Interuniversity Institute of Myology

UNIVERSITÀ DEGLI STUDI DI PERUGIA

16th IIM Meeting

1st HIGH TRAINING COURSE IN ADVANCED MYOLOGY

Assisi 17-20 October 2019

Pathogenesis and Therapies of Neuromuscular Diseases

TOPICS

- Biophysics and E-C coupling
- Genetics and epigenetic
- Muscle stem cells and regenerative medicine
- Muscle wasting and cachexia
- Exercise
- Signaling and metabolism
- Therapeutic approaches

Keynote Lectures

Bénédicte Chazaud (Institut NeuroMyoGène Lyon-France)
Bente Klarlund Pedersen (University of Copenhagen-Denmark)
Olivier Pourquié (Harvard Stem Cell Institute Boston-USA)
Alessandra Sacco (Sanford Burnham La Jolla-USA)

Venue

Hotel Il Cenacolo (Assisi-Italy)
http://www.hotelcenacolo.com/

Scientific Committee

Barbieri E, Blaauw B, Fulle S, Gabellini D, Gargioli C, Grassi F, Musarò A, Mammucari C, Moresi V, Puri PL, Sampaolesi M, Sandri M, Sorci G.

Thursday, October 17th

11:30-14:30 Registration

14:45 Welcome and opening of the meeting

15:00-15:40 Lecture 1
Alessandra Sacco (Sanford Burnham La Jolla-USA)
Dynamics of muscle stem cells during tissue growth and regeneration. Chair: Pier Lorenzo Puri

15:40-16:25 Session 1: Genetic and epigenetic alterations in muscle dystrophies and myopathies. Chair: Marianna Cosentino and Samuele Metti

Circulating microRNAs in ALS disease
Irene Casola

Epigenetic regulation of a splicing factor involved in Duchenne Muscular Dystrophy
Michela Lisi

New emerging role for HDAC4 on sarcolemma stability in DMD
Alessandra Renzini

16:25-16:55 Coffee break

16:55-17:30 Welcome from Authorities

17:30-18:45 Session 2: Satellite cells and muscle regeneration in healthy muscle and in diseases. Chair: Ester Di Filippo and Alessandra Renzini

Muscle stem cells from developing children with cerebral palsy: a pilot study
Marlies Corvelyn

Physiopathological characterization of the role of MCUb in skeletal muscle regeneration
Simona Feno

Splenic Ly6C^{hi} monocytes are critical players in dystrophic muscle injury and repair
Biliana Lozanoska-Ochser

Approaches to delay the progression of Muscular Dystrophy
Graziella Messina

Dissecting the secretome during muscle regeneration: role and kinetics of cytokines and extra-cellular vesicles.
Simone Vumbaca

19:30-21:30 *Aperitivo Umbro* and ROUNDTABLE DISCUSSIONS*

Friday, October 18th

9:00-9:40 Lecture 2
Bente Klarlund Pedersen (University of Copenhagen-Denmark)
Muscle as an endocrine organ. Chair: Elena Barbieri

9:40-10:25 Session 3: Biophysics and E-C coupling in the pathophysiology of neuromuscular diseases. Chair: Giosuè Annibalini and Biliana Lozanoska-Ochser

***Organ-on-a-chip* modelling of the human neuromuscular junction in both physiological and pathological conditions**
Ersilia Fornetti

Polyglutamine-expanded androgen receptor alters excitation-contraction coupling machinery and calcium dynamics
Caterina Marchioretti

Transverse Tubule Remodeling Enhances Orai1-dependent Ca^{2+} Entry in Skeletal Muscle
Antonio Michelucci

10:25-10:55 Coffee break

10:55-12:25 Session 4: Signaling in muscle growth, homeostasis and diseases. Chair: Giorgia Catarinella and Carles Sanchez Riera

The regulation of protein synthesis and muscle plasticity
Ana Georgia Dumitras

Drp1 promotes mitochondrial transport and repositioning in muscle by enhancing kinesin-1 activation
Matteo Giovarelli

The NF-Y regulome in mouse fetal myoblasts reveals a role of SREBP in differentiation
Roberto Mantovani

Unveiling novel and specific functions of protein kinase CK2 subunits in skeletal muscle differentiation and fusogenic activity
Giorgia Pallafacchina

