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Interuniversity Institute of Myology

IIM-Myology Meeting
13-16 October 2016

FOCUS ON PATHOGENESIS OF RARE DISEASES AND THERAPIES

Topics

- Signalling in muscle growth, homeostasis and diseases
- Satellite cells and muscle regeneration in healthy muscle and in diseases
- Genetic and epigenetic alterations in muscle dystrophies and myopathies
- Biophysics and E-C coupling in the physiopathology of neuromuscular diseases
- Stem Cells and therapy
- Metabolic alterations and muscle diseases
- Muscle wasting and cachexia
- Therapeutic approaches *tor* muscle diseases

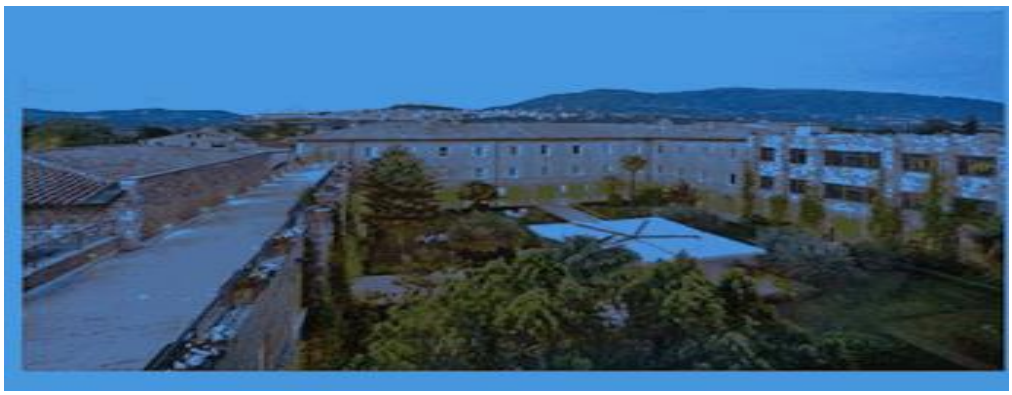
Scientific Committee:

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

Main Lectures

VINCENT MOULY (UPMC, PARIS, FRANCE); FRANCESCO MUNTONI (UCL-LONDON, UK); MARIO PENDE (LNSERM,PARIS,FRANCE); RUDIGER RUDOLF (UNIV.OF HEIDELBERG, GERMANY); VINCENZO SORRENTINO (UNIVERSITY OF SIENA,LTALY); LEESWEENEY(MYOLOGYINST.,UF-USA)

Venue:HotelIlCenacolo(Assisi-ltaly) http://www.hotelcenacolo .com/



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LECTURES

Cellular Therapies for Muscular Dystrophies: Frustrations and Clinical Successes

Vincent Mouly

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Cell-based therapy for muscular dystrophies was initiated in humans after promising results obtained in murine models. Early trials in DMD failed to show substantial clinical benefit, sending researchers back to the bench, which led to the discovery of many hurdles as well as many new venues to optimize this therapeutic strategy. New models have been generated to take into account the specificity of human cells, and new cell candidates have been explored. Epigenetics have been introduced within the paradigm, and attention has also been paid to the targets. The recent clinical trial using autologous myoblasts in OPMD patients showed some clinical benefit for the patients. However, the future of cell therapy will probably involve several combined approaches, and this will be discussed mainly in the context of OPMD.

1. *Périé S, et al. Autologous myoblast transplantation for oculopharyngeal muscular dystrophy: a phase I/IIa clinical study. Mol Ther 2014;22:219-25. doi: 10.1038/mt.2013.155. Epub 2013 Jul 8*
2. *Périé S, et al. Premature proliferative arrest of cricopharyngeal myoblasts in oculo-pharyngeal muscular dystrophy: Therapeutic perspectives of autologous myoblast transplantation. Neuromuscul Disord 2006;16:770-81.*
3. *Shadrin IY, et al. Striated muscle function, regeneration, and repair.Cell Mol Life Sci 2016;73:4175-4202. Epub 2016 Jun 6.*

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A molecular approach to understand assembly and organization of proteins of the SR and T-tubules at triads

