IIM Interuniversity Institute of Myology

21st IIM Meeting

Assisi, Italy • 4-7 September 2024

From Muscle Physiology to Pathogenesis and Therapies of Neuromuscular Diseases

Programme & Abstracts

https://IIM2024.azuleon.org

Topics

Muscle function and E-C coupling
Genetic, epigenetic, and metabolic regulation of muscle
Muscle plasticity and physical exercise
Muscle stem cells and regenerative medicine
Muscle aging
Muscle wasting and cachexia
Modeling and treating muscle diseases
Cardiac muscle and cardiomyopathy

Keynote Lectures

Leonardo
Ferreira

Denis C.
Guttridge

Michael
Rudnicki

Julia
von Maltzahn

IIM Scientific Committee

Sestina
Falcone

Stefania
Fulle

Davide
Gabellini

Lucia
Latella

Emanuele
Mocciaro

Antonio
Musarò

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Palacios

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Francesca
Riuzzi

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Anna
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Esposito

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Roccabianca

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RESEARCH PRODUCTS

ACQUA AI FIORI DI SPELLO
Umbria, Italia

Programme

Convegno *(Assisi, Hotel Cenacolo)*

L'ESERCIZIO FISICO COME PREVENZIONE

Dalla contrazione muscolare alla nutrizione

Moderatori: Guglielmo Sorci (Università di Perugia; Direttore IIM)
Anna Villarini (Università di Perugia)

9:30 **Saluti delle Autorità**

9:50 **Francesca Riuzzi** *(Università di Perugia; IIM)*
Evoluzione e proprietà del muscolo scheletrico

10:10 **Antonio Musarò** *(Università Sapienza Roma; IIM)*
Il muscolo come organo endocrino. Le miochine

10:30 **Massimo Raffaele Mannarino** *(Università di Perugia)*
Effetti benefici dell'esercizio fisico. Esercizio fisico come poli-pillola

10:50 **Raffaella Spada** *(Istituto di Medicina e Scienza dello Sport - CONI)*
Aspetti nutrizionali legati all'esercizio fisico

11:10 **Roberto Cammarelle** *(ex pugile, campione olimpico e campione del mondo)*
La mia esperienza da campione

11:40 **Discussione generale**

12:00 **Conclusioni**

Diretta streaming sul sito https://IIM2024.azuleon.org.

15:00-15:10 **Welcome and opening of the IIM meeting**

15:10-15:50 **Lecture 1**
Chair: Giacomo Rubini (Young IIM Committee)

Leonardo F. Ferreira *(Duke University, Durham, NC, USA)*
Molecular and biophysical basis of skeletal muscle dysfunction in heart failure

15:50-17:00 **Session 1: Muscle function and weakness**
Chairs: Dario Coletti, Sara Roccabianca

Stefano Perni *(Siena, Italy)*
EC-coupling and beyond. Dissecting the molecular interactions in the SR/ER-PM junctions of muscle and nerve

Charlotte Gineste *(SFM; Illkirch-Graffenstaden, France)*
Testing tamoxifen as a potential therapeutic approach in Stim1-related TAM/STRMK murine model

Federica Fiore* *(Siena, Italy)*
Evaluating miR-486 overexpression as a potential therapeutic approach in mouse models of RyR1-related myopathies

Jacqueline Ji* *(Illkirch-Graffenstaden, France)*
Gene replacement to cure BIN1 centronuclear myopathy

17:00-17:30 ***Coffee break***

17:30-18:55 **Session 2/a: Muscle diseases and regenerative medicine**
Chairs: France Pietri-Rouxel, Beatrice Biferali

Lorenzo Giordani *(Paris, France)*
Multimodal Single Cell spatial profiling of Duchenne Muscular Dystrophy

Caterina Boccia* *(Rome, Italy)*
Targeting IL-6 signalling to attenuate dystrophic muscle degeneration

Sonia Albini *(Evry, France)*
Disease exacerbation in MYOrganoids derived from Duchenne Muscular Dystrophy iPSC reveals limitations of microdystrophin therapeutic efficacy

Laura Lociuro* *(Milan, Italy)*
Enhancing micro-dystrophin gene therapy: the role of SRT2104, a new Sirtuin1-activating compound, for the treatment of Duchenne Muscular Dystrophy

Ilaria Zito *(Parent Project; Rome, Italy)*
The role of patients' associations in IHI European multi-partner projects

19:30 ***Dinner - Aperitivo Umbro***
and (only for participants registered for the Advanced Myology Update course)
Roundtable 1: "How to choose a lab for a Post-Doc experience"
with **M. Rudnicki** and **J. von Maltzahn**

Roundtable 2 "Approaches to optimize the Post-Doc experience"
with **L. Ferreira** and **D. Guttridge**

***Young Investigator. Eligible for Best Talk awards.**

9:00-10:10 **Session 2/b: Muscle diseases and regenerative medicine**

Chairs: Cesare Gargioli, Mariam Zouhair

Riccardo Gamberale* *(Monza, Italy)*

Characterization of the infiltrating polarized macrophages during the onset of heterotopic ossification in a mouse model of Fibrodysplasia Ossificans Progressiva

Cassandra Margotta* *(Milan, Italy)*

Enhancing skeletal muscle regeneration in ALS via intramuscular allosteric activation of the P2X7 receptor

Rodrigo D'Amico* *(Rome, Italy)*

Consequences of CNBP reduced expression in DM2 pathogenesis

Alexis Boulinguiez *(SFM; Egham, United Kingdom)*

Different outcomes of endurance and resistance exercise in skeletal muscles of Oculopharyngeal muscular dystrophy

10:10-10:35 ***Coffee break***

10:35-11:15 **Lecture 2**

Chair: Pier Lorenzo Puri

Michael Rudnicki *(Ottawa Hospital Research Institute, Ottawa, Canada)*

Molecular regulation of muscle stem cell function

11:15-12:25 **Session 3: Muscle stem cells and stem cell niche**

Chairs: Chiara Sassoli, Federica Esposito

Andrea Münsterberg *(Norwich, United Kingdom)*

Muscle stem cell function is impaired in absence of Talpid3 - a gene required for primary cilia formation

Cristina Parisi* *(Rome, Italy)*

Fibro-Adipogenic Progenitors (FAPs) facilitate skeletal muscle reinnervation via retinoic acid release

Stefano Cagnin *(Padua, Italy)*

Non-coding RNAs to Treat Skeletal Muscle Atrophy

Nikki Wanders* *(Maastricht, The Netherlands)*

Experimental autoimmune encephalomyelitis induced muscle loss in mice triggers engraftment of systemically administered mesoangioblasts

12:25-12:40 **Technical talk *(by Prodotti Gianni/Abcam)***

Danilo Lemos

Tackling reproducibility crisis with recombinant technology

12:40-12:50 **Poster blitz 1** (ODD numbers; selection)

13:00	***Lunch***
14:30-16:00	**Poster Session 1** (ODD numbers)
16:00	***Bus departure to Assisi: guided tour of the Basilica of Saint Francis or the "Rocca Maggiore" Fortress (free time after the visits)***
19:00	***Bus departure to the "Cantico di San Francesco" restaurant***

***Young Investigator. Eligible for Best Talk awards.**

9:00-9:40 **Lecture 3**

Chair: Maurilio Sampaolesi

Julia von Maltzahn *(Brandenburg University of Technology, Cottbus-Senftenberg, Germany)*

Muscle stem cells in age and disease

9:40-10:40 **Session 4: Muscle aging**

Chairs: Libero Vitiello, Katja Hönzke

Giacomo Bincoletto* *(Padua, Italy)*

Premature aging of skeletal muscle of Kennedy disease mouse models

Susanna Molinari *(Modena, Italy)*

Nanoparticle-mediated delivery to skeletal muscle cells of N-palmitoylethanolamide (PEA), an endocannabinoid-like molecule with anti-inflammatory properties

Clara Sciorati *(Milan, Italy)*

Sarcopenic obesity in the elderly: a dysfunctional crosstalk between tissues?

10:40-11:10 ***Coffee break***

11:10-12:20 **Session 5: Genetic and epigenetic regulation in muscle pathologies**

Chairs: Vanina Romanello, Alex Pezzotta

Paul Kemp *(London, United Kingdom)*

The H19/Let-7/myc axis regulates muscle inflammatory responses

Emanuele Mocciaro *(Milan, Italy)*

Pre-clinical development of a drug inhibiting the chromatin remodeling protein WDR5 in FSHD muscular dystrophy

Alexis Osseni *(SFM; Lyon, France)*

Inhibition of HDAC6 improves muscle integrity in Duchenne Muscular Dystrophy mouse model

Ashley (Ju-Wei) Wang* *(Leuven, Belgium)*

Investigating the potential of MICAL2 modulation for impeding rhabdomyosarcoma cancer progression

12:20-12:30 **Poster blitz 2** (EVEN numbers; selection)

13:00 ***Lunch***

14:30-16:00 **Poster Session 2** (EVEN numbers)

16:00-16:40 **Lecture 4**

Chair: Fabio Penna

Denis C. Guttridge *(Medical University of South Carolina, Charleston, SC, USA)*

Regulation of the inflammatory muscle microenvironment in cancer cachexia

16:40-17:00 ***Coffee break***

17:00-18:45 **Session 6: Muscle wasting and cachexia**

Chairs: Andy Judge, Sara Chiappalupi

Gabriele Guarnaccia* *(San Diego, CA, USA)*

Serum amyloid protein A1 (SAA1) impairs myogenesis and myotube size in pancreatic cancer cachexia

Martina Biglietto* *(Rome, Italy)*

Engineered exosomes as a therapeutic tool to counteract muscle degeneration

Giacomo Rubini* *(Turin, Italy)*

Immunomodulation via interleukin-4 improves energy metabolism in C26 tumor-bearing mice

Martina Paiella* *(Novara, Italy)*

Western diet worsens cancer-induced muscle wasting which is restrained by *Vaccinium macrocarpon* extract

Andy Judge *(Gainesville, FL, USA)*

Muscle directed nutrition for cancer cachexia

Andrea Ghiroldi *(Cinisello Balsamo, Italy)*

Unraveling the therapeutic potential of givinostat in muscle atrophy induction

18:45-19:30 **IIM General meeting**

20:00 ***Social Dinner - Awards and prizes***

22:00 ***Dance party***

***Young Investigator. Eligible for Best Talk awards.**

Reserved to participants registered to the
High Training Course in AADVANCED MYOLOGY UPDATE 2024

10:00 ***Bus departure to Palazzo Bernabei***

10:30 **J. von Maltzahn**
Driving rhabdomyosarcoma into myogenic differentiation

11:00 **L. Ferreira**
Assessment of muscle function in rodents: old approaches, modern applications

11:30 **M. Rudnicki**
Developing innovative regenerative therapies for neuromuscular diseases

12:00 **D. Guttridge**
Identifying limitations in mouse models of cancer cachexia

12:30 ***Light lunch***

Posters always on display during the meeting

Discussion

ODD numbers: Thursday, 5 September (14:30-16:00)

EVEN numbers: Friday, 6 September (14:30-16:00)

P.1 Valentina Guardascione** *(Siena, Italy)*
Evaluation of miR-486 expression levels in mice skeletal muscle under different metabolic conditions

P.2 Sara Roccabianca** *(Siena, Italy)*
Changes in CLIMP63 expression alter the organization of the microtubule network of mouse skeletal muscle fibers

P.3 Lucrezia Puccini** *(Siena, Italy)*
Gene expression analysis in skeletal muscles of mice carrying a deletion in a muscle-specific stretch/super enhancer region inside the ANK1 locus

P.4 Paul Kemp *(London, United Kingdom)*
Regulation of individual sensitivity to inflammation by myc contributes to muscle loss in COPD

P.5 Marco Simula** *(Rome, Italy)*
Long noncoding RNAs at the interface between muscles and nerves

P.6 Maxime Gelin** *(Paris, France)*
Exploring the role of GDF5 in neuromuscular system during growth and physical activity

P.7 Margaux Van Puyvelde** *(Leuven, Belgium)*
A meta-analysis of state-of-the-art *in vitro* generated skeletal muscle

P.8 Muhammad Dawood Amjad** *(Chieti, Italy)*
Complex Magnetic Fields (CMFs): harnessing electromagnetic symphony for muscle regeneration

P.9 Alessandro Arcari** *(Milan, Italy)*
Lights and shadows of neuropsychiatric drugs on skeletal muscle in Duchenne muscular dystrophy

P.10 Pietro Chiolerio** *(Padua, Italy)*
Exploring the role of extracellular matrix in neuromesodermal differentiation of human induced pluripotent stem cells

P.11 Aly Bourguiba Villeneuve** *(Paris, France)*
GDF5 therapeutic potential on neuromuscular junction defects

P.12 Sabrina D'Amore** *(Chieti, Italy)*
Cell adhesion and migration: an important determinant of GTP-dependent myogenesis

P.13 Beatrice Biferali** *(Milan, Italy)*
Understanding and treating inflammation in FSHD muscular dystrophy

P.14 Francesca De Paolis** *(Rome, Italy)*
Human mytendineous junction 3D *in vitro* modeling

P.15 Giorgia Cavioli** *(Rome, Italy)*
HDAC4 mediates the crosstalk between skeletal muscle fibers and fibro-adipogenic progenitors in Duchenne Muscular Dystrophy

P.16 Rebecca Deodati** *(Rome, Italy)*
Skeletal Muscle Tissue Engineering a promising technology in the field of food

P.18 Federica Esposito** *(Milan, Italy)*
Exploring the role of HMGB1 in nuclei dynamics during myogenesis

P.19 **Fabiana Fanelli**** *(Urbino, Italy)*
Effect of tunicamycin treatment on IGF1R production and IGF1R signaling pathway activation in C2C12

P.20 **Rachele Garella** *(Florence, Italy)*
New tools to evaluate satellite cell activation after eccentric contraction-induced damage at the muscle-tendon junction in mice: the light sheet microscopy

P.21 **Serena Germani**** *(Milan, Italy)*
CHOP/ERO1A pathway of unfolded protein response (UPR) in RYR1 and SEPN1-related myopathies

P.22 **Martina Parigi**** *(Florence, Italy)*
Red photobiomodulation promotes skeletal myoblast differentiation and counteracts anti-myogenic effects of TGF-β1: new perspective for muscle regenerative medicine

P.23 **Alex Pezzotta**** *(Milan, Italy)*
Improving the effects of HDAC8 inhibition by combining the activation of SIRT1 in a zebrafish model of Duchenne muscular dystrophy

P.24 **Elena Ruggieri**** *(Milan, Italy)*
High Mobility Group Box 1 recycling orchestrates regeneration in skeletal muscle

P.26 **Cristina Purcaro**** *(Chieti, Italy)*
Environmental pollutants impact on adult and pluripotent-derived myogenic progenitors

P.27 **Ilaria Versari**** *(Bologna, Italy)*
Nuclear Phospholipase C delta 4 is a crucial player in modulation of rhabdomyosarcoma cells proliferation

P.28 **Fabio Ferrini** *(Urbino, Italy)*
Hyaluronan improves the C2C12 murine myoblast proliferation and myogenic differentiation under oxidative and inflammatory conditions

P.29 **Francesco Millozzi**** *(Rome, Italy)*
Aptamer-conjugated gold nanoparticles enable selective oligonucleotide delivery into muscle stem cells to promote regeneration of dystrophic muscles

P.30 **Alessandro Antonioli**** *(Novara, Italy)*
Ketogenic diet mitigates liver damage but not muscle atrophy in western diet-induced obesity

P.31 **Paul Kemp** *(London, United Kingdom)*
AntagomiR inhibition of miR-424(322) increases muscle fibre diameter in old mice and in response to respiratory viral infection

P.32 **Bianca Bartoloni**** *(Florence, Italy)*
An autocrine loop of lactate sustains cancer cachexia in skeletal muscle

P.33 **Frida Karakashi**** *(Milan, Italy)*
Menin is a novel therapeutic target for FSHD muscular dystrophy

P.34 **Lorenza Bodo**** *(Turin, Italy)*
The role of Pde4d transcript variants in restraining cAMP signaling in cancer cachexia

P.35 **Martina Lupoli**** *(Rome, Italy)*
The role of Protein-Kinase C Theta in muscle-nerve communication: the case of Amyotrophic Lateral Sclerosis and Myasthenia Gravis

P.36 **Karolina Majchrzak**** *(Senftenberg, Germany)*
Role of Sbno2 in myogenic differentiation under cachectic conditions

P.37 Mariam Zouhair** *(Rome, Italy)*
3D skeletal muscle construct for *in vitro* recapitulation of Duchenne Muscular Dystrophy Progression

P.38 Michele Mannelli** *(Florence, Italy)*
Auranofin, a potential drug to counteract cancer cachexia in skeletal muscle

P.39 Giovanni Delli Carpini** *(Rome, Italy)*
In vivo restoration of dystrophin expression in mdx mice using antisense oligonucleotides (ASOs) conjugated to gold nanoparticles

P.40 Mark Griffiths *(London, United Kingdom)*
Metabolomic markers of mitochondrial dysfunction predict recovery of muscle mass in a human model of acute muscle wasting

P.41 Katja Hönzke *(Senftenberg, Germany)*
Investigation of the role of different IQgap proteins in myogenesis

P.42 Tommaso Raiteri** *(Perugia, Italy)*
Dietary and endogenous advanced glycation end-products (AGEs) induce muscle wasting in vitro which could be counteracted by *Vaccinium macrocarpon* extract

P.43 Libero Vitiello *(Padua, Italy)*
Targeting monoamine oxidase B (MAOB) in dystrophic mdx hearts dampens inflammation and fibrosis

P.44 Laura Salvadori *(Novara, Italy)*
Vaccinium macrocarpon extract restrains muscle wasting induced by Western diet-derived AGEs

P.45 Giorgia Maria Renna** *(Milan, Italy)*
Molecular characterization of ER stress mediators in the pathogenesis of SEPN1 and RYR1-related myopathies

P.46 Sara Chiappalupi *(Perugia, Italy)*
Proteomic analysis suggests novel mechanisms involved in the protection against cancer-induced muscle wasting in mice lacking RAGE at myofiber level

****Young Investigator. Eligible for Best Poster Blitz and Best Poster awards.**

Keynote Lecturers

Leonardo F. FERREIRA

Duke University
Durham, NC, USA

KEYNOTE LECTURE 1

Molecular and biophysical basis of skeletal muscle dysfunction in heart failure

Leonardo F. Ferreira
Duke University, Durham, NC, USA

Impairments in redox homeostasis and mitochondrial bioenergetics are evident in several diseases and aging and have been associated with loss of muscle contractile function. The sources of oxidants causing dysfunction include NADPH oxidases, xanthine oxidase, and mitochondria. Excitation-contraction coupling and sarcomeric proteins are susceptible to oxidation that impair function. The molecular basis of contractile dysfunction due to protein oxidation has been attributed largely to cysteine oxidation. However, our lab has profiled the redox proteome in heart failure and identified methionine oxidation as a putative post-translational modification responsible for contractile dysfunction. Moreover, we have found that methionine residues in skeletal muscle α-actin are hyperoxidized in heart failure with reduced ejection fraction and methionine oxidation contributes to contractile dysfunction induced by reactive oxygen species. In the context of mitochondrial bioenergetics, the link between metabolic and contractile dysfunction has not been established. Mitochondrial respiration impairments lead to accumulation of metabolites of the tricarboxylic acid cycle (TCA) that are upstream of the electron transport system. High fat diet, diabetes, hypoxia/ischemia, cancer, and even exercise disrupt metabolism resulting in several fold increase in TCA cycle metabolites. We have found that postmenopausal heart failure with preserved ejection fraction (HFpEF) causes muscle mitochondrial dysfunction that is accompanied by protein modification induced by a TCA metabolite that causes loss of myofiber force and power through diminished cross-bridge kinetics and calcium sensitivity. Overall, our findings suggest novel molecular mechanisms of contractile dysfunction mediated by redox and metabolic abnormalities.

