Report and Abstracts of the 18th Meeting of the Interuniversity Institute of Myology: Virtual meeting, October 21-24, 2021

Francesca Grassi, Sestina Falcone

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Report and Abstracts of the 18th Meeting of the Interuniversity Institute of Myology: Virtual meeting, October 21-24, 2021

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Abstract

In 2021, as the situation due to COVID-19 pandemic was still uncertain, the 18 th annual meeting of the Interuniversity Institute of Myology (IIM), took place on a virtual platform, following the same organization already tested for the previous edition. Participants from Italy, European countries, Canada and USA included clinicians, scientists, pharmaceutical companies and representatives of patient organizations. Four keynote speakers presented new insights into the modulation of muscle stem cell self-renewal in the treatment of neuromuscular disease, the role of nuclear positioning in muscle function, regeneration and tumorigenesis in the heart and advances on therapies of muscular dystrophies. Young PhD students and trainees presented oral communications distributed in five scientific sessions and posters in two poster sessions. On October 21, 2021, selected young scientists participated in the "High Training Course on Advanced Myology", organized with the University of Perugia, Italy. This course consisted of lectures on muscle regeneration and therapeutic perspectives by internationally recognized speakers, followed by roundtable discussions on "Omics technologies in myology" and "New therapeutic approaches", plus the meeting itself. Young trainees, winners of past IIM conferences, forming the Young IIM Committee, selected one of Keynote speakers and were involved in the organization of scientific sessions and roundtable discussions. The friendly welcoming of the meeting, which has strongly characterized this event and is of great help in facilitating scientific exchanges and stimulating novel collaborations, was the hallmark of the conference this year again, even on virtual platform. Breakthrough studies showing interdisciplinary works are fostering new avenues in the field of myology. This year again, scientists and students attended the meeting at the huger number, challenging the difficulties due to the COVID-19 pandemic. All participants shared the wish to continue and implement IIM meeting with new insights on muscle biology, perspectives in the understanding of the muscle-related diseases and in novel therapeutic approaches. We report here abstracts of the meeting describing basic, translational, and clinical research contributing to the large field of myology.

Key Words: Cachexia; clinical trials; dystrophy; epigenetics; ex-vivo; hear; homeostasis; in-vivo; metabolism; muscle development; neuromuscular; proof of concept; regeneration; sarcopenia; stem cells; translational; wasting.

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The Interuniversity Institute of Myology (IIM), founded in 2004 by several Italian Universities, held its 18th annual meeting from October 22 to 24, 2021.

Due to the uncertainties about travel possibilities imposed by COVID-19 pandemic, the meeting has been held online once again, but this has not discouraged participation. The coming of age of the association has been celebrated with the largest attendance ever recorded: 171 enrolled participants from several European countries, USA and Canada, *vs.* 153 in 2020. Four plenary lectures, 12 talks selected among submitted contributions and 47 posters have been the basis for lively scientific discussion, mainly aimed at promoting active participation of young attendees, as typical of all IIM meetings. The Keynote speakers were: Michael Rudnicki (Ottawa Hospital Research Institute, Canada); Edgar Gomes (Universidade de Lisboa, Portugal); Johnny Kim (Max Planck Institute for Heart and Lung

Research, Germany) and Elizabeth McNally (Northwestern Univ. Feinberg School of Medicine, Chicago, USA). The meeting was also part of the 3rd edition of the high training Course on Advanced Myology, organized through a collaboration between IIM and the University of Perugia, Italy. The course has been attended by 16 participants under the age of 35 years and consisted in lectures by and roundtable discussions with the keynote speakers, plus the meeting itself. This year, the roundtables were on *Omics technologies in myology* (discussants: Michael Rudnicki and Edgar Gomes) and *New therapeutic approaches* (discussants: Elizabeth McNally and Johnny Kim). To foster engagement of young participants, one of the keynote lecturers plus the topic of the roundtables were selected by the Young IIM Committee, composed by trainee winners of awards in the past IIM Meeting editions. The voice of patients affected by Duchenne and Becker Muscular Dystrophies was heard thanks to the contribution of Gloria Antonini, staff member of Parent Project Italy. Industry was represented by Prodotti Gianni. Both Parent Project and Prodotti Gianni sponsored the meeting. The topics covered by the various presentations were: Muscle biophysics and E-C coupling; Muscle genetics and epigenetics; Muscle stem cells and regenerative medicine; Muscle and exercise; Signaling and metabolism in muscle cells; Clinical studies and therapeutic approaches to muscle diseases. The first Keynote lecturer, Michael Rudnicki, is Senior Scientist and Director of the Regenerative Medicine Program and the Sprott Centre for Stem Cell Research at the Ottawa Hospital Research Institute. His talk was entitled: *Modulating muscle stem cell self-renewal to enhance regenerative myogenesis to treat neuromuscular disease*. He presented the most recent data obtained in his lab about the pathways regulating the response of satellite cells (muscle-specific stem cells) to muscle damage.[1,2] These results are potentially important to target the intrinsic defects of satellite cells in Duchenne's muscular dystrophy, which strongly reduce the generation of the myogenic progenitors needed for proper muscle regeneration. The second Keynote lecture was given by Edgar Gomes, Professor at the Faculty of Medicine of the Universidade de Lisboa and group leader at the Instituto de Medicina Molecular (IMM), Lisbon, Portugal. The title of his talk was: *The role of nuclear positioning in muscle function*. He presented the latest study of his team about the distribution of mRNAs in non-specialized regions of the myofibers.[3] These results include the surprising finding that huge mRNAs, encoding large proteins such as titin, spread throughout the cell; conversely, smaller mRNAs exhibit perinuclear accumulation. Both distribution patterns depend on microtubules. In summary, his data support the hypothesis that size-selective mRNA distribution in non-specialized regions of skeletal muscle ensures cellular compartmentalization and simultaneous long-range distribution of giant mRNAs. The third Keynote lecture was presented by the speaker invited by the Young IIM Committee, Johnny Kim, Senior Staff Scientist and research Group Leader at the Max Planck Institute for Heart and Lung Research in Bad Nauheim, Germany. In the talk entitled "*Regeneration and tumorigenesis at the heart of reversible programming*" he discussed the trade-off between availability of stem cells for regeneration and tumorigenesis. Heart is basically devoid of stem cells, thus unable to regenerate upon ischemic damage, the leading cause of death worldwide. At the same time, it is the only organ in our body not affected by cancer. Enabling heart regeneration would allow restoration of cardiomyocytes that are otherwise permanently lost after heart damage. Data from Kim's lab show that transient expression of well defined transcription factors able to induce de-differentiation of cardiomyocytes leads to heart repair, whereas sustained expression invariably gives rise to heart tumours.[4] This work provides exciting perspectives on the possibility to define strategies aimed at repairing infarcted hearts. The fourth Keynote lecture was given by Elizabeth McNally, Northwestern University Feinberg School of Medicine, Chicago, IL, USA and director of Northwestern's Center for Genetic Medicine. In her talk, entitled "*Therapeutically modifying muscular dystrophy*" she addressed the benefits and side effects of glucocorticoids, the gold standard for Duchenne muscular dystrophy. Works performed in her lab during the last few years have shown that weekly administration of glucocorticoids has definitely fewer negative effects than daily dosing, while reducing metabolic syndrome and improving the lean/fat mass ratio.[5,6] Thus, clinical trials are ongoing to ameliorate scheduling of glucocorticoids in DMD patients. Gloria Antonini, from the Parent Project association, has highlighted how COVID-19 has temporarily stopped their financial support for research on DMD. She then presented the activities of the association, which was founded in Italy in 1996 (after the USA and the Netherlands) and currently is in touch with over 800 families of Duchenne's and Becker's MD patients. Finally, she advertised their meeting, which will take place in February 2022, hopefully in presence. The other sponsor, Prodotti Gianni, introduced the Gene Recommender, an application based on Artificial Intelligence to perform literature mining aimed at speeding the identification of genes of interest in research. The 12 talks selected among submitted abstracts covered all the topics of the meeting, spanning from basic muscle function and repair to exercise effects to mechanisms of and possible therapeutic approaches to muscle diseases and cancer cachexia. Speakers came from Italy, UK and the USA. The overall quality of the talks was very high and elicited intense discussions among participants of all ages. The possibility to ask questions through the chat increased the number of questions that could be asked (and answered!) while respecting the tight time limits. Additionally, 18 poster presenters were selected to "advertise" their work

through poster blitzes, lasting 60 s each. Overall, 47 posters were discussed in virtual rooms that allowed good interactions between presenter and participants. At the end of the meeting, prizes were awarded to young participants (less than 35 years old) according to the evaluation by panels of experienced scientists attending the meeting. For the best talk the prize was awarded to Chiara Nicoletti, while Enrico Pozzo and Elena Ruggieri won *ex-aequo* the prize for best poster and Beatrice Biferali the one for the best poster blitz. This year Azuleon, the meeting management company, awarded an additional prize for the best poster blitz from an "industrial" perspective, which went to Carl Kutzner. Perhaps thanks to the great confidence with virtual meeting acquired during this pandemic period the 18th IIM Meeting has once again succeeded in putting together many myologists, having interests ranging from basic science to pre-clinical and even clinical studies. In keeping with the tradition of the Association, participation of young researchers has been promoted by means of discussion, dissemination of the most recent results, exchange of ideas, formulation of new hypotheses, and opening of new international collaborations. Taken together, the attendees of the IIM and of the Padua Muscle Days (PMDs, a meeting more oriented to advanced Translational Myology) and the authors of papers e-printed in the European Journal of Translational Myology (EJTM) are a substantial part of the international community of Myologists. Here, the abstracts of the 18th IIM Meeting show the relevant contribution of this community to the study of muscle function and therapeutic approaches to neuromuscular diseases.

List of acronyms

COVID-19 - Disease caused by SARS_CoV-2
DMD - Duchenne muscular dystrophy
E-C coupling - excitation-contraction coupling
IMM - Interuniversity Institute of Myology
MD - Muscular dystrophy
mRNAs – messanger RNA
PMDs – Padua Muscle Days

Author's contributions

Authors equally contributed to write the manuscript. They also approved the final version.

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Conflict of Interest

The authors declare they have no financial, personal, or other conflicts of interest.

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Topics

Biophysics and E-C coupling Genetics and epigenetics
Muscle stem cells and regenerative medicine Muscle wasting and cachexia
Exercise
Signaling and metabolism
Clinical studies and therapeutic approaches

Keynote Lectures

Edgar Gomes

Johnny Kim

ElizabethMcNally

MichaelRudnicki

Eur J Transl Myol 2021; 31 (4), 10270. doi: 10.4081/ejtm.2021.10270

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Invited Speakers

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Michael RUDNICKI
Sprott Centre for Stem Cell Research, Ottawa, Canada

Michael Rudnicki is a Senior Scientist and the Director of the Regenerative Medicine Program and the Sprott Centre for Stem Cell Research at the Ottawa Hospital Research Institute. He is Professor in the Department of Medicine at the University of Ottawa. Dr. Rudnicki is CEO and Scientific Director of the Canadian Stem Cell Network (SCN). Dr. Rudnicki's achievements have been recognized by numerous honours including being named a Tier 1 Canada Research Chair, an International Research Scholar of the Howard Hughes Medical Institute for two consecutive terms, a Fellow of the Royal Society of Canada, an Officer of the Order of Canada, and a Fellow of the Royal Society (London). He has been a founder in several spin-off biotechnology companies including Satellos Bioscience. Dr. Rudnicki is an internationally recognized thought leader in molecular genetics and regenerative medicine, whose research has transformed our understanding of muscle development and regeneration and has fueled the development of novel stem cell-based approaches to treat muscular dystrophy. His work is consistently published in top journals including Cell, Nature, Nature Cell Biology, Nature Medicine, and Cell Stem Cell. He holds major research grants from CIHR, NIH, SCN, and several health charities. For the past 16 years, Dr. Rudnicki has led the Stem Cell Network (SCN), a transformative initiative involving over 175 investigators across Canada. As Scientific Director of the SCN, he has forged a national community that transformed stem cell research in Canada and brought research to the point where regenerative medicine is impacting clinical practice.

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KEYNOTE LECTURE 1

Modulating muscle stem cell self-renewal to enhance regenerative myogenesis to treat neuromuscular disease

Michael A. Rudnicki

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Duchenne muscular dystrophy (DMD) leads to severe muscle wasting and death in the second or third decade of life. The disease gene dystrophin functions as an essential component of the dystrophinglycoprotein complex (DGC). In the absence of dystrophin DGC assembly is impaired which weakens the muscle fibers rendering them highly susceptible to injury. We discovered that dystrophin is highly expressed in activated muscle stem cells (also known as satellite cells), in which it associates with the serine-threonine kinase Par1b, an important regulator of cell polarity. Consequently, the number of asymmetric divisions is strikingly reduced in dystrophin-deficient satellite cells. These intrinsic defects strongly reduce the generation of myogenic progenitors that are needed for proper muscle regeneration. Through a small-molecule screen, we made the discovery of a novel signal transduction pathway involving epidermal growth factor receptor (EGFR) and Aurora kinase A (Aurka) as keyregulators of asymmetric satellite cell divisions. Inhibiting EGFR causes a substantial shift from asymmetric to symmetric division modes, whereas EGF treatment increases asymmetric divisions. EGFR activation acts through Aurka to orient mitotic centrosomes, and inhibiting Aurka blocks EGF stimulationinduced asymmetric division. In vivo EGF treatment markedly activates asymmetric divisions of dystrophin-deficient satellite cells in mdx mice, increasing progenitor numbers, enhancing regeneration, and restoring muscle strength. Therefore, activating an EGFR-dependent polarity pathway promotes functional rescue of dystrophin-deficient satellite cells and enhances muscle force generation. In collaboration with Satellos Bioscience, we have been developing small molecule drugs that stimulate asymmetric division by modulating the EGFR/Aurka pathway. Recent progress will be presented.
IIM Meeting 2021 • 22-24 October 2021

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Eur J Transl Myol 2021; 31 (4), 10270. doi: 10.4081/ejtm.2021.10270

Edgar GOMES
Faculty of Medicine of the University of Lisbon and Instituto de Medicina Molecular (iMM), Lisbon, Portugal.

Edgar GOMES is Professor at Facult is Professor at Faculty of Medicine of the University of Lisbon and Instituto de Medicina Molecular (iMM), Lisbon, Portugal. He is a biochemist by training and performed his PhD on cell biology in Coimbra, Portugal. Thereafter, he did his post-doc at Columbia University, NY, USA. In 2007, at the Institute of Myology, Paris, France, Edgar set up his own research group and became a Director of Research. Since 2014, he is heading a lab at iMM, where they are interested in how the cell architecture of skeletal muscle cells works, including in central core myopathies. Mechanisms of nuclear movement during cell migration and skeletal muscle formation are being studied and what is the role for nuclear positioning in skeletal muscle formation is a main topic of research. Recently he became a Professor of Histology and Developmental Biology at the Faculty of Medicine, where he works closely with Hospital Santa Maria, the main hospital in Lisbon, at the edge of diagnostics and therapeutics.

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KEYNOTE LECTURE 2
The role of nuclear positioning in muscle function

Edgar R. Gomes

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Skeletal muscle myofibers are large and elongated cells with multiple and evenly distributed nuclei. Nuclear distribution suggests that each nucleus influences a specific compartment within the myofiber and implies a functional role for nuclear positioning. Compartmentalization of specific mRNAs and proteins has been reported at the neuromuscular and myotendinous junctions, but mRNA distribution in non-specialized regions of the myofibers remains largely unexplored. We report that the bulk of mRNAs are enriched around the nucleus of origin and that this perinuclear accumulation depends on recently transcribed mRNAs. Surprisingly, mRNAs encoding large proteins - giant mRNAs - are spread throughout the cell and do not exhibit perinuclear accumulation. Furthermore, by expressing exogenous transcripts with different sizes we found that size contributes to mRNA spreading independently of mRNA sequence. Both these mRNA distribution patterns depend on microtubules and are independent of nuclear dispersion, mRNA expression level and stability, and the characteristics of the encoded protein. Thus, we propose that mRNA distribution in non-specialized regions of skeletal muscle is size selective to ensure cellular compartmentalization and simultaneous long-range distribution of giant mRNAs.
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Eur J Transl Myol 2021; 31 (4), 10270. doi: 10.4081/ejtm.2021.10270

Johnny KIM
Max Planck Institute for Heart and Lung Research in Bad Nauheim, Germany

Johnny Kim is a Senior Staff Scientist and research Group Leader at the Max Planck Institute for Heart and Lung Research in Bad Nauheim, Germany. A major goal of the Kim lab is to elucidate processes that govern the self-renewal and coordinated differentiation of stem cells during development and regeneration. To this end, the Kim lab pioneered the integration of genetics and functional genomics of the primary regenerative muscle compartment and his team has exploited these means to inform possibilities to enable heart regeneration. The Kim lab aims to reach a systems-level understanding of tissue regeneration and of how defects in these processes can lead to the manifestation of human disease including cancer, or to the progressive loss of regenerative potential during disease and physiological aging. On these grounds, the research theme of the Kim lab is that cell fate decisions made by cells in their past are not irrevocable but can be reverted and reprogrammed. This dynamic concept of a cell's fate corresponds with the established view that maintenance of a cellular phenotype does not reflect a static state but rather requires continuous regulation. This phenomenon enables to redirect and manipulate cells and enhance regenerative properties of tissues and organs provided that the molecular mechanisms in regulating cellular phenotype are known.