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The sarcoplasmic reticulum (SR) is a specialized form of the endoplasmic reticulum that in striated muscles is dedicated to support the release the high amount of Ca^{2+} required to activate muscle contraction. In skeletal muscle, the SR forms a network of longitudinal tubules and terminal cisternae that surround each myofibril. A defining characteristic of the SR is the regular repetition of areas of longitudinal tubules that alternate with terminal cisternae that are aligned with respect to specific regions of the sarcomere for the entire length of myofibrils. In the past years, we and others have shown that the longitudinal tubules of the SR are connected with the myofibrils through interactions mediated by two muscle-specific proteins - sAnk1.5 on the SR and obscurin on the sarcomere. In addition to establish connections with the myofibrils, the SR also participates to the assembly of junctional membrane complexes, known as triads, formed by the close apposition of one T-tubule derived from the sarcolemma and two terminal cisternae of the SR. Triads are required to allow the structural and functional association of the Ca^{2+} release channels (RyR1) on the SR and the voltage-gated dihydropyridine receptors (DHPR) channels on the T-tubule. Therefore, triads represent a structural platform that allows a functional coupling that translates the signal mediated by depolarization of the T-tubule into Ca^{2+} release from the SR. In addition to DHPRs and RyRs, all other proteins required for Ca^{2+} release from the SR are localized at triads. Development and maintenance of triads requires the presence of muscle-specific junctophilin-1 and 2 (JPH1 and JPH2) that physically tether the membrane of the T-tubules with that of the SR terminal cisternae. These interactions are mediated by eight phospholipid-binding modules (MORN) in the N-terminus and by a trans-membrane domain (TMD) in the C-terminus of JPHs. In addition to their role in mediating the assembly of triads, JPHs seem to play a role in the organization of several proteins, including DHPRs and RyRs, required for releasing Ca^{2+} from the SR to activate muscle contraction. Recent developments in understanding the mechanisms that regulate assembly and organization of proteins of the SR and T-tubules at triads will be presented.

1. *Barone V, et al. Organization of junctional sarcoplasmic reticulum proteins in skeletal muscle fibers. J Muscle Res Cell Motil 2015;36:501-15.*
2. *Rossi D, et al. Distinct regions of triadin are required for targeting and retention at the junctional domain of the sarcoplasmic reticulum. Biochem J 2014;458 (part 2):407-17.*
3. *Bagnato P, et al. Binding of an ankyrin-1 isoform to obscurin suggests a molecular link between the sarcoplasmic reticulum and myofibrils in striated muscles. J Cell Biol 2003;160:245-53.*

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Muscular dystrophy: new challenges and review of the current clinical trials

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There has been a rapid expansion of new experimental therapies for DMD. The field has experienced some successes; however numerous studies have missed their efficacy endpoints, indicating the complexity of developing DMD therapies. Here we analyse the outcome of recently completed clinical trials for which efficacy data are available. Analysis of recently completed DMD phase II and phase III clinical trials in which clinical efficacy was the primary outcome. There have been different reasons why the different studies have failed to meet their primary endpoints, and these are often intrinsic to the mechanism of action of the study drug and the expected size for the therapeutic benefit. Additional confounding factors have been the stage of the

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disease progression; the duration of several studies; and limitations of outcome measures used. In conclusion, Current DMD clinical trials teach us important lessons for the design of future clinical trials.

1. *Schartner V, et al. Dihydropyridine receptor (DHPR, CACNA1S) congenital myopathy. Acta Neuropathol. 2016 Dec 23. doi: 10.1007/s00401-016-1656-8. [Epub ahead of print].*
2. *Bachmann C, et al. Cellular, biochemical and molecular changes in muscles from patients with X-linked myotubular myopathy due to MTM1 mutations. Hum Mol Genet 2016;pii: ddw388. doi: 10.1093/hmg/ddw388. [Epub ahead of print].*
3. *Treves S, et al. Ca^{2+} handling abnormalities in early-onset muscle diseases: Novel concepts and perspectives. Semin Cell Dev Biol 2016; pii: S1084-9521(16)30212-9. doi: 10.1016/j.semcdb.2016.07.017. [Epub ahead of print] Review.*

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Rare diseases of nutrient/mTOR signal transduction

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The mammalian Target of Rapamycin is a master regulator of growth. mTOR is a serine/threonine protein kinase that exists in two distinct complexes in the cell (mTORC1 and mTORC2) and transduces virtually all anabolic signals from the environment: nutrients, such as glucose and amino acids, growth factor peptides, such as insulin and insulin like growth factors, oxygen, mitochondrial metabolites, energy status. mTOR is required to sustain cell responses to nutrient availability including cell growth, proliferation, macromolecule biosynthesis, and suppress autophagy. During the past ten years we have generated and characterized a wide panel of mouse mutants in the mTOR pathway. We were involved in revealing specific and interesting phenotypes that increased our knowledge of mTOR roles in pathophysiology: mutants with small cells, mutants resistant to tumorigenesis in specific tissues and after specific oncogenic, mutants with muscle dystrophy, mutants mimicking caloric restriction and promoting longevity, mutants with altered insulin action. I will present our progress on the molecular mechanisms of cell size control and organismal longevity. I will also detail our efforts to understand rare human genetic diseases that arise from pathological changes in the activity of the mTOR pathway or that may benefit from therapeutical intervention on this pathway. These diseases include Tuberous Sclerosis Complex, lysosomal storage diseases, lipin1 deficiency.

