSCs) isolated from progeroid mice were defective in proliferation and myogenic differentiation, establishing a stem cell defect associated with aging. Remarkably, wild type (WT) MDSCs rescued the proliferation and differentiation defects of the ERCC1-deficient MDSCs in a co-culture system. In vivo, intraperitoneal (IP) injection of WT MDSCs resulted in a significant extension of healthspan and lifespan of the progeroid ERCC1-deficient mice. Ercc1-/- mice display significantly reduced CD31+ blood vessels per muscle fiber as well as muscle fiber size compared to their WT littermates. Notably, IP and intramuscular (IM) injection of WT-MDSCs into Ercc1-/- mice also resulted in a significant increase in both vascular supply and average muscle fiber size when compared to the non-injected mutant animals. The level of vascularity was comparable to that of 9 wk-old WT mice. Collectively, these data suggest the therapeutic effect of post-natal stem cells in aging was the result of a paracrine mechanism mediated by a factor that promotes angiogenesis and growth.

Stem Cells

131. Damaged skeletal muscle is required for BMP9-induced muscle heterotopic ossification

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Heterotopic ossification (HO) is defined as the formation of lamellar bone inside soft-tissues. Symptoms include joint stiffness, swelling and pain. Apart from the inherited form, the common traumatic form occurs generally at the site of injured muscle and is often associated with brain injury, which suggests the presence of a circulating osteoinductive factor. We evaluated bone morphogenic protein 9 (BMP9), which possesses a strong osteoinductive capacity, for its potential involvement in muscle HO physiopathology. We found that BMP9 had an osteoinductive influence on mouse muscle resident stromal cells by increasing alkaline phosphatase activity and the expression of bone specific markers. Using miroCTscan and assessing for the expression of BMP9 receptor (ALK1), BMP9 was shown to induce bone formation in damaged muscle. The addition of the soluble form of ALK1 significantly inhibited the ostoinductive potential of BMP9 in cells and HO formation in the damaged muscles. BMP9 should thus be considered as a candidate for HO physiopathology.

Stem Cells

132. A Cre-Lox system based study of muscle cell plasticity after skeletal muscle injury in adult mouse

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In the skeletal muscle system of certain amphibians, dedifferentiation occurs after muscle injury where mononuclear myogenic progenitors are released from multinuclear myofibers at the local injury site. These mononuclear cells can then serve as an important source of muscle precursor cells for effective muscle regeneration; however, it still remains a mystery as to whether or not a similar dedifferentiation process occurs in the skeletal muscle of mammals. A great challenge to the study of dedifferentiation has been the availability of a model that utilizes myotubes that are definitively differentiated so that there is no potential contamination from non-fused mononuclear cells including stem cells within the cell population being studied. We have successfully solved this problem by setting up a Cre/Lox-b-galactosidase system to specifically tag differentiated myotubes as well as track the dedifferentiation process of the myotubes. In the current study, transgenic myoblasts transduced with this Cre-Lox system were transplanted into the skeletal muscle of adult mice to determine the plasticity of the differentiated myofibers after skeletal muscle injury. Our results demonstrate that the dedifferentiation of differentiated muscle cells and the release of mononuclear cells appear to occur after muscle injury in mouse skeletal muscle.

Stem Cells

133. Conditional TGF-beta1 treatment increases stem cell-like cell population in myoblasts

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The limitation in successfully acquiring large populations of stem cell has impeded their application. A new method based on the dedifferentiation of adult somatic cells to generate induced multipotent stem cells would allow us to obtain a large amount of autologous stem cells for regenerative medicine. The current work was proposed to induce a sub-population of cells with characteristics of muscle stem cells from myoblasts through conditional treatment of Transforming Growth Factor (TGF)-beta1. Our results show that a lower concentration of TGF-beta1 is able to promote C2C12 myoblasts to express stem cell markers as well as to repress myogenic proteins, which involves a mechanism of dedifferentiation. Moreover, TGF-beta1 treatment promoted the proliferation-arrested C2C12 myoblasts to re-enter the S-phase. We also investigated the multi-differentiation potentials of the dedifferentiated cells. TGF-beta1 pretreated C2C12 myoblasts were implanted into mice to repair dystrophic skeletal muscle or injured bone. In addition to the C2C12 myoblasts, similar effects of TGF-beta1 were also observed in the primary myoblasts of mice. Our results suggest that TGF-beta1 is effective as a molecular trigger for the dedifferentiation of skeletal muscle myoblasts and could be used to generate a large pool of progenitor cells that collectively behave as multipotent stem cell-like cells for regenerative medicine applications.

Stem Cells

134. Reducing the expression of NF-kappaB improved the success of muscle stem cell transplantation

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Recent research results demonstrated that blocking p65, a subunit of IKK/NF-kappaB, enhances muscle regeneration in injured and diseased skeletal muscle; however, it is unclear whether the inhibition of NF-kappaB/p65 directly increases muscle regeneration or does so indirectly by attenuating inflammation and fibrosis. In this study, muscle derived stem cells (MDSCs) were isolated from the skeletal muscles of heterozygote P65 knock-out (P65+/-) and wild type (WT) mice via a preplate technique. The results showed that the MDSCs from P65+/- muscle proliferate faster and differentiate better than the MDSCs from WT muscle. Moreover, P65+/- MDSCs have an enhanced muscle regeneration capacity when implanted into dystrophic mdx skeletal muscle and experience less inflammation and necrosis compared to WT MDSCs. We also found that selective pharmacological inhibition of IKK-beta enhanced MDSC differentiation into myotubes in culture. In addition, we demonstrated that when NF-kappaB activation was blocked in vivo using an adeno-associated viral vector that delivered a dominant-negative form of IKK- alpha and IKK-beta, it increased muscle regeneration. The data from these studies suggest that the IKK/NF-kappaB signaling pathway could potentially be a therapeutic target for treating muscle injuries and diseases. Clinical research should be conducted to test the efficacy of P65 inhibition therapy in patients suffering from muscle disorders.

Stem Cells

135. MyoD directs stem cells into the muscle lineage by activating preskeletal mesoderm genes

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The family of Myogenic Regulatory Factors (MRFs), including MyoD and myogenin, are unique in their ability to convert a wide variety of cells into skeletal muscle. Previously, it has been shown that MyoD requires cellular aggregation to direct P19 embryonal carcinoma stem cells into the skeletal muscle lineage. In order to determine the mechanism by which stem cells can be converted into skeletal muscle, a time course of P19 cell differentiation was examined in the presence and absence of MyoD. The first genes upregulated by MyoD in monolayer culture were Pax3 and Eya2. Subsequently, Meox1 and Pax7 were upregulated by days 2-3 during aggregation, followed by Six1, Myogenin, Myf-5, and MEF2C by day 5. To understand the temporal pattern of expression observed, chromatin immunoprecipitation experiments were performed to identify direct targets of MyoD in this system. Exogenous MyoD was observed associated with regulatory regions of Pax3/7, Eya2, Six1, Meox1 as well as myogenin. Finally, a dominant negative MyoD was created by replacing the transcriptional activation domain with the engrailed repression domain, termed P19(MyoD/En). P19(MyoD/En) cells did not differentiate into skeletal muscle, as expected, and downregulated Pax3, Eya2, Six1, and Meox1. Taken together, MyoD appears to direct stem cells into the skeletal muscle lineage by first binding and activating the expression of preskeletal mesoderm genes. This work was supported by the Canadian Institutes of Health Research.

Stem Cells

136. Impairment of the Notch signalling in Pax7 -/- satellite cells

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Stem cells have the unique ability to regenerate tissue. Satellite stem cells are found in adult skeletal muscle and are able to repair injured muscle fibres. Pax7 is a transcription factor with a pivotal role in satellite cell homeostasis where it is required for satellite cell self-renewal and proliferation. Indeed, transgenic mice lacking Pax7 show a dramatic postnatal decline in satellite cell number and consequently lose the ability to regenerate their muscles. To better understand the biological significance of Pax7, we performed microarray analysis on FACS purified satellite cells extracted from Pax7-/- mice. Using cluster analysis we demonstrated that Notch signalling is dramatically down-regulated in Pax7-/- stem cells. This result was supported by immunocytochemical analysis of muscle fibres isolated from Pax7-/- mice and siRNA knockdown of Pax7 in primary myoblasts followed by quantitative PCR. We show that, like primary myoblasts treated with the Notch inhibitor DAPT, Pax7 -/- satellite cells have defects in clonal expansion and differentiate precociously. Current experiments are underway to determine which phase of the cell cycle is misregulated and which level of the Notch pathway is interrupted in Pax7 -/- stem cells. It is noteworthy that, while genes responsible for the processing and internalization of Notch signals remain undisturbed, data from our microarray suggests that genes from the Notch receptor and Hes transcription factor families are highly affected in Pax7-/- satellite cells.