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KEYNOTE LECTURE 3

Regeneration and tumorigenesis at the heart of reversible programming

Johnny Kim

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Tissues that can regenerate like the skeletal muscle often rely on effective tissue-resident stem cell systems. Essentially, the mammalian adult heart cannot regenerate because cardiomyocytes do not divide and nor do regenerative heart (stem) cells exist. Notably, while the necessity of cardiomyocytes to retain cellular identity and the post-mitotic state for maintaining heart contractility makes cardiomyocyte replacement difficult it also prevents development of tumors. Cardiovascular diseases (CVDs) include coronary artery diseases and myocardial infarctions, commonly known as heart attack, which ultimately leads to the death of cardiomyocytes that are responsible for heart contraction. CVDs remain amongst the leading causes of death worldwide. Therefore, profound interest lies in identifying means that would enable heart regeneration through restoration of cardiomyocytes that are permanently lost after heart damage. We discovered that partial, cardiomyocyte-specific reprogramming instates regenerative potential of the mammalian heart by dedifferentiating adult cardiomyocytes to a proliferation-competent state that is similar to embryonic cardiomyocytes. Timely termination of pluripotency factor expression however enables re-differentiation of cardiomyocytes back to the mature and division-incompetent state. Remarkably, if cardiomyocyte-specific OSKM expression is sustained, this gives rise to heart tumors with 100% penetrance, providing the first and only model of heart tumor formation. Importantly, by controlling the degree of progressive cardiomyocyte de-differentiation we can instate proliferative and regenerative capacity and restore physiological heart function upon cardiac damage – without tumor formation – before and during a myocardial infarction. Our study provides perspectives to control cellular plasticity in a spatio-temporally defined fashion to synthesize tissue regenerative abilities by combinations of genetic and chemical interventions.
IIM Meeting 2021 • 22-24 October 2021

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Eur J Transl Myol 2021; 31 (4), 10270. doi: 10.4081/ejtm.2021.10270

Elizabeth McNALLY,
MD, PhD, FAHA, Chair, Council on Basic Cardiovascular Sciences of the American Heart Association (AHA).

Dr. Elizabeth McNally investigates genetics determinants and mechanisms of myopathies as they affect the heart and muscle. Her work has yielded new insights into the development and presentation of cardiomyopathy and muscular dystrophy. Dr. McNally earned her medical degree and doctorate in microbiology and immunology from Albert Einstein College of Medicine. She did her internship, residency and cardiovascular fellowship at Brigham and Women's Hospital, and genetics fellowship at Boston Children's Hospital. Before joining Northwestern University, Dr. McNally, a Chicago native, was on the faculty at the University of Chicago. McNally directs Northwestern's Center for Genetic Medicine, working towards the goal of transforming how genetic information is used clinically. The Center is capitalizing on advances in sequencing and leveraging the electronic clinical data to improve patient outcomes. Among her nearly 300 publications are studies on developing genetic profiling to identify mutations underlying cardiomyopathy, including a collaborative project associating a single mutation with a range of outcomes, and research demonstrating the utility of whole-genome sequencing to find individual mutations in cardiomyopathy patients, and of a supercomputer to facilitate wholegenome analysis. She also recently compared protein coding variation in the genomes of patients with hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM). The results obtained in this study support the concept that increased variation in cardiomyopathy genes creates a genetic background that predisposes to DCM and increased disease severity. Most recently, as part of a multidisciplinary collaborative group, she has been monitoring exposure to SARS-CoV2 and response to vaccination using a highly sensitive and specific serological assay, demonstrating high household spread of COVID-19, high exposure rate across the diverse areas in Chicago, and a stronger vaccine response in women compared to men.

KEYNOTE LECTURE 4
Therapeutically modifying muscular dystrophy

Elizabeth McNally

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The muscular dystrophy are genetically heterogeneous disorders that result in progressive loss of myofibers and muscle strength. Muscle degermation in muscular dystrophy is driven by many primary different mechanisms, including loss of membrane integrity and intracellular or even intranuclear defects. Many gene and allele specific therapies are advancing, with available drugs for a small number of patients with specific primary mutations. Despite this promise, there are many muscular dystrophies for which there are no therapies and most of the current approaches being tested are not expected to fully correct the disease process. We use a genetic approach to identify naturally occurring pathways that suppress aspects of the muscular dystrophy phenotype. In a mouse model of muscular dystrophy, an intercross strategy has been employed to identify genetic modifiers of muscular dystrophy. The first modifier identified is a TGFbeta binding protein, LTBP4, which modifies both mouse and human muscular dystrophy. Using data from mice, we developed an antibody that stabilizes the large latent complex which includes the LTBP4 protein, along with multiple forms of TGFbeta. This antibody can be delivered to mouse models of muscular dystrophy, where we found it stabilizes the sarcolemma, reduces TGFbeta signaling, improves muscle strength and performance, and reduces muscle fibrosis. We are now developing antibodies for the purposes of treating muscular dystrophy.
IIM Meeting 2021 • 22-24 October 2021

Eur J Transl Myol 2021; 31 (4), 10270. doi: 10.4081/ejtm.2021.10270

Abstracts of Selected Talks

IIM Meeting 2021 • 22-24 October 2021

SESSION 1

Chromatin architecture dynamics of satellite cells during physiological ageing

Emanuele Di Patrizio Soldateschi 1,2, Philina Santarelli 1, Gloria Pegoli 1, Valentina Rosti 1,2, Margherita Mutarelli 3, Roberto Quadri 1, Rosa Rescigno 4, Francesca Gorini 1, Federica Lucini 5, Cristiano Petrini 5, Giovanni Lembo 5, Francesco Ferrari 5,6, Chiara Lanzuolo 1,2

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The skeletal muscle regenerative capacity is primarily mediated by the muscle stem cells (satellite cells) that, upon damage, activate and differentiate to rebuild the damaged parts of .the muscle. Nonetheless, during ageing, the satellite cells ability to reconstruct the muscles declines due to a loss of myogenic potential caused by a drop of both cell identity and senescence. This regenerative impairment is caused by primary ageing hallmarks that impair homeostatic conditions and physiological functions. Several epigenetic alterations, as posts translational modifications, have been extensively studied to find molecular mechanisms at the basis of muscular ageing. However, how chromatin changes determine age-dependent muscular dysfunction is poorly understood. We recently developed the Sequential Analysis of MacroMolecules accessibilitY (SAMMY-seq), a new technology able to isolate multiple chromatin fractions, enriched for differences in accessibility. We successfully applied the SAMMY-seq on 10-50 thousand satellite cells at various stages of mouse ageing, separating euchromatin and heterochromatin. We will discuss chromatin characteristics, dynamics and reshaping from postnatal to adult, old, and geriatric. We created a picture of chromatin status associated with the muscle functionality.
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Apelin resistance contributes to muscle loss during cancer cachexia in mice

Andrea David Re Cecconi 1, Mara Barone 1, Mara Forti 1, Martina Lunardi 1, Alfredo Cagnotto 2, Mario Salmona 2, Davide Olivari 1, Lorena Zentilin 3, Andrea Resovi 4, Dorina Belotti 4, Nobuyuki Takakura 5, Hiroyasu Kidoya 5, Rosanna Piccirillo 1

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Cancer cachexia consists of dramatic body weight loss with rapid muscle depletion due to imbalanced protein homeostasis. We found that the mRNA levels of apelin decrease in muscles from cachectic hepatoma-bearing rats and three distinct mouse models of cachexia. Furthermore, apelin expression inversely correlates with MuRF1 in muscle biopsies from cancer patients. To shed light on the possible role of apelin in cachexia in vivo, we generated apelin 13 carrying all the amino acids in D isomers, ultimately extending plasma stability. Notably, apelin D-peptides alter cAMP-based signalling in vitro as the L-peptides, supporting receptor binding. In vitro apelin 13 protects myotube diameter from dexamethasone-induced atrophy, restrains rates of degradation of long-lived proteins and MuRF1 expression, but fails to protect mice from atrophy. D-apelin 13 given intraperitoneally for 13 days in C26-bearing mice does not induce anti-catabolic pathways in muscles, as occurred instead in vitro. Puzzlingly, circulating apelin increases in murine and human plasma during cachexia and adult muscle electroporation of a plasmid expressing APJ, but not apelin, preserves myofiber area from colon adenocarcinoma C26-induced atrophy, supporting apelin resistance in vivo. Altogether, we believe that during cachexia apelin resistance occurs contributing to muscle
wasting and nullifying any possible peptide-based treatment.
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SESSION 2

Identification of a druggable epigenetic target required for DUX4 expression and DUX4-mediated toxicity in FSHD muscular dystrophy

Emanuele Mocciaro, Roberto Giambruno, Stefano Micheloni, Cristina Consonni, Maria Pannese, Valeria Runfola, Giulia Ferri, Davide Gabellini

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Facioscapulohumeral muscular dystrophy (FSHD) is one of the most prevalent neuromuscular disorders. The disease is associated with loss of epigenetic repression of the double homeobox 4 (DUX4) transcription factor. DUX4 expression is physiologically restricted to early stages of embryogenesis while being silenced in most somatic tissues of the adult. In FSHD, DUX4 mis-expression triggers the activation of a pro-apoptotic transcriptional program leading to block of muscle differentiation and apoptosis. As of today, no cure or therapeutic option is available to FSHD patients. Our laboratory previously showed that the long non-coding RNA DBE-T is required for aberrant DUX4 expression in FSHD. We developed a reporter assays to dissect DBE-T functional domains. This allowed us to identify a single DBE-T fragment, which is mainly responsible for driving gene expression. Using affinity purification followed by proteomics, we identified the cellular factors specifically associated to this fragment. Among them, we focused on WD repeat-containing protein 5 (WDR5), a chromatin remodeling protein and a core component of the MLL/SET1 histone methyltransferase complexes. Intriguingly, WDR5 binding to specific lncRNAs is essential to maintain active chromatin. We found that WDR5 is required for DUX4 activation in FSHD muscle cells. Moreover, WDR5 knockdown rescues cell viability and myogenic differentiation of FSHD muscle cells without affecting healthy muscle cells. Remarkably, we obtained analogous results by WDR5 pharmacological inhibition. Our results further elucidate the players controlling DUX4 expression and identify a novel druggable target opening a new therapeutic perspective for FSHD.
IIM Meeting 2021 • 22-24 October 2021

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Reshaping cell identity: insights into a master Transcription Factor and insulator interplay during myogenic conversion

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Maintenance of cell identity is achieved through the preservation of the cell-type specific tridimensional chromatin organization and gene expression. Architectural proteins, such as CTCF and cohesin, ensure that once established, 3D genome organization is maintained, thus preventing aberrant activation of alternative transcriptional programs. Cell lineage determinant transcription factors, also known as master Transcription Factors (mTF), possess the distinctive ability to bind compact regions of chromatin, otherwise inaccessible, to activate expression of lineage-specific genes. While already shown, by our group and others, that mTFs are capable of remodel chromatin architecture to promote their cell-specific transcriptional programs, still little is known about the interplay between CTCF and mTFs and to what extent mTFs functional and structural domains contribute to this process. Here, we employ MYOD as paradigmatic mTF, given its unique ability to efficiently convert fibroblasts into skeletal muscle without the co-expression of other TFs, to investigate how mTFs affect CTCF binding throughout the genome, reconfiguring 3D chromatin organization to activate lineage-specific gene expression. We performed high-resolution CTCF and H3K27ac HiChIP, coupled with CTCF and H3K4me3 CUT&RUN, ATAC-seq, and RNA-seq, during the trans-differentiation process of human fibroblasts expressing either MYOD or MYOD deletion mutants (defective in their ability to engage with RNA PolII or other TFs and chromatin remodeling complexes), or MYF5 (a muscle-specific TF highly homologue to MYOD, but incapable of engaging RNA PolII). Deletion of key functional domains (i.e., transactivation domain and MYOD domains mediating the interaction with other TFs and/or chromatin remodeler complexes) impairs MYOD ability to reorganize chromatin interactions and to repress cell-of-origin transcriptional program, compromising its trans-differentiation potential.
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SESSION 3

The PolgD257A "mutator" mice as model to investigate aging-associated defective skeletal muscle regeneration

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PolgD257A constitutive knock-in mice feature a premature-ageing phenotype (8 to 12 months), involving anemia, kyphosis, alopecia, lipodystrophy as well as sarcopenia. The PolgD257A mutation selectively blunts DNA-proofreading activity, but not DNA polymerase activity, of Polg, a nuclear genome gene encoding a DNA polymerase involved selectively in mitochondrial DNA-repair. Although PolgD257A feature high mutation rate of mitochondrial DNA and mitochondrial dysfunction, the aging phenotype is driven by DNA replicative stress and nuclear genome DNA-double strand breaks (DSBs) caused by depletion of nucleotide pool in proliferating progenitor and stem cells. Sarcopenia is a major feature of aging and major negative determinant for quality of life in the elderly. Sarcopenia is defined as loss of skeletal muscle and is associated to neuromuscular junction (NMJ) degeneration and loss of muscle strength. In addition, defective muscle regeneration has been suggested to contribute to the associated sarcopenia in mice and humans, likely through non cell autonomous mechanisms. Thus, we set to investigate whether Polg D257A mice features defective cardiotoxin-induced muscle regeneration and skeletal muscle function. Consistently with previous data, PolgD257A feature extensive sarcopenia in several skeletal muscle. Finally, cardiotoxin-induced skeletal muscle regeneration of tibialis anterior was evaluated. Upon 7 days injury, the muscle of PolgD257A mice feature a highly disrupted phenotype, suggesting persistent inflammation. However, at 18 days upon injury, the cross-sectional area of centrally nucleated fibers was clearly reduced, thus suggesting defective regeneration. Finally, our data indicate PolgD257A mice as a suitable and accelerated model to investigate the molecular mechanisms underlying aging-associated sarcopenia and defective muscle regeneration.
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Clonal behaviour of myogenic precursor cells throughout the vertebrate lifespan

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Recent human genetic analyses have revealed the diverse embryonic origin, limited cell turnover and low clonal drift in the adult skeletal muscle cell population but, as yet, lack high spatial resolution. Here, we employ quantitative confocal time-lapse analyses of whole muscles at cellular resolution together with Musclebow clonal fluorescent protein labelling of myogenic precursor cells (mpcs) to track muscle growth and mpc behaviour over the lifetime of zebrafish. Musclebow consists of a somitic enhancer trap insertional transgene encoding three floxed fluorescent proteins that were recombined by heatshock-driven Cre. We find that: 1. A cohort of early non-proliferative mpcs generate the early myotome. 2. Marked mpc clones contribute little to muscle growth prior to hatching. 3. At 3 days post-fertilization (dpf) when larvae have hatched, around 25% the nuclei in a single somite are in mpcs. 4. From 3-6 dpf, dermomyotome-derived mpc clones rapidly expand while some progeny undergo terminal differentiation. 4. Neither fibre nor mpc death was observed in uninjured animals. 5. Individual marked muscle fibres persist across much of the lifespan indicating low rates of nuclear turnover. 6. In adulthood, early-marked clones label stable blocks of tissue comprising a significant fraction of either epaxial or hypaxial somite, in one or a few adjacent somites. 7. Fusion of cells from separate early-marked clones occurs in regions of clone overlap. 8. Wounds made next to marked mpc clones are regenerated partially from the marked cells, suggesting that many/most mpcs (both marked and unmarked) within a muscle block contribute to local wound repair. In conclusion, our data indicate that most mpcs in muscle tissue contribute to local growth and repair and suggest that cellular turnover is low in the absence of trauma.
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The transcription factor NF-Y is required for satellite stem cell myogenic progression and skeletal muscle tissue repair

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The fate of satellite cells, the skeletal muscle stem population responsible for post-natal muscle growth and regeneration, depends on the activation of specific transcriptional programs. Several transcription factors are involved in SCs maintenance, proliferation and differentiation. The transcription factor NF-Y, composed by NF-YA, NF-YB and NF-YC subunits, has an important role in the regulation of cellular proliferation and differentiation in different cell types, among which stem cells. In particular, the shorter NF-YA isoform (NF-YAs) seems majorly associated to proliferation and stemness, in opposition to the longer one (NF-YAl) that is linked to differentiation. The expression of NF-YA is high in proliferating myoblasts and decreases during differentiation, being absent in mature skeletal muscle. Here we demonstrate that satellite cells express uniquely NF-YAl, whose levels decrease during differentiation. Through the generation of a conditional knock out mouse model that selectively deletes NF-YA in Pax7+ SCs (NF-YA cKO), we show that NF-YA is crucial to preserve the pool of muscle stem cells and it ensures proper regenerative response to muscle injury. Cellular and molecular analyses carried out in vivo and ex vivo highlight that satellite cells that survive to NF-YA loss exit the quiescence and are rapidly committed to early differentiation, despite delayed in the progression towards later states. In vitro results show that NF-YA-depleted muscle stem cells accumulate DNA damage and cannot properly differentiate. Transcriptomic profiling and chromatin immunoprecipitation assays suggest that NF-YA expression is dispensable for proliferation of muscle progenitor cells, but it is required for direct and indirect activation of the differentiation program. To our knowledge, this is the first in vivo study showing that NF-Y activity in stem cells is majorly involved in quiescence and differentiation states, rather than proliferation.
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The voice of patients affected by Duchenne and Becker Muscular Dystrophy

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Parent Project aps is an association of patients and parents of children affected by Duchenne and Becker Muscular Dystrophies (DMD and BMD), considered the most common among rare diseases and for which there is still no cure. The association is committed to funding research and disseminating the multidisciplinary approach that has so far enabled doubling the patients' life expectancy and improving their quality of life. Research support is one of the key objectives of the association. Parent Project Scientific office manages all the activities related to the support of research and the dissemination of scientific information to patients, families and the outside world. The office also manages the Italian DMD/BMD patient registry, collecting demographic, genetic and clinical information about patients with DMD/BMD, necessary for the recruitment of patients for clinical trials, but also to improve the epidemiological information about these pathologies. The registry is an integral part of the Global Registry established by the Treat-NMD, a network of excellence whose objective is the coordination and harmonization of research in the field of neuromuscular diseases. Parent Project aps is committed to build a future of quality for thousands of children and young people living with DMD/BMD and identifies information as one of the key tools to achieve this important goal. For this reason the association runs an educational program that includes an annual international conference which is an unique opportunity to raise awareness, to share experiences and to learn about the latest progresses in the fight to end Duchenne and Becker. Families, physicians, researchers, caregivers, industry partners and patients living with Duchenne and Becker gather to connect, to share information and to discuss and debate the latest news and opportunities in DMD and BMD research. The next conference is scheduled from the 17th to 20th of February 2022.
IIM Meeting 2021 • 22-24 October 2021

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SESSION 4

Beneficial effects of horsetail (*Equisetum arvense*) in experimental models of sarcopenia and osteoporosis

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Osteosarcopenia is an age-associated and unresolved condition characterized by concomitant bone (osteoporosis) and skeletal muscle (sarcopenia) wasting increasing risk of fractures, loss of independence, morbidity and mortality.[1-3] Common factors, such as low-grade chronic inflammation and excessive treatment with glucocorticoids (GCs),[2,3] are responsible of bone and muscle loss. The imbalance between muscle protein breakdown and synthesis leading to reduction in muscle type II myosin heavy chain (MyHC-II), and between bone formation and resorption due to excess activity of osteoclasts, are the many causes of osteosarcopenia.[2,3]
We tested a standardized dry extract of horsetail (*Equisetum arvense*, EQ), traditionally recommended for the treatment of many conditions in virtue of its numerous pharmacological activities,[4] in *in vitro* experimental models mimicking muscle atrophy [i.e., C2C12 myotubes treated with proinflammatory cytokines (TNFα/IFNγ) or excess GCs (dexamethasone, Dex)][2] or osteoclastogenesis (i.e., RAW 264.7 cells treated with RANKL).[4] We found that EQ extract: i) counteracts MyHC-II degradation blunting the activity of different catabolic pathways depending on the applied atrophying stimuli, and ii) reduces RANKL-dependent osteoclast formation, as evidenced by decrease in TRAP-positive cells, TRAP enzymatic activity, and expression of osteoclastogenic markers. Consumption of EQ (500mg/kg/die for 2 months) preserved muscle mass and MyHC amounts, and strongly improved muscle performance in 22-month-old WT mice. Thus, thanks to its active compounds, EQ might be useful to preserve both muscle functionality and physiological bone remodeling during aging, ameliorating the quality of life and reducing health-care costs.

