iim
Interuniversity Institute of Myology

XI Annual Meeting
2- 5 October 2014

TOPICS

- *Genetic and epigenetic regulation of muscle homeostasis and differentiation*
- *Muscle atrophy*
- *Myology and sport medicine*
- *Muscle physiology, biophysics and E-C coupling*
- *Endocrinology of aging and Sarcopenia*
- *Muscle wasting and cancer cachexia*
- *Muscular dystrophies and related diseases*
- *Satellite cells, tissue engineering and muscle regeneration*
- *Novel routes towards the 'heart' of myogenesis*

Scientific Committee:
Barbieri E, Blaauw B, Fulle S, Gabellini D, Grassi F, Mammucari C, Musarò A, Protasi F, Puri PL, Sampaolesi M, Sandri M, Sorci G.

Main lectures:
Giuseppe De Vito (University College, Dublin)
Feliciano Protasi (University of Chieti)
Maurilio Sampaolesi (University of Pavia)
Marco Narici (University of Nottingham)

With the support and Scientific contribution of:
Duchenne Parent Project

Venue: Borgo San Luigi; Monteriggioni (Siena)

iim secretary
e-mail: fisiologia@unich.it
D'Alfonso Antonella

Session 1. NMJ, Signaling and Differentiation

01. LOCAL EXPRESSION OF SOD1^{G93A} MUTANT PROTEIN TRIGGERS NEUROMUSCULAR JUNCTION DISMANTLEMENT

G. Dobrowolny 1,2, M. Martini 1,2, V. Romanello 3, S. Boncompagni 4, L. Pietrangelo 4, M. Bouchè 2, R. Rudolf 5, F. Protasi 4, M. Sandri 3,6, A. Musarò 1,2,7

1 Center for Life Nano Science@Sapienza, Italian Istitute of Tecnology.
2 DAHFMO-Unit of Histology and Medical Embryology, Institute Sapienza University of Rome, Italy.
3 Dept Biomedical Sciences, University of Padova, Italy. Venetian Institute of Molecular Medicine, Dulbecco Telethon Institute, Padova, Italy.
4 CeSI – Center for Research on Aging – Dept Neuroscience, Imaging and Clinical Sciences, University of Chieti, Italy.
5 Institut fur Angewandte Informatik, Karlsruhe Institute of Technology, Institut fur Toxikologie und Genetik, Karlsruhe Institute of Technology, Institut fur Molekular und Zellbiologie, University of Applied Sciences Mannheim, Institut fur Medizintechnologie, University of Applied Sciences Mannheim and University of Heidelberg, Germany.
6 Institute of Neuroscience, Consiglio Nazionale delle Ricerche, Padova, Department of Medicine, McGill University, Montreal, Canada, Telethon Institute of Genetics and Medicine (TIGEM), Napoli, Italy.
7 Pasteur Cenci-Bolognetti, Rome Sapienza University, Italy.
E-mail: gabriella.dobrowolny@uniroma1.it

The alteration of Reactive Oxygen Species (ROS) homeostasis plays a causal role in several chronic pathology such as aging and neurodegenerative disease. such as Amyotrophic Lateral Sclerosis (ALS). Although it is recognized that axon and synapses are first cellular sites of degeneration in ALS disease, controversy exists on whether pathological events initially begin at the NMJs and then, in a dying back phenomena, contribute to motor neuron degeneration. Moreover, the precise molecular mechanisms of pathology-associated deterioration in neuromuscular system have remained elusive. Here we provide evidences that muscle specific accumulation of SOD1^{G93A} in the transgenic mice model MLC/SOD1^{G93A} induces mitochondria dysfunction and triggers NMJ dismantlement. Further, we demonstrate that treatment of MLC/SOD1^{G93A} mice with Trolox, a potent antioxidant, is sufficient to rescue mitochondria and NMJ defects in the MLC/SOD1^{G93A} mice, stabilizing muscle-nerve connection. The analysis of potential molecular mechanisms that mediate the toxic activity of SOD1 revealed the activation of specific Protein Kinase as a downstream player of NMJ dismantlement. Overall our data demonstrate that muscle specific expression of SOD1^{G93A} mutation causes mitochondrial impairment and NMJ dismantlement, suggesting that muscle defects and NMJs alteration precede motor neuron degeneration rather than resulting from it.

02. SIGNALING PATHWAYS AT THE NEURO-MUSCULAR SYNAPSE AND WITHIN MUSCLE FIBERS

S. Hashemolhosseini

Institut für Biochemie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany
E-mail: said.hashemolhosseini@fau.de

Myopathies are defined as disorders that end up in muscle weakness. At neuromuscular synapses CK2 phosphorylates MuSK, thereby stabilizing neuromuscular synapses. Conditional mice lacking the beta-subunit of CK2 in muscle fibers develop a decrease of grip strength. However, in mutant mice the reason for the severity of grip strength loss is not clear and its clarification should yield insights into mechanisms involved in myopathies. Here, we show that in mutant muscle fibers impaired mitochondrial metabolism contributes to muscle weakness. In disease, Wnt signaling pathways are associated with carcinomas, but are also involved in synaptic neurodegenerative disorders. Moreover, members of Wnt signaling pathways have been identified at neuromuscular synapses (Wnt3/3a, Wnt4, Wnt11r, APC, Dishevelled, beta-Catenin). Nevertheless, it is almost unknown which of the Wnt signaling pathways are active at either forming neuromuscular synapses, for their maintenance, or involved in neuromuscular disorders. The participation of the 'canonical' and/or 'planar cell polarity' pathway have been discussed, but up-to-now none of the others, like the 'calcium', 'frizzled nuclear import', or 'divergent canonical' pathway. In order to identify Wnt signaling pathways at neuromuscular junctions (NMJ) or within muscle fibers, we used a Wnt canonical reporter mouse line to detect full response of the Wnt canonical pathway at NMJs of mice.

*****

03. THE ROLE OF CONDUCTIN/AXIN2 AT NEURO-MUSCULAR JUNCTIONS

Danyil Huraskin, Nane Eiber, Bojana Kravic, Said Hashemolhosseini

Institut für Biochemie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany
E-mail: said.hashemolhosseini@fau.de

Wnt signaling in healthy organisms is essential for many developmental processes. Emerging evidence indicates its role in synapse formation and maintenance. Canonical Wnt signaling induces target gene transcription, due to inhibition of GSK3-beta-dependent beta-catenin phosphorylation and beta-catenin proteasomal removal, enabling beta-catenin to enter nucleus and stimulate LEF/TCF targets. GSK3-beta is part of a destruction complex additionally composed of APC, Axin 1 or Axin2. Here, we detected an increase of Axin2, a known target of LEF/TCF, while C2C12 cells transit from myoblasts to myotubes. Therefore, we investigate the role of Wnt canonical signaling for neuromuscular junction formation and maintenance by modulating Axin1 and Conductin/Axin2 expression.

04. THE TEMPORAL AND SPATIAL EXPRESSION PATTERN OF CONDUCTIN/AXIN2 IN SKELETAL MUSCLE FIBERS

Nane Eiber, Danyil Huraskin, Bojana Kravic, Said Hashemolhosseini

Institut für Biochemie, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany
E-mail: said.hashemolhosseini@fau.de

Next to APC and GSK3beta, ubiquitously expressed Axin1 is part of the destruction complex that leads to removal of beta-catenin by GSK3-beta mediated phosphorylation. Upon binding of Wnt to its receptor, Dishevelled disrupts this complex, beta-catenin accumulates and translocates to the nucleus where it stimulates LEF/TCF target gene transcription. Conductin/Axin2 expression pattern is known to be more restricted. Here, we aimed to identify the expression pattern of Conductin/Axin2 and to compare it with the expression pattern of Axin1 in skeletal muscle fibers. To this end, we analyzed diaphragms of embryonic and adult mice by in situ hybridization with riboprobes. Moreover, we analyzed Conductin/Axin2 expression using a Conductin/Axin2-lacZ-reporter mouse model.

*****

05. INTERACTION OF PINK1 AND TOM22 IS LINKED TO MYOPATHY

Bojana Kravic (1), Angelika Harbauer (2), Luca Simeone (1), Vanina Romanello (3), Andreas Buttgereit (4), Winfried Neuhuber (5), Marion Straubinger (1), Dieter Heuss (6), Rüdiger Rudolf (7), Oliver Friedrich (4), Marco Sandri (3), Chris Meisinger (2), Said Hashemolhosseini (1)

(1) Institut für Biochemie, (4) Medizinische Biotechnologie, (5) Anatomisches Institut, (6) Neurologische Klinik, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany; (2) Institut für Biochemie und Molekularbiologie, ZBMZ, Universität Freiburg, Germany; (7) University of Applied Sciences Mannheim, Germany; (3) Dulbecco Telethon Institute at Venetian Institute of Molecular Medicine, Padova, Italy
E-mail: said.hashemolhosseini@fau.de

Previously, our lab identified protein kinase CK2 interacting with the receptor tyorsine kinase MuSK at neuromuscular junctions (NMJ). In the absence of CK2-beta, conditional skeletal muscles of mice fail to phosphorylate MuSK which ends up in significant fragmentation of acetylcholine receptor aggregates at NMJs. Additionally, we now observed mutant muscles less able to phosphorylate Tom22, as part of the mitochondrial protein import translocase. Pink1, mutants of which are known to be the cause of Parkinsons disease, is also imported into healthy mitochondria, but accumulates and labels damaged mitochondria. Here, we detect a Pink1 and Tom22 interaction and mapped the responsible epitopes of both proteins. Moreover, Pink1-Tom22 binding is influenced by the CK2-dependent phosphorylation status of Tom22.

*****

Session 2. Muscle growth and atrophy

06. THE ROLE OF THE MITOCHONDRIAL CALCIUM UNIPORTER IN THE CONTROL OF SKELETAL MUSCLE MASS

G. Gherardi 1, A. Raffaello 1, G. Pallafacchina 1,2, I. Zamparo 1, A. Braga 1, D. De Stefani 1, L. Zentilin 3, M. Giacca 3, C. Mammucari 1, R. Rizzuto 1,2

1 Department of Biomedical Science, University of Padova, Padova, Italy.
2 Institute of Neuroscience, Consiglio Nazionale delle Ricerche, Padova, Italy.
3 Molecular Medicine Laboratory, International Centre for Genetic Engineering and Biotechnology, Trieste, Italy.
E-mail: rosario.rizzuto@unipd.it

The second messenger Ca^{2+} plays a key role in mitochondrial function: cytosolic Ca2+ transients, generated by physiological stimuli, elicit mitochondrial Ca2+ (mtCa2+) uptake which stimulates aerobic metabolism. Accordingly, high amplitude increases in mt [Ca2+] are detected in skeletal muscle mitochondria during contraction. The highly selective channel responsible for Ca2+ entry into mitochondria is the Mitochondrial Calcium Uniporter (MCU), whose molecular identity has been described three years ago. More recently the role of MICU1 and MICU2 has been disclosed. MICU1 and MICU2 are direct modulators of the pore-forming subunit (MCU) with opposite effects on channel activity, and form a regulatory dimer. Importantly, mutations of MICU1 have been identified in individuals with a disease phenotype characterized by proximal myopathy, learning difficulties and a progressive extrapyramidal movement disorder. Here we show that MCU is both required and sufficient for muscle mass maintenance and that MCU exerts a protective effect against atrophy. Thus we suggest that MCU plays a crucial role in muscle trophism and therefore represents a possible target of clinical intervention.

*****

07. ADAPTIVE RESPONSE OF SOLEUS MUSCLE AFTER CRUSH DENERVATION AND DURING REINNERVATION: PRELIMINARY RESULTS

Eleonora Bardi 1, Stefano Sartini 2, Riccardo Cuppini 2, Roberto Bottinelli 1, Maria Antonietta Pellegrino 1

1 Dept Molecular Medicine, University of Pavia, Pavia, Italy.
2 Dept Earth, Life and Environmental Sciences, Section of Physiology, Urbino, Italy.
E-mail: eleonora.bardi@unipv.it

BDNF is a neurotrophin known to be involved in axon sprouting in the CNS.It is known that exercise may alter the expression ofdifferent neurotrophins. Chronic treadmill mid-intensity running is able to increase multiple innervation percentage through BDNF. Possibly, others neurotrophinsmay contributeto sprouting induction. Peripheral nerve injury causes skeletal muscle inactivity and atrophy. Re-innervation promotes muscle mass recovery, but few data are available about the pathways supporting such process. BDNF stimulates protein synthesis byactivating Akt/mTOR/p70S6K pathway through TrkB receptor onmuscle membrane. In order to understand a) the

involvement of otherneurotrophins in multiple innervation 2) the potential role of neurotrophins in controlling skeletal muscle mass we investigated the time course (3,7,10 days) of denervation and reinnervation, which start at 4 days, with and without exercise.We found increased NT-4 and IGF-1 expression in exercised denervatedrats soleussuggesting their possible trophic role on motoneurons. Moreover, we found a loss of muscle mass after denervation and during re-innervation that persist also after exercise. Consistent with this, P-Akt, P-S6 and P-4E-BP1 levels were lower in denervated soleus compared to un-denervated control; reduction of P-4E-BP1 persisted during re-innervation also with exercise. Denervation induced an impairment of autophagy (beclin-1, Chatepsin-L) that persist during reinnervation. With exercise we observed a reestablishment of control values. Our results suggest a possible role of NT-4 and IGF-1, together with BDNF, in determining the increased multiple innervation observed with this exercise. The duration of our protocol probably was not enough to protect soleus from denervation andreinnervation atrophy.

*****

08. S6K IS REQUIRED FOR AKT-INDUCED INCREASES IN MUSCLE FUNCTION, BUT NOT FOR MUSCLE GROWTH

Manuela Marabita 1, Martina Baraldo 1, Francesca Solagna 1, Judith Ceelen 1, Roberta Sartori 1, Hendrik Nolte 2, Ivan Nemazanyy 3, Marcus Kruger 2, Mario Pende 3, Bert Blaauw1,4 #

1 Venetian Institute of Molecular Medicine (VIMM), Via Orus 2, 35129, Padova, Italy.
2 Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany.
3 Institut Necker-Enfants Malades, Inserm, Université Paris Descartes, CS 61431, Paris, France.
4 Department of Biomedical Sciences, 35137, University of Padova, Italy.
E-mail: manuela.marabita@gmail.com

One of the key intracellular pathways regulating adult skeletal muscle mass is the Akt-mTORC1-S6K1 pathway. We previously showed that activation of Akt is sufficient to rapidly induce a functional muscle hypertrophy. One of the key downstream mediators of Akt is the complex mTORC1, as its inhibitor rapamycin strongly reduces Akt-induced hypertrophy. While in myotubes it has been shown that S6K1 is the rapamycin-sensitive mTORC1 target regulating myotube size, the role for S6K1 in adult skeletal muscle hypertrophy is still unknown. We clearly show that S6K1, S6K2 or the phosphorylation of ribosomal protein S6 is not required for Akt-induced muscle hypertrophy. In order to better understand the functional role of S6K1 in adult muscle hypertrophy we generated a new transgenic mouse in which we can inducibly activate Akt in skeletal muscles of S6K1 k.o. mice. Activation of Akt in S6K1 k.o. mice leads to a 60% increase in fiber size which is accompanied by the presence of fiber degeneration. Furthermore, muscle force production in these hypertrophic Akt-S6K1 k.o. muscles is compromised. Possibly, part of this myopathic phenotype is due to the lack of ribosome biogenesis in the absence of S6K1, making it problematic to maintain elevated rates of protein synthesis and translation fidelity. Indeed, treatment of Akt-S6K1 mice with rapamycin reduces rates of protein synthesis, blunting muscle hypertrophy, but preventing the drop in normalized force. Overall we can conclude that S6K is not required for muscle mass, but for maintaining muscle function during hypertrophy.