Stem Cells

137. Alteration of the satellite cell niche impairs satellite cell self-renewal but preserves regeneration capacity

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Satellite cells (SCs) are muscle progenitors that account for postnatal muscle growth and regeneration. The SC niche comprises two different environments: the sarcolemma on the SC basolateral side and the basal lamina on the apical side. Disruption of the SC niche through genetic ablation of Syndecan-3, a transmembrane heparan sulfate proteoglycan expressed by SCs, results in impaired SC function. Upon muscle injury, Syndecan3 null (sdc3-/-) SCs show impaired self-renewal, nonetheless sdc3-/- muscles retain the ability to regenerate following three successive induced muscle injuries. To explain the unexpected capacity for muscle regeneration in sdc3-/- mice we explored three possible mechanisms: i) upon injury, sdc3-/- myoblasts delay re-acquisition of quiescence, remain activated and commit to myogenesis; ii) other progenitors compensate for impaired SC function and iii) Syndecan-3 loss establishes a pro-myogenic environment that

overcomes impaired SC function. In support of (i) sdc3-/- muscles analyzed 3 months post-injury show reduced Pax7+ SCs, larger myofibers and increased number of centrally nucleated myofibers. However, we also observe increased numbers of Sca1+ blood vessel-associated progenitors that correlates with increased muscle vascularization, in support of (ii) and (iii). We propose that a combination of all three mechanisms may be responsible for successful muscle regeneration in the absence of Syndecan-3.

Stem Cells

138. Protein phosphatase 1 interacts with Nkx2.5 and controls its subcellular localization

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Nkx2.5 is a transcription factor that is instrumental in regulating cardiomyocyte differentiation and heart morphogenesis. We have undertaken a proteomic screen to identify cofactors of Nkx2.5 during cardiomyogenesis in P19 embryonal carcinoma cells. The beta isoform of protein phosphatase 1(PP1) and the PP1 targeting subunit Mypt1 were both identified by the screen as being putative binding partners of Nkx2.5 and were confirmed by co-immunoprecipitation. Coexpression with PP1/Mypt1 results in decreased transcriptional activity of Nkx2.5. This effect is not observed in the presence of PP1 and its nuclear targeting subunit, NIPP1. Interestingly, the effect of PP1 on Nkx2.5 was independent of phosphatase activity. Further characterization of this interaction revealed that coexpression with PP1/Mypt1 resulted in exclusion of Nkx2.5 from the nucleus. We propose that PP1 interacts with Nkx2.5 in differentiating cells and thereby controls its subcellular localization.

Stem Cells

139. Genome-wide analysis of Pax3 and Pax7 targets reveals unique roles for Pax7 in adult myogenesis

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Pax3 and Pax7 belong to a family of conserved transcription factors that play important and diverse roles in development. In the embryo, they carry out similar roles in neural and somite development, but Pax7 fails to compensate for critical functions of Pax3 in the development of limb musculature. Conversely, in the adult, Pax7 is necessary for the maintenance and survival of muscle satellite cells, whereas Pax3 cannot effectively fulfill these roles in the absence of Pax7. To identify the unique roles of Pax7 in adult muscle cells, we have analyzed global binding of Pax3 and Pax7 by ChIP-seq. Here, we show that despite highly homologous DNA-binding domains, the majority of binding sites are uniquely recognized by Pax7 and are enriched for homeobox motifs. Genes proximal to conserved, unique Pax7 binding sites cluster into specific functional groups which may reflect the unique biological roles of Pax7. Combining Pax7 binding sites with gene expression data, we describe the regulatory networks directed by Pax7 and show that Pax7 binding is associated with positive gene regulation. Moreover, we show Myf5 is a direct target of Pax7 and identify a novel binding site in the satellite cell control region upstream of Myf5.

Stem Cells

140. Characterization of Pax7+Myf5- satellite stem cells in culture condition

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Previously we reported that freshly isolated Pax7+Myf5- satellite cells can generate both Pax7+Myf5+ satellite cells and mature myocytes in vitro and in vivo experiment. These result suggested that there is advantage to apply Pax7+Myf5- satellite cells in clinical usage; however, it is still unknown whether these cells can be expanded with keeping immature phenotype in culture condition or not. We tried several culture conditions to expand these cells efficiently. Though they proliferated only slowly in conventional myoblast culture method, 20% FBS media with LIF, FGF2, and EGF allowed Pax7+Myf5- cells to expand very rapidly. In media with LIF, FGF2, and EGF, cultured Pax7+Myf5- cells expressed similar level of myogenic transcription factor with freshly isolated ones. Without Myf5 expression, these cells could differentiate into fast MyHC+ myocytes as well as Pax7+Myf5+ cells. These result suggested that Pax7+Myf5- satellite cells can expand in vitro condition and they could differentiate into fast MyHC+ myocytes without becoming Pax7+Myf5+ satellite cells.

Stem Cells

141. Injection of WT and mdx heterozygous mouse induced pluripotent stem cells into muscular dystrophy blastocysts: testing pluripotent and corrective potentials in one assay

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We injected WT and mdx+/- eGFP-marked, mouse-induced pluripotent stem (iPS) cells into mdx, mdx:utrophin+/- and mdx:utrophin-/- blastocysts, which are predisposed to develop DMD with an increasing degree of severity (mdx <<< mdx:utrophin). iPS cells populated all tissues, confirming that the newly derived cells were pluripotent. Mice with at least 20% chimerism showed a significant amelioration of the disease at the morphological and functional levels, strengthening the notion that iPS cells are capable of effecting corrections in skeletal muscles. In the mdx or mdx:utrophin+/- rescue, dystrophin was evident in the skeletal muscle coincident with histopathological corrections. Although iPS cell incorporation was non-uniform, the whole extensor digitorum longus (EDL) muscle demonstrated tetanic/twitch stress functional recovery. In the mdx muscle (no iPS cell injection), utrophin was upregulated in the sarcolemma and ameliorated disease. Surprisingly however, in the rescued chimeric muscle (iPS cell injection), upregulation of utrophin was not apparent in areas devoid of dystrophin, although global corrections were apparent. This outcome suggested upregulation of utrophin was dispensable for functional corrections of the skeletal muscle, in the presence of at least 20% of iPS cell chimerism. The lack of utrophin upregulation was also observed in dystrophin negative cardiac myocytes in chimeric hearts. Our goal now is to use

human iPS cell-derived cardiac myocytes derived from a normal subject and a DMD patient, to identify potential factors that might be involved in the cell-to-cell regulation of utrophin by dystrophin. In the mdx:utrophin-/- mutant chimeras, dystrophin and utrophin were evident in the skeletal muscle (sarcolemma, neuromuscular junction and terminal axons). However, no tetanic/twitch stress recovery was observed in EDL muscles. Chimeric mice display kyphosis and poor skeletal muscle histopathology, suggesting that the corrections in this severe model of muscular dystrophy were incomplete. Thus, blastocyst injection of WT and mutant iPS cells into mutant blastocysts allows investigators to study the pluripotent character of newly derived iPS cells, while testing their corrective potential in skeletal muscle disease.

Stem Cells

142. Understanding morphogenic properties of Gli2 during stem cell differentiation

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Gli2 is a primary mediator of the sonic hedgehog (Shh) signaling pathway during myogenesis and neurogenesis in developing embryos. Previously, Gli2 has been shown to play a role in cardiomyogenesis and skeletal myogenesis in vivo and to induce cardiac and skeletal myogenesis in vitro in P19 cells, an established stem cell model for muscle and neuronal cell differentiation. We found that in addition to myogenesis, expression of Gli2 resulted in the induction of neurogenesis in P19 cells. This is the first observation of the neurogenic effect of Gli2 in stem cells. Similar to Gli2, Mef2C is also important in myogenesis and neurogenesis in vivo, and its expression resulted in induction of cardiac and skeletal muscle as well as neurons in P19 cells. We found that Gli2 and Mef2C upregulated each other's transcription by directly binding to their respective gene elements, indicating a novel positive regulatory loop between Gli2 and Mef2C. Moreover, we found that Gli2 and Mef2C form a protein complex during P19 cellular differentiation. Gli2 and Mef2C proteins showed synergistic activation of the Nkx2.5 promoter, a factor playing a major role in cardiomyogenesis both in vitro and in vivo. Our findings shed light on transcriptional regulation as well as protein complex formation and function during myogenesis and neurogenesis by Shh signaling in vitro.