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Iron supplementation is sufficient to rescue cancer-induced muscle wasting and function

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Cachexia is a wasting syndrome characterized by devastating skeletal muscle atrophy that dramatically increases mortality in various diseases, most notably in cancer with a penetrance up to 80%. Knowledge regarding the mechanism of cancer-induced cachexia remains very scarce, making cachexia an unmet medical need. In this study, we discovered strong alterations of iron metabolism in the skeletal muscle of both cancer patients and tumor-bearing mice, leading to reduced iron pool into the mitochondria. We found that modulation of iron levels directly influences muscle mass, as inadequate iron availability causes mitochondrial dysfunction in the skeletal muscle of tumor-bearing mice. Furthermore, iron supplementation was sufficient to preserve both muscle function, mass, prolong survival in tumor-bearing mice, and even rescue strength in human subjects within unexpectedly short frame. Importantly, promoting iron uptake in muscle by electroporation was sufficient to promote fiber hypertrophy, thus uncovering the direct effect of iron on muscle mass. Overall, our findings provide new mechanistic insights in cancer-induced skeletal muscle wasting, and support targeting iron metabolism as a potential therapeutic option for muscle wasting diseases.

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An original network analysis approach for tackling exerkines' world

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Physical exercise is a powerful stimulus for triggering the release of a multitude of humoral factors - called "exerkines" - by several organs. Such factors, directly secreted into circulation or transported by extracellular vesicles, spread their effect with autocrin, paracrin, or systemic patwhays throughout the whole body. The ever-expanding exercise-related secretome is a fertile area of research, and current analytic tools allow to improve the field with robust and original insights. In particular, network models are intriguing for exploring the metabolic cross-talk in response to acute and chronic exercise. Aiming to find any shared physiological pathways between the factors, accounting for exercise-related metabolic communication (Murphy et al., 2020), the STRING database Version 11.5 (string-db.org) was checked. Customizable protein–protein networks were set for IL-6, Follistatin, Irisin, Meteorin-like protein, FGF21, SPARC, Musclin, Cathepsin B, Adiponectin, IGF1, and BDNF. The full STRING network focused on homo sapiens was used, with the interaction sources of experiments, databases, co-expression, neighborhood, gene fusion, and co-occurrence; no more than 10 interactors per molecule were taken into account, and a minimum interaction score of 0.4 was required. A link of CCAAT/enhancer-binding protein beta (C/EBPβ) with both IL-6 and Adiponectin was found; C/EBPβ plays a key role in cardiomyocyte hypertrophy and proliferation, as well as in immune, inflammatory, and bioenergetic pathways. A link of Matrix Metalloproteinase 2 (MMP2) with both SPARC and IGF1 was also found; MMP2 plays a key role in myocardial and vascular pathways. Indeed, endurance exercise affects C/EBPβ, while MMP2 shows a differential response to resistance vs endurance exercise. Network models interestingly fit the framework of exerkines: bioinformatic tools and open databases shall provide novel insights for the networks of physiology, clinics and public health.
IIM Meeting 2021 • 22-24 October 2021

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SESSION 5

Psat1, an enzyme of the serine biosynthesis pathway, is required for Muscle Stem Cells activation

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Muscle stem cells (MuSCs) are critical for muscle growth and regeneration. Quiescent in homeostatic conditions, MuSCS are activated upon specific stimuli such as injury, re-enter the cell cycle and differentiate, allowing proper muscle regeneration. Recent studies revealed that metabolism can regulate transcription via metabolites, acting as a cofactor for epigenetic regulators. However, the molecular link between them is not well characterized. Here, we report that MuSCs activation is accompanied by increased expression of genes involved in the serine biosynthesis pathway: Phgdh, Psat1, and Psph. Psat1, produces alpha-ketoglutarate (aKG), which is used as a cofactor for histone and DNA demethylase enzymes. This led us to investigate if Psat1 could link metabolism and gene expression in MuSCs. To this end, we generated a conditional mouse model that allows Psat1 deletion in MuSCs (Pax7 positive cells). We show that Psat1-depleted myoblasts have a decreased proliferation and reduced intracellular aKG level. Moreover, Psat1 ablation is sufficient to alter gene expression and modify chromatin accessibility in primary myoblasts, suggesting changes in the epigenetic profile. In agreement, we detected an enrichment for H3K4me3 at higher expressed genes in Psat1KO. Those genes are involved in extracellular matrix organization and cell migration processes, suggesting that their expression influence proper myoblasts function. Finally, Psat1KO mice display an impaired muscle regeneration and decreased myofibers size at 28 days after injury. In summary, our data reveal an important role exerted by Psat1 on metabolism and epigenetics of MuScs.
IIM Meeting 2021 • 22-24 October 2021

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Defective dystrophic thymus determines degenerative changes in skeletal muscle

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Growing evidence demonstrates crosstalk between the immune system and the skeletal muscle in inflammatory muscle diseases and dystrophic conditions such as Duchenne Muscular Dystrophy (DMD) as well as during normal muscle regeneration. Immune system activation was traditionally considered as a consequence of muscular wasting, but recent recently we demonstrated a defect in central tolerance caused by thymus alteration and the presence of autoreactive T-lymphocytes in DMD. Since immunocompetent T-cells and Tregs are necessary for the maintenance of immune tolerance and mainly originate in the thymus, we recently showed that the architecture of thymus in DMD murine model, the mdx mice, is severely impaired, according to the expression of ghrelin and autophagy machinery. In particular, dysfunctions of the axis ghrelin (GHR)-GHR receptor (GHS-R) have been demonstrated to cause a reduction in the amount of naïve T cells and consequent defects in thymic output. These results hint at a potential involvement of GHS-R in the modulation of genes associated with dystrophic thymic stromal microenvironment changes and adipogenesis. Since GHR is also involved in T-cell maturation and emigration of T-cells from thymus, to test the prevalence of innate or adaptive immunity in DMD and the role of thymus in this framework, we showed that transplantation of dystrophic thymus (from mdx mice) in recipient nude mice determined the up-regulation of inflammatory/fibrotic markers and marked metabolic breakdown, determining muscle atrophy and loss of force. Our results indicated that involution of dystrophic thymus exacerbates muscular dystrophy by altering central immune tolerance and the balance between innate and adaptive immunity. This represent the first evidence that impaired immune system activation is a primitive feature of DMD independently from genetic muscle defects.

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Abstracts of Posters

IIM Meeting 2021 • 22-24 October 2021

P.1

The interplay between polyglutamine-expanded AR and its transcription co-factors in skeletal muscle

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Expansion over 38 repeats of the polymorphic CAG tandem repeat in the first exon in androgen receptor gene (AR) causes Spinal and Bulbar Muscular Atrophy (SBMA), an inherited, slowly progressive neurodegenerative disease that fully manifests only in males. For a long time, SBMA has been thought to primarily be a motor neuron disorder. However, in the last decade a key emerging aspect of this disease is the primary involvement of peripheral tissues, such as skeletal muscle. In detail, we provide evidence that polyglutamineexpanded AR alters the expression of genes involved in the excitation-contraction coupling, muscle contraction and metabolism. These gene expression changes are associated with early mitochondrial dysfunction and pathology. Using different models, we have found that specific AR co-regulators are overexpressed in SBMA skeletal muscle in an androgen-dependent manner. These AR co-activators enhance polyglutamine-expanded AR toxic gain-of-function and toxicity, and thus they are important pharmacological targets. These observations suggest that targeting overexpressed AR native co-regulators could be a possible therapeutic approach for SBMA to attenuate the AR toxic gain of function without exacerbating the AR loss of function.

IIM Meeting 2021 • 22-24 October 2021

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P.2

C26 and LLC tumor conditioned medium induce adrenergic resistance in C2C12 through a cAMP phosphodiesterases 4-dependent mechanism

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Cancer-associated cachexia consists in a severe loss of skeletal muscle mass and functionality and affects most of the cancer patients. Tumor communicates with the host tissues through the induction of several cytokines, leading to increased energy expenditure and negative energy balance. The Gαs-coupled β2-adrenergic receptor (β2AR) is a major regulator of skeletal muscle metabolism in both physiological and pathological conditions through the activation of the cAMP/PKA/CREB axis and consequent induction of several genes, including PGC-1α, thus counteracting protein degradation, regulating neuromuscular junction (NMJ) maintenance, and mitochondrial biogenesis. Skeletal muscles of cachectic mice display impairment of oxidative metabolism, defective NMJ integrity, and muscle atrophy, suggesting the presence of dysfunctional adrenergic responsiveness in muscle cells. To investigate this hypothesis, we treated C2C12 with tumor-conditioned medium (TCM) from colon carcinoma (C26) and Lewis lung carcinoma (LLC) cells and we analyzed the adrenergic responsiveness through isoproterenol (a β2-adrenergic agonist) treatment. The adrenergic-dependent PGC-1α induction is attenuated in C2C12 myotubes and myoblasts pre-treated with the TCM. Consistently, C2C12 myoblast cell exposure to the TCM reduces the adrenergic-dependent activation of the cAMP/PKA/CREB axis. Since the expression of thecAMP- hydrolyzing phosphodiesterase 4B and 4D (PDE4B and PDE4D) is increased in TCM- activation of cAMP/PKA/CREB pathway through treated C2C12, we raised the hypothesis that it may mediate adrenergic resistance. Indeed, treatment with the PDE4 inhibitor rolipram restores adrenergic signaling in C2C12 exposed to C26 TCM. These data indicate that the pro-cachectic environment impairs the adrenergic signaling in C2C12

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through a PDE4-dependent mechanism and suggest that, during cancer cachexia, the adrenergic signaling in skeletal muscle is impaired.
IIM Meeting 2021 • 22-24 October 2021

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P.3
Physical force drives in vivo skeletal muscle growth

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Muscle activity, or lack thereof, results in dramatic change in the size and strength of muscle fibres to accommodate functional demands. Substantial progress has been made in elucidating the signalling molecules that control muscle mass, although it remains unknown how activity sets in motion these molecular events to facilitate growth. One prominent hypothesis is that mechanical forces, generated by actomyosin contraction, drive mechanochemically transduced responses in muscle fibres and muscle stem cells (MuSCs). However, there is a paucity of direct evidence that mechanical factors per se explain the effects of activity on muscle, the issue being that it is difficult experimentally to separate the role of mechanical factors from other correlates of activity, such as neural input or cytoplasmic calcium rise. Here, I use a novel muscle growth assay in living zebrafish to examine the role of actomyosin activity per se in skeletal muscle growth in vivo. Inactive muscle exhibits reduced growth, which can be fully restored by brief electrically- or optogenetically-evoked activity. Activity-induced growth occurs by increase in muscle fibre size and muscle fibre number, suggesting an effect of MuSCs. Pharmacological blockade of myosin function, which inhibits force production and myosin ATPase activity, but does not affect upstream electrical events, completely prevented the rescue of growth. Asking whether myosin-dependence indicates a requirement for force, or a role for ATP hydrolysis by myosin ATPase, activators of ATP consumption and the energy-stress sensor AMPK, were employed, revealing that growth could not be rescued by energy stress. It is concluded that physical force is necessary, and that electrophysiological signals alone are insufficient, to stimulate skeletal muscle growth. Future work will the explore the molecular signalling mechanism(s) that link force to growth.
IIM Meeting 2021 • 22-24 October 2021

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P.4
Circular RNA role in Myotonic Dystrophy type 1

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Myotonic dystrophy type 1 (DM1), is a multisystemic disorder caused by expanded CTG repeats in the 3′ UTR of the DMPK gene leading to deregulated mRNA-splicing. Circular RNAs (circRNAs), a class of covalently closed RNAs produced by back-splicing events are emerging as important regulators of muscular disorders. Given the pronounced splicing dysregulation in DM1, we hypothesized circRNA levels and function may be deregulated in DM1. Accordingly, we have previously identified a subset of circRNAs that were significantly increased in muscle biopsies of DM1 patients. By analysing published datasets and applying a stringent selection pipeline, we identified 20 additional circRNAs candidates differentially expressed between DM1 and CTRLs, conserved in mouse, and displaying a high ratio between the circular and the linear isoforms. Validation of DM1 circRNAs was performed in biceps barchii biopsies of 24 DM1 and 16 CTRL subjects. Out of 15 circRNAs tested, 6 displayed an increased circular fraction in DM1 samples. Specifically, the levels of circARHGAP10 displayed a significant direct correlation with the number of CTG-repeats, and an inverse correlation with skeletal muscle strength of DM1 patients. Moreover, ROC curve analysis showed that circARHGAP10 discriminated DM1 patients from CTRLs. The circular structure of circARHGAP10 was confirmed by RT-qPCR analysis using divergent primers, Sanger sequencing, and by the resistance to the RNase R digestion. Bioinformatic analysis indicated that circARHGAP10 may act as a sponge for miR-409. Accordingly, miR-409/circARHGAP10 interaction was shown in a pull-down assay using biotinylated oligonucleotides targeting circARHGAP10 backsplice junction. Our results allowed the identification of new circRNAs dysregulated in DM1 patients that might be used as DM1 biomarkers, and highlight the need for further research to assess their implication in DM1.
IIM Meeting 2021 • 22-24 October 2021

Eur J Transl Myol 2021; 31 (4), 10270. doi: 10.4081/ejtm.2021.10270

P.5

Safety and efficacy of MATR3-based gene therapy in an animal model of FSHD

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Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common myopathies, affecting an estimated 1 in 8,000 individuals. FSHD is caused by aberrant myogenic expression of the transcription factor double homeobox 4 (DUX4). In FSHD, DUX4 mis-expression activates a pro-apoptotic transcriptional program leading to muscle wasting and weakness. For long time, the lack of an animal model with FSHD features in which to study DUX4- mediated muscle pathology and test therapeutic interventions has been a major roadblock in developing treatments for this important disease. Recently, a novel mouse with inducible DUX4 expression (FLExDUX4) was reported. When crossed to the skeletal muscle-specific and tamoxifen inducible ACTA1-MCM driver, the mice reveal several features of the human pathology. In our lab, MATR3 was identified as the first endogenous protein inhibitor of DUX4. MATR3 binds DUX4 directly and block DUX4-induced toxicity in cell culture by counteracting DUX4 dependent gene expression. Specifically, we identified a short MATR3 fragment that is sufficient to directly block DUX4-induced toxicity. The aim of my project is to test safety and efficacy of MATR3-based gene therapy in vivo in the FLExDUX4 animal model. We are characterizing the mouse model to optimize the future step in which the FLExDUX4 and control mice will be transduced systemically with the myotropic AAVMYO vector containing a muscle-specific promoter driving the expression of MATR3 or its minimal DUX4-inhibiting fragment. Animals will be analyzed at molecular, functional and histological levels. We expect MATR3 or its minimal DUX4-binding fragment to significantly rescue aberrant expression of DUX4 targets and muscle degeneration in FSHD animal model with negligent side effects. This would open a new possibility to future drug development for FSHD patients.

