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Report and Abstracts of the 20th Meeting of IIM, the Interuniversity Institute of Myology: Assisi, October 12-15, 2023

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Abstract

The 2023 represented a milestone for the Interuniversity Institute of Myology (IIM) since it marked twenty years of IIM activity joined with the 20th annual meeting organized by the association. The 20th IIM meeting took place in the fascinating town of Assisi, in the heart of central Italy, from 12 to 15 October. The commemorative 20th edition of the meeting represented a success in terms of participation and contributions as it brought together 160 myologists, clinicians, pharmaceutical companies, and patient organization representatives from Italy, several European countries (especially France), the United Kingdom, Brazil, and the USA. Four main scientific sessions hosted 36 oral communications and 54 always-on-display posters reporting original and unpublished results. Four main lectures from internationally renowned invited speakers and talks from delegates of the Societé Française de Myologie gave particular interest and emphasis to the scientific discussion. In line with the traditional policy of the IIM to encourage the participation of young researchers, about 50% of the attendees were under 35 years old. Moreover, the 20th IIM meeting was part of the high-training course in "Advanced Myology Update 2023", reserved to young trainees and managed by the University of Perugia (Italy) in collaboration with the IIM. In addition to the meeting scientific sessions, the 29 attendees to the course had a dedicated round table and dedicated lessons with the IIM invited speakers as teachers. Awards for the best talk, best poster blitz, and best poster have been conferred to young attendees, who became part of the IIM Young Committee, involved in the scientific organization of the IIM meetings. To celebrate the 20th IIM anniversary, a special free-access educational convention on "Causes and mechanisms of muscle atrophy. From terrestrial disuse to Space flights" has been organized, in which IIM experts in the field have illustrated the current knowledge about the muscle atrophy process in several atrophying conditions, and the former Italian astronaut, Paolo Nespoli shared his incredible experience in Space fascinating the large audience attending both in presence and online live stream. The meeting was characterized by a vibrant, friendly, and inclusive atmosphere, and stimulated discussion on emerging areas of muscle research, fostering international collaborations, and confirming the IIM meeting as an ideal venue to discuss around muscle development, function, and diseases pointing to the development of efficacious therapeutic strategies. Here, the abstracts of the meeting illustrate the most recent results on basic, translational, and clinical research in the myology field. Some abstracts are missing as per authors' decision due to the patentability of the results.

Key Words: muscle; genetics, epigenetics, development and regeneration; wasting; neuromuscular diseases; clinical trials.

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The Interuniversity Institute of Myology (IIM; https://iim-myology.it) was founded in 2004 starting from the idea of Giorgio Fanò-Illic (University of Chieti-Pescara, Italy, at that moment) to bring together scientists of Italian universities involved in the myology field and with the main scope to organize an annual meeting on muscle research. Over time, the IIM has become an increasingly international reality, and now it is a scientific association open to scientists from all over the world belonging to universities or research centers involved in the study of the mechanisms at the basis of muscle physiology and diseases, ranging from myogenesis to muscle regeneration, muscle function, muscle atrophy, and muscular dystrophy. Aims of the IIM are to promote the study of biological, physiological and pathological aspects of muscles; to foster collaboration between myologists from different centers and countries; to stimulate the mobility of students, researchers and teachers; and, to promote knowledge on muscle biology through seminars, conventions, meetings, and high-training courses. The activity of the IIM culminates in the organization of an annual international meeting, which has reached the twentieth edition this year. The 20th IIM meeting was held in Assisi from October 12 to 15, 2023, and brought together 160 researchers, clinicians, pharmaceutical companies, and patient organization representatives from Italy, Europe (with a consistent French delegation), the United Kingdom, Brazil, and USA, leading to a fruitful discussion on the main areas of muscle research. The following four sessions were structured: i) muscle function, muscle stem cells and regenerative medicine; ii) muscle plasticity, aging and exercise; iii) muscle wasting and cachexia; and, iv) modeling and treating muscle diseases. Attendees had the opportunity to show their most recent and unpublished results through 36 oral communications selected by the IIM scientific committee, and 54 posters which were always on display during the meeting and were object of discussion in two planned poster sessions. Four keynote lectures were given by Philippos Mourikis (Paris Est University, Paris, France) about the self-made quiescent niche of muscle stem cells (MuSCs);[1,2] Dada Pisconti (College Arts and Sciences, Stony Brook, USA) about the unifying mechanism regulating muscle stem cell quiescence entry, maintenance and exit;[3,4] Shahragim Tajbakhsh (Institut Pasteur, Paris, France) about the skeletal muscle stem and niche cell dynamics in developmental and regenerative myogenesis;[5,6] and, Saverio Tedesco (University College of London, UK) about engineering human skeletal muscle for advanced tissue, disease, and therapy modeling.[7,8]

Thanks to a collaboration with the Societé Française de Myologie (SFM), the IIM invited three SFM representatives, Bruno Allard, Vincent Gache, and Capucine Trollet to take talks in the meeting.

The IIM puts strong effort in maintaining the meeting registration fees as lower as possible for young trainees to encourage their participation; actually, about 50% of attendees were under-35-year people. Many young researchers chose to attend the university high-training course in Advanced Myology Update, which represents a very peculiar formula in the scenario of the university education. Indeed, the course is managed by the University of Perugia in collaboration with the IIM and included the entire IIM meeting, a dedicated round table on career in science, and dedicated lessons given by the invited speakers of the meeting. Philippos Mourikis discussed about new single-cell transcriptomics technologies and their relevance to muscle research; Saverio Tedesco illustrated the development of new *in vitro* models of muscle tissue; Shahragim Tajbakhsh gave an interactive lesson about the temporal evaluation of myogenic cell states; and, Dada Pisconti explained how to design a translationally meaningful pre-clinical trial for muscle disorders. The overall scientific program of the course, together with the residential formula of this latter giving the possibility to share opinions and perspectives with leading international myologists, resulted in a very valuable experience for both young and senior scientists, and it has been particularly appreciated by early post-docs and Ph.D. students, which contributed enthusiastically to the discussions.

During the meeting, two technical talks were provided by Bio-Techne (Milan, Italy) about spatial biology, single cell characterization and omics, and Bi/ond (Delft, The Netherlands) about dynamic in vitro muscle models. A slot was dedicated to Parent Project association (https://parentproject.it) whose representative, Ilaria Zito illustrated the aim and scope of this association made of parents of patients suffering from Duchenne or Becker muscular dystrophy, the effort of the association in sustaining the patients' families and the research, and the projects intended to make this complex pathology easily understandable.

To celebrate the 20th anniversary, the 2023 IIM meeting hosted the convention "Causes and mechanisms of muscle atrophy. From terrestrial disuse to Space flights" (13th October) in the auditorium of the Department of Medicine and Surgery of the University of Perugia. The convention was focused on muscle atrophy, a biological process associated with a multitude of conditions including disuse, denervation, malnutrition, aging (sarcopenia), prolonged use of steroid anti-inflammatory drugs, chronic inflammatory pathologies, the presence of certain cancer types (cachexia), and the absence of gravity as experienced during long-lasting space flights. Experts in the field such as Marco Sandri (University of Padua, Italy),[9] Paola Costelli Sandri (University of Turin, Italy),[10] Sestina Falcone (Sorbonne University Center of Research in Myology, Paris, France)[11] and Stefania Fulle (University G. d'Annunzio Chieti-Pescara, Italy)[12] discussed with a lay language about several conditions of muscle atrophy in the light of the latest knowledge in this topic. The presence of the former Italian astronaut, Paolo Nespoli as a special guest represented a great element of attraction; he fascinated the large audience with stories about his experiences in various missions in Space. More than 500 people attended the convention in presence and an additional 300 people attended via the dedicated live

streaming connection. At the end of the conference, the participants in the meeting had a guided tour at the "Casa del Cioccolato Perugina", with a visit to the factory where the well-known chocolate is produced.
On Saturday, 14th October, there was a commemorative session during which Giorgio Fanò-Illic, the first IIM director, told in a funny way the new affiliated researchers about the origins of the IIM and its evolution in a scientific association in 2019, the directors the IIM has had over time (2004-2007, Giorgio Fanò-Illic, University G. d'Annunzio Chieti-Pescara, Italy; 2007-2010, Roberto Bottinelli, University of Pavia, Italy; 2010-2016, Antonio Musarò, University Sapienza Rome, Italy; 2016-2022, Davide Gabellini, San Raffaele Institute, Milan, Italy; 2022-present, Guglielmo Sorci, University of Perugia, Italy), and the future perspectives of the association. During the same session, the new logo and the new web site (https://iim-myology.it) of the IIM were presented as well as the new X (Twitter's rebranded identity) (https://twitter.com/IIM_myology) and Instagram (https://www.instagram.com/iim_myology/) pages that, together with the existing LinkedIn page, completed the new social media panel of IIM.
At the end of the meeting, under a convivial and commemorative environment, special awards were assigned based on the evaluation by international panels composed by IIM members. In particular, prizes were assigned for the best oral communications (Andrea Bracaglia, Rome, Italy; Eloisa Turco, Padua, Italy; Noora Pöllänen, Helsinki, Finland), the best posters (Silvia Codenotti, Brescia, Italy; Gaia Laurenzi, Rome, Italy; Giorgia Piccoli, Padua, Italy), and the best poster blitzes (Giacomo Rubini, Turin, Italy; Martina Paiella, Novara, Italy; Caterina Boccia, Rome, Italy). This latter prize has been decided by the engagement of the audience with a live vote through a QR Code provided at the end of the blitzes presentation. A highly participated dance party concluded the meeting, in accordance with the important role of physical exercise in maintaining health and skeletal muscle performance during adulthood.
The 20th IIM meeting resulted successful in bringing full-time together so many myologists in a friendly and vibrant atmosphere, favoring discussion on the main areas of muscle research, providing protected dissemination of the most recent results, and fostering collaboration among researchers. The attached abstracts of the 20th IIM meeting reveal the relevant contribution of this scientific community to the myology field. The next IIM meeting is scheduled for September 4-7, 2024, to be held in Assisi, Italy again.

List of abbreviations

IIM, Interuniversity Institute of Myology
SFM, Societé Française de Myologie

Conflict of interest

The authors declare no potential conflict of interest, and all authors confirm accuracy.

Ethics approval and informed consent

Not applicable.

Patient consent for publication

Not applicable.

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Disclaimer

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2 years anniversary

IIM Interuniversity Institute of Myology

20th IIM Meeting

Assisi, Italy • 12-15 October 2023

Pathogenesis and Therapies of Neuromuscular Diseases

Programme & Abstracts

https://IIM2023.azuleon.org

Topics

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

Keynote Lectures

Philippos **Mourikis**

Dada **Pisconti**

Shahragim **Tajbakhsh**

Saverio **Tedesco**

IIM Scientific Committee

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Programme

11:30-14:30 **Registration**

14:30-14:40 **Welcome and opening of the meeting**

14:40-15:20 **Lecture 1**

Chair: Lucia Latella

Philippos Mourikis *(Paris Est University, France)*

A self-made quiescent niche of muscle stem cells

15:20-16:20 **Session 1/a: Muscle function, muscle stem cells and regenerative medicine**

Chairs: Biliana Lozanoska-Ochser, Giosuè Annibalini

Elena Ruggieri *(Milan, Italy)*

Lorenza Esposito *(Rome, Italy)*

Injury-experienced satellite cells retain long-term enhanced regenerative capacity

Federica Esposito *(Rome, Italy)*

Study of IntegrinB1 transported by FAPs derived extracellular vesicles in correcting asymmetric division of dystrophic satellite cells

Carmen Santangelo *(Chieti, Italy)*

Post-mortem human muscle satellite cells: understanding of fundamental regenerative mechanism

16:20-16:50 ***Coffee break***

16:50-17:05 **Technical talk *(by Bio-Techne)***

Dissecting Muscles: from spatial biology to single cell characterization and Omics. The Bio-Techne approach

17:05-18:20 **Session 1/b: Muscle function, muscle stem cells and regenerative medicine**

Chairs: Libero Vitiello, Emilie Venereau

Amélie Vergnol *(Paris, France)*

Regulation of CaVβ1 isoform expression in skeletal muscle

Vincent Gache *(SFM, Lyon, France)*

Microtubule detyrosination is essential for neuromuscular junction stability and muscle stem cell function

Bruno Allard *(SFM, Lyon, France)*

Zebrafish, an animal model with ultra-high-performance skeletal muscle E-C coupling

Vladimir Krylov *(Prague, Czech Republic)*

The effect of Sertoli cells on striated muscle regeneration in the new model of *Xenopus tropicalis*

Peter Zammit *(London, United Kingdom)*
Identifying Satellite Cell-opathies: neuromuscular disorders caused by muscle stem cell dysfunction

18:20-18:30 **Poster blitz 1** (odd numbers)

19:00-20:30 ***AperiPoster* - Poster Session 1** (odd numbers) **and *Aperitivo Umbro***

9:00-9:40 **Lecture 2**

Chair: Sestina Falcone

Dada Pisconti *(College of Arts and Sciences, Stony Brook, NY, USA)*

A unifying mechanism regulating muscle stem cell quiescence entry, maintenance and exit

9:40-10:40 **Session 2: Muscle plasticity, aging and exercise**

Chairs: Iena Barbieri, Danilo Bondi

Eloisa Turco *(Padua, Italy)*

Giulia Ferrarese *(Padua, Italy)*

Andrea Bracaglia *(Rome, Italy)*

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

Ugo Carraro *(Padua, Italy)*

It's never too early, it's never too late to counteract muscle decay with the Full-Body In-Bed Gym

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

11:10-12:10 **Session 3/a: Muscle wasting and cachexia**

Chairs: Nicoletta Filigheddu, Vanina Romanello

Giulia Gentili *(Perugia, Italy)*

Tumor or muscle RAGE: which one sustains cancer-induced cachexia?

Mariam Zouhair *(Rome, Italy)*

3D bioengineered skeletal muscle tissue as a tool for testing therapeutics for cancer-associated cachexia

Tommaso Raiteri *(Novara, Italy)*

Vitamin D binding protein induces skeletal muscle atrophy and contributes to cancer-associated muscle wasting

Andrea David Re Cecconi *(Milan, Italy)*

The p97-Nploc4 ATPase complex plays a role in muscle atrophy during cancer and amyotrophic lateral sclerosis

12:10-12:25 **Technical talk *(by Biond)***

MUSbit: Towards functional and dynamic *in vitro* muscle models

12:30-13:30 ***Lunch*** and (only for participants registered to the Advanced Myology Update course) **Roundtable Discussions** with the Invited Speakers: **P. Mourikis, D. Pisconti, S. Tajbakhsh, S. Tedesco**

"A career in science. Options and opportunities"

14:00 ***Bus departure to Perugiar***

Convention
CAUSES AND MECHANISMS OF MUSCLE ATROPHY
From terrestrial disuse to Space flights

Chairs: Antonio Musarò (Sapienza University of Rome, Italy), Guglielmo Sorci (University of Perugia, Italy)

15:00 **Welcome from authorities**

15:30 **Marco Sandri** *(University of Padua, Italy)*
Causes and mechanisms of muscle atrophy

15:50 **Paola Costelli** *(University of Turin, Italy)*
Cancer-associated muscle atrophy

16:10 **Sestina Falcone** *(Paris Est University, France)*
Age-related muscle atrophy

16:30 **Stefania Fulle** *(University of Chieti-Pescara, Italy)*
Muscle atrophy. Lessons from Space

16:50 **Paolo Nespoli** *(former ASI/ESA astronaut)*
My experience in Space

17:20 **General discussion**

18:00 **Conclusions**

The convention will be in Italian with simultaneous translation into English. A live streaming connection will be activated.