Stem Cells

143. Myosphere stem cells contribute to the satellite cell niche

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Cell-based therapy to treat muscle disease has been hampered by difficulties in isolating, maintaining and propagating the stem cells that are needed for treatment. Here we report the isolation of muscle stem cells from both young and old mice and their propagation over extended periods of time in culture as myospheres. Analysis of these sphere-forming cells showed that they express Sca-1, CD29, CD90, and CD34, but did not express CD45, CD31, or myogenic markers (Pax7, Myf5, and MyoD). We found that cells derived from myospheres and then grown adherently (MDACs) behaved similar to primary myoblasts, in that these cells expressed myogenic markers and were able to easily form multinucleated myotubes. Unlike the parental myopspheres but analogous to primary myoblasts, MDACs expressed Pax7, Myf5, and MyoD, indicating that the parent myosphere cells are a more primitive type of cell. In support of this we found that myopsheres were able to differentiate into adipogenic and osteogenic cells in culture and that donor myosphere cells were able to contribute to injured muscle in vivo including the satellite cell niche (indicated by Pax7/Laminin staining). This research was supported by a grant from NIH-NIAMS, Grant Number KO1AR052372.

Therapy

144. IMPAIRED RESPIRATORY FUNCTION IN MDX AND MDX/UTRN+/- MICE

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Duchenne muscular dystrophy (DMD) is the most common and lethal genetic muscle disease. The DMD mouse models, mdx mice and mdx mice with eterozygous insufficiency of the utrophin gene (mdx/utrn+/-), display progressive diaphragm fibrosis with impaired muscle contractility. To address whether mdx and mdx/utrn+/- mice have significantly impaired respiratory function, we performed unrestrained whole-body plethysmography in mdx and mdx/utrn+/- mice, and compared with wild-type controls. Respiratory function parameters, including respiratory frequency, tidal volume, minute volume, peak inspiratory flow, and peak expiratory flow, were all significantly reduced in mdx mice at 3 and 6 months of age as compared with wild-type controls. Consistent with more severe diaphragm fibrosis seen in mdx/utrn+/- mice, respiratory function was worse in mdx/utrn mice than in mdx mice at 6 months of age. We conclude that whole-body plethysmography can be used to monitor in vivo respiratory function of mdx and mdx/utrn+/- mice and may serve as a valuable outcome measurement of therapies targeting diaphragm fibrosis. With more severe diaphragm fibrosis and respiratory function impairment, mdx/utrn+/- mice may represent a better model than mdx mice for testing antifibrotic therapies, especially at a severe stage. Supported by K08 NS049346 (LZ) and MDA#91682 (LZ).

Therapy

145. Lithium chloride protects against the toxicity of mutant poly(A) binding protein nuclear 1 in oculopharyngeal muscular dystrophy

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Oculopharyngeal muscular dystropgy (OPMD) is an adult onset disorder characterized by progressive eyelid drooping, swallowing difficulties, and proximal limb weakness. OPMD is caused by a small expansion of a short polyalanine tract in the poly (A) binding protein nuclear 1 protein (PABPN1). No medical treatment is available for OPMD. Here, we demonstrate for the first time that lithium chloride (LiCl)can protect against the toxicity caused by mutant PABPN1 protein in our established OPMD cell models. The enhancement of cell survival was measured at different days post treatment of LiCl using the in vivo microscopy and FACS methods. We also show

that these protective effects can be partly accounted for by LiCl acting through Wnt/beta catenin pathway, as elevated level of beta catenin protein was detected. Our results suggest that LiCl should be considered as a new therapeutic intervension for OPMD.

Therapy

146. Nitric oxide donors reduce prednisone side effects in mdx diaphragm and promote exercise-induced muscle growth and cytoskeletal modifications in aged mice

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Sarcolemmal disruption in Duchenne dystrophy is ultimately lethal, and palliative prednisone (P) therapy can induce myopathy or calcification. Since nitric oxide (NO) activates satellite cells and promotes muscle regeneration, we tested a new NO donor MyoNovin (guaifenesin dinitrate, MN) and isosorbide dinitrate (ISDN) for potential to improve P effects on mdx dystrophy. Groups (n=8) [control, MN (80 mg/kg po), ISDN (66 mg/kg po), P (1 mg/kg sc), MN+P, ISDN+P] were treated for 18 days and injected with Evans dye (EBD, ip). ISDN increased heart mass; MN increased quadriceps mass. In diaphragm, P increased the %EBD+ fibers and calcification; MN+P and ISDN+P reduced the %EBD+ fibers. ISDN suppressed calcification. Dystrophy tended to be less severe after NO-donor treatment. In 18mo normal mice, 6wks ISDN increased quadriceps weight, fiber size, NOS-1:beta-dystroglycan ratio, and the hypertrophy induced by 3 wks voluntary exercise. Results suggest that NO donors have potential to suppress two important side effects of prednisone treatment in mdx dystrophy and to improve the impact of exercise in attenuating age-related sarcopenia in mice. Support: Canadian Space Agency--Life Sciences, Manitoba Institute of Child Health, NSERC-USRAs

Therapy

147. TAT-microUtrophin improves the phenotype of dystrophin and utrophin double-knockout mice

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Mice lacking both dystrophin and utrophin (DKO) suffer from severe muscle wasting, weakness, and premature death. Recently, we showed that recombinant TAT-microUtrophin improved EDL muscle force and resistance to eccentric contraction-induced injury in mdx mice. Here, the effects of TAT-microUtrophin replacement therapy on DKO mouse survival, quality of life, muscle contractility, and susceptibility to eccentric contraction-induced force loss was assessed. Treated mice (TAT-mUtr) were injected twice weekly with TAT-microUtrophin (8.5 ug/kg BM) and control DKO mice were injected twice weekly with PBS (PBS). Quality of life measurements were improved in TAT-mUtr mice compare to PBS mice. Specifically, food consumption (3.1 vs 1.9g per 24 hr, respectively) and ambulatory distance (272 vs 177 m per 24 h, respectively) were both increased in the TAT-mUtr mice. TAT-mUtr EDL muscles also had greater specific force in vitro (8.3 vs 6.4 N/cm2) and modest protection from contraction-induced injury both in vitro (TAT-mUtr 48% vs PBS 66% force loss post eccentric contractions) and in vivo (TAT-mUtr 56% vs. PBS 66% force loss post eccentric contractions). Lastly, TAT-microUtrophin treatment improved lifespan survival by ~50%. These data indicate that the benefits of TAT-microUtrophin replacement therapy are repeatable in the DKO mouse and may represent a therapy to mitigate muscle force loss in patients with DMD.

Therapy

148. L-arginine ameliorates muscular dystrophy in mdx mice without utrophin upregulation

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Rationale: Duchenne muscular dystrophy (DMD) is caused by genetic mutations which result in the absence of cytoskeletal protein known as dystrophin. The lack of dystrophin leads to muscle weakness and necrosis by progressive muscle destruction, fibrosis, and cell infiltration Studies have shown in mdx neonatal mice, the murine model of DMD, that utrophin, a closely related protein, is able to prevent the development of muscle necrosis and weakness when overexpressed. More recently, L-arginine has shown to induce utrophin upregulation in mdx adult mice. In order to prevent the oncet of muscle destruction, mdx neonatal mice were treated with l-arginine. Objective: Our goal was to investigate if the early L-arginine treatment in mdx neonatal mice could ameliorate muscular dystrophy. Methods:Then seven days old animals were treated IP daily 800m/kg of L-arginine or saline. Six weeks after treatment, TA contractility, centronucleation evaluation, utrophin immunostaining and western blot, creatine kinase activity and, nitric oxide (NO) production were performed. Results. Our results show that: 1) TA weight and percentage of centronucleation in l-arginine treated animals were significantly lower than saline treated animals despite the fact that body weights were not different; 2) l-arginine improved TA ability to resist injury caused by high-stress contractions; 3) CK level was two time higher in saline treated animals compared to l-arginine treated animals. However, this difference was not statistically significant; 4) NO production was significantly higher in l-arginine treated mice 5) there was no evidence that the reduction of TA weight, the low level of centronucleation and the ability of TA to resist to eccentric contractions were associated to utrophin upregulation induced by l-arginine. Supported by: The Muscular Dystrophy Association (USA), the Canadian Institutes of Health Research, the Fonds de la Recherche en Sante du Quebec, and the Association Francaise contre les Myopathies.