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P.6

Pde4 targeting rescues tonic β-adrenergic signaling in cachectic C26-bearing mice

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The sympathetic nervous system innervates the skeletal muscles and controls the continuous release of noradrenaline under physiological conditions. This tonic signal activates the β2 adrenergic receptor and the downstream cAMP/PKA/CREB axis, playing a crucial role in the maintenance of the muscle homeostasis and the neuromascular junction structure. The local resection of the sympathetic nervous system has been proven to induce loss of muscle mass, NMJ disruption and loss of muscle functionality. Our in vitro preliminary data in C2C12 cells, demonstrate that C26 tumor condition medium (TCM) impairs β-adrenergic dependent activation of cAMP/PKA/CREB pathway through the induction of Pde4 cAMP phosphodiesterase. Indeed, Pde4 pharmacological targeting rescues th a reduction in the total number of NMJs and in the amplitude of evocated potentials. C26 TCM-induced defective cAMP/PKA/CREB axis activation in C2C12 cells. Thus, we raised the hypothesis that cancer cachexia in vivo induces muscle resistance to tonic β-adrenergic signaling from the autonomous nervous system. Indeed, PDE4B mRNA, the most abundant PDE4 isoform in skeletal muscle, was induced in skeletal muscles of C26- bearing mice. The intraperitoneal administration of Rolipram, a selective inhibitor of Pde4 isoforms, increases cAMP levels, decreases atrogenes induction thus contributing to revert cachexia. Altogether, these data suggest that defective adrenergic signaling through cAMP/PKA/CREB signaling contributes to skeletal muscle wasting, and indicate that PDE4B targeting, may represent a strategy to revert the muscle wasting in cancer cachexia.
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P.7

Modulation of the cyclin inhibitor p27 to ameliorate Merosin Deficient Congenital Muscular Dystrophy (MDC1A)

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MDC1A (or LAMA2 disease) is a severe disease, mostly presenting within the first decade of life, and characterized by a progressive impairment of motor functions and is one of the most frequent recessively inherited-neuromuscular disorders among rare diseases. MDC1A is due to mutations in the LAMA2 gene causing lack of laminin211 in Schwann cell and muscle basal lamina. Lack of laminin211 is responsible for tissue degeneration resulting in relentless neuropathy and muscular dystrophy. The disease is recapitulated in some Lama2 mouse models including the Lama2dy2J/dy2J mouse. Our previous findings suggest that tissue degeneration in Lama2 mice may be consequence of dysregulated, increased, levels of the cyclin inhibitor p27KIP1. For this reason we evaluated consequences of p27KIP1 down-regulation by genetic deletion of p27KIP1 in Lama2 mice by cross-breeding Lama2dy2J/dy2J into p27-null background. Treadmill analysis and grip test, significantly deteriorated Lama2dy2J/dy2J mice, did not show amelioraton in double mutants (Lama2/p27KO). Pathology showed, as expected, fiber atrophy, necrotic fibers and fibrosis in Lama2dy2J/dy2J mice. Double mutants did not show amelioration of fiber size, but a reduced number of necrotic fibers and fibrosis. Inflammatory (macrophage) infiltrate was also reduced in double mutants as compared to Lama2dy2J/dy2J mice. Finally, we observed in double mutants a significant increase of fibers with central nuclei and of pax7 positive satellite cells. Our data suggest that muscle degeneration in LAMA2 disease could be, at least in part, consequence of dysregulated high level of the cyclin inhibitor p27KIP1, and that p27KIP1 downregulation may constitute pharmacological strategy to ameliorate some aspects of the disease.
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P.8

Transcriptional and epigenetic regulation in age-dependent progressive decline of skeletal muscle regenerative capacities

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This project aims to identify the transcriptional and epigenetic events that mark senescent cells and to depict the chromatin state that distinguishes competent and incompetent chromatin landscape toward muscle lineage. Eventually, this will help to understand the molecular mechanisms of muscle stem cell (MuSCs) functional decline and to find new stem cell-based rejuvenation strategies. In skeletal muscle, aging is characterized by gradual muscle loss, resulting from the failure of MuSCs activation. The functional antagonism between replicative senescence and activation of the myogenic program, due to DNA damage signaling (DDR), impairs muscle regeneration in aged muscles. The DDR-resistant MyoD mutant (MyoD-Y30F) overcomes the senescence barrier leading cells throughout the cell cycle, restoring a more permissive chromatin state. Given the importance of the cell cycle for histones production and that decreased histone levels have been observed in aged MuSCs, we speculate that histone depletion might contribute to the inhibition of muscle gene expression in senescent cells. Yet, MyoD-Y30F is essential for synthesis of histones, histones chaperones and several chromatin-bound proteins that make the chromatin of MuSCs compatible with the expression of muscle-specific genes and for myogenic conversion. To study the composition of chromatin, we performed Mass Spectrometry experiments on chromatin-bound proteins in young and old fibroblasts expressing MyoD-WT or Y30F. Our data show that when old cells express MyoD-Y30F, besides proteins regulating skeletal muscle regeneration, there is an upregulation of those involved in nuclear bodies formation. These membraneless compartments have important roles in several processes: alternative splicing, RNA maturation and retention and histones mRNA processing. Therefore, we think that studying nuclear bodies formation and involvement in myogenesis is essential to understand how to restore the myogenic potential of old MuSCs.
IIM Meeting 2021 • 22-24 October 2021

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P.9

MiR-152-3p, -193a-3p and -193b-3p impact on NMJ functionality and cause fast to slow myofiber shift

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MiRNAs are established as crucial modulators of processes involved in muscle and neuronal development and differentiation, suggesting that their aberrant expression can contribute to the onset of neuromuscular diseases. Loss of axon and muscle contact leads to neuromuscular junction (NMJ) alterations and muscle atrophy. With the aim of identifying non-coding RNAs involved in the modulation of NMJ functions, we identified miRNAs and genes regulating NMJ structure that were altered in SOD1 G93A ALS mouse model and after the sciatic nerve cut. We identified a network of miRNAs involved in the modulation of NMJ. Using the luciferase assay we confirmed the interaction among three miRNAs and their targets. In particular, we confirmed the interaction among a) miR-193a-3p and Chrna1 and Musk b) miR-193b-3p and Chrna1, Chrnb1, Chrnd, Chrng, Emb, and Musk; c) miR-152-3p and Chrne and Prima1. After the in vivo modulation of miRNAs previously cited we evidenced a reduced expression of their targets and a reduction of gastrocnemius and tibialis anterior muscle mass. In association with these observations we evidenced a fast to slow fiber shift with a reduction in the total number of NMJs and in the amplitude of evocated potentials.These results support the role of 3 miRNAs in the maintenance of NMJ function and in the regulation of skeletal muscle atrophy.
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P.10

Alterations of regulatory chromatin interactions leading to aberrant patterns of gene expression in Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is a lethal disease caused by mutations in the Dystrophin gene. Absence of dystrophin leads to increase membrane fragility upon contraction and aberrant signaling cascade. Current clinical trials have focused on dystrophin expression restoration using AdenoAssociated Vectors (AAV) to deliver truncated, functional version of dystrophin (microdystrophin, μDys) in muscle fibers and protect the sarcolemma from contraction stress. Additionally, applications of CRISPRCas9 genome editing are being explored in vitro as potential therapeutic approaches. Here we want to understand whether Dystrophin deficiency causes pathogenic epigenetic and transcriptional alterations, and whether Dystrophin restoration can revert these alterations to a healthy status, or if once caused by dystrophin deficiency become refractory to current therapeutic approaches. To address the above-mentioned issues, we have differentiated WT hiPSCs and the isogenic DMD hIPSCs line - DMDΔex8-9, a mutation found in a DMD patient - into skeletal muscle cells by ectopic expression of MYOD and BAF60c and performed high-resolution 3D chromatin interaction map by H3K27ac HiChIP, coupled with ATAC-seq and RNA-seq to investigate the epigenome and transcriptome of DMD human muscles. To understand whether alteration found in DMD muscles are reverted to “healthy” status upon dystrophin restoration we either delivered μDys using lentiviral system either in myoblasts or in myotubes derived from DMD hiPSCs or we differentiated DMDRΔex6-9 - a line in which dystrophin reading frame is restored - into myotubes and analyzed the epigenomic landscape and transcriptional output. Thus, information derived from this study will provide insights into the therapeutic potential of Dystrophin restoration approaches.
IIM Meeting 2021 • 22-24 October 2021

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P.11

Circulating myomiRs in muscle denervation: from surgical to ALS pathological condition

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ALS is a fatal neurodegenerative disorder, characterised by muscle atrophy, motoneuron degeneration and denervation. Different mechanisms are involved in the pathogenesis of the disease; among those, microRNAs have been described as biomarkers and potential pathogenetic factors for ALS. MicroRNAs produced by skeletal muscle, namely myomiRs, play a crucial role in tissue homeostasis; furthermore, they can be released in blood circulation under pathological conditions, including ALS. However, the functional role of myomiRs in muscle denervation has not yet been completely clarified. In the present study, we analysed the levels of two myomiRs, miR-206 and miR-133a, in skeletal muscle and blood samples of denervated mice. We demonstrated that surgical denervation reduced the expression of both miR-206 and miR-133a, while miR-206 but not miR-133a was upregulated during the re-innervation process. Moreover, we quantified miR-206 and miR-133a levels in serum samples of two ALS mouse models, characterized by different disease velocities. We observed a different modulation of circulating myomiRs during ALS disease, according to the velocity of disease progression. Finally, taking into account surgical and pathological denervation, we described a different response to increasing amounts of circulating miR-206, suggesting a hormetic effect of miR-206 in relation to changes in neuromuscular communication.
IIM Meeting 2021 • 22-24 October 2021

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P.12

Role of STAT3-mediated autophagy in driving muscle regeneration during aging

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Age-related reduced regenerative potential and muscle wasting (sarcopenia) has been in part associated with a decline in the number and function of adult muscle stem cells (MuSCs), due to intrinsic and niche/systemic alterations. We and others have demonstrated a key role of STAT3 in regulating MuSCs expansion and differentiation. In addition, we described the essential role of autophagy in driving MuSCs function toward efficient muscle regeneration. The established role of autophagy in maintaining muscle mass and tissue homeostasis together with the emerging role of STAT3 in regulating the autophagic process inspired the rationale behind this project which resides in the study of the STAT3-mediated autophagy toward skeletal muscle repair. Our hypothesis is that STAT3 might have a role in regulating myogenic lineage and regeneration process by affecting the autophagic process thereby restoring the bioenergetic demand of the old myogenic niche to support muscle regeneration. We show that STAT3 inhibitor (STAT3i) treatment induces the autophagic process upon muscle regeneration both in vitro and in vivo. STAT3-mediated autophagy during muscle regeneration is associated with eIF2α phosphorylation that is conceivably achieved by PKR that is no longer sequestered by STAT3. Intriguingly, STAT3i treatment is able to resume the autophagic process in old MuSCs, otherwise characterized by low levels of autophagy, leading to proficient muscle regeneration. Our findings highlight the key role of the STAT3- dependent autophagy in driving muscle regeneration, revealing potential biological targets in MuSCs that may restart an efficient regenerative response in aged mice.
IIM Meeting 2021 • 22-24 October 2021

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P.13

Sertoli cells induce utrophin expression in human DMD myotubes with different mutations and exert promyogenic and antifibrotic effects

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Sertoli cells (SeC) are the major component of the seminiferous tubules, where they secrete trophic and immunomodulatory factors necessary to protect developing germ cells.[1] Based on their peculiar properties, many preclinical studies have reported the successful employment of SeC in different pathologies, including Duchenne muscular dystrophy (DMD).[1-3] We demonstrated that a single i.p. injection of microencapsulated porcine SeC, in the absence of pharmacological immunosuppression, induces the recovery of muscle morphology and performance in dystrophic mice, thanks to two independent effects: the release of antiinflammatory/trophic factors, and the release of heregulin β1 that upregulates the dystrophin paralogue, utrophin at the sarcolemma.[3,4] The direct effects of SeC on myoblasts/myotubes have not been investigated so far. We found that SeC-derived factors i) stimulate cell proliferation in the early phase of the differentiation process in C2C12 and human healthy and DMD myoblasts; ii) delay the expression of differentiation markers in the early phase, and stimulate terminal differentiation in DMD myoblasts; iii) restrain the fibrogenic phenotype in human fibroblasts, and inhibit myofibroblast transdifferentiation in C2C12 and healthy and DMD myoblasts; iv) induce the upregulation of utrophin at the sarcolemma in DMD myotubes regardless of the mutation in a heregulin β1/ErbB2/ERK1/2-dependent manner. Altogether, our results suggest that the SeC-based treatment is equivalent to a combinatorial approach with beneficial effects on the myoblast and fibroblast components of dystrophic muscles, and represents a universal (i.e., mutation-independent) approach to DMD. Moreover, SeC might be beneficial during the early phase of muscle regeneration when myoblasts have to proliferate to efficiently replace damaged myofibers.

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P.14

DLL4 and PDGF-BB regulate migration of human iPSC-derived skeletal myogenic progenitors

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Skeletal muscle growth and regeneration is sustained by muscle satellite stem cells (MuSCs). Despite their self-renewal and differentiation potential, human MuSCs have limited in vitro amplification and in vivo migration potential, restricting their use in cell therapies for muscular dystrophies affecting multiple skeletal muscles. To obtain myogenic progenitors with controllable

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proliferation capacity and differentiation potential, numerous methods have been developed to generate MuSC-like cells from human induced pluripotent stem cells (hiPSCs). However, similarly to tissue-derived MuSCs, hiPSC-derived myogenic cells also have limited cell migration, negatively impacting on clinical translation. To overcome this challenge, we tested whether activation of NOTCH and PDGF pathways with DLL4 and PDGF-BB improve migration of hiPSC-derived myogenic progenitors. Transcriptional and functional profiling showed that response to treatment is conserved in rodents, humans and across several hiPSC lines, including Duchenne muscular dystrophy hiPSC-derived progenitors which underwent genetic correction. DLL4 and PDGF-BB treated cells could be expanded in culture and maintained their myogenic capacity. RNAseq analyses of treated myogenic progenitors showed significant modulation of cell migration signalling pathways, consistent with data from cell motility analyses at single cell resolution. Importantly, transwell assays highlighted improved trans-endothelial migration in DLL4 and PDGF- BB treated cells. This study establishes the foundations of a transgene-free strategy inspired by developmental biology to achieve an important translational milestone for intravascular delivery of myogenic progenitor cells, advancing hiPSC technology for future gene and cell therapies.
IIM Meeting 2021 • 22-24 October 2021

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P.15

Contingent intramuscular boosting of P2XR7 axis improves motorfunction in transgenic ALS mice

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Muscle weakness plays an important role in neuromuscular disorders comprising Amyotrophic Lateral Sclerosis (ALS), a fatal neurodegenerative disorder that leads to progressive degeneration of motor neurons and severe muscle atrophy without effective treatment. Most research on the disease has been focused on studying motor neurons and supporting cells of the central nervous system. Strikingly, recent observations have shown that the expression of the SOD1G93A mutation in skeletal muscles causes denervation of the neuromuscular junctions, inability to regenerate and consequent atrophy, all clear symptoms of ALS, suggesting that these morpho-functional alterations in skeletal muscle precede motor neuron degeneration, bolstering the interest in studying muscle tissue as a potential target for the delivery of therapies. We previously showed that the systemic administration of the P2XR7 agonist, 2′ (3′) - O - (4 - benzoylbenzoyl) adenosine 5 - triphosphate (BzATP), enhanced the metabolism, improved the innervation and promoted the myogenesis of new fibres in the skeletal muscles of SOD1G93A mice. Here we further corroborated this evidence showing that intramuscular administration of BzATP improved the motor performance of ALS mice by enhancing satellite cells and the muscle pro-regenerative activity of infiltrating macrophages. The preservation of the skeletal muscle retrogradely propagated along with the motor unit, suggesting that backward signalling from the muscle could impinge on motor neuron death. In addition to providing the basis for a suitable adjunct multisystem therapeutic approach in ALS, these data point out that the muscle should be at the centre of ALS research as a target tissue to address novel therapies in combination with those oriented to the CNS.
IIM Meeting 2021 • 22-24 October 2021

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P.16

Role of mitochondrial calcium in the activation and differentiation of satellite cells during skeletal muscle regeneration

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Skeletal muscle shows a remarkable ability to regenerate upon injury, due to the presence ofsatellite cells. In resting conditions these cells reside under the basal lamina of myofibers in aquiescent state. However, upon muscle injury, they became activated, proliferate and eitherdifferentiate to form new myofibers or return to the quiescent state to replenish the stem cells pool. Satellite cells are characterized by a different metabolic preference depending on their state, being predominantly oxidative in quiescent state and predominantly glycolytic upon activation. A return to oxidative metabolism is associated to the differentiation phase.However, still unclear is how metabolism can affect the activation and differentiation ofsatellite cells. Since mitochondrial

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calcium controls the rate of oxidative metabolism, ourhypothesis is that it is a key player of satellite cells activation and differentiation. To test thishypothesis, we asked whether satellite cells activation is characterized by changes onmitochondrial calcium homeostasis. To this end, we used as working model cardiotoxin (CTX)-induced muscle damage on wild-type mice. Three days after CTX injection, we isolatedsatellite cells from regenerated and non-regenerated mice and analyzed the expression of thedifferent MCU complex components. Our results show that the expression of the pore-formingsubunit MCU does not change between quiescent and activated cells, while we observed atrend of increase of the dominant-negative subunit MCUb and of the gatekeeper MICU2, and a trend of decrease of the positive modulator of the channel MICU1 upon activation. Moreover, to understand whether satellite cells proliferation and differentiation is sensitive to mitochondrial calcium homeostasis, we are generating a mouse model where MCU expression is blunted specifically in satellite cells. We will soon characterize its muscle regeneration capacity, calcium homeostasis and perform transcriptomic analysis.
IIM Meeting 2021 • 22-24 October 2021

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P.17

The effect of exosomes isolated after training from older people on skeletal muscle regeneration

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Sarcopenia is the age-related loss of muscle mass, strength and function; one of the causes is the decreased activity of satellite cells (SCs). Both fibers and SCs are important sites for the release of nanovesicles including exosomes (EXOs), suggesting that skeletal muscle plays the role of a secretory organ. The EXOs can represent a way of communication between close cells but even between distant cells since they can also be delivered by the circulation playing an important role of cross-talking. Recent studies suggest that proper physical activity slow down sarcopenia progression. It is known that following exercise, the muscle releases factors that can be conveyed through EXOs. The aim of the present study is to elucidate the role of EXOs released by myoblasts and myotubes pre- and post-training and those present at the systemic level, on muscle regeneration in the elderly. To achieve this goal, healthy male elderly subjects (71±4 years; n=54 volunteers were recruited and randomly assigned to different training (endurance and resistance) and control groups; training protocol consisted in 12 weeks, 3 sessions/w. Furthermore, at the volunteer were done the Vastus Lateralis skeletal muscle biopsy before (Pre-) and after (Post-) training. In detail: 1) Pre- and Post-SCs were isolated and characterized; 2) EXOs were isolated and characterized by both culture medium and blood serum collected Pre- and Post- training; 3) microRNAs (miR-1, miR206, miR133, miR182, miR-183) were analyzed in EXOs isolated; 4) Sedentary elderly SCs proliferative rate and differentiation ability, were evaluated after EXOs Post-training incubation. In conclusion, our data suggested that miRNAs transported by the EXOs, show a different expression in subjects trained with endurance protocol compared to resistance or sedentary subjects. The miRNAs released with the EXOs following endurance training appear to be correlated with positive effects on muscle regeneration.
IIM Meeting 2021 • 22-24 October 2021

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P.18

Study of extracellular vesicles (EVs) protein content released by dystrophic fibro-adipogenic progenitors (FAPs) treated with HDAC inhibitors (HDACis)