Michael RUDNICKI

Ottawa Hospital Research Institute
Ottawa, Canada

KEYNOTE LECTURE 2

Molecular regulation of muscle stem cell function

Michael A. Rudnicki
Ottawa Hospital Research Institute, Ottawa, Canada

Satellite cells and their progenitors are thought to be organized hierarchically with functional heterogeneity existing withing different subsets of quiescent cells. How satellite cells balance the generation progenitors while maintaining self-renewal can be posited as either stochastic fate acquisition, or a hierarchical organization of asymmetric divisions with determined cell fates. Previously, we identified a putative stem cell within the satellite cell population using Cre-LoxP lineage tracing. We found committed satellite myogenic cells express high levels of *Myf5-Cre* (YFP^{+}), whereas 8 % of satellite stem cells have never expressed *Myf5-Cre* (YFP^{-}). Engraftment experiments established that satellite stem cells ($Pax7^{+}/Myf5^{low}$) reconstitute the self-renewing satellite cell population following transplantation whereas satellite myogenic cells cannot efficiently reconstitute this population. Satellite stem cells are multipotential and can generate both muscle and brown fat. Thus, this sub-compartment fulfill the defining criteria of adult stem cells in that they exhibit long-term self-renewal and are multipotential. Therefore, we performed single-cell RNA-seq and gene expression analysis on YFP-($Myf5^{low}$) populations enriched for the satellite stem cell population and defined a novel cluster. We identified unique cell surface markers that facilitate prospective isolation of $Myf5^{low}$ cells. Engraftment experiments demonstrate that this subset of MuSCs exhibits superior stem-like characteristics, enhanced self-renewal, and are deeply quiescent. They exhibit low metabolic activity, markedly reduced mitochondrial membrane potential (MMP), and smaller fragmented mitochondria with high levels of pDRP-1. We conclude that $Myf5^{low}$ MuSCs represent a distinct subpopulation of satellite cells with very low metabolic requirements, which represent long term self-renewing muscle stem cells (LT-MuSCs).

Julia
VON MALTZAHN

Brandenburg University of Technology
Cottbus-Senftenberg, Germany

KEYNOTE LECTURE 3

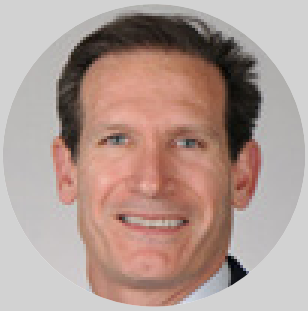
Muscle stem cells in age and disease

Julia von Maltzahn
Brandenburg University of Technology Cottbus-Senftenberg, Germany

Skeletal muscle has diverse functions in the organism and a remarkable ability to adapt to physiological demands such as growth, training and injury. Furthermore, it is one of the organs with the highest ability to regenerate, a process depending on muscle stem cells. During aging muscle stem cell (MuSC) numbers are reduced, but most importantly their functionality decreases resulting in impaired regeneration of skeletal muscle. The reduced regenerative capacity of skeletal muscle can be attributed to intrinsic changes in muscle stem cells, changes in their niche as well as systemic changes and changes in supporting cells. Changes in different signaling in muscle stem cells, changes in systemic factors as well as changes in the extracellular matrix are examples for age-related changes affecting muscle stem cell functionality and thereby regeneration of skeletal muscle in the aged. However, inhibition of aberrantly active signaling pathways or replenishing missing systemically delivered factors or factors in the immediate muscle stem cell niche allow the improvement of regeneration in the aged.
During aging alterations in innervations of skeletal muscle occur. Acute loss of innervation causes a significant increase in the total number of MuSCs, as well as proliferating, activated and differentiating MuSCs . This aberrant activation and premature commitment of MuSCs to the myogenic lineage is accompanied by profound alterations on the mRNA and protein level after denervation. Interstingly, acute loss of innervation causes changes in the secretome of myofibers which then lead to alterations in MuSC behavior and regenerative capacity. This example emphasizes the need to investigate the interplay of different cell types in regeneration of skeletal muscle.

Denis C. GUTTRIDGE

Medical University of South Carolina
Charleston, SC, USA



KEYNOTE LECTURE 4

Regulation of the Inflammatory Muscle Microenvironment in Cancer Cachexia

Denis C. Guttridge
Medical University of South Carolina, Charleston, SC, USA

Cancer cachexia is a syndrome characterized by weight loss largely due to the depletion of adipose and skeletal muscle. Cachexia occurs in over half of cancer patients, and it is estimated to be responsible for greater than 20% of all cancer deaths. Studies have shown that loss of muscle mass derives from tumor and host factors that signal to myofibers to activate the ATP dependent ubiquitin proteasome and lysosomal autophagy systems, which in turn induces catabolism of muscle proteins leading to muscle wasting. Mitochondrial dysfunction also plays a significant role in exacerbating wasting during cancer progression. A distinction between wasting that occurs in cachexia versus muscular dystrophy is the lack of myofiber degeneration that is prevalent in dystrophy resulting from sarcolemma damage and clearance of debris by inflammatory immune cells. Although not as severe, our laboratory showed that in cachexia, tumor-induced myofiber damage occurs leading to an activation of a regenerative program. More recently, we have been interested in understanding how this damage relates to inflammation in the muscle microenvironment. Specifically, we aim to determine how this inflammation is defined in cachectic muscle, the factors that mediate this inflammation, and whether this inflammation is causal to wasting. Using mouse models and patient samples, our findings show that cachectic muscle is marked by enhanced innate immunity, predominated by accumulation of macrophages. This accumulation is regulated by an NF-kB activity localized to multiple cells, including satellite cells, myofibers, and fibro-adipogenic progenitors. Dependent on their subtype, macrophages exhibit diverse functions in regulating cachexia, either inhibiting muscle regeneration to promote atrophy, or maintaining myofiber integrity to counter atrophy. We propose that NF-kB functions in the muscle microenvironment to regulate inflammation to both promote and protect against muscle wasting in cancer.

Selected Talks Abstracts

EC-coupling and beyond. Dissecting the Molecular Interactions in the SR/ER-PM junctions of muscle and nerve

Stefano Perni[1,2], K Beam[2]
[1]Dept. Molecular and Developmental Medicine. Univ. degli studi di Siena. Siena, Italy
[2]Dept. Physiology and Biophysics. Univ. of Colorado. Denver (CO), USA

Sarco/Endoplasmic reticulum-plasma membrane junctions (SR/ER-PM junctions) are regions at which the plasma membrane comes in close apposition with the endoplasmic reticulum, allowing the cross-talking between proteins in the PM and those in the ER, and governing local or whole-cell Ca^{2+} signaling events. In skeletal muscle SR-PM junctions (triads), which are formed and stabilized by the proteins Junctophilin 1 and 2, the voltage-gated Ca^{2+} channel $Ca_V1.1$ in the PM and the calcium-releasing channel RyR1 in the SR interact to execute excitation-contraction coupling (ECC). In about 30 years of studies on ECC, knock-out models have revealed several additional proteins necessary for the process besides $Ca_V1.1$ and RyR1. Still, they couldn't provide a definitive answer as to whether other undiscovered players were also required. A complementary approach to knock-out models is represented by *de-novo* reconstitution. This approach aims at recapitulating a specific cellular process by inducing the expression of proteins known to participate in it into undifferentiated, naïve cells. In the past few years, we have used the reconstitutional approach to identify key structural and functional interactions between the molecules that populate the SR/ER-PM junctions. This led us to define the minimum set of proteins required for skeletal muscle ECC and provided insights into the role of junctophilins in muscle and in the much less explored neuronal ER-PM junctions. Here, I present an overview of past and ongoing research aimed at understanding the molecular interactions among proteins populating the SR/ER-PM junctions of skeletal muscle and neurons.

Testing tamoxifen as a potential therapeutic approach in *Stim1*-related TAM/STRMK murine model

Charlotte Gineste, D. Reiss, J. Laporte
IGBMC, Dpt Translational Medicine and Neurogenetics, Illkirch, France

Tubular Aggregate Myopathy (TAM) and Stormorken Syndrome (STRMK) form a clinical continuum affecting the skeletal muscle, platelets, spleen, skin, bones, and eyes. One of the main forms of the disease is caused by dominant gain-of-function mutation in *STIM1*, encoding a key player of the store-operated Ca^{2+} entry (SOCE) pathway, resulting in intracellular Ca^{2+} overload. To date, no therapy exists. Interestingly, tamoxifen showed beneficial effects on muscle function in other myopathies presenting similar Ca^{2+} homeostasis defects than TAM/STRKM. We characterized the effects tamoxifen (10 mg/kg/d) on muscle function in mice harboring the most common TAM/STRMK mutation R304W in 4-month-old Stim1 mice (*Stim1*$^{R304W/+}$) after a 2-month period of tamoxifen exposure. Force of the tibialis anterior muscle (TA) was measured *in situ* at incremental frequencies (1 to 150 Hz) and during fatiguing exercise (40 Hz, 1 sec on, 3 sec off). Tamoxifen-treated *Stim1*$^{R304W/+}$ mice were compared to placebo-treated (untreated) *Stim1*$^{R304W/+}$. Wild-type (WT) mice served as control and received either tamoxifen or placebo at similar age and dose than *Stim1*$^{R304W/+}$ mice. Tamoxifen had no effect on the muscle-atrophy related muscle weakness or the higher submaximal force, relaxation time of contraction and muscle fatigue observed in untreated *Stim1*$^{R304W/+}$ mice compared to WT mice. Overall, our results indicate that this dose of tamoxifen did not improve the defective skeletal muscle function, which suggests no positive effects of tamoxifen on the SOCE-related excessive Ca^{2+} influx within cells in *Stim1*$^{R304W/+}$. Thus, tamoxifen may not serve as a strategy to counteract muscle defects in TAM/STRMK. Further investigations will be performed in order to assess the effect of tamoxifen on other affected tissues in TAM/STRMK.

Evaluating miR-486 overexpression as a potential therapeutic approach in mouse models of RyR1-related myopathies

Federica Fiore[1], A. Laurino[1], M. Serano[1], E. M. Rubino[1], D. Rossi[1, 2], E. Pierantozzi[1], V. Sorrentino[1, 2]
[1]Department of Molecular and Developmental Medicine, University of Siena, Siena, Italy
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Mutations in the *RYR1* gene constitute the most frequent causes of non-dystrophic congenital muscle disorders, collectively referred to as RyR1-related myopathies. Among the non-dystrophic congenital myopathies associated with *RYR1* mutations, the most common is central core disease (CCD), which exhibits a variable phenotype and is histologically characterized by large areas devoid of mitochondria and oxidative enzyme activity, known as cores. Additionally, mutations in the *RyR1* gene predispose individuals to malignant hyperthermia susceptibility (MHS), a rare lethal pharmacogenetic disorder that manifests as a hypermetabolic state with elevated muscle contraction and body temperature.
Currently, there are no approved treatments for RyR1-related myopathies. Two distinct knock-in mice models, $Ryr1^{Y524S/+}$ and $Ryr1^{I4895T/+}$, which correspond to the human variants Y522S and I4898T, respectively, are widely recognized as valuable models to study the pathophysiology of MHS and CCD. To evaluate possible therapeutic approaches, we focused on mir-486, a microRNA enriched in skeletal muscle. Previous studies in mdx mice, a model of muscular dystrophy, demonstrated that mir-486 overexpression ameliorated the dystrophic phenotype. Based on this evidence, we investigated skeletal muscle-specific mir-486 overexpression as potential treatment strategy for RyR1-related myopathies.
For this study, we generated two new mouse lines, in $Ryr1^{YS/mir-486}$ and $Ryr1^{IT/mir-486}$, by crossing $Ryr1^{YS/+}$ and $Ryr1^{IT/+}$ mice with transgenic mice ($Tg^{mir-486/+}$) overexpressing mir-486 specifically in skeletal muscle tissue. We then conducted functional assessments on the offspring using different tests to evaluate muscle force and endurance. Our result indicate that mir-486 overexpression significantly enhances muscle function in both $Ryr^{Y524S/+}$ and $Ryr1^{I4895T/+}$ models, suggesting that it may represent an effective strategy for treating RyR1-related myopathies.

Gene replacement to cure *BIN1* centronuclear myopathy

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Centronuclear myopathies (CNM) are a group of severe genetic disorders characterized by generalized muscle weakness associated with abnormal nuclear centralization in muscle fibers. Most CNM cases are caused by mutations in genes involved in muscle membrane remodeling, including *BIN1* encoding amphiphysin 2. Currently, there is no curative treatment for CNM. Here, we aimed to cure the *Bin1*-CNM mouse model (*Bin1*$^{mck-/-}$) via an adeno-associated virus (AAV)-based gene replacement strategy.
We investigated the therapeutic efficacy of both preventive and curative approaches of exogenous BIN1 (amphiphysin 2) expression in *Bin1*$^{mck-/-}$ mice. Initially, we evaluated the benefit of the delivery of muscle-specific (mBIN1) and ubiquitous (uBIN1) BIN1 isoforms in *Bin1*$^{mck-/-}$pups. Muscle force and muscle mass were rescued in *Bin1*$^{mck-/-}$ mice injected at day 1 postnatal with mBIN1, whereas only a slight improvement was observed with uBIN1. Based on these findings, mBIN1 was selected to test disease reversion. To improve muscle specific gene delivery, we performed an in-depth analysis of myotropic AAV serotypes recently published, including AAVMYO and MyoAAVs, for their muscle transduction efficiency under different conditions and ages. Comparative analysis showed that MyoAAV4A appeared the most suitable serotype for specific transduction of the entire muscular system in adult mice. Therefore, MyoAAV4A-mBIN1 was administered into affected Bin1$^{mck-/-}$ mice at 8 weeks. Four weeks after treatment, total rescue of muscle force and fatigue, as well as restoration of organelle positioning and the t-tubule network, were observed.
Overall, our study provides a first proof-of-concept for an efficient gene therapy strategy to prevent disease progression and revert muscle weakness in *BIN1*-related CNM.

Multimodal single cell spatial profiling of Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is one of the most severe pediatric degenerative myopathies. In the initial phase of the disease, muscle is exposed to continuous cycles of degeneration and regeneration; over time, regenerative potential is exhausted, and necrosis prevails. As of today, the cellular and molecular determinants responsible for this functional exhaustion remain largely uncharacterized.
Adult tissue repair requires the activation of resident stem cells that can both self-renew and generate differentiated progeny. To establish and maintain their properties, stem cells require constant interactions with their microenvironment and their neighboring cells that altogether constitute the niche. The stem cell and its niche form as a whole the minimum functional unit of adult tissue repair. Any given perturbation affecting either the stem cell or the molecular/cellular components of the niche will invariantly impact repair potential. Therefore, in DMD the changes hindering the correct execution of the repair process must therefore occur either in the stem cell or in its niche.
Here we present a multi-omic Spatial strategy to elucidate the determinants interfering with regeneration in the dystrophic muscle and study the niche-stem cell interactions. By leveraging multi-modal data integration (Spatial transcriptomics, snRNAseq and snATACseq), we assessed changes in cell-type compositions, their spatial relationships and dependencies on other cell types, and the evolution of their respective crosstalk. Through our approach, we highlight the changes that occur in the transcriptome and muscle epigenome during disease progression in those regions associated with injury, regeneration, and degeneration. In conclusion, our study delivers an integrative molecular map of dystrophic muscle and lays the groundwork for future studies aimed at the identification of novel biomarkers and potential therapeutic approaches to promote muscle regeneration.

Targeting IL-6 signalling to attenuate dystrophic muscle degeneration

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Duchenne Muscular Dystrophy (DMD) is a muscle wasting genetic disease caused by mutations in the dystrophin gene. The absence of dystrophin protein triggers degenerative events in muscle, including necrosis, inflammation and fibrosis. A curative treatment for the disease is not currently available, despite the development of different therapeutical approaches aimed to restore dystrophin expression. Since dystrophic muscle is dominated by pro-oxidant and pro-inflammatory conditions, our hypothesis is that this hostile environment might interfere with the efficacy of therapeutic strategies. Mounting evidence support the role of IL-6 in fostering degenerative events in DMD muscle, triggering chronic inflammation. Thus, we propose to selectively interfere with the pro-inflammatory functions of the cytokine without compromising its homeostatic activity in order to slow down disease progression. The data collected suggest that the modulation of IL-6 activity during the necrotic phase of the disease could be a good strategy to stabilize dystrophic muscle, attenuating muscle inflammation, necrosis and preserving muscle functionality. Furthermore, the attenuation of later-stage muscle degeneration by IL-6 signalling modulation highlights the protective action of our approach against the grave loss of functional muscle tissue over time.