Therapy

149. Alpha7 integrin mediated alleviation of merosin-deficient congenital muscular dystrophy

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Merosin-deficient congenital muscular dystrophy (MDC1A) is a neuromuscular disease caused by mutations in the LAMA2 gene which encodes laminin alpha2 protein. Laminin alpha2 is necessary for the assembly of laminin 211 and 221 (merosin); laminin 211 is a major component of skeletal muscle extracellular matrix. Patients with MDC1A display hypotonia, severely delayed motor milestones and decreased life spans. MDC1A patients have a secondary reduction in alpha7beta1 integrin, however it is unclear to what extent this contributes to pathology. The dyW mouse model for MDC1A has altered expression of alpha7 integrin. Overexpression of alpha7 integrin improves the phenotype of other dystrophic mice. Therefore, we investigated if transgenic overexpression of alpha7 integrin would be beneficial in the dyW mouse. Laminin alpha2 deficient mice which overexpress alpha7 integrin in skeletal muscle were generated and showed improved muscle pathology and increased lifespan. Our data indicate that alpha7 integrin is a major modifier of disease progression in dyw mice and that compounds which target alpha7 integrin expression in the skeletal muscle may have therapeutic potential for MDC1A patients. Supported by the NIH.

Therapy

150. RNA-based gene therapy for myotonic dystrophy type 1 (DM1)

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DM1 is caused by the expansion of CTG repeats in the 3' untranslated region of a protein kinase (DMPK) transcript. The current favorite hypothesis of the pathogenesis promotes that dominant-negative pathogenic effects of RNA with CUG expansions, including gain-of-function mutation, cause DM1. Targeting the mutant DMPK transcripts could then be a major approach for the development of a gene therapy for DM1. The proof of principle regarding this approach was originally demonstrated in human DM1 myoblasts. We have shown that ribozyme and antisense RNAs are more efficient than shRNAs to target mutant RNAs and to restore a normal behavior to DM1 myoblasts. The antisense therapy, delivered IM with rAAVs, decreased mutant DMPK RNAs by 55% ± 20 and increased muscle volume by 30%, in a DM1 mouse model with 500 CTGs. Systemic delivery failed to destroy mutant transcripts. The use of AAV by systemic injection in human is not presently possible. To overcome these difficulties we developed an antisense phophorothioate oligonucleotides (ASO) approach. We showed that ASO destroys up to 90% of human mutant transcripts following intramuscular injection. Destruction of mutant DMPK transcripts with systemic injection was achieved at high doses. These data support the potential of a RNA-based gene therapy for DM1, but it needs improvement. Different strategies are presently tested and will be discussed.

Therapy

151. Oxidative stress and RYR1 related myopathies

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RYR1 is the major skeletal muscle calcium release channel. Mutations in RYR1 are associated with core myopathies and are the commonest genetic cause of congenital myopathies. The primary function of RYR1 is to regulate excitation-contraction coupling (ECC), and defective ECC is a key aspect of the pathogenesis of RYR1-related myopathies. However, other aspects of RYR1 function likely impact disease. RYR1 has been shown to be an important sensor of oxidative stress. We hypothesized that reduction of RYR1 expression, as seen in recessive core myopathies, is associated with increased oxidative stress, and that anti-oxidant treatment will improve disease outcome. We tested this hypothesis using a combined approach. Using a zebrafish model of recessive core myopathy, we found that loss of RYR1 results in increased levels of basal oxidative stress and increased susceptibility to pro-oxidants. We next found similar increases using myotubes from human RYR1 myopathy patients. We lastly treated the RYR1 zebrafish with anti-oxidants, and found that treated fish had significant improvements in motor function. In all, we uncovered an association between loss of RYR1 function and increased oxidative stress, and determined that anti-oxidants can rescue aspects of the clinical phenotype. We believe that anti-oxidant therapy can thus be a viable potential therapeutic option for patients with RYR1 related myopathies.

Therapy

152. AAV-mediated SERCA2a therapy ameliorates ECG defects in the mouse model of Duchenne cardiomyopathy

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Duchenne muscular dystrophy (DMD) is a lethal muscle disease caused by dystrophin deficiency. A major cause of death in DMD is dilated cardiomyopathy and subsequent heart failure. Recent studies suggest that AAV-mediated sarcoplasmic reticulum Ca2+ ATPase (SERCA2a) expression be a very promising strategy to treat heart failure. Here we hypothesize that AAV-mediated SERCA2a expression may improve cardiomyopathy in DMD. To test this hypothesis, we injected AAV9-SERCA2a into 12-month-old mdx mice (n=5) via tail vein. Mdx mice are the most commonly used animal model for DMD. These mice develop characteristic dilated cardiomyopathy when they reach 20 months of age. Eight months after AAV9-SERCA2a gene therapy, we examined cardiac function by electrocardiography (ECG). The results were compared with age- and sex-matched untreated mdx (n=9) and normal (n=7) mice. We observed significant improvement in most of the ECG parameters ($P<0.01$), specifically, heart rate 541\'ac\'b14 bpm, PR interval 41.4\'ac\'b11.5 msec, QT 20.6\'ac\'b11.2 msec, and cardiomyopathic index 0.69\'ac\'b10.06. Interestingly, QRS duration and Q wave amplitude were not altered by SERCA2a therapy. In summary, our results show that AAV-mediated SERCA2a expression improves ECG performance in DMD animal model. Future hemodynamic studies are warranted to further corroborate

therapeutic benefit of SERCA2a in end-stage heart disease in aged mdx mice.

Therapy

153. AAV9-mediated catalase expression in mitochondria improves exercise performance in mdx mice

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Duchenne muscular dystrophy (DMD) is a lethal degenerative muscle disease caused by dystrophin deficiency. The precise mechanisms by which absence of dystrophin results in the muscular dystrophy remain not fully understood. It has been suggested that increased oxidative stress may contribute to myofiber necrosis and degeneration. Here we tested whether reducing mitochondrial free radical could ameliorate muscle disease in the mdx model of DMD. The human catalase gene was engineered with the mitochondria-leading sequence for targeted expression in the mitochondria (Schriner et al Science 308:1909, 2005). The mitochondrial catalase gene was packaged in adeno-associated virus serotype-9 (AAV9) and delivered to neonatal mdx mice. Histopathology and muscle force were examined three months later. We did not detect morphology improvement in muscle section, neither was serum creatine kinase level reduced. However, the eccentric contraction-induced damaged was mitigated in the extensor digitorium longus muscle. When challenged with downhill treadmill running, AAV-infected mice significant outperformed untreated mice. Taken together, our results suggest that the mitochondria-derived reactive oxygen species may contribute to muscle weakness in mdx mice. Strategies to reduce oxidative stress in mitochondria may represent a viable approach to treat muscular dystrophy.

Therapy

154. MRI Evaluation of Systemic Myostatin Inhibition in Skeletal Muscle of Golden Retriever Muscular Dystrophy Dogs

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The purpose of this study was to evaluate the effects of systemic myostatin inhibition on skeletal muscle volume and magnetic resonance proton transverse relaxation time (T2) in a canine model of Duchenne muscular dystrophy. Myostatin inhibition by liver-targeted gene transfer was performed in four Golden Retriever muscular dystrophy (GRMD) dogs (9-10 months old). 3D-gradient echo and fast spin echo axial images were acquired of the lower hindlimbs using a 1.5T GE scanner at monthly intervals for 6 months and at an additional time point at 21-25 months of age. Also, images were acquired on three untreated GRMD dogs (23-25 months old). Muscle volume and T2 were measured in the anterior compartment (AC) of lower hindlimbs. Following treatment, muscle volume in the AC increased ($p<0.05$) over 6 months (17±11%), with no changes in muscle T2 (pre 29.4±1.9ms; 6 months: 29.7±2.2ms). Also, the AC volume was greater in treated GRMD dogs (27.2±4.7cm3) than untreated GRMD dogs (19.1±3.4cm3) at 21-25 months of age. The findings of this study indicate that systemic myostatin inhibition increases skeletal muscle mass in GRMD dogs. Furthermore, the maintenance of T2 with age suggests that the increase in muscle size in the treated GRMD dogs was not accompanied by a disproportionate elevation in fatty tissue infiltration. Supported by the Wellstone Muscular Dystrophy Center (1U54RO52646-01A1) and Muscular Dystrophy Association (MDA4170).