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Duchenne muscular dystrophy (DMD) is the most common and severe neuromuscular dystrophy caused by mutations in the dystrophin gene that caused a loss of the protein. The absence of dystrophin involved an alteration of the structural and functional integrity of skeletal muscles. Unfortunately, there is not a cure for DMD but only treatments focused on an improvement of DMD patients' lives. In the last few years has been highlighted

the enormous potential of Extracellular Vesicles (EVs) as cell-free treatment in regenerative medicine. Our previous data have shown that the communication between two skeletal muscle cell populations, fibroadipogenic progenitors (FAPs) and muscle stem cells (MuSCs), have been mediated by EVs released by FAPs and directed to MuSCs. Furthermore, we have demonstrated how a pharmacological treatment of dystrophic mice (MDX) with an epigenetic drug, the HDAC inhibitor Trichostatin A (TSA), changes the miR expression profile toward a myogenic one inside FAPs-EVs isolated from dystrophic muscles. We have performed a proteomic analysis showing a change in the protein expression profile of EVs TSA compared to EVs CTR. This analysis revealed that the most up-regulated protein in EV-TSA is Integrinb1. Itgb1 is a very promising protein, localized along myofibers in sarcolemma. As described in the literature, Itgb1 is necessary for the maintenance of MuSCs quiescence in homeostasis, and to drive MuSCs proliferation and self-renewal during muscle regeneration after injury. We aim to investigate the role of the amount of Itgb1 transported by the FAPs- EVs TSA to guide the proliferation and differentiation of MuSCs by in vitro and ex vivo experiments. Additionally, by in vivo analysis on MDX mice, we will evaluate the effects on muscle regeneration, fibrosis and inflammation linked to transplant of EVs in which Itgb1 is modulated. In future, we will analyze if Itgb1 is also necessary for a specific biodistribution of EVs injected systemically, in muscles.
IIM Meeting 2021 • 22-24 October 2021

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P.19

Role of HMGB1 redox isoforms in cancer cachexia

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Cancer cachexia is a multifactorial syndrome characterized by severe skeletal muscle wasting associated to systemic inflammation that significantly reduces prognosis, therapeutic response, and quality of life, leading to death in about 20-30% of all cancer patients. There is no available treatment for cachexia, making it an urgent medical need. Our group is interested in the High Mobility Group Box 1 (HMGB1) protein as a possible diagnostic biomarker and/or therapeutic target for cancer cachexia. This nuclear protein can be released in the extracellular space to act as a danger signal. We previously demonstrated that HMGB1 promotes regeneration or inflammation in skeletal muscle by switching between mutually exclusive redox states. The fully-reduced isoform (frHMGB1), which contains all the three cysteines in a reduced state, promotes tissue repair while the disulfide isoform (dsHMGB1), containing a disulfide bond between the two first cysteines, sustains inflammation. We recently uncovered that oxidation of HMGB1 is a finely regulated process both in time and in space, by employing mouse models of acute muscle injury, muscular dystrophies and wellestablished models of cancer cachexia. We found that dsHMGB1 was highly expressed in skeletal muscle upon acute injury and in dystrophic muscles, but not in cachectic muscles. Moreover, tumor microenvironment was enriched in this pro-inflammatory isoform, in contrast to in vitro culture of tumor cells. These results unveiled the tight correlation between an inflammatory state of the tissue and the presence of the dsHMGB1 isoform. Accordingly, we identified leukocytes as reservoir and carrier of dsHMGB1. We are currently investigating the role of HMGB1 redox isoforms in the pathogenesis of cancer cachexia, by using different knockout strategies, and we will evaluate the potential of dsHMGB1 as biomarker for cancer cachexia.
IIM Meeting 2021 • 22-24 October 2021

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P.20

Deletion of C1q ameliorates the dystrophic muscle phenotype observed in a mouse model of Duchenne Muscular Dystrophy

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Elevated WNT-signaling has been shown to play a detrimental role in muscle regeneration and to promote the accumulation of fibrotic tissue in dystrophic muscles. However, the molecular and cellular pathways responsible for this process are poorly characterized. The initiating molecule of the classical complement pathway, C1q was reported to activate canonical WNT-signaling

in aged mice. We hypothesized that the C1 complex (C1q with serine proteases C1r and C1s) induces WNT-signaling in Duchenne muscular dystrophy to exacerbate the disease. Consistently, we found that the classical complement protein levels were up to 10-fold upregulated as early as one month of age and remain elevated up to one year of age in the dystrophic mdx muscles compared to the healthy controls. In support of our hypothesis, these enhanced complement protein levels positively correlate with the increased expression of WNT-target proteins in the mdx regenerating areas. Furthermore, C1 complex induces the expression of the WNT-signaling in murine fibroblasts and myoblasts in vitro and we show that macrophages and fibro-adipogenic progenitors are increased in the mdx muscles proximal to the regenerating areas and secrete distinct subunits of the C1 complex. Therefore, they can act as a combinatorial source of WNT-activity. Initial in vivo loss of function observations support the idea that complement C1q has a detrimental role in dystrophic muscles. Our data support the idea that complement is detrimental in the dystrophic environment. Our data increase the comprehension of the link between complement levels and degenerative diseases and support further investigation of novel therapeutic strategies to delay the progression of Duchenne muscular dystrophy.
IIM Meeting 2021 • 22-24 October 2021

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P.21

A novel technique to measure neuromuscular junction functionality in isotonic conditions

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The neuromuscular junction (NMJ) is a specialized chemical synapse that plays a crucial role in transmitting and amplifying information from spinal motor neurons to skeletal muscles [Punga A. R. et al., 2012]. Impaired NMJ functionality can be involved in several pathological conditions, like aging, acute denervation, Duchenne Muscular Dystrophy (DMD) and Amyotrophic Lateral Sclerosis (ALS) [Gonzalez-Freire M. et al., 2014, Rudolf R. et al., 2014, Dupuis L. et al., 2009, Hadj-Saïd W. et al., 2012]. Despite NMJ functionality has been investigated in different animal models of neuromuscular diseases in isometric conditions [Fogarty M. J. et al., 2020, Fogarty M. J. et al., 2019, Personius K. E. et al., 2006], the situation that better mimics the physiological muscle activity is the isotonic one, with a particular reference to fatigue, known to enhance the impairments in the synaptic transmission. In this work, we proposed a novel technique to characterize NMJ functionality in murine Tibialis Anterior (TA) muscles in isotonic conditions together with an extensive testing protocol for the measurement of NMJ functionality in isometric and isotonic conditions. Experimental results demonstrated the feasibility of the technique, in terms of accuracy of all the parameters measured both for direct and indirect stimulations. To characterize the alterations of synaptic transmission during isotonic fatigue test, we also devised a novel parameter, namely Isotonic Neurotransmission Failure (INF), and computed it for TA muscles of SOD1G93A ALS mouse models at the end-stage of the disease. Results showed that the parameter properly expressed the NMJ impairment during isotonic fatigue. The devised technique might be used for studying the NMJ functionality during ALS progression in SOD1G93A mouse model, aiming at providing new insights on the communication between muscle and nerve at different stages of the disease.
IIM Meeting 2021 • 22-24 October 2021

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P.22

Niacin supplementation restores NAD+ levels and counteract muscle wasting in cancer- and chemotherapy-induced cachexia

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Cancer cachexia is a complex multifactorial syndrome that affects more than half of all cancer patients. It is characterized by involuntary loss of body weight, depletion of adipose tissue, metabolic abnormalities and muscle wasting, impairing patients' quality of life and survival. Several studies demonstrated the activation of muscle proteolysis, accompanied by reduced protein synthesis and mitochondrial alterations, both in cancer patients and in experimental models. Recently, we observed in an experimental model of cancer cachexia a decline in muscle NAD+ levels. Since NAD+ takes part in mitochondrial energy metabolism reactions, alterations of its levels may affect mitochondrial homeostasis and lastly muscle function. Aim of this study was to exploit the potential role of NAD+ precursor,

niacin, administrated to C26 tumor-bearing (TB) mice with ongoing chemotherapy (FOLFOX), in reducing body and muscle weight loss, in improving protein synthesis and in restoring mitochondrial alterations, in order to propose niacin as a possible new approach for the treatment of cancer cachexia. Preliminary data collected from healthy control mice and two groups of C26 TB mice, one treated with niacin (150 mg/Kg) and one with a vehicle, suggested that niacin administration counteracts body and muscle weight loss, rescuing protein synthesis in TB skeletal muscle, without affecting tumor mass. Moreover, we observed in skeletal muscle that niacin supplementation improved the expression of proteins involved in mitochondrial biogenesis (PGC-1α, SDH, CytC), while reduced autophagy and mitochondrial stress markers (LC3B, p62, PINK1), compared to C26 TB mice treated with the vehicle. Despite further analyses will be performed on the capability of niacin to influence the host's response to the tumor, the results obtained so far suggest niacin as a novel therapeutic strategy to prevent cancer cachexia, improving patients' body weight and supporting whole-body energy metabolism.
IIM Meeting 2021 • 22-24 October 2021

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P.23

The effect of cocoa polyphenol extract on myogenic differentiation in oxidatively injured murine myoblasts

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In this study we describe the effect of cocoa polyphenol extract (CPE, from flavanols-richcocoa) on myogenic differentiation in murine myoblasts (C2C12 cells) exposed to H2O2. The myogenic program was monitored using morphological, ultrastructural, and molecular approaches. Treatment with 100 uM of H2O2 for 1 h given 24 h following the differentiation commitment, decreased cell viability. C2C12 exposed to H2O2 showed a significant number of apoptotic and necrotic cells, and mitochondria appeared emptied, with cristae heavily damaged. To evaluate the effect of CPE on myoblast viability and myotube formation, 10 μg/ml of CPE were added at the beginning of the differentiation; then the medium was changed with a new one containing fresh CPE every 24 h. CPE protected C2C12 myoblasts from H2O2-induced oxidative damage both at early and late (immediately or six days postoxidative challenge, respectively) phases of differentiation, preventing cell death and mitochondrial damage. Indeed, after 6 days the number of mitochondria in myotubes (per area of cell surface) increased 2-fold in both control and in CPE-supplemented/H2O2-treated C2C12, and mitochondria showed a greater extension of cristae, as compared to the beginning of differentiation. CPE-supplemented monolayers showed surface and inner cell features comparable to the untreated ones both at early and late stages, suggesting that CPE supplementation significantly mitigated the effect of H2O2. Preliminary data obtained calculating the myogenic index (Giemsa staining) suggested that CPE-supplemented cells were partially protected from H2O2-induced myogenesis inhibition. More thorough studies atthe biochemical level are being performed to unravel the molecular mechanism(s) of CPEprotecting activity.On the whole, CPE supplementation seems to preserve the mitochondrial integrity and the myogenic differentiation ability of oxidatively injured C2C12 ensuing further nutraceutcal perspectives.
IIM Meeting 2021 • 22-24 October 2021

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P24

Platelet rich plasma affects voltage- dependent gap junctional features in TGFβ1- induced myofibroblasts via Vascular Endothelial Growth Factor-A signaling in vitro

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Increasing evidence supports the antifibrotic potential of Platelet Rich Plasma (PRP), but currently there is not a clear consensus and the cellular and molecular mechanisms underpinning PRP action need to be clarified. We have recently demonstrated the capability of PRP to prevent the in vitro differentiation of fibroblasts towards myofibroblasts induced by transforming growth factor (TGF)-β1. In addition PRP is able to reduce the expression of α-smooth muscle actin (sma), a well-known myofibroblastic marker, engaging the vascular endothelial growth factor (VEGF)-A/ VEGF

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Receptor-1-Mediated signaling. Finally PRP can abolish the occurrence of the TGF-β1-induced voltage-dependent gap junction (GJ) currents, while preventing the expression of connexin (Cx)43, the typical Cx isoform forming voltage-dependent connexons. By the dual whole cell-patch clamp technique and the parallel morphological analysis, we evaluated the involvement of VEGF-A/VEGF receptor in the PRP-induced response of NIH3T3 fibroblasts cultured in the presence of TGF-β1 in vitro, focusing on GJ currents and Cx43 expression. Our results have demonstrated that the VEGF-A neutralization, by blocking antibodies, or the VEGFR inhibition by KRN633 during differentiation are able to prevent the PRP-promoted effects on the GJ currents and Cx43 expression. At last we have confirmed the role of VEGF-A signaling in inhibiting these events by the treatment of fibroblasts with soluble VEGF-A. This study contributes to provide new insights into the mechanisms behind PRP action on preventing fibroblast to myofibroblast differentiation, and recognizes GJ communication as a crucial target of the VEGF-A mediated pathway.
IIM Meeting 2021 • 22-24 October 2021

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P.25
Mitochondrial metabolism regulates muscle homeostasis in adulthood and aging

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Upon physiological stimuli, skeletal muscle mitochondria efficiently accumulate Ca2+ via an electrogenic pathway, that relies on the driving force of a steep electrochemical gradient. A [Ca2+]mt peak occurs in parallel to agonist-induced [Ca2+]cyt increases, thanks to the activity of the Mitochondrial Calcium Uniporter (MCU), the selective channel responsible for mitCa2+ (mitochondrial Ca2+) accumulation. We have demonstrated that skeletal muscle-specific MCU deletion inhibits mitCa2+ uptake, impairs muscle force and exercise performance. MitCa2+ uptake is required for effective glucose oxidation, as demonstrated by the impaired oxidative metabolism in muscle-specific MCU-/- myofibers. The decreased pyruvate dehydrogenase activity is the main trigger of this metabolic rewiring. Although defective, mitochondrial activity is partially sustained by increased fatty acid oxidation. Here, we have investigated the role of mitCa2+ uptake during skeletal muscle aging. We show that mitochondrial Ca2+ accumulation decreases in old mice and this condition is accompanied by a decreased pyruvate dehydrogenase (PDH) activity. We demonstrate a rewiring of skeletal muscle metabolism toward fatty acids rather than glucose oxidation. Finally, we targeted pharmacologically the pyruvate dehydrogenase kinase to modulate PDH activity in aged muscles restoring the normal oxidative metabolism.
IIM Meeting 2021 • 22-24 October 2021

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P.26
Structure-function characterization of a DUX4 inhibitor in FSHD muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is a progressive myopathy due to complex genetic and epigenetic mechanisms. The disease is associated to loss of repression of the transcription factor double homeobox 4 (DUX4), which is toxic to skeletal muscle causing muscle wasting. DUX4 expression is normally confined to the cleavage stage of embryonic development where it activates the expression of coding and non-coding genes required for implantation and early development. DUX4 expression is shut off at the 8-cell stage and remains silenced in most somatic tissues. In FSHD, DUX4 gain of expression leads to impaired muscle differentiation, increased sensitivity to oxidative stress and activation of cell death via apoptosis causing muscle wasting. Therefore, blocking DUX4 expression or activity represent plausible therapeutic options for FSHD. Previous analyses from our group identified MATRIN 3 (MATR3) as the first endogenous protein able to inhibit both DUX4 expression and activity. MATR3 directly binds to DUX4 and prevents his toxicity in FSHD muscle cells. The aim of our project is to characterize the interaction between DUX4 and MATR3 and identify the minimal MATR3 region necessary and sufficient to inhibit DUX4. To this purpose, we expressed and purified different DUX4 and MATR3 fragments to test their interaction using in vitro pull-down experiments. Next, MATR3 fragments were tested for their ability to block activation of DUX4 target genes, and rescue cell death and myogenic defects of FSHD muscle cells. Though this work, we mapped the MATR3 activity to a small peptide. We are currently combining protein engeneering with ultrastructural studies to generate a drug-like DUX4 inhibitory molecule that will be tested in cellular and animal models of FSHD.
IIM Meeting 2021 • 22-24 October 2021

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P.27

Myogenesis stimulation of pluripotent stem cell-derived mesodermal progenitors in a Notch-dependent manner by valproic acid

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Muscular dystrophies are debilitating neuromuscular disorders affecting both cardiac and skeletal muscles for which no cure exists. Thus, patients would benefit from a cellular therapy that can simultaneously regenerate both tissues. The current protocol to derive bipotent mesodermal progenitors relies on the spontaneous formation of embryoid bodies, thereby hampering further clinical translation. Additionally, as skeletal muscle is the largest organ in the human body, a high myogenic potential is necessary for successful regeneration. Here, we have optimized a protocol to generate chemically defined induced pluripotent stem cellderived mesodermal progenitors (cdMiPs). We demonstrate that cdMiPs contribute to myotube formation and still differentiate into cardiomyocytes, both in vitro and in vivo.[1] Furthermore, the addition of valproic acid, a clinically approved small molecule, increases the potential of the cdMiPs to contribute to myotube formation without compromising their ability to differentiate towards cardiomyocytes. Moreover, dystrophic mice injected with valproic acid pre-treated cdMiPs show a milder dystrophic phenotype and increased muscle strength and amelioration in functional performances. This effect is mediated through the activation of the Notch signalling pathway. In conclusion, we provide a novel protocol to generate mesodermal progenitors with enhanced myogenic potential using clinically approved reagents, which can be further explored for the treatment of preclinical animal models of muscular dystrophy.

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IIM Meeting 2021 • 22-24 October 2021

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P.28

Beneficial effects of boosting skeletal muscle metabolism by SIRT1activator in DMD mouse model

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Duchenne muscular dystrophy (DMD) is a severe and relentlessly progressive myopathy caused by out-of-frame or nonsense mutations in the X-linked DMD gene. DMD is a complex disease and multiple approaches are needed to target pathological processes, both the underlying genetic mutations and the secondary complications. Despite several therapeutic options have been developed with good results, more effective treatment options are essential and may be generated through the definition of novel therapeutic targets. A muscular metabolic dysregulation is an established DMD feature with mitochondrial dysfunctions as one of the earliest deficits that arise from multiple cellular stressors. Among metabolic regulators, Sirtuin 1 (SIRT1) represents an intriguing candidate since it acts on different aspects of cellular metabolism, regulating energy homeostasis, mitochondrial biogenesis, and inflammation. SIRT1 overexpression represents an important counter-mechanism to alleviate the dystrophic phenotype and its pharmacological modulation could be relevant as well in DMD conditions. Consistently, SIRT1 activation by selective compound, i.e., SRT2104, has already been proven to reinforce muscular structure, mitochondrial functionality, and to reduce inflammation. SRT2104 has never been tested in muscular diseases; therefore, considering its metabolic and immunomodulatory effects, we tested SRT2104 as an attractive candidate for DMD treatment. Accordingly, in our preliminary data, long-term SRT2104 administration improved muscle force and stimulated oxidative capacity. This was paralleled by reduced fibrosis and inflammatory infiltrate and increased regeneration in mdx muscle. In conclusion our results demonstrate the efficacy of SRT2104 as a new SIRT1 activator in DMD and highlight that considering DMD also as a metabolic disease and treating it as such, could provide important therapeutic strategies additional to gene therapies.