1. *Liang N, et al. Regulation of YAP by mTOR and autophagy reveals a therapeutic target of tuberous sclerosis complex. J Exp Med 2014;211:2249-63. doi:10.1084/jem.20140341*
2. *Nemazanyy I, et al. Defects of Vps15 in skeletal muscles lead to autophagic vacuolar myopathy and lysosomal disease. EMBO Mol Med 2013;5:870-90. doi: 10.1002/emmm.201202057.*
3. *Aguilar V, et al. S6 kinase deletion suppresses muscle growth adaptations to nutrient availability by activating AMP kinase. Cell Metab 2007;5:476-87*

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Sympathetic coinnervation of NMJs and its importance for synaptic homeostasis

(1) Muzamil Majid Khan, (2) Danilo Lustrino, Willian Silveira (2), Franziska Wild (1), (3) Evelyne Benoit, (3) Jordi Molgo, Veit Witzemann (4), Hanns Lochmuller (5), Isis Kettelhut (2), Luiz Navegantes (2), Tullio Pozzan (6), **Ruediger Rudolf (7)**

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The vertebrate neuromuscular junction (NMJ) has been considered as a purely cholinergic synapse. Sympathetic agonists like salbutamol or ephedrine have recently shown high efficiency in treating several forms of congenital myasthenic syndromes (for review see e.g. Cruz et al., 2014[1] and Engel et al., 2015[2]), but the underlying mechanism has remained elusive. We have found[3] that sympathetic neurons regularly approach NMJs in different mouse skeletal muscles and often form a network of connections with blood vessels, motor neurons, muscle fibers and NMJs. Direct stimulation of sympathetic neurons in combination with simultaneous in vivo-imaging of muscles transfected with molecular biosensors revealed activation of postsynaptic beta2-adrenergic receptors and cAMP production. Furthermore, sympathetic neuron stimulation induced rapid nuclear import of the transcriptional coactivator PGC1alpha. Treatment with the sympathicomimetic clenbuterol corrected electrophysiological and morphological deficits of NMJs upon local chemical sympathectomy and in myasthenic mice. This study identifies the NMJ as a target of direct sympathetic innervation, which is crucial for synapse maintenance and function.

1. *Rodríguez Cruz PM, et al. Congenital myasthenic syndromes and the neuromuscular junction. Curr Opin Neurol 2014;27:566-75. doi: 10.1097/WCO.0000000000000134.*
2. *Engel AG, et al. Congenital myasthenic syndromes: pathogenesis, diagnosis, and treatment. Lancet Neurol 2015;14:420-34. doi: 10.1016/S1474-4422(14)70201-7.*
3. *Khan MM, et al. Sympathetic innervation controls homeostasis of neuromuscular junctions in health and disease. Proc Natl Acad Sci US A 2016;113:746-50. doi: 10.1073/pnas.1524272113. Epub 2016 Jan 5.*

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Translational Myology: from bench to bedside

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Duchenne muscular dystrophy (DMD) is caused by loss of the force transmitting and membrane complex organizing protein, dystrophin. DMD is characterized by progressive muscle deterioration with failed regeneration and replacement with a fatty-fibrous matrix. Dystrophin replacement therapies have been developed that target specific subsets of DMD patients with either deletions or nonsense mutations as a therapeutic strategy to slow disease progression. The development of the nonsense suppression drug, ataluren (translarna), led to the use of the 6-minute walk test as the primary outcome for the initial therapeutic trials in DMD, which has presented a number of challenges. However, it is clear that to date the dystrophin restoration therapies have shown only limited ability to slow the disease process. Thus therapeutics targeting other aspects of DMD disease progression, which can be used in combination, are needed. One potential target that we have investigated is tadalafil (a PDE5 inhibitor), which potentially can target exercise-induced ischemia in skeletal muscle and improve calcium handling in the heart. Tadalafil failed to slow decline in the 6-minute walk test in a recent DMD clinical trial, but did show signs of cardiac impact. Cardiomyopathy is a leading cause of mortality among DMD patients and is well modeled by the golden retriever muscular dystrophy (GRMD) dog model of DMD. Prophylactic use of the PDE5 inhibitor, tadalafil, improved GRMD histopathological features of the hearts, decreased levels of the pathogenic cation channel TRPC6, increased phosphorylation of TRPC6, decreased m-calpain levels and indicators of calpain target proteolysis, and elevated levels of the dystrophin ortholog, utrophin. The progressive loss of cardiac function was significantly slowed in the GRMD dogs by these effects. These data demonstrate that prophylactic use of tadalafil can potentially delay the onset of dystrophic cardiomyopathy in DMD. Another potential therapeutic that has recently entered the clinic in DMD is a small molecule to inhibit nuclear factor κB (NFκB), which is upregulated in DMD muscles. We examined this novel class of NF-κB inhibitors in mdx mouse and golden retriever muscular dystrophy (GRMD) dog models of DMD. These orally bioavailable compounds improved the phenotype of voluntarily run mdx mice, in terms of amount of activity, muscle mass and function, inflammation, and fibrosis. Surprisingly, the muscles were also more resistant to contraction-induced damage, which we demonstrated was significant increases in dysferlin, a protein required for membrane damage repair. Thus nonsense suppression, PDE5 inhibition, and NF-κB inhibition are all potential therapeutics to consider in developing a combinatorial approach to the treatment of DMD. However, the path to approval for these and other DMD therapeutics has been slowed because of the modest benefit of the therapeutics as mono-therapies, as well as the difficulty in finding age-appropriate outcome measures to demonstrate benefit.[2,3]