Disease exacerbation in MYOrganoids derived from Duchenne Muscular Dystrophy iPSC reveals limitations of microdystrophin therapeutic efficacy

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Duchenne muscular dystrophy (DMD) is a lethal muscle-wasting disease caused by the absence of Dystrophin, a protein essential to preserve muscle integrity continuously challenged by contractions. Gene therapy utilizing adeno-associated virus (AAV) to deliver truncated forms of dystrophin (µDys) is currently the most promising therapeutic approach. However, the therapeutic outcome in treated patients has not been as successful as anticipated by animal studies, underscoring the need for improved and high-throughput models for fast and accurate prediction of human response. Here, we describe the generation of MYOrganoids, an in vitro 3D muscle platform based on direct myogenic conversion of human induced pluripotent stem (iPSC) cells including fibroblasts to ensure proper muscle structure and function. We also exploited the secretory activity of fibroblasts to provide microenvironmental cues, essential for pathophysiological studies. Remarkably, MYOrganoids derived from DMD-iPSC including DMD fibroblasts, show exacerbated pathogenic hallmarks such as extracellular matrix remodeling, muscle force loss and fatiguability, across the different DMD iPSC cell lines employed. As proof of the suitability of our system for gene therapy screening, we employed AAV9-mediated µDys gene transfer in DMD-MYOrganoids. We showed that µDys delivery, partially improved muscle resistance and environmental stress but failed to significantly restore dystroglycan components at the membrane. Transcriptomic analysis confirmed an amelioration of mechano-stability and inflammatory hallmarks but, more importantly, revealed that only a partial correction of the DMD signature is achieved after microDys restoration. This evidence highlights the necessity to identify additional therapeutic targets and places our bioengineering approach at the forefront of exploring complementary strategies beyond gene therapy with the potential to accelerate the discovery of more effective therapeutics.

Enhancing micro-dystrophin gene therapy: the role of SRT2104, a new Sirtuin1-activating compound, for the treatment of Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy(DMD) is an X-linked recessive disorder caused by mutations in the *dystrophin* gene. The lack of dystrophin protein disrupts the dystrophin-glycoprotein complex eliciting structural degeneration and functional impairments of myofibers. Currently, there's no cure for DMD. However, progress has been made in gene therapies to restore dystrophin and among the different trialed strategies, the approach based on AAV-delivered micro-dystrophin (MD) is the last to be approved. Nevertheless, it still presents some limits. Indeed, the dystrophic muscle milieu exhibits chronic inflammation and early sarcolemmal fragility that do not support MD engraftment and its preservation over time. Therefore, to enhance gene therapy efficacy, it is crucial to develop conservative therapies that preserve dystrophic muscles.
For this purpose, the NADH-dependent deacetylase Sirtuin1 (SIRT1) might be the suitable target. In *mdx* mice, SIRT1 overexpression tends to counteract the dystrophic muscular and cardiac phenotype.
Among the new SIRT1-activating compounds, SRT2104 has never been tested in DMD. We assessed its efficacy on *mdx* mice demonstrating that, after 12 weeks of administration, treated mice show functional, metabolic and histological improvements compared to the controls.
Overall, given its effects on crucial hallmarks of DMD, SRT2104 could be the proper candidate to sustain MD-based gene therapy in a combined treatment. Noteworthy, this dual approach could also allow to reduce the required AAV dose and, consequently, reduce the dangerous adverse effects related to viral vector immunogenicity.
In a dose-response study, we selected the minimum sub-optimal doses of AAV-MD able to restore at least 20% of dystrophin expression in *mdx* mice and two of these doses have been injected into mice previously treated with SRT2104 to assess whether this can promote a recovery superimposable to the optimal dose of MD, thus demonstrating the advantages of a combined therapy.

The role of patients' associations in IHI European multi-partner projects

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Parent Project (PP) is an association of patients with Duchenne and Becker Muscular Dystrophy. With the main goals of improving quality of life, prolonging life expectancy and finding a cure, we accompany patients and their families throughout their journey with a multidisciplinary approach.
The scientific office is involved in many activities that work in this direction, including funding research and organizing our annual International Conference. Other less direct yet extremely powerful ways by which we aim to speed up the development of new therapies and supporting research are being generated thanks to the growing number of partnerships with different stakeholders. In fact, as our association expanded, the same happened with connections with various partners and we are now well established in an international network rotating around the pathology that sees us also involved in different, important IHI European Projects, among which the MAGIC and the PaLaDIn Project.
The MAGIC project is a multi-partner ambitious scientific project aiming at developing advanced models of human skeletal muscle and innovative gene therapy approaches. In addition to several scientists, different patients' associations (PAs) are involved in this project, to highlight the importance of the partnership among scientists and PAs, which becomes evident in our role of disseminating the scientific findings in a family-friendly language and in bringing the patients' voice to scientists to guide them in the best direction.
PaLaDIn is a multi-stakeholder project, coordinated by PP, that aims to align registry-reported data with Patient Reported Outcome Measures and other data, e.g. wearable devices, to be collected in an omni-interoperable-platform (the Interactium) to improve outcomes and decision-making, and accelerate innovation for a range of stakeholders. Once again the role of PAs is crucial to the outcome of such a project due to our high-level of experience with patient data collection.

Characterization of the infiltrating polarized macrophages during the onset of heterotopic ossification in a mouse model of Fibrodysplasia Ossificans Progressiva

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Fibrodysplasia Ossificans Progressiva (FOP) is a rare congenital disease that results in heterotopic ossification (HO) of skeletal muscles. It arises from a mutation (R206H) in the Acvr1 gene encoding for the activin type I receptor, leading to an aberrant activation of the bone morphogenetic proteins and activin A signalling pathways.
FOP patients develop HO in response to flare-ups in skeletal muscles. In this context, macrophages still have an unclear role and require a better characterization.
To model FOP, we used the Acvr1(R206H)loxP;Gt(ROSA26)SorCreERT2 conditional transgenic FOP mouse strain. Tamoxifen induced FOP mice received muscle injury in the gastrocnemius to trigger local inflammation. Computerized tomography (CT) showed that FOP mice formed HO at 14 and 21 days post injury.
To investigate the role of macrophages during HO, we depleted circulating monocytes by injecting clodronate liposomes intravenously. CT revealed that macrophage-depleted FOP mice had lower HO at 14 and 21 days after injury.
To explore the early signalling leading to HO, we performed single-cell RNA sequencing on the gastrocnemii of control and FOP mice at 5 and 7 days after injury. We analysed the differentially expressed genes in macrophages and fibroadipogenic precursors (FAPs), the cells mainly responsible for HO in FOP.
We observed an upregulation of ossification genes in FOP FAPs and an upregulation of glycolysis/OXPHOS in FOP macrophages. Moreover, both clusters were enriched in hypoxia and extracellular matrix remodelling pathways.
Next, we studied the interactions between FAPs and macrophages in vitro. We tested whether FAPs and macrophages interaction could affect HO formation by culturing FAPs with the conditioned medium (CM) derived from polarized macrophages. Both control and FOP FAPs treated with CM from FOP macrophages showed increased mineralization.
Overall, our data indicate that macrophages and FAPs interaction promotes bone formation since early timepoints in FOP mice.

Enhancing skeletal muscle regeneration in ALS via intramuscular allosteric activation of the P2X7 receptor

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Amyotrophic Lateral Sclerosis (ALS) is a fatal motor neuron disorder characterized by early skeletal muscle failure and irreversible atrophy at the periphery. We recently found that systemic intramuscular administration of the P2X7 agonist (BzATP), improved motor performance in ALS mice by enhancing satellite cells and the muscle pro-regenerative activity of infiltrating macrophages.
Here, we translated this evidence into a therapeutic strategy in ALS by investigating the disease-modifying effects of enhancing the P2X7 pathway in the skeletal muscle of ALS mouse models using a highly selective P2X7 positive allosteric modulator, the ginsenoside compound K (CK). CK has shown neuroprotective, anti-inflammatory, and pro-regenerative effects in chronic diseases, and it has high specificity for the P2X7 receptor in humans, making it a safe and effective drug for clinical use.
Preliminary data shows that CK stimulates, via the P2X7 pathway, the proliferation and the differentiation of muscle satellite cells isolated from ALS mice. Additionally, CK polarizes primary macrophage cells towards an M2 anti-inflammatory phenotype, which is crucial for effective muscle regeneration.
In parallel, a targeted pharmacokinetic and pharmacodynamics study was conducted to determine the optimal CK dose regimen. These data allowed us to obtain primary *in vivo* evidence in fast (PrP-FUS) and slow (SOD1G93A) transgenic mice. Intramuscular treatments with CK increases muscle mass, which correlates with more mature muscle fibers in early-stage ALS mice undergoing muscle atrophy.
Our data provide the first evidence to show that selective activation of the P2X7 pathway with CK exerts positive effects on skeletal muscle regeneration in ALS mice. Ongoing studies will systematically validate this therapeutic strategy to promote muscle regeneration that can be used alone or in combination with CNS-targeted drugs to improve the effectiveness of potential clinical treatments in ALS.

Consequences of CNBP reduced expression in DM2 pathogenesis

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Myotonic dystrophy type 2 (DM2) is a dominant autosomal disease that primarily affects skeletal muscle, causing degeneration and dysfunction of muscle fibers. DM2 is due to a CCTG repeat expansion mutation in intron one of CNBP (Cellular Nucleic acid Binding Protein) gene. The pathogenic mechanism involved in DM2 development is still uncertain.
Our studies demonstrated that a decrease of CNBP expression in muscles can affect locomotor activity by causing a reduction in polyamine levels, linked to a decrease of the key polyamine biosynthesis enzyme ODC (Ornithine Decarboxylase) translation in Drosophila. We demonstrated that the locomotor defects caused by CNBP depletion can be rescued by polyamine supplementation or restoring dOdc expression.
CNBP deficiency correlates with a reduction of polyamine levels in muscle cells from DM2 patients, which are both downregulated compared to healthy individuals.
We investigated the effect of CNBP depletion in the constitutive KO CNBP mouse model. We have observed that mice with heterozygous deletion of CNBP show a late onset phenotype of impaired locomotor activity reminiscent of DM2, associated with morphological alterations of muscle tissues.
The exact mechanism linking the impairment of CNBP/ODC/polyamine axis to the observed muscle dysfunction is currently unknown and it is not clear if polyamine supplementation might provide therapeutic benefit to other animal models and, most relevantly, to DM2 patients. Emerging observations are pointing at the translation factor eIF5A, whose activity is strictly dependent on the polyamine levels. We analyzed eIF5A hypusination, in dCNBP-depleted larvae, that resulted reduced compared to controls, suggesting that dCNBP may regulate eIF5A activity, through its translational control of ODC.
Our results revealed a new role of CNBP in muscle diseases, linked to polyamine metabolism. Further studies may provide innovative approaches to treat DM2 patients.

Different outcomes of endurance and resistance exercise in skeletal muscles of Oculopharyngeal muscular dystrophy

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Background. Exercise is widely recognized for its beneficial effects on skeletal muscle aging and several muscular dystrophies. Oculopharyngeal muscular dystrophy (OPMD) is a late-onset autosomal dominant disorder caused by PAPBN1 gene mutations. These mutations lead to PABPN1 intranuclear aggregates. This study aims to evaluate the effects of endurance and resistance exercise on the OPMD skeletal muscle phenotype using a murine model.

Methods. Two exercise protocols were tested. In the endurance protocol, wild-type and OPMD mice underwent a 6-week motorized treadmill program (3 sessions/week, 20cm/s for 20 minutes). In the resistance protocol, induced by chronic mechanical overload (OVL), surgical removal of gastrocnemius and soleus muscles was performed to induce plantaris muscle hypertrophy. Muscles of OPMD and wild-type mice were compared to respective sedentary controls. Force measurement, muscle histology, and molecular analyses were conducted.

Results. Endurance exercise showed no significant changes in muscle physiological parameters but increased PABPN1 intranuclear aggregates in both tibialis anterior and gastrocnemius, along with enhanced collagen deposition in OPMD mice. The resistance OVL protocol resulted in increased collagen deposition in the plantaris muscle of OPMD mice, larger muscle mass and fiber cross-sectional area, increased absolute maximal force, and reduced PABPN1 aggregate number.

Conclusions. Running and mechanical overload produced distinct outcomes in OPMD mice skeletal muscles. Both exercises increased collagen deposition; however, running increased aggregates, while OVL reduced them. Importantly, OVL reversed muscle atrophy and enhanced maximal force in OPMD mice. This study suggests differential effects of exercise types on OPMD muscle, warranting further evaluation in humans for lifestyle recommendations in OPMD individuals.

Muscle stem cell function is impaired in absence of Talpid3 - a gene required for primary cilia formation

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Skeletal muscle stem cells (MuSC) are crucial for tissue homeostasis and repair after injury. Following activation, they proliferate to generate differentiating myoblasts. A proportion of cells self-renew, re-enter the MuSC niche under the basal lamina outside the myofiber and become quiescent. Quiescent MuSC have a primary cilium, which is disassembled upon cell cycle entry. Ex vivo experiments suggest cilia are important for MuSC self-renewal, however, their requirement for muscle regeneration in vivo remains poorly understood. Talpid3 (TA3) is essential for primary cilia formation and Hedgehog (Hh) signalling. We used tamoxifen-inducible conditional deletion of TA3 in MuSC (iSC-KO) and showed that regeneration is impaired in response to cytotoxic injury. Depletion of MuSC after regeneration suggested impaired self-renewal. This was consistent with an exacerbated phenotype in TA3iSC-KO mice after repeat injury. Single cell transcriptomics of MuSC progeny isolated from myofibers identified components of several signalling pathways, which were deregulated in absence of TA3, including Hh and Wnt pathways. Pharmacological activation of Wnt restored muscle regeneration in vivo, while purmorphamine, an activator of the Smoothened (Smo) co-receptor in the Hh pathway, had no effect. Together, our data show that TA3 and primary cilia are important for MuSC self-renewal and that pharmacological treatment can efficiently restore muscle regeneration in the absence of cilia.

Fibro-Adipogenic Progenitors (FAPs) facilitate skeletal muscle reinnervation via retinoic acid release

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Fibro-Adipogenic Progenitors (FAPs) are a population of interstitial cells that resides in skeletal muscle tissue and participate in muscle homeostasis under physiological and pathological conditions. Following nerve injury, such as denervation, FAPs are among the first cells activated in skeletal muscle. Through next-generation sequencing techniques and classical molecular biology we found that after denervation FAPs synthesize and release Retinoic Acid (RA). Therefore, to determine the role of FAPs in this context, we used in vitro models of RA-sensitive reporter cells. By this strategy we demonstrated that FAPs produce and release RA after denervation. Furthermore, we generated a mouse model where we can eliminate Rdh10 - a key factor in RA synthesis – expression only in FAPs. These findings can provide the basis for studying the mechanisms of muscle reinnervation under pathological denervation conditions and the role of FAPs in mediating muscle reinnervation.

Non-coding RNAs to Treat Skeletal Muscle Atrophy

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Skeletal muscle atrophy occurs due to malnutrition, age, genetics, lack of physical activity, or certain medical conditions. The most harmful effect of muscle atrophy is decreased quality of life due to functional disability, increased risk of fractures, decreased basal metabolic rate, and reduced bone mineral density. Increasing evidence suggests the central role of non-coding RNAs in genetic and epigenetic modulation of muscle function. We analyzed coding and non-coding RNAs in different conditions of muscle atrophy to identify signatures based on non-coding RNAs involved in the maintenance of muscle mass. We focused on a specific process associated with muscle aging and loss of nerve function to identify short and long non-coding RNAs capable of inducing myotube formation and enhancing muscle mass. Conclusions. We have shown that satellite cells overexpressing a specific miRNA (patent process) produce thicker myotubes and secrete the same miRNA to affect other non-engineered muscle cells. These cells are also able to reverse muscle atrophy induced by a pathological condition such as ALS. In addition, overexpression of a specific long non-coding RNA, which counteracts miRNA activity and is overexpressed in pathological conditions in muscle, is able to limit the beneficial effect of miRNA.

Experimental autoimmune encephalomyelitis induced muscle loss in mice triggers engraftment of systemically administered mesoangioblasts

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Disuse-induced muscle atrophy often results in muscle weakness, causing decreased mobility and self-sufficiency. We are developing a stem-cell-based recovery of muscle mass without the need to exercise, as this is often not feasible. We tested our approach in an experimental autoimmune encephalomyelitis (EAE) mouse model in which disuse of the hindlimbs is observed due to paresis and paralysis. We first determined if healthy muscle stem cells, called mesoangioblasts (MABs), engraft in the atrophic muscle. To investigate this, we determined atrophy-associated expression patterns and evaluated MABs homing in the hind-limb muscles.
EAE was induced in female C57BL/6 OlaHSD mice (n=15) by an emulsion of MOG/CFA, followed by an injection of PTX, with a follow up of 8-23 days. Muscle mass loss (21.1±6.4% SEM, $p<0.05$) was observed at end point in the m. gastrocnemius (GN) in EAE compared to controls (n=12). Increased IκBα mRNA levels (2.7±0.07 SEM fold change, $p<0.0001$) were detectable at day 23, indicating inflammatory signaling in the GN in the preceding days. Based on this data, day 16 after EAE-induction was chosen to transplant the mice intra-arterially with vehicle-control or ~$2.5x10^5$ luciferase-expressing MABs (n=2/group). After 24h, mice were injected i.p. with D-luciferin to measure bioluminescence. Total photon flux was captured with an ROI average of $9,9*10^3$ p/s in the transplanted leg, indicating a positive signal for MAB homing. Analysis of relative gene expression within the GN is currently being performed to confirm luciferase-expression in the muscle fibers.
We conclude that systemically injected MABs engraft in the atrophic muscles of EAE mice. Our next experiment will determine if this engraftment leads to an increase in muscle mass and functional recovery. These studies will provide the first preclinical data of muscle stem cell therapy for disuse-induced muscle atrophy. If positive, this will open-up new therapeutic options for treating muscle disuse.