18:30 ***Guided tour of Casa del Cioccolato Perugina and AperiChocolate***

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

Chair: Anna Urciuolo

Shahragim Tajbakhsh *(Institut Pasteur, Paris, France)*

Skeletal muscle stem and niche cell dynamics in developmental and regenerative myogenesis

9:40-10:40 **Session 3/b: Muscle wasting and cachexia**

Chairs: Bert Blaauw, Valentina Saccone

Noora Pöllänen *(Helsinki, Finland)*

NAD^+ depletion as a disease mechanism and therapy target for cancer cachexia

Davide Acquarone *(Turin, Italy)*

Giorgia Careccia *(Milan, Italy)*

Enrico Pierantozzi *(Siena, Italy)*

Deletion of the muscle-specific internal promoter of the *Ank1* gene results in a pre-diabetic phenotype

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

11:10-12:40 **Session 4/a: Modeling and treating muscle diseases**

Chairs: Cesare Gargioli, Chiara Mozzetta

Quentin Giraud *(Illkirch-Graffenstaden, France)*

MTM1 overexpression efficiently rescues BIN1-related centronuclear myopathy

Laura Pérez i Guàrdia *(Illkirch-Graffenstaden, France)*

ORAI1 inhibition as an efficient preclinical therapy for tubular aggregate myopathy (TAM) and Stormorken syndrome (STRMK)

Nefele Giarratana *(Leuven, Belgium)*

Targeting rhabdomyosarcoma cancer progression by MICAL2 regulation

Lucas Duvert *(Marseille, France)*

Laser-assisted bio-printing and structuring for muscle modeling

Beatrice Auletta *(Padua, Italy)*

Capucine Trollet *(SFM, Paris, France)*

Investigating the molecular, cellular and mechanistic differences of muscle fibrosis in human myopathies to determine to what extent fibrosis is a unique feature or results from different contributing factors

12:40-12:50 **Poster blitz 2** (even numbers)

13:00-14:30 ***AperiPoster* - Poster Session 2** (even numbers) **and *lunch***

14:30-15:10 **Lecture 4**

Chair: Valeria Runfola

Saverio Tedesco *(University College London, United Kingdom)*

Engineering human skeletal muscle for advanced tissue, disease, and therapy modelling

15:10-16:25 **Session 4/b: Modeling and treating muscle diseases**

Chairs: Graziella Messina, Alessandro Fanzani

Beatrice Biferali *(Milan, Italy)*

Sora Han *(Seoul, South Korea)*

CTRP1, a myogenic protein in skeletal muscle differentiation and mitochondrial function

Michela Gloriani *(Rome, Italy)*

Giovanni Delli Carpini *(Rome, Italy)*

Clara De Palma *(Milan, Italy)*

SRT2104, a new SIRT1 activator, is an effective metabolic enhancer that promotes muscle recovery in DMD

16:25-16:55 ***Coffee break***

16:55-17:55 **Session 4/c: Modeling and treating muscle diseases**

Chairs: Roberta Sartori, Luca Madaro

Giorgia Cavioli *(Rome, Italy)*

Unveiling the HDAC4 functions in mediating the cross-talk between skeletal muscle fibers and fibro-adipogenic progenitors in Duchenne Muscular Dystrophy

Francesca De Paolis *(Rome, Italy)*

In vitro modeling of the human neuromuscular junction in a microfluidic device for the study of facioscapulohumeral dystrophy

Anne Forand *(Paris, France)*

Assessment of cardiac structure and function in a $Dys^{-/-}$;$Utr^{-/-}$ mouse model of DMD treated with long term dystrophin replacement therapies

Ilaria Zito *(Parent Project, Rome, Italy)*

The voice of patients affected by Duchenne and Becker muscular dystrophy

18:00-19:00 **IIM General meeting**

Giorgio Fanò-Illic *(Free University of Alcatraz, Italy)*
20 years of IIM: no ifs, no buts

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

22:00 ***80's-90's Dance party with Tony Ross Dee Jay***

Reserved to participants registered to the
High Training Course in ADVANCED MYOLOGY UPDATE 2023

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

10:30-12:30 **Dedicated lessons**

Philippos Mourikis
New single-cell transcriptomics technologies and their relevance to muscle research

Saverio Tedesco
Development of new *in vitro* models of muscle tissue

Shahragim Tajbakhsh
Temporal evaluation of myogenic cell states

Dada Pisconti
How to design a translationally meaningful pre-clinical trial for muscle disorders

12:30 ***Light lunch***

Posters always on display during the meeting

Discussion

Odd numbers: Thursday, 12 October (19:00-20:30)

Even numbers: Saturday, 14 October (13:00-14:30)

P.1 **Vittoria Marini** *(Leuven, Belgium)*
3D human cardiac models of Duchenne cardiomyopathy generation and NOX4 inhibition

P.2 **Adriano Ciena** *(Rio Claro, Brazil)*
Telocytes morphology at the myotendinous junction after muscle injury

P.3 **Delia Verucci** *(Chieti, Italy)*

P.4 **Gaia Giuriato** *(Verona, Italy)*
Intrinsic Skeletal muscle contractility and Nrf2 in humans

P.5 **Danilo Bondi** *(Chieti, Italy)*
A pilot application of integrated procedures to accelerate, deepen, and guide genetic investigation on the myokinome

P.6 **Elisabetta Meacci** *(Florence, Italy)*
Sphingosine-1-phosphate/sphingosine-1-phosphate receptor (S1P/S1PR) axis modulates irisin signaling in skeletal muscle cells

P.7 **Ozlem Kartal** *(Padua, Italy)*
Targeting mitochondrial calcium and metabolism by RNA-based therapy in sarcopenia

P.8 **Anna Pedrinolla** *(Trento, Italy)*
Effect of high-intensity exercise training combined with dark chocolate rich in polyphenols and vitamin-E on muscle mass and strength in elderly people with dementia. Preliminary results from the Choko-Age study

P.9 **Lorenzo Marramiero** *(Chieti, Italy)*
Extracellular 500 μM GTP enhances myogenesis inducing specific gene and protein pathways

P.10 **Giorgia Piccoli** *(Padua, Italy)*

P.11 **Francesco Millozzi** *(Rome, Italy)*

P.12 **Jurandyr Pimentel Neto** *(Rio Claro, Brazil)*
Muscle injury protocol and adjacent cells at the neuromuscular junction region: a new aspect view

P.13 **Emanuele Mocciaro** (*Milan, Italy)*

P.14 **Martina Biglietto** *(Rome, Italy)*
Engineered exosomes as a therapeutic tool to counteract muscle degeneration

P.15 **Veronica Ruggieri** *(Rome, Italy)*
The emerging role of polyamine pathway in skeletal muscle during ALS progression

P.16 **Desiree Genovese** *(Rome, Italy)*
Characterization of *ex-vivo* muscle engineered tissue's (X-MET) functional remodelling

P.17 **Valeria Runfola** *(Milan, Italy)*
Identification of MATR3 as endogenous inhibitor of DUX4 for the treatment of FSHD muscular dystrophy

P.18 **Francesco Palmieri** *(Florence, Italy)*
Role of HIF-1α/MMP-9 axis in promoting skeletal myoblast differentiation under normoxia condition

P.19 **Caterina Boccia** *(Rome, Italy)*

P.20 **Cristina Purcaro** *(Chieti, Italy)*
Environmental pollutants and muscle stem cells: findings of potential interactions

P.21 **Silvia Casati** *(Milan, Italy)*
Targeting mitochondrial dynamics to tackle duchenne muscular dystrophy progression

P.22 **Lucia Rossi** *(Padua, Italy)*

P.23 **Silvia Codenotti** *(Brescia, Italy)*
The Src/Akt1/caveolin-1/catalase signaling axis is critical for radioresistance of rhabdomyosarcoma cells

P.24 **Stefano Testa** *(Marseille, France)*
Development of an innovative extrusion-based 3D bioprinting system and its characterization for Skeletal Muscle Tissue Engineering applications

P.25 **Raffaele Epis** *(Milan, Italy)*

P.26 **Alessandro Antonioli** *(Novara, Italy)*
Ad libitum ketogenic diet reverts western diet-pathological effects in liver, but not in skeletal muscle in mice

P.27 **Francesca Gasparella** *(Padua, Italy)*
Elucidating the effects of MAO-B inhibitors in DMD: rasagiline treatment in mdx mice dampens the expression of pro-inflammatory genes in multiple cell types

P.28 **Mara Barone** *(Milan, Italy)*

P.29 **Gaia Laurenzi** *(Rome, Italy)*

P.30 **Sara Chiappalupi** *(Perugia, Italy)*
Effects of real microgravity on human muscle precursor cells and skeletal muscle tissue. The *MyoGravity* project

P.31 **Paola Mantuano** *(Bari, Italy)*
Antifibrotic potential of growth hormone secretagogues in Duchenne muscular dystrophy: effects of JMV2894 in the D2-*mdx* mouse model

P.32 **Pietro Chiolerio** *(Padua, Italy)*

P.33 **Meriem Matouk** *(Montigny le Bretonneux, France)*
The compartmentalized distribution of proteins of the dystrophin-associated protein complex in the muscle fiber syncytium

P.34 **Martina Lunardi** *(Milan, Italy)*

P.35 **Maria Pannese** *(Milan, Italy)*

P.36 **Martina Paiella** *(Novara, Italy)*
Western diet enriched in advanced glycation end-products (AGEs) induces muscle wasting which could be counteracted by *Vaccinium macrocarpon* extract

P.37 Cristiana Perrotta *(Milan, Italy)*
What nutraceuticals can do for Duchenne muscular dystrophy: the plumbagin experience

P.38 Federica Palo *(Milan, Italy)*

P.39 Andrea Pirrottina *(Rome, Italy)*
Development of efficient gene delivery systems for the treatment of Duchenne muscular dystrophy

P.40 Ilaria Piazza *(Padua, Italy)*

P.41 Gabriele Rovetta *(Milan, Italy)*

P.42 Rosanna Piccirillo *(Milan, Italy)*

P.43 Emilia Skafida *(Rome, Italy)*
Dynamic changes in transcriptional and epigenetic profile of fibro-adipogenic progenitors in dystrophic skeletal muscles and their modulation by epigenetic drugs (HDACi)

P.44 Giacomo Rubini *(Turin, Italy)*
Immunomodulation via interleukin-4 improves cachexia in C26 tumor-bearing mice

P.45 Giosuè Annibalini *(Urbino, Italy)*
N-glycosylation inhibition reduced IGF-1 serum level and muscle IGF-1R signalling pathway activation

P.46 Laura Salvadori *(Novara, Italy)*
Vaccinium macrocarpon restrains myotube atrophy in experimental models mimicking cancer cachexia

P.47 Elena Conte *(Bari, Italy)*
Insight into the role of calcium homeostasis and store-operated-calcium-entry dysfunction in sarcopenia: effect of supplementation with BCAA-based formulation in aged mice

P.48 Andrea Scircoli *(Novara, Italy)*
RNAseq analysis revealed a lower inflammation in tumor-bearing mice lacking VDBP resulting in a milder cachectic phenotype

P.49 Evgeniia Motanova *(Padua, Italy)*
Morphological alterations of human neuromuscular junction in ageing

P.50 Elisabeth Wyart *(Turin, Italy)*

P.51 Leonardo Sandrini *(Brescia, Italy)*
Putative contribution of caveolin-1 in satellite cells senescence

P.52 Rachele Agostini *(Urbino, Italy)*
Extracellular vesicle release and protein cargo are altered by caveolin-1-overexpression and contribute to cancer dissemination in a model of rhabdomyosarcoma

P.53 Alessandra Guidi *(Rome, Italy)*
Role of PRDM16 in maintaining nuclear integrity and genomic stability in fibro-adipogenic progenitors

P.54 Suvham Barua *(Turin, Italy)*
Non-Autonomous Muscle Stem Cells mediated defective muscle regeneration in the accelerated ageing Polg Mutator Mouse Model

Keynote Lecturers

Philippos MOURIKIS

East Paris University, Creteil, France

I am Research Director of the French research organisation CNRS (Centre national de la recherche scientifique), deputy director of a 110-members research team working on the neuromuscular system lead by Prof F. Relaix, and leader of a group working on muscle stem cells-niche interactions, situated at the Mondor research Institute, Paris-East University, France.
I have a long-standing interest in Notch signalling and the regulation of stem cell quiescence. We are using the murine skeletal muscle as a model system and our experimental approach is based on high-throughput techniques coupled with mouse genetics to identify and study novel regulators of muscle stem cell establishment and maintenance. I conducted my PhD in the laboratory of Prof Artavanis-Tsakonas at the Harvard Medical School, a leading team in the field since Prof Artavanis cloned the Notch locus. My introduction to the field of muscle research was done during my postdoctoral fellowship at the Pasteur Institute, Paris, and later at the Institute of Myology also in Paris.
My research is addressing fundamental aspects of muscle stem cell biology. I am a strong advocate for basic research that I consider the essential core of discoveries from which biomedical knowledge and therapies can stem. In addition, I am committed to teaching and mentoring trainees at all levels, including graduate and medical students.
I firmly support collaborative research for exchanging and generating ideas, learning new skills, and higher quality results. For this, I have been actively involved in the organisation of international meetings in my two main fields of expertise: muscle biology and Notch signalling.

A self-made quiescent niche of muscle stem cells

Philippos Mourikis
East Paris University, Creteil, France

The maintenance and repair of numerous adult tissues depend on the presence of resident stem cells. To preserve these cells' regenerative potential, a finely orchestrated interplay of intrinsic and extrinsic factors is essential. These factors work in tandem to suppress differentiation and regulate various processes such as chromatin state, RNA maturation and processing, metabolism, and adhesion. I will discuss the specific role of these factors in the context of skeletal muscle stem cells, commonly referred to as satellite cells, with a primary focus on Notch signaling and its interaction with the extracellular matrix. Notch serves as a cell-to-cell communication pathway, acting as a sensor of the microenvironment and playing a pivotal role in the maintenance of satellite cells. This is achieved by inhibition of the muscle differentiation factors MyoD and Myogenin, but also by directly regulating the production of components of the extracellular matrix niche. Therefore, in addition to the notion of intrinsic and extrinsic factors, our working model is based on a self-sustaining system of stem cell maintenance. This system is triggered by Notch activation within the satellite cell and incorporates both inherent factors and external cues, which are autonomously produced by the satellite cells. The role of specific collagens and other Notch-regulated ECM factors will be discussed in the context of satellite cell maintenance and activation.

Dada PISCONTI

Department of Biochemistry and Cell Biology, SUNY Stony Brook, Stony Brook, NY, USA

Dr. Dada Pisconti earned her PhD from the University of Bari School of Medicine and undergraduate degrees from the University of Perugia. She is currently an Associate Professor in the Department of Biochemistry and Cell Biology at SUNY Stony Brook, New York, where she moved in late 2018 from the University of Liverpool, UK. Her research aims to understand how muscle stem cells interact with their microenvironment, also known as niche, and how niche remodeling during growth and regeneration is mechanistically linked to stem cell fate decisions, homeostasis and regenerative potential. The Pisconti lab also studies the molecular pathogenesis of Duchenne muscular dystrophy and Myalgic Encephalomyelitis/ Chronic Fatigue Syndrome. More recently the lab has started a collaboration with colleagues at Nofima in Oslo, Norway, to study the chicken myopathy commonly known as wooden breast, which afflicts a significant fraction of fast-growing broilers. Together with long-term collaborator Dr. Hugo Olguin, at the PUC in Santiago, Chile, the Dr. Pisconti studies the mechanisms that regulate the choice between differentiation and quiescence in muscle stem cells, as well as the mechanisms that maintain quiescence.

A unifying mechanism regulating muscle stem cell quiescence entry, maintenance and exit

Addolorata Pisconti
Department of Biochemistry and Cell Biology, SUNY Stony Brook, Stony Brook, NY, USA

Muscle stem cells (also known as satellite cells or MuSCs) were discovered over 60 years ago and since their discovery over 5,000 articles have been published, therapeutic approaches based on boosting MuSC function have been developed and clinically trialed, and numerous patents on using MuSC for all sort of applications, including meat production, have been granted. However, to this date, some fundamental questions about MuSC biology remain open. A fascinating open question is: how do proliferating MuSCs decide whether to differentiate or become quiescent upon exiting the cell cycle? Our recent data strongly suggest that the tumor suppressor p53 is at the center of a molecular mechanism regulating the balance between quiescence and differentiation. Specifically, our data suggest that regulation of p53 protein levels by ubiquitination and deubiquitination plays a central role in the control of all three aspects of MuSC quiescence regulation: entry, maintenance and exit. To test this hypothesis, we have used transgenic mice where the gene encoding p53 (Trp53) and the gene encoding its main regulator at the protein level, the E3 ligase MDM2, were knockdown or knocked out only in MuSCs. We then used a muscle injury model to study how this MDM2-p53 molecular network regulates MuSC quiescence. Concomitantly, we have used a validated in vitro system to model MuSC quiescence to dissect the underlying molecular mechanism(s) of p53-mediated regulation of MuSC quiescence entry, maintenance and exit. In my talk I will present unpublished data showing that MuSC activation in response to injury involves p53 destabilization via induction of MDM2. Consistently, MDM2 knockout impairs muscle regeneration in a p53-dependent manner. Additionally, I will show that p53 expression is periodically induced in proliferating myoblasts leading to cell cycle exit and that cell fate and cell cycle are molecularly linked via p53 and MyoD reciprocal levels

Shahragim TAJBAKHSH

Institut Pasteur, Paris, France

Prof. Shahragim Tajbakhsh obtained a Ph.D. in Biology, Carleton U., Canada on molecular biology of viruses. Following postdoctoral research at Institut Pasteur he established an independent group in 2001 called “Stem Cells & Development” to study how stem cells establish and regenerate organs and tissues, with a focus on skeletal muscle. The aim of his laboratory is to investigate stem cell properties during development and postnatally to understand how skeletal muscle is established, and how it regenerates during disease, and after injury. Areas of focus include quiescence, niche, self-renewal, symmetric/asymmetric cell divisions, regeneration and ageing. His lab used mouse genetics to show that muscle stem cell populations are remarkably diverse in function leading to the hypothesis that the modular design in the embryo might in part be responsible for the mosaic response in pathology of muscles in myopathies.

ST is an EMBO member, former Head of Dept. of Developmental & Stem Cell Biology, and co-Director of “Laboratory of Excellence” Consortium, REVIVE (28 labs working on stem cells; 2011-2024). He is member of different scientific councils (e.g., French myopathy organisation, vice-President), several SABs and presides on editorial boards of 4 scientific journals. He has participated in several EU consortia (FP6, EuroStemCell; FP7, EuroSyStem, Optistem, NotchIT) and received several awards including Chair of Excellence Louis Pasteur (Institut Pasteur, 2017) and the French Academy of Sciences. He received numerous competitive grants (e.g., EU, ANR, FRM, ARC, 2-time awardee of ERC Advanced Grant). In 2010, 2015, and 2020 the national HCERES ranked the ST laboratory as A^+, outstanding.

Skeletal muscle stem and niche cell dynamics in developmental and regenerative myogenesis

Shahragim Tajbakhsh
Stem Cells & Development Unit, UMR CNRS 3738, Institut Pasteur, Paris

Muscle stem cells are heterogeneous in properties, both within a single muscle, and in different anatomical locations. This heterogeneity is manifested at multiple levels including distinct gene regulatory networks and modes of cell divisions. For example, following muscle injury, MuSCs can self-renew and differentiate through symmetric (SCD) and asymmetric (ACD) cell divisions and the relative frequency of these modes of cell division are dynamic during muscle regeneration. We developed several static and live imaging pipelines to assess MuSC fate decisions in different microenvironments to record cell divisions and respective cell fates in normal and dystrophic conditions. Our observations lead us to propose that MuSCs can adopt different strategies to execute ACDs and that they are not engaged in obligate modes of cell division. Further, these cell fate decisions implicate both intrinsic and extrinsic factors.
To further investigate the diverse properties of MuSCs we examined the proliferation and differentiation properties of extraocular and limb muscles. We performed transcriptional profiling and functional assays of satellite cells isolated from these and other cranial and limb muscles and identified core gene regulatory networks that are common and unique to each population. How satellite cell properties are modulated in these different contexts will be discussed.

Saverio TEDESCO

University College London and The Francis Crick Institute, London, United Kingdom

Prof. Tedesco is a clinician-scientist with expertise in paediatric neuromuscular diseases and muscle regeneration. He graduated in Medicine and Surgery with honours at the Sapienza University of Rome (Italy). Before his doctorate he was a visiting scientist at the Institut Pasteur (Paris, France) studying muscle stem cell biology. He obtained his PhD investigating novel gene and cell therapies for muscular dystrophy at the San Raffaele Scientific Institute of Milan (Italy). Prof. Tedesco received the 2015 Young Investigator Award by the European Society of Gene and Cell Therapy. He was then awarded an NIHR Academic Clinical Fellowship, followed by a Clinical Lectureship in Paediatric Neurology, and a prestigious European Research Council (ERC) Starting Grant. He received the 2020 Simon Newell Investigator of the Year award by the Royal College of Paediatrics and Child Health and the 2021 MacKeith Prize by the British Paediatric Neurology Association.