1. *Willcocks RJ, et al. Multicenter prospective longitudinal study of magnetic resonance biomarkers in a large Duchenne muscular dystrophy cohort. Ann Neurol 2016;79:535-47.*
2. *Hammers DW, et al. Tadalafil Treatment Delays the Onset of Cardiomyopathy in Dystrophin-Deficient Hearts. J Am Heart Assoc 2016;5(8). pii: e003911.*
3. *Hammers DW, et al. Disease-modifying effects of orally bioavailable NF-κB inhibitors in dystrophin-deficient muscle. JCI Insight 2016;1(21):e90341.*

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SESSION 1.
SATELLITE CELLS AND MUSCLE REGENERATION IN HEALTHY MUSCLE AND IN DISEASES

CD11bDTR-mdx mouse: a mouse model to dissect the role of macrophages in muscular dystrophy

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Our project aims at defining the role of macrophages in muscular dystrophy progression of mdx mice focusing on the crosstalk between macrophages and muscle resident cells. To this end, we took advantage of a mouse model recently generated in our laboratory, by crossing CD11bDTR mice with mdx mice in order to generate dystrophic mice (CD11bDTR-mdx) where we can transiently remove macrophages in an inducible manner.[1] The experimental design includes macrophage depletion from dystrophic muscles of CD11bDTR-mdx mice by intramuscular injection of diphtheria toxin (DT). Histopathological, molecular and ex-vivo cell culture analyses will be performed in order to characterize the role of macrophages in muscular dystrophy.
We set the protocol to get substantial and prolonged macrophage depletion in CD11bDTR-mdx mice by intramuscular DT injection. The histopathological and molecular analyses revealed an exacerbation of dystrophic phenotype upon macrophage depletion, both in terms of fibrosis and fat deposition. Moreover, macrophage depletion influences also the number and the differentiative behavior of muscle resident cells, specifically satellite cells (SC) and Fibro-Adipogenic Progenitors (FAPs) purified from PBS or DT-injected CD11bDTR-mdx mice, by cell sorting. Furthermore, we performed genome-wide expression analysis of sorted cells as a starting point to characterize the molecular pathways perturbed by macrophage depletion in SC and FAPs. Considering that chronic inflammation is an important hallmark of muscular dystrophy,[2] and that macrophages represent one of the main players involved in this process,[3] the characterization of the role of this cell population may represent a crucial point for therapeutic approaches.

1 *Arnold L, et al. Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory*

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macrophages to support myogenesis. J Exp Med. 2007;204:1057-69.
2 *Madaro L, Bouché M. From innate to adaptive immune response in muscular dystrophies and skeletal muscle regeneration: the role of lymphocytes Biomed Res Int 2014;2014:438675*
3. *Kharraz Y1, et al. Macrophage plasticity and the role of inflammation in skeletal muscle repair. Mediators Inflamm 2013;2013:491497. Doi: 10.1155/2013/491497. Epub 2013 Jan 30.*

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Unacylated ghrelin enhances satellite cell function and relieves mdx dystrophic phenotype

Simone Reano (1), Elia Angelino (1), Michele Ferrara (1), Hana Sustova (1), Valeria Malacarne (1), Emanuela Agosti (1), Sara Clerici (1), Andrea Graziani (2), Nicoletta Filigheddu (1)