Adipogenesis of skeletal muscle Fibro/Adipogenic Progenitors is controlled by the Wnt5a/GSK3/β-catenin axis
Alessio Reggio

Evo-Devo approach to study Pax3/7 functions
Valentina Taglietti

13:00 lunch

14:30-16:00 POSTER DISCUSSION

16:00-16:30 Coffee break

16:40 Bus departure: Guided Tour of Montefalco and Dinner at *Cantina Lungarotti*

Saturday, October 19th

09:00-09:40 Lecture 3
Bénédicte Chazaud (Institut NeuroMyoGène Lyon-France)
Roles of macrophages in normal skeletal muscle regeneration versus muscle disease. Chair: Davide Gabellini

09:40-10:40 Session 5: Metabolic alterations and muscle diseases. Chair: Valentina Taglietti and Marlies Corvelyn

Mitochondrial adaptation in parvalbumin knockout muscle fibers
Gaia Butera

Regulation of oxidative metabolism and substrate preferences in aged skeletal muscle by mitochondrial calcium uptake
Gaia Gherardi

Metabolic reprogramming of Fibro/Adipogenic Progenitors facilitates muscle regeneration
Marco Rosina

Identification of a novel TFEB and exercise dependent gene
Davide Steffan

10:20-11:10 Coffee break

11:10-12:55 Session 6: Muscle fibrosis, sarcopenia and cachexia. Chair: Sara Chiappalupi and Giacomo Valli

Direct effects of vitamin D_3 (cholecalciferol) on skeletal muscle
Maraiza Alves Teixeira

rGDF5, an unexpected treatment against age-related muscle mass loss
Sestina Falcone

The role of vitamin D binding protein in the onset of cancer cachexia - beyond vitamin D transport
Nicoletta Filigheddu

A metabolic shift drives cancer cachexia in myotubes
Michele Mannelli

PIN1: a putative molecular target to protect skeletal muscle against age-related muscle loss
Susanna Molinari

Involvement of CerK in skeletal muscle atrophy
Federica Pierucci

Receptor for advanced glycation end-products (RAGE) as a biomarker of muscle wasting in cancer conditions
Aleksandra Vukasinovic

13:00 lunch

14:30-16:00 POSTER DISCUSSION

16:00-16:40 IIM young committee invited lecture
Olivier Pourquié (Harvard Stem Cell Institute Boston-USA)
***In vitro* modeling of human muscle development and disease.** Chair: Gaia Gherardi

16:40-17:10 Coffee break

17:10-18:55 Session 7: Therapeutic approaches for muscle diseases. Chair: Giulia Terribile and Emanuele Mocciaro

A novel 3D culture system as an *in vitro* model for muscle disease
Marianna Cosentino

Use of Sertoli cells to treat DMD patients is supported by their immunomodulatory rather than immunosuppressive effect
Sara Chiappalupi

The voice of patients affected by Duchenne and Becker Muscular Dystrophies
Gloria Antonini

Pterostilbene induces autophagic flux in Collagen VI-deficient muscle
Samuele Metti

Inhibition of Acid Sphingomyelinase as Novel Alternative Therapy for Duchenne Muscular Dystrophy
Paulina Melania Roux-Biejat

A novel wet-spinning system for 3D bioprinting of an artificial skeletal muscle tissue
Stefano Testa

Hitting DMD With a One-Two Punch: a Novel MAOB/VAP1 Inhibitor Greatly Improves the Pathological Phenotype in mdx Mice
Libero Vitiello

19:00-20:00 IIM General meeting

20:30 Social Dinner - Awards and prizes

22:00 Dance Party

Sunday, October 20th

09:30-12:30 High Training lectures*

Departure

*Roundtable discussions and High Training lectures are reserved to participants registered to the High Training Formula.