The Tedesco laboratory studies skeletal muscle regeneration, focusing on the development of novel therapies for incurable neuromuscular disorders of childhood. They work pioneered the use of cutting-edge technologies such as human induced pluripotent stem (iPS) cells, artificial chromosomes and tissue engineering for advanced disease modelling and gene/cell therapies of muscle diseases. Current projects investigate iPS cell-derived myogenesis for complex neuromuscular disease and therapy modelling, as well as the use of small molecules to improve myogenic cell delivery. The overall goal of the Tedesco laboratory is the translation of the aforementioned regenerative strategies into novel therapies to improve outcomes for children with neuromuscular disorders.

Engineering human skeletal muscle for advanced tissue, disease, and therapy modelling

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Skeletal muscle is a complex tissue composed of multinucleated myofibres supported by a variety of cell types and a specialised extracellular matrix (ECM), compromised in severe incurable neuromuscular diseases such as muscular dystrophies. Limitations in animal models and lack of reliable, human(ised) in vitro models currently pose hurdles towards development of novel neuromuscular therapies. To address these limitations, we developed three-dimensional (3D) human skeletal muscle constructs to model different morphological and functional defects in tissue compartments impaired in muscular dystrophies (e.g., sarcolemma, nuclear envelope, ECM). 3D muscles were developed from human myoblasts, fibroblasts or induced pluripotent stem cells (iPSCs) differentiated into myogenic, neural and vascular progenitor cells, and then combined with biomaterials to generate aligned myofibre scaffolds containing ECM, vascular networks and motoneurons. Engineered muscles recapitulated molecular, morphological, and functional characteristics of human skeletal muscle, providing a high-fidelity platform to study muscle pathology, such as the emergence of nuclear abnormalities in muscular dystrophies caused by mutant nuclear lamins. To further validate this approach in another severe, congenital muscle disease with abnormal myonuclear features, we developed a new 3D model of X-linked centronuclear (myotubular) myopathy, identifying morphological and functional disease-associated readouts for therapeutic development. Moreover, we extended this technology to another incurable congenital muscular dystrophy characterised by abnormal ECM, detecting disease-associated readouts at macro- and micro-scopic scale. Finally, this 3D platform and its disease-associated in vitro outcome measures were utilised to test both mutation-independent and -specific genetic therapies, laying the foundations for a multi-functional platform for precision medicine in neuromuscular diseases.

Selected Talks Abstracts

Injury-experienced satellite cells retain long-term enhanced regenerative capacity

J. Morroni, A. Benedetti, Lorenza Esposito, M. De Bardi, G. Borsellino, C. Sanchez Riera, L. Giordani, M. Bouche, B. Lozanoska-Ochser
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Background. Inflammatory memory or trained immunity is a recently described process in immune and non-immune tissue resident cells, whereby previous exposure to inflammation mediators leads to a faster and stronger responses upon secondary challenge. Whether previous muscle injury is associated with altered responses to subsequent injury by satellite cells (SCs), the muscle stem cells, is not known.
Methods. We used a mouse model of repeated muscle injury, in which intramuscular cardiotoxin (CTX) injections were administered 50 days apart in order to allow for full recovery of the injured muscle before the second injury. The effect of prior injury on the phenotype, proliferation and regenerative potential of satellite cells following a second injury was examined in vitro and in vivo by immunohistochemistry, RT-qPCR, and histological analysis.
Results. We show that SCs isolated from muscle at 50 days post- injury ('injury-experienced SCs (ieSCs)) enter the cell cycle faster and form bigger myotubes when cultured in vitro, compared to control SCs isolated from uninjured contralateral muscle. Injury-experienced SCs were characterised by the activation of the mTORC 1 signalling pathway, suggesting they are poised to activate sooner following a second injury. Consequently, upon second injury, SCs accumulate in greater numbers in muscle at 3 and 10 days after injury. These changes in SC phenotype and behaviour were associated with accelerated muscle regeneration, as evidenced by an earlier appearance of bigger fibers and increased number of myonuclei per fiber at day 10 after the second injury.
Conclusions. Overall, we show that skeletal muscle injury has a lasting effect on SC function priming them to respond faster to a subsequent injury. The ieSCs have long-term enhanced regenerative properties that contribute to accelerated regeneration following a secondary challenge.

Study of IntegrinB1 transported by FAPs derived extracellular vesicles in correcting asymmetric division of dystrophic satellite cells

Federica Esposito[1,2], M. Sandonà[2], V.C. Carregari[3], L. Pieroni[2], S. EL Andaloussi[4], M. De Bardi[2], D.F. Angelini[2], P.L. Puri[5], V. Saccone[2,6]

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Duchenne muscular dystrophy (DMD) is a rare neuromuscular disorder caused by mutations in the dystrophin gene, resulting in the absence of the corresponding protein, which leads to progressive muscle degeneration. There is currently no cure for DMD, and only palliative treatments are available to extend the lifespan of affected individuals. Our group has uncovered the potential of epigenetic drugs, such as Trichostatin A, which can inhibit Histone Deacetylases and promote muscle regeneration. Moreover, recent advancements in regenerative medicine have highlighted the therapeutic potential of Extracellular Vesicles (EVs) as a cell-free treatment option capable of avoiding side effects. In our experiments on dystrophic mice, we observed that communication between fibroadipogenic progenitors (FAPs) and muscle stem cells (MuSCs) is mediated by EVs. We demonstrated that pharmacological treatment with TSA fine-tunes the content of FAPs-derived EVs, making them pro-regenerative. The genetic material carried within these EVs can be transferred to MuSCs, influencing their activation, differentiation, and fate. Notably, the link between Itgb1 and DMD is robust, as dystrophic MuSCs show an unbalance in the integrin dimer a7b1 on their surface, with a decrease only in the b1 chain that contributes to the breakdown of the muscle membrane. Our finding indicates that Itgb1 loaded onto the external membrane of TSA-EVs is essential in preventing the spurious and aberrant MuSCs proliferation and differentiation, thereby correcting their polarization and restoring the correct asymmetric cell division defying their correct fate. Moreover, we discovered that Itgb1 released by EVs to dystrophic MuSCs contributes to the beneficial effects of TSA-EVs on the muscle environment. These significant results pave the way for profoundly understanding the role of Itgb1 loaded in TSA-EVs, which could help to restore muscle architecture, stabilize the MuSCs niche, and preserve muscle tissue.

Postmortem Human Muscle Satellite Cells: understanding of fundamental regenerative mechanism

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[6]Interuniversity Institute of Myology (IIM), Chieti, Italy

Human postmortem skeletal muscles are a unique source of satellite cells (SCs) able to proliferate and differentiate *in vitro*. Interestingly, presomite and somite SCs from postmortem skeletal muscles are an easily accessible source of SCs, potentially applicable for muscle physiology and diseases understanding and for drug testing. Aiming to explore and understand the regenerative potential of postmortem SCs, the postmortem human muscle precursor cells (hMPCs) were isolated from the bioptised thyrohyoid (THY) pharyngeal arches and the iliopsoas (IL) muscles, obtained from 40, 43, 45, and 71 years old corpses. Postmortem hMPCs migrated out of the explant within 15 days (alive donors 3-4 months) and showed an increased proliferation rate compared with living volunteers. Postmortem THY hMPCs proliferate faster than IL hMPCs. The [Ca2+]i in postmortem THY hMPCs was significantly less than [Ca2+]i in IL hMPCs. Not all the hMPCs were fused into myotubes at 7 days, but at 10–12 days about 90% of hMPCs differentiated. Similar [Ca2+]i in presomite and somite myotubes were found under resting conditions. The investigation of the acetylcholine channels revealed different responsiveness between somite and presomite-differentiated hMPCs to the nicotine stimulation. In perspective, our work is in line with the recent advances in regenerative and precision medicine. Moreover, our study addresses the need of understanding the fundamental mechanisms in presomite and somite SCs models and their role in microphysiological systems, and possibly contributes to enrich new biobank for physiological and pathological studies on skeletal muscle.

Regulation of CaVβ1 isoform expression in skeletal muscle

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*Equally participated

CaVβ1, encoded by *Cacnb1* gene, exists as several transcript variants in skeletal muscle. Our recent work demonstrates CaVβ1D as the constitutive adult isoform, localized with CaVα1 at the triad. On the other hand, we show that CaVβ1E and CaVβ1A are expressed at late embryogenesis and peri-natal stages. Interestingly, our results revealed the existence of another undescribed CaVβ1 isoform: at early embryogenesis stages (E12.5), *Cacnb1* exon 7A is excluded leading to a premature stop codon in exon8 and giving rise to the Cacnb1_early mRNA. Interestingly, we were able to show Cacnb1_early expression at protein level. Between E12.5 and E16, a progressive inclusion of exon7A leads to a gradual switch toward adult Cacnb1 mRNAs. These results support the existence of a new CaVβ1 isoform, expressed early during embryogenesis and for which function remains to be characterized.
In adult muscle, the re-expression of these embryonic CaVβ1E and/or CaVβ1A isoform(s) plays a crucial role in muscle mass homeostasis when electrical activity is impaired by preventing loss of muscle mass. We showed that both the embryonic CaVβ1E/CaVβ1A and the newly discovered CaVβ1_early transcripts begin at exon1 while the mRNA of the adult constitutive isoform CaVβ1D begins at exon3. We found that the expression of these isoforms derives from the activation of two distinct promoters at exon1 and exon3, respectively. Finally, we showed that the epigenetic marks H3K4me3 and H3K9ac, characteristics of active promoters, are increased at exon1 and reduced at exon3 following denervation. This suggests that the exon1/exon3 promoter switch induced by denervation can be regulated through the restoration of an embryonic epigenetic program.
These results bring insights on the mechanism behind the induction of embryonic CaVβ1 isoform expressions in adult skeletal muscle after impaired electrical activity and rise questions regarding the role of CaVβ1 isoforms both in embryonic and adult skeletal muscles.

Microtubule detyrosination is essential for neuromuscular junction stability and muscle stem cell function

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Microtubule cytoskeleton is essential for many biological processes, such as cell shape maintenance, motility, intracellular trafficking or organelle positioning. Pathological muscle fibers frequently exhibit alteration of the microtubule network architecture, that strongly impacts myonuclear domain features. Microtubule stability and dynamics are finely tuned by a set of posttranslational modifications. Among them, detyrosination of the C-terminus tail of the alpha-tubulin, essentially mediated by the Small Vasohibin-Binding Protein (SVBP), is associated with more stable microtubules. Interestingly, detyrosinated microtubules are abundant in skeletal myofibers. Thus, we hypothesize that SVBP-mediated detyrosination regulates myofiber homeostasis. Analyzing *Svbp*-knockout (*Svbp*$^{-/-}$) mice revealed that myofibers have decreased myonuclear domains, suggesting increased content of myonuclei that could arise from newly fused myogenic progenitors. Using *in vitro Svbp*$^{-/-}$ satellite cell-derived myoblasts, we confirmed that detyrosination of microtubules controls myoblast fusion events. Additionally, using *ex vivo*isolated and cultured myofibers, we showed that loss of SVBP enhances quiescent satellite cell capacity to enter the cell cycle and promotes their differentiation at the expense of self-renewal. Moreover, we find that *Svbp*$^{-/-}$ myofibers are denervated and exhibit fragmented neuromuscular junctions (NMJs). It was previously shown that disruption of NMJs can trigger fusion of a subset of satellite cell-derived progenitors. Collectively, our data suggest that diminished microtubule detyrosination intrinsically disturbs satellite cell function, which could be further affected by the disruption of neuromuscular junction and myofiber integrity.

Zebrafish, an animal model with ultra-high-performance skeletal muscle E-C coupling

Bruno Allard
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The zebrafish has emerged as a very relevant animal model for probing the pathophysiology of human skeletal muscle disorders. This vertebrate animal model displays a startle response characterized by contraction of fast skeletal muscle fibers excited at extremely high frequencies, critical for escaping predators and capturing prey. Such intense muscle performance requires extremely fast properties of the contractile machinery but also of excitation-contraction coupling. However, thus far, the fastest Ca^{2+} transients evoked by vertebrate muscle fibers has been described in muscles used to produce sounds, such as those in the toadfish swim bladder, but not in muscles used for locomotion. By performing intracellular Ca^{2+} measurements under voltage control in skeletal muscle fibers from adult zebrafish and mouse, we demonstrate that fish fast muscle fibers display superfast kinetics of action potentials, intramembrane charge movements and action potential-evoked Ca^{2+} transient, allowing fusion and fused sustained Ca^{2+} transients at excitation frequencies much higher than in mouse fast skeletal muscle fibers and comparable to those recorded in muscles producing sounds. In parallel, we characterized at the single channel and macroscopic level and cloned a mechano-and heat-gated two-pore domain K^+ channel in adult zebrafish skeletal muscle that is absent from mammalian muscle. We show that heat and stretch in zebrafish muscle lead to acceleration of action potentials repolarization suggesting that heat production and membrane deformation associated with muscle activity can control muscle excitability through this K^+ channel activation. This two-pore domain K^+ channel may represent a teleost-specific evolutionary product contributing to improve skeletal muscle performance.

The effect of Sertoli cells on striated muscle regeneration in the new model of *Xenopus tropicalis*

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Renewal of striated muscle after injury or muscular dystrophy is limited in adult mammals, including humans. The amphibian Xenopus tropicalis has long served as a model for studying genetics, developmental processes, and the cell cycle. Injection of human choriogonadotropin hormone (hCG) induces the production of hundreds of oocytes by individual female frogs, ensuring an ample supply of tadpoles for experimental work. X. tropicalis tadpoles are semi-translucent, allowing for the tracking of fluorescently labeled cells using confocal or light-sheet microscopy in whole-mount preparations. Muscle regeneration following tail amputation in X. tropicalis resembles the process observed in mammals. It involves the proliferation and differentiation of Pax7-positive satellite cells to restore the damaged tissue. Muscle regeneration is closely linked to the innate immune response, including M1 pro-inflammatory and M2 pro-regenerative macrophages (myeloid) cell populations. Enhancing the M2 phenotype holds promise to boost striated muscle regeneration using native cells. Both our research and that of others have demonstrated that Sertoli cells possess this immunomodulatory capacity. We successfully established a culture of common progenitors of Sertoli and peritubular myoid cells (XtiSCs) derived from the testes of juvenile male froglets. When injected into the dorsal vein of tadpoles, XtiSCs migrate significantly to the injury site following tail amputation. Additionally, our studies have shown that XtiSCs support the proliferation of Pax7 satellite cells and replenish the myeloid cell population following their depletion through administration of Clodronate liposome, up to seven days after the treatment. Our published and preliminary results indicate two potential mechanisms for myeloid cell restoration. The first involves mitochondrial transfer from XtiSCs to impaired myeloid cells. The second scenario suggests the direct trans differentiation of XtiSCs into myeloid cells.

Identifying Satellite Cell-opathies: neuromuscular disorders caused by muscle stem cell dysfunction

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Muscle health relies on muscle stem cells that enable postnatal muscle growth, maintenance and repair. However, satellite cell function is gradually compromised in many muscular dystrophies and congenital myopathies.
We have recently advanced the concept of Satellite cell-opathies, where the genetic mutation underlying pathogenesis also affects satellite cell function. Primary Satellite Cell-opathies we define as where myopathogene mutations directly affect only satellite cell function: generally characterised by congenital onset, hypotonia, involvement of respiratory, trunk and facial muscles, but normal serum CK levels. Archetypes include mutations in *PAX7* causing Progressive Congenital Myopathy with Scoliosis and *MYMK* in Carey-Fineman-Ziter Syndrome. Secondary Satellite Cell-opathies have pathogenic mutations that directly affect both satellite cells and muscle fibres and are generally more heterogeneous in terms of onset and muscle groups affected. Examples are *LMNA* mutations underlying laminopathies and alterations at the D4Z4 macrosatellite in FSHD. Non-satellite cell-opathy neuromuscular disorders are caused by mutations in myopathogenes that do not directly affect satellite cells. A key issue is whether a pathogenic mutation in a myopathogene directly affects satellite cell function. We assess whether genes associated with hereditary neuromuscular conditions are differentially expressed during adult murine satellite cell activation. Next, we determine whether such myopathogenes are controlled by PAX7, a master regulator of skeletal myogenesis. Finally, we consider satellite cell numbers/function in the associated neuromuscular disease and animal models. We use our multimodal approach to identify muscle disorders in which satellite cell dysfunction directly contributes to pathology.

Ganassi, Muntoni and Zammit (2022). ***Experimental Cell Research*** **411:**112906
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Transcriptional and proteomic regulation in age-dependent progressive decline of skeletal muscle regenerative capacities

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During aging, skeletal muscle faces a gradual loss in its functionality as a result of the failure of Muscle Stem Cells (MuSCs) activity. This includes the progressive inability of MuSCs to activate the myogenic program and the acquisition of senescence features. During MuSCs ageing, MyoD mediates the functional antagonism between replicative senescence and the activation of the myogenic program. Indeed, when DNA damage accumulates in muscle precursors, the DNA Damage Response (DDR) leads to MyoD phosphorylation in tyrosine 30 (Y30) and the activation of the Differentiation Checkpoint (DC). The DC halts the execution of the myogenic program until the lesions have been repaired, impairing muscle regeneration in aged muscle. The DDR-resistant mutant MyoD Y30F overcomes the DC and the senescence-associated barrier. It was already demonstrated that MyoD Y30F ability to bypass the DC is associated with the transit of the cells throughout the cell cycle that is a necessary event to allow MuSCs to execute the myogenic program.
Our data demonstrate that MyoD Y30F stimulates the synthesis of histones and generates important changes in the chromatin environment of senescent cells. In particular, mass spectrometry of chromatin-bound proteins revealed that MyoD Y30F expression in senescent cells leads to the enrichment of proteins involved in the regulation of nuclear bodies formation. These nuclear compartments have important roles in several processes involving RNAs: maturation, retention, alternative splicing and histones mRNAs processing. In particular, the regulation of histone locus bodies driven by MyoD Y30F in senescent cells highlights a novel mechanism to increase histone amount in senescent fibroblasts. These results reveal the molecular and cellular events at the base of the inability of senescent cells to execute the myogenic program upon MyoD expression.