*****

09. THE ROLE OF RAPTOR IN ADULT SKELETAL MUSCLE

Martina Baraldo, Marco Sandri, Bert Blaauw
Venetian Institute of Molecular Medicine (VIMM), Via Orus 2, 35129, Padova, Italy.
E-mail: : martina.baraldo@studenti.unipd.it, marco.sandri@unipd.it, bert.blaauw@unipd.it

Mammalian target of rapamycin (mTOR) plays a central role in cell growth. mTOR assembles into two distinct multiprotein complexes, namely the rapamycin-sensitive complex mTORC1 and the rapamycin-insensitive complex mTORC2. One of the key members of the mTORC1 complex is a. 150kDa protein called Raptor, which has been shown to be able to recruit mTOR substrates S6K1 and 4EBP1 on mTORC1. Mice lacking Raptor only in skeletal muscle from birth show a pronounced myopathy leading to a premature death. However, treating adult mice with the specific mTORC1 inhibitor rapamycin does not lead to a myopathic phenotype, and even improves muscle physiology in aged mice. Here we want to examine the role of Raptor and mTORC1 using a new CreER-inducible transgenic mouse in which we can delete Raptor in muscles of adult mice (Raptor k.o.). Activation of Cre by treatment with tamoxifen leads to a rapid loss of Raptor transcript and protein levels. Also the phosphorylation levels of ribosomal protein S6, a known mTORC1 target, are strongly reduced in Raptor ko mice. One month after Raptor deletion, muscle weight and basic histology are unchanged. Considering the important role of mTORC1 in the regulation of adult skeletal muscle mass, we are currently examining the role of Raptor both in muscle atrophy and hypertrophy. From preliminary experiments it seems that Raptor plays an important role in the regulation of autophagy, but might not be required for muscle hypertrophy.

*****

10. UNACYLATED GHRELIN INHIBITS DEXAMETHASONE-INDUCED SKELETAL MUSCLE ATROPHY IN MICE

Agoni Valentina 1, Minetto Marco 2, Bottinelli Roberto 1, Canepari Monica 1

1 Department of Molecular Medicine, University of Pavia, Italy.
2 Department of Internal Medicine, Division of Endocrinology, Diabetology and Metabolism, Molinette Hospital, University of Turin, Italy.
E-mail: valentina.agoni@unipv.it

To understand the triggering mechanisms of steroid myopathy we studied the adaptations to dexamethasone (DEX) administration (5mg/Kg/day) in vastus lateralis (VL) and soleus (Sol) muscles of adult mice at different times (1, 3, 10 hours and 3, 7 and 15 days). It has been demonstrated that the unacylated form of ghrelin (UAG), a peptide hormone that stimulates GH release, is able to counteract

dexamethasone effects on cultured myoblasts. Therefore, the effects of administration of UAG (100ug/Kg/day) during the onset of steroid myopathy was evaluated. Significant fibers atrophy in VL was found after 7 (13%) and 15 days (31%) of DEX treatment, whereas in Sol atrophy (20%) was found only after 15 days. Significant up-regulation of MuRF-1 was found after 1, 3 and 10 hours of DEX treatment in VL and after 1 hour in Sol. A significant up-regulation of myostatin was found after 1, 3 and 10 hours of DEX treatment in both VL and Sol. UAG administration blunted the atrophy observed after 15 days of DEX treatment in VL (14% lower) and reversed atrophy in Sol. In both muscles UAG prevented MuRF-1 and myostatin up-regulation after 1 and 3 hours, but failed to prevent the induction after 10 hours of DEX treatment. Results suggest that: a) in the early phases of steroid myopathy atrophy is supported by the activation of the ubiquitin-proteasome system and by the induction of myostatin b) UAG exhibits effects capable to counteract steroid atrophy c) UAG has short-term effects.

*****

11. HSP60 EXPRESSION IN SKELETAL MUSCLE INCREASES AFTER ENDURANCE TRAINING

Barone Rosario 1,2, Macaluso Filippo 1,2, Sangiorgi Claudia 1,2, Moresi Viviana 3, Coletti Dario 3,4, Adamo Sergio 3, Francesco Cappello 1,2, Farina Felicia 1, Zummo Giovanni 1, Di Felice Valentina 1.

1 Dipartimento di Biomedicina Sperimentale e Neuroscienze Cliniche, Università degli Studi di Palermo, Italy.
2 Euro-Mediterranean Institute of Science and Technology (IEMEST), Palermo, Italy;
3 Dipartimento di Scienze Anatomiche, Istologiche, Medico-legali e dell'Apparato Locomotore, SAPIENZA Università di Roma, Italy.
4 Laboratory of Genetics and Physio-pathology of Muscle Tissues, UR4 - Ageing, Stress, Inflammation, University Pierre et Marie Curie Paris 6, France.
E-mail: rusbarone@hotmail.it

Adult skeletal muscle is remarkably plastic. Increased contractile activity, such as endurance exercise, elicits multiple signals to activate a large set of genes, leading to phenotypic changes in skeletal muscle, including IIb-to-IIa fiber type switching, enhanced mitochondrial biogenesis, and angiogenesis, to match physiologic capability to functional demand. Heat shock protein (Hsp) 60 is a mitochondrial protein, which plays a key role in the translocation of proteins from the cytoplasm to mitochondria. Apart from its mitochondrial localization, Hsp60 has been detected in the cytoplasm, in the cellular membrane and inside exosomes [1]. In the skeletal muscle the expression of Hsp60 is fibre type specific, being expressed more in type IIa and I fibers compared to IIx and IIb. Upon endurance training Hsp60 increases particularly in type I fibers (unpublished data). To investigate the role of Hsp60 in skeletal muscle plasticity and mitochondrial biogenesis, we used three experimental models: 1) Forty-eight trained young healthy male mice; 2) in vitro C2C12, where Hsp60 was over expressed by plasmids or repressed by siRNAs; 3) in vivo transfected muscles where Hsp60 was over expressed by plasmids and inhibited by siRNAs.

Reference: [1] Campanella et al. (2012) The odyssey of Hsp60 from tumor cells to other destinations includes plasma membrane-associated stages and Golgi and exosomal protein-trafficking modalities. PLoS One 7: e42008.
This work was supported by MIUR - PRIN 2012 grant.

*****

Scientists meet Companies

Isolation of functional satellite cells using automated tissue dissociation and magnetic cell separation

Dr. Christoph Hintzen

Miltenyi Biotec srl
E-mail: christophh@miltenyibiotec.de

Keynote Lecture

Sport Medicine and Myology: The Exercise Prescription for the Older Individual

Giuseppe De Vito
University College Dublin, Ireland, UCD Institute for Sport and Health.
E-mail: giuseppe.devito@ucd.ie

According to the most recent projections the number of older European citizens (>65 years) will increase from 87 million in 2010 to 148 million in 2060. Aging unfortunately is associated with a generalized deterioration of all physiological functions and in general between the age of 30 and 70 it is normal to observe a 25-30% decline in most functional capacities. In this context it is imperative to implement a strategy directed to maintain and improve both health and independence in the entire life span. The concept of independence includes the ability to remain mobile and to perform proficiently all the activities of daily living, such as: raising from a chair, climbing stairs or carrying and lifting shopping bags, all activities which require adequate level of muscle strength, cardio-respiratory fitness and also of postural balance and flexibility. In addition, there is now also a large and mounting evidence showing that increased fitness is associated to better cognitive function. Luckily, also in advanced age, an appropriate exercise-training could bring at least a partial recovery of the lost functional capacities. The most adopted guidelines recommend for an adult to perform about 30 minutes of moderate physical activity 4-5 times per week. It has also emerged that, on a given day, these 30 minutes may be accumulated in several short bouts dispersed throughout the day rather than in one continuous bout, without reducing training efficacy. This is a particularly relevant point to consider as accumulating short periods of exercise during the day is more palatable to the general population, hence improving long term adherence to this lifestyle change. Although endurance/aerobic exercise has been the more traditional method of improving cardiovascular fitness, resistance training is presently recommended as an important component of a broad-spectrum fitness program. This is particularly relevant for the older individual, in whom loss of muscle mass and weakness are major deficits. In term of resistance training it is now demonstrated that even 2 training sessions a week are

sufficient to induce an increase in muscle strength and power. This increase following resistance training can be the result of both an increase in the muscle mass (hypertrophy) and/or in the level of neural activation. For a similar training stimulus the hypertrophic response seems to be blunted in the older and this phenomenon seems to be more evident in the female subjects. In this respect, it is not clear the role of diet especially in term of protein intake in determining the muscle adaptations. In conclusion, the regular practice of physical activity can counteract the natural age-related decline but not stop it completely. This is exemplified by the observation of master athletes who despite having maintained good level of training across the life span exhibit an age related decline in their maximal performance. Thus, it is important to emphasize that these older athletes present, in their specific event, performance values not only superior in respect to sedentary individuals of the same age but also to sedentary subjects 30-40 years younger.

Session 3. Sarcopenia, cachexia and muscle wasting

12. AGING OF SKELETAL MUSCLE, P53 AND PLIN2: AN UNUSUAL RELATIONSHIP

M. Conte 1, E. Bertaggia 2, A. Armani 2, F. Vasuri 3, A. D'Errico-Grigioni 3, M. Franchi 4, M.V. Narici 4, F. Dela 5, E. Müller 6, M. Sandri 2, C. Franceschi 1, S. Salvioli 1

1 Dept of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Italy.
2 Dulbecco Telethon Institute, Venetian Institute of Molecular Medicine, Padua, Italy.
3 Addarii Institute of Oncology and Transplant Pathology, S.Orsola-Malpighi Hospital, University of Bologna, Italy.
4 School of Graduate Entry Med. and Health, Derby Royal Hospital, University of Nottingham, UK.
5 Center for Healthy Aging, University of Copenhagen, Copenhagen, Denmark.
6 Department of Sport Science and Kinesiology, University of Salzburg.
E-mail: m.conte@unibo.it

Aging is characterised by skeletal muscle atrophy and sarcopenia, whose etiology is still not clear. Present knowledge indicates a role for physical inactivity and inter-fibre fat infiltration, while little is known about the possible role of intra-muscular triglycerides (IMTG) accumulation. In skeletal muscle the levels IMTG are positively correlated with Plin2 and Plin5 that surround lipid droplets. Recently, we found that Plin2 expression in Vastus lateralis increases with age and inactivity, is inversely associated with muscle mass and strength, and is accompanied by activation of p53 in young and old healthy donors and patients with lower limb mobility impairment. The exact role of p53 in muscle atrophy is not totally clear, and in particular, a relationship between activation of p53 and accumulation of IMTG has never been reported. We extended our analysis to other subjects with different level of physical activity, and found that there's a general concordance between the levels of p53 and Plin2. To corroborate these data, we used a mouse model of skeletal muscle atrophy induced by denervation and found that in the muscle of the denervated leg, the expression of Plin2 and p53 resulted higher with respect to the contralateral non-denervated one. Preliminary data from experiments of in vivo transfection with sh-Plin2 indicate that transfected fibres display down-regulated Plin2 and a concomitant decrease in p53 expression. If confirmed, these results suggest that Plin2, beside its role as a structural protein for IMTG storage, could also promote the transcription of a crucial master gene such as p53.

*****

13. ELECTRICAL STIMULATION (FES) COUNTERACTS MUSCLE DECLINE IN SENIORS

Helmut Kern 1,2†, Laura Barberi 3†, Stefan Löfler 2, Simona Sbardella 3, Samantha Burggraf 2, Hannah Fruhmann 2, Ugo Carraro 2, Simone Mosole 2, Nejc Sarabon 4, Michael Vogelauer 1,Winfried Mayr 5, Matthias Krenn 5, Jan Cvecka 6, Vanina Romanello 7, Laura Pietrangelo 8, Feliciano Protasi 8, Marco Sandri 7, Sandra Zampieri 2,9 and Antonio Musaro 3,10*

1 Institute of Physical Medicine and Rehabilitation,Wilhelminenspital, Vienna, Austria.
2 Ludwig Boltzmann Institute of Electrical Stimulation and Physical Rehabilitation, Vienna, Austria
3 DAHFMO-Unit of Histology and Medical Embryology, Institute Pasteur Cenci-Bolognetti, IIM, Sapienza University of Rome, Rome, Italy.
4 Science and Research Centre, Institute for Kinesiology Research, University of Primorska, Koper, Slovenia.
5 Center of Medical Physics and Biomedical Engineering, Medical University of Vienna, Vienna, Austria.
6 Faculty of Physical Education and Sport, Comenius University, Bratislava, Slovakia.
7 Dulbecco Telethon Institute at Venetian Institute of Molecular Medicine, Padua, Italy.
8 Department of Neuroscience and Imaging, CeSI – Center for Research on Ageing, University Gabriele d'Annunzio, Chieti, Italy.
9 Laboratory of Translation Myology, Department of Biomedical Sciences, University of Padova, Padova, Italy.
10 Center for Life Nano Science@Sapienza, Istituto Italiano di Tecnologia, Rome, Italy.
E-mail: sanzamp@unipd.it

The loss in muscle mass coupled with a decrease in specific force and shift in fiber composition are hallmarks of aging. Training and regular exercise attenuate the signs of sarcopenia. However, pathologic conditions limit the ability to perform physical exercise. We addressed whether electrical stimulation (ES) is an alternative intervention to improve muscle recovery and defined the molecular mechanism associated with improvement in muscle structure and function. We analyzed, at functional, structural, and molecular level, the effects of ES training on healthy seniors with normal life style, without routine sport activity. ES was able to improve muscle torque and functional performances of seniors and increased the size of fast muscle fibers. At molecular level, ES induced up-regulation of IGF-1 and modulation of MuRF-1, a muscle-specific atrophy-related gene. ES also induced up-regulation of relevant markers of differentiating satellite cells and of extracellular matrix remodeling, which might guarantee shape and mechanical forces of trained skeletal muscle as well as maintenance of satellite cell function, reducing fibrosis. Our data provide evidence that ES is a safe method to counteract muscle decline associated with aging.

Frontiers in Aging Neuroscience July 2014 doi: 10.3389/fnagi.2014.00189

*****

14. WHAT IS THE EFFECT OF AGEING ON MYOMIR EXPRESSION?

Ester Sara Di Filippo 1,3,4, Rosa Mancinelli 1,3,4, Rita Maria Laura La Rovere 1,2,3,4, Mattia Quattrocelli 2,3, Tiziana Pietrangelo 1,3,4, Maurilio Sampaolesi 2,3, Stefania Fulle 1,3,4

1Dept. Neuroscience Imaging and Clinical Sciences, University "G. d'Annunzio" of Chieti-Pescara, Italy.
2Dept. Development and Regeneration, Stem Cell Institute, University of Leuven, KU Leuven Belgium.
3IIM- Interuniversity Institute of Myology.
4Ce.S.I. - Center for Research on Ageing, "G. d'Annunzio" Foundation, Chieti, Italy.
E-mail: es.difilippo@unich.it

In humans, ageing is characterized by a progressive loss of muscle mass and strength associated with a decline in functional ability, defined as Sarcopenia. The muscle mass regeneration is promoted by satellite cells, muscle stem cells. These cells act in different ways after an injury in young and in old skeletal muscle. In young subjects, local signals induce satellite cells proliferation and differentiation for tissue repair. On the contrary, in elderly subjects, intrinsic stem-cell aging, ageing of the niche or ageing of the systemic environment induce a reduced functionality of satellite cells. Furthermore, we previously demonstrated that there is an increase of apoptosis in myoblasts and a difficulty to term the differentiation program in elderly subjects compared to young. This difficulty could be due to the failure of the activation of myogenic transcriptional factors together with miRNA dysregulation. MicroRNAs (miRNAs) are important regulators of cell proliferation, differentiation, apoptosis and gene expression at the post-transcriptional level. We investigated the muscle specific miRNAs, miR-206, miR-1, and miR-133, called myomiRs. We also performed RT-PCR on specific pathways (such as proliferation, atrophy and ubiquitin proteasome system). We suggested a correlation between sarcopenia, apoptosis and the dysregulation of specific myomiRs and genes expression. In conclusion we hypothesized that miRNAs interfere negatively with myogenic differentiation, sustaining the intrinsic mithocondrial apoptosis and the impaired differentiation ability in old skeletal muscle.