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Adult skeletal muscle undergoes regeneration after injury or in pathological states such as muscular dystrophies. Muscle regeneration depends on satellite cells (SCs), quiescent precursors that, in consequence of injury, activate, proliferate, and differentiate to repair the damaged tissue. A subset of SCs undergoes self-renewal, thus preserving the SC pool and its regenerative potential. Unacylated ghrelin (UnAG) is a circulating hormone that protects muscle from atrophy, promotes myoblast differentiation, and enhances ischemia-induced muscle regeneration.[1-3] Here we show that UnAG increases SC activity and stimulates atypical PKC/p38-mediated SC asymmetric division, fostering both SC self-renewal and myoblast differentiation. Because of those activities on different steps of muscle regeneration, we hypothesized a potential beneficial effect of UnAG in mdx dystrophic mice, in which the absence of dystrophin leads to chronic muscle degeneration, defective muscle regeneration, fibrotic tissue deposition, and, at later stages of the pathology, SC pool exhaustion. Upregulation of UnAG levels in mdx mice reduces muscle degeneration, improves muscle function, and increases dystrophin-null SC self-renewal, maintaining the SC pool. Our results suggest that UnAG has significant therapeutic potential for preserving the muscles in dystrophies.This study was supported by the Muscular Dystrophy Association (MDA294617).

1. *Porporato PE, et al. Acylated and unacylated ghrelin impair skeletal muscle atrophy in mice. J Clin Invest 2013;123:611-22.*
2. *Filigheddu N, et al. Ghrelin and des-acyl ghrelin promote differentiation and fusion of C2C12 skeletal muscle cells. Mol Biol Cell 2007;18:986-94.*
3. *Ruozi G, et al., AAV-mediated in vivo functional selection of tissue-protective factors against ischaemia. Nat Commun 2015;6:7388.doi: 10.1038/ncomms8388.*

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Autophagy regulates satellite cell ability to regenerate normal and dystrophic muscles

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Autophagy is emerging as a key regulatory process during skeletal muscle development, regeneration and homeostasis, and deregulated autophagy has been implicated in muscular disorders and age-related muscle decline. We examined the autophagic process in muscle biopsies isolated from Duchenne Muscular Dystrophy patients at different stages of disease and in muscles of mdx mice. We show that autophagy is activated during the early, compensatory regenerative stages of DMD. A progressive reduction was observed during mdx disease progression, in coincidence with the functional exhaustion of satellite cell-mediated regeneration and accumulation of fibrosis.[1] Moreover, pharmacological manipulation of autophagy can influence disease progression in mdx.[2] Studies performed in regenerating muscles of WT mice revealed an essential role of autophagy in the activation of satellite cells upon muscle injury. Collectively our data reveal a central role of autophagy in MuSCs activation during regeneration of normal and dystrophic muscles.[3] Indeed autophagy is induced in MuSCs during regeneration of healthy muscles, and inhibition of autophagy delays the regenerative response. This evidence indicates that autophagy is a key role in regulating MuSCs activity supporting the notion that regeneration-associated autophagy contributes to the early compensatory stage of DMD progression, and extended activation of autophagy might be beneficial in the treatment of DMD. As MuSCs are the cellular effectors of the compensatory regeneration at early stages of DMD, and their long-term activity is compromised by dystrophin deficiency, we speculate that autophagy could be a potential "disease modifier" exploitable by new interventions aimed at extending the compensatory stage in DMD progression.

1 *Serrano AL, et al. Cellular and molecular mechanisms regulating fibrosis in skeletal muscle repair and disease. Curr Top Dev Biol 2011;96:167–201.*
2. *De Palma C ,et al. Autophagy as a new therapeutic target in Duchenne muscular dystrophy. Cell Death Dis 2012;3:e418.*
3. *Fiacco E, et al. Autophagy regulates satellite cell ability to regenerate normal and dystrophic muscles. Cell Death Diff 2016;10.1038.*

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Physiopathological characterization of the role of MCUb in skeletal muscle regeneration

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Mitochondrial calcium uptake plays a key role in modulating cell metabolism, cell survival and other cell specific function. Calcium accumulates into the mitochondrial matrix through the mitochondrial calcium uniporter (MCU). Few years ago a MCU homolog has been discovered, which has been called MCUb. MCU and MCUb shares a 50% sequence and structure similarity although some conserved differences in the primary sequence prevent MCUb from forming a Ca2+ permeable channel, thus acting as a dominant-negative subunit. Interestingly, MCUb/MCU expression ratio varies greatly between tissues, suggesting that it might contribute to the spatiotemporal control of mitochondrial calcium uptake. RT-PCR experiments demonstrated that MCUb expression levels dramatically increase after 48, 96 and 72h of skeletal muscle regeneration after cardiotoxin-induced injury. Since MCUb is poorly expressed in skeletal muscle in physiological conditions, we hypothesized that MCUb might play a role in skeletal muscle regeneration. In addition, high MCUb expression levels have been detected in anti-inflammatory macrophages (M2). The latter are one of the most important effectors of the later stages of tissue repair. We thus hypothesize that MCUb overexpression, occurring during skeletal muscle regeneration, might be crucial for the differentiation of M2 macrophages. We are confirming this hypothesis by measuring MCUb mRNA levels in M2 extracted from regenerating muscle after sorting. We are performing regenerating muscle experiments in total MCUb knockout animals in order to see if the lack of MCUb might impair skeletal muscle regeneration and lead to the formation of fibrosis.