It's never too early, it's never too late to counteract muscle decay with the Full-Body In-Bed Gym

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The elderly tend to devote very little time to physical activity every day, thus aggravating their mobility difficulties. In fact, muscles and mobility deteriorate with age, while muscle contractions are the only demonstrated countermeasures. It is useful to offer them a safe rehabilitation regime: Full-Body In-Bed Gym, easy to learn and do independently at home. We suggest a 10-20 minutes daily routine of voluntary physical exercises that can improve the top 200 skeletal muscles used for daily activities. Many of the exercises can be performed in bed (Full-Body In-Bed Gym), so patients can learn this light workout before leaving the hospital. The routine consists of series of repetitions of 15 bodyweight exercises to be performed one after the other without breaks during and between sets. Alternating sequences of arm and leg exercises are followed by moving body parts, while lying or sitting in the bed. These are followed by a series of standing-up off the bed (including tiptoeing). The progressive improvements can be tested with a set of push-ups on the floor. Starting with 3 to 5, the number of repetitions increases by adding 5 more each week. To maintain or even reduce the total daily training time, each movement could be accelerated weekly. The time devoted each morning to train all the major muscles of the body can be less than 10 minutes. Since there are no breaks during and between sets, the final push-ups become very demanding: at the end of the daily workout the heart rate, depth and number of spontaneous breaths and frontal sweating increase for a few minutes. We will present an educational case of an 80-year-old person. Even if performed in bed, the full-body workout is a resistance training equivalent to a short jog. An optimized routine to improve the gluteal muscles will also be presented along with the quantitative effects determined by muscle ultrasound of the Full-Body In-Bed Gym workout.

Tumor or muscle RAGE: which one sustains cancer-induced cachexia?

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We reported that RAGE (receptor for advanced glycation end-products) signaling in mice sustains all hallmarks of cachexia, a highly debilitating multifactorial syndrome affecting more than 50% of patients with advanced cancer. Indeed, tumor-bearing mice lacking RAGE (*Ager*$^{-/-}$ mice) showed reduced systemic inflammation and tumor-derived cachectic factors, delayed loss of skeletal muscle mass and strength, and dramatically increased survival. Here, we investigated the specific contribution to cachexia of RAGE expressed at tumor and muscle levels by using: i) WT and *Ager*$^{-/-}$ mice subcutaneously injected with LLC (Lewis lung carcinoma) cell clones stably transfected with full-length (fl) RAGE, RAGEΔcyto (RAGE lacking the transducing cytosolic domain) or empty vector; and, ii) a newly generated tamoxifen-inducible conditional *Ager*^{mKO} mouse model, in which the RAGE gene is selectively ablated in skeletal muscles, injected with LLC cells. We found that: i) fl-RAGE overexpression in LLC cells increased their tumorigenic and cachectic potential in WT mice; ii) LLC-injected *Ager*$^{-/-}$ mice maintained body and muscle masses and did not activate catabolism in muscles irrespective of the LLC clone injected, suggesting that RAGE overexpression in the tumor is not sufficient *per se* to induce muscle atrophy; and, iii) LLC-injected *Ager*^{mKO} mice showed increased survival, and resistance to body and muscle weight loss and muscle protein degradation compared with LLC-bearing control (*Ager*$^{flx/flx}$) mice, although to a lesser extent than LLC-*Ager*$^{-/-}$ mice. Thus, RAGE engagement at myofiber rather than tumor level is a determinant in sustaining muscle atrophy and mortality in cancer conditions. Nevertheless, total ablation of the receptor maximally protects against cancer-induced muscle wasting, indicating that systemic targeting of RAGE might represent a promising strategy to counteract the cachexia syndrome in cancer patients.

3D bioengineered skeletal muscle tissue as a tool for testing therapeutics for cancer-associated cachexia

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Cancer-associated cachexia is a complex metabolic syndrome characterized by weight loss, atrophy, and a consequent decline in muscle force-generating capacity. Currently no therapeutic approaches are available to completely reverse the cachectic phenotype, thus more research is needed to better define causes and mechanisms underlying this pathological process. Interleukin-6 (IL-6) has been extensively described as a key factor in skeletal muscle physiopathology, exerting opposite roles through different signalling pathways. We evaluated the effectiveness of selective inhibition of IL-6 transignaling in counteracting the cachectic phenotype using a three-dimensional ex-vivo engineered muscle tissue (X-MET). Firstly, we demonstrated how our 3D model recapitulates fundamental hallmarks of cancer-associated cachexia upon treatment with medium derived from C26 adenocarcinoma cells, a cellular model frequently used *to establish peripheral tumors in mice* for the study of cancer cachexia. Selective inhibition of IL-6 transignaling prevented the direct action of IL-6 cytokine in inducing cachexia, reducing the activation of proteolytic pathways, and preserving muscle homeostasis. Remarkably, X-MET model has proven to be a reliable drug-screening tool to identify novel therapeutic approaches and to test them in preclinical studies, significantly reducing the use of animal models.

Vitamin D binding protein induces skeletal muscle atrophy and contributes to cancer-associated muscle wasting

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The maintenance of skeletal muscle tissue is of pivotal importance in health and disease, as its loss is often associated with chronic or progressive pathologies, generally worsening the prognosis. Increased levels of vitamin D binding protein (VDBP) in biological samples were reported to co-occur with diseases susceptible to muscle wasting, including several tumors. We, therefore, hypothesized that VDBP may participate in muscle wasting and investigated its direct effects on skeletal muscle homeostasis. Here we demonstrate that VDBP induces atrophy independently of vitamin D and we identified the perturbation of the intracellular actin dynamic and subsequent mitochondrial dysfunction as the main molecular mechanisms at the basis of VDBP-induced atrophy. Indeed, in accordance with its physiological role in the G-actin scavenging system, we found that in C2C12 myotubes, VDBP sequesters the intracellular G-actin, causing the alteration of actin cycling between the G- and F- states, which is fundamental for many cellular processes involved in muscle homeostasis, including the control of mitochondrial dynamic.

Furthermore, the ectopic introduction of VDBP in mice lacking the protein (*Gc*-knockout mice; *Gc*-KO) through injection of the protein or adeno-associated virus (AAV)-induced gene expression induced muscle atrophy, the dismantling of neuromuscular junctions, and decreased strength. Finally, we present proof-of-concept evidence that VDBP contributes to cancer-associated muscle wasting. Altogether, these findings provide novel insights into the biological function of VDBP as a pro-atrophic hormone and have potential implications for the treatment of muscle wasting in humans.

The p97-Nploc4 ATPase complex plays a role in muscle atrophy during cancer and amyotrophic lateral sclerosis

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The p97 complex participates in the degradation of muscle proteins during atrophy upon fasting or denervation interacting with different protein adaptors. We investigated if it was involved in muscle wasting in cancer or amyotrophic lateral sclerosis (ALS).
As cancer cachexia models, we used mice bearing colon adenocarcinoma C26, human renal carcinoma RXF393, or Lewis lung carcinoma, with breast cancer 4T1-injected mice as controls. As ALS models, we employed 129/SvHsd mice carrying the mutation G93A in human SOD1. The expression of p97 and its adaptors was analysed in muscles by qPCR and western blot. We electroporated plasmids into muscles or treated mice with disulfiram (DSF) to test the effects of inhibiting p97 and nuclear protein localization protein 4 (Nploc4), one of its adaptors, on atrophy.
The mRNA levels of p97 were induced in tibialis anterior (TA) of all the cachectic models but not in the non-cachectic 4T1-mice. Similarly, p97 was high both in mRNA and protein in TA from 17-week-old SOD1G93A mice. Electroporation of a shRNA for murine p97 in TA reduced the fibre atrophy caused by C26 or ALS. In a microarray for p97 adaptors, we found Derl1, Herpud1, Nploc4, Rnf31, and Hsp90ab1 induced in cachectic TA from C26-mice. By qPCR, we validated their inductions in TA of cachectic and ALS models and selected Nploc4 as the one also induced at the protein level. Electroporation of a CRISPR/Cas9 vector against Nploc4 in TA reduced the fibre atrophy caused by C26 or ALS. Because DSF uncouples p97 from Nploc4, we treated atrophying myotubes with DSF, and found accumulated mono and polyubiquitinated proteins and reduced degradation of long-lived proteins, including actin. DSF halves Nploc4 in the soluble muscle fraction and given to C26-mice limited the muscle weight loss, with no effect on tumour.
Overall, the p97-Nploc4 complex appears to have a crucial role in muscle atrophy during cancer and ALS and disrupting this complex might serve as a novel drug strategy.

NAD$^+$ depletion as a disease mechanism and therapy target for cancer cachexia

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Cachexia, a syndrome with involuntary muscle and body weight loss, is a detrimental comorbidity in cancer patients. Although cancer cachexia (CC) creates resistance to anti-cancer treatments and is responsible for 20-30% of cancer deaths (1), we still fail to identify and treat cachectic patients. CC manifests with muscle mitochondrial and energy metabolism alterations that promote tissue wasting. We recently discovered the loss of redox cofactor, nicotinamide adenine dinucleotide (NAD$^+$), to contribute to the cachectic muscle mitochondrial dysfunction (2). This study aimed to perform a deeper investigation of NAD$^+$ metabolism disturbances in CC (3).
The examination of NAD$^+$ metabolism in different CC mouse models confirmed that NAD$^+$ depletion and downregulation of the NAD$^+$ biosynthetic gene *Nrk2* are common features of CC. Furthermore, NAD$^+$ repletion therapy with vitamin B3 niacin improved NAD$^+$ and mitochondrial metabolism and ameliorated cachexia symptoms, such as muscle wasting, and typical biochemical alterations in CC. Interestingly, cachectic colorectal and pancreatic cancer patients showed muscle *NRK2* downregulation in comparison to healthy controls. Low *NRK2* gene expression correlated with metabolomic abnormalities in the muscles of these cancer patients. To conclude, our findings highlight the role of NAD$^+$ metabolism aberrations in CC pathophysiology and encourage to investigate NAD$^+$ repletion therapies for cachectic cancer patients.

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Deletion of the muscle-specific internal promoter of the *Ank1* gene results in a pre-diabetic phenotype

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A number of genetic studies identified an association between multiple single nucleotide polymorphisms (SNPs) in the ANK1 gene and type 2 diabetes (T2D). However, the causative role of these SNPs has never been identified. We identified a T2D-associated SNP (rs508419) in the 3' region of the ANK1 gene. This SNP is located in the internal promoter (P2) of the ANK1 gene, and the C/C variant of rs508419 was found to increase the expression of sAnk1.5, a striated muscle-specific ankyrin1 isoform, and miR-486, a striated muscle-enriched microRNA. However, skeletal muscle overexpression of sAnk1.5 and miR-486 in transgenic mice did not predispose to type 2 diabetes.
On the other hand, parallel bioinformatic analysis also noticed that the ANK1 P2 promoter is located in the middle of a muscle stretch-/super-enhancer region. Stretch-/super-enhancers are chromatin domains characterized by a high content of gene regulatory elements capable of coordinating the transcription of different genes over large genomic distances. To evaluate whether this stretch-/super-enhancer may have a potential role in T2D susceptibility, KO mice carrying a deletion of the P2 *Ank1* promoter were characterized.
The *Ank1* P2 KO mice show increased fasting blood glucose and impaired glucose tolerance starting after 4 months of age. Transcriptomic analyses of EDL and Soleus muscles and single myofibers revealed that pathways involved in glucose homeostasis and lipid metabolism are significantly altered in *Ank1* P2 KO mice. Altogether, these data support a causative involvement of the ANK1 locus in the development of T2D. Further experiments are in progress to validate and complete this analysis, and to better classify the metabolic changes underlying the pre-diabetic phenotype observed in these mice.

MTM1 overexpression efficiently rescues *BIN1*-related centronuclear myopathy

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Centronuclear myopathies (CNM) are severe muscle disorders characterized by muscle weakness, hypotonia, and abnormal organelle positioning in myofibers. Two major forms of CNM are caused by mutations in *MTM1* and *BIN1*. As both corresponding proteins conjointly act in membrane dynamics, disruption of their interplay in muscle cells is a likely cause of CNM. This is supported by our previous experiments demonstrating an amelioration of the murine *Mtm1*-CNM phenotype through BIN1 overexpression. However, it remains to be determined if MTM1 overexpression can compensate for the lack of BIN1.
Here we assessed the therapeutic potential of MTM1 overexpression in a *Bin1* KO mouse model faithfully reproducing autosomal recessive CNM. A systemic injection of AAV-MTM1 into *Bin1* KO neonates restored normal motor function, *in situ* maximal muscle force, organelle positioning, and partially rescued myofiber size 10 weeks post-injection thus efficiently preventing disease progression. Similarly, intramuscular injection of AAV-MTM1 at a late disease stage improved myofiber size and corrected organelle positioning but had limited effect on the muscle force, suggesting that MTM1 overexpression is more effective when achieved at an early disease stage. To determine the molecular effect of MTM1 overexpression in the *Bin1* KO we used a catalytically inactive mutant to treat *Bin1* KO neonates. We found once again an improvement of the muscle force, but no recovery of the myofibers size. These results suggest that MTM1's phosphatase activity specifically affects muscle atrophy in *Bin1* KO animals, while other phenotypes likely rely on protein-protein interactions.
Altogether, this study demonstrates that MTM1 overexpression during postnatal muscle maturation efficiently antagonizes disease progression in the *Bin1* KO model, and suggests that both the phosphatase activity and non-enzymatic functions of MTM1 significantly contribute to this therapeutic effect.

ORAI1 inhibition as an efficient preclinical therapy for tubular aggregate myopathy (TAM) and Stormorken syndrome (STRMK)

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Tubular aggregate myopathy (TAM) and Stormorken syndrome (STRMK) are clinically overlapping disorders characterized by childhood-onset muscle weakness and a variable occurrence of multi-systemic signs including short stature, thrombocytopenia, and hyposplenism. TAM/STRMK is caused by gain-of-function mutations in the Ca2+ sensor STIM1 or the Ca2+ channel ORAI1, both regulating Ca2+ homeostasis through the ubiquitous SOCE (store-operated Ca2+ entry) mechanism. Functional experiments in cells have demonstrated that the TAM/STRMK mutations induce SOCE overactivation, resulting in excessive influx of extracellular Ca2+. There is currently no treatment for TAM/STRMK, but SOCE is amenable to manipulation. Here we crossed Stim1R304W/+ mice harboring the most common TAM/STRMK mutation with Orai1R93W/+ mice carrying an ORAI1 mutation partially obstructing Ca2+ influx. Compared with Stim1R304W/+ littermates, Stim1R304W/+Orai1R93W/+ offspring showed a normalization of bone architecture, spleen histology, and muscle morphology, an increase of thrombocytes, and improved muscle contraction and relaxation kinetics. Accordingly, comparative RNA-sequencing detected more than 1200 dysregulated genes in Stim1R304W/+ mice and revealed a major restoration of gene expression in Stim1R304W/+Orai1R93W/+ mice. Altogether, we provide physiological, morphological, functional, and molecular data highlighting the therapeutic potential of ORAI1 inhibition to rescue the multi-systemic TAM/STRMK signs, and we identified myostatin as a suitable biomarker for TAM/STRMK in human and mouse.

Targeting rhabdomyosarcoma cancer progression by MICAL2 regulation

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MICAL2, a member of the Microtubule Associated Monooxygenase, Calponin And LIM domain containing protein family, has the capacity to regulate F-actin depolymerization and cytoskeletal dynamics via oxidation-reduction reactions, modulating cell motility and division (1). Our recent findings emphasize the significance of MICAL2 in regulating muscle filament dynamics, highlighting its essential role in muscle tissue regeneration (2). Due to the implication of MICAL2 in myogenic lineage commitment and its dysregulation in cancer cells, including rhabdomyosarcoma (RMS), the most common soft tissue sarcoma afflicting children, we sought to investigate the role of MICAL2 in RMS malignancy. We demonstrated that reduction of migratory and invasive properties of RMS cells are observed upon MICAL2 knockdown (KD), *in vitro*. To determine if cell migration and invasion capacities were affected, different markers of epithelial-to-mesenchymal transition (EMT) were examined, and we demonstrated their decreased expressions as a result of MICAL2 silencing. Substantiated by more evidence of reduced migratory potential of MICAL2 KD RMS cells with transwell migration assays, we generated inducible TET ON lentiviral constructs to assess the impact of stable MICAL2 KD RMS cells *in vivo*. Our results indicate that MICAL2 silencing engenders beneficial outcomes as evidenced by reduced primary tumor size, absence of metastatic sites and improved muscle functional performance in tumour- bearing mice. Therefore, this project marks the initiation of comprehensive inquiries into the role of MICAL2 in RMS, shedding light on its potential as a promising molecular target for the treatment of RMS.

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Laser-assisted bio-printing and structuring for muscle modeling

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Bio-printing techniques have begun to develop in the 2000s and have since been greatly improved and perfected. Based on interdisciplinary approaches they aim at the creation and patterning of organized 2D and 3D cell scaffolds. In that scope, it has been more than a decade since Laser-Induced Forward Transfer (LIFT) is studied in lab scale for its ability to transfer biomaterials, and more specifically living cells, on a substrate. This technique uses a short laser pulse to transfer tiny amounts of material from a thin donor film to the desired location with high precision, resolution and reproducibility. In this context, thanks to an interdisciplinary collaboration between the LP3 lab [LIFT process] and the MMG [primary and IPS derived neuro-muscular progenitors] we are able to print bio-inks for the creation of biological models.
Here, we will present the LIFT process and its optimisation allowing us to achieve a controlled, reliable, precise printing of muscle progenitor cells, ensuring a high post-printing cell survival rate and proliferation. Making use of the resolution and reproducibility of the LIFT process, we will present the potential of co-culture printing that further improves the fidelity of the bio-models created and open the door for neuro-muscular disease modelling with precise printing of different cell type on a single substrate.
In parallel, laser structuration by direct laser ablation of hydrogels was developed and combined to the printing process. Muscle progenitors are now printed precisely in pre-made micro-structured channels that improves their differentiation rate while showing an alignment of the resulting myotubes according to the channels orientation. Besides being able to print living cells, we can reproducibly produce muscle fibres of about 200µm width and several millimetres long from affected and non-affected human progenitors. Once validated, these models will be a useful tool for personalised medicine applications.