*****

15. DISSECTING THE POSSIBLE ROLE OF CXCR4 PATHWAY IN CANCER-INDUCED MUSCLE LOSS

Giulia B. Martinelli*, Laura Talamini*, Sara Previdi and Rosanna Piccirillo

* these authors contributed equally to this project
Oncology Department, Laboratory of Cancer Cachexia AIRC Start-Up.
IRCCS - Istituto di Ricerche Farmacologiche "Mario Negri", via La Masa, 19, 20156 Milan, Italy.
E-mail: rosanna.piccirillo@marionegri.it

Cancer cachexia is a life-threatening syndrome characterized by severe body weight loss, due to depletion of adipose tissue and skeletal muscle, and affects up to 80% of patients with advanced cancers. The rapid loss of muscle mass is the main cause of function impairment, fatigue and respiratory complications, leading to death in 20-48% of cases. To date, no effective treatment is available. Interestingly, previous microarray analysis has identified a subset of genes whose expression is specifically altered in cachectic muscles of hepatoma-bearing rats. The recent development of novel softwares to analyze long list of genes, like Ingenuity Pathways Analysis software, enabled us to find a gene signature suppressed specifically in rat muscles atrophying because of cancer (and not because of diabetes or fasting or disuse): CXCR4 pathway. To test if suppressing this pathway is sufficient to drive muscle atrophy, we treated fully differentiated C2C12 myotubes with the inverse agonist AMD3100 and measured fiber diameter as index of muscle atrophy. Importantly, neither 48h-treatment with AMD3100 (up to 1 ug/ml) nor with the agonist SDF1 (up to 200 ng/ml) of CXCR4 receptor causes evident myotube toxicity. Interestingly, myotubes treated for 24 or 48h with 0.25, 0.5 or 1 ug/ml AMD3100, but not with SDF1, displayed decreased diameter in a dose- and time-dependent way, supporting its action through a saturable pathway (i.e. CXCR4). Ongoing experiments in cultured myotubes and in vivo adult mouse muscles aim at dissecting the possible role of this pathway in muscles during cancer cachexia.
In conclusion, our preliminary data show that attenuating CXCR4 pathway recapitulates muscle atrophy at least in cell culture.

*****

16. MARKERS OF AUTOPHAGY ARE INCREASED IN MUSCLE OF CANCER PATIENTS

*Fabrizio Pin 1,2, *Zaira Aversa 3, Fabio Penna 1,2, Simone Lucia 3, Roberto Verzaro 4, Giuseppe Vuolo 5, Filippo Rossi Fanelli 3, Maurizio Muscaritoli 3, Paola Costelli 1,2

1Department of Clinical and Biological Sciences, University of Turin, Turin, Italy.
2Interuniversitary Institute of Myology.
3Department of Clinical Medicine, Sapienza, University of Rome, Rome, Italy, 4M.G. Vannini Hospital, Rome, Italy.
5St. Maria alle Scotte Hospital, Siena, Italy.
E-mail: paola.costelli@unito.it

Autophagy is the major process for degradation of cellular constituents. Its rate is enhanced under stress conditions marked nutrients restriction, to recycle biomolecules to synthetize essential constituents. Recently autophagy was shown to contribute to muscle wasting not only in myopathies due to intrinsic muscle defects, but also in conditions such as sepsis, COPD, experimental cancer cachexia and aging. Aim of the present study was to evaluate if autophagy is induced also in the skeletal muscle of cancer patients. Upon written informed consent, 29 cancer and 11 control patients undergoing abdominal surgery were enrolled. Biopsies of the rectus abdominis were obtained during surgery. The expression of autophagic markers was evaluated by real-time PCR and western blotting. Statistical analysis was performed by nonparametric tests ($p<0.05$: significant). Beclin-1 protein levels increased by about 124% in the skeletal muscle of cancer patients with body weight loss

(WL)>5%. In addition, LC3B-II protein levels increased by about 108%, while p62 increased in all patients, irrespective of weight loss. Beclin-1, p-62, BNIP3 and TFEB mRNAs were not significantly modulated; by contrast, LC3B mRNA increased of about 50% in cancer patients with WL >5%. Results obtained suggest that autophagy contributes to the complicated network leading to muscle wasting in cancer patients. Further studies are needed to better understand the role played by this proteolytic pathway in the pathogenesis of cancer cachexia and to clarify if interference with autophagy could be useful to prevent cancer-associated muscle wasting.
*F. Pin and Z. Aversa equally contributed.

*****

17. EPIGENETIC THERAPY BY JQ1 ADMINISTRATION PREVENTS SKELETAL MUSCLE LOSS DURING CANCER CACHEXIA

Marco Segatto 1, Raffaella Fittipaldi 1, Claudio Fenizia 1, Paola Costelli 2, Giuseppina Caretti 1

1 Dipartimento di Bioscienze, Università degli Studi di Milano, via Celoria 26, 20133, Milano.
2 Dipartimento di Scienze Cliniche e Biologiche, Università degli Studi di Torino, Corso Raffaello 30, 10125 Torino.
E-mail: marco.segatto@unimi.it

Skeletal muscle wasting is a hallmark of cancer cachexia. This metabolic syndrome is a major clinical problem in oncology and it is responsible for about 25% of cancer deaths. In particular, muscle loss in cachectic patients often leads to increased morbidity and mortality rates, decreased beneficial effects from chemotherapy treatment, and poor quality of life. For these reasons, the development of novel strategies to prevent muscle wasting during cancer cachexia is attracting increasing clinical interest. To date, no effective therapies for cachectic muscle are available. Recently, our research group reported that the small bromodomain inhibitor JQ1 enhances muscle fiber size and protects from dexamethasone-induced muscle atrophy in C2C12 myotubes, by counteracting skeletal muscle pro-atrophic pathways. Hence, in the present work we evaluated the effect of JQ1 treatment in skeletal muscle wasting during cancer cachexia. To this aim, C26 (adenocarcinoma cell line) bearing mice were chronically treated with JQ1 or vehicle. After 12 days, body weight, skeletal muscle weight and the anabolic/catabolic pathways involved in skeletal muscle homeostasis were analyzed. The results show that JQ1 treatment protects tumor-bearing mice from body weight loss and muscle wasting, suggesting that the epigenetic modulation mediated by bromodomain inhibitors may represent a promising therapeutic approach in the management of muscle wasting during cancer cachexia.

*****

18. GASTROINTESTINAL TUMOR CELLS IMPAIR SKELETAL MUSCLE PHYSIOLOGY

Emanuele Berardi and Maurilio Sampaolesi

Laboratory of Translational Cardiomyology, Department of Experimental Medicine, University of Pavia, Italy and Department of Development and Regeneration, KU Leuven, Belgium.
E-mail: emanuele.berardi@med.kuleuven.be

Cancer cachexia is a syndrome characterized by a progressive muscle wasting occurring in response to tumor pro-inflammatory stimuli. Gastrointestinal tumor (GIST) is the most diffuse tumor of gastrointestinal tract, characterized by high expression of DOG1, a calcium-dependent chloride channel. Advanced GISTs may be associated with metastases and patients frequently develop a muscle-wasting syndrome. By inoculating of tumor biopsies from same donors, we found that around one third of human GIST-xenograft bearing mice develop cancer-related muscle wasting, resulting in sudden death. Skeletal muscle tissue was highly affected in all bearing mice and atrophic phenotype correlated with high expressions of DOG1 in transplanted tumor. Gene expression profile analysis showed that the cachexia process was not mediated by inflammatory cytokines produced by tumor or by the presence of inflammatory cells in muscle microenvironment. In addition, we found human nuclei in muscle tissues of tumor bearing mice and loss of function studies revealed that DOG1 is involved in cell migration. These results suggest a direct role of DOG1 in GIST-mediated cachexia and provide evidences that DOG1 positive cells can migrate from transplanted area to distal muscles and locally induce Atrogin1 expression.

*****

19. ARTESUNATE INDUCES ROS-MEDIATED APOPTOSIS AND COUNTERACTS TUMOR GROWTH IN VIVO IN EMBRYONAL RHABDOMYOSARCOMA CELLS

Sara Beccafico, Angelo Sidoni, Rosario Donato and Guglielmo Sorci

Department of Experimental Medicine, University of Perugia, Perugia, Italy.
E-mail: guglielmo.sorci@unipg.it

Embryonal rhabdomyosarcoma (ERMS) is the most common soft tissue sarcoma in childhood, and is characterized by the expression of muscle-specific transcription factors and overexpression of PAX7 [1]. Thus, ERMS has been suggested to have an origin in muscle precursor cells that fail to exit the cell cycle and terminally differentiate. Artesunate (ARS) is a semi-synthetic derivate of artemisinin, a Chinese-medicine compound long known as a very effective anti-malarial drug. More recently, artemisinin and its derivatives have been found effective as anticancer drugs since they induce cell cycle arrest or apoptosis in several kinds of cancer [2]. Here we demonstrate that ARS dose-dependently induces cell cycle arrest and apoptosis in the ERMS cell lines, TE671 and RD18. Production of reactive oxygen species (ROS) and activation of p38 MAPK have a central role in triggering ARS-mediated apoptosis in ERMS cells, since either the antioxidant and ROS scavenger, N-acetylcysteine (NAC), or the p38 inhibitor, SB203580, protects ERMS cells from ARS-induced apoptosis. Moreover, ARS treatment in ERMS cells ROS-dependently induces the expression of the specific myo-miRs, miR-133a and miR-206, which are typically down-regulated in ERMS, and downregulates PAX7 expression. Finally, ARS upregulates the expression of the adhesion molecules, NCAM and integrin β1, and reduces migration and invasiveness in ERMS cells in vitro, and ARS treatment reduces ERMS tumor growth in vivo. Our results suggest

ARS as a potential candidate for the therapeutic treatment of ERMS. 1. Parham D.M. and Ellison D.A. (2006) Rhabdomyosarcomas in adults and children. An update. Arch. Pathol. Lab. Med. 130:1454-1465. 2. Crespo-Ortiz M.P. and Wei M.Q. (2012) Antitumor activity of artemisinin and its derivatives: from a well-known antimalarial agent to a potential anticancer drug. J. Biomed. Biotechnol. 2012:247597

*****

I Lecture

Sarcopenia and Loss of Muscle Quality: Why Is Aging Muscle Intrinsically Weak?

Marco Narici
University of Nottingham, School of Medicine, MRC-ARUK Centre for Musculoskeletal Ageing Research, Derby, UK
E-mail: marco.narici@googlemail.com

Sarcopenia, the age-related loss of muscle mass, affects >50% of the population aged 75 yr and over and is a main cause of impaired physical performance and reduced mobility. Amongst the several factors contributing to sarcopenia neuroendocrine changes are regarded as primary drivers of this condition (1) and responsible for α-motoneurons and neuromuscular junction (NMJ) degeneration, for muscle fibre denervation which, also fuelled by mitochondrial dysfunction and oxidative damage, leads to loss of motor units and muscle weakness. One of the major functional characteristics of sarcopenia is the disproportionate loss of muscle strength: at the age of 80 yrs, the loss of muscle strength is about 4-fold greater than that of muscle size. This intrinsic muscle weakness, also known as a deterioration in 'muscle quality' has traditionally been reconducted to a decrease in fibre specific tension, reduced excitation-contraction coupling and reduced neural drive. However, new evidence suggests that this disproportionate loss of force also arises from changes in the extracellular matrix (ECM) and of associated proteins, leading to a decrease in lateral force transmission (2), which in young muscle normally contributes to >50% of muscle force output. Indeed, as recently reported for rat muscle, lateral force transmission is markedly reduced in old animals (-20%) and even more in very old ones (-44%) (3). This phenomenon seems to originate from alterations at focal adhesion sites (costameres) responsible for the transmission of force from the z-discs of skeletal muscle fibres to the ECM and also for mechanotransduction via the dystrophin glycoprotein complex and focal adhesion kinase (FAK) protein. Hence, the muscle weakness associated with sarcopenia may not only take origin from muscular changes but also from alterations in costamere morphology and biochemical composition resulting in a decrease in lateral force transmission.

1. Narici MV, Maffulli N. Br Med Bull. 2010;95:139-59.
2. Zhang C, Gao Y. J Biomech. 2014, 47:944-8.
3. Ramaswamy et al. J Physiol 2011; 589: 1195–1208.

*****

Session 4. Muscle Differentiation, Homeostasis and Regeneration

20. CONSTITUTIVE INHIBITION OF MUSCLE DIFFERENTIATION BY SENESCENCE-ACTIVATED DNA DAMAGE SIGNALING

Lucia Latella 1,3, Chiara Nardoni1, Pasquale Fasanaro 1, Pier Lorenzo Puri 1,2
1 Santa Lucia Foundation Rome, Italy.
2 The Burnham Institute for Medical Research, San Diego, USA.
3 Institute of Translational Pharmacology, National Research Council Rome, Italy.
E-mail: lucia.latella@ift.cnr.it

Upon exposure to genotoxic stress, skeletal muscle progenitors coordinate DNA repair and the activation of the differentiation program, by the DNA damage-activated "differentiation checkpoint" (DC), which prevents the transcription of differentiation genes during the DNA repair. We have shown that in myoblasts, DNA damage-activated cAbl phosphorylates a tyrosine (Y30) at the N-terminal activation domain of MyoD that transiently inhibits MyoD-dependent transcription following DNA damage, and is reversed possibly upon the successful repair of the lesion.[1] The presence of a cAbl consensus site discriminates MyoD from the functional paralog Myf5 and from other muscle bHLH proteins in executing the DC during development.[2] Our recent observations reveal that the DNA damage-activated ABL-MyoD signaling contributes to DNA repair in skeletal myoblasts, conferring a dual role on MyoD in the differentiation checkpoint, as transcription factor and component of the repair machinery.[3] In this study we show that the constitutive, endogenous DNA damage signaling associated with cellular senescence triggers a persistent differentiation checkpoint that constitutively inhibits the myogenic program induced by MyoD. Indeed, human primary fibroblasts show a progressively resistance to MyoD-mediated myogenic conversion, along with the increased number of passages in culture. This resistance coincided with the acquisition of phenotypic and molecular markers of replicative senescence, including the activation of constitutive senescence-activated DNA damage signaling. A cAbl phosphorylation resistant MyoD mutant - Y30F – that is insensitive to DNA damage signaling,[1,2] overrides the block to myogenic conversion imposed by replicative senescence. Interestingly, the ability of MyoD Y30F to bypass senescence-mediated inhibition of myogenesis coincided with the resumption of the cell cycle in senescent fibroblasts, indicating that DNA replication is required for nuclear reprogramming toward the activation of the myogenic program. This evidence reveals the intimate relationship between cell cycle, transcriptional machinery and DNA damage signalling as key components of the functional antagonism between cellular senescence and terminal differentiation. RNA-seq analysis revealed the identity of transcriptional networks that mediate resistance to activation of the myogenic program in senescent cells, as well as interesting pathways activated upon bypass of senescence by MyoD Y30F, that might provide new molecular insight into the relationship between aging, cellular senescence and

decline of muscle regenerative potential. 1. Puri PL, et al. Nat Genet. 2002; 2. Innocenzi A, Latella L, et al. EMBO REP 2011; 3. Simonatto M, et al. Cell Death & Diff 2013.