1. *Raffaello A, et al. The mitochondrial calcium uniporter is a multimer that can include a dominant-negative pore-forming subunit. EMBO J 2013; 32:2362–76.*
2. *De Stefani D, et al. A 40 kDa protein of the inner membrane is the mitochondrial calcium uniporter. Nature. 2011; 476(7360):336–40.*

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Divergent Progenitor Populations in the Dystrophic Skeletal Muscle and their Relocation towards Regenerative Fibers

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A continuous build-up and breakdown of skeletal muscle fibres dictates the pathological features of muscular dystrophies. Several progenitors resident in the skeletal muscle have been described to play a role in this degeneration/regeneration process of muscle fibres. As of yet, hardly anything is known about native mechanisms for attraction of myogenic progenitors towards regenerative fibres. We found a subpopulaton of Lineage negative/Sca1+ progenitors positive for tissue non-specific Alkaline Phosphatase (Alpl) that is increased by 20 fold in dystrophic muscle compared to wild type. These cells were characterized as pericytes with myogenic properties mainly acting via fusion with myofibers, especially the first month after birth as reported in Dellavalle et al. in 2011. We aspire to determine which factors, released by regenerative fibres, attract these progenitors and relocate them so that they can exert their pro-myogenic mechanisms. Several options have been explored pinpointing towards growth factor release and chemotactic gradients. This study aims to widen the understanding about progenitor residence and activity in a regenerative muscle environment with the potential to lead towards a therapeutic intervention to support muscle regeneration.

1. *Dellavalle A, et al. Pericytes resident in postnatal skeletal muscle differentiate into muscle fibres and generate satellite cells. Nat Commun 2011;2:499.*
2. *Joe A, et al. Muscle injury activates resident fibfo/adipogenic progenitors that facilitate myogenesis. Nat Cell Biol 2010;12:153-163.*
3. *Cossu G, et al. Intra-arterial transplantation of HLA-matched donor mesoangioblasts in Duchenne muscular dystrophy. Embo Mol Medicine 2015;7:1513-28.*

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The potential of mass cytometry to reveal the complex interplay between muscle resident mononuclear cells during regeneration

Filomena Spada, Claudia Fuoco, Lucia L. Petrilli, Elisa Micarelli, Stefano Pirrò, Alessio Reggio, Serena Paoluzi, Cesare Gargioli, Luisa Castagnoli, Gianni Cesareni

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Adult skeletal muscle is a relatively complex tissue, which has the ability to self- renew and to self-repair in response to mechanical or chemical damage, stress caused by genetic mutations or increased workload. The regenerative process is orchestrated by different populations of resident mononuclear cells, which directly or indirectly contribute to maintain myofiber homeostasis. The stability of the muscle mononuclear populations and the integrity of the satellite cell niche can be affected physiologically during ageing, resulting in a decreased regeneration capacity. Moreover, in pathological conditions, as in Duchenne muscular dystrophy (DMD), the repeated cycles of muscle degeneration-regeneration exhaust the satellite cell pool and decrease their regeneration potential. Understanding which populations contribute to the regenerative process and how signals control the activation and differentiation of these cells in physiological and pathological conditions are still open

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issues with important translational implications. The ability to probe the heterogeneity and the dynamic of the muscle tissue is fundamental to achieve a complete understanding of muscle regeneration.
In this context, we analysed different models of muscle regenerations (cardiotoxin- induced injury, mdx mice) in order to investigate the role and the function of the different muscle mononuclear populations. Since skeletal muscle is a complex heterogeneous system, we have invested in a novel approach exploiting mass cytometry technology (CyTOF2 platform), a recently developed multi-parametric single-cell technique. By this approach, we described the time-dependent dynamics at a single-cell level of muscle regeneration after cardiotoxin-induced injury by mass cytometry, and we estimated their abundance and activation state. Furthermore, we studied the distribution at single-cell level and the abundance of the main muscle mononuclear populations of mdx mice, in the degeneration/regeneration processes, highlighting the activation state of each cell type.