Investigating the molecular, cellular and mechanistic differences of muscle fibrosis in human myopathies to determine to what extent fibrosis is a unique feature or results from different contributing factors.

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Fibrosis is described in many organs as an excessive accumulation of extracellular matrix (ECM) proteins that replace tissue and alter its function. In skeletal muscle, fibrosis is a pathological feature common to many clinically different muscle myopathies, such as Duchenne muscular dystrophy (DMD), oculopharyngeal muscular dystrophy (OPMD) or inclusion body myositis (IBM). Excessive accumulation of ECM alters the muscular function and the potential innovative therapeutic strategies. Several cellular actors are known to be implicated in the establishment and the maintenance of the fibrosis: macrophages, fibroadipogenic progenitors (FAPs) as well as satellite cells. The ECM, apart from its essential role as an architectural scaffold, has also a pivotal role in this process influencing muscle-resident cells through biochemical and biomechanical signals.
Combining mass cytometry, transcriptome profiling, secretome analysis, in vitro co-culture experiments and in vivo transplantation in immunodeficient mice, we investigated the role and nature of FAPs isolated from human fibrotic muscles and compared them to FAPs from healthy muscle. Our results show that human FAPs from fibrotic muscles display a strikingly different profile than FAPs from non fibrotic muscles; fibrotic FAPs show an exacerbated proliferation and ECM secretion, and when activated, have a detrimental effect on muscle differentiation. In pharyngeal muscles, we also demonstrated the role of endothelin, a new targetable regulator involved in this process. Then using mass spectrometry, we characterized the ECM composition of DMD, OPMD and IBM human skeletal muscle biopsies. We identified shared ECM protein components as well as many specific ones for each pathology, highlighting differences in the nature of ECM components.
This work on human muscle biopsies will lead the way to the identification of key components and targetable pathways for anti-fibrosis therapies in humans.

CTRP1, a myogenic protein in skeletal muscle differentiation and mitochondrial function

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The levels of C1q/TNF-α-Related Protein 1 (CTRP1) within skeletal muscle exhibit marked reductions in patients diagnosed with polymyositis, dermatomyositis, and Duchenne muscular dystrophy, in comparison to those in healthy individuals. Notably, CTRP1 levels demonstrated a progressive decline in tandem with the severity of those muscular diseases. However, the precise mechanistic contributions of CTRP1 to skeletal muscle functionality remain largely unexplored. This study delves into the novel role of CTRP1 in muscular activities closely associated with mitochondrial function. CTRP1 showed its significant expression within skeletal muscle tissues, displaying a positive correlation with myogenic differentiation. CTRP1 depletion in C2C12 cells by CRISPR-Cas9 system resulted in delays in myogenic differentiation, collectively implying a pivotal role for CTRP1 in this process. Notably, mice with skeletal muscle-specific CTRP1 deficiency showed diminished muscle dimensions, reduced weight, and compromised grip strength. Intriguingly, the absence of CTRP1 prompted disturbances in muscular mitochondrial dynamics and impaired COX IV activity, intricately linked with the process of myogenesis. This effect was partially mitigated by the introduction of a *Ctrp1*-overexpressing adenovirus in primary myoblasts, which effectively restored CTRP1 levels, leading to improved mitochondrial function, enhanced COX IV activity, and facilitated myogenesis. Furthermore, in vivo experiments demonstrated that CTRP1 deficiency resulted in delayed muscle regeneration subsequent to cardiotoxin-induced injury. Collectively, these findings substantiate the multifaceted role of CTRP1 in governing critical aspects of mitochondrial function, muscular differentiation, and regenerative processes. The implications of these discoveries extend to the realm of muscular pathologies, positioning CTRP1 as a promising therapeutic target for conditions characterized by compromised muscular integrity.

SRT2104, a new SIRT1 activator, is an effective metabolic enhancer that promotes muscle recovery in DMD

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Duchenne Muscular Dystrophy (DMD) is a degenerative disorder caused by mutations in the DMD gene encoding dystrophin protein. Despite remarkable progress has been made in genetic approaches to restore dystrophin or its function, targeting secondary pathological mechanisms remains an important issue. Sirtuin 1 (SIRT1) is a NAD+-dependent class III histone deacetylase belonging to the Sirtuin family that controls several key cellular processes. Different attempts have been made to increase SIRT1 expression or activation in mdx mice, and to date, the most promising one seems to be resveratrol. However, more potent and selective activators exist, and among these SRT2104 is the most promising and advanced in clinical studies. We performed, a series of molecular dynamics simulations on SIRT1 available structures, proving that a conformational selection mechanism was responsible for the activity of SRT2104, i.e., the open inactive conformation of SIRT1 explored a more compact intermediate state that is stabilized by the drug, then converted into its active form.
Even more potent and specific than resveratrol, it has never been tested in DMD therefore we challenged SRT2104 effects in mdx mice.
We orally administered SRT2104 for 12 weeks in 8-week-old mdx mice obtaining promising results. SRT2104 promoted muscle OxPhos capacity and improved muscle performances and phenotype. The proteomic profile of SRT2104 treated muscle revealed the specific enrichment of fatty acid oxidation and mechanotransduction signals, both contributing to muscle recovery.
In conclusion, SRT2104 can be considered a good metabolic enhancer for dystrophic muscles and an interesting treatment for DMD.

Unveiling the HDAC4 functions in mediating the cross-talk between skeletal muscle fibers and fibro-adipogenic progenitors in Duchenne Muscular Dystrophy

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Histone deacetylase 4 (HDAC4) is a class IIa HDAC member that exerts protective functions in the skeletal muscles of mdx mice, a model of Duchenne Muscular Dystrophy (DMD). The deletion of HDAC4 in the skeletal muscle of mdx mice increases muscle damage and compromises regeneration, overall affecting muscle function of mdx;KO mice. In addition, the deletion of HDAC4 leads to intramuscular fibrous and adipose tissues in an early phase of the disease. Fibro-adipogenic progenitors (FAPs) are the main source of fibrotic and adipose infiltration in skeletal muscle and influence muscle stem cells (MuSCs) differentiation; thus, FAPs are directly involved in the progression of DMD. Strikingly, FAPs isolated from mdx;KO mice show higher adipogenic potential, if compared to FAPs isolated from mdx littermates, despite expressing similar levels of HDAC4. Moreover, FAPs isolated from mdx;KO mice negatively affect mdx MuSC differentiation and viability. These data suggest that HDAC4 from skeletal muscle is mediating a paracrine signal that influences surrounding cells. To prove this hypothesis, we isolated single myofibers and tested the conditioned media (CM) on mdx FAPs. Strikingly, CM from mdx;KO single myofibers increases mdx FAP adipogenic potential, if compared to CM from mdx myofibers. Further, extracellular vesicles (EVs) isolated from mdx;KO myofiber CM are sufficient to increase the adipogenic potential of mdx FAPs, confirming that HDAC4 mediates the release of soluble factors from skeletal muscle via EVs. Current investigations are aimed at defining the soluble factors responsible for the above phenotype. Defining the secretome modulated by HDAC4 in DMD skeletal muscle will shed light on the mechanisms underpinning this disease and provide the experimental basis for new therapeutic approaches to counteract the pathological features of DMD.

In vitro modeling of the human neuromuscular junction in a microfluidic device for the study of facioscapulohumeral dystopy

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NMJ is a chemical synapse that forms between the axon of a spinal motor neuron and a skeletal muscle fibre. While the complexity of this highly specialized structure makes in vitro modelling a very challenging task, NMJ co-culture systems have been developed over the past two decades to address the problems encountered in animal models. A further improvement has been provided by microfluidic chip which, unlike the classical co-culture, allows the spatial and temporal control of different microenvironments allowing to independently manipulate neuronal and muscle cells. This allows to study the mechanisms involved in the formation and maintenance of NMJ. Therefore, by exploiting an organ on-a-chip approach, our aim is to obtain a reliable and predictive human NMJ in vitro model in both physiological and pathological conditions in order to unravel the interplay between muscle and motor neuron leading to synapse damage and to neuromuscular diseases. In this work, we mainly focus on FSHD as it is identified as one of the most common forms of muscular dystrophy, affecting 1/8000 people. Despite its frequency, the mechanisms that leads to the development and progression of the disease are still not fully understood as the involvement of NMJ given the neural impairment. For this purpose, motor neurons deriving from hiPSCs and myogenic progenitors from healthy and FSHD patient could be seeded in two separate chambers of a microfluidic device. The two cell populations are separated by microchannels that allow axonal growth but not cell bodies migration, allowing the compartmentalization of the two populations without interrupting cell-cell communication. Hence employing this approach, we can study the cellular and molecular mechanisms underlying the FSHD pathology and the way in which the muscular and motor neuron components influence each other. The configuration is versatile enough to accommodate patient-specific cells and perform functional and molecular analysis.

Assessment of cardiac structure and function in a Dys$^{-/-}$;Utr$^{-/-}$ mouse model of DMD treated with long term dystrophin replacement therapies

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Duchenne muscular dystrophy (DMD) is a severe and progressive inherited muscular dystrophy, affecting children with an incidence of 1:3,500 - 1:5,000 live male births. It is one of the most severe pediatric degenerative myopathies. DMD is caused by X-linked mutations in the *DMD* gene leading to the loss of dystrophin, a structural protein located at thesarcolemma. Patients display progressive muscle weakness starting at a young age, lose ambulation around the age of 10–12 years old, and die from cardiorespiratory failure during the second or third decade of life. With improved disease management, cardiomyopathy has emerged as a leading cause of death in patients. Extensive research over the last three decades has shown promising results, notably the capacity of micro-dystrophin, expressed using AAV-based gene therapy, to rescue heart function. We here studied the long-term effect of dystrophin replacement strategies to assess the structural and functional benefits of replacement therapy as well as cardiac consequences in a severe model of DMD, the dKO (*Dys$^{-/-}$;Utr$^{-/-}$*) mice. The AAV-micro-dystrophin treatment restored normal weight gain and remarkably improved survival of DMD mice. We next assessed cardiac structure and function. While the treatment led to a significant improvement in cardiac function after 1 year post treatment, we were able to reveal an increased septum thickness, which could be the result of tissue remodeling. We further asked what cellular population could participate in this cardiac remodeling and identified the presence of leukocytes in AAV-micro-dystrophin treated dKO animals. Our data warrant consideration that micro-dystrophin replacement therapy in the dKO mouse model may be associated with cardiac muscle inflammation despite improved cardiac function and survival.

The voice of patients affected by Duchenne and Becker muscular dystrophy

Ilaria Zito
Parent Project aps

Parent Project aps is an association of patients and parents of children affected by Duchenne and Becker Muscular Dystrophy (DMD and BMD). As of today there is no effective cure for this debilitating disorder.
With the main goals of improving the quality of life, prolonging life expectancy and, eventually, finding a cure to end Duchenne, we accompany the patients and their families in all the steps of the pathology, from the diagnosis through its evolution, in a multidisciplinary approach. This becomes possible thanks to a structured organigram with different competence areas working together towards the objective.
The Scientific Office is one of the pillars of the association, operating as a connecting link between the scientific world and the patients world. Supporting the scientific research, disseminating the scientific information, curating the Italian DMD/BMD patient registry, facilitating access to clinical management and to clinical trials for patients, promoting the adherence to the standards of care are among the main activities of the scientific office. To work effectively in this direction, we engage with all key stakeholders, including patients, caregivers, scientists, clinicians, industry partners, regulators.
Parent Project aps runs an educational program that includes an annual International Conference on Duchenne and Becker muscular dystrophy, which is a unique opportunity to raise awareness, to share experiences and to learn about the latest progresses in the fight to end these diseases. During the conference all the aforementioned stakeholders gather to connect, to share information and to discuss and debate the latest news and opportunities in DMD and BMD research.
Our next International Conference on Duchenne and Becker muscular dystrophy is scheduled from the 16th to the 18st of February 2024.
For more information, please visit our website: http://parentproject.it

Poster Abstracts

P.1

3D human cardiac models of Duchenne cardiomyopathy generation and NOX4 inhibition

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Duchenne muscular dystrophy (DMD) is the most prominent muscle-wasting condition, and it is driven by mutations in the X-linked dystrophin gene that result in DYSTROPHIN deficiency. Currently, cardiomyopathy is the leading cause of mortality in late-stage DMD patients. Mechanistically, the pathogenesis of DMD-cardiomyopathy is attributed to cardiomyocyte degeneration caused by calcium overload, mitochondrial dysfunction, and excessive production of reactive oxygen species due to the dysregulation of NADPH oxidase (NOX) family enzymes. Notably, NOX4 is predominantly expressed in cardiomyocytes where it is constitutively active at low levels, inducing cardioprotective effects under chronic stress. It has been demonstrated that high levels of NOX4 in dystrophic cardiomyocytes have severe detrimental effects thus contributing to the development of cardiovascular diseases[1]. We recently developed patient-derived 3D cardiac organoids (DMD-COs) based on induced pluripotent stem cells and the relative CRISPR/Cas9 corrected control (DMD-Iso-COs), able to reproduce cardiomyopathic features and the disease progression phenotypes in long-term cultures[2]. Over time DMD-COs showed higher cell death rate, endoplasmic reticulum stress, and the formation of fibrotic and adipose tissues. Upon treatment with NOX4 inhibitors developed from the crystal structure of the NOX4 enzyme[3], flow cytometry analysis showed higher cell viability as well as an improved calcium handling of the dystrophic cardiomyocytes. In conclusion, we generated patient-derived cardiac organoid models that displayed DMD-related cardiomyopathy features and disease progression. Notably, the inhibition of NOX4 appeared to significantly enhance dystrophic cardiomyocyte survival and contractility.

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Telocytes morphology at the myotendinous junction after muscle injury

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The myotendinous junction (MTJ) is a microregion between skeletal muscle and tendon tissue composed by prolongations of the sarcomeres denominated sarcoplasmic evaginations and by extracellular matrix extensions intersected to the basal lamina and formed by collagen fibers, denominated sarcoplasmic invaginations. The MTJ main function is to promote the force transmission between skeletal muscle and tendon; it is one of the focal areas of predisposition to injury. Telocytes are interstitial cells with distinct genes compared to the fibroblasts.The telocytes are characterized by prolongations denominated telopodes and pods, that are terminal structures that promote the interconnection and network between telocytes with neighboring cells. A telocytes niche was recently discovered at the MTJ acting in the support and maintenance of this region after an experimental model of ladder-based training exercise.The aim of the present study was to analyze the adaptations of telocytes after acute muscle injury. For the muscle injury protocol 8 *Wistar* rats were used, their gastrocnemius muscles were dissected for immunofluorescence and electron microscopy protocols. For immunostaining CD34 antibody was used to positive stain telocytes and phalloidin to the skeletal muscle fibers. The present study demonstrates the telocytes accrue at the myotendinous interface against muscle commitment and presents the interaction of this cellular element to stabilize this area of tissue intersection in the face of morphological and indirect functional impairment. We concluded that the telocytes can be a “tool cell” for maintenance of the MTJ in the face of direct and indirect stimuli that affects this interface.

Intrinsic Skeletal muscle contractility and Nrf2 in humans

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The process of skeletal muscle contraction relies on precise molecular orchestration. Recent studies have clarified the multifaceted role of the nuclear factor erythroid 2-related factor 2 (Nrf2), a protein known for its antioxidant properties. Evidence suggests that the Nrf2 signaling pathway plays a crucial role in maintaining redox homeostasis, which is essential for preserving skeletal muscle structure and function. Indeed, the upregulation of Nrf2 in mice improved muscle contractility and overall function. However, research in humans is limited, especially the role of Nrf2 on intrinsic muscle contractility is not clear. Therefore, we aimed to understand whether Nrf2 levels influence human intrinsic muscle contractility.
Single potentiated twitches were electrically delivered on the resting dominant leg' femoral nerve of 26 young healthy subjects (13 M + 13 F), to assess intrinsic muscle contractile properties. A skeletal muscle biopsy was donated and Nrf2 levels were assessed. The muscle cross-section area (CSA) was evaluated using the panoramic ultrasound technique. An International Physical Activity Questionnaire (IPAQ) questionnaire was used to assess physical activity status.
We observed a strong correlation between Nrf2 and the maximal evoked muscle contraction ($r=0.60$, $p= 0.004$). Moreover, the rate of muscle contraction ($r=0.63$, $p=0.002$) and relaxation ($r=-0.59$, $p=0.005$) were also strongly correlated with Nrf2. No correlation was seen between Nrf2 and the muscle CSA.
We showed that Nrf2 levels are associated with the electrically induced force-generating capacity and relaxation rates in humans. This association was not driven by the muscle CSA, confirming research on mice. Usually, higher Nrf2 levels are associated with higher physical activity: in this study, our participants had similar IPAQ, meaning that the Nrf2 is likely associated with intrinsic skeletal muscle force, rather than the level of physical activity.

P.5

A pilot application of integrated procedures to accelerate, deepen, and guide genetic investigation on the myokinome

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The skeletal muscle secretome is ever-expanding and current bioinformatic tools allow to fasten new discoveries within the field. However, these methods still need epistemological criticism, as well as standardized procedures. The current study dealt with the combined expert-assisted and data science-assisted approaches to tackle this issue. Our idea applied to the “myokinome” was to present a combined expert-driven and data-driven approach for enlarging the use of bioinformatic tools by non-bioinformaticians in medicine and physiology. Data science tools were used to fix the literature research, suggest investigation targets, and predict possible scenarios through a novel workflow. Three recognized groups with expertise on myokines were invited to provide independently a list of the most important myokines, that were used as input for GeneRecommender, GeneMANIA, HumanNet, and STRING tools. Networks were built on STRING and GeneMANIA. The outcomes included the top 5 recommendations from each tool. Then, from these results the experts led a discussion that was finally integrated with a data science-assisted approach to provide further perspectives. Among the results, 11 molecules had already been described as bona-fide myokines in literature, and 11 molecules were putative myokines. Most of the myokines and the putative myokines recommended were described as present in the cargo of extracellular vesicles. Encompassing algorithms focused on both protein interaction and gene represent a comprehensive approach to tackle the complex world of myokines. Further studies would compare in silico, in vitro, ex-vivo and in vivo analyses to optimize the algorithms. Data science-assisted methods for reviewing existent evidence, recommending targets of interest, and predicting original scenarios, coupled with experts' ideas is a worth exploring method for fastening and improving molecular studies, enough that novel groundbreaking insights are likely to emerge from this paradigm.