*****

21. OVEREXPRESSION OF HSOD1G93A IN C2C12 CELL LINE INDUCE AN ALTERATION OF THE MYOGENIC PROGRAM

1 Gabriella Dobrowolny, 2 Michela Aucello, 1 Martina Martini, 1 Antonio Musarò

1 DAHFMO-Unit of Histology and Medical Embryology, IIM, Sapienza University of Rome, Via A. Scarpa 16, 00161 Rome, Italy; Center for Life Nano Science@Sapienza, Istituto Italiano di Tecnologia, Viale Regina Elena 291, 00161 Rome, Italy.
2 DAHFMO-Unit of Histology and Medical Embryology, IIM, Sapienza University of Rome, Via A. Scarpa 16, 00161 Rome, Italy.
E-mail: martina.martini@uniroma1.it

Amyotrophic lateral sclerosis is a fatal adult onset disorder characterized by progressive muscular paralyses reflecting degeneration of motor neurons in the primary cortex, brainstem and spinal cord. One of the most studied gene related to ALS is the gene encoding the Superoxide Dismutase 1 (SOD1). To define whether the overexpression of human mutant SOD1 proteins cause an alteration on skeletal muscle, in particular on the myogenic program, we stably transfected C2C12 cell line with the mutated SOD1 cDNA driven by the MLC promoter. Morphological and immunofluorescence analysis showed myoblast elongation at day0 and the formation of myotube at late stage of differentiation in control C2C12 cells. On the contrary MLC/SOD1G93A C2C12 cells completely failed to form multinucleated myotubes under identical experimental conditions. In addition, we analyzed the expression of the major myogenic regulatory factors. RT-PCR analysis revealed a significant reduction in MyoD, Myf5, MRF4 and Myogenin expression in MLC/SOD1G93A cells compared to C2C12 control. The altered expression of myogenic regulatory factors was also associated with the upregulation of Pax3 in the transfected cell line compared to the control cells, suggesting that overexpression of SOD1G93A may induce a change in C2C12 differentiative cell fate.

*****

022. NOTCH SIGNALING REGULATES MYOGENIC COMMITMENT OF MURINE AND HUMAN MESOANGIOBLASTS

Quattrocelli M, Sampaolesi M

Translational Cardiomyology Lab, Dept Development and Regeneration, KU Leuven, Belgium;
E-mail: mattia.quattrocelli@med.kuleuven.be

Somatic stem cells hold attractive potential for the treatment of muscular dystrophies (MDs). Mesoangioblasts (MABs) constitute a myogenic subset of muscle pericytes and have been shown to efficiently regenerate dystrophic muscles in mice and dogs. In addition, HLA-matched MABs are currently being tested in a phase 1 clinical study on Duchenne MD patients (EudraCT #2011-000176-33). Many reports indicate that the Notch pathway regulates muscle regeneration and satellite cell commitment. However, little is known about Notch-mediated effects on other resident myogenic cells. We therefore asked whether Notch signaling played a pivotal role in regulating MAB myogenic capacity. Through different approaches of loss- and gain-of-function in murine and human MABs, we determined that the interplay between Delta-like ligand 1 (Dll1)-activated Notch1 and Mef2C supports MAB commitment in vitro and ameliorates engraftment and functional outcome after intra-arterial delivery in dystrophic mice. Furthermore, using a transgenic mouse model of conditional Dll1 deletion, we demonstrated that Dll1 ablation, either on the injected cells, or on the receiving muscle fibers, impairs MAB regenerative potential. Our data corroborate the perspective of advanced combinations of cell therapy and signaling tuning to enhance therapeutic efficaciousness of somatic stem cells.

*****

23. USE OF A NON-OXIDIZABLE FORM OF HMGB1 TO PROMOTE MUSCLE REPAIR

Mario Tirone 1, Andrea Gorzanelli 2, Valentina Conti 1, Chiara Ceriotti 2, Marielle Saclier 3, Sylvain Cuvellier 3, Bénédicte Chazaud 3, Maura Casalgrandi 4, Alessandro Preti 4, Silvia Brunelli 1,5, Marco E. Bianchi 2,6 and Emilie Vénéreau 2,4

1 Division of Regenerative Medicine, Stem Cells and Gene Therapy, San Raffaele Scientific Institute, Milan, Italy.
2 Chromatin Dynamics Unit, San Raffaele Scientific Institute, Milan, Italy.
3 Institut Cochin, INSERM U1016, Paris, France.
4 HMGBiotech srl, Milan, Italy.
5 Department of Health Sciences, University of Milano-Bicocca, Monza, Italy.
6 San Raffaele University, Milan, Italy.
E-mail: venereau.emilie@hsr.it

High mobility group box 1 (HMGB1) is a nuclear protein that acts extracellularly as an alarmin to modulate inflammation and tissue repair by recruiting cells and promoting their migration and activation. Recently, we showed that HMGB1 orchestrates both processes by switching among mutually exclusive redox states. Fully reduced HMGB1 acts as a chemoattractant, whereas a disulfide bond makes it a proinflammatory cytokine and further cysteine oxidation by reactive oxygen species (ROS) abrogates both activities. The fully reduced HMGB1 is prevalent in the extracellular environment immediately after acute muscle injury, and disulfide- HMGB1 appears a few hours later. Thus, the generation of ROS during muscle damage might modulate the redox status of the protein and eventually limit its lifespan and functions. We created a mutant (3S-HMGB1) not susceptible to redox modifications and we evaluated its regenerative activity in a model of acute muscle injury induced by cardiotoxin. We demonstrated so far that HMGB1 has beneficial effects in skeletal muscle regeneration after acute injury by dramatically increasing the number of healthy fibers and the number of satellite cells and M2c macrophages, two cell types essential for muscle repair. Moreover, HMGB1 acts directly on primary myoblasts by inducing their migration and their fusion to form large myotubes. Remarkably, 3S-HMGB1 behaves

as a superagonist of HMGB1 in vivo, suggesting that it is a promising candidate for muscle repair therapies. Our study will be extended to other models of muscle damage, in particular dystrophies, in order to evaluate the therapeutic potential of 3S-HMGB1 in chronic conditions.

*****

24. LEUCINE SUPPLEMENTATION HAS EFFECTS ON FIBROSIS AND FUNCTIONAL RECOVERY ON REGENERATION OF PHENOTYPICALLY DISTINCT SKELETAL MUSCLES

Marcelo G. Pereira 1, Meiricris T. Silva 1, Eduardo O. C. Carlassara 1, Paulo A. Abrahamshon 2, Anselmo S. Moriscot 1, Marcelo S. Aoki 3, Elen H. Miyabara 1

1 Department of Anatomy, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, SP, Brazil.
2Department of Cell and Developmental Biology, Institute of Biomedical Sciences, University of Sao Paulo, SP, Brazil.
3 School of Arts, Sciences and Humanities, University of Sao Paulo, Sao Paulo, SP, Brazil.
E-mail: pereiramg@gmail.com

Although the regenerative process has been investigated in detail, studies on the strategies to improve it are still on going. We investigated the effects of leucine supplementation on the skeletal muscle regenerative process, focusing on the remodeling of connective tissue (CT) and functional recovery in the phenotypically distinct muscles. Male Wistar rats were subjected or not to leucine supplementation (1.35 g/kg per day), which started 3 days prior to cryolesion of soleus and tibialis anterior (TA) muscles from left hind limb, and continued receiving leucine until 10 days later. Muscles from right hind limb were used as control. Muscle cross-sections were used to reveal the overall morphology, and determine myofiber cross-sectional area (CSA). Quantification of the CT area density was analyzed under light and polarized microscopes. Neonatal myosin heavy chain (MyHC-n) and the content of MyHC-I and II were analyzed by immunostaining and Western Blot, respectively. Phosphorylation of transforming growth factor-β receptor type I (TβR-I), and the positive nuclei for Smad2/3, were assessed by immunostaining. Muscle function was assessed by in vivo electrostimulation. Leucine supplementation promoted an increase in the CSA of regenerating muscles, which was more pronounced in the soleus ($p<0.05$) than in the TA. Leucine also reduced the amount of CT and the activation of TβR-I and Smad2/3 on both regenerating muscles ($p<0.05$); and accelerates the shift of MyHC-n to MyHC-I and II on soleus and TA, respectivelly ($p<0.05$). In addition, prevented the development of fatigue in regenerating soleus and the decrease in the tetanic strength in regenerating TA ($p<0.05$). Leucine supplementation accelerates CT repair and consequent functional recovery of regenerating soleus and TA through the attenuation of TβR-I and Smad2/3 activation. These results suggest that leucine supplementation can be used as a nutritional strategy to prevent or attenuate several events in muscle diseases.

*****

25. MIR RETROPOSON EXONIZATION IN IGF-1 GENE CAN EXPLAIN THE IGF-1EB AND IGF-1EC/MECHANO GROWTH FACTOR ISOFORM PRODUCTION IN MAMMALS AND THEIR SPECIES-SPECIFIC PATTERN OF EXPRESSION

Giosuè Annibalini 1, Elena Barbieri 1, Pamela Bielli 2, Deborah Agostini 1, Mauro De Santi 1, Rosa Mancinelli 3, Stefania Fulle 3, Giorgio Brandi 1, Claudio Sette 2, Vilberto Stocchi 1

1 Department of Biomolecular Sciences, University of Urbino Carlo Bo, Urbino, Italy.
2 Department of Biomedicine and Prevention, University of Rome Tor Vergata, Rome, Italy.
3 Department of Neurosciences and Imaging, University of Chieti-Pescara, Chieti, Italy.
E-mail giosue.annibalini@uniurb.it

The IGF-1 gene comprises 6 exons showing alternative splicing (AS) patterns. AS occurring at the 3' of the IGF-1 pre-mRNAs, gives rise to three different IGF-1 isoforms called IGF-1Ea, IGF-1Eb and IGF-1Ec, which contain exons 4-6, 4-5, and 4-5-6 respectively. Since the expression of IGF-1Ec in muscle has been linked to mechanical stimuli, this splice variant is sometimes referred to as mechano growth factor (MGF). In this study, we have analyzed the expression of IGF-1 mRNA splice variants in different vertebrate species in order to trace the evolutionary history of IGF-1 isoforms. Our analysis showed that only IGF-1Ea isoform is conserved among all vertebrates, whereas exon 5, included in IGF-1Eb and IGF-1Ec/MGF, is a evolutionary novelty that appeared in an ancestor of mammals. Interestingly, exon 5 is part of a region which displays approximately 60% similarity to mammalian interspersed repetitive-b (MIR-b) element. The splice acceptor site of exon 5, determining the inclusion of exon 5, is embedded within the MIR sequence and is extremely conserved in different mammals. On the contrary the splice donor site of exon 5, producting IGF-1Ec/MGF, lays outside the MIR sequence and it displays a variable strength ranging from strong (in rodents) to weak (in human). This variability may underlie the species-specific pattern of IGF-1Eb and IGF-1Ec/MGF expression observed in the mammalian species examined herein. In conclusion, our study suggests that "exonization" of a MIR element in the IGF-1 gene might explain the species-specific AS of IGF-1Eb and IGF-1Ec/MGF isoforms in mammals.

*****

26. HDAC4 IS NECESSARY FOR SATELLITE CELL DIFFERENTIATION AND MUSCLE REGENERATION.

Nicoletta Marroncelli 1, Chiara Noviello 1, Silvia Consalvi 2, Valentina Saccone 2, Pier Lorenzo Puri 2, Eric N. Olson 3, Sergio Adamo 1 and Viviana Moresi 1

1 Dept. of Anat., Histol., Forens. & Orthop. Sciences, Sapienza University of Rome, Italy.
2 Istituto di Ricovero e Cura a Carattere Scientifico (I.R.C.C.S.) Fondazione Santa Lucia, 00143 Rome, Italy.
3 Dept. of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA.
E-mail: viviana.moresi@uniroma1.it

In response to injury, skeletal muscle exhibits high capacity to regenerate and epigenetics controls multiple steps of this process. In vitro has been demonstrated that completion of muscle differentiation requires shuttling of histone deacetylase 4 (HDAC4), a member of class IIa HDACs, from the nucleus to the cytoplasm and consequent activation of MEF2-dependent differentiation genes. In vivo, HDAC4 expression is up-regulated in skeletal muscle upon injury, suggesting a role for this protein in muscle regeneration. With the aim to elucidate the role of HDAC4 in skeletal muscle regeneration, we generate mice lacking HDAC4 in the satellite cells (HDAC4fl/fl;Pax7CE Cre). Lack of HDAC4 inhibits satellite cell differentiation. Despite having similar amount of sorted cells, HDAC4 KO satellite cells proliferate less and have less pax7 than controls. Importantly, muscle regeneration in vivo is impaired in HDAC4fl/fl;Pax7CE Cre mice. These results are confirmed by molecular analyses of the expression of myogenic markers. All together, these data delineate the importance of HDAC4 in muscle regeneration and suggest a protective role in response to muscle damage.