Therapy

155. IGF-1 Ameliorates Pathology of Congenital Muscular Dystrophy 1A

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Type 1A (MDC1A) is the second most common form of congenital muscular dystrophy and results in early childhood death because it lacks an effective treatment. It is caused by mutations in LAMA2, the gene coding for the laminin alpha 2 chain. LAMA2 deficiency results in accelerated muscle degeneration with little or no regeneration. The Lama2Dy-w is a mouse model used to study MDC1A. Anti-apoptotic measures led to a marked improvement in muscle and nerve phenotype in the mouse model. However, these measures only partly corrected the pathology: the mice were still notably smaller than wild type mice with clear onset of paralysis with age. We have also found that the in vivo and in vitro proliferation of satellite cells is drastically decreased in Lama2Dy-w muscles compared to the wild type muscle. To evaluate the effect of increased levels of IGF-1 in muscles of laminin-deficient mice, we crossed transgenic mice over-expressing a precursor of muscle-specific Igf-1 in skeletal muscle (mIGF-1) with lama2Dyw mice. Our preliminary findings demonstrate a marked increase in the survival of Lama2Dy-w mice expressing the IGF-1 transgene. IGF-1 over-expressing mice also have significant improvement in their body weight and muscle function. In addition, preliminary data indicate that IGF-1 activation when combined with inhibition of bax, result in a significant increase in body weight of Lama2Dy-w mice that is not achieved with either treatment alone.

Therapy

156. Prevention of muscle aging by myofiber-associated satellite cell transplantation

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Sarcopenia is characterized as the inevitable loss of muscle mass and function with age where changes in the local systemic environment inhibiting the regenerative capacity of satellite cells is believed responsible. Uniquely positioned between the myofiber plasma membrane and basal lamina, satellite cells are acutely sensitized to changes in the surrounding environment. We demonstrate that engraftment of myofiber associated satellite cells, coupled with muscle injury, profoundly alters the adult muscle environment whereby donor satellite cell expansion and engraftment causes a 50% increase

in mass and a 2-fold increase force generation that is resistant to aging and which persists for the lifespan of the mouse. This change in the transplanted muscle is accompanied by an overall 3-fold increase in satellite cell numbers due exclusively to donor cells. Further, donor cells possess extensive self-renewal capabilities as demonstrated via serial transplantation. These lifelong, physiological changes present a new paradigm for the amelioration of muscle atrophy and diminished functionality that arise with aging through myofiber-associated satellite cell transplantation. Future work is aimed at further elucidating the mechanisms regulating this response.

Therapy

157. Laminin-111, promising therapy for the Duchenne Muscular Dystrophy population

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Laminin-111 is a 900kDa heterotrimeric protein found in the basal lamina of the kidney while the related protein laminin-211 is found in normal skeletal muscle. A single systemic administration of EHS laminin-111 (LAM-111) to 10 day old dystrophin-deficient mdx mice distributed to cardiac and skeletal muscles and resulted in a substantial protection of skeletal muscles from the damaging effects of downhill treadmill exercise. LAM-111 has a very high affinity for the laminin receptor alpha7beta1 isoform of integrin, the predominant integrin isoform found on skeletal muscle, and the mechanism of action of LAM-111 appears to be through high affinity binding to preexisting alpha7beta1 integrin as well as transcriptional activation of new alpha7 integrin message. We have administered single and multiple doses of LAM-111 to older and downhill treadmill exercised mdx mice. The consequences in blood chemistry, functional outcome and histology will be presented.

Therapy

158. BMP antagonists enhance myogenic differentiation and ameliorate the dystrophic phenotype in a DMD mouse model

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Duchenne Muscular Dystrophy (DMD) is an X-linked lethal muscle wasting disease characterized by muscle fiber degeneration and necrosis. Secondary processes such as inflammation trigger fibrosis and in addition impair muscle regeneration, thus contributing to the progressive pathology of DMD. It is therefore important to elucidate the molecular pathways involved in these seconday processes. In a previous study we found increased expression of BMP4 in DMD patient myoblasts. The aim of the current study was to investigate whether inhibition of BMP signaling is beneficial for myoblast differentiation and muscle regeneration process in a DMD context. All tested BMP inhibitors; Noggin, dorsomorphin and LDN-193189, were able to accelerate and enhance myogenic differentiation and induce protein levels of myogenin in vitro. However, dorsomorphin inhibited both BMP- and TGFbeta-signaling and was found to be toxic to primary myoblast cell cultures. Noggin was found to be a potent and selective BMP-inhibitor and was therefore tested in vivo in a DMD mouse model. Local overexpression of Noggin in muscle of mdx utrn+/- mice resulted in a two-fold increase of Myog expression and a twenty percent decrease in the percentage of fibrotic/nectrotic area. In conclusion, our results suggest that repression of BMP signaling may constitute an attractive adjunctive therapy for DMD patients. This research was financially supported by the Dutch Duchenne Parent Project.

Therapy

159. High throughput screening identifies small molecules that synergize with antisense oligonucleotides to enhance exon skipping on the DMD gene

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Duchenne muscular dystrophy (DMD) is primarily caused by out of frame multiexon deletions in the DMD gene that ablate dystrophin protein expression. Internal domain deletions that preserve the reading frame produce a truncated dystrophin protein that retains actin and DGC binding and can result in a less severe phenotype. Therapies utilizing antisense oligos (AO) target exons for exclusion from mRNA to change out-of-frame mutations into in-frame DMD deletions. AOs have corrected DMD gene mutations in mice, dogs and humans, yet the skipping process remains inefficient. To improve efficiency and identify modulators of DMD exon skipping, we performed a small molecule highthroughput screen on 1622 drugs using a fluorescent human DMD exon 50 reporter myoblast cell line. Of 113, 20 have been validated in a 16-point dose response with the cell reporter to confirm efficacy in cell culture. Drug titrations with and without AO in primary mouse myotubes validated 12 drugs that synergize with AO to induce DMD exon 23 skipped mRNA. One of these FDA approved drugs was tested in mdx mice in replicate in vivo studies and was shown to synergize with AO to increase levels of DMD exon 23 skipped mRNA and sarcolemmal dystrophin protein expression. These findings describe novel drug synergy with AO and identify drugs and screening strategies for potentiating DMD exon skipping therapies and elucidating mechanisms regulating splicing.

Therapy

160. Activin receptor type IIB inhibition increases utrophin expression and improves function in dystrophic muscle

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Utrophin has been identified as a potential Duchenne muscular dystrophy (DMD) therapeutic based on its structural homology to dystrophin and ability to restore myofiber stability in dystrophic muscle. Activin receptor type IIB (ActRIIB) signals for negative regulators of muscle growth and treatment with a non-signaling ActRIIB (RAP-031) improves muscle strength in mdx mice, a murine DMD model. To determine if utrophin mediates the RAP-031 effect on dystrophic muscle, utrophin expression and localization were assessed in mdx5cv mice treated with RAP-031 (10 mg/kg, s.c.) or vehicle. After 20 treatment weeks, pectoralis and extensor digitorum longus muscles were assayed for utrophin protein expression and membrane localization, respectively. Consistent with previous work, RAP-031 increased muscle mass in dystrophic mice. RAP-031 also increased utrophin protein expression 2-fold

compared to the vehicle-treated cohort. Immunofluorescence localized utrophin expression to the sarcolemma, consistent with utrophin acting as a dystrophin replacement to stabilize the myofiber. These results demonstrate that RAP-031 increases sarcolemmal utrophin in dystrophic muscle, making it a possible therapeutic for DMD. Funded by Acceleron Pharma.

Therapy

161. Inhibition of the activin receptor type IIB produces transient increases in body mass, strength, and survival in myotubularin deficient mice

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X-linked myotubular myopathy (XLMTM) is a severe congenital myopathy caused by a deficiency of the phosphoinositol phosphatase, myotubularin. Muscle biopsies from patients with XLMTM display excessively small fibers with increased numbers of central nuclei and central aggregates of organelles. We postulated that therapeutically increasing muscle fiber size may lead to an improvement of symptoms resulting from myotubularin deficiency. Recent studies have found an important role of activin receptor type IIB (ActRIIB) in muscle growth regulation, which can be inhibited with the soluble ActRIIB analog, RAP-031, to produce myofiber hypertrophy. Treatment of wild type animals with RAP-031 produced persistent increases in body mass and forelimb grip force through the hypertrophy of both oxidative and glycolytic fibers. Treatment of myotubularin-deficient mice with RAP-031 produced transient increases in body mass and forelimb grip strength, with a 10-day (18%) extension in lifespan. This response was due to the selective hypertrophy and delayed atrophy of glycolytic fibers, with little apparent therapeutic effect on oxidative fibers. These findings support a role for RAP-031 in ameliorating the effects of myotubularin deficiency.