Premature aging of skeletal muscle of Kennedy disease mouse models

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The androgen receptor (AR) is a nuclear receptor activated by ligand binding (androgenic hormones). Activation leads to AR translocation into the nucleus, DNA binding, cofactors recruitment and ultimately regulation of androgen responsive genes' expression. Expansions over 38 repeats of the polymorphic glutamine (Q)-encoding CAG tandem repeat in the first exon of the androgen receptor gene (*AR*) gene cause Kennedy disease, also known as spinal and bulbar muscular atrophy (SBMA): an inherited, slowly progressive neurodegenerative disease that fully manifests only in males, due to their higher levels of testosterone. SBMA is characterized by both a loss of function (LOF) and toxic gain of function (GOF) of AR, leading to motor neuron loss, muscle atrophy, mild androgen insensitivity syndrome and metabolic syndrome. 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 of extensive activation of pathways linked to DNA damage, inflammation and cellular senescence in muscles of our mouse models and biopsies from SBMA patients. The alteration of these pathways starts at disease onset and is further increased at the late stage of the disease in mice, mirroring the progressive worsening of the muscle phenotype in the model. Castration results in an amelioration of the phenotype, highlighting the androgen-dependency of the pathology, driven by the toxicity of the polyQ-expanded AR. These observations suggest that senolytics or senomorphics might be an effective therapy to clear damaged cells from the muscle, therefore attenuating the detrimental consequences of the toxic GOF without exacerbating the LOF of the receptor.

Nanoparticle-mediated delivery to skeletal muscle cells of N-palmitoylethanolamide (PEA), an endocannabinoid-like molecule with anti-inflammatory properties

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Much evidence supports the idea that chronic low-grade inflammation contributes to sarcopenia, that is the loss of muscle function and mass observed during aging, suggesting the potentiality of daily anti-inflammatory strategies to counteract age-related muscle wasting. In this work we assessed whether N-palmitoylethanolamide (PEA), a natural endocannabinoid like molecule, might represent an anti-inflammatory compound of putative relevance to treat sarcopenia as its use is not linked to serious side effects. The use of PEA as a drug is highly limited due to its extremely low solubility in biological fluids and consequent poor bioavailability. We developed nanotechnology-based formulations (nanoparticles, NPs) to deliver PEA to muscle cells in culture. We found that PEA loaded NPs are efficiently internalized in muscle cells and they do not interfere with cell viability or muscle cell terminal differentiation. Furthermore, our preliminary results indicate that PEA-loaded NPs reduced lipopolysaccharides (LPS)-mediated increase of the level of transcripts encoding interleukin (IL)-6 and Tumor Necrosis Factor (TNF)-alpha. Our results will contribute to clarify clinical potential of PEA-loaded NPs to evaluate further *in vivo* development of this preclinical study.
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Sarcopenic obesity in the elderly: a dysfunctional crosstalk between tissues?

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Sarcopenic obesity (SO) is a growing health problem, especially in the elderly. The mechanisms linking obesity, sarcopenia, and inflammation are largely unknown. To unravel their interplay at cellular and molecular levels, we developed a mouse model of SO in aging using 12-month old female mice fed for 7 months with high fat diet (HFD, 60% of kCal derived from fat) or standard diet (SD). We monitored the onset of obesity and sarcopenia measuring weight, fat/lean mass ratio and muscle strength. At selected time points, we performed histological and molecular analyses of blood, skeletal muscle, and subcutaneous and visceral adipose tissue.
HFD induced a rapid increase of body weight and fat, and a loss of muscle strength. Muscle mass was reduced after 7 months of HFD when appeared a significant inflammatory infiltrate in muscle tissue. Adipose tissues showed an increase in adipocyte size after 1 month of HFD. The metabolic profiles of skeletal muscle, adipose tissue, and plasma revealed significant alterations starting from 1 month of diet (e.g. reduced capacity to metabolize fatty acids and decreased oxidative stress buffering). Accordingly, skeletal muscle mitochondrial respiratory capacity was reduced in HFD.
To dissect mechanisms underlying the cross-talk between adipose tissue, skeletal muscle, and blood, we also characterized circulating extracellular vesicles (EVs). Preliminary results demonstrate that EVs of leukocyte, macrophage, endothelial, platelet or adipocyte origin were present in both mouse groups. EVs of adipocyte and endothelial origin decreased in mice receiving HFD indicating a possible uptake by the tissues.

The H19/Let-7/myc axis regulates muscle inflammatory responses

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H19 is negatively associated with fat free mass index (FFMI) in COPD and older individuals. One potential mechanism suggested for this association has been the production of miR-675 from H19 leading to suppression of myoblast proliferation and thereby regeneration. However, the association between H19 and FFMI is with whole muscle H19 RNA rather than myoblast RNA raising the possibility that the relevant function of H19 is in the myofiber.
H19 is a multi-functional long non-coding RNA that contributes to an H19/Let-7/myc regulatory loop by binding and inactivating Let-7 miRNAs. Our recent analysis has suggested that patients with severe COPD have higher expression of myc regulated inflammatory response genes raising the possibility that this loop regulates the muscle inflammatory response and thereby muscle loss.
We compared H19 expression with other genes in microarray data from COPD patients and found that H19 was associated with genes from the epithelial mesenchymal transition (EMT) and with myc target genes. To determine whether H19 regulated myc and inflammatory responses in muscle cells via binding Let-7 miRNAs, we designed locked nucleic acid (LNA) oligos to disrupt the interaction between H19 and the Let-7e and Let-7g (LNA7g and LNA7e respectively), an LNA designed to knock down H19 (LNA-KD) and a control LNA that bound H19 in a region with no known function (LNA-C). In C2C12 cells LNA-KD, LNA7e suppressed H19 expression. All 3 test LNAs suppressed myc and MCP-1 expression compared to LNA-C. LNA7e also inhibited the increase in IL-6 expression in response to TNFa stimulation. We electroporated LNA7e and LNA7g into one tibialis anterior (TA), LNA-C into the other, then infected the mice with respiratory syncytial virus. 5 days later the muscles containing LNA7e/g had lower expression of inflammatory response genes than those containing LNA-C. These data suggest that H19 regulates muscle inflammation via the H19/let-7/myc axis.

Pre-clinical development of a drug inhibiting the chromatin remodeling protein WDR5 in FSHD muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is one of the most prevalent neuromuscular disorders. Weakness is slowly progressive with high variability among patients. The disease is caused by the aberrant expression of the transcription factor DUX4, which is normally confined to early embryonic development. In FSHD, DUX4 mis-expression activates a pro-apoptotic transcriptional program leading to block of differentiation and muscle wasting. Given its pivotal role in FSHD, blocking DUX4 expression with small molecule drugs is an attractive solution.
Previously, by combining proteomics with genetic and pharmacological targeting, we identified the chromatin remodeling protein WDR5 as a key activator of DUX4 expression in FSHD. By testing various compounds, we identified a novel WDR5 inhibitor (WDR5i) showing higher potency and better pharmacological properties, which are important for pre-clinical testing. To this aim, we evaluated WDR5i safety and efficacy in preclinical models of FSHD. We confirmed WDR5i ability to inhibit DUX4 expression using muscle cells isolated from multiple FSHD patients. We also found that an intermittent WDR5i treatment if sufficient to obtain long-term DUX4 repression. Importantly, long-term WDR5i treatment does not significantly affects muscle cells proliferation or differentiation. To perform in vivo tests, we set up a humanized animal model of FSHD that will allow us to evaluate WDR5i safety and efficacy in a relevant setting.
Results from our work could provide a novel therapeutic opportunity for FSHD patients that, up to now, have no cure.

Inhibition of HDAC6 improves muscle integrity in Duchenne Muscular Dystrophy mouse model

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Duchenne muscular dystrophy (DMD) is the most common and fatal form of muscular dystrophy. The absence of dystrophin in DMD disrupts the dystrophin-associated glycoprotein (DGC) complex resulting in fiber fragility and atrophy, associated with disorganization of microtubules and of the neuromuscular junction (NMJ) as well as to fibrosis. The non-conventional cytoplasmic histone deacetylase 6 (HDAC6) was previously shown to impede acetylcholine receptor distribution and promote muscle atrophy.
Here we show that administration of the specific HDAC6 inhibitor (tubastatin A) to the *mdx* mouse model for DMD improves muscle strength, restores microtubule, NMJ and DGC organization and protects against muscle atrophy and fibrosis. Unexpectedly, we found that the beneficial effects of HDAC6 inhibition also involve the downregulation of transforming growth factor-beta (TGF-β) signaling by increasing acetylation of the downstream effector Smad3 in the cytoplasm, thereby preventing their phosphorylation, nuclear translocation, and transcriptional activity. Moreover, a series of additional experiments were performed based on the therapeutic relevance of HDAC6 inhibitor in *mdx* mice.
These results shed new light on the mechanisms involved in muscle cell development, while revealing the therapeutic potential of targeting HDAC6 for the treatment of neuromuscular diseases. Consequently, inhibition of HDAC6 by pharmacological agents represents an attractive therapeutic target for DMD, offering numerous advantages, including impact on all muscles and benefits for all patients, regardless of dystrophin mutations.

Investigating the potential of MICAL2 modulation for impeding rhabdomyosarcoma cancer progression

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MICAL2 is a member of the Microtubule Associated Monooxygenase, Calponin And LIM domain containing protein family that is involved in regulating F-actin depolymerization and actin cytoskeleton rearrangement, which underlie cell motility and division, via oxidation-reduction reactions. The overexpression of MICAL2 in rhabdomyosarcoma (RMS), among many other cancers, prompted us to examine the role this gene may play in RMS as it represents the most common soft tissue sarcoma afflicting children and young adults and is characterized by dysregulated myogenesis. Initial *in vitro* investigations involving MICAL2 knockdown (KD) using plasmid constructs containing short hairpin RNAs (shRNAs) designed to target *mus musculus* (*Mm*) MICAL2 mRNA demonstrated significant reductions in migratory and invasive properties of murine fusion negative RMS (FN-RMS) cell lines. Upon transitioning to *in vivo* experiments by loading these shRNAs into inducible TET ON lentiviral constructs to assess the impact of stable MICAL2 KD, our results corroborated the *in vitro* observations, wherein MICAL2 silencing gave rise to positive outcomes including reduced primary tumor size, absence of metastatic sites, and improved functional performance. Subsequent analyses of the muscle tissues from the *in vivo* experiments using bulk RNA-sequencing also confirmed the beneficial effects of MICAL2 KD, and proteomic analyses are now in progress in order to further enhance our understanding of the molecular alterations that contribute to RMS pathophysiology. The results obtained thus far motivate more in-depth examinations into the role of MICAL2 in FN-RMS and highlight targeting MICAL2 as a potential therapeutic strategy for RMS treatment.

Serum amyloid protein A1 (SAA1) impairs myogenesis and myotube size in pancreatic cancer cachexia

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Cancer cachexia is a multifactorial syndrome characterized by a progressive loss of skeletal muscle mass, leading to increased mortality in cancer patients. The complexity of cachexia makes diagnosis challenging, with major clinical manifestations including systemic inflammation and muscle wasting. This syndrome can reduce body weight by up to 30%, negatively impacting patient response to treatments and decreasing life expectancy. Cachexia occurs in individuals with various types of cancer, with the highest incidence reported in pancreatic cancer. Understanding the systemic factors influencing muscle in cachexia may lead to novel therapeutic targets. A currently debated issue is whether cachexia also negative affects the ability of skeletal muscle to regenerate. To assess if circulating factors negatively affect muscle stem cells (MuSC), I treated human MuSCs (hMuSCs) with serum from cachectic patients. The results show reduced self-renewal potential and upregulated levels of Serum Amyloid A1 protein (SAA1), a protein released during acute phase response. Treatment of hMuSC with SAA1 protein alone produces effects comparable to those of the cachectic serum. In an *in vivo* model of pancreatic cancer, we found that mice exhibites muscle loss (~10%) and a reduced MuSC self-renewal. Proteomic analysis of plasma from these cachectic mice show increased SAA1 levels. Further, RNAscope and ELISA tests demonstrate high production of SAA1 by skeletal muscles in cancer cachexia, suggesting SAA1 as a potential target for treatment. I am investigating the molecular mechanisms by which SAA1 affects the myogenic process and causes muscle loss. An increased understanding of the processes underlying cancer cachexia will accelerate the discovery of novel treatments, ultimately improving the survival and quality of life for cancer patients.

Keywords: cancer cachexia, SAA1, muscle stem cells, muscle wasting.

Engineered exosomes as a therapeutic tool to counteract muscle degeneration

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Duchenne muscular dystrophy (DMD), caused by mutations in the dystrophin gene, is the most prevalent form of muscular dystrophies (MDs). Other MDs involve mutations in components of the dystrophin-associated protein complex (DAPC), such as the sarcoglycan complex, where mutations in the SCGB gene result in autosomal recessive limb-girdle muscular dystrophy type 2 (LGMD2E). Furthermore, muscle degeneration is also associated with aging and various chronic pathologies such as diabetes and cancer. Recent studies highlight the importance of paracrine factors in sustaining muscle homeostasis. Extracellular vesicles (EVs), carrying specific host factors such as microRNAs (miRNAs), can play a role in muscle physiological growth, development, and regeneration (1). We have identified miRNAs that can boost myogenic differentiation of mesodermal progenitors derived from human induced pluripotent stem cells (hiPSCs) (2). Our research focuses on developing custom-engineered EVs as therapeutic tools to counteract muscle degeneration. To this end, we are developing in vitro 2D and 3D models to mimic different types of muscle degeneration, using patient-derived DMD hiPSCs (3), bSGC null hiPSCs, and human immortalized myoblasts. EVs from DROSHA knockout HEK-293T cells have been enriched with selected miRNAs or combinations thereof. These EVs will be tested on our cell models to evaluate their efficacy in improving myogenic differentiation. The generation and delivery of EVs with custom-engineered cargos, either alone or in combination with other therapies, may offer innovative approaches for treating muscle wasting, providing new hope for patients with various muscle degeneration conditions.

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Immunomodulation via interleukin-4 improves energy metabolism in C26 tumor-bearing mice

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Introduction. Cancer cachexia is a complex syndrome featuring loss of body weight and skeletal muscle mass, systemic inflammation, mitochondrial alterations and altered energy metabolism. Pro-inflammatory cytokines are central in the pathogenesis of cancer cachexia, suggesting the use of antagonistic cytokines as a potential therapeutic strategy. Indeed, previous studies proved that treating tumor-bearing (TB) mice with interleukin-4 (IL4) improves muscle function, myogenesis and survival. Whether IL4 administration improves energy metabolism in the skeletal muscle is unknown.
Methods. Eight-week-old Balb/c male mice were inoculated with 5 x 10^5 C26 colon carcinoma cells. Daily IL4 treatment (66.5 µg/kg) was performed by intraperitoneal injection. The gastrocnemius muscle was used to assess mitochondrial proteins (western blot) and respiration (high-resolution respirometry, Oroboros Instruments). Liver and gastrocnemius muscle were used to perform mass spectrometry based metabolomic analyses (University of Colorado, Aurora, USA).
Results. Treatment with IL4 counteracted the loss of body weight, muscle mass and muscle strength in TB mice. Spleen enlargement was found in treated TB mice. The protein levels of the oxidative phosphorylation (OXPHOS) complexes II and III, cytochrome c and PGC-1α in the skeletal muscle were significantly increased in treated vs untreated TB mice, while trends to decrease were found for BNIP3 and TFAM. The activity of OXPHOS complex II was significantly increased in treated vs untreated TB mice. Tumor and IL4 dependent modulations in liver and skeletal muscle metabolomic profiles were observed.
Conclusion. Treatment with IL4 improves energy metabolism in the skeletal muscle of TB mice, possibly exerting an exercise-mimetic role. Spleen enlargement suggests that IL4 modulates the immune response. Whether IL4 effectiveness also results from modulation of the immunological milieu in the tumor microenvironment remains to be investigated.

Western diet worsens cancer-induced muscle wasting which is restrained by *Vaccinium macrocarpon* extract

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Cancer cachexia (CC) is a complex and still poorly understood syndrome characterized by progressive loss of muscle mass and strength (*i.e.* muscle wasting, MW) and negatively influencing anti-tumor therapies, leading to poor outcomes. The worldwide consumption of Western diet (WD) foods rich in sugars and fats, and containing high levels of advanced glycation end-products (dietary AGEs, dAGEs) has contributed to increasing systemic inflammation and oxidative stress promoting several diseases. AGEs induce tissue damage by directly cross-linking proteins or by binding their receptor, RAGE. High levels of AGEs are found in plasma and primary tumors of cancer patients, and RAGE sustains cachexia in tumor-bearing mice. The direct role of WD-derived dAGEs in the onset and progression of CC is unknown. C2C12 myotubes exposed to tumor necrosis factor-α or pro-cachectic factors secreted by Lewis lung carcinoma (LLC) cells, in the absence or presence of cisplatin, to mimic CC *in vitro* showed AGE accumulation in concomitance with ubiquitin-proteasome-dependent degradation of myosin heavy chain (MyHC)-II and reduction of myotube diameters. Male mice fed with dAGE-enriched WD *vs* standard diet (SD) and injected subcutaneously with LLC cells exhibited a more severe CC as revealed by greater loss of body weight, higher presence of atrophic myofibers, and increased MyHC-II degradation and induction of *Trim63* expression. The administration of a standardized *Vaccinium macrocarpon* (*VM*) extract, which reduces dAGE accumulation/activity, counteracted MW in both *in vitro* models of CC and LLC tumor-bearing mice fed with WD. The active metabolites in *VM* extract and their potential molecular targets were investigated by *in silico* target fishing. Our results indicate that CC is worsened by the consumption of unhealthy WD and that *VM* might represent a food supplement useful to restrain MW in cancer patients.