*****

27. UNACYLATED GHRELIN ENHANCES SKELETAL MUSCLE REGENERATION

Simone Reano, Elia Angelino, Michele Ferrara, Omar Sabry, Andrea Graziani, Nicoletta Filigheddu

Deptment of Translational Medicine, University of Piemonte Orientale, Novara, Italy.
E-mail: simone.reano@med.unipmn.it

Acylated and unacylated ghrelin (AG and UnAG, respectively) are peptide hormones released by the stomach during fasting. AG, but not UnAG, stimulates growth hormone release, appetite, and positive energy balance through binding to its receptor GHSR1a. Both peptides act on the skeletal muscle: they promote differentiation of C2C12 myoblasts and prevent skeletal muscle atrophy by acting directly on the skeletal muscle through signaling pathways involving p38 and mTORC2-mediated activation of Akt without the involvement of GHSR1a. To further investigate the role of ghrelin peptides in the biology of skeletal muscle, we exploited Myh6/Ghrl transgenic mice, over-expressing the ghrelin gene Ghrl under a myocardial promoter and featuring constitutively high UnAG circulating levels, and we investigated if UnAG affected skeletal muscle regeneration triggered by cardiotoxin intramuscular injection. We observed that skeletal muscle regeneration was enhanced in Myh6/Ghrl mice, with new-formed myofibers characterized by increased area and number of central nuclei. Evaluating the quiescent pool of satellite cells (SCs) in uninjured mice, surprisingly we found that skeletal muscles of Myh6/Ghrl mice have a greater number of quiescent Pax7+ve SCs compared to WT mice, suggesting that UnAG could affect SCs proliferation and/or self-renewal. Indeed, treatment of SCs -either in culture or on isolated myofibers- with UnAG enhances their proliferation and self-renewal while maintaining their differentiative ability. These data, together with the finding that UnAG improves SCs muscular engraftment, suggest that UnAG might represent a novel therapeutic approach to treat muscular dystrophies, characterized by the progressive loss of SCs and irreversible muscle degeneration. [Project funded by MDA]

Scientists meet Companies

PARENT PROJECT and IIM

Maria Teresa Moscato
E-mail: m.moscato@parentproject.it

*****

Session 5. Muscular Dystrophies and Related Diseases

28. GENERATION OF AN MDX/AGER$^{-/-}$ DOUBLE MUTANT MOUSE. PRELIMINARY DATA ON SKELETAL MUSCLE ARCHITECTURE

Roberta Sagheddu*, Sara Chiappalupi*, Laura Salvadori, Rosario Donato and Guglielmo Sorci

* These Authors contributed equally to this work

Dept Experimental Medicine, University of Perugia, Italy.
E-mail: guglielmo.sorci@unipg.it

Duchenne muscular dystrophy (DMD) is a lethal X-linked pathology affecting 1:5000 male birth and characterized by progressive muscle degeneration due to lack of dystrophin, a protein crucial for the stabilization of myofiber sarcolemma during contraction.[1] Muscles of DMD patients are characterized by chronic inflammation, which plays a major role in the progression of the pathology. RAGE (receptor for advanced glycation end-products) is a multiligand receptor of the immunoglobulin superfamily involved in physiological and pathological processes including inflammation and myogenesis.[2] Whereas RAGE is not expressed in adult muscle tissue, it is expressed in immune cells, regenerating myofibers during muscle regeneration, and dystrophic muscle tissue. The *mdx* mouse represents the best characterized animal model of DMD. To have information about the role of RAGE in the pathophysiology of DMD we generated a double mutant *mdx*/*Ager*$^{-/-}$ mouse lacking both dystrophin and RAGE. We analyzed *mdx*/*Ager*$^{-/-}$ mice in comparison with those of age-matched *mdx* mice, since this age corresponds to the acute phase of the pathology in the *mdx* model. Haematoxylin/eosin analysis revealed significantly reduced numbers of necrotic myofibers, reduced areas of immune cell infiltrate, and similar percentages of centrally-nucleated myofibers in muscles of *mdx*/*Ager*$^{-/-}$ mice compared with mdx mice. Moreover, *mdx*/*Ager*$^{-/-}$ muscles showed strongly reduced MAC3^{+} areas and reduced MAC3 expression as analyzed by immunohistochemistry and Western blotting, respectively. These preliminary results suggest that RAGE might have a role in the progression of DMD pathology.

1. D.J. Blake, A. Weir, S.E. Newey, K.E. Davies. (2002) Function and genetics of dystrophin and dystrophin-related proteins in muscle. Physiol. Rev. 82, 291-329
2. G.P. Sims, D.C. Rowe, S.T. Rietdijk, R. Herbst, A.J. Coyle. (2010) HMGB1 and RAGE in Inflammation and Cancer. Annu. Rev. Immunol. 28:367-388

*****

29. TARGETING PKCΘ IN MDX TO AMELIORATE MUSCULAR DYSTROPHY

V. Marrocco, L. Madaro, P. Fiore, M. Bouchè

Dipartimento SAIMLAL-sezione di Istologia ed Embriologia Medica. Sapienza università di Roma, Italy.
E-mail: valeria.marrocco@uniroma1.it

Protein kinase C θ (PKCθ) is a member of the PKCs family highly expressed in both immune cells and skeletal muscle; given its crucial role in adaptive, but also innate, immunity, it is being proposed as a valuable pharmacological target for immune disorders. We show that targeting PKCθ in mdx, the mouse model for Duchenne muscular dystrophy (DMD), by both genetic manipulation (in the bi-genetic mdx/θ-/- mutant) and by pharmacological approach, improves healing and regeneration, preventing massive inflammation. The observed phenotype was primarily due to the lack of PKCθ in hematopoietic cells, as revealed by BM transplantation experiments. However, PKCθ plays also a role in muscle maintenance, and we recently showed that PKCθ is involved in ER-stress induced autophagy in muscle cells. Several muscular dystrophies are characterized by alteration in autophagy activation, due to the loss of signalling from the extracellular matrix components to the inside of the cell and we show that PKCθ is involved in the regulation of ER-stress in dystrophic skeletal muscle. We are currently dissecting the role of PKCθ in both skeletal muscle and immune cells during DMD progression, in order to verify whether it can be proposed as a valuable pharmacological target to ameliorate the disease.

*****

30. MITOCHONDRIAL IMPAIRMENT IN LIMB GIRDLE MUSCULAR DYSTROPHY 2D: INVOLVEMENT OF BIOGENESIS PROCESS

Sarah Pambianco 1, Federica Morisi 1, Emilio Clementi 1,2, Clara De Palma 1

1 Department of Clinical and Biomedical Sciences, Unit of Clinical Pharmacology "Luigi Sacco" University Hospital, University of Milano, Italy; 2 E.Medea Institute, Bosisio Parini, Lecco, Italy.
E-mail : sarah.pambianco@unimi.it

Skeletal muscle is a major site of metabolic activity and changes in mitochondrial function might be an important player, causing myopathy. Mitochondria are essential for energy production, but, if damaged, they become a source of proapoptotic factors and reactive oxygen species, as occurs in some kinds of muscular dystrophy. Here we characterized the mitochondrial profile of α-Sarcoglicand-null (αSG-/-) mice and we found out a severe reduction in mitochondrial content in both tibialis anterior and diaphragm accounting for a lower OxPhos capacity of these muscles. However the respiratory rates relative to mitochondrial DNA suggested that mitochondrial content was the major determinant of the lower oxidative capacity of αSG null muscles. Since defect of nitric oxide (NO) generation is a key pathogenic event in muscular dystrophies and NO donors have been explored as new therapeutics for this disease, we treated dystrophic animal with NO-donor Molsidomine to evaluate a possible impact on mitochondrial content and activity. Moreover it has already been demonstrated that Molsidomine is able to slow down the progression of muscular dystrophy in the $\alpha SG^{-/-}$ - mice, improving muscle function and that NO is able to promote mitochondrial biogenesis. Unexpectedly, the treatment with the NO-donor Molsidomine did not affect mitochondrial content in $\alpha SG^{-/-}$ mice, but it was able to improve significantly their oxidative capacity, triggering a therapeutic fiber switch and stimulating fatty acid oxidation rather than improving mitochondrial function per se. Molsidomine indeed enhanced the deacetylation and activation of PGC1α, the principal transcriptional co-activator involved in muscle fiber type determination. The NO-dependent deacetylation of PGC1α in $\alpha SG^{-/-}$ mice was due to a significant increase in SIRT-1 expression, the deacetylase responsible for PGC1α activation. Altogether these results highlight the important role of mitochondrial metabolism in Limb Girdle Muscular Dystrophy and point out the NO–dependent switch toward more oxidative muscle fibers, as a rescue mechanism.

*****

31. D4Z4 REPEAT AS THE FIRST POLYCOMB RESPONSE ELEMENT INVOLVED IN A HUMAN GENETIC DISEASE

Valentina Casa 1,2, Maria Victoria Neguembor 1, Giulia Ferri 1,2, Roberta Caccia 1, Jeffrey Dilworth 3 and Davide Gabellini 1

1 Dulbecco Telethon Institute at Division of Regenerative Medicine, San Raffaele Scientific Institute, Milano, Italy.
2 Universita' Vita-Salute San Raffaele, Milano Italy.
3 Regenerative Medicine, Sprott Center for Stem Cell Research, Ottawa Hospital Research Institute
E-mail: casa.valentina@hsr.it

FSHD (1:15000) is a genetic disease characterized by complex interplay of genetic and epigenetic events. FSHD is not due to mutations in protein-coding genes. Instead it is linked to contraction of a macrosatellite repeat called D4Z4 on 4q35. By an unknown mechanism, D4Z4 contraction is associated with loss of gene silencing of 4q35 genes. Polycomb group (PcG) proteins are essential epigenetic regulators of development and differentiation. In Drosophila, PcG is recruited to genetic elements called Polycomb Response Elements (PREs). Less is known about PcG recruitment to target genes in vertebrates. The FSHD locus displays similarities with PcG targets. A sequence inside D4Z4 called DBE is identical to a consensus motif shared by Drosophila PREs. By ChIP we found specific enrichments on D4Z4 for core components of the two main PcG complexes PRC1 and PRC2, and relative histone marks. Importantly, muscle cells from patients display a reduced binding of Polycomb to the FSHD locus. Moreover, RNAi experiments indicate that Polycomb is required for repression of FSHD candidate genes. One cardinal feature of Drosophila PREs is their ability to recruit PcG complexes when ectopically inserted. Accordingly, we found that D4Z4 is capable to mediate robust ectopic recruitment of PRC1, PRC2 and associated histone marks. Intriguingly, DBE element inside D4Z4 is sufficient to recruit Polycomb and is able to mediate a copy number-dependent Polycomb silencing. These results indicate that loss of PcG silencing is responsible for de-repression of 4q35 genes inFSHD and suggest a possible role for D4Z4 as a PRE.

32. DIRECT INTERPLAY BETWEEN TWO CANDIDATE GENES AS UNIFYING MOLECULAR PATHOGENESIS FOR FSHD MUSCULAR DYSTROPHY

Giulia Ferri 1,2, Davide Gabellini 1

1 Dulbecco Telethon Institute and Division of Regenerative Medicine, San Raffaele Scientific Institute, 20132 Milan, Italy.
2 Vita-Salute San Raffaele University, 20132 Milan, Italy.
E-mail: ferri.giulia@hsr.it

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disorder with a strong epigenetic component with a poorly understood molecular pathogenesis of FSHD. Unlike the majority of genetic diseases, FSHD is not caused by mutation in a protein-coding gene, but it is linked to decrease in copy number of a 3.3-Kb tandem repeated macrosatellite (D4Z4), located on chromosome 4q35. D4Z4 deletion alters chromatin structure of the locus leading to inappropriate de-repression of nearby 4q35 genes. The two leading FSHD candidate genes are FRG1 (FSHD region gene 1) and DUX4 (Double homeobox 4). The unusual nature of the mutation that causes FSHD and its complex effects on the chromatin surrounding the 4q35 region makes it unlikely that the root cause of the disease can be attributed to a single gene. Accordingly, it has been suggested that FSHD could be a contiguous gene syndrome where multiples 4q35 genes contribute to the final result. DUX4 is a transcriptional activator and we located several putative DUX4 binding sites in the FRG1 genomic area. We show DUX4 association to these regions by chromatin immunoprecipitation. We also found that ectopically expressed DUX4 up-regulates the endogenous FRG1, while DUX4 knockdown leads to a decrease in FRG1 expression. Moreover, DUX4 specifically transactivates constructs containing FRG1 genomic regions in luciferase assays. Finally, by EMSA we show a direct binding of DUX4 to its binding site inside the FRG1 gene. Collectively, our data indicate that FRG1 is a direct DUX4 transcriptional target pointing towards a unifying molecular pathway for FSHD pathogenesis.

*****

33. A POSSIBLE PHARMACOLOGICAL APPROACH FOR THE TREATMENT OF FSHD MUSCULAR DYSTROPHY

Roberta Caccia 1, Maria Victoria Neguembor 1, Clara Sciorati 2, Emilio Clementi 3, Clara De Palma 4 and Davide Gabellini 1

1 Dulbecco Telethon Institute and Division of Regenerative Medicine, San Raffaele Scientific Institute, Milan, Italy.
2 Division of Regenerative Medicine, San Raffaele Scientific Institute, Milan, Italy.
3 E. Medea Scientific Institute, Bosisio Parini, Italy.
4 Department of Biomedical and Clinical Sciences, "Luigi Sacco" University Hospital , University of Milano, Italy.
E-mail: caccia.roberta@hsr.it

In classical forms of muscular dystrophy, onset of the disease is with muscle fiber necrosis. As a secondary effect, inflammation and myogenic satellite stem cells are activated to repair the muscle. Cycles of muscle degeneration/regeneration continue until the satellite cells are no longer able to repair the damage and the muscle tissue is substituted by connective and fat tissue. Facioscapulohumeral Muscular Dystrophy (FSHD), the second most common muscular dystrophy in the adult, displays an autosomal dominant inheritance with an incidence of 1/14000 and no cure. The only available murine model, reproducing key features of the disease, is the transgenic mouse overexpressing FSHD Region Gene 1 (FRG1) specifically in the skeletal muscle. We recently reported that, unlike classical form of dystrophy, inflammation and satellite cell impairment are primary defects in FRG1 mice. Gene expression profiling, performed before the muscular dystrophy development, shows that the transcriptome of FRG1 mice is remarkably similar to that of FSHD patients. This analysis highlighted the involvement of the immunity system in the onset of the disease and identified a Nos1 downregulation in FRG1 mice. Nitric oxide (NO) synthase 1 (Nos1) is expressed in skeletal muscle and regulates satellite cells activation and differentiation. Based on our results, we designed a pre-clinical trial with anti-inflammatory drugs (Ibuprofen) and NO donors (Molsidomine) to address the phenotypic consequences of FRG1 overexpression. The combined treatment leads to histological and functional amelioration of the phenotype. This study could open a potential route to clinical application and treatment for individuals affected by FSHD.

*****

34. EXTRACELLULAR COLLAGEN VI MODULATES CELL SURVIVAL AND AUTOPHAGY IN MOUSE EMBRYONIC FIBROBLASTS

S. Castagnaro 1, M. Chrisam 1, M. Cescon 1, P. Grumati 1,2, P. Bonaldo 1

1 Dept. of Molecular Medicine, University of Padova, Via Ugo Bassi 58/B – 35131 Padova, Italy.
2 Institute of Biochemistry 2, Goethe University - Faculty of Medicine, University Hospital, Bldg. 75, Theodor- Stern-Kai 7 - 60590 Frankfurt am Main, Germany.
E-mail: silvia.castagnaro@studenti.unipd.it

Collagen VI (colVI) is a major extracellular matrix protein broadly distributed in skin, cartilage and skeletal muscle. Mice lacking colVI (Col6a1$^{-/-}$) display a dystrophic phenotype due to apoptosis and defective activation of autophagy in skeletal muscles, leading to accumulation of dysfunctional mitochondria in myofibers.[1,2] Fibroblasts are the main cell type producing colVI, nevertheless little is known about alterations in Col6a1$^{-/-}$ fibroblasts. We established mouse embryonic fibroblast lines (MEFs) from wild-type and Col6a1$^{-/-}$ mice and analyzed alterations due to ablation of colVI. TUNEL and flow cytometric analysis showed increased apoptosis in Col6a1$^{-/-}$ MEFs, which was rescued when cells were grown onto purified colVI, but not on control collagen I. When cultured under nutrient stress, Col6a1$^{-/-}$ MEFs showed increased cell death. Moreover, Col6a1$^{-/-}$ MEFs displayed a block in autophagic response both in basal and under serum withdrawal. In Col6a1$^{-/-}$ MEFs autophagosomes are formed but their fusion with lysosomes was impaired, leading to autophagosome accumulation. Col6a$^{1-/-}$ lysosomes appeared highly enlarged. Western blot

showed increased AMPK activation in Col6a1$^{-/-}$ MEFs, suggesting an energy imbalance, and persistent Akt and Ulk1 phosphorylation under serum withdrawal. Fibroblasts lacking colVI displayed impaired autophagy and failed to remove damaged fragmented mitochondria, leading to increased cell death. These results provide the first evidence that not only muscle fibers, but also connective tissue cells producing collagen VI are affected by the lack of this protein. Furthermore these defects are reminiscent of those detected in muscle fibers, thus enabling further mechanistic studies in patients affected by collagen VI myopathies.