HIGH TRAINING COURSE IN "ADVANCED MYOLOGY"

Thursday October 17, 2019
Hotel Il Cenacolo

19:30-21:30 *Aperitivo Umbro* and ROUNDTABLE DISCUSSIONS
with keynote speakers and selected IIM PIs

Sunday October 20, 2019
Palazzo Bernabei

9:00 bus to Palazzo Bernabei

9:30-9:55
Olivier Pourquie (Harvard Stem Cell Institute Boston-USA)
Skeletal muscle development in vertebrates

9:55-10:00 Discussion

10:00-10:25
Alessandra Sacco (Sanford Burnham La Jolla-USA)
Exercise-induced injury muscle-stem cell responses

10:25-10:30 Discussion

10:30-10:55
Benedicte Chazaud (Institut NeuroMyoGène Lyon-France)
Exercise-induced injury non-muscle cell responses

10:55-11:00 Discussion

11:00-11:25
Bente Klarlund Pedersen (University of Copenhagen-Denmark)
Hormonal responses to exercise

11:25-11:30 Discussion

11:30-11:55
Marco Narici (University of Padova-Italy)
Neuromuscular adaptation to exercise and inactivity

11:55-12:00 Discussion

12:00-12:30
Lunch

13:00 bus to hotel Il Cenacolo

POSTERS

ALWAYS ON DISPLAY – DISCUSSION:
ODD numbers: Friday, October 18th (14:30-16:00)
EVEN numbers: Saturday, October 19th (14:30-16:00)

P. 01. Lurbinectedin delays the onset of splenomegaly and extends survival of C26 tumor-bearing mice
Giorgio Aquila

P. 02. Oxytocin as a physiological anti-cachectic agent?
Alexandra Benoni

P. 03. Role of STAT3-mediated autophagy in driving muscle regeneration during aging
Giorgia Catarinella

P. 04. Novel approaches to counteract aging by physical intervention
Ester Sara Di Filippo

P. 05. Inhibition of N-glycosylation impairs myoblast differentiation and IGF-1 receptor signalling pathways activation
Laura Di Patria

P. 06. Differential expression and epigenetic modulation of TGF-β target genes in healthy and DMD skeletal muscle cells
Monica Forino

P. 07. miRNAs as potential molecular biomarkers of cancer cachexia
Lorena Garcia-Castillo

P. 08. SCA1 expression variability governs fibro/adipogenic progenitor (FAP) behaviour
Giulio Giuliani

P. 09. SRF is a mechano-transductor in response to exercise and may play a role in the exercise-mediated rescue of muscle homeostasis in cancer cachexia
Hassani Medhi

P. 10. Sphingolipids regulate VDR expression in skeletal muscle cells
Maria Chiara Iachini

P. 11. High levels of circulating Interleukin-6 affect the redox balance in skeletal muscle, inducing the expression of inflammatory-related factors
Carmen Miano*, Laura Forcina*

P. 12. The chromatin remodeling protein WDR5 is required for the aberrant expression of DUX4 in facioscapulohumeral muscular dystrophy
Emanuele Mocciaro

P. 13. Extremely low frequency electromagnetic fields modulate the strength in skeletal muscle in sedentary adult mice
Caterina Morabito

P. 14. Inflammation in dystrophic heart and diaphragm: a comprehensive study
Jacopo Morroni

P. 15. Intracellular attenuation of thyroid hormone influences energy metabolism by reducing mitochondria biogenesis and inducing mitochondrial dysfunction
Annarita Nappi

P. 16. Musclin, a myokine induced by aerobic exercise, retards muscle atrophy during cancer cachexia
Andrea David Re Cecconi

P. 17. The transcription factor NF-Y is required for satellite stem cell proliferation and skeletal muscle tissue repair
Giovanna Rigillo

P. 18. The Thyroid Hormone activating enzyme, Type 2 deiodinase, induces myogenic differentiation by regulating mitochondrial metabolism and reducing oxidative stress.
Serena Sagliocchi

P. 19. Unraveling endurance differences on dystrophy affected muscle groups
Carles Sanchez-Riera

P. 20. Epigenetic tuning of miR in FAP-derived Extracellular vesicles promotes regeneration and inhibits fibrosis in dystrophic muscles
Martina Sandonà

P. 21. Dissecting the role of heterochromatin conformation in muscle aging and sarcopenia
Philina Santarelli

P. 22. Evidence supporting a morpho-functional interaction between telocytes and satellite cells in damaged skeletal muscle
Chiara Sassoli

P. 23. Dissecting the possible role of p97 in muscle wasting during cancer
Giulia Terribile

P. 24. The RNA binding protein FRG1 controls transcription landscape regulating muscle maturation and metabolism
Rossella Tupler