IIM Meeting 2021 • 22-24 October 2021

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P.29

Possible mechanisms underlying the dynamic assembly of Calcium Entry Units: the role of temperature and pH

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The function of skeletal muscle fibers is finely regulated by intracellular Ca2+ levels. Two main mechanisms control movements of Ca2+ ions from intracellular stores (i.e. the sarcoplasmic reticulum, SR) and from extracellular space: i) excitation-contraction (EC) coupling; and ii) store-operated Ca2+ entry (SOCE). SOCE is a mechanism that allows recovery of extracellular Ca2+ during prolonged muscle activity, when the SR undergoes depletion. We recently discovered that prolonged exercise leads to formation of Calcium Entry Units (CEUs), intracellular junctions located at the I band formed by two elements: SR-stacks and transverse tubules (TTs). Assembly of CEUs during exercise promotes the interaction between STIM1 and Orai1, the two main proteins that mediate SOCE, and increases muscle resistance to fatigue in presence of extracellular Ca2+. However, the molecular mechanisms underlying the exercise-dependent remodeling of SR and TT leading to CEU assembly remain to be fully elucidated. Here, we first verified whether CEUs can assemble ex-vivo (in absence of blood supply and innervation) subjecting excised EDL muscles from mice to an ex-vivo incremental fatigue protocol (80Hz tetanus stimulation lasting 45 minutes). The data collected demonstrate that CEUs can assemble ex-vivo in EDL muscles isolated from the animals. We then evaluated if intracellular parameters that are affected by exercise, such as temperature and pH, may influence the assembly of CEUs. We found that higher temperature (25°C vs. 36°C) and lower pH (7.4 vs. 7.2) promotes the formation of CEUs increasing the % of fibers containing SR stacks, the n. of SR stacks/area, and also the elongation of TT at the I band. Importantly, increased assembly of CEUs at higher temperature (36oC) or at lower pH (7.2) correlated with increased fatigue resistance of EDL muscles in presence of extracellular Ca2+, suggesting that CEUs assembled ex-vivo are functional.
IIM Meeting 2021 • 22-24 October 2021

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P.30

Regulation of vascular cells in Duchenne Muscular Dystrophy

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Skeletal muscle is highly vascularized. During normal muscle regeneration, endothelial cells (ECs) play important roles, by interacting with muscle stem cells, therefore sustaining myogenesis. Duchenne Muscular Myopathy (DMD) is characterized by permanent cycles of myofiber necrosis/regeneration, triggering myofiber loss, fibrosis and adiposis. In that context, the role of ECs remains poorly known. Interestingly, alterations were shown in the vascular bed of the mdx mouse model of DMD. In human, studies from the 70's reported alterations of the capillary basal membrane. Our aim is to define the interactions that ECs develop with their environment in DMD and to identify the molecular pathways at work. Using ECs isolated from mouse muscle and the Matrigel plug assay in vivo, we observed that mdx-derived ECs have a higher capacity to proliferate and to colonize the plugs. In vivo we observed an increase of capillary density and capillary volume in both human and mouse DMD. However, analysis of the vessel coverage by pericytes showed an increase of uncovered vessels in mdx muscle, as compared with WT, the uncoverage being increasing in the fibrotic areas. FibroAdipoPrecursors (FAPs) are more numerous in the DMD muscle and participate to fibrosis. Since fibroblastic cells have angiogenic properties, we investigated in FAP:EC interactions. The Matrigel plug assay showed that FAPs derived from mdx muscle stimulated angiogenesis better than WT-FAPs, this stimulation being emphasized in Mdx recipient as compared with WT. Moreover, analysis of gene expression in ECs isolated from mdx and DMD muscles showed a dysregulation of genes involved in EC:pericyte interactions and in basal lamina production, as compared with healthy muscle. These results suggest alterations of FAP:EC interplay in DMD muscle, that may lead to a dysregulation of vessel maturation and organization. The next step is to dissect the interactions between FAPs, ECs and pericytes at the molecular level.
IIM Meeting 2021 • 22-24 October 2021

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P.31
Understanding the crosstalk between cancer and skeletal muscle

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IIM Meeting 2021 • 22-24 October 2021

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P.32
Inducible CRISPR/Cas9 strategy mediates efficient gene editing of trinucleotide repeat expansion in DMPK locus

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Myotonic dystrophy type 1 (DM1) is an autosomal dominant disorder, characterized byprogressive myopathy, myotonia and multiorgan involvement, for which no cure is yet available. The pathogenic mechanism of the disease involves a CTG-repeats expansion in the 3' untranslated region of the DMPK (dystrophia myotonica protein kinase) gene. The mRNAs transcribed from the expanded allele are trapped in cell nuclei, where they form abnormal hairpin-like structures that accumulate as foci in the nucleus and bind with high affinity proteins of the Muscleblind-like (MBNL) family. The loss of function of MBNL proteins leads to aberrant alternative splicing of many transcripts, resulting in a fetal-like splicing pattern in patient with DM1. To identify a therapeutic strategy aimed at eliminating the pathogenic mutation, we have developed a gene modification strategy using a drug-inducible and tissuespecific CRISPR/Cas9 system to delete CTG repeats in the human DMPK locus. We have demonstrated that this strategy determines time-limited gene editing in proliferating and post-mitotic myogenic cell models generated by fibroblasts derived from patients with DM1. Removal of CTG expansion is accompanied by reduction of ribonuclear foci and partial recovery of normal splicing. Furthermore, we have evaluated this approach in a wellcharacterized DM1 mouse model carrying a mutated human DMPK gene with >1,000 CTG repeats. We demonstrated that a single intramuscular injection of recombinant AAV9 vectors expressing CRISPR-Cas9 components into the tibialis anterior muscle of DM1 mice was sufficient to achieve inducible gene editing in vivo. Given that this treatment results in mutation removal from the genome, reduces the accumulation of expanded transcripts and minimizes the potential occurrence of unintended events in off-target genomic loci, it could open the way for future gene therapy application in humans.
IIM Meeting 2021 • 22-24 October 2021

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P.33
AMPKα2 is a satellite cell intrinsic regulator of myonuclear accretion

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Skeletal muscle satellite cells (MuSCs) are activated in response to injury, and differentiate and fuse to contribute to the regeneration of skeletal muscle fibers (i.e., myogenesis). This cell fate progression is accompanied by a change in cellular metabolism, which is coordinated by intra- and extracellular metabolic cues that are integrated through the metabolic sensor AMPK. We hypothesized that AMPKα2 plays an important role in the regulation of myogenesis. To study myogenesis in vivo, skeletal muscle injury was provoked by cardiotoxin (CTX) injection in mouse tibialis anterior (TA), and regeneration was assessed 14 days post injury (d.p.i.). Individual steps of myogenesis were further dissected in vitro, using FACS sorted MuSCs. In line with a role for AMPKα2 in myogenesis, mice lacking AMPKα2 had a lower relative TAmass and mass-specific force at 14 d.p.i. This myogenesis defect was recapitulated in vitro, asMuSCs isolated from AMPKα2 KO mice had a lower fusion index after 48h in differentiationmedium. Conversely, wildtype (WT) MuSCs treated with the AMPKα2 activator 991 show anincreased fusion index. The relative MyoG mRNA expression and the percentage of MyoG+cells were unaltered in AMPKα2 KO MuSCs after 24h in differentiation medium, showing nodefect of differentiation. Nevertheless, a specific fusion assay showed a decreased fusion inAMPKα2 KO MuSCs. Interestingly, live imaging during 12h of fusion assay revealed nodifference in the number of myoblast-

myoblast fusion events. However, in a specificmyonuclear accretion assay we observed a lower rate of fusion between AMPKα2 KOmyoblasts and WT myotubes than between WT myoblasts and WT myotubes. Finally, fusion ofsatellite cells labelled with EdU during 5-14 d.p.i. (i.e., after the formation of myofibers) was diminished in AMPKα2 deleted mice. Together, this shows a role for AMPKa2 in skeletal muscle regeneration, specifically through the regulation of myonuclear accretion.
IIM Meeting 2021 • 22-24 October 2021

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P.34

Pathogenic variants in the myosin chaperone UNC-45B cause progressive myopathy with eccentric cores

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The myosin-directed chaperone UNC-45B is essential for sarcomeric organization and muscle
function from Caenorhabditis elegans to humans. The pathological impact of UNC-45B in muscle disease remained elusive. We report ten individuals with biallelic variants in the UNC45B gene, who exhibit childhood onset progressive muscle weakness. We identified acommon UNC45B variant which acts as a complex hypomorph splice variant. PurifiedUNC-45B mutant proteins showed changes in folding and solubility. In situ localizationstudies further demonstrated reduced expression of mutant UNC-45B in muscle combined with abnormal localization away from the A-band towards the Z-disk of the sarcomere. Thephysiological relevance of these observations was investigated in C. elegans by transgenicexpression of conserved UNC-45 missense variants, which showed impaired myosin bindingfor one and defective muscle function for three. Together, our results demonstrate thatUNC-45B impairment manifests as a chaperonopathy with progressive muscle pathology,which discovers the previously unknown, conserved role of UNC-45B in myofibrillar organization.
IIM Meeting 2021 • 22-24 October 2021

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P.35

Extracellular matrix protein tenascin-c is required to maintain the muscle stem cell pool and promotes skeletal muscle regenerative potential

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Aging is associated with a decline in skeletal muscle mass and function. Aged individuals are confronted with a reduction of the regenerative potential due to a decrease of muscle stem cell (MuSC) function, and dysregulation of the tissue microenvironment, including extracellular matrix (ECM) components. ECM components provide structural integrity and stiffness to the tissue and modulate MuSC myogenic potential. However, how ECM-related mechanisms regulate such decline in mass and function in aged muscles is poorly understood. We have identified tenascin-c (TNC) as an ECM protein expressed by MuSC and relevant to promote expansion of fetal MuSC. It is known that TNC levels decrease with aging. However, whether this reduction affects MuSC function is currently unexplored. Here, we show that genetic deletion of TNC induces a postnatal decrease in MuSC numbers and migration. After birth, the muscle is growing and reaching homeostasis, while MuSC progressively enter a stem-like stage. Such decline in MuSC numbers is more pronounced during muscle repair, and the muscle-regenerative response is delayed in the TNC-knockout mouse. This reduction of the MuSC pool size is due to premature myogenic commitment and impaired self-renewal capacity. Our transplantation experiments indicate that TNC in the MuSC-microenvironment plays a major role in regulating MuSC myogenic capacity. Overall, mice lacking TNC recapitulate a premature aging phenotype. Furthermore, in vitro TNC treatment promotes MuSC migration and delays differentiation, suggesting that TNC participates in maintaining the MuSC pool. Since TNC expression is reduced in aged muscle, ongoing experiments will determine whether restoring TNC levels will rejuvenate the myogenic and migratory properties of aged MuSC. These findings will improve the understanding of the complex interplay between MuSC and their microenvironment and aid in developing new therapeutic approaches using TNC to enhance aged muscle repair.
IIM Meeting 2021 • 22-24 October 2021

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P.36

Studying acetylcholine receptors and muscle regeneration in ALS models to develop prognostic markers and potential therapies hampering disease progression

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Amyotrophic lateral sclerosis (ALS) is a heterogeneous disease with high variability in the speed of progression even in cases with a defined genetic cause such as superoxide dismutase 1 (SOD1) mutations. SOD1G93A mutation on mice with distinct genetic backgrounds (C57 and 129Sv) show consistent differences in speed of disease progression and lifespan resembling what is observed in ALS patients. We recently hypothesized that the difference in the peripheral neuromuscular system rather than the extent of spinal motor neuron loss reflects the phenotypic difference between these two mouse models. Therefore, we redirect our attention to the skeletal muscle as an early component of ALS pathogenesis aiming to discover the molecular mechanisms contributing to the distinct phenotypes and to identify factors underlying fast and slow disease progression. In this work, we compare the functional, morphological and molecular profiles of the gastrocnemius muscle (GCM) from these two SOD1G93A mouse strains at the pre-symptomatic and onset stage of the disease. Data collected clearly defined the extent of muscle denervation, NMJ stability and muscle regeneration as a discriminator between rapidly and slowing progressing ALS mice. Notably, these results demonstrated that slow-progressing mice, despite the premature muscle atrophy, activate different compensatory mechanisms including the expression and clustering of the AChR, myogenesis and inflammatory response, that delay the onset of their symptoms. On the contrary, in fast-progressing mice initial muscle denervation and atrophy were concomitant with muscle strength loss. This study highlights a set of key gene and molecular pathway indices of fast or slow disease progression, which may prove useful in identifying potential disease modifiers responsible for the heterogeneity of human amyotrophic lateral sclerosis and which may represent valid therapeutic targets for ameliorating the disease course in humans.

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P.37

Human cardiac organoids as a model for Duchenne muscular dystrophy cardiomyopathy

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Duchenne Muscular Dystrophy (DMD) is an X-linked recessive neuromuscular disorder characterized by DYSTROPHIN (DYS) deficiency. DYS is a sarcolemmal protein which mainly links the cytoskeleton to the extracellular matrix (ECM), by interacting with the dystrophin glycoprotein complex (DGC). Dilated Cardiomyopathy (DC) represents the major cause of death in late-stage DMD patients. To model the cardiac DMD degeneration, we generated self-aggregated DMD cardiac organoids (DMD-COs), by differentiating DMD-hiPSCs into DMD cardiomyocytes (DMD-CMs) within agarose microwells (1). Experiments of histological examination, gene expression and protein localization were performed focussing on genes and markers involved in proliferation, maturation, stress and apoptosis. DMD isogenic cardiac organoids (DMD-Iso-COs) previously generated by CRISPR-Cas gene editing were employed as controls. In this study, we found that DMD-COs were able to exhibit DMD hallmarks, including the loss of the contractile function, fibrosis, myocyte partial replacement with connective tissue and fat and the downregulation of epigenetic regulators involved in DC onset. On the basis of these findings, we envision to develop a more complex, realistic and reliable in vitro 3D model to study DMD-related cardiomyopathies.

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P.38
Aptamer-conjugated gold nanoparticles for selective microRNAs delivery in dystrophic muscles

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Duchenne Muscular Dystrophy is the most common and severe form of genetic dystrophy affecting around 1 in every 5000 new-born boys. It is caused by the lack of dystrophin protein, that leads to a progressive loss of muscle mass that is then substituted by fibrotic and adipose tissue. Patients usually die within the third decade of life due to diaphragm failure or cardiac complications. Current strategies aimed at restoring functional dystrophin in DMD boys are facing several limitations, including the vast extension of the target tissue and the length of the dystrophin gene. Within this context, we have recently developed a novel approach based on gold nanoparticles (AuNPs) for targeted delivery of oligonucleotidesand drugs into muscle stem (satellite) cells in dystrophic mice. To obtain specific delivery, we have functionalized the therapeutic AuNPs with an aptamer against the a7/b1 integrin dimer, a protein dimer highly enriched in the surface of skeletal muscle, including satellite cells. In addition, to show their efficiency as a therapeutic delivery system we conjugated the AuNPs to microRNA-206, a potent regulator of satellite cells function and also a paracrine molecule in regenerating muscle. Our functional experiments in dystrophic mice showed that systemic delivery of AuNPs containing microRNA-206 efficiently target satellite cells leading to improved skeletal muscle regeneration in vivo, indicating our AuNPs as a good future candidate for a new specific therapy in DMD.
IIM Meeting 2021 • 22-24 October 2021

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P.39
A novel approach to target BMP-resistance in cancer cachexia skeletal muscle atrophy

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IIM Meeting 2021 • 22-24 October 2021

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P.40
Dysregulation of heme synthesis-export axis in skeletal muscle reshapes energetic metabolism and results in impaired motor performance

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Heme metabolism plays an essential role in the maintenance of skeletal muscle health. Feline leukemia virus subgroup C receptor 1a (FLVCR1a) is a plasma membrane heme exporter that, by removing the intracellular excess of heme, limits the feedback inhibitory effects of heme on its own production, thus sustaining heme biosynthesis. Being part of the heme synthesisexport functional axis, heme export by FLVCR1a has been reported to sustain the tricarboxylic acid cycle (TCA) flux and the electron transport chain (ETC) activity in tumors. Here we generate skeletal muscle-specific Flvcr1a-null mice and analyze the impact of disrupted heme synthesis-export axis in this tissue. Metabolic data obtained in gastrocnemius show that, upon deletion of Flvcr1a, the activity of TCA cycle enzymes and ETC complexes is increased, along with glutaminolysis and fatty-acid beta oxidation. Conversely, the activity of glycolytic enzymes is reduced. Flvcr1a deletion also affects muscle morphology. The number of fibers expressing the slow-oxidative myosin heavy chain isoform is increased in Flvcr1anull mice compared to controls and the average cross-sectional fibers' area is reduced. In motor behavior tests, Flvcr1a knockout mice show worse performance compared to controls, a phenotype exacerbated by aging. Collectively, these results hint that FLVCR1a is involved in the regulation of energetic metabolism in skeletal muscle, and that the heme synthesis export system is important to maintain proper muscle function.
IIM Meeting 2021 • 22-24 October 2021

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P.41

Is the development of the neuromuscular junction altered in dystrophic mice?