1. Irwin et al. Nature Genet. 35, 2003. [2] Grumati et al. Nature Med. 16, 2010.

*****

35. NEW PERSPECTIVES IN LGMD2D THERAPY: SMALL MOLECULES "TO CURE" THE MUTATED ALPHASARCOGLYCAN

Elisa Bianchini 1, Romeo Betto 2 and Dorianna Sandonà 1

1 Dipartimento di Scienze Biomediche, Università di Padova.
2 Istituto di Neuroscienze, Padova, CNR, Consiglio Nazionale delle Ricerche.
E-mail: dorianna.sandona@unipd.it

LGMD2D is a rare genetic disorder affecting mainly the limb girdle musculature. It is due to defects of the gene coding α-sarcoglycan (SG) that, together with β-, γ- and δ-SG, forms a tetramer conferring structural stability to the sarcolemma of striated muscles. Most of the mutations associated to LGMD2D are missense mutations. The primary event in this disease is the premature degradation of the folding-defective mutant, with a secondary loss of the wild-type partners, operated by the Endoplasmic Reticulum Associated Degradation (ERAD). Interestingly, the entire complex can be rescued at the proper cellular site by reducing the degradation of the mutated-SG. This means that the pathogenic mechanism underlining LGMD2D is a loss of function because of the lack/reduction of the complex, instead of a mutant functional-impairment and opens a new perspective for the therapy of this neglected disease. We have designed two small molecule-based strategies aimed at either "save" mutants from degradation or "assist" mutant in the folding process. The pharmacological inhibition of the E3 ligase HRD1, key element of the degradative route, leads to the quantitative and functional rescue of the mutant both in a cell model and in primary myotubes from a LGMD2D patient. Regarding the "protein assisting" strategy, we are testing several small molecules, known as pharmacological chaperones that, promoting folding not only preserve α-SG mutants from degradation but also permit their proper localization. Altogether our results constitute the proof of concept for the development of innovative pharmacological therapies for the cure of a rare muscle disease.

*****

36. MIR-206 AS A BIOMARKER OF DMD

Sandonà M. 1, Saccone V. 1, Consalvi S. 1 and Puri PL1 2

1 Fondazione Santa Lucia. Rome-Italy.
2 Sanford-Burnham Institute for Medical Research, La Jolla, CA. USA.
E-mail: martinasandona2@gmail.com

Duchenne muscular dystrophy (DMD) is a rare neuromuscular disease characterized by degeneration of muscles, impaired locomotion and premature death. During DMD progression there is a decreased ability of muscle stem cells (satellite cells) to replace the regenerating muscles and also there is an increase of fibroadipogenic activity of a population of interstitial cells, named fibrodipogenic progenitors (FAPs). In our recent study we observed that treatment with HDACi exerts beneficial effect in DMD targeting FAPs and promoting their "latent" myogenic phenotype (Mozzetta et al., 2013) (Saccone et al., 2014). A big trouble in DMD is to find a reliable biomarker to follow the progression of the disease and also to evaluate beneficial effects of the treatment with HDACi. In our recent discovery we observed, after treatment with HDACi, an increase of expression of myogenic miRNAs in FAPs and DMD muscles. Focusing our attention on miR-206, classified as a regeneration microRNAs (Greco et al., 2008), and using an immunohistochemistry technique, we observed a correlation between miR-206 expression and the stage of the disease and also a correlation with the efficacy of the treatment with HDACi. This study will define novel reliable biomarkers of disease progression and response to pharmacological interventions that will have a tremendous impact on optimal selction of patients and evaluation of outcomes of clinical trial in DMD boys with the novel therapeutic intervention such as HDACi.

*****

37. AUTOPHAGY DURING DMD PROGRESSION

Fiacco Elisabetta 1, Bianconi Valeria 1, Madaro Luca 1, Francesca Nazio 1, Puri Pier Lorenzo 1, Latella Lucia 1,2

1 Santa Lucia Foundation Rome, Italy.
2 Institute of Translational Pharmacology, National Research Council Rome, Italy
E-mail: elisabettafiacco@tiscali.it

Autophagy is a degradative process involved in the replacement of cellular components and the turn-over of whole organelles but also in the removal of these components during starvation or stress. Autophagy contributes to the pathogenesis of many neurodegenerative diseases and muscular dystrophies. Duchenne muscular dystrophy (DMD) is an aggressive form of dystrophy leading to progressive muscle weakness associated with a loss of muscle mass that is eventually replaced with fat and connective tissue .We decided to monitor the changes of autophagic flux during DMD progression in mdx mice at different ages The overall goal of this study was to establish a relationship between autophagy, regeneration and DMD progression. Our data show that autophagy is modulated during the progression of disease in mdx mice. We found an increased flux of autophagy in the early stages of the disease in coincidence

with high levels of regeneration; however, a progressive decline in the autophagic flux was observed along with the progression of the disease. Autophagy seems therefore an essential character in the activation and mobilization of muscle progenitors that drive regeneration. We isolated MSC and FAPS by flow cytometry analysis and cultured in vitro. We performed a trans-well co-culture with FACS and MSC to assess the crosstalk between FAPS and MSC in driving muscle regeneration. We speculate that autophagy may play a role to create the perfect environment for regeneration in the early stages of the disease, and may help to prevent the deposition of fat and fibrous tissue in the later stages of the disease. Autophagy is actively contributing to DMD pathogenesis and modulation of this process can be used to enhance the efficacy of pharmacological treatments

*****

38. INTERSTITIAL CELL ACTIVATION DURING ACUTE MUSCLE DENERVATION AND ALS

Madaro Luca 1,2, Passafaro Magda 1, Fasanaro Pasquale 1, Berghella Libera 1, Puri Pier Lorenzo 3

1 IRCCS Fondazione Santa Lucia. Rome, Italy; 2 CNR Institute of Cellular Biology and Neurobiology, Rome, Italy; 3 Sanford-Burnham Institute for Medical Research, La Jolla, CA. USA.
E-mail: l.madaro@hsantalucia.it

Upon denervation or disuse, skeletal muscles undergo atrophy, leading to reduced myofibers size, impaired contractile and metabolic activities. Despite of the recent identification of some components of the catabolic network that mediates degradation of sarcomeric proteins, there is a lack of knowledge on the contribution of muscle neighboring cells in the atrophic process. Here we show that denervation-induced muscle atrophy is associated to the increase of interstitial cells as well as Fibro-Adipogenic Precursor Cells (FAPs) and Macrophages. RNA-sequencing analysis, reveal a specific modulation of a gene expression program in FAPs during denervation, suggesting a recruitment of those cells in these events. Similar result was obtained in a mouse model of ALS in which neuronal degeneration is associated with muscle atrophy. We have observed an increasing number of FAPs in SODG93A at different stages of disease progression, by immunostaining and cytofluorimetric analysis. Of note, similarly to acute denervation, muscle-derived FAPs show an alteration of genes expression in SODG93A mice.

*****

Session 6. Genetic and Epigenetic Regulation of Muscle Homeostasis and Differentiation

39. HISTONE DEACETYLASE 4 IS PROECTIVE IN AMYOTROPHIC LATERAL SCLEROSIS AND MODULATES THE RESPONSE TO OXIDATIVE STRESS

Pigna Eva 1, Coletti Dario 1,2, Mancinelli Rosa 3, Adamo Sergio 1 and Moresi Viviana 1

1 Sapienza University of Rome, Department of Anatomical, Histological, Forensic & Orthopaedic Sciences, Histology & Medical Embryology Section, 00161 Rome, Italy.
2 Pierre and Marie Curie University - Paris 6 Vieillissement, stress, inflammation (UR 4), Paris, France.
3 Department of Neuroscience and Imaging, University G. d'Annunzio Chieti-Pescara, Chieti, Italy.
E-mail: eva.pigna@gmail.com

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by motor neuron degeneration, muscle atrophy and paralysis. Several factors account for the development of ALS, including accumulation of oxidative stress in skeletal muscle. A correlation between the histone deacetylase 4 (HDAC4) expression and the progression of the disease has been recently reported, but the role of HDAC4 in the disease is unknown. In this study we investigate HDAC4 role in ALS and in response to oxidative stress by deletion of HDAC4 in skeletal muscle in a mouse model of ALS, SOD1G93A mice. Lack of HDAC4 anticipates body weight loss in SOD1G93A HDAC4 mKO mice, inducing more pronounced muscle atrophy compared with SOD1G93A mice. To study the molecular mechanisms underlying HDAC4 function in response to a chronic denervation, such as in ALS, we cut the sciatic nerve of one limb of HDAC4 mKO mice and analyse muscles over time. HDAC4 mKO mice are resistant to muscle atrophy until two weeks following denervation, but at later time point muscles degenerate. Moreover, HDAC4 mKO muscles present ultrastructural defects in myofiber organization and higher levels of ROS in contralateral innervated muscle, while muscle architecture and the molecular responses to oxidative stress are blunted following denervation. From our results, we conclude that HDAC4 is important to maintain muscle mass and integrity upon oxidative stress, thus, the administration of HDAC4 inhibitors may be deleterious for ALS patients. Further studies are necessary to delineate the role of HDAC4 in skeletal muscle following chronic denervation.

*****

40. REGULATORY MECHANISM DRIVING AB-CRYSTALLIN INDUCTION IN SKELETAL MUSCLE CELLS UPON REDOX IMBALANCE

Neri Mercatelli, Simona Fittipaldi, Ivan Dimauro, Maria Paola Paronetto, Daniela Caporossi

Department of Movement, Human and Health Sciences, University of Rome "Foro Italico", Italy
E-mail: daniela.caporossi@uniroma4.it

The cytoprotective and antioxidant role of αB-crystallin (αB-cry) is mediated by the ability to upregulate itself, but the molecular pathway driving αB-cry expression in muscle cells during oxidative stress still remains unknown. We analyzed changes in αB-cry expression in C2C12 myogenic cells at different time post-treatment (0, 2, 6 and 18 hours) with two distinct oxidative stress inducers: sodium meta-arsenite NaAsO2 (50 μM) and hydrogen peroxide H2O2 (100 μM). A significant and rapid increase in αB-cry mRNA and protein levels was observed in NaAsO2 treated cells. On the contrary, H2O2 treatment showed a slight increase, at 18 hours only. Activation of JNK and AKT were evident for both oxidants. The AKT phosphorylation induced by NaAsO2 was always much less intense than that resulting by H2O2, while a stronger activation of p38 kinase was observed after NaAsO2 compared to H2O2 treatment. Only NaAsO2 exposure induced an almost complete oxidation of both Thioredoxin-1 and Thioredoxin-2, with a complete recovery of their redox status at 6 hours from treatment. To get a further insight into this regulatory mechanism, we searched for putative redox-sensitive transcription factors (RSTFs) that could drive the αB-cry mRNA induction. Interestingly, the RSTFs c-jun and Nrf2 were found specifically up-regulated after NaAsO2 treatment. Moreover, our data regarding their binding activity on αB-cry promoter strongly support the direct role for c-jun and Nrf2 in the regulation of this gene upon NaAsO2-induced redox imbalance in skeletal muscle cells.

*****

41. CHARACTERIZATION OF THE EXOSOME-LIKE VESICLES RELEASED BY C2C12 CELLS DURING THE EARLY PHASE OF MYOGENIC DIFFERENTIATION PROCESS

Michele Guescini 1, Serena Maggio 1, Michela Battistelli 2, Giosuè Annibalini 1, Michele Menotta 1, Luciana Vallorani 1, Sara Salucci 2, Elisabetta Falcieri 2, Vilberto Stocchi 1

1 Department of Biomolecular Sciences, 2 Department of Human, Environment and Nature Science, University of Urbino, Urbino, Italy.
E-mail: michele.guescini@uniurb.it

Muscle is a highly plastic tissue able of adapting to different stresses, in part due to its remarkable regenerative capacity. In addition to soluble proteins, it has been found that myoblasts and myotubes release exosome-like vesicles in the extracellular environment during myogenic differentiation. Cells can release different types of extracellular vesicles (EVs): exosomes, shedding vesicles, and apoptotic bodies. The aim of this study was to investigate the type of EVs released by C2C12 cells in the early phases of myogenic differentiation process. Myoblasts were grown to confluence and then shifted to low-serum medium to induce myotube formation. EVs were purified by serial ultracentrifugation. The EVs collected during myogenic differentiation process were characterized using TEM, atomic force microscopy, western blot, density gradient and real-time PCR analyses. The EVs from myoblasts showed a mean size of approximately 53±8 nm in diameter with the outer dense wall and the inner less dense region. At the early stage of myogenic differentiation the most abundant EVs had a larger diameter if compared to those of myoblasts and contained an electron-dense material. At late differentiation stage, size distribution analysis of EVs revealed a mean diameter size of 205±86 nm with an abundant quantity of vesicles containing electron transparent material. Moreover, at the first days of differentiation, using density gradient separation it was possible to identify two sub-populations of EVs. The results herein reported suggest that differentiating C2C12 cells may release different types of EVs which could have different effects on neighboring cells.

*****

42. SAM68 REGULATES ALTERNATIVE SPLICING AT THE ONSET OF MYOGENIC DIFFERENTIATION

Maria Paola Paronetto 1,2, Piergiorgio La Rosa 3, Elisabetta Volpe 2, Laura Pelosi 4, Daniela Caporossi 1, Carmine Nicoletti 4, Antonio Musarò 4 and Claudio Sette 2,3

1 University of Rome "Foro Italico", Piazza Lauro de Bosis 15, 00135, Rome, Italy.
2 CERC Fondazione Santa Lucia, Via del Fosso di Fiorano 64, 00143, Rome, Italy.
3 University of Rome "Tor Vergata", Via Montpellier 1, 00133, Rome, Italy.
4 University of Rome "La Sapienza", Rome, Italy.
E-mail: mariapaola.paronetto@uniroma4.it

Alternative splicing (AS) is a key process that allows generation of multiple distinct mRNAs isoforms from a limited number of genes, thus contributing to protein diversity. Extensive modulation of AS occur during myogenic differentiation, suggesting a major role for this process in the acquisition of the muscle protein repertoire. SAM68 is a RNA binding protein (RBP) involved in several steps of RNA metabolism with implications in cell differentiation and cancer. *Sam68*$^{-/-}$ mice display defects in gametogenesis, bone metabolism, adipogenesis, mammary gland development and motor coordination. *Sam68*$^{-/-}$ mice are smaller than wild type littermates. Muscle analysis revealed a specific reduction in mass, which correlated with a higher number of fibers of smaller size. Maturation of the fibers and increase in cross section area between 4 and 12 weeks of age was particularly impaired, associated with a specific defect in muscle force. Skeletal muscle contains several types of progenitor cells involved in muscle differentiation and homeostasis, including satellite cells and fibro-adipogenic progenitors (FAPs). FACS analysis showed that Sam68-/- muscles have a higher number of FAPs, while satellite cells are less affected. Notably, *Sam68*$^{-/-}$ FAPs displayed impaired capacity to differentiate into adipocytes, suggesting a specific defect in their differentiation potential. At the molecular level, we have identified a number of AS events in genes involved in myogenic differentiation that are altered in Sam68$^{-/-}$muscles. Collectively, our studies provide

a new function for SAM68 in skeletal muscle and suggest that this RBP plays a direct role in the execution of the myogenic differentiation program.