P. 25. Effects of aerobic, resistance and combined exercises on 24-hr glucose variability, metabolism and muscle signalling pathways regulation in Type 1 Diabetics
Giacomo Valli

P. 26. N6-Methyladenosine as a novel pharmacological target involved in muscle regeneration
Francesco Millozzi

POSTER ABSTRACTS

P. 01. Lurbinectedin delays the onset of splenomegaly and extends survival of C26 tumor-bearing mice

Giorgio Aquila[a], Andrea David Re Cecconi[a], Giulia Terribile[a], Roberta Frapolli[b], Ezia Bello[b], Deborah Novelli[c], Lidia Staszewsky[c], Roberto Latini[c], Maurizio D'Incalci[b] , Rosanna Piccirillo[a]
a. Dept. of Neuroscience – **b.** Dept. of Oncology – **c.** Dept. of Cardiovascular Medicine, Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy

Lurbinectedin (PM01183, PM) is a synthetic alkaloid derivate of trabectedin (ET743, ET), a marine-derived anticancer agent. PM is a DNA minor groove covalent binder that has been tested in different Phase I-III trials. It affects tumor microenvironment by limiting the production of inflammatory cytokines. Some of these cytokines are elevated in various cancers characterized by rapid body wasting with muscle, fat and cardiac tissue depletion (i.e. cachexia). Differing from ET, PM displays less liver toxicity, and less endothelial inflammation at the site of injection. Mice injected with murine colon adenocarcinoma C26 cells display cachexia, increased circulating levels of inflammatory cytokines, acute phase response activation and subsequent splenomegaly. Thus, we tested whether PM, at doses with no antitumoral activity on C26 colon adenocarcinoma, has any beneficial effects in mice against C26-induced cachexia. 10-weeks old BALB/c mice were injected subcutaneously with C26 cells and three days later randomized to receive into their tail vein, three times a week for three weeks 0.07 mg/kg PM (n = 8) or vehicle (n = 8). C26-carrying mice showed decreased body weights and premature death. Strikingly, PM was able to extend the lifespan of C26-bearing mice by about 85% from a median survival time of 20 days (range day 10-31) to a median survival time of 37 (range day 14-41). This occurred without affecting tumor growth or food intake or the number of lung metastases. Another set of mice were sacrificed at 10-13 days from C26 implant. PM did not grossly protect multiple tissues (fat, muscle, heart) from cachexia. Preliminary data showed that C26-induced splenomegaly was inhibited by PM administration as long as PM treatment lasted. In C26-bearing mice, this effect exerted by PM seems to be associated also to restrained circulating levels of M-CSF, but not of other inflammatory cytokines (i.e. IL-6, G-CSF or GM-CSF). Further studied are necessary to correlate the improved survival with the pharmacodynamic effect observed.

P. 02. Oxytocin as a physiological anti-cachectic agent?

Alexandra Benoni[1,2]; Medhi Hassani[1,2]; Viviana Moresi[1]; Li Zhenlin[2]; Dario Coletti[1, 2]; Xue Zhigang[2]; Sergio Adamo[1]
1. DAHFMO Unit of Histology and Medical Embryology, and Interuniversity Institute of Myology, Sapienza University of Rome, Italy – **2.** Dept. Of Biological Adaptation and Ageing B2A (CNRS UMR 8256 - INSERM ERL U1164 - UPMC P6), Sorbonne University, France

Oxytocin, classically known for its effects on uterus, lactation, CNS, is also a powerful regulator of myogenic differentiation and muscular homeostasis. Previous works have shown that the addition of OT stimulates differentiation of myogenic precursors and induces myotube hypertrophy. Interestingly, in sarcopenia (senile muscular atrophy) exogenous OT antagonizes skeletal muscle atrophy and restores skeletal muscle trophism in mice. Neoplastic cachexia has a strong negative prognostic significance in cancer patients, being associated with a reduction in quality of life and response to therapies. Several pharmacological and hormonal treatment have been proposed to counteract cachexia, but this syndrome is still incurable. Oxytocin (OT) plays a physiological role in the maintenance of muscle homeostasis in aging. Therefore, we propose to study whether the administration of OT counteracts skeletal musculature atrophy in cancer-cachexia. To mimic cancer cell-mediated effects on muscle cells, we incubated L6 myoblasts with C26 tumor-conditioned medium (C26 CM). Preliminary observations suggest that *in vitro* the inhibition of differentiation by C26 CM is reversed by the addition of OT in the culture medium of myogenic cells. We also showed that *in vivo*, OT counteracts the TNF effects on muscle regeneration of the Tibialis muscle following freeze-injury. Since hampered muscle regeneration and satellite cell function is a key feature of cachexia, contributing to muscle wasting, our preliminary data suggest that indeed OT treatment may have a beneficial effect on muscle homeostasis in tumor bearing mice. Thanks to the fact that OT is already approved for clinical use, this work seems promising to prevent cancer-cachexia skeletal muscle atrophy in patients.