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Duchenne muscular dystrophy (DMD) is characterized by the absence of dystrophin, a cytoskeletal protein that connects several protein complexes, including those maintaining the proper organization of the neuromuscular junction (NMJ). Denervation or partial overlap of pre- and post-synaptic structures are observed, and the endplate acquires a fragmented structure in DMD patients and in all animal models of the disease. Functional changes are also observed (Ng and Ljubicic, 2020), with reduced postsynaptic response to ACh and a compensatory increase in quantal content (van der Pijl et al., 2016). The damage to the NMJ can impair neuromuscular transmission and contribute to muscle weakness, exacerbating disease progression (Lovering et al., 2020). Since NMJ fragmentation is also observed in acutely injured or ageing mouse muscles, it is still debated whether the damage to the NMJ is due to the absence of dystrophin or to cycles of damage and regeneration of muscle fibres (see Kong et al., 2012 vs. Haddix et al., 2018 for recent contributions on the two opposing views). In either case, in the dystrophic muscle, ongoing reinnervation of newly formed muscle fibres is required to maintain muscle function. Even in animals transplanted with wt satellite cells, NMJs in muscle fibres containing donor derived cells are often fragmented, in line with experiments showing no impact of dystrophin levels up to 20% of normal (van der Pijl et al., 2018). It is assumed that reinnervation follows the same steps occurring in the development of the NMJ in young animals. These events are altered in mdx mice, at least in terms of synapse elimination (Miniatel et al., 2003). To try and address this controversy, we have studied the functional changes of the NMJ in the first 3 weeks of animal life, when muscle damage is reportedly minimal, but structural changes are detected.
IIM Meeting 2021 • 22-24 October 2021

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P.42

The Bromodomain and Extra-terminal domain inhibitor JQ1 ameliorates muscle function in aged mice

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During aging, skeletal muscle progressively reduces its mass, leading to a loss of muscle strength and function, defined as sarcopenia. Previous reports from our group showed that bromodomain and extra-terminal domain (BET) protein BRD4 plays a role in promoting muscle wasting in DMD and cachexia. Here, we evaluated the role of BRD4 in sarcopenia, by blocking its action through administration of the BET inhibitor JQ1, to old mice. Young (3 months) or old (24 months) mice were treated daily with JQ1+ (20mg/kg) or the inactive enantiomer JQ1- , for 24 days. During treatment, mice were weighed, and muscle performance was evaluated with the treadmill and grip tests. After sacrifice, different muscles and several tissues were isolated and collected for morphological and molecular analysis, by RNA-seq, Western Blot and IHC. Our data showed a marked reduction in mouse weight in old-JQ1+-treated mice. Moreover, we observed a beneficial effect mediated by JQ1+ administration in muscle performance of old mice, when compared to muscle function in old mice treated with JQ1. In addition, we observed a significant increase in the number of oxidative fibres in old mice following JQ1+ treatment, and a marked reduction in fibrosis. RNA-seq data confirmed a decrease in the expression profile of pro-fibrotic as well as pro-inflammatory genes. Immunoblot analysis revealed a rescue of autophagy and the activation of the AMPK pathway. In conclusion, our data suggest that JQ1+ treatment induces a functional rescue in muscle of old mice, and may be beneficial in the treatment of sarcopenia.
IIM Meeting 2021 • 22-24 October 2021

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P.43

Genome wide DNA binding profile of DUX4 in facioscapularhumeral muscular dystrophy

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FacioScapuloHumeral muscular Dystrophy (FSHD), is linked to reduction in copy number and/or epigenetic alterations of the D4Z4 macrosatellite repeat. These changes lead to aberrant reactivation of the D4Z4-encoded double homeobox4 (DUX4), a transcription factor highly toxic to skeletal muscle. In FSHD, DUX4 is expressed only in a minority of myonuclei. For this reason, so far direct DUX4 targets have been defined by ectopically overexpressing the protein. To identify direct targets of the endogenous DUX4, we adapted Cleavage Under Targets and Tagmentation (CUT&Tag) to human primary myonuclei extracted from FSHD muscle cells. In CUT&Tag, DUX4 is bound in situ by a specific antibody, which then tethers the A-Tn5 transposase fusion protein. Activation of the transposase generates DNA fragments, which are directly amplified and sequenced. CUT&Tag is characterized by high resolution and low background, and is amenable to very lower inputs. This is relevant since FSHD is due to burst of DUX4 expression in a small number of myonuclei. Initial studies in which CUT&Tag fragments were analyzed by qPCR showed a positive signal by DUX4 antibodies on selected target genes. Importantly, the DUX4 CUT&Tag-qPCR signal was strongly decreased by DUX4 knockdown. Supported by these findings, we performed RNA-seq and CUT&Tag-seq in primary FSHD muscle cells following control or DUX4 knockdown. Preliminary data from RNA-seq experiments revealed 130 significantly deregulated genes. The genes identified showed 40% of overlap with DUX4-regulated genes described across distinct cellular contexts indicating that this model recapitulates important aspects of FSHD biology. Results from this pilot study will provide, for the first time, the direct targets of the endogenous DUX4 in primary FSHD muscle cells opening the possibility to study the biology of the protein in its natural disease contest to better understand the mechanism behind DUX4-mediated cytotoxicity and develop possible treatments.
IIM Meeting 2021 • 22-24 October 2021

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P.44

Ageing causes ultrastructural modification to calcium release units and mitochondria in cardiomyocytes

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The function of striated muscles (skeletal fibers and cardiomyocytes) relies on appropriate supply of ATP and Ca2+ ions to the contractile elements. ATP is provided by mitochondria during cellular respiration, and Ca2+ needed for contraction is released by the sarcoplasmic reticulum (SR) during excitation–contraction (EC) coupling. The intracellular units deputed to EC coupling are named Ca2+ release units (CRUs) and are formed by the association of the SR with transverse (T)-tubules. We analyzed mitochondria and CRUs in hearts of adult (4 months) and aged (≥24 months) mice. Analysis by confocal and electron (EM) microscopy revealed that: (a) in aged hearts mitochondria are improperly disposed and often damaged (severely damaged mitochondria: adults 3.5 ± 1.1%; aged 16.5 ± 3.5%); (b) CRUs are often misoriented and/or misplaced from the correct position. A quantitative EM analysis of CRUs points to a decrease in their frequency (adult: 5.1 ± 0.5 / 50 μm2; aged: 3.9 ± 0.4 / 50 μm2) and size (adult: 362 ± 40 nm; aged: 254 ± 60 nm). Disarray of the EC coupling system could be in part caused by the decreased expression of Cav-3 and JP-2 detected by western blot (WB), two proteins involved in formation of T-tubules and in docking SR to T-tubules in dyads. By WB analysis, we also detected increased levels of 3-NT in whole hearts homogenates of aged mice, a recognized marker of oxidative stress. The changes in morphology and disposition of mitochondria and CRUs highlighted by our results may underlie an inefficient supply of Ca2+ ions and ATP to the contractile elements, and possibly contribute to cardiacdysfunction in ageing.
IIM Meeting 2021 • 22-24 October 2021

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P.45

Mitochondrial calcium signaling in duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a progressive and X-linked muscle wasting disease caused by the lack of dystrophin, essential protein for sarcolemma stability. Calcium dysregulation, a central event in dystrophic muscles, contributes to muscle degeneration, altered regeneration and mitochondrial dysfunction. Indeed, high cytosolic calcium levels determines the opening of the mitochondrial permeability transition pore and eventually cell death. Previous studies demonstrated that in mdx mice, a widely used animal model of DMD, cytosolic calcium transients and mitochondrial calcium uptake are increased compared to wild type mice, suggesting that the restoration of physiological Ca2+ transients may protect from muscle degeneration. However, in healthy skeletal muscles, downregulation of MCU, the highly selective channel responsible for mitochondrial Ca2+ uptake, triggers atrophy. Thus, we wish to clarify whether, in mdx skeletal muscle, MCU downregulation exerts a beneficial effect or rather triggers muscle dysfunction. For this purpose, we used different genetic strategies, including MCU silencing by AAV9 particles injection or deletion of MCU allele(s) in the mdx mouse.
IIM Meeting 2021 • 22-24 October 2021

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P.46

Jab1 deletion in muscle lineage causes a muscular dystrophy that resembles LAMA2 disease

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Merosin deficient congenital muscular dystrophy (or LAMA2 disease) is an autosomal recessive disorder characterized by progressive wasting muscular dystrophy, dysmyelinating neuropathy and brain abnormalities. This disorder is caused by mutations in the LAMA2 gene, which encodes for the laminin211, the primary component of the Schwann cell and muscle basal lamina. We recently identified Jab1 as a nuclear molecule downstream the laminin211 pathway and showed that Jab1 regulates Schwann cell behavior through the modulation of p27Kip1 levels. Interestingly, loss of Jab1 in Schwann cells results in a dysmyelinating neuropathy that phenocopies the neuropathy of LAMA2 disease. To evaluate whether Jab1 plays also a role in the pathogenesis of LAMA2 muscular dystrophy, we generated mice with conditional inactivation of Jab1 in the muscle lineage through the MyoDi-cre transgene. Mice with deletion of Jab1 in skeletal muscle (named Jab1-MscKO) showed progressive motor deficits, reduced lifespan and overt muscular dystrophy phenotype. Muscles appeared smaller in size and presented myopathic features such as reduced fiber diameter, presence of necrotic fibers, inflammatory cells and fibrosis. The evaluation of satellite cell number showed a reduction of proliferating satellite cells, suggesting a defect of cell cycle progression. Muscle regeneration, induced by cardiotoxin injection, was significantly impaired in Jab1-MscKO mice. Jab1-MscKO muscles showed increased of p27Kip1 expression. Genetic deletion of p27Kip1 in muscles of Jab1-MscKO mice revealed a mild, but not significant, amelioration of motor performances and muscle pathology, while reduced inflammatory infiltrates and ameliorated satellite cells cycling. Our results suggest that Jab1 is involved in the pathogenesis of LAMA2 muscular dystrophy. The role of p27Kip1 is less clear.
IIM Meeting 2021 • 22-24 October 2021

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P.47

Metabolomic, transcriptomic and epigenomic effects of oncosuppressor microRNAs in pediatric rhabdomyosarcoma

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Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma of childhood arising from undifferentiated skeletal muscle cells. Currently used therapies are poorly tumor-specific and fail to tackle the molecular machinery underlying the tumorigenicity and uncontrolled proliferation of RMS1. We recently described two miRNAs that have a positive impact on the myogenic commitment of RMS2. Here, we aimed at assessing the

effects of oncosuppressor miRNAs in RMS. We first identified a selection of miRNAs poorly present in samples from patients diagnosed with RMS. We then performed RNAseq and metabolomic after miRNA perturbation in both human and mouse RMS models which showed a wide shift at the transcriptome and metabolome level of miRNA-treated samples. The in vitro and in vivo experiments showed beneficial effects on several oncogenic hallmarks, including reduced proliferation and migration both in vitro and in vivo. Thus, we checked the possible effects at the epigenomic level, and observed a shift towards hypomethylated state of miRNA-treated RMS cells. To further validate the transcriptome-epigenome interaction after miRNA treatment, we are now perfoming multiome scATACseq/RNAseq to identify the effects at the single cell level. In conclusion, in our study we identified a novel miRNA combination tackling the tumorigenic features of RMS by reducing its cancer stemness, proliferation and metastatic potential.

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P.48

Muscle extracellular matrix engineered with mesenchymal stem cells derived extracellular vesicles leads to functional muscle recovery in a murine model of volumetric muscle loss

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Decellularized muscle tissue are the best support to improve muscle recovery when a serious muscle mass loss occurs. However, at long term, the development of fibrosis jeopardizes the muscle functional recovery. This work aims at evaluating the muscle regeneration effect of Mesenchymal Stromal Cells Extracellular vesicles (MSC EVs) when used as stimuli in an in vivo murine model of Volumetric Muscle Loss (VML). Healthy mouse muscles were decellularized using a detergent enzymatic treatment and used as scaffold in the damaged muscle. MSC EVs isolated from Wharton Jelly cells were characterized. An in vivo murine model (C57Bl/6 wild type mice) was created after ablation of tibialis anterior muscle. As muscle reconstruction treatment, the decellularized scaffold was inserted in the site of damage and firstly embedded with MSC EVs. Secondly, a new administration of MSC-EVs was performed after 72h after damage via intraperitoneal injection. The control group was treated with PBS alone. After 7 and 30 days from the surgical procedure, animals were euthanized and TA recovered for multiple analysis. The effect of MSC-EVs was evaluated analyzing (1) the macrophage compartment, (2) the angiogenesis, (3) the myogenic and the fibrosis processes by immunofluorescence, qRT-PCR and with cytofluorimetric analysis from freshly isolated cells. 30 days after damage, functional analysis was performed. The pro-regenerative macrophages population was increased after MSC EV treatment, as well as angiogenesis, myogenesis improved. Fibrosis decreased at later time point and force recovery was ameliorated only in MSC EV treated mice. Thus muscle regeneration and strength functional recovery was triggered by the combination of decellularized muscle scaffold embedded with human MSC EVs.

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P.49

The dominant-negative mitochondrial calcium uniporter subunit MCUb drives macrophage polarization during skeletal muscle regeneration

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Mitochondrial calcium uptake plays a key role in modulating cell metabolism, cell survival and other cell specific functions. Calcium accumulates into the mitochondrial matrix through the mitochondrial calcium uniporter (MCU). Few years ago, a MCU homolog has been discovered, which has been called MCUb. MCU and MCUb shares 50% sequence and structure similarity although some conserved differences in the primary sequence prevent MCUb from forming a Ca2+-permeable channel, thus acting as a dominant-negative subunit. RT-PCR experiments demonstrated that MCUb expression levels dramatically increase during skeletal muscle regeneration after cardiotoxin-induced injury. In addition, high MCUb expression levels have been detected in pro-inflammatory M1 macrophages. The latter are one of the most important effectors of the earlier stages of muscle repair. To study the role of mitochondrial calcium in macrophages during regeneration, we performed skeletal muscle regeneration experiments on a MCUb KO mouse model, and we analysed the effect of specific MCUb ablation in macrophages by bone marrow transfer experiments. Our results demonstrate that the lack of MCUb causes a delay in skeletal muscle regeneration that occurs in parallel with a reduction of the expression level of known markers of M2 macrophages. Moreover, macrophages from MCUb KO animals show lower phagocytic capacity compared to wild type animals. MCUb ablation alters macrophage metabolism by promoting glycolysis and the accumulation of TCA cycle intermediates accompanied by the stabilization of HIF-1α, the master transcriptional regulator of the macrophage proinflammatory program. This defect ultimately leads to delayed regeneration of damaged fibers and exhaustion of the satellite cell pool. Our data demonstrate that MCUb expression is tightly controlled in macrophages to perform satellite cell functional differentiation.
IIM Meeting 2021 • 22-24 October 2021

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P.50

Cytoplasmic HDAC4 grants skeletal muscle adaptation and function in Duchenne Muscular Dystrophy by mediating the membrane repair mechanism

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Histone deacetylase 4 (HDAC4) is a stress-responsive factor that mediates multiple cellular responses. As a member of the class IIa HDACs, HDAC4 shuttles from the nucleus to the cytoplasm; however, the cytoplasmic functions of HDAC4 in skeletal muscle are poorly characterized. We found that HDAC4 expression is upregulated in skeletal muscles of Duchenne Muscular Dystrophy (DMD) patients and in a mouse model of DMD, suggesting a role for HDAC4. DMD is a genetic, progressive, lethal disorder, characterized by muscle degeneration and weakness. Pan-HDAC inhibitors are presently used for the treatment of DMD, since their efficacy in preventing fibrosis and adipogenesis, while promoting compensatory regeneration, despite presenting several important limitations. By characterizing the specific functions of different HDAC members, more selective drugs or combined approaches can be proposed as therapeutic treatment for DMD. To dissect HDAC4 functions in skeletal muscle in DMD, we generated dystrophic mice with a skeletal muscle-specific deletion of HDAC4 (mdx;KO mice). Deletion of HDAC4 in skeletal muscle worsens the pathological features of DMD, increasing muscle damage and compromising muscle regeneration and function in mdx mice. Impaired membrane repair mechanism underpins the mdx;KO phenotype; consistently, ectopic expression of Trim72, a major player in the membrane repair mechanism, prevents mdx;KO satellite cell death and increases myogenic fusion in vitro, while reduces myofiber damage and improves mdx;KO muscle function in vivo. The mdx;KO phenotype is also rescued by restoring the cytoplasmic levels of HDAC4, both in vitro and in vivo. We demonstrated that HDAC4 plays crucial functions in the cytoplasm of dystrophic muscles, allowing a response to muscle damage in the DMD context. These findings shed new light on a novel function of HDAC4 restricted to the cytoplasm that should be considered for the development of future therapeutic approaches.
IIM Meeting 2021 • 22-24 October 2021

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P.51

The Acid Sphingomyelinase Pathway as a promising target of inflammation and oxidative stress in Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD), caused by loss-of-function mutations in the dystrophin gene, is the most common and severe muscular dystrophy. Although DMD is a genetic disease, two other mechanisms overlapping each other play an important role in the pathophysiology: inflammation and oxidative stress. Sphingomyelinases are intertwined between these two processes being stimulated during inflammation and in response to oxidative stress. Their importance in inflammatory-associated disorders and their therapeutic potential are interesting. Functional inhibitors of A-SMase (FIASMAs), work by inducing the lysosomal degradation of A-SMase and have a good safety profile. As repositioned drugs they have cost and time benefits and could synergize with gene-based therapies. Therefore, we administered amitriptyline, a TCA and FIASMA, to mdx mice (the murine model of DMD) and noticed a lowered inflammation and reduced damage along with the decrease in A-SMase activity. Interesting data on inflammation and oxidative stress were obtained after we treated mdx mice with fluoxetine an SSRI and FIASMA having fewer side effects. To dissect the role of A-SMase in muscle injury, we did experiments on A-SMase$^{-/-}$ mice and discovered its crucial role in M1 macrophage polarization. This is especially interesting knowing that M1 macrophages act early in muscle injury and their quick elimination in mdx mice lessens muscle lesion. We also found in A-SMase$^{-/-}$ M1 macrophages an increase in the expression of NRF2, a master player of the antioxidant and anti-inflammatory response. After acute muscle injury, A-SMase$^{-/-}$ mice presented an improved muscle regeneration with less inflammation in the M1/M2 ratio, and an increase in the number of satellite cells. We are currently trying polytherapy to target a broader range of pathways with lower doses and minimal side effects by combining fluoxetine and an antioxidant such as quercetin or plumbagin.
IIM Meeting 2021 • 22-24 October 2021