Muscle directed nutrition for cancer cachexia

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Cancer-associated cachexia is a multifactorial syndrome characterized by the involuntary loss of body and skeletal muscle mass which negatively impacts tolerance to cancer treatments, increases complications following cancer surgery, and is strongly predictive of reduced survival. Unfortunately, there are currently no approved FDA or EMA approved therapies to preserve, or reverse the loss of, muscle mass in cancer patients. However, one potential therapy that holds significant potential is ursolic acid, a natural dietary compound found in several edible herbs and fruits. Ursolic acid has been shown to protect against muscle loss in various pre-clinical models that induce muscle wasting, including fasting, muscle disuse and aging. To test the hypothesis that ursolic acid might prevent cancer cachexia, we injected pancreatic, colon, or lung cancer cells into mice, or PBS as a control and, when tumors first became palpable, switched a subset of mice to a diet supplemented with ursolic acid. Mice were tracked until those in the tumor bearing control diet group reached IACUC mandated endpoint, at which time multiple tissues were collected. Data will be presented on our findings from these studies.

Unraveling the therapeutic potential of givinostat in muscle atrophy induction

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Muscle atrophy is a debilitating condition characterized by muscle degradation and subsequent weakness. It arises from several conditions ranging from temporal muscle disuse to genetic pathologies, including Duchenne Muscular Dystrophy (DMD). In healthy muscles, homeostasis alteration by atrophic stimuli is transient, and satellite cells contribute to muscle repair. In pathological conditions such as DMD, persistent inflammation and fibrotic tissue accumulation is coupled with reduced satellite cell function and to irreversible muscle loss. While corticosteroids represent the standard for the treatment of DMD, their efficacy is often hindered by undesirable side effects, and many alternative therapeutic strategies have been explored. Recently, our proprietary small molecule HDACs inhibitor givinostat has been approved by FDA as the first nonsteroidal drug to treat DMD patients with all genetic variants. This study aimed at elucidating the effect of givinostat on muscle atrophy using an *in vitro* model based on differentiated human primary skeletal myotubes (HSkM). Cells were treated with TNF-α, both in the presence and absence of Deflazacort (DZ) and/or givinostat, followed by transcriptomic analysis to unravel the molecular mechanisms. Givinostat alone reduced the activation of inflammatory and cell cycle pathways and upregulated metabolic and intracellular vesicles trafficking pathways. These modulations were confirmed by a proteomic analysis on murine myotubes that showed a mRNA-protein correlation of 80.3%. Moreover, givinostat reversed the TNF-α deleterious effects, in contrast to DZ, downregulating key pathways associated with muscle degradation, as inflammation, proteasome core complex and necroptosis. Furthermore, givinostat increased protein deubiquitination, both independently and in combination with DZ. Remarkably, DZ induced ferroptosis that was restored by givinostat. These findings further support the role of givinostat in reducing muscle damage in DMD.

Poster Abstracts

P.1

Evaluation of miR-486 expression levels in mice skeletal muscle under different metabolic conditions

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Skeletal muscle is a dynamic organ primarily responsible for voluntary movement and support to body posture. Additionally, it is also critically involved in regulating glucose metabolism and maintaining overall metabolic homeostasis. Moreover, skeletal muscle has remarkable regenerative and reparative capabilities. These properties are largely due to changes in gene expression, which can be governed by noncoding RNAs, particularly microRNAs (miRNAs). A series of miRNAs, known as myomiRs, have been identified and shown to be muscle-enriched, playing key functional roles in myogenesis and muscle function. miR-486 is a muscle-enriched miRNA encoded by intron 40 of the Ankyrin-1 (Ank-1) gene and involved in post-transcriptional gene regulation by acting in different molecular pathways. Among the targets of miR-486 are the mRNAs encoding PTEN and FOXO1, negative regulators of phosphoinositide-3-kinase (PI3K)/Akt signaling cascade which is involved in the regulation of glucose handling by skeletal muscle. It has been reported that in vitro exposure to high glucose levels can modulate the expression of microRNAs (miRNAs), currently, there is insufficient evidence from whole-animal studies to confirm the potential impact of various diets on miR-486 levels in skeletal muscle.
For this reason, we aim to evaluate miR-486 expression levels in mice skeletal muscle under different metabolic conditions.
To achieve our objective, we measured miR-486 expression levels by qPCR in the gastrocnemius, tibialis anterior, and diaphragm of C57BL/6 mice in a fasting state and/or fed with a standard diet (SD) or high-fat diet (HFD).
We found a significantly decrease in miR-486 expression levels in mice fed with HFD compared with SD. Our preliminary data suggests that different diet regimens may modulate miR-486 expression in skeletal muscle.

Changes in CLIMP63 expression alter the organization of the microtubule network of mouse skeletal muscle fibers

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The endoplasmic reticulum (ER) is a highly specialized organelle, responsible for protein synthesis and Ca^{2+} handling. It establishes contacts with several other organelles and is closely associated with microtubules (MTs). MTs act as a scaffold for ER positioning and remodeling, playing a central role in ER morphogenesis. Recent data revealed of a role of ER dynamics in regulating MT network distribution and stability, providing novel insights on the extent of interconnection between ER and MTs. In skeletal muscle, the sarcoplasmic reticulum (SR) is a specialization of the ER, dedicated to Ca^{2+} storage and release necessary for muscle contraction. Recent data suggested that the SR protein triadin is involved in MT-dependent organization of SR membranes by interacting with the cytoskeleton-linking membrane protein 63 (CLIMP63), an ER/SR-shaping protein able to directly bind MTs. Research conducted in our laboratories identified CLIMP63 as an interactor of two additional SR proteins, junctophilin 1 and 2. To further evaluate the role of CLIMP63 on the SR and MT organization, we performed experiments of either overexpression or downregulation of CLIMP63 in skeletal muscles from newborn and adult mice. Analysis of expression of SR proteins and MT organization suggest that, in newborn mice, changes in CLIMP63 expression alter the architecture of the MT network, confirming the role of this protein as a structural tether between the ER and MT.

P.3

Gene expression analysis in skeletal muscles of mice carrying a deletion in a muscle-specific stretch/super enhancer region inside the ANK1 locus

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The *ANK1* gene, encoding for ankyrin-1, is characterized by the presence of a first promoter (P1), driving the ubiquitary expression of the full length ANK1 protein, and a second muscle-specific internal promoter (P2), located in the 3' region, driving the expression of a small muscle specific isoform, sAnk1.5. Analysis of tissue-specific transcriptome data and chromatin accessibility indicated that the 3' region of the *ANK1* gene, including P2 promoter, has the features of a stretch/super enhancer (SSE), characterised by a strong enrichment of transcriptional coactivators binding regions that can regulate long distance genes by 3D chromatin remodelling. To elucidate the role of this regulatory region we took advantage of sAnk1.5 (P2) KO mouse model, carrying a 941 bp deletion in the P2 region, that we found to have altered glucose homeostasis. Thus, we hypothesized that the deleted region could exert a role in transcriptional control on genes related to metabolism. Therefore, we performed a microarray analysis of gene expression in soleus and EDL muscles of sAnk1.5 (P2) KO mice. Genes involved in glucose or lipid metabolism or genes with higher differential expression levels between WT and sAnk1.5 (P2) KO muscles were prioritized for the analysis. sAnk1.5 (P2) KO mice showed a reduced expression of several genes participating in glucose metabolic pathways and an overexpression of fatty acids transporters type 4 and 5. This different expression suggests that sAnk1.5 (P2) KO mice could have a shift in the energy source towards fatty acids utilization. Additionally, genes involved both in myogenic differentiation and muscle regeneration were found altered in sAnk1.5 (P2) KO mice. Further investigations are ongoing to better unravel the molecular mechanisms altered in the sAnk1.5 (P2) KO mice and the correlation with altered glucose homeostasis.

P.4

Regulation of individual sensitivity to inflammation by myc contributes to muscle loss in COPD

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Inflammation is a likely contributor to muscle loss. However, studies comparing inflammatory cytokines with muscle loss in patients have mixed results. The picture is further complicated by the inter-relationship of inflammation and inactivity. We compared Fat free mass index (FFMI), physical activity and inflammatory cytokines in controls, patients with mild (n=28) or severe COPD (n=51). FFMI was similar in mild COPD vs controls but lower in severe patients vs both other groups. Neither activity nor circulating inflammatory cytokines differed between patient groups, but activity was lower, and inflammatory cytokines higher, in both patient groups vs controls. Consequently, neither activity nor inflammatory cytokines alone explain the difference in FFMI.
Comparison of quadriceps gene expression quantified by microarray with lung function (Transfer capacity of the lung for CO) showed genes associated with the epithelial mesenchymal transition, inflammatory pathways and myc targets increased with disease severity. Further analysis showed the expression of multiple cytokine receptors (e.g. OSMR and IL31RA) and signalling intermediates increased with disease severity. Comparison of circulating cytokines with the array data in mild and severe disease groups separately showed that proinflammatory cytokines associated positively with inflammatory gene sets strongly in severe COPD but weakly in mild COPD. Anti-inflammatory cytokines were negatively associated with inflammatory gene-sets in mild disease but positively associated in severe disease. Comparison of myc expression with the microarray showed positive association for inflammatory genes including OSMR, IL31RA, CCL2 and IL6. Together our data suggest that the muscle of patients with severe COPD is more sensitive to inflammatory signals than those with mild COPD leading to greater muscle loss with myc as a potential regulator.

P.5

Long noncoding RNAs at the interface between muscles and nerves

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Extensive high-throughput analyses and innovative biochemical approaches have unveiled, beyond protein factors, the additional and essential contribution of RNA-mediated molecular mechanisms in the regulation of myogenic gene expression. In particular, tissue-specific long noncoding RNAs (lncRNAs) were shown to play pivotal roles in muscle physiology and development, although the knowledge concerning their mechanisms of action is still far from being complete. Our aim is to study the contribution of lncRNAs to muscular and neuromuscular physiology through the integration of gene editing, induced pluripotent stem (iPS) cell technology and interactome analyses. Our investigations include Charme (Taliani V. et al., Elife 2023), previously identified in mice as a myogenic lncRNA, and currently under investigation in human skeletal muscle differentiation. Emphasis is also placed on the impact of lncRNA on muscle-nerve communication, specifically focusing on the spinal cord and skeletal muscle functional interactions at the neuromuscular junction (NMJ). By applying CRISPR-cas9 genome editing we generated human iPSCs lines knocked out for either Charme or the motoneuronal lncRNA nHotairM1 (Tollis P. et al., Cell Death & Dis 2023). We exploit co-cultures of iPSC-derived spinal motoneurons and myotubes and 3D model systems (neuromuscular organoids) to untangle the contributions of neural and muscular lncRNAs to the structural and functional properties of the NMJ.

P.6

Exploring the role of GDF5 in neuromuscular system during growth and physical activity

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GDF5 Growth and differentiation factor 5 (GDF5), also called bone morphogenetic protein-BMP14, belongs to the BMP family activating SMAD (fusion of *Caenorhabditis elegans* Sma-*Drosophila* Mad) 1/5 complex phosphorylation and transcription of inhibitor of differentiation (Id) genes. BMPs are involved in bone and cartilage development and regeneration, however, genetic polymorphisms and *GDF*5 mutations are associated not only with osteoarthritis susceptibility and joint and bone disorders, but also with decreased muscle strength in humans over 65 years of age. GDF5 is a pleiotropic factor, modulating physiological processes to shape skeletal tissues, control angiogenesis, neuronal development and repair. In addition, after nerve damage, increased GDF5 expression has been shown to be essential to counteract atrophy and sustain re-innervation in mice. Recently, we showed that age-related muscle mass loss is associated with the alteration of GDF5 axis in mice and humans and that boosting this signalling prevented aged muscle wasting. Despite increasing evidence on the importance of this protein in muscle mass homeostasis, there is very few data on its physiological function during growth and physical activity. To better define its role in the neuromuscular system we deeply characterized muscle physiology in GDF5 deficient mice (GDF5-KO) in sedentary and exercised conditions. Our preliminary data show that constitutive absence of GDF5 leads to premature muscle atrophy due to increase of small fiber and decrease of large fiber percentages. This phenotype is associated with a significantly increased Myostatin expression and decreased Akt protein phosphorylation, canonically proteins linked to reduced protein synthesis. In addition, we explored fiber type composition and we did not observe any significant difference induced by GDF5 deficiency. To further investigate the role of GDF5 in skeletal muscle function, we are analyzing the response to exercise of WT and GDF5-KO mice.

P.7

A meta-analysis of state-of-the-art *in vitro* generated skeletal muscle

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Skeletal muscle makes up nearly half of the human body mass and is prone to numerous genetic and metabolic disorders, as well as cancer- and ageing-related wasting conditions. Therefore, generating skeletal muscle in vitro through methods such as differentiation of human pluripotent stem cells or transdifferentiation of somatic cells by overexpression of MYOD1 towards the myogenic fate, is a valuable tool for disease modelling, investigation of myogenesis and development of regenerative muscle therapies. However, the myogenic cells generated from these protocols have been proven to poorly mimic skeletal muscle identity. To delineate the differences in transcriptomic identities between the state-of-the-art in vitro generated skeletal muscle and its in vivo counterpart, we conducted a large meta-analysis on 400 RNA sequencing samples, covering over 60 published datasets. Various cell types were included, ranging from primary muscle cells from human biopsies to in vitro differentiated or transdifferentiated myogenic cells and immortalized cell lines. Our analyses reveal aberrant expression of genes related to metabolism, epigenetic complexes, transcription factors and specific signaling pathways in the in vitro models, acting as a robust guide for future research of in vitro skeletal muscle models to improve their quality and highlighting the need to study the gene networks of regulating the induction of this cell fate.

Complex Magnetic Fields (CMFs): harnessing electromagnetic symphony for muscle regeneration

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Sarcopenia is a physiological condition in which loss of muscle mass and function, presents a significant challenge in aging populations, due to the decline in ability of satellite cells to proliferate and differentiate into new muscle fiber. This study highlights Complex Magnetic Fields (CMFs) as a non-invasive approach to enhance muscle regeneration utilizing human myogenic precursor cells (hMPC). We applied varying intensities and configurations of CMFs to assess their impact on cellular proliferation, differentiation, myotube formation and wound healing. The results demonstrated that exposure to CMFs enhances cell viability and proliferation as evidenced by MTT assay. Moreover, the characterization showed increase in the fusion index, indicated improved myotube formation. The wound healing assay demonstrated accelerated wound closure, suggesting enhanced regenerative properties of the CMFs. In conclusion, CMFs being a non-invasive approach enhances muscle regeneration and exhibit the potential for regenerative medicine.
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P.9

Lights and shadows of neuropsychiatric drugs on skeletal muscle in Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is the most severe muscular dystrophy caused by a mutation in the dystrophin gene, leading to progressive muscle atrophy and weakness. Moreover, patients with DMD are at elevated risk of developing psychiatric disorders, including anxiety and depression as well as autism spectrum disorder and hyperactivity disorder.
With prolonged life expectancy, due to the new therapies for DMD, behavioral disorders will become central to address in order to enhance the overall quality of life for both patients and their families. Preclinical testing of drugs used in the treatment of these diseases is therefore crucial to find the best therapeutic strategies avoiding detrimental effects at muscular level.
Initially, we focused our attention on two drugs used in the treatment of depression, i.e fluoxetine and sertraline, administered to 1 month old mdx mice in the drinking water for 3 months. None of the treatments affected the food and water intake and consequently the weight of the animals. No signs of distress or pathology attributable to drug adverse reactions were observed in treated mice. Moreover, the two drugs did not worsen the dystrophic phenotype, as demonstrated by the performance in the treadmill until exhaustion assay and by the analysis of the skeletal muscles.
A drug particularly used in children and adolescent with neurobehavioral disorders is the second generation antipsychotic risperidone. However, weight increase and metabolic syndrome are common adverse reaction of this kind of drug. So far, little is known about its effect on muscles of DMD patients. Therefore, we started with a pilot in vitro study treating mdx satellite cells and fibroadipogenic precursors with risperidone and assessing the drug toxicity and the effect on proliferation and differentiation.
With this study we intend to evaluate the effect of neuropsychiatric drugs on DMD muscles in order to help in finding the correct therapeutic for this population of patients.

P.10

Exploring the role of extracellular matrix in neuromesodermal differentiation of human induced pluripotent stem cells

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During development, cells orchestrate intricate cell-cell and cell-extracellular matrix (ECM) interactions to regulate essential processes such as cell commitment, differentiation, migration and morphogenesis[1,2]. In particular, neuromesodermal progenitors (NMPs), which are found in an early embryonic structure called the primitive streak, and later at the tail-end of the embryo, show dual neurectoderm and mesoderm fate, contributing to the formation of the spinal cord and of the skeletal muscle[3-6]. Recent studies demonstrated that human NMPs can be generated in vitro from human induced pluripotent stem cells (hiPSCs) through the activation of WNT and FGF and the inhibition of BMP signaling pathways[6]. Despite ECM has been shown to be crucial during myogenic commitment and differentiation of the neuromuscular system in the developing embryo, there is a lack of knowledge on the role that the ECM can exert on hiPSC commitment and differentiation toward the neuromesodermal fate.
In this study, we hypothesized that the ECM proper of the skeletal muscle regulates the commitment and differentiation of hiPSCs. To investigate this, we studied the effect of different ECM-enriched substrates, including decellularized SkM (dSkM), Matrigel droplets and photosensitive hydrogels, on pluripotency and neuromesodermal potetial of hiPSCs. Our findings demonstrated the crucial role of the ECM during hiPSCs commitment and differentiation, highlighting the relevance of the ECM biochemical composition, mechanical properties and topography in triggering the exit of hiPSCs from pluripotency, promoting the the neuromesodermal fate.