P. 03. Role of STAT3-mediated autophagy in driving muscle regeneration during aging

Giorgia Catarinella[1,2], Andrea Bracaglia[1], Elisa Bisicchia[1], Francesca Di Felice[1], Alessandra Sacco[3], Lucia Latella[1,2]
1. Epigenetics and Regenerative Medicine, IRCCS Fondazione Santa Lucia, Rome – **2.** Institute of Translational Pharmacology, National Research Council of Italy, Rome, Italy – **3.** Development, Aging and Regeneration Program, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA

Age-related neuromuscular diseases are associated with a decline in muscle stem cell (MSC) function and an age-dependent muscle wasting, also called sarcopenia. Despite the clinical and social impact of sarcopenia, the age-related decline of muscle mass and function in not entirely deciphered. It has been demonstrated a key role of STAT3 in regulating MSC expansion and differentiation, extending its use as therapeutic target to

in dystrophic patient is the use of corticosteroids, supporting an important role of the inflammatory compartment in the disease progress. The several and severe adverse effects of long-term corticosteroid treatment also highlight the need for other anti-inflammatory approaches. Our group recently found that pharmaceutical inhibition of Protein Kinase C theta prevented damage in limb skeletal muscle and ameliorated disease when administered in young (2week-old) *mdx* mice, acting predominantly through inhibition of early T cell infiltration of dystrophic muscle. We now plan to study in detail the inflammatory compartment of dystrophic heart and DIA using the mouse model of DMD, *mdx*. We will characterize in detail the kinetics and quality of inflammatory cell populations in *mdx* heart and DIA, using our established 9 color cytofluorimetric protocol, analysing mice from 4 weeks of age up to 11 months. Morphological analysis on muscle fibrosis, necrosis and organization will be performed, as well as molecular analyses to assess cytokines levels and fibrosis markers. Given that *mdx* mice often show a poor heart pathology, we are currently defining an exercise protocol in order to worsen the dystrophic phenotype, in order to mirror the human pathology. The data we will collect in this part of the study will be instrumental to design new pharmacological treatments using specific inhibitors (as we did for skeletal muscle) instead of broad-spectrum anti-inflammatory compounds. We hope that our study will help developing more effective and fine targeted treatments, with less adverse effects for patients

P. 15. Intracellular attenuation of thyroid hormone influences energy metabolism by reducing mitochondria biogenesis and inducing mitochondrial dysfunction

Annarita Nappi[a], Annunziata Gaetana Cicatiello[a], Serena Sagliocchi[a], Caterina Miro[a], Emery Di Cicco[a], Giuseppina Mancino[a], Domenico Salvatore[b], Monica Dentice[a]

a. Dept. of Clinical Medicine and Surgery – **b.** Dept. of Public Health, University of Naples Federico II, Italy