Therapy

162. DM Registry of Volunteers: Suggesting a Few Key Principles to Manage Confidentiality Issues

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In 2009, the Registry of volunteers for research on Myotonic Dystrophy (hereinafter Registry) was made available to connect volunteers and researchers around the world. The Registry is a tool to facilitate research. It contains the information necessary to reach individuals interested in participating in different research projects on DM. Throughout the development of the project, we faced and solved many unseen challenges pertained to the protection of confidentiality and management of personal information. These issues are the most common and important regarding the development of any disease-related registry. Ethical norms (Canadian and international) related to the protection of information are abundant and various. Thus, making sense of all of them is no easy task. However, the protection of personal information responds to certain general key elements that are grouped into 10 principles in the Model Code for the Protection of Personal Information of the Canadian Standards Association. We will explain how these 10 basic principles may be referred to during the development stage of many volunteer registries. We will also examine two mechanisms that should ensure good governance of registries: (1) adoption of management policy, and (2) implication of research ethics boards. Finally, considering that the risk of over-solicitation is a very specific concern to any volunteer registry, we will discuss the approaches to manage this risk.

Therapy

163. Relaxin regulates the expression of MMPs and promotes satellite cell mobilization during muscle healing in both young and aged mice

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Our previous research has proven the beneficial attributes of the polypeptide hormone relaxin of preventing excessive collagen deposition in fibrosis formation in injured skeletal muscle which results in improved skeletal muscle regeneration. However, the molecular and cellular mechanisms by which relaxin regulates myogenic cell differentiation and muscle healing are still unclear. In the current study, the potential effect that relaxin has on myoblasts in vitro was studied. Relaxin was also injected into the injured muscles of the mice to observe its function in vivo in young and aged muscle. Our results showed that relaxin administration can improve muscle healing, not only by accelerating muscle regeneration and preventing fibrotic scar tissue formation, but also by regulating the expression of MMPs (MMP 1, 2 and 9), activation of muscle precursors, promoting angiogenesis, and inhibited inflammation. Moreover, relaxin is beneficial for regenerating both young and aged skeletal muscle. Since Phase II/III clinical trials of relaxin are moving forward, understanding the mechanisms behind relaxin's action in the muscle healing process could be helpful to guide its clinical application and will further benefit relaxin's use in patients suffering with skeletal muscle injuries and diseases.

Therapy

164. Genetic ablation of CC class chemokine receptor 2 (CCR2) improves myofiber structure and function in the dystrophic (mdx) diaphragm

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Background: In the mdx mouse model of Duchenne Muscular Dystrophy (DMD), the diaphragm most closely resembles the human phenotype. The infiltration of inflammatory cells into dystrophic muscles propagates the pathology of DMD. We have previously reported that CC class chemokines, which attract macrophages and other inflammatory cell types found within dystrophic muscles, are highly upregulated in the mdx diaphragm. Objectives: We hypothesized that genetic ablation of CCR2, a CC class chemokine receptor which is important for monocyte/macrophage trafficking, would attenuate pathological

changes within the mdx diaphragm. Methods and Results: Mdx/CCR2-/- mice were generated and evaluated at 2 ages (6 weeks and 3 months). Histological analysis revealed that mdx/CCR2-/- diaphragmatic myofibers were significantly larger and had fewer central nuclei than their mdx counterparts. In mdx/CCR2-/- mice, multiple proinflammatory genes were downregulated by quantitative RT-PCR, and immunostaining revealed a significant reduction in the number of macrophages contained within the diaphragm. In addition, maximal force production and the ability to maintain force following eccentric contractions were both significantly improved in mdx/CCR2-/- diaphragms compared to mdx mice. Conclusions: In the severely affected mdx diaphragm, which has a high level of inflammation and resembles human DMD, the loss of CCR2 function has beneficial effects upon the structure and function of dystrophic muscle fibers. Therefore, these findings suggest that CCR2 and/or its ligands could be useful therapeutic targets in DMD.

Therapy

165. Trichostatin A rescues motor defects and promotes growth and survival independent of Smn expression in a mouse model of intermediate SMA

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Spinal muscular atrophy (SMA) is a motor neuron disease caused by deletions or mutations of the Survival of Motor Neuron 1 (SMN1) gene. Previous studies have shown that trichostatin A (TSA), a histone deacetylase inhibitor, increases SMN expression and survival in an SMA mouse model harboring a human SMN2 transgene. Whether TSA has effects specifically through the SMN2 gene and whether other off-target molecules also respond to TSA and in turn provide protection to the SMA mice is unclear. In the present study, we are investigating the effects of TSA in the Smn2B/- mouse model. We found that TSA significantly promotes survival and attenuates weight loss of Smn2B/- mice in comparison to vehicle treated SMA mice. Motor behavior, as assessed by a grip strength test and a time to right test, was also improved in TSA-treated SMA mice, albeit with variability amongst the individuals. Of note is that TSA treatment did not result in increased expression of the mouse Smn gene at the mRNA and protein levels, suggesting that TSA targets genes other than Smn. Thus the improvements observed in the Smn2B/- mice are independent of the Smn gene. We are currently undertaking further analyses to gain an insight into the pathological changes in TSA-treated SMA mice and to identify TSA target genes that may be having a positive effect.

Therapy

166. Increase beta1D Integrin Alleviates Muscular Dystrophy in mdx mice

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Duchenne muscular dystrophy is caused by mutations in the dystrophin gene and their consequence on the formation of the dystrophin glycoprotein transmembrane complex. In normal muscle the dystrophin complex connects the myofiber cytoskeleton with laminin in the extracellular matrix. In the absence of this linkage the integrity of muscle fiber membranes is compromised and severe muscle wasting develops. One potential therapy for addressing muscular dystrophy is to augment the connections between laminin and the actin cytoskeleton by increasing the amount of other laminin receptors. The alpha7beta1 integrin is the major laminin binding integrin in skeletal muscle. We previously demonstrated that transgenic over-expression of alpha7 chain in skeletal muscle alleviates development of severe muscular dystrophy and extends the lifespan of mdx/utrn-/- mice. In contrast, enhanced expression of alpha7 integrin in mdx mice provided relatively little additional integrin at the muscle membrane and little improvement to muscle; the additional alpha7 protein was localized within fibers. This suggested that the normal level of beta1 chain in mdx fibers might limit formation and localization of the heterodimer to the membrane and thereby restrict effectiveness of the integrin in compensating for the absence of dystrophin. We demonstrate here that increasing beta1D integrin in mdx mice results in more functional integrin at the sarcolemma, more matrix laminin and decreased damage of muscle fibers. These results suggest that increasing both alpha and beta integrin subunits is necessary to achieve the maximal therapeutic effect of enhancing integrin-mediated connections in dystrophic muscle fibers and that increasing the amount of beta1D integrin may be sufficient to promote this.

Therapy

167. Exon skipping for DMD: translating a promising experimental therapy into effective treatment

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Frameshift and nonsense mutations in the dystrophin gene cause Duchenne muscular dystrophy (DMD). Skipping the mutated exon(s) with antisense oligonucleotides (AON) can restore reading-frame and production of dystrophin proteins. Our early studies demonstrated that functional levels of dystrophin can be induced by intramuscular and systemic delivery of 2'-O-methyl phosphorothioate AONs (2'O MePS) and morpholino oligomers (PMO) in the mdx mouse and the dystrophic dogs. More recently, we showed that peptide and polymer tagged PMO (PPMO and Vivo-PMO) can induce and maintain near normal levels of dystrophin for 3 months in all skeletal and cardiac muscles with significant improvement in functions in the mdx model. The therapeutic applicability of AO therapy is supported by recent Phase I clinical trials with both 2'O MePS and PMO targeting human dystrophin exon 51 in local muscles of DMD patients. However, systemic effects of AO therapy in patients remain to be demonstrated. We have now established that long-term therapeutic effect can be achieved in the animal models with the antisense therapy. Reduction in acute toxicity of PPMO and Vivo-PMO is crucial for its safe and successful application in clinics. Our results also indicate that dose, regimen and efficiency of selected AO are critical factors for achieving systemic therapeutic effect. Antisense therapy offers a realistic hope for the treatment of majority of DMD patients.

Therapy

168. Over-expression of sarcospan increases glycosylation in dystophin deficient mice

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Over-expression of sarcospan, a core-component of the dystrophin-glycoprotein complex, has been shown to ameliorate the pathological phenotype of the mdx mouse model of Duchenne Muscular Dystrophy by increasing levels of extrasynaptic utrophin. Interestingly, we have found that mild over-expression of sarcospan in mdx mice increases glycosylation of alpha-dystroglycan as evaluated by binding of the lectin, WFA. It has been previously reported that the over-expression CT GalNAc (Galgt2) transferase in mdx mice ameliorates dystrophy in a mechanism that involves increased glycosylation of alpha-dystroglycan and the CT2 antigen as well as elevated levels of utrophin. Similarly, we observe an increase in the CT2 antigen upon mild over-expression of sarcospan in mdx mice. This study demonstrates that the up-regulation of sarcospan in mdx mice is linked to an increase in glycosylation of alpha-dystroglycan that is associated in a complex with utrophin. This work was supported by grants NIH/NIAMS (R01 AR048179) to RHC, USPHS National Research Service Award (GM07104) to JLM, and Swedish Research Council (524-2009-619) to KJH.