Sphingosine-1-phosphate/sphingosine-1-phosphate receptor (S1P/S1PR) axis modulates irisin signaling in skeletal muscle cells

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Irisin, a hormone-like myokine produced in abundance by skeletal muscle (SkM), is involved in many signaling pathways related to metabolic processes. Despite much evidence on the regulators of irisin, the contribution of bioactive lipids, such as sphingolipids, to the modulation of this myokine in SkM is missing. We demonstrate the existence of distinct intracellular pools of sphingosine 1-phosphate (S1P) able to affect the expression of the irisin precursor FNDC and the role of S1P/S1P receptor axis in irisin release as well as in its autocrine/paracrine action on myoblast proliferation and myogenic differentiation. Altogether, these findings provide the evidence for a functional crosstalk between the S1P/S1PR axis and irisin signaling, which may open new windows for potential therapeutic treatment of SkM dysfunctions.

P.7

Targeting mitochondrial calcium and metabolism by RNA-based therapy in sarcopenia

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Mitochondrial Ca^{2+} ($mitCa^{2+}$) homeostasis links oxidative metabolism to muscle function. Ca^{2+} enters into the mitochondrial matrix through a highly selective channel (Mitochondrial Calcium Uniporter, MCU) located in the inner mitochondrial membrane. The MCU complex is composed of pore-forming subunits (MCU, MCUb and EMRE) and of regulatory subunits (MICU1, MICU2, and MICU3). Within the mitochondria, Ca^{2+} activates key dehydrogenases of the TCA cycle, i.e. pyruvate dehydrogenase (PDH), isocitrate dehydrogenase and oxoglutarate dehydrogenase. PDH activation occurs upon dephosphorylation by pyruvate dehydrogenase phosphatase. On the contrary, pyruvate dehydrogenase kinase (PDK) phosphorylates and inactivates PDH. Sarcopenia is an age-related loss of skeletal muscle mass and strength. Mitochondria are negatively affected by ageing and the most prominent age-associated mitochondrial dysfunction includes reduced overall volume density, oxidative capacity and ATP production. In skeletal muscle, $mitCa^{2+}$ uptake positively regulates muscle trophism and metabolism by impinging on hypertrophic pathways and on PDH activity respectively. Thus, we decided to target $mitCa^{2+}$ uptake and PDH activity by using the novel RNA-based therapy to prevent muscle loss in sarcopenia. While the effect of MCU overexpression on skeletal muscle trophism is already established, the effect of the silencing of MICU2 and of PDK isoforms mainly expressed in skeletal muscle (PDK1, PDK2, PDK4) has to be analysed. For this purpose, we will test different short hairpin RNA (shRNA) for PDK1, PDK2, PDK4 and MICU2 and we will select the most effective ones for the ability to increase myofiber size. In parallel we will test different delivery methods for shRNA in skeletal muscle.

Effect of high-intensity exercise training combined with dark chocolate rich in polyphenols and vitamin-E on muscle mass and strength in elderly people with dementia. Preliminary results from the Choko-Age study

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Introduction. Demented people (Dem) are characterized by poor muscle mass, and poor muscle strength seriously affecting independency and quality of life. No studies have explored neuromuscular function in this population, addressing all the causes of this conditions to hormonal dysregulation and daily habits. Also, no studies examined the effect of exercise combined with nutritional supplementation on muscle strength and mass in Dem. Thus, first aim of this study is to compare neuromuscular function in demented and non demented individuals. Second aim is to assess the effect of high intensity aerobic and strength training, combined with vitamin-E-functionalized dark chocolate on muscle mass and strength.
Methods. Dem individuals (68±5 yrs, 23±4 MMSE) and non demented elderly (Ctrl, 68±7 yrs) were tested for body composition. Maximal voluntary contraction (MVC), voluntary activation (VA), and potentiated twitch force (Qtw, pot) were determinated. Dem were randomly allocated to (EX), exercise + chocolate with or without Vitamin E (CH1 or CH2, double-blinded) group. Three sessions a week were performed for 12 weeks, consisting of 4 x 4' @90% of HRmax walking on a treadmill (HIIT) and 4 x 4 repetition @85% 1-RM at the leg press. Right after the treatment subject were evaluated again.
Results. Compared to Ctrl, Dem exhibited a reduced muscle mass (-8%, p=0.037), MVC (-19%, p=0.048), and VA (-23%, p=0.0394). Qtw,pot was similar. After treatment lower limb muscle mass seems unchanged in EX (+0.1%) and increased in CH1 (+2.7%) and CH2 (+4.2%). All groups seemed to show an increase in MVC (+6.9%, +3.5%, and +25.0%), and %VMA (+2.5%, +11.5%, and +14.5%)
Conclusions. Results show how in Dem, the central component of the muscle strength is twisted… failing the efficacy of the signal from the brain to the muscles. Concerning the treatment, despite the preliminary nature of these results, CH2 reached better improvements in muscle mass, MVC and VA compared with the other 2 groups.

Extracellular 500 µM GTP enhances myogenesis inducing specific gene and protein pathways

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Extracellular Guanosine 5′-triphosphate(GTP) at 500µM concentration in C2C12 mouse myotubes binds to specific P2Y-receptor-like sites, inducing an increase in intracellular Ca^{2+}concentrations due to its release from intracellular inositol-1,4,5-trisphosphate-sensitive stores thus causing both membrane hyperpolarisation through Ca^{2+}-activated K^{+} channels and nucleus gene transcription modulation. These events enhance C2C12 myoblasts fusion into multinucleated myotubes due to the expression of myosin heavy chain. In fact, further gene expression analysis show that extracellular 500µM GTP in differentiating C2C12 cells already after 24h, leads to an upregulation of genes involved in several pathways associated with myogenic process such as cytoskeleton structure, the respiratory chain, myogenesis, chromatin reorganization and the Jak/Stat pathway, and a downregulation of MAPK pathway. Genomic data were confirmed testing myogenic key genes through RT-PCR as Pp3ca, Gsk3b, and Pax7 (Mancinelli R.2012). A more recent proteomics analysis was performed on proliferating and 3 days differentiating C2C12 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. Notably, all dysregulated proteomic pathways related to respiratory chain and D-myo-inositol (1,4,5)-trisphosphate degradation pathway agree whit genomic results in differentiating C2C12 cells. Shortly, key proteins modulated in pathway of interest in proteomic analysis will be confirmed in cytofluorimetry. In conclusion, extracellular 500µM GTP in C2C12 cell line is able to enhance the myogenic process as demonstrated by the recent proteomic analysis thus confirming previous gene expression profile data.

Muscle injury protocol and adjacent cells at the neuromuscular junction region: a new aspect view

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The neuromuscular junction (NMJ) was composed by the axonal motor neuron, the muscle fiber sarcolemma and the terminal Schwann cells that contribute to the synapses, maturation, muscle contraction and regeneration. The telocytes (TC) are interstitial cells with prolongations denominated telopodes, that element promotes the interconnection between TCs and neighboring cells forming a network that contribute to the maintenance of the tissue accruing to the extracellular matrix. Given the importance of NMJ function and TCs at skeletal muscle tissue, the present study demonstrates the possible presence of the TCs as an adjacent cell at NMJ in a muscle injury protocol. For this, 8 adult Wistar rats were divided into 2 groups Control (C), 24h post injury (24hPI). The animals were submitted to euthanasia and the gastrocnemius muscle was dissected from the Control Group at the protocol day and from the 24hPI Group 24h post injury. The samples were cryo-fixed and sectioned for immunofluorescence (longitudinal) and light microscopy (transverse). The TCs were immunostained with CD34, the NMJ with a-bungarotoxin conjugated and the nuclei with DAPI, for light microscopy the sections were colored with hematoxylin and eosin. The TCs are found neighboring the NMJ region cells in both groups and the inflammatory process was determined by the light microscopy with a specific region of fibrosis and high cellular activity at 24hPI Group. We concluded that TCs can be found for the first time adjacent to the NMJ region and can be explored for their capacity to help with cell communication and region maintenance.

P.14

Engineered exosomes as a therapeutic tool to counteract muscle degeneration

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The muscular dystrophies (MDs) represent a group of several different inherited diseases, all involving progressive weakness and degeneration of muscle. Among MDs, sarcoglycanopathies are a family of muscle disorders due to genetic defects in one of four cell membrane glycoproteins, α-, β-, γ- or δ- sarcoglycan (SG) which are closely linked to the dystrophin-associated protein complex. Mutations in the beta-sarcoglycan gene causes limb-girdle muscular dystrophy type 2E (LGMD 2E), characterized by a childhood to adolescent onset of progressive pelvic- and shoulder-girdle muscle weakness. To better characterize the role of β-SG on muscle physiology and to find new therapeutic approaches to counteract the muscular alteration provoked by its deficiency, the availability of human pluripotent stem cells (iPSC) has proven to be useful. To this purpose, we generated iPSC-derived myotubes lacking β-SG (HC1), using CRISPR/Cas9 technology, to study the muscular genetic disease in a human context in both 2D and 3D cell models.
Recent studies documented the importance of paracrine factors in sustaining muscle homeostasis and as such, extracellular vesicles (EVs) carrying specific host factors such as microRNAs (miRNAs), are able to play a role in muscle physiological growth, development and regeneration (1). We identified miRNAs that can boost myogenic differentiation of mesodermal progenitors derived from human iPSCs (2). EVs from DROSHA knockout HEK-293T cells have been enriched with selected miRNAs or several combinations of them. These custom-engineered EVs will be tested on the HC1-derived myotubes to check the efficacy of the miRNA(s).
The generation and delivery of EVs with custom-engineered cargos alone, or in combination with other therapies, may represent novel approaches for the treatment of muscle wasting.

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P.15

The emerging role of polyamine pathway in skeletal muscle during ALS progression

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by the degeneration of upper and lower motor neurons resulting in **atrophy** and weakness of voluntary muscles. **Polyamines** (PA), such as **putrescine**, **spermidine** and **spermine**, are compounds with amino groups at both ends of a hydrocarbon that play a pivotal role in many biological functions, including cell growth, differentiation and gene regulation. Using **spatial transcriptomics (ST)**, a novel technique that could contribute to a revolution in the field of muscle ''omics'' as a fusion of recent sequencing technologies and classical histology, we demonstrated that genes encoding for key enzymes of PA synthesis pathway tend to be more expressed in the **glycolytic fibers** of healthy muscle. Furthermore, we observed **altered** expression of these genes and the **polyamine balance** during **ALS progression** in a $SOD1^{G93A}$ mouse model, suggesting a possible role of this metabolic pathway in ALS pathogenesis. In conclusion, despite the intense efforts in recent years to identify the pathogenic mechanisms of ALS, its etiology remains elusive, therefore, exploring the contribution of skeletal muscle polyamine metabolism in ALS could lead to a better understanding of the pathogenic mechanism of the disease and help define new therapeutic targets.

P.16

Characterization of ex-vivo muscle engineered tissue's (X-MET) functional remodelling

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Mechanical stimuli have a critical role for the development and maintenance of both cardiac and skeletal muscle. The application of an appropriate biomechanical environment is essential for the successful recapitulation of *in vivo* behavior in 3D muscle models. X-MET is a three-dimensional engineered muscle tissue that possesses all morphological and biomechanical properties of adult skeletal muscles. Recently, we demonstrated that mechanical stimuli applied on X-MET structure trigger a functional remodeling of the 3D skeletal muscle system toward a cardiac muscle-like structure. This was supported by molecular and functional analyses, demonstrating that remodeled X-MET expresses the capacity to form gap junctions, guaranteeing electrical integration of the myotubes into a functional syncytium and preserving heart function in a murine model of chronic myocardial ischemia (Cosentino et al., 2023). The aim of this study is to investigate the molecular signature underlying the functional remodelling of X-MET, focusing on the change in cellular and molecular conformations linking mechanical forces with biochemical signals. We investigated the cellular factors that can be involved in this process, crucial for muscle regeneration but also electrophysiology and cardiac conduction, due to the expression of connexin-43. Interestingly, we found an enrichment of genes related to phagocytosis and immune response-regulating gene expression program in stretched X-MET compared to unstretched and 2D primary culture, supporting the view that macrophages are involved in the cardiac remodelling of X-MET and their interaction with other important cell types is involved in the pathology and resolution of inflammation after MI.

P.17

Identification of MATR3 as endogenous inhibitor of DUX4 for the treatment of FSHD muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is one of the most prevalent neuromuscular diseases and no treatment is currently available. The disease is caused by gain of expression of the double homeobox 4 (*DUX4*) gene, encoding for a transcription factor normally silent in most adult somatic tissues. In FSHD, DUX4 aberrant activation triggers a pro-apoptotic transcriptional program resulting in muscle wasting. DUX4 has been recently implicated also in the pathogenesis of solid tumors, leukemia and herpes viral infection. Hence, blocking DUX4 activity is a plausible therapeutic option for FSHD and other diseases associated with aberrant DUX4 activity.

We identified MATR3 as the first direct endogenous inhibitor of DUX4. MATR3 is a nuclear protein involved in regulation of gene expression, RNA metabolism and DNA repair and mutated in dominant distal myopathy, frontotemporal dementia and ALS. We found that MATR3 binds to DUX4 DNA-binding domain and blocks DUX4 expression and activity. We have also characterized a MATR3 N-terminal fragment that is necessary and sufficient to block DUX4-induced toxicity and rescue myogenic differentiation of FSHD muscle cells, without affecting healthy muscle cells. We are currently performing structural-functional studies aimed to generate a drug-like DUX4 inhibitory molecule that will be tested in cellular and animal models of FSHD. In parallel we are working on the design of a MATR3-based PROTAC strategy to mediate direct DUX4 degradation. Our final goal is to generate a therapeutic option for the treatment of FSHD, that in perspective might be applied to related but currently incurable diseases.

P.18

Role of HIF-1α/MMP-9 axis in promoting skeletal myoblast differentiation under normoxia condition

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Satellite cells (SCs) are the resident muscle stem cells that crucially affect skeletal muscle regeneration after injury. SCs remain quiescent without dividing or differentiating, await activating signals from damaged tissue. These signals trigger the asymmetric self-renewal in which a SC divides into one stem cell and one differentiating daughter cell. During differentiation, SCs undergo also metabolic reprogramming, from a mainly glycolytic metabolism in myoblasts to a mainly oxidative one in more differentiated stages. In this regard, the Hypoxia-inducible factor (HIF)-1 has been recognized as SCs function regulator. HIF-1 is a transcription factor having an oxygen-responsive subunit named HIF-1α, that is usually degraded in normoxia. In contrast, in hypoxia HIF-1α is stable and can translocate to the nucleus to regulate target gene expression. Several studies in skeletal muscle have highlighted an oxygen level-independent regulation of HIF-1α expression and activation, suggesting a role of such a protein in skeletal muscle physiological conditions. To date, the spatio-temporal expression and function of HIF-1α in myoblasts during differentiation need to be clarified, as well as the molecular targets of this factor. To this aim, we here deeply investigated the role of HIF-1α in differentiating myoblasts, C2C12 and murine satellite cells, under normoxia, using a multidisciplinary approach. Moreover, based on the known involvement of matrix metalloproteinase (MMP-9) in myogenesis, we intended to consider MMP-9 as a possible HIF-1α downstream effector. We found that: 1) HIF-1α expression synchronises with that of MMP-9 and of the myogenic activation marker MyoD, with a comparable increase after 24 h of differentiation; 2) MMP-9 is a target of HIF-1α and 3) HIF-1α/MMP-9 axis is required for myoblast myogenic commitment. In conclusion, we here demonstrated HIF-1α/MMP-9 axis involvement in the early phases of skeletal myoblast differentiation under normoxia condition.

Environmental pollutants and muscle stem cells: findings of potential interactions

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The literature of the last decades highlighted the environmental pollution as a worsening serious threat toward human health. As components of the wide category of environmental pollutants, ozone and polystyrene nanoparticles have been extensively studied due to their toxic effects on cells and tissues. Among other tissue, muscle tissue could also be affected. In this context, aim of the study is to observe the effect of ozone and polystyrene nanoparticles on muscle tissue using human muscle stem cells (hMSCs). At the beginning, hMSCs were firstly isolated from muscle biopsies derived from n=15 healthy male volunteer subjects between 20-80 years and then characterized. After characterization, hMSCs were exposed to ozone at 120Ppb in the Reaction Chamber and/or hMSCS were stimulated with polystyrene nanoparticles at different sizes and dilutions. The two pollutants were tested at first alone and then in combination to determine their impact on cells viability and differentiation capability. Superoxide anion levels and microRNA (miRNA) expression have been also evaluated on hMSCs upon all the cited conditions. Regarding miRNA, miR-1, miR133, miR-206 and miR-23 have been studied. The results obtained showed negative effects upon viability and differentiation of hMSCs of both ozone and nanoparticles, in particular with small-sized nanoparticles used at lower dilution. Superoxide anion levels increased in treated hMSCs, while miRNA expression seemed to be downregulated compared to controls. Moreover, differences in the release of volatile metabolites from hMSCs were observed in the volabolomic analyses. Thus, the results obtained so far underscore the ability of environmental pollutants to negatively impact on skeletal muscles and muscle stem cells due to a diffusive behaviour into the human organisms and the interaction with cellular and molecular structures altering local homeostasis.

P.21

Targeting mitochondrial dynamics to tackle duchenne muscular dystrophy progression

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Duchenne muscular dystrophy (DMD) is a severe, progressive, muscle-wasting disorder caused by the lack of dystrophin, a crucial protein that maintains muscle integrity during contraction. Mitochondrial impairment is one of the earliest dysfunction of *mdx* muscles and a plethora of metabolic defects have been identified over the years. However the organization of the mitochondrial network is also compromised, but few studies have addressed the involvement of mitochondrial dynamics in the pathophysiology of DMD. We discovered that adult dystrophic muscle showed high levels of Drp1 and a less interconnected mitochondrial network.
Consistently, the interaction between Drp1 and its mitochondrial receptors increased indicating an enhanced Drp1 activity. Unbalanced fission could promote UPR induction culminating in myokine release and, as expected, in parallel with Drp1 activity, dystrophic muscle displayed increased FGF-21 production.
After inhibiting Drp1 with MDIVI-1 we observed overall beneficial effects at functional, morphological and molecular level. Specifically, fibrosis, inflammation, and necrosis were remarkably reduced and regeneration was promoted by the treatment. According to this, MDIVI-1 was able to maintain the myogenic capacity of dystrophic muscle stem cells, improving in vitro myotubes formation. Dystrophic muscle ameliorations after MDIVI-1 administration are also associated with reduced FGF-21 levels, suggesting a role of this myokine in establishing dystrophic damage.
In summary, Drp1 is emerging as a relevant target in DMD and the modulation of its activity is a promising approach to counteract muscular dystrophy progression.