Thyroid hormone (TH) has a major role in the control of systemic metabolism, influencing carbohydrate, protein and lipid metabolism. Moreover, T3 regulates mitochondrial function and turnover controlling mitochondrial biogenesis, proton leak, OXPHOS and ROS generation. The deiodinases enzymes enable intracellular TH activation or inactivation, regardless of circulating hormone levels. To address the physiological function of deiodinases in skeletal muscle metabolism, we used muscle-specific gain-of-function approaches. Here, we show that muscle-specific hypothyroidism in mice by D3 overexpression (TG-D3 mice) induces a metabolic reprograming of muscle fibers reducing glycolytic and lipid metabolism. D3 overexpression in muscle also reduces mitochondrial dynamics. Immunofluorescence analysis revealed a structural alteration of mitochondria. FACS analysis highlighted a reduced mitochondrial density with increased size. However, the increased mitochondrial size did not correlate with increased activity. Consistently with these findings, mitochondria turnover genes DRP-1, MFN-1 and OPA-1, as well as PGC-1α, were reduced in TG-D3 muscles. These data suggest that metabolic differences between TG-D3 and control muscles are associated with an alteration in number and function of the mitochondria. Interestingly, the overexpression of PGC-1α in TG-D3 muscles rescued the mitochondrial defects, reactivating the expression of genes involved in mitochondrial fusion and fission. Altogether these results indicate that the mitochondrial dysfunction of TG-D3 muscles is mediated by the downregulation of PGC-1α, the master regulator of mitochondrial biogenesis. Our findings indicate that muscle-specific hypothyroidism via D3 overexpression potently impacts on mitochondrial metabolism, identifying the deiodinases as critical metabolic regulators. Understanding the mechanisms of action of deiodinases in metabolism might be relevant for therapeutic treatment of metabolic disorders.

P. 16. Musclin, a myokine induced by aerobic exercise, retards muscle atrophy during cancer cachexia

Andrea David Re Cecconi[a], Mara Forti[a], Michela Chiappa[a], Luigi Cervo[a], Luca Beltrame[b], Sergio Marchini[b], Rosanna Piccirillo[a]

a. Dept. of Neurosciences – **b.** Dept. of Oncology, Mario Negri Institute for Pharmacological Research IRCCS, Milan, Italy.

Background: Physical activity ameliorates the prognosis of cancer patients, also by contrasting the associated muscle wasting (i.e. cachexia). Since aerobic exercise seems to be the most effective to preserve muscles during cancer, we asked whether it promotes secretion of proteins by muscles (i.e. myokines) that may contrast cachexia. Methods: To mimic aerobic exercise, we infected C2C12 myotubes with PGC1α expressing adenoviruses. *In vitro* we evaluated the effects of supernatants from GFP or PGC1α-overexpressing cells on protein synthesis and degradation of atrophying myotubes and in LucAssay. By microarray, we identified putatively secreted proteins inducible by PGC1α and confirmed by Q-PCR. We measured by Q-PCR their expression in *Tibialis Anterior* (TA) muscle of C26-bearing mice during cachexia and plasma levels by ELISA. To induce aerobic exercise adaptations, mice were run on treadmill. Anaerobic exercise-like effects were obtained in vivo in overloaded Plantaris muscle and *in vitro* in myotubes expressing myristoylated AKT. Results: Our microarray and Q-PCR analyses showed musclin as a PGC1α-induced myokine. Conversely, its expression was unchanged in myotubes hypertrophying because of activated AKT. Dexamethazone-treated myotubes or constitutively active (ca)FoxO3-expressing ones undergo atrophy as measured by increased

proteolysis and MuRF1 induction. Unlike to GFP, musclin restrained the dexamethazone-induced MuRF1 expression in Luciferase assays. Consistently, musclin-containing supernatants reduced the caFoxO3-induced rates of long-lived protein degradation. Among the newly identified PGC1α-induced myokines, we found that only musclin (and its receptor Npr3) was strongly downregulated in cachectic muscles and plasma of C26-bearing mice. Thus, we electroporated TA of C26-bearing mice with musclin or Npr3-encoding plasmids and found either musclin or Npr3 to preserve fiber area. Interestingly, treadmill exercise protected C26-bearing mice from muscle loss, with no effect on tumor growth, and rescued the C26-induced downregulation of musclin in muscles and plasma. By contrast, musclin expression did not change in overloaded Plantaris of adult mice. Conclusions: Musclin is a myokine induced specifically by PGC1α, typically increased upon aerobic exercise and preserves muscles from wasting during C26 growth or myotubes from atrophy. Overall, musclin could be beneficial to cancer patients that cannot exercise and are at risk of developing cachexia.