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3. Sambasivan R et al. 2021
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P.11

GDF5 therapeutic potential on neuromuscular junction defects

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Neuromuscular junction (NMJ) is a tripartite structure essential for neuromuscular communication and supports motor function of the muscle. Communication between the nervous system and the muscle will be impacted if one of the NMJ components is damaged. In this context, Growth Differentiation Factor 5 (GDF5) appears to be a potential candidate for the treatment of NMJ defects. Indeed, GDF5 has been described to support neuronal survival and neurite growth *in vitro.* In addition, GDF5 has been demonstrated as required in promoting skeletal muscle re-innervation after nerve crush and limiting denervation-related atrophy. Notably, our previous study described that its overexpression is important to limit age-related muscle mass loss and NMJ defects. Here, we hypothesize that GDF5-based treatment could preserve innervation, muscle mass and function in neuromuscular diseases. We used, for pre-synaptic damage indication the $SOD1^{G93A}$ mice one of the best characterized amyotrophic lateral sclerosis mouse model, and for post-synaptic damage, the mdx mice well described Duchenne muscular dystrophy mouse model. We first characterized muscle mass evolution and NMJ alterations in these two models.Then, we overexpressed GDF5 in $SOD1^{G93A}$ mice by systemic adeno-associated virus (AAV) injection and revealed a therapeutic benefit of this treatment on the maintenance of skeletal muscle mass, reinnervation markers NMJ integrity. To further understand the role of GDF5 at NMJ, we investigated the potential interaction between GDF5 and muscle-specific kinase MuSK and we showed, with *ab initio* molecular docking, a strong affinity between GDF5 and MuSK. We will confirm this interaction by biochemical experiments. In addition, we will study the impact of GDF5 treatment on post-synaptic damage in the mdx mice. These studies will generate important insights on the beneficial effect of GDF5 on neuromuscular system and paving the way for the possibility of a GDF5-based treatment for NMJ defects.

P.12

Cell adhesion and migration: an important determinant of GTP-dependent myogenesis

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Extracellular Guanosine 5′-triphosphate (GTP) at 500µM concentration in C2C12 cells enhances in vitro myogenesis. New observation shows that when cells are trypsinized, GTP-stimulated cells take more time to detach respect to control cells. Indeed, through a spectrophotometric assay it was demonstrated that in presence of trypsin, the number of the detached GTP-stimulated cells is lower respect to control cells as the measured absorbance, directly proportional to detached cells, is lower in GTP-stimulated cells respect to control cells. Supposing an involvement of adhesion proteins, a wound healing assay was done revealing that the cell-covered area was 67.8% in GTP-stimulated cells respect to 70.7% in control cells suggesting a slower migration process in stimulated cells. To investigate the differential proteins expression due to GTP stimulation, proteomic analysis was performed on proliferating and 3 days differentiating C2C12 cells in GTP-stimulated cells respect to control cells. Data analysis showed an upregulation of the following pathways: D-myo-inositol 1,4,5-trisphosphate degradation, tRNA charging, glycolysis I, microRNA biogenesis signaling pathway and TCA Cycle II in both conditions. Conversely, only in differentiating C2C12 cells was observed and upregulation of Aspartate Degradation II pathway and a downregulation of AMPK Signaling. The expression of key proteins (COL1A1, ERK2 and CPT2) involved in the aforementioned pathways was confirmed in western blotting analysis. Finally, the expression and the localization of adhesion and cytoskeletal proteins were analysed through confocal microscopy. In conclusion, extracellular 500 µM GTP in C2C12 cell line is able to enhance the myogenic process through an array of proteins mainly involved in cell adhesion.
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P.13

Understanding and treating inflammation in FSHD muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is caused by aberrant expression of the transcription factor double homeobox 4 (DUX4), which is typically not expresses in healthy somatic cells. Muscle inflammation is a prominent FSHD feature. However, the molecular mechanism responsible for inflammation in FSHD is currently unknown and all clinical trials testing anti-inflammatory drugs have failed so far. In FSHD, muscle inflammation anticipates muscle loss and its fibro-fatty substitution, suggesting that inflammation directly contributes to FSHD pathogenesis and is not just a non-specific event, unlike other muscular dystrophies in which inflammation is generally secondary to muscle wasting. It is tempting to speculate that the nature of the inflammatory insult in FSHD is different from that of other muscular dystrophies and should be hence treated differently.
The main direct targets of DUX4 are endogenous retroviruses and other repetitive sequences which, through the accumulation of double strand RNA (dsRNA), might lead to the induction of a “viral mimicry” status encompassing innate and adaptive immune responses. We found that dsRNA, viral mimicry, IFN-γ, STAT-1, STAT-3 and IL-6 are activated by DUX4 in primary muscle cells of FSHD patients and in muscle tissues of the FSHD mouse model. In vivo, this is associated with the expansion of fibro-adipogenic progenitors and their conversion to a pro-fibrotic state. Notably, in vitro and in vivo, IFN-γ treatment is sufficient to activate the same pathway. Importantly, in vivo treatment with an anti-IFN-γ neutralizing antibody significantly reduces inflammation, decreases FAPs accumulation, and preserves muscle tissue. Collectively, our results indicate that DUX4 directly activates a pro-inflammatory pathway contributing to muscle wasting in FSHD. Importantly, this pathway can be efficiently blocked underscoring its therapeutic importance.

P.14

Human mytendineous junction 3D *in vitro* modeling

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Tendons are specialized connective tissues that transfer force from muscles to bones, promoting overall body motility and stability. The interface between muscle and tendon is called Myotendinous Junction (MTJ), that represents the crucial tethering point between theese two tissue. Given its junctional nature, it is often difficult to obtain MTJ-containing biopsies and to prepare comparable samples for imaging studies, due to technical difficulties in proper orientation and sectioning. In this context, 3D constructs represent a reliable tool for mimicking MTJs, based on the use of myogenic (muscle-derived pericytes) and tendon progenitors in combination with biomimetic matrix scaffold. By relying on 3D biocompatible printing technologies, we will generate 3D bioengineered functional constructs by depositing in one step cells and a supporting material, with the highest biomimetic and architectural spatial arrangement.
This novel approach enables the creation of compact and precisely organized three-dimensional constructs, which can aid the scientific community in studying junction-related diseases in a patient-specific manner and serve as an experimental platform for drug testing.

P.15

HDAC4 mediates the crosstalk between skeletal muscle fibers and fibro-adipogenic progenitors in Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is an X-linked, progressive disease due to mutations in the *DMD* gene, which encodes for the dystrophin protein. The absence of dystrophin compromises muscle integrity leading to substitution of skeletal muscle fibers with fibrotic and adipose tissues. This is mainly due to the altered activity of a mesenchymal stem cell population, named fibro-adipogenic progenitors (FAPs). FAPs are the main cellular target of Givinostat, a pan-HDAC inhibitor, recently approved by the FDA as a treatment for DMD patients. However, we have previously shown that histone deacetylase 4 (HDAC4) exerts protective functions in DMD. Indeed, deletion of HDAC4 in skeletal muscle of mdx mice increases muscle damage and compromises regeneration, overall decreasing muscle performance.
To further characterize HDAC4 functions in DMD, we investigated the intramuscular accumulation of fibrous and fatty tissues in mdx;HDAC4mKO (hereby mdx;KO) mice. FAPs isolated from mdx;KO mice display higher fibrotic and adipogenic potential, and negatively affect mdx muscle stem cell differentiation and viability, despite expressing similar levels of HDAC4 as compared to FAPs isolated from mdx mice. We demonstrated that the HDAC4-mediated paracrine effect that muscle fibers have on FAPs is mediated by extracellular vesicles (EVs), while it is independent of HDAC4 deacetylase activity. Importantly, the expression of the cytoplasmic-restricted form of HDAC4 reduces the levels of fibrotic and adipose tissues in mdx;KO muscles, in addition to reducing muscle degeneration, as previously published. All of the above confirms the importance of preserving the cytoplasmic functions of HDAC4 in DMD. Current investigations are aimed at defining the soluble factors responsible for the above phenotype. By defining the secretome modulated by HDAC4 in mdx skeletal muscle, we will provide a novel experimental basis for therapeutic approaches to ameliorate the pathological features of DMD.

P.16

Skeletal Muscle Tissue Engineering a promising technology in the field of food

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Awareness for the need for alternative food sources has become more and more widespread in recent years. In this regard, several companies have started to produce cultivated meat by growing myogenic and adipogenic progenitors. However, developing cultivated meat is a daunting challenge, overall when talking about structured cultured meat. Scaffolding and bioprinting are the most investigated technologies already used in skeletal muscle biomedical applications. The basic idea of 3D bioprinting is patterning the cells and extra-cellular matrix in a layer-by-layer additive manner.
Based on this idea core/shell 3D printing technology and PEG-Fibrinogen (PF) matrix have been exploited to develop a structured cultured meat prototype. The 3D-bioprinter is characterized by a microfluidics printing head (MPH) that allows a stable and quick controlled deposition of the bioink. The bioink is a specific formulation based on PF and myogenic and adipogenic progenitor cells which will be extruded in the form of hydrogel fibers with a predetermined alignment pattern to create a layer-by-layer 3D meat prototype. Due to its rheological properties, PF cannot be deposited alone, therefore, alginate is necessary for the formulated bioink. The presence of a bath containing bivalent ions ($CaCl_2$) in the printer system allowed the instantaneous physical gelation of the alginate simultaneously with the bioink extrusion, exploiting the MPH outlet coaxial nozzles. The highly organized skeletal muscle architecture is replied in our system by collecting the hydrogel fibers on a rotating drum, without any need for ad-hoc printing code and resulting in a quick and stable technology, successfully obtaining a 3D printed steak prototype composed of muscle and fat derived by cell agriculture.

P.18

Exploring the role of HMGB1 in nuclei dynamics during myogenesis

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The position of nuclei within the cell is essential for multiple cellular processes in development and regeneration, a particular example is myogenesis. Nevertheless, the field of nuclear dynamics during myogenesis remains largely unexplored. Our laboratory has developed an *ex vivo* live-cell imaging technology to analyze nuclear dynamics during myogenesis by exploiting H2B-GFP-expressing myoblasts. Unlike traditional techniques that rely on fixed cells and impose numerous limitations, our method overcomes these constraints. Our preliminary data show a link between nuclear dynamics and the ability of myoblasts to form myofibers. It is already known that cells regulate their nuclear stiffness by compacting their chromatin during cell migration but also to allow the peripheral migration of nuclei within a mature myofiber. These findings demonstrate that nuclei within myofibers exhibit distinct characteristics, including variations in positioning and chromatin compaction. We have identified the High Mobility Group Box 1 (HMGB1) protein, a nuclear protein that acts as a DNA chaperone, as a possible candidate to modulate nuclei dynamics in muscle. We found that HMGB1 promotes muscle regeneration and that the lack of the protein leads to defects in myogenesis. In addition, our preliminary data show that HMGB1 is involved in modulating chromatin compaction in muscle and thus plays an essential role in nuclear dynamics. The main goal of the project is to provide an unprecedented characterization of nuclear dynamics and features, focusing on chromatin compaction, modulated by HMGB1 in both healthy and pathological myogenesis.

P.19

Effect of tunicamycin treatment on IGF1R production and IGF1R signaling pathway activation in C2C12

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The insulin-like growth factor receptor (IGF1R) signaling pathway plays and important role on myoblast proliferation and muscle cell growth [1]. The IGF1R is synthesized as a single polypeptide precursor (proreceptor), which undergoes proteolytic cleavage into α (130 KDa) and β (97 kDa) chains that form a tetramer (α-β-β-α). Each α and β subunit contains 11 and 5 N-glycosylation sites, respectively [2]; therefore, the α-β-β-α tetramer structure may enclose 32 glycosylation positions. Here, we evaluate the effect of N-glycosylation inhibition by tunicamycin (TUN) on IGF1R proreceptor maturation, localization and signaling pathway activation in C2C12 myoblasts. C2C12 myoblasts were treated with non-toxic doses of TUN (0.01 µg/mL) for 24 h and cells were harvested on day 1 after the induction of differentiation. The IGF1R and IGF1R proreceptor production was evaluated by western blotting and the sub-cellular localization by immunofluorescence staining. Finally, C2C12 were treated with recombinant mouse IGF-1 (100 ng/mL) for 30 min, and the level of IGF1R, AKT, and ERK1/2 phosphorylation was quantified by western blotting. A marked reduction of IGF1R and increased mobility shift of IGF1R proreceptor in TUN-treated C2C12 cells was found. Immunofluorescence analysis of untreated C2C12 cells showed a perinuclear and diffuse cytoplasmic staining of IGF1R, which was markedly reduced after TUN treatment. Finally, the IGF-1-induced IGF1R, AKT, and ERK1/2 phosphorylation was markedly reduced in TUN-treated C2C12 cells. Altogether, these results showed that N-glycosylation inhibition affects the IGF1R signaling pathway activation due to the reduction of IGF1R proreceptor maturation and activity. This study provides new molecular mechanism through which diseases associated with aberrant N-glycosylation, such as Congenital Disorders of Glycosylation (CDG), affect muscle development and function.

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P.20

New tools to evaluate satellite cell activation after eccentric contraction-induced damage at the muscle-tendon junction in mice: the light sheet microscopy

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Eccentric contraction can induce traumas which are frequently responsible for injuries to the muscle-tendon junction. Following a focal lesion, adult skeletal muscle is able to ensure tissue regeneration thanks to resident satellite cells (SC). The signals from the damaged tissue induce the SCs to emerge from quiescence and trigger tissue self-renewal: each SC asymmetrically divides originating a stem cell and a differentiating cell, ensuring the muscle fiber differentiation and the maintenance of the stem cell niche.
The aim of this feasibility study was to test the light sheet microscopy (LSM) as a tool to identify the SC activation in a three-dimensional reconstruction of a damaged muscle and, possibly, to compare the outcomes with the healthy contralateral muscle. To this end, we used murine EDL muscles: from each mouse we explanted one muscle serving as healthy control (HC) and one was subjected to forced eccentric contraction (EC) to cause the damage. The injury was achieved by stretching and exposing the muscles to a series of contractures in high K^+ solution (1 min) alternating with relaxation cycles in physiological solution (4 min). The extent of SCs' activation was evaluated across the whole sample by using tissue clearing and high-resolution LSM. In particular, after clearing the entire muscle, we performed a whole-mount immunostaining against MyoD to label activated SCs. The high-resolution volumetric reconstructions can enable the quantification and the evaluation of the spatial arrangement of SC. Due to the possibility of examining the whole muscle, this technique minimizes potential sampling errors that might arise in conventional 2D histology.
Although very preliminary, this study proves the feasibility of the adopted technique in this kind of investigation offering new tools for the identification of SCs' activation after muscle injury. This can be useful to test novel therapeutic approaches to enhance tissue regeneration at the muscle-tendon injury.

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CHOP/ERO1A pathway of unfolded protein response (UPR) in RYR1 and SEPN1-related myopathies

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The most common mutations in core myopathies are those of the gene encoding for RYR1 calcium channel, although more rarely, also mutations in the gene encoding for SEPN1 lead to the disease. Due to their low incidence in the population, both are classified as rare diseases. RYR1-RM and SEPN1-RM overlap the clinical signs of infancy onset, delayed motor development, and muscle weakness. They also overlap for histological features on muscle biopsies. In the mice models of these two congenital myopathies it has been observed the presence of ER stress at the muscular level, but how it is involved needs more studies. All of these suggest a potential common underlying pathological mechanism, which is worth investigating, given the potentiality of setting a target therapy for these untreatable diseases. Using a genomic approach, we crossed the RYR1^{I4898T} mice with the CHOP KO mice, to evaluate the recovery rate of the pathological phenotype and we crossed SEPN1-KO mice with the ERO1 KO mice. On their collected diaphragms we performed histological analysis, ran RNA-Seq analysis and RT-PCR. To pharmacologically mimic the effect of ERO1 deletion, we tested a chemical chaperone (TUDCA) a pan ER stress inhibitor. We observed a reduction of UPR markers levels in the skeletal muscles of the double mutant pups, but CHOP deletion neither restored RYR1 function nor protected the pups from perinatal lethality. The SEPN1 KO/ERO1 KO mice showed improved muscle function. RNA-Sequencing analysis identified a downregulation of UPR in DKO diaphragms compared to SEPN1 KO diaphragms, suggesting UPR as a disease pathway rescued by the ERO1 deletion. TUDCA treatment reflected the beneficial effects of ERO1 genetic deletion in SEPN1 KO mouse muscles, paving the way for a novel pharmacological treatment. This study suggests ER stress/UPR and specifically the branch CHOP/ERO1 as a disease mechanism of RYR1-RM and SEPN1-RM and opens the possibility of treatment with chemical chaperones.

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Red photobiomodulation promotes skeletal myoblast differentiation and counteracts anti-myogenic effects of TGF-β1: new perspective for muscle regenerative medicine

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Adult skeletal muscle regenerates lost damaged tissue mainly thanks to the activity of satellite cells (SCs). In the case of chronic or severe damage, SCs' functionality may be compromised by the occurrence of an aberrant fibrotic reparative response. Strategies aimed to improve the muscle intrinsic regenerative capacity while limiting the excessive deposition of fibrotic tissue are attractive. In this perspective, photobiomodulation (PBM) (i.e. application of light with 400-1100 nm wavelength using different laser or LED devices, power density less than 100 mW/cm^2 and energy density less than 10 J/cm^2 at target) may offer promises based on its well-known pro-regenerative effects in different organs and increasing evidence of its antifibrotic potential. However, PBM's effects on skeletal muscle are controversial, univocal guidelines for its use are missing and light-tissue interactions need further study. To this aim we evaluated the effects of red PBM treatment (laser diode 635±5 nm, energy density: 0.4, 4 and 8 J/cm^2, single exposure) on myoblastic cells undergoing differentiation in the presence or absence of transforming growth factor (TGF)-β1 (2ng/ml) and on differentiated myotubes.
Morphological and electrophysiological analyses revealed that red PBM, particularly with 4 J/cm^2 energy density, positively affected myoblast differentiation and did not alter myotube viability and features. Red PBM was also able to counteract the anti-myogenic action of TGF-β1.
This study provides experimental compelling evidence supporting red PBM' pro-myogenic effects and offers new cues for further investigations.