Therapy

169. Preventing phosphorylation of dystroglycan improves muscle pathology in the mdx mouse model of dystrophin deficiency

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Phosphorylation is a key regulatory step in targeting proteins for ubiquitin mediated recycling or degradation. Beta-dystroglycan (DG), an integral component of the dystrophin glycoprotein complex (DGC), gets phosphorylated on tyrosine residues which results in its internalisation and targeting to an endosomal compartment. The internalisation of DG could be part of the mechanism underlying the loss of DG and the whole DGC in conditions such as DMD. The ability of beta-DG to interact with both dystrophin and utrophin is also regulated by phosphorylation of a key tyrosine residue. Mutation of this residue to a phenylalanine prevents its phosphorylation and presumed internalisation and increases levels at the cell membrane. Therefore, the mutated beta-DG is likely to be resistant to ubiquitination and would be maintained at the membrane where it could still support dystrophin and utrophin binding. We propose that restoration of dystroglycan at the membrane in dystrophin-deficient muscle e.g. DMD, could prevent loss of DGC components. Other compensatory proteins such as utrophin and plectin would then be able to bind to and stabilise the complex, thus ameliorating the DMD phenotype. Therefore, we have generated a gene targeted mouse where the equivalent tyrosine residue has been mutated to phenylalanine. Mice homozygous for the mutation have no obvious muscle pathology and when crossed with the mdx mouse a reduction in muscle pathology was observed. This research is supported by an MRC grant awarded to S Winder and G Miller.

Therapy

170. The Jain Foundation: A New Paradigm for Funding Disease Research

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The Jain Foundation's mission is to accelerate and develop a therapy for Limb Girdle Muscular Dystrophy 2B/Miyoshi Myopathy, a rare, autosomal recessive muscular dystrophy caused by mutations in the dysferlin gene. We accomplish this through funding, coordinating, and managing global research projects. Since 2005, we have awarded over $6 million USD in research support and currently fund 34 research projects in the following areas of study: dysferlin structure/function, ferlin proteins, membrane repair, high-throughput drug screening, gene, protein and cell therapy, and other therapeutic strategies. Prior to the establishment of the Jain Foundation, LGMD2B/MM received little attention from researchers due to the lack of funding and resources. To eliminate roadblocks, we developed dysferlin specific resources, cDNAs, antibodies, cell lines, and animal models, and make them available to all researchers. Furthermore, researchers discuss their challenges and share their expertise, protocols, and resources on our online scientific forum, research sharing network, and at our annual dysferlin conference. We also maintain a patient registry with over 600 registrants and facilitate mutational analysis of dysferlin to identify patients for future clinical studies and further our understanding of the disease. Our multi-faceted program and hands on approach to project management establishes a novel paradigm for enabling disease research.

Therapy

171. TGF beta antagonists improve respiratory function of mdx mice and increase regenerative capacity of diaphragm muscle

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TGF beta plays a key role in the pathophysiology of Duchenne Muscular Dystrophy. An animal model of DMD (the mdx mouse) lacking dystrophin was treated either with a neutralizing antibody (1D11) against TGF-beta or an antagonist to the angiotensin receptor (losartan). Significant increases in muscle fiber density were observed in mdx mice treated with 1D11, losartan, and a combination of the 2 drugs when compared to vehicle-treated controls. The 1D11-treated animals showed a slight increase in diaphragm muscle fiber density when compared with those treated with losartan. All treatment groups showed an increase in the total number of centrally nucleated fibers, suggesting an increase in fiber regeneration. In addition, respiratory function was normalized in all three treatment groups. Combination therapy with 1D11 and losartan was not more effective than treatment with either agent alone. Other indicators of improved function in treated animals included decreased serum creatine kinase and hydroxyproline

levels, indicating improved sarcolemmal integrity and decreased fibrosis, respectively; increased number of myogenin-positive nuclei, suggesting more late-stage differentiated satellite cells; and normalized forelimb grip strength (for 1D11-treated animals only). In conclusion, our studies indicate that TGF beta antagonism improves muscle regeneration and function in mdx mice.

Therapy

172. Weak muscles translate to compromised bone strength and geometry in dystrophic mice

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Diminished muscle function in DMD reduces the frequency and magnitude of contraction-induced loads applied to bone. Consequently the skeleton undergoes deleterious bone loss following reduced muscle stimuli, thereby predisposing it to fracture. The severity of these effects on mdx bone and the underlying mechanisms have been minimally assessed. Therefore, the purpose of this study was to 1) confirm the weakened state of dystrophic muscle, 2) assess tibial bone strength 3) determine the mechanism of altered bone strength (bone geometry or material properties), and 4) assess functional relationships between muscle and bone. EDL contractile function was assessed in vitro on wildtype, mdx, and dko mice (n=8, 7 and 7). Tibial bones were excised and bone strength and geometry were assessed by 3-point bending and microCT imaging. Boneâ€™s material properties were analyzed by bone density and collagen content. Muscle and bone weakness in dystrophic mice was confirmed, as EDL specific force was reduced 41-51% and bone strength by 18-50% (P=0.01). Low bone strength was primarily attributed to dystrophic mice having smaller cortical cross-sectional areas and cross-sectional moment of inertias (6-57%, P=0.018), rather than altered material properties. These data establish that dystrophic mice are a suitable model for investigating further mechanistic and therapeutic studies in DMD research aimed at improving bone health and decreasing fracture risk.

Therapy

173. Establishing pre-clinical models of therapeutic stem cell transplantation for Duchenne muscular dystrophy

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Muscle-derived cell transplantation has the potential to effectively treat many human diseases, including muscular dystrophy. Studies in mdx mice demonstrate that normal muscle-derived cells engraft into skeletal muscle, effectively restore dystrophin expression and reconstitute the satellite cell pool. However, immune rejection of donor cells prevented long-term engraftment in human trials. We have induced immune tolerance in cxmd canines using a clinically relevant regimen of bone marrow transplantation, and show donor muscle-derived cell engraftment and survival for at least 24 weeks in the absence of immunosuppression. In addition to the immune tolerant cxmd model, we have developed a canine-to-mouse xenotransplantation model to rapidly and quantitatively compare canine muscle cell engraftment. The canine-to-mouse model allows us to quantitatively compare cell populations and modulating factors, and establish priority for canine-to-canine transplantation experiments. Canine models of hematopoietic cell transplantation and solid organ transplantation have been demonstrated to accurately predict clinical outcomes in humans. Our studies will establish the best protocols for muscle cell transplantation and lead to future human clinical trials of muscle cell transplants for the treatment muscular dystrophies.

Therapy

174. Development of a small molecule drugs to improve muscle regeneration, decrease inflammation, and improve muscle function in dystrophin-deficient muscle

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Glucocorticoids are among the most highly prescribed drugs worldwide, and are considered standard of care for Duchenne muscular dystrophy but are associated with significant side effects. Emerging evidence suggests that many of the anti-inflammatory effects, such as suppression of NF-kB, are via protein-protein signaling pathways, whereas deleterious side effects are through the better studied classic receptor-mediated transcriptional action on GRE promoter elements. A series of non-hormonal steroid analogues, VBP compounds, are being developed as an alternative to traditional glucocorticoids. These compounds lack the traditional transcriptional activities of glucocorticoids (binding the glucocorticoid receptor and GRE-dependent gene transcription) but maintain the non-transcriptional signaling (NF-kB inhibition, increased myotube contractility, and acute reduction in ATP and Hsp90 phosphorylation) at levels similar to prednisone. Pre-clinical efficacy of VBP drugs were tested in both mdx and dysferlin mouse models of muscular dystrophy by oral delivery. The drugs improved functional and histological endpoints similar to or superior to prednisone, but did not show the side effects (weight loss, cardiac fibrosis) seen with chronic prednisone. VBP drugs show promise as small molecules able to improve regeneration and decrease inflammation in muscular dystrophy.