P. 17. The transcription factor NF-Y is required for satellite stem cell proliferation and skeletal muscle tissue repair

Giovanna Rigillo, Valentina Basile, Silvia Belluti, Carol Imbriano
Dept. of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy

Reconstruction of the skeletal muscle tissues after damage relies on the activation of Satellite Cells (SCs), a population of resident muscle stem cells. Several transcription factors are involved in SCs activation and proliferation and ensure proper temporal and spatial expression of muscle-specific genes during muscle regeneration. The transcription factor NF-Y, composed by NF-YA, NF-YB and NF-YC subunits, has an important role in the regulation of cellular proliferation and differentiation in different cell types, among which muscle cells. While NF-YA, the DNA binding subunit of NF-Y, is down-regulated in the adult muscle of WT mice, its expression is observed in the mdx mouse and correlates with euchromatic markers and expression of NF-Y target genes controlling cell growth. With the aim to investigate the role of NF-YA in the SCs proliferation and differentiation, we generated and characterized a conditional knock out mouse model in which NF-YA is deleted in Pax7+ SCs by Tamoxifen induction in adult NF-YA$^{flox/flox}$:Pax7^{CreER} mice (NF-YA cKO). Cellular and molecular analysis carried out on isolated myofibers and SCs from WT and NF-YA cKO mice highlighted that NF-Y activity is important for the maintenance of SCs homeostasis. NF-YA loss depletes Pax7+ SCs pool and impairs their proliferation. Moreover, SCs-mediated regeneration following muscle damage induced by cardiotoxin is delayed in NF-YA cKO. The effect of NF-YA abrogation was also explored in post-natal muscle growth. Immunohistological analysis showed defects in muscle morphology and a decrease in SCs number in 3 weeks aged NF-YA cKO mice, period of major increment of muscle mass by SCs-mediated myonuclear accretion. The molecular mechanism underlying the impairment of SCs activity following NF-YA loss was investigated by gene expression profiling. Overall, our results highlight a role of NF-Y in muscle regeneration and in SCs fate, whose modulation could be useful to improve stem cell based therapies to treat muscular dystrophies.

P. 18. The Thyroid Hormone activating enzyme, Type 2 deiodinase, induces myogenic differentiation by regulating mitochondrial metabolism and reducing oxidative stress.

Serena Sagliocchi[a], Annunziata Gaetana Cicatiello[a], Emery Di Cicco[a], Caterina Miro[a], Annarita Nappi[a], Giuseppina Mancino[a], Domenico Salvatore[b], Monica Dentice[a].
a. Dept. of Clinical Medicine and Surgery – **b.** Dept. of Public Health, University of Naples Federico II, Naples, Italy

Thyroid hormone (TH) is a key metabolic regulator that acts by coordinating short-term and long-term energy needs. Accordingly, significant metabolic changes are depending on thyroid status. Although it is established that hyperthyroidism augments basal energy consumption, thus resulting in enhanced metabolic state, the net effects on cellular respiration and generation of reactive oxygen species (ROS) remain unclear. To elucidate the effects of augmented TH signal in muscle cells, we generated a doxycycline-inducible cell line, in which the expression of the TH-activating enzyme, type II deiodinase (D2) is reversibly turned on by the “Tet-ON” system. Interestingly, increased intracellular TH caused a net shift from Oxidative Phosphorylation (OXPHOS) to glycolysis and a consequent increase in extracellular acidification rate. As a result, the mitochondrial ROS production, and both the basal and doxorubicin-induced production of cellular ROS were reduced. Importantly, the expression of a set of antioxidant genes was up-regulated, and, among them, the mitochondrial scavenger SOD2 was specifically induced at transcriptional level, by D2-mediated TH activation. Finally, we observed that the attenuation of the oxidative stress and increased levels of SOD2 are key elements of the differentiating cascade triggered by the TH and D2, thereby establishing that D2 is essential in coordinating metabolic reprogramming of myocytes during myogenic differentiation. In conclusion, our findings indicate that TH plays a key role in oxidative stress dynamics by regulating ROS generation. Our novel finding that TH and its

P. 23. Dissecting the possible role of p97 in muscle wasting during cancer

Giulia Terribile[a], Andrea David Re Cecconi[a], Giorgio Aquila[a], Andrea Degiorgi [b], Mara Forti[a], Rosanna Piccirillo[a]

a. Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy – **b.** Università degli Studi di Parma, Italy