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Improving the effects of HDAC8 inhibition by combining the activation of SIRT1 in a zebrafish model of Duchenne muscular dystrophy

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Despite the challenges that hinder the application of gene and cell therapies for Duchenne Muscular Dystrophy (DMD), pharmacological treatments that mitigate skeletal muscle loss, inflammation, and fibrosis offer a promising and immediate approach. Among these treatments, histone deacetylase inhibitors (HDACi) are being investigated extensively, with the pan-HDACi givinostat recently gaining FDA approval for DMD patients.
Our previous research demonstrated that inhibiting HDAC8, an enzyme overexpressed in DMD patients, restored normal muscle function in DMD zebrafish by remodeling the cytoskeleton. In the *mdx* mice, we found that activating SIRT1, a NAD+ dependent class III HDAC, enhanced muscle performance by promoting fatty acid oxidation and fiber type switch towards a more oxidative phenotype.
This study evaluated a novel combination therapy for DMD patients that involves HDAC8 inhibition using the HDAC8-selective inhibitor PCI-34051 and SIRT1 activation, through SRT2104 administration. Our data show that DMD zebrafish embryos treated with PCI-34051 and SRT2104 exhibited reduced muscle loss and inflammation. Notably, the combination therapy allowed for lower doses of each drug, maximizing the effects of the single treatment and minimizing possible side effects. In parallel, since it has been shown that HDAC8 inhibition might influence the proliferation and differentiation of zebrafish neurons and murine neural stem cells, we excluded the toxicity of PCI-34051 and SRT2104 on the neural system.
In conclusion, our findings support the potential benefits of combining HDAC8 inhibition with SIRT1 activation, demonstrating a synergistic approach to target modulation in DMD treatment.

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High Mobility Group Box 1 recycling orchestrates regeneration in skeletal muscle

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High mobility group box 1 (HMGB1) is a nuclear protein that acts as a DNA chaperone inside the cell and as a redox-sensitive danger signal outside the cell. It plays a crucial role in myogenesis, and more specifically, we showed that the level of HMGB1 protein was important for muscle regeneration, as heterozygous mice showed a delay while administration of recombinant HMGB1 was able to promote the regeneration process. Although the implication of HMGB1 has been reported in numerous physiological and pathological conditions, its fate after release remains largely unexplored. Our recent findings unraveled the recycling of extracellular HMGB1 inside the nuclei of regenerating myofibers, both upon acute injury or in a model of Duchenne muscular dystrophy, and its contribution to the control of myofiber size. In addition, RNAseq experiments revealed that HMGB1 recycled in the nucleus modulates the expression of genes related to muscle function. Notably, we found that the recycling of non-muscle cell-derived HMGB1 into regenerating myofibers acts as a compensatory mechanism in muscle-specific KO mice, ensuring muscle regeneration. Conversely, we observed that whole-body HMGB1 KO mice displayed a severe impairment in muscle regeneration. We identified heparan sulfate proteoglycans (HSPGs) as mediators of HMGB1 internalization and multiple endogenous molecules modulating its internalization, unraveling the existence of physiological mechanisms to regulate this process. Finally, we generated truncated HMGB1 proteins as internalization-resistant form to demonstrate the contribution of internalization to the myogenic properties of HMGB1. These findings might be of great importance for medical conditions associated to HMGB1 release, such as inflammation-related disorders, including muscular dystrophies, and more in general regenerative medicine, as targeting the recycling of HMGB1 might represent an innovative and much more efficient and safer therapeutic approach.

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Environmental pollutants impact on adult and pluripotent-derived myogenic progenitors

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Environmental pollutants are known to exert toxic effects on organisms. Their burden on public health is a significant concern. Among these pollutants, ozone and polystyrene nanoparticles exhibit diffusive behaviour, leading to accumulation and toxicity in many tissues at both the foetal and adult stages. Notably, pollutants appear to alter microRNA (miRNA) expression, including myo-miRNAs involved in muscle function and tissue regeneration. This study aims to evaluate the effects of ozone and polystyrene nanoparticles on human myogenic cells. Adult muscle stem cells (MuSCs) and pluripotent-derived myogenic cells (iPSCs-MuSCs), used as a foetal model, were treated with the two pollutants, either alone or in combination. MuSCs and IPSCs-MuSCs were exposed to ozone at 120 Ppb in the Reaction Chamber and stimulated with polystyrene nanoparticles of 100 nm size at a 1:1000 dilution. Cell viability was assessed via MTT assays and differentiation capacity was evaluated using immunochemistry protocols and qRT-PCR. Additionally, the expression of miR-1, miR-133, miR-206 and miR-23 was measured in myogenic cells exposed to the pollutants. The results indicated a negative impact of both ozone and nanoparticles on myogenic progenitors, highlighting the detrimental effects of these pollutants on skeletal muscle tissues.

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Nuclear Phospholipase C delta 4 is a crucial player in modulation of rhabdomyosarcoma cells proliferation

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Human rhabdomyosarcoma (RMS) is a common pediatric soft tissue sarcoma and, due to its aggressivity, current treatments are often unsuccessful. RMS originates from mesenchymal precursors of skeletal muscle cells which have impaired differentiation ability due to the presence of chromosomal translocations and driver mutations, leading to uncontrolled cell growth. A crucial player in RMS aberrant proliferation could be the nuclear protein Phospholipase C delta 4 (PLCδ4), whose role in driving proliferative processes in mesenchymal stromal stem cells has already been described. Our molecular and morpho-functional analyses reveal that PLCδ4 is mainly expressed in A204 embryonal RMS cells, whereas it is only slightly detected in RD rhabdomyosarcoma cells. To better characterize the role of PLCδ4, rhabdomyosarcoma RD cell line was stably transfected with wild-type PLCδ4. When PLCδ4 is overexpressed in RD cell line, its localization is purely nuclear. Proteome profiler array analysis demonstrates that enhanced expression of PLCδ4 in RD cells positively influences the phosphorylation of PRAS40 (T246), Chk2(T68), WNK1(T60) and Akt 1/273 (S473). Overexpression of PLCδ4 in RD cells results in G2/M phase cell cycle arrest, enhancing cyclin B1 expression. Overall, our study identifies a novel role for nuclear PLCδ4 to block proliferative functions via inducing cyclin B1 phosphorylation by Akt. Therefore, the modulation of PLCδ4 expression and of its downstream targets could represent a crucial strategy to block embryonal RMS cells proliferation.

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Hyaluronan improves the C2C12 murine myoblast proliferation and myogenic differentiation under oxidative and inflammatory conditions

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Hyaluronan (HA) is a non-sulfated glycosaminoglycan widely used for medical and pharmaceutical applications, including tissue muscle repair. A recent study demonstrates that HA activates muscle stem cells to repair damaged muscle: when muscle damage occurs, stem cells start producing and coating themselves with hyaluronic acid and demethylase JMJD3-driven hyaluronic acid synthesis that allows muscle stem cell adaptation to inflammation and the initiation of muscle repair [(1)]. In this study, we analyzed HA's effect on myoblast rescue under stress and inflammatory conditions using C2C12 murine muscle cell proliferation and differentiation. First, we evaluated the wound healing of myoblast cell monolayer at (t0) and after 24 hours (t1) in the presence/absence of an HA blend of 2 to 1000 KDa (0.3 mg/ml, Regenflex T&M Regenyal Laboratories SRL) and with/without pro-inflammatory agents (IL-1β, TNF-α, LPS); with/without pro-oxidants (H_2O_2) that slow their proliferation. The wound healing assay revealed an improvement in reparative mechanisms even under oxidative and inflammatory stimuli. Secondly, the C2C12 myogenic properties treated with HA were characterized based on MyoD Mrf4, myogenin, and IGF-1 expression under the previously described stress and inflammatory conditions. From the preliminary results obtained, it appears that HA possesses significant pro-proliferative activity, improving wound healing 24 hours after injury in both stress and inflammatory conditions and inducing an upregulation of the myogenic biomarker as a beneficial effect on the differentiation program, indicating that this HA formulation could be used as a promising treatment for muscle tissue regeneration.

1.Nakka K, et al., Science. 2022 Aug 5.

P.29

Aptamer-conjugated gold nanoparticles enable selective oligonucleotide delivery into muscle stem cells to promote regeneration of dystrophic muscles

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Inefficient targeting of muscle stem cells (MuSCs) represents a major bottleneck of current therapeutic strategies for muscular dystrophies, as it precludes the possibility of promoting compensatory regeneration. Here, we describe a novel delivery platform based on gold nanoparticles that enables the release of therapeutic oligonucleotides into MuSCs. We demonstrate that AuNPs conjugation to muscle-specific aptamer directs either local or systemic delivery of microRNAs to MuSCs, thereby promoting muscle regeneration and improving muscle functionality in a mouse model of Duchenne Muscular Dystrophy. We show here that this platform is biocompatible, non-toxic, non-immunogenic, and adaptable for the release of therapeutic oligonucleotides into diseased muscles.

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Ketogenic diet mitigates liver damage but not muscle atrophy in western diet-induced obesity

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The Western diet (WD), marked by elevated levels of sugars and saturated fats, significantly contributes to obesity and its related health complications. Its influence on insulin resistance and inflammation has been associated with several conditions, such as type 2 diabetes mellitus (T2DM), metabolic-associated steatotic liver disease (MASLD), and metabolic syndromes (MetS).
Ketogenic diets (KDs) represent dietary approaches characterized by minimal carbohydrate intake, high fat, and adequate protein levels. Energy is sourced from ketone bodies (KBs), derived from fat oxidation and protein metabolism. Given the increasing evidence supporting the effectiveness of KDs in reducing inflammation, oxidative stress, and improving mitochondrial function, we hypothesized that KDs could hold promise in addressing obesity-related conditions. To test this hypothesis, we subjected mice to a Western diet (WD) for 16 weeks, followed by a transition to an *ad libitum* KD, or continued adherence to WD for an additional 4 or 8 weeks.
Our finding demonstrated that within the muscle, KD affect the expression of genes associated with atrophy, autophagy and mitophagy. KD induced ketosis, leading to weight loss and improvements in hepatic inflammatory response. Additionally, KD partially ameliorated the metabolism of myofibers, but did not produce positive effects on muscle strength, mass, and morphology after WD.
To further investigate the impact of fatty acids and KBs on muscle cells, we examined various doses of palmitate (PA) and butyrate (BU) on C2C12-derived myotubes. PA significantly reduced myotube diameter in a dose-dependent manner but also induces ROS production, and mitochondrial membrane depolarization. In contrast, low doses of BU protected against PA-induced atrophy in myotubes, while high doses appear to be ineffective or may even contribute to atrophy. Our findings suggest that tailored doses of ketone bodies could offer a non-pharmacological approach to treating MetS.

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AntagomiR inhibition of miR-424(322) increases muscle fibre diameter in old mice and in response to respiratory viral infection

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Muscle loss is a common complication of many diseases that can lead to frailty. We have shown that miR-424 is elevated in the muscle of patients with COPD, ICUAW and in those about to undergo aortic surgery. Furthermore, pre-surgical miR-424 was directly proportional to muscle loss 7 days later in those undergoing surgery. Mechanistically we have also shown that miR-424 inhibits protein synthesis by reducing ribosomal RNA synthesis thereby suppressing the capacity to make new protein. Finally, over-expression of miR-424 (known as miR-322 in mice but referred to here as miR-424) in mouse muscle promoted rapid muscle loss. Together these data suggest that inhibiting miR-424 in muscle would inhibit muscle loss. We developed an antagomiR to miR-424. Transfection of this antagomiR into mouse C2C12 cells inhibited the effects of miR-424, preventing the suppression of UBTF a key component of ribosomal RNA synthesis and relieving the suppression of protein synthesis compared to transfection with a control oligonucleotide. Indeed, in the absence of exogenous miR-424 the antagomiR increased basal protein synthesis. We have recently found that infection of old (>24 month) mice with respiratory syncytial virus provides a useful model of muscle wasting. To determine whether inhibition of miR-424 could increase muscle mass in vivo we electroporated the antagomiR into the left tibialis anterior (TA) of mice a control into the right TA of female old mice with and without infection with RSV and male mice infected with RSV. Quantification of UBTF mRNA showed increased expression in the left TA compared to the right. Quantification of fibre diameter by histological analysis showed increased fibre diameter in the left TA compared to the right TA in all groups. This difference was largest in the female mice infected with RSV.
Together these data show that inhibition of miR-424 promotes muscle fibre maintenance/growth under atrophic conditions.

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An autocrine loop of lactate sustains cancer cachexia in skeletal muscle

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Our previous studies demonstrated that muscle wasting is associated with high production of lactate that, behind the metabolic role, acts as signalling molecule through the binding to the GPR81 receptor. Our aim is to dissect the role of the secreted lactate in the onset and maintenance of the cachectic condition in skeletal muscle. Our results show that lactate in the extracellular environment is involved in myotube wasting, since the inhibition of the lactate transporter MCT1 impedes the formation of the cachectic conditions. Interestingly, the inhibition of MCT1 transporter blocks the decrease of pyruvate dehydrogenase activity which is greatly inhibited in cachectic muscles. Moreover, increased amount of lactate and enhanced level of lactate dehydrogenase has been observed in mitochondria of cachectic myotubes. Interestingly, the abdominal muscle of cachectic patients contains increased level of lactate associated with an upregulation of lactate dehydrogenase in comparison to the noncachectic counterpart. Increased amount of lactate and upregulated lactate dehydrogenase and GPR81 receptor have been detected in human fibroblasts isolated from the abdominal muscle of cachectic patients in comparison to the fibroblasts from non-cachectic individuals. Although preliminary, these findings suggest that in skeletal muscle an autocrine loop of lactate could drive the onset and maintenance of the cachectic condition.

P.33

Menin is a novel therapeutic target for FSHD muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD), a major neuromuscular disorder with no cure or treatment available, is caused by aberrant expression of the transcription factor DUX4.
Our laboratory previously showed that the chromatin remodeling protein WDR5 is a key activator of DUX4 expression in FSHD. However, considering that it is part of several complexes, WDR5 targeting could lead to unwanted side effects. Therefore, the identification of the specific WDR5-containing complex required for DUX4 expression could point to a target with decreased safety concerns.
To pursue our goal, we performed focused genetic and pharmacological screens targeting factors uniquely present in WDR5-containing complexes including SET1A/B, MLL1/2 MLL3/4 and MYC using primary FSHD muscle cells. From our results we identified Menin, which belongs exclusively to the MLL1/2 complex, as the main factor required for DUX4 expression. Notably, Menin targeting recapitulates the effects of targeting WDR5. Specifically, Menin knockdown inhibits the expression of DUX4 and DUX4 target genes, and rescues both cell viability and myogenic differentiation of primary FSHD muscle cells.
Several small molecule drug inhibitors of Menin are available. They exhibit high picomolar to low nanomolar activity, possess a favorable pharmacokinetic profile, and demonstrate high oral bioavailability. In vivo, these compounds are well tolerated, with no signs of toxicity in healthy mice. Remarkably, several of them are currently in clinical trial and initial results support safety and efficacy also in humans, indicating that a sufficient therapeutic window exists for these inhibitors.
Recently, we set up a humanized mouse model of FSHD to evaluate safety and efficacy of Menin drugs in vivo.
With our work we could provide a novel therapeutic strategy for FSHD patients.

P.34

The role of Pde4d transcript variants in restraining cAMP signaling in cancer cachexia

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In muscle fibers, the activation of the cAMP/PKA/CREB axis extensively modulates the transcription of mitochondrial-related genes, acting as a major regulator of skeletal muscle metabolism. Our previous work identified impaired cAMP/CREB signaling in skeletal muscle as a driver of mitochondrial dysfunction in cancer cachexia. We showed that targeting phosphodiesterases 4 (PDE4), the enzymes that hydrolyze cAMP, counteracts cancer cachexia-induced metabolic dysfunction and muscle wasting. Within the PDE4 family, Pde4b and Pde4d are upregulated in cachectic muscles of mice and in our *in vitro* models of cancer cachexia. We set to investigate their role in cAMP resistance using CRISPR-Cas9 to knock out Pde4b and Pde4d in C2C12 myoblasts, revealing the central role of Pde4d in mediating tumor-conditioned medium-induced cAMP resistance. Notably, only PDE4D mRNA expression is induced in skeletal muscle biopsies from cachectic patients. Pde4 genes produce different transcript variants via alternative promoter usage. Interestingly, we found in cachectic mice muscles significant changes in the transcriptional composition of Pde4d variants, but not Pde4b, with a strong induction of Pde4d-213 and Pde4d-204 and a downregulation of Pde4d-205 at both early and late cachexia stages. Our goal is to characterize the Pde4d transcript variants, which differ in N-terminal sequences and for the inclusion or exclusion of UCR1 and UCR2 domains, and to investigate their role in mediating cAMP dysfunction in the pro-cachectic environment.

P.35

The role of Protein-Kinase C Theta in muscle-nerve communication: the case of Amyotrophic Lateral Sclerosis and Myasthenia Gravis

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The impairment of muscle-nerve communication is a common feature of several neuromuscular diseases, including Amyotrophic Lateral Sclerosis (ALS) and Myasthenia Gravis (MG).
ALS is a neuromuscular disorder characterized by muscle atrophy and degeneration of motor neurons. It has been described that a transgenic mouse, that over-expresses the human mutated SOD1 gene selectively in skeletal muscle, exhibits typical alterations of the pre-symptomatic stage of ALS disease. We have demonstrated that NMJ impairment is causally linked to the aberrant activation of a Protein-Kinase C, namely PKCθ. PKCθ has a crucial role in the regulation and function of immune cells, and interestingly it has been observed that macrophage recruitment at the NMJ is responsible for the dismantlement of the endplate. MG is a neuromuscular and autoimmune disease characterized by postsynaptic defect of neuromuscular transmission, associated to the presence of specific antibodies targeting NMJ components. ALS and MG have a different aetiopathogenesis, however they share some clinical features that often lead to a prior diagnosis of MG in ALS patients. Nowadays, it does not exist an efficient strategy for the treatment of ALS, and MG therapies are responsible of many severe side effects.
Therefore, here we aim to evaluate whether PKCθ aberrant activation is a common feature of ALS and MG pathogenesis. By histological and molecular analysis, we highlight that ALS and MG share several pathological features like muscle metabolic defects and oxidative stress. Moreover, we prove that PKCθ is aberrantly activated in both ALS and MG mice, and we provide evidence that pharmacological inhibition of PKCθ activity can attenuate the progression of both diseases, reducing macrophages recruitment at the NMJ and inducing their anti-inflammatory phenotype. In all, our data demonstrate that PKCθ can be considered as a therapeutical target to fight ALS and MG.