P.52

High Mobility Group Box 1 orchestrates regeneration in skeletal muscle

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Skeletal muscle regeneration is a well-orchestrated process in which inflammation represents a key step. High Mobility Group Box 1 (HMGB1) is a ubiquitous nuclear protein that is released by injured cells to serve a soluble message of tissue damage and to trigger inflammation. We previously demonstrated that HMGB1 promotes inflammation or regeneration in muscle, both upon acute injury (Tirone et al. J Exp Med 2018) and in muscular dystrophies (Careccia et al. Sci Transl Med. 2021), by switching among mutuall exclusive redox states. To identify the source(s) of HMGB1 during muscle repair, we generated cell-specific HMGB1 knockout mouse models with deletion in myogenic cells, endothelial cells or platelets. In addition, we generated a whole body inducible HMGB1 KO mouse model to further investigate the contribution of HMGB1 to muscle regeneration. Our data indicate that HMGB1 derived from different sources plays distinct roles in muscle regeneration, suggesting that a timely and/or spatially regulated release of HMGB1 is required for optimal regeneration. Overall, our findings identified HMGB1 as a key player in muscle regeneration.
IIM Meeting 2021 • 22-24 October 2021

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P.53

Identification of the first endogenous inhibitor of DUX4 in FSHD muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is one of the most prevalent neuromuscular diseases affecting children and adults of all ages and both sexes. Unfortunately, no treatment is currently available. FSHD is caused by gain of expression of the double homeobox 4 (DUX4) gene, encoding for a transcription factor normally silent in most adult somatic tissues. In FSHD, DUX4 aberrant activation triggers a pro-apoptotic transcriptional program resulting in muscle wasting. DUX4 has been recently implicated also in the pathogenesis of solid tumors, leukemia and herpes viral infection. Hence, blocking DUX4 activity is a plausible therapeutic option for FSHD and other diseases associated with aberrant DUX4 expression or activity. We have identified MATR3 as the first direct endogenous inhibitor of DUX4. MATR3 is a nuclear protein involved in regulation of transcription and splicing, RNA metabolism and DNA repair, that has been associated with dominant distal myopathy, frontotemporal dementia and ALS, diseases showing molecular overlaps with FSHD. We found that MATR3 directly binds to DUX4 DNA-binding domain and blocks DUX4-mediated gene expression. As a result, MATR3 administration rescues cell viability and myogenic differentiation of FSHD muscle cells, while it does not affect muscle differentiation in healthy cells. Notably, we characterized a short MATR3 fragment that is necessary and sufficient to directly block DUX4-induced toxicity to the same extent of the full-length protein. We are currently evaluating the genome-wide effects of MATR3-fragment administration to more broadly evaluate safety and efficacy at molecular level. In summary, we identified the first endogenous inhibitor of DUX4 for the treatment of FSHD, that in perspective might be applied to a spectrum of related and currently incurable diseases.
IIM Meeting 2021 • 22-24 October 2021

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P.54

Increased basal metabolic rate in mice susceptible to malignanthyperthermia and heat stroke

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Ryanodine receptor type-1 (RYR1) and Calsequestrin-1 (CASQ1), two proteins located in the sarcoplasmic reticulum (SR) of skeletal muscle fibers, are key players in excitationcontraction (EC) coupling. Mutations in the human RYR1 gene (encoding for the SR Ca2+ release channel), and ablation in mice of CASQ1 (a SR Ca2+ binding protein) causes hypersensitivity to halogenated anesthetics (malignant hyperthermia susceptibility, MHS), and to heat (heat stroke, HS). As both MH and HS are characterized by excessive cytosolic Ca2+ levels and by hypermetabolic responses, here we studied the metabolism of 4-month-old mice from two different lines that are MH/HS susceptible: knock-in mice carrying a human MH mutation (RYR1Y522S) and CASQ1-knockout(ko) mice. Using indirect calorimetry (OxyletPro Calorimeter, PanLab), we found that RYR1YS and, to a lesser degree, CASQ1-ko mice show a significantly increased oxygen consumption (VO2) and a lower respiratory quotient (RQ) when compared to WT mice (a lower RQ is suggestive of a metabolism that relies more on lipids). Analysis of the body fat mass indicated that both MH/HS susceptible lines have indeed a reduction in adipose tissue. In addition, we found that RYR1YS and CASQ1- ko mice consume on the average more food (respectively +26.04% and +25.58% grams/day vs. WT) and have a higher basal core temperature (+0.57°C and +0.54°C vs. WT). Finally, western blots and electron microscopy indicated that, in hyperthermic mice a) SERCA (used to remove myoplasmic Ca2+) and UCP3 (responsible of a thermogenic process that dissipates mitochondrial H+ gradient) are overexpressed; b) mitochondrial volume and percentage of damaged mitochondria are both increased. In conclusion, the hyperthermic phenotype of RYR1YS and CASQ1-ko mice seems to be associated to an intrinsically increased basal metabolism, possibly due to the need of additional ATP required to remove the excess of cytosolic Ca2+.
IIM Meeting 2021 • 22-24 October 2021

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P.55

Expansion of human iPSCs-derived muscle progenitor cells in vitro by inhibition of the JAK2/STAT3 pathway

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Duchenne muscular dystrophy (DMD) is a muscle degenerative disease caused by mutations in the dystrophin gene. In the absence of dystrophin, myofibers experience damage caused by contraction, membrane leakage, and progressive replacement by fibrotic and adipose tissue. The myofiber damage coupled with constant regenerative pressure contribute to the depletion of the muscle stem cell (MuSC) pool in DMD patients. Previous studies from our laboratory have shown that the JAK2/STAT3-FAM3A pathway promotes MuSC differentiation, and its transient inhibition enhances mouse and human MuSC expansion. As a current challenge in the field is the generation and expansion of MuSC while retaining their selfrenewal properties, the goal of this study is to assess whether JAK2 inhibition can lead to the expansion ex vivo of human induced pluripotent stem cell (iPSC)-derived myogenic progenitors. We used the JAK2 inhibitor AG490 on both embryonic stem cell (ESC) lines as well as human iPSCs to inhibit the activation of STAT3. At 30 days of myogenic derivation culture, we observed that AG490 treatment increased the expansion of cells positive for PAX7, a muscle progenitor marker, in a dose-dependent manner in both ESCs as well as iPSCs. We confirmed the increase in expansion of PAX7+ cells, assessed by immunofluorescence as well as qPCR. We utilized human-derived iPS cell lines with different DMD mutations and demonstrated that JAK2 inhibition expands human PAX7+ myogenic progenitors also in DMD cells. We are planning transplantation experiments to evaluate regenerative and self-renewal potential of expanded human iPSC-derived myogenic progenitors in vivo. The optimization of protocols for the efficient expansion of Pax7+ myogenic progenitors from healthy and diseased patients will serve as a useful platform for disease-in-a-dish modeling, drug screening, as well as aid in the generation of sufficient number of progenitors for cell-based therapeutic approaches for muscle diseases.
IIM Meeting 2021 • 22-24 October 2021

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P.56

Enhancing late stage myogenesis in 3D skeletal muscle constructs by electromagnetic stimulation

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Skeletal muscle tissue engineering focuses on creating muscle tissue in vitro to repair muscle loss. In addition, engineered muscle can serve as a model system to study muscle diseases or towards in-vitro testing of drugs. To enable functional 3D skeletal muscle constructs, efficient maturation strategies are warranted. Although skeletal muscle are defined as a dynamic tissue, most culturing approaches still focus on static culturing of these constructs. Yet, sparse evidence of stimulation protocols indicates a positive effect on gene regulation, protein expression and myogenesis. Most groups working on stimulation protocols either use mechanical or electrical stimulation or a combination thereof. Pulsed electromagnetic field (PEMF) stimulation has been already extensively used in bone tissue engineering but its impact on tissue engineered skeletal muscle is poorly studied. In this work, we evaluated the effects of PEMF stimulation, at 75 ± 2 Hz of signal frequency and magnetic induction of 2 ± 0.2 mT, on human skeletal muscle cells both in 2D and 3D. First, PEMF stimulation was applied on 2D cultured human myoblasts during the differentiation phase. Here, enhanced myotube formation and diameter could be observed. Next, results were translated towards 3D engineered muscles. For this, bio-artificial muscles were subjected to PEMFs for varying periods of time and 2 hours of stimulation a day was found to be most efficient in advancing myotube formation. Lastly, applying this 2 hours PEMF stimulation for the complete culture period (16 days) was compared to starting the PEMF stimulation only once myotubes were already formed, meaning after 8 days. The latter was found to result in a significantly higher myotube diameter, fusion index and myosin heavy chain 1 expression. Taken together, this work shows the potential of PEMF stimulation for advancing myotube formation both in 2D and 3D and warrants its further consideration for dynamic culturing techniques.
IIM Meeting 2021 • 22-24 October 2021

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P.57

Impact of type 1 diabetes and exercise intervention on markers of skeletal muscle remodelling

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The aim of this study was to compare markers of inflammation, growth, and skeletal muscle remodelling between participants with type 1 diabetes (T1D) and healthy control (CNT) subjects to characterise the diabetes myopathy. Moreover, we analysed if these markers were affected by 12 weeks of combined (COMB) exercise intervention. Ten sedentary T1D (4M/6F; 32±4 y) and ten CNT (4M/6F; 28±6 y) performed high-intensity COMB exercise sessions (resistance + aerobic exercise) 3 times per week. Muscle biopsies were collected before and after training to quantify markers of inflammation (TNF-α and IL6), growth (IGF-1Ea/Eb/Ec, AKT, eEF2) and remodelling (p38, MuRF1, Atrogin-1). The effect of training on muscle strength (1RM), aerobic capacity (VO2max) and parameters of glycaemic control (standard deviation (SD) and coefficient of variance % (CV%) of interstitial glucose (IG)) was also evaluated. At baseline, the expression of TNF-α and MuRF1 mRNA, the total AKT protein level and the eEF2 and p38 protein phosphorylation was higher in T1D than CNT subjects. The other markers did not differ. After the training period, the expression of TNF-α remaine, elevated while MuRF1 decreased at values comparable to the CNT group. The expression of the IGF-1 isoforms increased only in T1D after training while p38 phosphorylation increased, only in the CNT group. Pre-training SD and CV% of IG were higher in T1D than CNT while baseline muscle strength and VO2max did not differ. The training program led to better, glycaemic control in T1D and increased strength and aerobic capacity in both groups; however, the improvement was smaller in T1D compared to CNT. This study highlights an alteration of markers of inflammation and growth/atrophy in the muscle of participants with T1D. These molecular differences were accompanied by a reduced exercise adaptation in T1D compared to CNT. There might be a relationship between molecular impairments and the response to exercise.
IIM Meeting 2021 • 22-24 October 2021

P.58

Rebalancing expression of HMGB1 redox isoforms to counteract muscular dystrophy

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Muscular dystrophies (MDs) are a group of genetic diseases characterized by progressive muscle wasting associated to oxidative stress and persistent inflammation. It is essential to deepen our knowledge on the mechanism connecting these two processes because current, treatments for MDs have limited efficacy and/or are associated with side effects. Here, we, identified the alarmin high mobility group box 1 (HMGB1) as a functional link between, oxidative stress and inflammation in MDs. This nuclear protein acts as a DNA chaperone, inside the cells and as a signal of tissue damage when extracellularly released. We previously demonstrated that the oxidation of HMGB1 cysteines switches its extracellular activities from, the orchestration of tissue regeneration to the exacerbation of inflammation. We now found, that extracellular HMGB1 is present at high amount and undergoes oxidation in patients with, MDs and in mouse models of Duchenne muscular dystrophy (DMD) and limb-girdle muscular, dystrophy-3 (LGMDR3) compared to controls. Genetic ablation of HMGB1 in muscles of DMD, mice leads to an amelioration of the dystrophic phenotype as evidenced by the reduced, inflammation and muscle degeneration associated to the improvement in muscle functionality,

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indicating that HMGB1 oxidation is a detrimental process in MDs. Pharmacological treatment with an engineered non-oxidizable variant of HMGB1, called 3S, improves functional performance, muscle regeneration and satellite cell engraftment in dystrophic mice, while reducing inflammation and fibrosis. Overall, our data demonstrate that the balance between HMGB1 redox isoforms dictates whether skeletal muscle is in an inflamed or regenerating state, and that the non-oxidizable form of HMGB1 is a possible therapeutic approach to counteract the progression of the dystrophic phenotype. Rebalancing the HMGB1 redox isoforms may also be a therapeutic strategy for other disorders characterized by chronic oxidative stress and inflammation.
IIM Meeting 2021 • 22-24 October 2021

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P.59

The non-muscle ADF/cofilin-1 controls sarcomeric actin filament integrity and force production in striated muscle laminopathies

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Emery-Dreifuss muscular dystrophy is characterized by skeletal muscle atrophy and weakness, associated with dilated cardiomyopathy and cardiac conduction disorders. Although the genetic causes of this disease have been identified since the 1990s, the molecular and cellular mechanisms that underlie the loss of muscular strength in patients are still a riddle. We have recently shown that abnormal activation of the ERK1/2 pathway in striated muscle from patients and study models of the pathology, triggered the phosphorylation and activation of cofilin-1 at Thr25, a protein known to participate in the actin network depolymerization. However, the role of this Thr25 phosphorylated form of cofilin-1 in the structure and function of pathological muscle remained unknown. We have demonstrated that cofilin-1 abnormally phosphorylated at Thr25 is protected from degradation by the proteasome, leading to its abnormal accumulation in striated muscle. This results in actin depolymerization at the sarcomere, the contractile unit of striated muscle, and a loss of force production. These results are important both for a better understanding of the pathophysiology of Emery- Dreifuss muscular dystrophy, but also in terms of therapeutic approaches.
IIM Meeting 2021 • 22-24 October 2021

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P.60

Study of skeletal muscle responses to denervation through scRNA-seq and snATAC-seq technology

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In vertebrate animals, skeletal muscle mass and function are finely controlled by both contractile units – myofibers and non-contractile heterogeneous cellular components of skeletal muscles – muscle-resident cells. Upon acute injury, coordination of muscle-resident cells facilitates the repair of damaged muscles. While in chronic conditions such as complete loss of neuromuscular junctions (NMJ) integrity, damaged muscle cannot be resolved, ultimately leading to muscle atrophy and fibrosis. Our lab has demonstrated that these outcomes are caused by the persistent activation of IL-6/STAT3 signaling in Fibro-Adipogenic Progenitors (FAPs). To characterize the regulation of IL-6 in FAPs upon loss of NMJ integrity and to better understand the functional interplay between different muscle-resident cells, we have used single cell RNA sequencing (scRNA-seq) and single nucleus ATAC sequencing (snATAC-seq) for a longitudinal analysis of transcriptomic profiles and chromatin accessibility in cells isolated from control and denervated skeletal muscles after sciatic nerve transection. We found out that denervation selectively induced the expansion of two cell types – muscle glial cells and activated fibroblasts. Interactome analysis suggested that activated fibroblasts probably activate muscle glial cells via the nerve growth factor (NGF) and NGF receptor signaling pathway. Selected effectors downstream of NGFR are currently investigated to verify the activation of muscle glial cells and to characterize their functional changes upon denervation. Moreover, via CICERO analysis, we identified several potential IL-6 cisregulatory elements – enhancers that distinctly interact with IL-6 promoter in different cell types or under different conditions. 3C and 4C assays are undergoing to verify the potential interactions.
IIM Meeting 2021 • 22-24 October 2021

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P.61

Exploring the effect of microRNAs identified in extracellular vesicles circulating in hypertrophic mice

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The distinct characteristics of skeletal muscle, namely growth and regeneration under physiological conditions or injury due to physical activity or degenerative diseases, upholds the significant network dynamic underlying this organ. Generally, chronic muscle disorders are associated with the main characteristic of a significant loss in muscle mass. Progressive muscle wasting is also a feature of aging. Given the importance of paracrine factors in upholding homeostasis in the muscular niche, extracellular vesicles (EVs) have been studied as hosts for factors capable of communicating with target cells through the immediate action of lipids and/or proteins or by altering gene expression through nucleic acids. Due to their role in post-transcriptional gene regulation, microRNAs (miRNAs) have emerged as potential modulators of stem cell differentiation. In this study, we scrutinized the content of EVs from mouse models of muscular dystrophy, aged, and hypertrophic mice. Through thorough omics, effective EV miRNA signatures from hypertrophic mouse models were unraveled. We tested several combinations of mimics and antagomirs of identified miRNAs upon the myogenic differentiation of human mesoangioblasts (hMABs) in vitro and in vivo and found that overall, miRNA-1 and miRNA-208a ameliorate myogenesis. Furthermore, the combination of these miRNAs was tested on a multitude of other cell types, however, the same effect was not evident. This implies that the combination of miRNA-1 and miRNA-208a may have significantly different roles depending on the cell type in question. On the other hand, the field of EV research is on the rise, with the possibility of overshadowing cell therapy. Therefore, we aim to generate miRNA custom-engineered EVs to tackle muscle wasting conditions.

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P.62

Drosophila as a myotonic dystrophy model: Insights into CELF family function during myofibril formation

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To understand how CELF1 misregulation contributes to myotonic dystrophy (DM1), it is important to examine how RNA processing functions in healthy muscle. As sarcomere structure and muscle protein composition, as well as many molecular aspects of CELF-family protein function, are highly conserved, we use Drosophila melanogaster flight muscle (IFM) as a tractable genetic model. We have previously shown using RNAi knockdown that the CELF1 homologue Bruno-1 (Bru1) controls flight muscle specific alternative splicing, regulating both sarcomere growth and myosin contractility. We generated new CRISPRmediated deletions in Bru1 that knock-out all Bru1 isoforms, resulting in stronger phenotypes in mutant flies that revealed defects at the earliest stages of myofibril formation, notably disorganization of the actin cytoskeleton in differentiating IFM. Using temporally-restricted RNAi knockdown, we can demonstrate that there are distinct requirements for Bru1 mediated splicing during early and late stages of myofibril formation. After sarcomere formation, while thin-filament growth in length is arrested, thin-filament lateral growth is dramatically misregulated leading to formation of hollow myofibrils. We performed mRNA-Seq and mass spectrometry and identified misregulation of both sarcomeric proteins and other RNAprocessing factors. Moreover, our temporal transcriptomics data reveal a progressive misregulation of gene expression and splicing as IFM development proceeds. Taken together, our data indicate that during muscle differentiation, Bru1 regulates cytoskeletal rearrangement necessary for myofibril formation as well as the balance in length versus lateral growth of the thin-filament. Defective RNA processing causes sarcomeric structural defects and progressive malfunction of dystrophic muscle.

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