Half of patients with malignancies develops muscle wasting (i.e. cachexia) and aerobic exercise ameliorates their prognosis. The p97/VCP ATPase complex interacts with multiple binding proteins, estimated to be around 60, and mainly extracts proteins from multimeric structures. In particular, by interacting with Ufd1 or p47, it facilitates the rapid degradation of myofibrillar proteins during muscle atrophy caused by denervation or fasting. The aim of this study was to investigate if p97 (and through which of its adaptors) plays a role also during cancer cachexia and if this is modulated by physical exercise. To induce cachexia, we injected subcutaneously one million of colon adenocarcinoma (C26) cells in BALB/c mice. This tumour causes massive muscle depletion with premature death in mice. Interestingly, by microarrays, we found that 8 out of 58 p97-binding proteins were induced in cachectic Tibialis Anterior (TA) from C26 mice, while 10 were reduced ($p<0.05$). Further analyses of these adaptors are in progress. To understand if aerobic exercise improves cancer cachexia through p97 modulation in muscle, C26-bearing mice were run on treadmill for 5 days at 12 m/min and +15° inclination for 45 min/day. By Q-PCR or Western Blotting, we measured the expression of p97 and its main adaptor proteins (Ufd1, Ufd2, p47) in cachectic TA muscle. *In vivo*, we found that the mRNA levels of p97, Ufd1, Ufd2 and p47 were induced in cachectic TA muscle from C26-carrying mice, undergoing body weight loss (i.e. cachexia). Interestingly, treadmill exercise protected C26-bearing mice from gastrocnemius muscle loss, with no effect on tumour growth, and rescued the C26-induced upregulation of p97 transcripts but not of these adaptors in muscles. Our preliminary data suggest that p97/VCP ATPase may play a role in muscle wasting also during cancer in mice. It remains to address which adaptor may be implicated and whether shRNA for p97 is able to recapitulate the beneficial effects of aerobic exercise *in vivo*.

P.24 The RNA binding protein FRG1 controls transcription landscape regulating muscle maturation and metabolism.

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Facioscapulohumeral muscular dystrophy (FSHD), the third most common hereditary myopathy, is not due to a classical mutation within a protein-coding gene. Instead, almost all FSHD patients carry deletions of an integral number of tandem 3.3-kilobase repeat units, termed D4Z4, located on chromosome 4q35. D4Z4 deletion leads to modifications of chromatin structure and inappropriate overexpression of 4q35 genes. Studies have proposed several candidate genes within this genomic region and several mouse models have been generated. Among these models, mice overexpressing *Facioscapulohumeral muscular dystrophy Region Gene 1 (FRG1)* present a progressive myopathy that recapitulate features of human disease. *FRG1* encodes for an RNA binding protein whose biological function is not well understood. To investigate the molecular mechanism triggered by *FRG1* overexpression leading to overt myopathy we analyzed molecular changes occurring during disease development. At first, gene expression profiles of skeletal muscles of mice overexpressing increasing levels of *FRG1* were examined at 28 days (dystrophy onset) and at 96 days (full dystrophy). We found a profound transcriptional deregulation correlating the severity of the muscle phenotype and *FRG1* expression. Gene Set Enrichment Analysis and Gene Ontology revealed alterations in pathways related to muscle function, energy metabolism and inflammation. Indeed, genes associated with adult and normal myogenesis were down-regulated with a significant enrichment of genes specifically expressed during embryogenesis. By contrast, we observed the incremental activation of inflammatory pathways through time. We found that *FRG1* overexpression causes the global perturbation in the mechanisms that guide postnatal muscle maturation. This process includes the anomalous expression of embryonic/neonatal proteins fundamental for muscle structures and metabolic pathways, the lack of mature proteins and the lag of muscle growth throughout postnatal life. In particular at 7 days and 14 days the expression of the embryonic isoforms of myosin remain high in *FRG1* mice instead of following the physiological down-regulation occurring in WT mice, meanwhile the expression of the mature isoforms is reduced. Starting from 14 days we observed the deceleration of body weight growth curve and a reduction of muscle cross-sectional area. Moreover, *FRG1* muscles displayed the significant reduction of ATP and the phosphocreatine in association with the transcriptional downregulation of Glut4, HK2 and AldoA. Our results indicate that *FRG1* overexpression induce

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Assisi, 20/10/2019

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This is to certify that **Giulia Terribile** attended the IIM Meeting 2019 held in Assisi, Italy, from 17 to 20 October 2019.

On behalf of the Organising Committee

Dr Davide Gabellini
Legale Rappresentante Istituto Interuniversitario di Miologia