P.36

Role of Sbno2 in myogenic differentiation under cachectic conditions

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Cancer-related cachexia, a condition characterized by progressive skeletal muscle wasting, is one of the common complications in cancer patients, leading to increased mortality. It has been reported that cancer cachexia is responsible for about 20-30% of all cancer related deaths worldwide. This metabolic wasting syndrome leads to atrophy of skeletal muscle as well as impaired muscle stem (MuSC) functionality and so far cannot be reversed by nutritional or medical support.
We compared the changes in gene expression in MuSCs from EDL, soleus and extraocular muscles from cachectic and healthy control mice and thereby identified Sbno2 (strawberry notch homologue 2) as one of the genes being specifically upregulated in cachectic mice. We hypothesized that the aberrant expression of Sbno2 in MuSCs from cachectic mice causes impaired differentiation. To model cancer cachexia in a cell culture system, we used conditioned medium from different cachexia inducing cancer cell lines and verified the aberrant expression of Sbno2 in myoblasts. Of note, when we reduced the aberrant expression of Sbno2 which is caused by the different cancer cell supernatants, we could restore myogenic differentiation under cachectic conditions. To unravel the mechanism by which Sbno2 is impairing myogenic differentiation under cachectic conditions, we are searching for interactions partner of Sbno2 followed by functional analyses.

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3D skeletal muscle construct for *in vitro* recapitulation of Duchenne Muscular Dystrophy Progression

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Duchenne muscular dystrophy (DMD) is a genetic disease characterised by progressive muscle degeneration, depletion of the muscle stem cell compartment and various side effects that severely affect the patient's quality of life. The mild progression of DMD in mice involves the use of a large number of animals and time-consuming experiments to study molecular processes at different stages of the disease. Furthermore, although conventional in vitro culture has greatly advanced our knowledge of cell behaviour, there are still several areas in which it differs from the in vivo response. Overcoming these limitations has prompted research to create bioengineered three-dimensional models that closely resemble the intricate architecture of muscle while allowing to accurately assess key functional characteristics. In this study, we developed a heterogeneous 3D skeletal muscle model of Duchenne Muscular Dystrophy (DMD) that replicates the pathological progression observed in native DMD muscle. This model effectively recapitulates critical hallmarks such as muscle deterioration, fibrosis, and impaired regeneration. Our 3D model exhibits sustained spontaneous contractility, essential in mimicking the contraction-associated damage characterizing DMD. Moreover, leveraging the decontextualization of our in vitro model, we explored how muscle-specific pathological secretions contribute to disease progression. This innovative approach provides new insights into the underlying mechanisms of DMD and offers a valuable platform for testing potential therapeutic interventions. Indeed, the possibility of identifying new molecular mechanisms involved in the muscle pathology of DMD may be of undisputed value in the field of public health, both for the identification of new therapeutic targets and diagnostic markers.

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Auranofin, a potential drug to counteract cancer cachexia in skeletal muscle

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Cancer cachexia is a complex multifactorial syndrome characterized by body weight loss in response to tumour growth. The pathology plays a key role in oncologic patients since it is responsible for half cancer death worldwide. Moreover, it limits the therapeutic options due to low reduced tolerance and response to antineoplastic treatments. The pivotal role of cancer-driven inflammation in cachexia onset has clearly emerged and the use of a molecule with anti-inflammatory properties could be useful for the block or amelioration of the pathology. We used Auranofin (AF), a gold-based drug with proven anti-inflammatory action, that has undergone clinical trials for the treatment of various malignancies, as ovarian cancer. Our results show that AF prevents the onset of cancer cachexia in myotubes, by blocking both the phenotypic and the metabolic modifications typical of the pathology. Moreover, AF induces the reversion of a pre-existing cachectic condition. Regarding the mechanism used by AF for counteracting cancer cachexia, the results show increased thioredoxin reductase activity in cachectic condition in comparison to control. Moreover, AF does not affect STAT3 phosphorylation, a signalling cascade involved in the induction of muscle wasting. Although the mechanism used by AF to block the onset of the pathology is not known so far, our results highlight a key role of AF in the counteraction of cancer cachexia, suggesting AF as a powerful tool for improving the severe inflammatory condition underlying the pathology.

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In vivo restoration of dystrophin expression in mdx mice using antisense oligonucleotides (ASOs) conjugated to gold nanoparticles

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Duchenne muscular dystrophy (DMD) is a severe muscle wasting disease emerging from defects in the dystrophin gene, such as nonsense or frameshift mutations, that preclude the synthesis of a functional protein. The lack of dystrophin leads to a progressive loss of muscle mass that is then replaced by fibrotic and adipose tissue. Exon skipping therapy using synthetic oligonucleotides called antisense oligonucleotides (ASOs) is a promising therapeutic prospect for overcoming the dystrophin mutation that causes DMD. This treatment involves splicing out the frame-disrupting segment of the dystrophin mRNA, which restores the right reading frame and produces a truncated -yet functional- dystrophin protein. In this context we have recently developed a novel biocompatible, non-toxic and non-immunogenic approach based on gold nanoparticles for targeted delivery of oligonucleotides and drugs into dystrophic muscles. By using muscle-targeting AuNPs conjugated to chemically modified ASOs we were able to restore around 30% of dystrophin in vivo in a mouse model of DMD, the mdx mice. This approach should reduce the severity of DMD by allowing the production of a partially functioning dystrophin protein.

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Metabolomic markers of mitochondrial dysfunction predict recovery of muscle mass in a human model of acute muscle wasting

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Understanding the recovery phase of ICU acquired weakness (ICUAW) is crucial because patients with disease related sarcopenia usually present with the established condition. We have previously characterised the muscle response of patients undergoing elective aortic surgery demonstrating approximately 10% loss of rectus femoris cross sectional area (RFcsa) within 7 days in half of the patients with corresponding weakness. Metabolomic analysis of plasma samples from these patients associated muscle wasting with pre-existing mitochondrial dysfunction, indicated by elevated concentrations of acylcarnitines, and a failure to inactive circulating glucocorticoids (DOI: 10.1002/jcsm.12597). In multiple conditions, growth and differentiation factor-15 (GDF-15) a putative mediator of sarcopenia, has coexisted with mitochondrial dysfunction.
In this prospective study (NCT03714399), we followed up patients (n=31 with complete physiological data) beyond hospital discharge to their first outpatient visit to characterise muscle injury and recovery, and to uncover associated pathogenic pathways in the circulating metabolome. Half of the patients lost muscle bulk in the first 7 days after surgery and roughly 50% of patients continued to waste, whereas measurements of strength (hand grip and knee dynamometry) and function (SPPB: short physical performance battery) returned to base-line levels.
At their peak on day 3 post-op, acylcarnitines (biomarker of mitochondrial dysfunction) were inversely associated with proportional (pre-op/ follow-up RFcsa) muscle mass after aortic surgery. GDF-15 correlated most closely with pre-operative indices of muscle bulk and strength.
This is a useful model of studying acute sarcopenia and early recovery. Our data suggest a central role for mitochondrial function in both processes.

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Investigation of the role of different IQgap proteins in myogenesis

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Skeletal muscle regeneration is a finely controlled process which is critically depending on muscle stem cells (MuSCs). Aging is associated with decreased stem cell function, poor regeneration and loss of skeletal muscle mass. Several intrinsic and extrinsic factors that regulate muscle stem cell functions are perturbed during aging. Therefore, there is a need to identify factors that contribute to the decline in stem cell functionality observed during aging. Through analysis of the transcriptome we identified an downregulation of IQGAP1, 2 and 3 mRNAs in MuSCs from aged compared to young mice. Since IQGAPs are in involved in cell adhesion, migration, proliferation and differentiation – all processes which are affected during aging in MuSCs, we further investigated their role in myogenic cells. Through loss of function studies through siRNA mediated reduction of the different IQGAPS (namely IQGAP1-3) in C2C12 cells and primary murine myoblasts, we analyzed their contribution to myogenesis, in particular proliferation of myoblasts and myogenic differentiation. While reduction of IQGAP1 expression resulted in an elevation in proliferation of myoblasts suggesting a possible role in myogenesis, reduction of IQGAP3 caused a decrease in cell proliferation suggesting that the different IQGAPs fulfill different functions in myogenesis. Interestingly, reduction of IQGAP2 and 3 resulted in an increased expression of myosin heavy chain suggesting enhanced differentiation, which will be investigated next.

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Dietary and endogenous advanced glycation end-products (AGEs) induce muscle wasting *in vitro* which could be counteracted by *Vaccinium macrocarpon* extract

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Advanced glycation end products (AGEs) are a heterogeneous group of non-enzymatic adducts formed when the reactive carbonyl group of a reducing sugar reacts with cellular macromolecules, contributing to their structural and functional alteration. AGEs, both endogenously formed or provided by exogenous sources, accumulate in the body and, by inducing the production of reactive oxygen species (ROS), oxidative stress, and inflammation, can lead to tissue/organ failure. The natural compound Vaccinium macrocarpon (VM) has been identified, in a preliminary ad hoc screening, as a compound able to reduce AGE accumulation and activity. In this study, we investigated if VM was efficacious at contrasting the harmful effects of AGEs in an in vitro model of skeletal muscle. C2C12 myotubes were treated with 200 mg/mL AGE-BSA, produced by incubating bovine serum albumin with glucose at 50°C for 4 days and representing the most common endogenous AGE, or 200 uM methylglyoxal (MG), the most representative of exogenous diet-derived AGEs, in the presence or absence of 100 mg/mL standardized dry extract of VM for 24 h. At the end of the treatments, myotube trophism, mitochondrial (mt) function, and ROS production were assessed. AGEs induced atrophy in C2C12 myotubes, which was seen as a reduction of cell thickness, induction of atrogenes, and reduction of myosin heavy chain. In addition, they induced ROS production and mitochondrial dysfunction, which was assessed as inhibition of mitochondrial respiration, mitochondrial membrane depolarization, and increased mitophagy. VM counteracted AGEs-induced myotube atrophy and mt dysfunction. Overall, our findings suggest that VM not only exhibits the capacity to reduce AGE formation, but also exhibits the ability to mitigate the detrimental impact of AGEs on mitochondrial function. By modulating these effects, VM could help preserve muscle mass and function, making it a potential natural food supplement to counteract AGE-induced muscle wasting.

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Targeting monoamine oxidase B (MAOB) in dystrophic mdx hearts dampens inflammation and fibrosis

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Background Cardiomyopathy is a major cause of death for Duchenne muscular dystrophy (DMD) patients. We previously showed that targeting mitochondrial MAOB with specific inhibitors (iMAOB) improves skeletal muscle function in dystrophic mice by lowering oxidative stress, inflammatory infiltrate and fibrosis.
Aim Explore the therapeutic potential of iMAOB to alleviate DMD cardiomyopathy using dystrophin-deficient *mdx* mice treated at an age when cardiac disfunction is not yet clinically evident.
Methods Three-month-old *mdx* mice were orally treated with iMAOB or vehicle for one month (n≥6). Heart ventricular mononucleated cells were obtained by enzymatic digestion. Myeloid, endothelial and fibroblast cells were then isolated by FACS and analysed by RT-PCR. Oxidative stress, inflammation and fibrosis were measured in tissue cryosections by fluorescent probes, immunofluorescence and immunochemistry, respectively.
Results The expression of proinflammatory and profibrotic genes was increased in myeloid (Il-1b, Il-6, Tgf-b and Spp1), endothelial (Il-1b, Il-6, mmp2 and nos3) and cardiac fibroblast cells (Tgf-b, Spp1, Timp1 and Col1) isolated from *mdx* hearts, as compared to wild type mice. Expression of all these genes was significantly dampened by iMAOB treatment. Noticeably, the differences were not linked to changes in the percentage of the various cell types, as they were unmodified amongst wild type, mdx and iMAOB-treated mdx hearts. In parallel, iMAOB treatment decreased the levels of oxidative stress, inflammation and fibrosis observed in *mdx* myocardium sections. Similar results were also seen in skeletal muscles, considering both mononucleated cells and single fibers.
Conclusions We show that iMAOB can positively affect the phenotype of cells that are important in cardiac tissue remodeling. Our data suggest that iMAOB could be a viable therapeutic tool in DMD cardiomyopathy. As iMAOB are already in clinical use, such approach could be easily translated to patients.

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Vaccinium macrocarpon extract restrains muscle wasting induced by Western diet-derived AGEs

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Western diet (WD) is a modern and diffuse dietary style characterized by high intake of fatty, sugary, ultra-processed and pre-packaged foods containing elevated advanced glycation end-products (dietary AGEs; dAGEs). WD increases the development of insulin resistance and metabolic inflexibility predisposing to muscle wasting (MW), i.e., loss of muscle mass and strength. AGEs are non-enzymatic products that can be endogenously formed in hyperglycemia conditions or exogenously sourced from diet. AGEs induce tissue damage by altering protein function or binding their receptor, RAGE, thus sustaining systemic/local inflammation and oxidative stress, as typically observed in subjects consuming WD. dAGE accumulation in skeletal muscle, blood, and skin has been reported in sarcopenia conditions in the elderly, and in diabetic subjects, and RAGE signaling sustains MW in several conditions. The mechanisms underlying WD-dependent MW and the potential role of dAGEs/RAGE axis have not been investigated so far. Here, we demonstrate that male adult mice fed for 20 weeks with WD containing high dAGEs *vs* standard diet (SD), despite eating the same amount of food, showed increased body, adipose tissue, and liver weights, and signs of steatosis in liver. Moreover, in concomitance with AGE accumulation in muscles and plasma, WD-fed mice showed increased numbers of thin myofibers, reduced amounts of myosin heavy chain (MyHC)-II, increased expression of RAGE, and activation of the ubiquitin-proteasome system in muscles, together with reduced muscle performance. The administration of a *Vaccinium macrocarpon* (*VM*) standardized extract (250 or 500 mg/kg/die) to WD-fed mice reduced the AGE content in muscles and improved muscle parameters. Thus, dAGE accumulation/activity as a consequence of WD consumption induces multi-organ detrimental effects, including MW, which might be restrained by the administration of *VM* extract.

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Molecular characterization of ER stress mediators in the pathogenesis of SEPN1 and RYR1-related myopathies

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SEPN1 and RyR1-related myopathies belong to the common class of multi-mini core diseases and are caused by mutations in *SELENON* and *RYANODINE RECEPTOR type1* genes, encoding SEPN1 and RyR1 proteins, respectively. SEPN1 is a type II protein of the endoplasmic reticulum (ER) which regulates the activity of calcium pump SERCA. RYR1 is a calcium channel of the ER involved in calcium efflux from the ER. These myopathies are characterized by impaired redox and calcium homeostasis accompanied by endoplasmic reticulum stress (ER) at molecular levels. Here, we analyze the pathogenic role of the ER stress mediator ER oxidoreductase-1 (ERO1A) and the ER stress-mediated defect in protein synthesis in these two diseases.
ERO1A is upregulated in SEPN1-devoid preclinical models, suggesting that ERO1 might be a biomarker of SEPN1-RM. Combined immunoprecipitation and mass spectrometric analysis identify a disulfide-bonded mediated physical interaction between SEPN1 and ERO1A. Moreover, ERO1A promotes the formation of redox inactive oligomeric SEPN1, suggesting cross-regulation of their respective activities.
The activity of the ER calcium channel RYR1 is critical for muscle contraction and I4895T mutated form leads to excitation-contraction uncoupling, thus enfeebling muscle contraction. $RYR1^{I4895T}$ models exhibit ER stress with augmented expression of ER stress markers. SUrface SEnsing of Translation (SUnSET) on $RYR1^{I4895T}$ models shows a defect in protein translation, suggesting attenuation of protein translation associated with ER stress. Moreover, treatment with ISRIB, a small molecule that restarts protein synthesis in conditions of ER stress, rescues such a defect. This study sheds light on the pathogenic role of specific ER stress/ ER stress response mediators in SEPN1 and RyR1-related myopathies and it is pivotal for implementing a targeted therapy for such diseases.

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Proteomic analysis suggests novel mechanisms involved in the protection against cancer-induced muscle wasting in mice lacking RAGE at myofiber level

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Cancer cachexia (CC) is a debilitating syndrome characterized by progressive muscle wasting and responsible for about half of cancer patients' deaths. The receptor RAGE (receptor for advanced glycation end-products) is re-expressed in myofibers of tumor-bearing mice undergoing cachexia, where it is stimulated by high amounts of cachexigenic RAGE ligands amplifying catabolic pathways. Tumor-bearing RAGE-null (*Ager*$^{-/-}$) mice showed reduced hallmarks of CC, delayed muscle atrophy, and increased survival. To understand the specific role of RAGE expressed at myofiber level in CC, we generated a conditional tamoxifen-inducible mouse model in which the RAGE gene is selectively deleted in skeletal muscles (*Ager*^{mKO} mice). Following subcutaneous injection of Lewis lung carcinoma (LLC) cells, *Ager*^{mKO} mice showed almost complete maintenance of muscle mass and performance at 25 dpi, similar to *Ager*$^{-/-}$ mice, the opposite being observed in *Ager*^{flox} mice. Moreover, the absence of RAGE in muscles of tumor-bearing mice (LLC/*Ager*^{mKO} mice) slowed down body weight loss and increased survival, although to a lesser extent than in the complete absence of the receptor (*Ager*$^{-/-}$ mice). Restrained degradation of fast myosin heavy chain (MyHC)-II, which is typically degraded in cancer conditions, and increased expression of slow isoform MyHC-I, which confers resistance to cancer-induced atrophy, characterized muscles of LLC-*Ager*^{mKO} and LLC-*Ager*$^{-/-}$ mice. Proteomic analysis revealed the absence of terms related to cell death and apoptosis, and several proteins modulated in common in muscles of *Ager*^{mKO} and *Ager*$^{-/-}$ mice compared with the *Ager*^{flox} mice, in the presence of cancer. In particular, SUMO-conjugating enzyme UBC9, whose expression is associated with slow-twitch myofibers, emerged among the upregulated proteins in muscles of LLC/*Ager*$^{-/-}$ and LLC/*Ager*^{mKO} *vs* LLC/*Ager*^{flox} mice, suggesting a novel RAGE-dependent mechanism involved in the resistance against CC.

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