P.23

The Src/Akt1/caveolin-1/catalase signaling axis is critical for radioresistance of rhabdomyosarcoma cells

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Rhabdomyosarcoma (RMS) is the third most common extracranial solid tumor of childhood and shares histopathological features with the skeletal muscle, originating from cell precursors of the skeletal muscle niche including satellite cells, muscle progenitors, and myoblasts. The management of RMS requires a multimodal approach, which involves surgery, chemotherapy and radiotherapy, the latter being the standard therapeutic option for patients with RMS. However, local failures and recurrence frequently occur.
This work aimed at dissecting the molecular mechanisms underlying radioresistance in RMS by employing the use of various radioresistant cell models and a phosphoproteomic approach to identify novel strategies to improve therapy efficacy.
We found that constitutive activation of the PI3K/Akt pathway in RD cell line was able to promote a radioresistant behavior, as shown by the enhanced survival capability to ionizing radiation (IR) treatment sustained by a faster and more efficient capacity to repair IR-induced DNA damages. These radioresistant cell lines exhibit also increased expression of the Src kinase, of the cholesterol-binding protein Caveolin-1 (Cav-1), and of the antioxidant enzyme Catalase. We confirmed a crucial role of the Src/Akt1/Cav-1/Catalase signaling axis in RMS radioresistance by using a model of RD cells carrying Cav-1 overexpression and two radioresistant RD and RH30 cell lines obtained through exposure to several rounds of IR treatment.
Furthermore, we found the cholesterol-lowering drugs statins as effective treatment strategies to restore the sensitivity to radiotherapy in RMS, by affecting the expression and activation of Akt and Cav-1 that are influenced by the content of cholesterol and isoprenoid derivatives at the plasma cell membrane. Thus suggesting statins as putative candidates to improve radiotherapy cytotoxicity and prevent the risk of recurrence in RMS patients.

Development of an innovative extrusion-based 3D bioprinting system and its characterization for Skeletal Muscle Tissue Engineering applications

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Nowadays, 3D printing technology is becoming more and more present in biology, especially in the field of tissue engineering. Among the different 3D bioprinting approaches, the extrusion-based one has proven to be the most suitable for skeletal muscle tissue engineering (SMTE). The organization of skeletal muscle tissue, composed of bundles of aligned muscle fibers, is well reproduced by this bioprinting approach, through the continuous deposition of printing fibers in a parallel pattern, layer by layer. Moreover, the extrusion mechanism forces the cells in orienting along the printing axis, allowing the differentiation of muscle progenitors in properly organized bundles of myotubes.
Crucial for this technique is the choice of the right biomaterial, which must possess the key requirement of “printability”, a quality often related to poor biological compatibility. Over the years, researchers have dealt with it by formulating "bioinks" composed of a combination of different biomaterials, one good for the machine and one for the cells, a strategy that has proved to be effective, while revealing limitations in the results, especially *in vivo*.
Here we report the development of a novel extrusion 3D bioprinting system based on PEG-Fibrinogen (PF), a biomaterial with excellent biocompatibility ideal for SMTE approaches. The printer is designed to compensate for the very low printability of PF, making it possible to use it as the only component of the bioink.
The experiments carried out demonstrated the ability of this innovative system to generate highly organized muscle constructs *in vitro*, capable of restoring volumetric muscle damage (VML) when implanted in mice, as revealed by the presence of properly organized, vascularized and innervated newly formed muscle tissue.
Overall, this novel bioprinting system can be considered a useful tool for SMTE, both for the creation of biological substitutes and for regenerative medicine applications.

Ad libitum ketogenic diet reverts western diet-pathological effects in liver, but not in skeletal muscle in mice

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Western diet (WD), characterized by high sugar and saturated fat content, plays a crucial role in contributing to obesity and its associated health issues. Its impact on insulin resistance and inflammation has been linked to various conditions, including type 2 diabetes mellitus (T2DM), metabolic-associated fatty liver disease (MAFLD), and metabolic syndromes (MetS). Ketogenic diets (KDs) are dietary approaches characterized by minimal carbohydrate intake, high-fathigh fat, and sufficient protein levels. Energy is provided by ketone bodies (KBs), like beta-hydroxybutyrate, derived from fat oxidation and protein metabolism. Since growing evidence consider KDs effective in mitigating inflammation, oxidative stress, and enhancing mitochondrial function, we estimated that KDs could be promising in countering obesity-related ailments. To test this hypothesis, we fed mice with WD for 16 weeks, followed by a transition to a standard diet (SD), an *ad libitum* KD, or continued adherence to WD for an additional 2 to 4 weeks.
Our findings revealed that KD induced ketosis, contributing to weight loss and ameliorating blood sugar control and hepatic inflammatory response. However, KD failed to exert positive effects on skeletal muscle strength, mass, and morphology after WD-feeding. Although KD effectively mitigated WD-induced liver damage and excessive weight gain, we hypothesize that muscles need a more protracted recovery phase than the liver.
To further explore fatty acid and KB effects on muscle cells, we performed in vitro experiments testing various palmitate (PA) and butyrate (BU) doses on C2C12 myotubes. PA not only significantly reduces myotube diameter in a dose-dependent manner, but also triggers lipid droplet accumulation. Conversely, low BU doses protect against PA-induced atrophy in myotubes, while high doses seem to be ineffective or even atrophic themselves. Our data suggest that appropriate KB doses could offer a non-pharmacological approach to treating MetS.

P.27

Elucidating the effects of MAO-B inhibitors in DMD: rasagiline treatment in *mdx* mice dampens the expression of pro-inflammatory genes in multiple cell types

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Oxidative stress is known to play a crucial role in the pathophysiology of Duchenne muscular dystrophy (DMD). We previously reported that the reactive oxygen species produced by the mitochondrial enzyme monoamine oxidase B (MAO-B) are causally involved in Duchenne pathophysiology, as demonstrated by the results obtained *in vivo*, in *mdx* mice, and *in vitro*, in human DMD myoblasts, with the MAO-B specific inhibitor safinamide.
In order to elucidate the mechanisms underlying the therapeutic effects of MAO-B inhibition and assess the possible role of this enzyme in dystrophic hearts, we treated *mdx* mice with the MAO-B inhibitor rasagiline for one month and then collected different cell types from both cardiac and skeletal muscle. From the former we isolated myeloid, endothelial and fibro-adipogenic progenitor cells thorough FACS, while from the latter we obtained myeloid cells with the use of magnetic beads and single muscle fibres through enzymatic digestion.
So far, our analyses have been completed only for heart-derived cells, showing that while the proportion of the various cell types was not affected by the treatment, the expression of pro-inflammatory genes was clearly decreased in myeloid (IL-1b, IL-6, TNF and SPP1) and endothelial cells (IL-1b, IL-6, MMP2 and NOS2).
Once completed, our study will provide further indications towards the clinical use of MAO-B inhibitors in DMD.

Effects of real microgravity on human muscle precursor cells and skeletal muscle tissue. The *MyoGravity* project

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Exposure to microgravity (μG) alters the homeostasis of several organs and tissues, representing a major obstacle in view of long-duration space flights. In response to diminished mechanical load, skeletal muscle tissue undergoes progressive mass loss and metabolic alterations, compromising astronauts' motor function. Only limited data are available about the effects of real μG on human skeletal muscles and muscle precursor cells (huMPCs). With the *MyoGravity* project, we had the opportunity to evaluate the cellular and transcriptomic alterations induced by real μG on board the International Space Station (ISS) in huMPCs and muscle tissue of the same astronaut. HuMPCs isolated from *Vastus lateralis* biopsies (pre-flight astronaut and age-matched volunteer) differentiated on board the ISS showed downregulation of genes related to sarcomere organization, and muscle-specific microRNAs, in comparison with cells cultured on ground. RNA-Seq analysis of post- *vs* pre-flight astronaut muscle tissue revealed that genes involved in muscle structure and remodeling are promptly activated after landing following a long-duration (5-months) space flight, while genes involved in the myelination process and neuromuscular junction organization appeared downregulated, suggesting readaptation of muscle mechanical components to the normal gravity but compromised myelination/innervation pattern. Finally, prolonged exposure to real μG strongly affects the biology and functionality of astronaut's huMPCs, since we observed reduced responsiveness to activating stimuli and proliferation rate, morphological changes, and almost inability to fuse into myotubes in post- *vs* pre-flight cells, suggesting that a condition of inefficient regeneration is likely to occur in muscles of post-flight astronauts following a damage. These results may contribute to defining approaches to counteract muscle wasting in astronauts involved in long-duration space missions, and rehabilitation protocols after landing.

P.31

Antifibrotic potential of growth hormone secretagogues in Duchenne muscular dystrophy: effects of JMV2894 in the D2-*mdx* mouse model

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The use of growth hormone secretagogues (GHSs) as a pharmacological strategy for Duchenne muscular dystrophy (DMD) may possibly represent an exclusive therapeutic opportunity in virtue of their wide profile and reported ability to contrast damaging signals in other muscle-wasting conditions (Conte et al., *IJMS* 2020). In this framework, putative clinically relevant effects of GHSs in DMD need to be validated via robust preclinical investigations. In our recent multidisciplinary study (Boccanegra et al., *Front Immunol* 2023), two selected GHSs (EP80317, JMV2894), resulted to be beneficial in terms of functional recovery and control of muscle inflammation and fibrosis, when administered to classic *mdx* mice (320µg/kg/d, s.c.). Importantly, preliminary docking studies disclosed a potential binding ability of JMV2894 on metalloproteases ADAMTS-5 and MMP-9, overactivated in DMD. This pushed us to test JMV2894 in the novel dystrophic D2.B10-*Dmd*^{mdx}/J (D2-*mdx*) mouse model, featuring a severe profibrotic genetic background. 4-week-old D2-*mdx* mice were treated with JMV2894 at two doses (640 and 1280µg/kg/d, s.c.) for 6 weeks. *In vivo*, JMV2894 partially improved hind limb plantar flexor torque in a dose-dependent manner, with recovery scores (R.S.) towards D2-WT up to 20%. This was paralleled by a decrease in gastrocnemius muscle ultrasound echodensity, a fibrosis-related index (R.S. 25% and 34%, for each dose, respectively). Diaphragm (DIA) echodensity was also partly reduced (R.S. 20% and 15%), whilst DIA amplitude was ameliorated, especially by the lower dose (R.S. 62%). However, DIA muscle force *ex vivo* was not improved. Focused histology and molecular biology experiments are ongoing aimed at better defining the antifibrotic potential of JMV2894 in D2-*mdx*. Moreover, results of PK analyses disclosed limited drug exposure in muscle tissue, supporting the need for novel formulation/delivery systems to improve GHSs bioavailability [Supported by AFM-Téléthon Grant #22199].

P.33

The compartmentalized distribution of proteins of the dystrophin-associated protein complex in the muscle fiber syncytium

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The dystrophin-associated protein complex (DAPC) is a transmembrane protein complex of the myofiber sarcolemma, which is required for muscle fiber integrity. We recently demonstrated a compartmentalized distribution of the DAPC member dystrophin in skeletal muscle fibers in vivo and ex vivo using the DmdEGFP reporter mouse model. We found dystrophin being highly enriched in myotendinous and neuromuscular junctions. Here, we studied the distribution of sarcoglycans and dystroglycans, two major families of DAPC proteins. These DAPC members are also strongly enriched at the MTJ and NMJ in myofibers from wild-type mice and rats. Furthermore, they persisted at reduced levels in the *mdx* mouse model for Duchenne muscular dystrophy. Restoration of dystrophin in mdx mouse, following treatment of mice with triclo-DNA to skip mutated Dmd exon 23, normalized levels of β-sarcoglycan. We ask whether DAPC members always enrich when colocalized with dystrophin. To answer this question, we currently explore the distribution of DAPC members in state of dystrophin mosaicism in female heterozygous carrier mice for the DmdEGFP transgene.

Western diet enriched in advanced glycation end-products (AGEs) induces muscle wasting which could be counteracted by *Vaccinium macrocarpon* extract

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A nutritional transition to “Western diet” (WD), characterized by excessive consumption of saturated fats, sugars, and grilled or fried foods, and containing high levels of advanced glycation end-products (AGEs), has been spreading worldwide, predisposing to several diseases. AGEs are a heterogeneous group of non-enzymatic adducts, especially glycosylated proteins, whose formation occurs also endogenously during aging, hyperglycemia, and oxidative stress conditions. AGE accumulation causes tissue damage by altering protein function or increasing oxidative stress and inflammation through the AGE receptor, RAGE. High AGEs have been correlated with loss of muscle mass and functionality (muscle wasting; MW) in diabetic and geriatric patients, and RAGE signaling sustains MW in several conditions. However, the impact of WD consumption and the involvement of the dietary AGEs/RAGE pathway in MW have not been investigated so far. We report that muscles of adult mice fed with high-AGE WD showed reduction of myosin heavy chain (MyHC)-II expression, AGE accumulation, upregulation of RAGE and activation of ubiquitin-proteasome system, in comparison with standard diet-fed mice. In accordance, AGEs induced atrophy in C2C12 myotubes. Starting from thirty standardized dry extracts or compounds, *Vaccinium macrocarpon* (*VM*), *Camellia sinensis*, and chlorophyll showed surprising ability in counteracting AGE formation. When tested for their biological effects on C2C12 myotubes, *VM* (100µg/ml), but not *C. sinensis* or chlorophyll, sustained myotube trophism and viability, and counteracted myotube atrophy (i.e., reduction of myotube diameter, and MyHC-II degradation) induced by AGEs. Our data suggest that consumption of high-AGE WD predisposes to MW, and that *VM* extract might be useful to prevent accumulation/activity of detrimental dietary AGEs in muscles.

P.37

What nutraceuticals can do for Duchenne muscular dystrophy: the plumbagin experience

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Duchenne muscular dystrophy (DMD), is a progressive muscle-wasting disease. Despite the recent approval of molecular treatments, the need to develop supportive therapies aimed at improving the quality of life of patients remains.
Over the years, a plethora of bioactive compounds derived from plants have been used for human healthcare owing to their antimicrobial, antioxidant, anticancer, and anti-inflammatory effects. Plumbagin (PLU), a plant-derived product, has shown interesting counteracting effects in many inflammation models.
This work was undertaken to evaluate the effects of PLU on DMD. To this end we tested PLU administer orally in two model of DMD, the dystrophic mutant of the fruit fly Drosophila melanogaster, the homozygous Dys^{E17}, and the mdx mouse. In both model, PLU, at the administered doses, did not show any particular side effects.
PLU improved the climbing ability of the dystrophic flies, their muscle morphology and reduced oxidative stress by increasing the expression of enzymes involved in the antioxidant defense system. In mdx mice, PLU enhanced the running performance on the treadmill and the muscle strength along with muscle morphology. Moreover, PLU reduced and modulated inflammation towards an anti-inflammatory state.
The effect of PLU was associated with the activation of cncC/NRF-2 pathway. This may explain the potential for plumbagin to therapeutically exploit key different targets involved in the pathogenic regulation of DMD.
Overall, these data indicate that use of PLU may have clinical implications for the treatment of patients with DMD.

P.39

Development of efficient gene delivery systems for the treatment of Duchenne muscular dystrophy

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Duchenne Muscular Dystrophy (DMD) is an X-linked genetic disorder caused by mutations in the dystrophin gene. The loss of dystrophin results in sarcolemma instability and progressive muscle wasting, leading to the loss of ambulation and respiratory failure. Despite considerable efforts to develop therapeutic strategies, the complexity and size of the dystrophin gene have posed challenges in finding effective treatments. To address this issue, the concept of employing smaller versions of the gene that contain essential domains has emerged, leading to the synthesis of micro-dystrophin variants that are better suited for gene delivery applications. In fact, since nucleic acids are prone to alterations and rapid degradation, they require encapsulation within delivery systems to facilitate cell internalization and enable the expression of therapeutic potential. In this context, the development of lipid-based nanoparticles (LNPs) could be a promising alternative to traditional viral vectors, as they offer several benefits as high biocompatibility, payload protection and easy control over their physio-chemical properties.
In this study we designed and characterized a library of LNPs for the delivery of plasmid DNA (pDNA) into muscle cells, which are notoriously hard to transfect. The LNPs were loaded with a gene reporter-coding pDNA using both the bulk mixing method and the microfluidic process. Following physio-chemical characterization, the cellular uptake efficiency was evaluated using fluorescently labelled lipids on both the C2C12 cell line and mdx^{4cv}-derived primary muscle cells, whereas the intracellular localization was assessed through confocal microscopy. Among all formulations employed, one resulted in a superior transfection efficiency and uptake, highlighting the potential of LNPs as gene delivery platforms for targeted treatment of muscular diseases, including DMD.

P.43

Dynamic changes in transcriptional and epigenetic profile of fibro-adipogenic progenitors in dystrophic skeletal muscles and their modulation by epigenetic drugs (HDACi)

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Duchenne Muscular Dystrophy (DMD) is a rare disorder characterized by progressive muscle wasting, weakness, and premature death. The pharmacological treatment with histone deacetylase inhibitors (HDACi) has been shown to stimulate muscle regeneration and to decrease both fibrosis and inflammation in mdx mice, the mouse model of DMD. Fibro-adipogenic progenitors (FAPs) are a heterogenous population of mesenchymal precursors and a significant signalling source in skeletal muscles. Besides their supportive role in muscle regeneration and homeostasis, they are the primary cellular source of fibrofatty infiltration in dystrophic muscles. We have illustrated that FAPs are the key cellular mediators of HDACi treatment. However, this beneficial impact is limited to the early disease stages. Aiming to elucidate the underlying mechanisms of responsiveness and resistance to HDACi, we performed single-cell multiome sequencing to simultaneously assess transcription and chromatin accessibility profiles of skeletal muscle cells before and after HDAC inhibition during disease progression. Profiling ~42,000 nuclei, we demonstrate that the treatment-driven chromatin landscape changes are both cell type and age specific. Furthermore, we identify distinct FAP, distinguished by their stemness-related, fibrogenic or myogenic gene signatures. Of note, their cell composition and chromatin conformation alter significantly during aging and upon HDAC inhibition in a type-specific manner. Further investigation will provide a valuable insight into the role of FAPs and their cross-talk with the muscle stem cell niche in every stage of DMD and will reveal in big scale the so far unknown molecular basis of HDACi effect to disease treatment.