1. *Bentzinger CF, et al. Cellular dynamics in the muscle satellite cell niche. EMBO reports 2013;14:1062-72.*
2. *Bendall SC, et al. (). Single-cell mass cytometry of differential immune and drug responses across a human hematopoietic continuum. Science (New York, NY) 2011;332:687-96.*
3. *Joe AW, et al. Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. Nature cell biology 2010;12:153-63.*

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Notch signalling controls FAPs differentiation in skeletal muscle

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Skeletal muscle regeneration is mediated by a complex crosstalk between several resident mononuclear cell populations. Satellite cells are the main source of new myoblasts and play a pivotal role during regeneration. However, their function relies on environmental cues shaped by other cell types such as macrophages, pericytes, and fibro-adipogenic progenitors (FAPs). FAPs have a leading role in the regeneration process by positively regulating satellite cells differentiation. However, in pathological conditions, they are responsible for fibrosis and fat infiltrations. Despite the established importance of FAPs in both regeneration and degeneration, the signals that regulate these opposing roles are not fully characterized. Since the Notch signaling pathway is responsible for maintaining satellite cells in the quiescent state, thus preventing their differentiation, we investigated whether this pathway also modulates FAPs adipogenesis. To answer this question we used DAPT, an inhibitor of γ-secretase, and DLL1, a Notch ligand, as inhibitor and activator, respectively, of the Notch pathway. Our results support a conclusion whereby Notch plays an important role in the regulation of FAPs differentiation both ex vivo and in vivo. However, the cells producing the signals that in vivo activate the Notch pathway in FAPs are not firmly established. In vitro, the direct contact with myotubes inhibits the adipogenic differentiation of FAPs. This inhibition is Notch dependent. However, in vivo, FAPs are not in direct contact with myotubes. It is therefore possible that the physiologically relevant Notch ligand is synthesized by a different cell type. We are currently investigating this issue. Interestingly, this control mechanism is impaired in FAPs isolated from young dystrophin-deficient, mdx mice.

1. *Bentzinger CF, et al. Cellular dynamics in the muscle satellite cell niche. EMBO Rep 2013;14:1062-72.*
2. *Uezumi A, et al. Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle. Nat Cell Biol 2010;12:143-52.*
3. *Bjornson CR, et al. Notch signaling is necessary to maintain quiescence in adult muscle stem cells. Stem Cells 2012;30:232-42.*

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Unraveling the exosome-bore miRNA network for enhancing skeletal muscle regeneration

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Striated muscle homeostasis is a fine balance between anabolic and catabolic signals strictly regulated during skeletal muscle development and regeneration. However, when the damage is sustained, as in the case of Muscular Dystrophies (MDs), repairing signals are not sufficient to counteract the progression of the disease and muscle degeneration cannot be rescued. In MD animal models the use of adult stem cells namely mesoangioblasts (MABs) has proven beneficial in the treatment of the disease and a phase I/II clinical trial proved MAB safety. However, the molecular players that can guide the beneficial effect of MAB-based treatments are largely unknown. Furthermore, the dual role of muscle hypertrophy, essentially beneficial on skeletal muscle, while burdensome on the heart, must be kept in mind when approaching therapeutic treatments. In our work we have focused on the bivalent effect of muscle hypertrophy while studying exosome-bore miRNAs, emerging players in intercellular communication. We have screened the miRNA content of serum-derived exosomes from hypertrophic and dystrophic mouse models. In addition, exosome-derived miRNA contents have been analyzed in MABs isolated from heart and hind limb muscle of mouse models. Mir1, mir206 and mir208 are differentially expressed in our samples and hypertrophic and dystrophic-associated miRNA compositions were established. Moreover, we have detected symmetry in terms of exosome-bore miRNAs between the serum and the MABs isolated from skeletal muscles. Interestingly, we have detected the skeletal muscle specific mir206 also in exosomes of MABs isolated from dystrophic heart. In conclusion, our data points at exosome-bore miRNAs as possible targets to modulate MAB myogenic potential.

1. *Cossu G, et al. Intra-arterial transplantation of HLA-matched donor mesoangioblasts in Duchenne muscular dystrophy. Embo Mol Medicine 2015;7:1513-28.*
2. *Quattrocelli M, et al. Mesodermal iPSC-derived progenitor cells functionally regenerate cardiac and skeletal muscle. J Clin Invest 2015;125:4463-82.*
3 *Giacomazzi et al. Unconventional Players on the Striated Muscle Field: microRNAs, Signaling Pathways and Epigenetic Regulators. Curr Stem Cell Res Ther 2016;11:554-60.*

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Inflammatory myopathies: preliminary evaluation of a blood miRNA signature as diagnostic marker