*****

43. RBFOX FACTORS REGULATE A TISSUE-SPECIFIC ALTERNATIVE SPLICING EVENT ESSENTIAL FOR MUSCLE DIFFERENTIATION

V. Runfola 1,2, J. Dilworth 3, D. Gabellini 1

1 Dulbecco Telethon Institute and Division of Regenerative Medicine, San Raffaele Scientific Institute, Milan.
2 PhD program in Cellular and Molecular Biology, Università Vita---Salute San Raffaele, Milan.
3 The Sprott Center for Stem Cell Research, Regenerative Medicine Program, Ottawa Hospital Research Institute, Canada.
E-mail: runfola.valeria@hsr.it

Alternative splicing of pre-mRNAs is a major contributor to proteomic diversity and regulation of gene expression in higher eukaryotic cells. For this reason, it is tightly regulated in different tissues at different developmental stages and its disruption can lead to a wide range of human disorders. Interestingly, skeletal muscle is one of the tissues with the highest number of differentially expressed alternative exons, revealing a previously unappreciated degree of alternative splicing complexity in muscle-specific transcripts. Transcription factors of Mef2 family are essential transducers of cell signalling that modulate differentiation of many cell types. Among Mef2 family members, Mef2D is unique, as it undergoes tissue-specific splicing to generate a muscle-specific isoform essential for muscle differentiation. Through bioinformatics analysis and expression profiling, we identified the Rbfox family of splicing factors as putative Mef2D muscle-specific splicing regulators. We developed a quantitative assay to monitor the expression level of Mef2D splicing variants finding that it parallels Rbfox1 and 2 expression during muscle differentiation. CLIP experiments indicate specific and direct Rbfox1 and 2 binding to Mef2D pre-mRNA in vivo. Gain and loss of function experiments demonstrate that Rbfox1/2 synergize in promoting Mef2D muscle-specific splicing and myogenesis. Our results elucidate the molecular underpinning regulating muscle differentiation at post-transcriptional level.

*****

44. EPIGENETIC DRUGS TREATMENT FOR DISUSE AND DENERVATION ATROPHY

Passafaro M. 1, Madaro L. 2, Berghella L. 2, Puri P.L. 2

1 Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Fondazione Santa Lucia, 00143 Rome, Italy.
E-mail: Magda.passafaro@gmail.com

Skeletal muscle is a dynamic tissue that can respond to external stimuli through both anabolic and catabolic processes. In a variety of conditions, including immobilization, AIDS and neuromuscular disorders, skeletal muscle mass is decreased (atrophy). Previous studies have identified key molecular pathways that lead to protein breakdown and degradation of sarcomeric proteins, and have suggested a key role of histone deacetylases (HDACs) in the control of machinery that promotes catabolic muscle atrophy. Our data show efficacy of HDACs inhibitors in two different mouse models of muscle atrophy: disuse (by immobilization) and neurogenic (by denervation). Treatment with HDACi prevented the reduction of muscle fiber cross-sectional area (CSA), which is typically observed upon immobilization and denervation. We will present data that predicts the molecular network(s) implicated in the protective effect of HDACi treatments on muscle mass loss.

*****

45. FSHD MUSCULAR DYSTROPHY REGION GENE 1 BINDS SUV4-20H1 HISTONE METHYLTRANSFERASE AND IMPAIRS MYOGENESIS

Maria Victoria Neguembor, Roberta Caccia and Davide Gabellini

Dulbecco Telethon Institute and Division of Regenerative Medicine, San Raffaele Scientific Institute, Milano, Italy.
E-mail: neguembor.marie@hsr.it

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant myopathy characterized by selective muscle wasting. The disease is caused by deletion of tandem repeats located at the subtelomeric region of chromosome 4 (4q35). Upon contraction, the region undergoes an epigenetic deregulation that causes the over-expression of nearby genes. Among them, we focused on FRG1 (FSHD Region Gene 1) since its over-expression leads to muscular dystrophy-like defects in mice, Xenopus laevis and Caenorhabditis elegans, suggesting that FRG1 plays a relevant role in muscle biology. Interestingly, we found that, when over-expressed, FRG1 binds and interferes with the activity of the histone methyltransferase Suv4-20h1, responsible for the di- and tri-methylation of H4K20. Accordingly, FRG1 over-expression or Suv4-20h1 knockdown inhibits myogenesis, and muscle specific Suv4-20h-/- mice develop signs of muscular dystrophy. We found that Suv4-20h1 expression inversely correlates with the severity of the phenotype in different skeletal muscles, providing a plausible molecular explanation for the differential muscle susceptibility in FSHD. Finally, we identified Eid3 as an epigenetic Suv4-20h1 target and a novel myogenic inhibitor, specifically upregulated in FSHD patients. Our study describes a novel role of FRG1 as epigenetic regulator of gene expression and shows that Suv4-20h1 has a gene-specific function in myogenic differentiation. These findings suggest that epigenetic defects contribute to FSHD and identify possible therapeutic targets.

*****

II Lecture

Link Between Malignant Hyperthermia (Mh) And Environmental Heat Stroke (Ehs): Just A Medical Hypothesis?

Feliciano Protasi

CeSI - Center for Research on Ageing & DNICS – Dept. Neuroscience, Imaging, and Clinical Sciences, University G. d'Annunzio of Chieti, I66100 Italy.
E-mail: fprotasi@unich.it

Background. Mutations in the gene encoding for ryanodine receptor type-1 (RYR1), the SR Ca2+ release channel, underlie debilitating, life-threatening muscle disorders such as central core disease (CCD) and malignant hyperthermia (MH). To date, MH is only seen as a clinical syndrome in which genetically predisposed individuals respond to volatile anesthetics in the operating room with potentially lethal episodes characterized by elevations in body temperature and rhabdomyolysis of skeletal muscle fibers. However, virtually identical over-heating episodes have been reported in individuals also after exposure to environmental heat, physical exertion, or even during febrile illness. The life-threatening nature of EHS underscore the critical need for a deeper mechanistic understanding of these syndromes and for the development of new and effective treatments. Specific Gaps of Knowledge. A) Mutations in RYR1 have been found in many, but not all, MH cases suggesting the potential involvement of additional genes in the pathogenesis of this syndrome. B) The relationship between classic MH and over-heating episodes triggered by different stressors (heat, exertion, fever, etc.) is not yet widely recognized. C) The cascade of molecular mechanisms that from SR Ca2+ leak leads to rhabdomyolysis of muscle fibers are still unclear and needs to be fully elucidated. Recent breakthroughs. In the last years, thanks to the support of Telethon (GGP08153 and GGP13213), we have moved significant steps forward. We have demonstrated in animal models that: A) MH episodes can result not only from mutations in RYR1, but also from mutations in proteins that interact with RYR1 (such as Calsequestrin-1, CASQ1); B) the mechanisms underlying hyperthermic episodes triggered by anesthetics and by heat and exertion are virtually identical, suggesting that these syndromes could be possibly treated/prevented using similar treatments; C) during lethal MH/EHS crises Ca2+ leak from intracellular stores results in a feed-forward mechanism mediated by excessive production of oxidative species of oxygen and nitrogen (ROS and RNS), which eventually will lead to depletion of the SR and to massive activation of Store Operated Ca2+ Entry (SOCE).

*****

Session 7. Muscle Physiology

46. INTEGRATED ELECTROMYOGRAPHIC ACTIVITY OF ANTIGRAVITATIONAL MUSCLES INCREASES IN STANDING VS. SUPINE POSTURE: CORRELATION WITH TOTAL BODY OXYGEN UPTAKE

Alessandro Rubini, Antonio Paoli

Department of Biomedical Sciences, Section Physiology, University of Padova, Italy
E-mail: alessandro.rubini@unipd.it

We measured the integrated electromyographic activities of two antigravitational muscles of the lower limb, soleus and gastrocnemius, on twelve young healthy subjects in supine and standing positions at rest. Total body oxygen uptake and carbon dioxide production were also determinated. As expected, we found statistically significant increments of integrated electromyographic activities in standing vs. supine position. A significant positive correlation was found between total body oxygen uptake and carbon dioxide production and integrated electromyographic activities of antigravitational muscles. These results indicate that, in comparison with supine posture, standing position is associated with an increased total body metabolic rate which is directly linked to antigravitational muscle tone increments.

*****

47. EVALUATION OF THE RESTING CHLORIDE CONDUCTANCE, SARCOLEMMA EXCITABILITY AND CALCIUM HOMEOSTASIS IN SKELETAL MUSCLE OF A SOD-1 RELATED AMYOTROPHIC LATERAL SCLEROSIS (ALS) MOUSE MODEL

Pierno S. 1, Fonzino A. 1, Liantonio A. 1, Conte E. 1, Camerino G.M. 1, Cannone M. 1, Dobrowolny G. 2, Musarò A. 2, Conte Camerino D 1

1 Section of Pharmacology, Dept. of Pharmacy & Drug Sciences, University of Bari, Bari, Italy. 2 DAHFMO-Unit of Histology and Medical Embryology, IIM, Sapienza University of Rome.
E-mail: sabata.pierno@uniba.it

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by muscle atrophy. Most of the familial cases are due to mutations within the gene encoding for the superoxide dismutase 1 (SOD-1) protein, involved in the detoxification of reactive oxygen species. Transgenic animals carrying mutations in the SOD-1 gene develop similar symptoms than those observed in clinic. In this animal model skeletal muscle has been demonstrated to be primarily involved in SOD-1-mediated toxicity (Dobrowolny et al., Cell Metab, 8:425, 2008). In this context, sarcolemma ion channels play a crucial role for muscle function. Resting chloride conductance (gCl), sustained by the ClC-1 channel, controls the resting membrane potential and excitability. Calcium channels are important for proper contractility. In this study we measured the resting gCl, muscle excitability and calcium homeostasis in extensor digitrorum longus (EDL) muscle of SOD-1 animals (Pierno et al., Brain 125:1510, 2002). We found that resting gCl was strongly

reduced in SOD-1 mice compared to wild-type (WT), being it 1593±100 µS/cm2 (19 fibers) and 2522±88 µS/cm2 (16 fibers), respectively. Also sarcolemma excitability, evaluated as the maximum number of action potentials, was accordingly increased from 5.7±0.3 in WT to 11.2±1.9 in SOD-1 muscle fibers. In these animals the resting intracellular calcium level was significantly increased and the response to caffeine was altered. We are currently evaluating the mRNA expression of ion channels by Real Time-PCR analysis. Thus, skeletal muscle ion channel function is modified in the SOD-1 transgenic animals suggesting their contribution to muscle damage and potential pharmacological modulation.

*****

48. FUNCTIONAL CHARACTERIZATION OF THE COMMUNICATION BETWEEN MUSCLE AND NERVE IN A MURINE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

Simona Pisu 1, Emanuele Rizzuto 2, Zaccaria Del Prete 2 and Antonio Musarò1

1 DAHFMO-Unit of Histology and Medical Embryology, Sapienza University of Rome, Italy.
2 Department of Mechanical and Aerospace Engineering, Sapienza University of Rome.
E-mail: simona.pisu@uniroma1.it

Recent studies in murine models of Amyotrophic Lateral Sclerosis (ALS) showed the primary role of skeletal muscle in the pathogenesis of the disease[1], pointing out the key role of the communication between nerve and muscle. To better address which element of the motor-unit is initially affected by this pathology [2], we developed a protocol to measure, in vitro the neuromuscular junction (NMJ) functionality of SOD1G93A murine model[3]. The protocol is based upon the comparison between the contractile response of the muscle stimulated through its nerve with that of the same directly simulated on the membrane. Since this latter stimulation by-passes the neuromuscular junction, any difference in the two responses can be related to defects in this crucial element of the muscle nerve communication. We investigated soleus-sciatic nerve preparation on transgenic and control mice at the very end stage of the disease. Experimental results revealed that transgenic muscles present a decrease in the capability of generating force and a significant slowdown of the kinetic parameters when stimulated through the nerve, in comparison to the response obtained stimulating the muscle directly on the membrane. The analysis of neurotransmission failure [4] and intratetanic fatigue [5] pointed out a significant increase of the NMJ fatigue in transgenic muscles. Taken all together these results show a prominent defect in the SOD1G93A soleus muscle NMJ and suggest a relationship with an intrinsic damage of muscle fibres and with an altered muscle fibres composition.

*****

49. ASSESSMENT OF EMG MAPS BY MEANS OF ELECTRODE MATRIX RECORDINGS

Filligoi G. 1, Sergi A. 2, Monteneri A. 2, Verrelli V. 2, Carlino S.2, Pescosolido E. 2

1Dept D.I.E.T. (Electronic and Telecommunication Engineering), Faculty I3S (Information Engineering, Informatics and Statistics), Via Eudossiana 18, 00184 Univ. of Roma Sapienza, Italy –
2 Students of the course on "Biomedical Signal Processing", Degree on Clinical & Biomedical Engineering, Via Eudossiana 18, 00184 Univ. of Roma Sapienza, Italy.
E-mail: giancarlo.filligoi@uniroma1.it

Recently, the possibility of increasing the knowledge about the physiopathology of the neuro-muscular system was shown to be feasible by the use of advanced detection systems and algorithms specifically designed to process information from multi-channel recordings. In particular, the adoption of electrode arrays for the EMG surface (sEMG) recordings has proven to be a valuable technique able to separate single MU activities as well as estimate single MU properties, thanks to the application of spatial sampling and spatial filtering techniques of the surface potentials, also from bipinnate muscles. Moreover, the use of two-dimensional spatial sampling allowed also to get a reliable estimate of the muscle fiber conduction velocity. In this work, we will present some results of our targeted experiments devoted to obtain topographical maps of both the myo-electrical activity below the electrode matrices placed over the skin and the relative maps of parameters further extracted from sEMG, from multi-channel recordings. These parameters have been evaluated either in the time- or the frequency-domain (thus expanding the approach usually applied for twin electrodes recordings) with possible applications to the detection of early signs of muscle fatigue, to ergonomics, to neuro-muscular diagnosis, to muscular rehabilitation monitoring, to sport medicine. The results we are going to present here concern a preliminary study, developed by a group of students of the Faculty of Clinical & Biomedical Engineering of the University of Roma Sapienza, applied to control subjects with no signs of pathologies, but with evident applications to neuromuscular physiopathology.

*****

50. CHARACTERIZATION OF CIRCULATING MUSCLE-DERIVED EXTRACELLULAR VESICLES

Serena Maggio 1, Michele Guescini 1, Barbara Canonico 2, Giosuè Annibalini 1, Francesco Lucertini 1, Elena Barbieri 1, Francesca Luchetti 2, Stefano Papa 2, Vilberto Stocchi 1

1 Department of Biomolecular Sciences - Division of Exercise and Health Sciences, University of Urbino Carlo Bo, 61029 Urbino, Italy.
2 Department of Human, Environment and Nature Science, University of Urbino Carlo Bo, 61029 Urbino, Italy.
E-mail: serena.maggio@uniurb.it

In recent years several research groups described the release of humoral factors from contracting muscle cells. Furthermore, recent studies reported that muscle could also release microRNAs (miRNAs) in circulation in response to physical exercise. Although an association between

circulating miRNA and extracellular vesicles (EVs) such as microvesicles, exosomes or apoptotic bodies has been demonstrated, the mechanisms by which muscle releases miRNAs in plasma has yet to be determined. The main aim of this study was to investigate whether muscle tissue releases EVs carrying miRNAs in circulation under physiological conditions. To achieve this goal we used a combination of three techniques: cytometric analysis, density gradient separation, and immuno-capturing. The possible role of exercise in the modulation of miRNAs loaded into EVs was also evaluated. Two important messages emerge from this study. Firstly, in line with data available from in-vitro studies, muscle tissues release EVs in the extracellular environment and, at least in part, the muscle-enriched miRNAs (MyomiRs) detected in circulation are engulfed into these EVs. Secondly, the physiological stimulus of exercise seems to modulate the levels of the MyomiRs contained into EVs. Future studies may be designed to deeply explore the content of muscle extracellular vesicles in physiological (e.g. exercise) or pathological (e.g. inactivity, atrophy, diabetes, etc.) conditions. Such efforts could provide insight into the role of EVs as not only as useful biomarkers, but also as regulators of the body's homeostasis.