Therapy

175. Postnatal PGC-1alpha over-expression reduces acute injury in mdx mice

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In animal models, utrophin replacement has shown great promise as a therapeutic strategy for DMD, however, a practical means of delivery in human patients is lacking. It has been previously shown that PGC-1alpha increases utrophin, as well as expression of oxidative proteins, which may be of additional benefit to dystrophic muscle. Further, transgenic PGC-1alpha over-expression reduced eccentric injury in mdx

mice. As dystrophin deficiency results in developmental abnormalities potentially corrected by transgenic PGC-1alpha over-expression, it is imperative to determine the extent to which PGC-1alpha over-expression induced postnatally blunts acute eccentric injury in dystrophin-deficient muscle. Neonatal mdx mice were injected in the left hind limb with null AAV6 and in the right hind limb with an AAV6 driving expression of PGC-1alpha (1x10^11 gc). At six weeks of age, animals ran downhill (-17 degrees, 10 m/min, 60 min) and were sacrificed three days later. PGC-1alpha over-expression reduced total damaged area by 50% ($p<0.05$). More specifically, the areas of hypercontracted cells, immune cell infiltration, and missing cells were reduced by 90%, 60%, and 66%, respectively ($p<0.05$). Centralized nuclei were reduced by 25% ($p<0.05$). Given that DMD patients are sensitive to eccentric injury, the degree of protection conferred to dystrophic muscle in this investigation justifies further exploration of PGC-1alpha as a therapy for DMD.

Therapy

176. Competitive primer extension PCR to genotype animal models in muscular dystrophy

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Many commonly used muscular dystrophy models carry point mutations. A robust, easy-to-use and low-cost genotyping protocol would significantly facilitate their use for deciphering pathogenesis and developing novel therapies. Compared to models caused by large deletion/insertion, idenityfing normal and diseased alleles has been quite challenging for single point mutations. Current methods often require multiple PCR reactions, post-PCR manipulations and/or special equipment/reagents. Here we report a simple and highly reproducible point mutation detection protocol using single PCR. It is based on the commonly used amplification resistant mutation system (ARMS). Specifically, PCR reaction was carried out with a common forward primer, a mutant allele-specific reverse primer and a wild-type allele-specific reverse primer. A stretch of non-specific nucleotides engineered at the 5' end of the wild-type allele differentiates the wild type allele PCR product. Application of this protocol allows reliable and rapid genotyping of mdx and mdx3cv models of Duchenne muscular dystrophy. (Research funding source: NIAMS, Muscular Dystrophy Association)

Therapy

177. Adenovirus capsid pIX: a promising platform for addition of large targeting ligands

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Adenovirus-based vectors (Ads) have many attractive features, which has made them a popular delivery system for gene therapy. Ads can infect a wide range of cells, both dividing and non-dividing, however, for effective therapeutic use, it is desirable for Ads to target specific cell types or tissues. We are exploring the use of pIX, a minor capsid protein, as a platform for the presentation of targeting ligands. pIX has several functions in the Ad lifecycle and has been implicated in transcriptional activation of early genes, reorganizing nuclear proteins, as well as strengthening the capsid by stabilizing the hexon-hexon interactions. In addition, capsids deficient of pIX can only package sub-genomic DNA. Recently, pIX has been used as a platform for the addition of large polypeptides to the Ad virion, such as green fluorescent protein (GFP) for virus tracking studies and targeting ligands. However, it is important to determine if addition of these large molecules affect pIX's normal function in maintaining the integrity of the capsid. In this study, we tested the effect of large polypeptide addition to pIX on capsid stability and genome packaging capacity of the resulting adenovirus. We demonstrated that Ad capsids containing pIX-GFP were able to package at least 37.1 kb of DNA (103% of wildtype Ad) without affecting capsid stability and that these viruses displayed similar growth kinetics and yield as wildtype Ads. Taken together, these data indicate that addition of large polypeptides to pIX does not affect its normal function in the Ad lifecycle. Therefore, pIX is an excellent platform for addition of large targeting ligands to redirect virus binding.

Therapy

178. Physiological drug screening with skeletal muscle tissue engineered from human DMD muscle cells

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A tissue-based approach to in-vitro drug screening allows the determination of the cumulative positive and negative effects of a compound at the physiological rather than the cellular or subcellular level. Immortalized mdx and patient DMD myoblasts were tissue engineered into three-dimensional muscle constructs with characteristics of functional tissue i.e. parallel arrays of striated and contractile myofibers. These miniature BioArtificial Muscles (mBAMs) were grown attached to two flexible microposts acting as artificial tendons in 96 microwell plates, and when electrically stimulated, generated maximal tetanic forces measured with an automated micropost motion tracking system. Tissue engineering and drug screening were performed with customized robotic hardware. Assays were developed to determine a compound's effect on mBAM strength, fatigability, and contraction-induced injury. Thirty-five and twenty-eight compounds were screened individually with mdx mBAMs and DMD mBAMs, respectively. Many, but not all, compounds that were effective in increasing muscle strength in the mdx mBAMs were also effective in the DMD mBAMs. Highly effective compounds included IGF-1, creatine, deflazacort, and prednisone. Other compounds such as melatonin and combinations were effective in reducing the rate of muscle fiber fatigue and injury, respectively. This new high content drug screening technology will be a useful tool for the identification of combinations of compounds for treating the multiple facets of muscle dysfunction in DMD. Supported by the NIH (NIA, NINDS, NIAMS), the NSF, AFM, Charley's Fund, Parent Project Muscular Dystrophy, and the Hood, Sharp, Quest and Jett Foundations.

Therapy

179. Identification and characterization of small molecules

for treatment of muscular dystrophy

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Identifying treatments for progressive, severely disabling neuromuscular disorders that lead to premature death is a major effort at PTC Therapeutics. We are currently focused on discovering treatments for Duchenne muscular dystrophy (DMD), spinal muscular atrophy (SMA) and myotonic dystrophy (DM). In the case of DMD, PTC Therapeutics, Parent Project Muscular Dystrophy (PPMD), and the University of Pennsylvania are collaborating to discover new treatments. DMD is the most common and most severe form of muscular dystrophy in children and is characterized by progressive muscle wasting. We are working on three key therapeutic targets (utrophin, myostatin, and IGF1) that may compensate for the loss of dystrophin protein. Myostatin is a negative regulator of muscle growth while IGF1 is a positive regulator, and appropriate alteration of their expression has been shown to improve mdx mouse muscle. Utrophin is a dystrophin ortholog that when up-regulated can functionally compensate for the lack of dystrophin. We have used our proprietary drug discovery platform technology called GEMSTM (Gene Expression Modulation by Small-molecules) to discover small molecules that post-transcriptionally up-regulate (in the case of utrophin and IGF1) or down-regulate (in the case if myostatin) protein production. Several molecular scaffolds that demonstrate dose-dependent and specific regulation have been identified for each target. Through structure activity relationship (SAR) studies compounds for each target with improved activity have been discovered. These compounds also have very good pharmaceutical properties (low cytotoxicity, low metabolism, and high exposure in animals). We have been able to demonstrate alteration of target protein level in animals for all three targets. Presently, we are focused on further optimizing the activity, potency and pharmacological properties of the molecules. The ultimate goal of this drug discovery and development effort is to identify small molecules that can specifically modulate the production of a number of proteins that can be used as monotherapy or as part of a combination therapy to treat muscular dystrophy.

Therapy

180. Exon skipping morpholino oligos restore cyclical power to limb and respiratory muscles of mdx mice

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In order to effectively perform cyclical activities such as locomotion and ventilation, skeletal muscles must maximize work during shortening (positive work) while minimizing work absorbed during lengthening (negative work). We tested if morpholino oligo exon skipping restored cyclical power (defined as (positive work - negative work) / unit time) to limb and respiratory muscles of the dystrophin-deficient mdx mouse. Treatment with a morpholino oligo (Mor23), designed to skip normal processing of the mutated exon 23, restored dystrophin expression to the periphery of muscle fibers of mdx mice. Power of the extensor digitorum longus (EDL) and a strip of the diaphragm (DIA) were assessed in vitro (35°) using the work loop technique. Work loop frequency, amplitude, and stimulation were chosen to optimize power output of the C57BL/6 EDL (111 ± 6 W/liter muscle) and DIA (71 ± 6 W/liter). When studied under these conditions, cyclical power was 32% and 40% lower for the mdx EDL and DIA, respectively. Mor23 treatment restored power of the EDL and DIA to C57BL/6 levels (109 ± 4 and 67 ± 2 W/liter, respectively). Five eccentric contractions caused power to fall over 4-fold more for the mdx EDL compared to the C57BL/6 EDL. The response of the mdx EDL to eccentric activity was normalized by Mor23 treatment. In general, the effectiveness of Mor23 treatment in restoring cyclical power was attributed to an increase in positive work rather than a decrease in negative work.

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