P.44

Immunomodulation via interleukin-4 improves cachexia 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 cachectic tumor-bearing mice with interleukin-4 (IL4) improves muscle mass and function, body weight and survival. Whether IL4 administration improves energy metabolism in the skeletal muscle has not been investigated yet.
Methods: Eight-week-old Balb/c male mice were divided into four groups: untreated (*C*) and treated (*IL4*) controls, untreated (*C26*) and treated (*C26+IL4*) tumor-bearing mice, implanted with C26 colon carcinoma cells. Daily IL4 treatment (1.66 µg/mouse) was performed by intraperitoneal injection. Mitochondria-related proteins were assessed in the skeletal muscle by western blotting.
Results: Treatment with IL4 significantly reduced body weight loss in tumor-bearing mice. Trends to increase were observed for skeletal muscle mass and strength comparing treated and untreated mice. The spleen weight significantly increased in *C26+IL4 vs C26*. The protein levels of the oxidative phosphorylation complexes II and III, cytochrome c and PGC-1α in the skeletal muscle were significantly increased in *C26+IL4 vs C26*, while trends to decrease were found for BNIP3 and TFAM.
Conclusion: The results obtained show that IL4 treatment partially improves energy metabolism in the skeletal muscle of tumor-bearing mice, possibly exerting an exercise-mimetic role. Spleen enlargement also 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.

P.45

N-glycosylation inhibition reduced IGF-1 serum level and muscle IGF-1R signalling pathway activation

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Recent studies have demonstrated that protein glycosylation is essential in the aetiology and development of muscle diseases [1]. Here, 6 wk old wild-type (WT) and MLC/mIgf1 transgenic mice, overexpressing the *IGF1Ea* isoform in muscle fibres, were chronically challenged with a low dose (0.1 mg/kg for 15 days) of the protein N-glycosylation inhibitor tunicamycin (TUN). Liver and tibialis anterior (TA) tissues were collected for mRNA quantification of ER stress-related genes (*Chop*, *Hspa5*, *Xbp1u* and *Xbp1s*), lectin blotting and IGF1 signalling pathway activation. Serum IGF1 level was also analysed by an ELISA assay.
WT and MLC/mIgf1 mice treated with TUN showed increased *Chop* and *Hspa5* and decreased *Xbp1u* and *Xbp1s* mRNA expression in the liver, while the ER stress-related gene expression did not change in TA muscle. TUN treatment slightly decreased the ConA lectin binding in the liver and TA muscle, while PHA-L and AAL lectins decreased only in TA muscle of WT mice. The mRNA expression of *IGF1* isoforms decreased after TUN treatment in liver and TA muscle of WT mice, while the overexpression of *IGF1Ea* isoform in the MLC/mIgf1 mice was unaffected by TUN treatment. Western blotting analyses showed that TA muscle of untreated MLC/mIgf1 mice mainly expressed the IGF1Ea prohormones and three immunoreactive bands were observed at approximately 22, 17 and 12 kDa. Two immunoreactive bands at 17 and 7 kDa were detected in the liver. TUN treatment decreased the IGF1Ea prohormones expression in the liver and TA muscle. MLC/mIgf1 mice showed increased IGF1R, AKT and ERK1/2 phosphorylation compared to WT mice, which was inhibited by TUN treatment. Finally, chronic TUN treatment decreased serum IGF1 levels.
These results showed that aberrant N-glycosylation dampens the IGF1 signalling and suggest that the impairment of muscle development and function observed in Congenital disorders of N-glycosylation (CDG) could be due to a dysfunction of the IGF1 hormonal axis.

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P.46

Vaccinium macrocarpon restrains myotube atrophy in experimental models mimicking cancer cachexia

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Cancer cachexia (CC) is an unresolved multifactorial syndrome affecting 50% of patients with advanced cancer and leading to a fatal prognosis. The key hallmark of CC is muscle wasting (MW), *i.e.* loss of skeletal muscle mass and strength, mainly due to an excessive muscle catabolic state induced by circulating cytokines and tumor-released factors. Ultra-processed foods rich in sugars and saturated fats, typical of unhealthy "Western diet", contain high levels of advanced glycation end-products (AGEs), which are non-enzymatic adducts, mainly glycosylated proteins. AGEs induce deleterious tissue damage and sustain the progression of many diseases, including cancer, by altering the protein function or interacting with their receptor RAGE, which strongly contributes to CC in mice. We hypothesized that dietary AGEs (dAGEs) could exacerbate MW, and that targeting dAGEs could restrain cachexia, in cancer conditions. Treatment of C2C12 myotubes with the most common dAGE, methylglyoxal (MG) resulted in reduction of myotube diameters, and myosin heavy chain (MyHC)-II degradation by activation of the proteolytic ubiquitin-proteasome system, and increased the cachectic potential of Lewis lung carcinoma (LLC) cell-secreted factors. The natural compound *Vaccinium macrocarpon* (*VM*), which reduces AGE accumulation and activity, counteracted MG-induced myotube atrophy. Interestingly, *VM* dramatically restrained myotube atrophy in *in vitro* experimental models mimicking CC, *i.e.*, C2C12 myotubes treated with tumor necrosis factor (TNF)-α or exposed to LLC cell-secreted factors, also in the presence of cisplatin, a common chemotherapy agent which causes MW *per se*. Thus, *VM* might represent a potential food supplement useful to reduce dAGE formation and to counteract MW in cancer conditions worsened by the consumption of WD.

Insight into the role of calcium homeostasis and store-operated-calcium-entry dysfunction in sarcopenia: effect of supplementation with BCAA-based formulation in aged mice

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During aging, sarcopenia and the decline of physiological processes lead to partial muscle strength loss and atrophy. Functional and structural alterations may be related to an incorrect intake of essential amino acids playing a key role in proteostasis. Furthermore, alterations in Ca^{2+} homeostasis and store-operated-calcium-entry (SOCE) process may be involved in aged muscle alterations (Brotto et.al.,2008). We demonstrated that Branched Chain Amino Acids (BCAAs) supplements with known anabolic properties, ameliorated muscle atrophy and strength in murine models of muscle disuse and aging (Mantuano et al 2021, 2023). On these bases, we here investigated the mechanisms underlying Ca^{2+}-related alterations in aged muscle, meanwhile assessing the potential benefit of BCAAs supplementation. 17-months-old male C57BL/6J mice received a 12-weeks-supplementation with BCAAs alone or boosted with two equivalents of L-Alanine (2-Ala) or with dipeptide L-Alanyl-L-Alanine (Di-Ala), in drinking water. Outcomes were evaluated on *ex vivo* indices *vs* adult 6-month-old male C57BL/6J mice. Ca^{2+} imaging confirmed a SOCE decrease and an increase of resting Ca^{2+} concentration in aged *vs* adult mice without alteration in STIM1/Orai1/TRPC1/TRPC4 levels, the key SOCE components. Importantly, aged muscles vs adult ones were characterized by a decrease in the expression of RyR1, SERCA pump, and sarcalumenin together with an alteration of the expression of Mitsugumin 29 and Mitsugumin 53, two new recently recognized players in SOCE mechanisms. Interestingly, BCAAs, particularly the formulation BCAAs+2-Ala, were able to ameliorate all these alterations. These results provide evidence that Ca^{2+} homeostasis dysfunction plays a key role in the functional deficit observed in aged muscle and provides further translational support of dietary BCAA supplementation in elderly subjects to counteract sarcopenia-related SOCE mechanism dysregulation. (Supporter by MUR-PNRR AGE-IT; PRIN2020 n.20202YAY9B_004)

RNAseq analysis revealed a lower inflammation in tumor-bearing mice lacking VDBP resulting in a milder cachectic phenotype

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Vitamin D binding protein (VDBP) is a multifunctional serum glycoprotein belonging to the albumin gene family encoded by the gene *Gc*. In addition to transporting vitamin D metabolites in the bloodstream, it is a cofactor for neutrophil migration, contributing to the inflammatory response.
Proteomic analyses revealed the upregulation of VDBP in biological fluids from patients affected by pathologies susceptible to progressive muscle wasting or cachexia, including several types of cancer, suggesting a potential role for VDBP in skeletal muscle homeostasis. Moreover, data from our lab show that VDBP increases in Lewis lung carcinoma (LLC)-bearing mice and that LLC-bearing mice lacking VDBP (*Gc* KO) are partially protected from cachexia-induced atrophy. To further understand the impact of VDBP in cancer-associated muscle wasting, we performed RNAseq analysis on the gastrocnemii from tumor-bearing mice compared to the corresponding controls in WT and *Gc* KO mice. We found that most DEGs in WT cachectic muscles were related to enhanced inflammation and immune response. Coherently, only cachectic WT mice showed the increased expression of atrogenes. Interestingly, the RNAseq analysis revealed a strong down-regulation of different collagen genes, two serpins, and *Arg1* in LLC-bearing *Gc* KO mice.
We hypothesize that the substantial loss of muscle mass in WT compared to *Gc* KO mice can be a consequence of the pro-inflammatory activity of VDBP, i.e., the selective recruitment of neutrophils. This hypothesis is further supported by the results of the gene set enrichment and the IPA upstream regulator analyses, which indeed proposed the pathways revolving around TNFα and IL6 to be central in VDBP-mediated atrophy *in vivo*. Overall, our data indicate that partial protection from cachexia-induced muscle wasting in *Gc* KO mice may rely on an altered inflammatory profile at least in muscles.

Morphological alterations of human neuromuscular junction in ageing

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Ageing negatively affects the morphology and function of the neuromuscular junction (NMJ). Most data concerning physiology and morphology of NMJs come from animal models (mostly rodents). Aged rodents' NMJs show fragmentation of the post-synaptic terminal, reduction in junctional folds, pre-synaptic axonal blebbing, nerve terminal branching, reduction in the number of synaptic vesicles. Research on human NMJs has been primarily conducted using post-mortem tissue analysis (Wokke J.H. et al., 1990) or surgical discard material from patients after lower limb amputation due to complications caused by peripheral vascular disease and diabetes mellitus (Jones R.A. et al, 2017). These studies reported controversial results. While Wokke et al. observed NMJ fragmentation and reduction in junctional folds, Jones at al. showed that human NMJ morphology is not altered in ageing. Additional studies involving ageing human NMJs are needed to resolve this controversy. Thus, the aim of our study was to assess the morphological alterations of human NMJ in ageing. For that, a vastus lateralis muscle biopsy was obtained in 37 healthy young individuals (18–35 years old) and 52 older individuals (>70 years old) followed by immunochemical staining of NMJs in separated fiber bundles. 7 young and 6 older individuals' samples were found positive for the presence of NMJs. The preliminary results obtained so far show distinct differences in NMJ morphology with ageing, such as a decrease in axon thickness, axonal blebbing, fragmentation, and partial denervation; fragmentation of the post-synaptic terminal and reduction in the endplate area; reduction in the acetylcholine receptor area.

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P.51

Putative contribution of caveolin-1 in satellite cells senescence

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Sarcopenia is the loss of skeletal muscle mass, strength and function occurring with age or with other muscle wasting disorders such as muscular dystrophies.
Interestingly, a great abundance of senescent satellite cells were found in all the pathological conditions associated with sarcopenia but targeting them with senomorphics or senolytics led to controversial results making necessary to search for more specific proteins changing during senescence in the complex anatomy of muscle fibers.
Caveolin 1 (Cav-1) is not only a structural protein involved in the assembly of caveolae, but is also a regulator that plays a role in signal transduction. Moreover, several studies performed in different cellular models showed an up-regulation of Cav-1 during cellular senescence making it a putative gatekeeper of this phenomenon.
Since Cav-1 is expressed in satellite cells but not in muscle fibers, we decided to take advantage of the murine C2C12 cell line to understand the possible contribution of this protein during senescence of satellite cells.
As expected, C2C12 undergoing multiple rounds of replication showed clear signs of senescence as demonstrated by reduced growth rate, increased oxidative stress and higher levels of β-Gal. All this was accompained by an up-regulation of Cav-1 protein level, an increase of Src, a decrease of p-Erk and a reduced ability to differentiate. Of note, accelerated models of aging such as the administration of galactose and stress-induced premature senescence (SIPS) led to changes of Cav-1 protein level and differentiation.
Starting from these preliminary data obtained in models of aged satellite cells, we can hypothesize that Cav-1 might represent a novel object of study to better understand the changes occurring during satellite cells senescence and the role in sarcopenia.

Extracellular vesicle release and protein cargo are altered by caveolin-1-overexpression and contribute to cancer dissemination in a model of rhabdomyosarcoma

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Rhabdomyosarcoma (RD) is a malignant tumor arising from striated muscle cells; many studies have shown that Caveolin-1 (CAV1) overexpression cooperates with tumor growth and metastatic potential in RD cells. CAV1 is an integral membrane protein required to generate caveolae and cholesterol-enriched lipid rafts, which are pivotal structures in extracellular vesicle (EV) secretion.
The present work aims to investigate if the increased aggressiveness of RD-cells overexpressing CAV1 (RD-CAV1) correlates with an altered extracellular vesicle release and cargo and if RD-CAV1 EVs contribute to cancer dissemination.
Large (lEVs) and small (sEVs) extracellular vesicles were isolated from RD-ctrl and RD-CAV1 conditioned media by sequential ultracentrifugation and characterized by Nanoparticle Tracking Analysis (NTA), Western Blot Analysis, and Flow Cytometry Analysis. Proteomic Analysis has been performed on both EV subpopulations.
The obtained data show that RD-CAV1 cells release more EVs, particularly more sEVs, than RD-Ctrl cells. Western Blot analysis highlighted that sEVs exhibit the typical exosomal markers, whereas lEVs are positive for Calnexin. Interestingly, RD-CAV1 sEVs are negative for the tetraspanins CD63, CD81 and CD9, unlike the control ones. All these data suggest that CAV1 overexpression induces an alteration of EV biogenesis and secretion. Moreover, the treatment of HUVEC with RD-CAV1 EVs shows an increase in cell proliferation and migration in a dose-dependent manner.
Altogether, these data demonstrate that CAV1 overexpression critically affects RD-EV release and cargo; moreover, RD-CAV1 EVs can alter the behaviour of the tumor microenvironment cells. Future studies will focus on the characterization of RD-EV cargo in terms of lipid- and miRNA-loading and the evaluation of RD-EV effects in other cells, typical of tumor niche.

Role of PRDM16 in maintaining nuclear integrity and genomic stability in fibro-adipogenic progenitors

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Prdm16 is a regulator of heterochromatin assembly to the nuclear envelope particularly enriched in mesenchymal, multipotent cells named fibro-adipogenic progenitors (FAPs). Prdm16 localizes at the nuclear lamina (NL) where it mediates interaction with the genome and orchestrates lamina associated domains (LADs) deposition. In FAPs, ablation of Prdm16 compromises the peripheral localization of H3K9me2-marked chromatin inducing NL defects and aneuploidy.
To establish the role of Prdm16 a specific conditional knockout mouse model FAPs ($Prdm^{16cKO}$) was generated. Our data showed that isolated FAPs$Prdm^{16cKO}$ present alteration in the NL organization such as invaginations, micronuclei formation and defects in H3K9 distribution. Moreover, they expanded much slowly in culture compared to $FAPs^{Ctrl}$. Time lapse analysis revealed that the mitotic process was impaired in FAPs lacking Prdm16 with the majority of cells failing to complete cell division or having extremely prolonged phases. In addition, immonofluorescence analysis highlighted a pronounced increase of phosphorylated H2AX foci in $FAPs^{Prdm16cKO}$ thus indicating the presence of DNA double-strand breaks (DSBs).
The morphological studies described above suggest the critical role of Prdm16 in maintaining nuclear architecture and genome stability. In particular, Prdm16 absence can promote genomic abnormalities by impairing stable LADs-NL interaction during cell division, thus driving mitotic errors. Loss of Prdm16 can thus promote oncogenic mutations and acquisition of (pre)-malignant features.

Keywords:
epigenetics, muscle dystrophy, tumors

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Non-Autonomous Muscle Stem Cells mediated defective muscle regeneration in the accelerated ageing Polg Mutator Mouse Model

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Polg^{D257A} mice display premature aging symptoms, including anaemia, kyphosis, alopecia, lipodystrophy, and sarcopenia. The Polg^{D257A} mutation impairs DNA proofreading but not its DNA polymerase activity, leading to an accumulation of mitochondrial DNA mutations. Sarcopenia, the age-related loss of muscle mass and strength, significantly impacts the quality of life for the elderly. It's thought to involve defective muscle regeneration, also through non-cell autonomous mechanisms. We observed that Polg^{D257A} mice exhibit strong impairment of muscle regeneration following cardiotoxin injury at 3rd month-of-age which is exacerbated at the 11th month, prompting further investigation into the cause of this regeneration defect.
Skeletal muscle of Polg^{D257A} mice displayed a reduced number of satellite cells (SCs) in basal conditions and at 7day post injury(dpi). Additionally, the gene expression of myogenic regulatory factors Pax7, MyoD, MyoG and Myf5 is reduced in Polg^{D257A} as compared to wild-type(WT), suggesting possible defective myogenesis. Ex-vivo analysis of SC proliferation and differentiation revealed that SCs from Polg^{D257A} background behave as the WT ones, ruling out possible cell autonomous defects.
To investigate the involvement of SC extrinsic factors, we transplanted WT-GFP^{+} myoblasts in WT vs. Polg^{D257A} recipient mice. Notably, the number of GFP^{+} myofiber after 30 days from transplant is lower in Polg^{D257A} recipient mice compared to WT. Preliminary histological analysis suggests increased macrophage infiltration into the regenerating muscle of Polg^{D257A} at 7 dpi. Reduced fibro-adipogenic progenitor-related gene expression in regenerating muscle further support the idea that SC extrinsic factors contribute to defective regeneration in Polg^{D257A} mice.

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