*****

III Lecture

NOVEL ROUTES TOWARDS THE 'HEART' OF MYOGENESIS

Maurilio Sampaolesi

Human Anatomy Institute, Department of public health, experimental and forensic medicine, University of Pavia, Italy and Laboratory of Translational Cardiomyology, Department of Development and Regeneration, KU Leuven, Belgium.

E-mail: maurilio.sampaolesi@unipv.it

Skeletal and cardiac muscle regeneration still constitutes a thrilling and ambitious frontier for conditions such as muscular dystrophies (MDs) where both tissues are affected. The ability of skeletal muscle to restore after injury is well established, and in contrast, heart regeneration is inadequate and scar tissues appear after injury. In the embryonic life skeletal and cardiac progenitors display a complete different hierarchical gene networks. Pax3 and Pax7 activate the muscle regulatory factors, MyoD, Myf5, Mrf4, and Myogenin, which subsequently drive the skeletal myogenesis. Mesp1 and Mef2c activate cardiac transcription factors, Nkx2.5, Gata4 and Myocardin that control the expression of the heart contractile apparatus genes, e.g. actin, myosin and troponins. While MyoD, is able to convert fibroblasts into myoblasts, the forced expression of Gata4, Tbx5 and Mef2C (and Hand2) are needed to turn fibroblasts into functional cardiomyocytes. Induced pluripotent stem (iPS) cell technology and epigenetic cell memory allow us to isolate mesodermal progenitors able to harness the simultaneous differentiation potential of cardiac and skeletal muscle progenitors. We generated mesodermal iPS cell-derived progenitors (MiPs) able to differentiate towards cardiac and skeletal lineages. We demonstrated the impact of murine iPS cell memory on chimerism in fetal development and in adult tissues, and on MiP-based combined regeneration of cardiac and skeletal muscle in a murine model of MDs. Finally, proofs of concept with TALEN-based genome editing for genetic correction of dystrophic dog iPS cells and with isogenic human settings qualify MiPs as candidates for combined cell therapy of cardiac and skeletal muscles.

*****

Session 8: Cardiac and skeletal myogenesis

51. INSIGHTS ON THE ROLE OF PKCε IN CARDIO-PROTECTION AND VESSEL FORMATION

Galli D., Di Marcantonio D., Carubbi C., Queirolo V., Masselli E., Gobbi G., Mirandola P., Vitale M.

Dipartimento di Scienze Biomediche, Biotecnologiche e Traslazionali (S.Bi.Bi.T).
Università degli Studi di Parma-c/o Ospedale Maggiore- Via Gramsci 14-43126 Parma.
E-mail: dgalli05@yahoo.it

Protein Kinase C epsilon (PKCε) plays a pivotal role in stem cell proliferation and differentiation and exerts cardio-protective effects in ischemia-reperfusion injury. On the other side, this isoform seems to be involved in endothelial and smooth muscle cell proliferation. Although there are studies suggesting a role for PKCε in vascular restenosis and aortic aneurism, molecular data on the function of PKCε in vessel formation are still lacking. We addressed the molecular role of PKCε in mouse heart vessels and vascular differentiation of adipose Sca1+ cells derived from tissues localized between mouse aorta and pulmonary artery. We found that in vivo PKCε over-expression determined significant down-regulation of SMA and PECAM in vascular heart cells. Also, pharmacological activation of PKCε in VEGF-differentiating progenitors strongly reduced vascular marker expression and tubule formation in vitro. The Ser144 phosphorylated isoform of P21-Activated Kinase (p-PAK1) is involved in this pathway, indeed pharmacological activation of PKCε in VEGF-differentiating vascular progenitors increases the expression level of p-PAK1 in vitro. Moreover, in vivo, PKCε co-immunoprecipitates with p-PAK1 confirming the partnership of these two proteins in vessel wall cells. The results on the role of PKCε in differentiation of mesenchymal Sca1+ cells towards smooth muscle and endothelial fate, together with in vivo results, open new insights for cardiac vessel disease therapy.

*****

52. INNOVATIVE STRATEGY TO GENERATE A COMPLETE AND FUNCTIONAL SKELETAL MUSCLE IN VIVO

Claudia Fuoco 1, Roberto Rizzi 2,3, Sergio Bernardini 1, Roberto Bottinelli 4, Claudia Bearzi 2,3, Stefano M. Cannata 1, Dror Seliktar 5, Giulio Cossu 6,7 and Cesare Gargioli 1,2.

1 Department of Biology, Tor Vergata Rome University, Via Ricerca Scientifica, 00133 Rome, Italy.
2 IRCCS MultiMedica, Via Fantoli 16/15, 20128 Milan, Italy.
3 Cell Biology and Neurobiology Institute, National Research Council of Italy, Via del Fosso del Fiorano, 00100 Rome, Italy.
4 Department of Physiology and Interuniversity Institute of Myology, University of Pavia and Fondazione Salvatore Maugeri (IRCCS), Scientific Institute of Pavia, Via Salvatore Maugeri 8-10, 20700 Pavia, Italy.
5 Faculty of Biomedical Engineering, Technion – Israel Institute of Technology, 32100 Haifa, Israel
6 Department of Cell and Developmental Biology, University College London. WC1E 6DE, UK.
7 Institute of Inflammation and Repair, University of Manchester, Michael Smith Building, D.4316. Oxford Road M13 9PL Manchester, UK.
E-mail: : cesare.gargioli@uniroma2.it

tions and severe loss of function. In vitro generated artificial muscles undergo necrosis when transplanted in vivo before host angiogenesis may provide oxygen for fibre survival. Here we report a novel strategy based upon the use of mouse or human mesoangioblasts encapsulated into PEG-fibrinogen hydrogel. Once engineered to express Placenta derived Growth Factor, mesoangioblasts attract host vessels and nerves, contributing to in vivo survival and maturation of newly formed myofibres. When the graft was implanted underneath the skin on the surface of the tibialis anterior, mature and aligned myofibres formed within several weeks as a complete and functional extra muscle. Moreover, replacing the fully ablated tibialis anterior with PEG-Fibrinogen embedded mesoangioblasts also results in an artificial muscle identical to a normal tibialis anterior. This strategy opens the possibility for patient-specific muscle creation for a large number of pathological conditions involving muscle tissue wasting.

*****

53. MICRO-PATTERNED GELATIN-GENIPIN HYDROGEL FOR SKELETAL MUSCLE TISSUE ENGINEERING

Francesca Gattazzo 1,2, Gianni Orsi 1, Giovanni Vozzi 1, Paolo Bonaldo 2

1 Research Center ‘‘E. Piaggio’’, University of Pisa, 56126 Pisa, Italy.
2 Department of Molecular Medicine, University of Padova, 35131 Padova, Italy.
E-mail: francesca.gattazzo@gmail.com.

Skeletal muscle tissue engineering aims to the reconstruction of skeletal muscle to replace muscle loss by traumatic injury and congenital defects. Skeletal muscle can be engineered using biodegradable and biocompatible 3D scaffolds that favour myogenic cell adhesion and subsequent tissue organization and formation.[1,2] Optimal scaffolds should behave as ECM analogue and reproduce the specific elastic moduli of skeletal muscle around 12 kPa.[3,4] In addition scaffolds must be modified in defined microarchitectures in order to control the spatial organization of cells. In fact, when cultured in vitro, myoblasts and myotubes lose their native organization and adopt random distributions. In the present study we produced natural scaffolds using gelatin cross-linked with genipin. Genipin (GP) is a natural derived cross-linking agent, obtained from gardenia fruit, with low cytotoxicity and high biocompatibility.[5] The surface of structures was topographically patterned through soft lithography which permits to topographically pattern the surface of structures in a highly reproducible manner, and in a much easier way compared to external stimuli such as mechanical stretch or electrical impulse. Myogenic cells were seeded and cultured on patterned scaffolds and the effects of patterning was evaluated on cell adhesion and differentiation. Further studies will involve the use of these materials for the study of cells derived from healthy and Collagen VI deficient myopathic mice.[6]

1. Rossi, C. A., Pozzobon, M. & Coppi, P. De. Advances in musculoskeletal tissue engineering Moving towards therapy. Organogenesis. 2010. 6:167–72. 2. Gattazzo F, Urciuolo A, Bonaldo P. Extracellular matrix: A dynamic microenvironment for stem cell niche. Biochim Biophys Acta. 2014. 1840(8):2506-2519. 3. Gilbert PM, Havenstrite KL, Magnusson KE, Sacco A, Leonardi NA, Kraft P, Nguyen NK, Thrun S, Lutolf MP, Blau HM. Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. Science. 2010. 27;329(5995):1078-81. 4. Engler AJ, Griffin MA, Sen S, Bönnemann CG, Sweeney HL, Discher DE. Myotubes differentiate optimally on substrates with tissue-like stiffness: pathological implications for soft or stiff microenvironments. J Cell Biol. 2004. 13;166(6):877-87.
5. Chiono V., Pulieri E, Vozzi G, Ciardelli G, Ahluwalia A, Giusti P. Genipin-crosslinked chitosan/gelatin blends for biomedical applications. J Mater Sci Mater Med. 2008. 19:889–898. 6. Urciuolo, A., Quarta, M., Morbidoni, V., Gattazzo, F., Molon, S., Grumati, P., et al. 2013. Collagen VI regulates satellite cell self-renewal and muscle regeneration. Nat. Commun. 2013. 4, 1964.

*****

54. THE METABOLIC MODULATOR TRIMETAZIDINE ENHANCES MYOBLAST DIFFERENTIATION AND POTENTIATES SKELETAL MUSCLE REGENERATION

Francesca Molinari 1, Sergio Chiandotto 2, Lucia Gatta 1, Fabrizio Pin 3, Paola Costelli 3, Giuseppe Rosano 1 and Elisabetta Ferraro 1.

1 Laboratory of Skeletal Muscle Development and Metabolism, IRCCS San Raffaele Pisana, Rome, Italy.
2 Department of Molecular and Clinical Medicine, University of Rome "La Sapienza", Rome, Italy.
3 Department of Experimental Medicine and Oncology, University of Torino, Italy.
E-mail: elisabetta.ferraro@sanraffaele.it

Trimetazidine (TMZ) is a modulator of cell metabolism, which optimizes energy production. It acts by blocking fatty

acid β-oxidation and by shifting ATP production towards glucose oxidation. We investigated the metabolic effect of TMZ on skeletal muscle cells and its role during myogenic differentiation and during muscle regeneration. In the present study we incubated C2C12 and satellite myoblasts with TMZ during differentiation and we administered TMZ to mice during regeneration following muscle injury. Our results show that TMZ significantly stimulates glucose and glycogen consumption in C2C12 myotubes. We also found that it transcriptionally down-regulates PDK. TMZ induces hypoglycemia in mice, suggesting that treated animals are using more glucose than the untreated ones. Moreover, we found that the administration of TMZ potentiates myogenic differentiation in both C2C12 and satellite cells. In fact, TMZ up-regulates MyoD, Myogenin, MyHC and PGC-1α and increases myoblast fusion in differentiating cells. In order to study the effectiveness of TMZ on muscle regeneration in vivo, we administered TMZ to mice following focal injury on Tibialis anterior (TA). TA analysis after the injury showed that TMZ increases the expression of Pax7 and Desmin, used as markers of satellite cell activation. TMZ also potentiates the expression of MyoD, Myogenin and of neonatal MyHC (neoMyHC) expressed by nascent regenerating myofibers. Our finding strongly suggest that TMZ stimulates myoblast differentiation and muscle regeneration following injury; this makes this drug appealing for its possible use in treatment of several skeletal muscle pathologies.

*****

55. FALL PREVENTION AND THROMBOEMBOLIC RISKS IN SURGERY: RESCUING STANDING OF BED CONSTRAINED ELDERLIES SUFFERING BORDERLINE MOBILITY IMPAIRMENTS BY FES AND VOLUNTARY FREE-BODY EXERCISE

Andrea Marcante 1,2, Stefano Masiero 1, Ugo Carraro 3, Francesco Piccione 2

1 Physical Medicine and Rehabilitation Unit, Dept. Neuroscience, University Hospital of Padova; 2 San Camillo Hospital, I.R.C.C.S., Venezia-Lido; and 3 IIM & CIR-Myo Translational Myology Lab, Dept. Biomedical Sciences, Padova, Italy
E-mail: andrea.marcante@studenti.unipd.it

Anticoagulation treatment and early mobilization after surgery are mandatory practice to reduce thromboembolic risks, in particular in bed constrained senior subjects. Standing and mobility impairments in the oldest old (fractures repair, arteriopathy surgical managements and oncologic surgery are now common in 80-90 year-old subjects) are major limitations of early mobilization after surgery, often worsened by obesity and related disuse muscle atrophy that are common risk factors of falls and their complications. We are testing FES-induced muscle training combined with a minimal program of free-body voluntary physical exercise that can be introduced before elective hospitalization or soon after urgent intervention after traumatic events. In both cases, the suggested muscle activity can be performed by patient lying in bed. Examples of the training exercises (and their results) for bed constrained subjects will be presented.

Special Talk

Engineering a self-organizing 3D model of DMD skeletal muscle for the study of pathological mechanisms and therapeutic approaches

Mohsen Afshar Bakooshli 1, Nimalan Thavandiran 1, Libero Vitiello 1,2, Penney Gilbert 1

1 Institute of Biomaterials and Biomedical Engineering (IBBME), University of Toronto, Toronto, Ont, Canada. 2 Department of Biology, University of Padova, Padova, Italy.
E-mail: libero.Vitiello@unipd.it

Currently, the two most commonly used in vitro and in vivo preclinical models for studying Duchenne muscular dystrophy (DMD) are cultures of patients' cells and mdx mice, respectively. However, the results they provide do not always translate in patients, since the murine dystrophy is known to differ from the human pathology in several respects and standard monolayer cultures cannot recapitulate all the phenomena present in the complex three-dimensional organization of skeletal muscle tissue. For these reasons we are now designing a self-organizing, 3D microtissue model of healthy and DMD skeletal muscle. The core of our system is a 96-well microfabricated silicon device, in which each well contains a gently inclining hill that culminates in two vertical posts. When a cell/ECM mixture is deposited in each well, the cells quickly remodel the matrix, so that the tissue contracts and slides up the hill, ultimately producing a uniform tissue 'doughnut' across the posts. In preliminary studies with murine cells we found that optimal microtissue maturation and stability required the use of a specific myoblasts/fibroblasts ratio (both cell populations were obtained by FACS). In these conditions, constructs showed the presence of well-organized sarcomeres and could be paced by electrical stimulation. Now we are optimizing the same approach for constructing human muscle microtissues, starting from normal and DMD cells.

*****

INDEX