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Inflammatory myopathies are a group of rare diseases involving chronic muscle inflammation with associated weakness. Most of them are idiopathic and classified according to their physiopathology, symptoms and signs. The main classes of idiopathic inflammatory myopathy (IIM) are polymyositis (PM), dermatomyositis (DM), inclusion-body myositis (IBM) and the immune-mediated necrotizing myopathies. The IIM managing needs of diagnosis improvement in terms of timeliness, accuracy, non-invasiveness (avoiding muscle biopsies), aiming at a better individualized treatment. The aim of the study was to find a blood myopathy signature analyzing circulating microRNA (miRs). A cohort of 16 subjects (age: from 30 to 87 years) were included in the study, 8 patients with different types of myopathies, enrolled at St. Orsola-Malpighi Hospital in Bologna, and 8 healthy controls (age and sex matched). Plasma samples from 8 subjects, 4 cases and 4 controls, were screened by means of card arrays (about 754 miRs, Applied Biosystem) to obtain circulating miRs profiling. The most changed miRs by profiling and those most interesting from literature were investigated by RT-qPCR in the larger cohort of 16 subjects.On the whole, miR-146a-5p (inflamma-miR), miR-223-3p (IGF-1 signaling; inflammasome inhibition), miR-206 (myomiR) and miR-409-3p (new identified miR) appear the most promising to classify myopathies and eventually IIM respect to healthy status. Likely, these miRs in addition with other circulating markers, such as hemato-biochemical parameters, could be used as signature to improve diagnosis, but further studies need to ascertain the power of this signature.

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1. *Tieu J, Lundberg IE, Limaye V. Idiopathic inflammatory myositis. Best Pract Res Clin Rheumatol 2016;30:149-68. doi: 10.1016/j.berh.2016.04.007.*
2. *Parkes JE, et al. The role of microRNAs in the idiopathic inflammatory myopathies. Curr Opin Rheumatol 2015;27:608-15. doi: 10.1097/BOR.0000000000000225.*
3. *Georgantas RW, et al. Inhibition of myogenic microRNAs 1, 133, and 206 by inflammatory cytokines links inflammation and muscle degeneration in adult inflammatory myopathies. Arthritis Rheumatol 2014;66:1022-33. doi: 10.1002/art.3829.*

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SESSION 2: THERAPEUTIC APPROACHES FOR MUSCLE DISEASES

3D bio-printing and muscle derived pericytes for artificial skeletal muscle human-like size

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The skeletal muscle tissue exhibits good regenerative capabilities, which are however limited by injury size. As a matter of fact, large muscle lesions are characterized by poor recovery accompanied by scar formation and functional detriment, condition common to people suffering from volumetric muscle loss and needing reconstructive therapeutic approaches. Even if surgical autologous transplantation is a standardized procedure, the outcomes are often unsatisfactory. Hence, the pressing need to develop engineered artificial tissues to replace wasted muscle. Tissue engineering (TE), exploiting stem cells embedded in biomimetic scaffolds, aims to mimic organogenesis by building artificial tissues to replace the damaged ones. Skeletal muscle TE is an up-and-coming biotechnology with great potential for muscle repair, but no conclusive strategy has been demonstrated yet. Reconstructing the skeletal muscle architecture and function is still a challenge requiring the parallel alignment of myofibrils arranged into organized sarcomeres. Recently we demonstrated the great potential of a hybrid biomimetic matrix, namely PEG-Fibrinogen, for enhancing the engraftment of myogenic cell progenitors by providing a suitable 3D environment for mouse muscle reconstruction. Starting from these observations, we developed a novel approach for the regeneration and/or reconstruction of skeletal muscle tissue segments of human-like size by exploiting a population of adult myogenic stem cells, namely pericytes, in combination with 3D bio-printing technology to guarantee a functional architecture. In vitro characterization of cell-laden constructs showed enhanced myogenesis and positive myostructure alignment. Thanks to the enhanced control over cell deposition and alignment, the presented technology has the potential to support skeletal muscle repair and regeneration

1. *Kang HW, et al. Nat Biotechnol A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. 2016;34:312-9.*
2. *Fuoco C, et al. In vivo generation of a mature and functional artificial skeletal muscle. EMBO Mol Med 2015;7:411-22.*
3. *Fuoco C, et al. Matrix scaffolding for stem cell guidance toward skeletal muscle tissue engineering J Orthop Surg Res. 2016;11:86.*

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Perturbation of muscle multipotent stem cells differentiation trajectories to counteract muscle myopathies

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Following damage, skeletal muscle displays a great regeneration potential. However, several pathological conditions result in a reduction of muscle function as a consequence of the decline in regeneration capabilities. Eventually this culminates in severe muscle tissue deterioration with detriment to quality of life and in many cases resulting in death. Besides Muscle Satellite Cells (MuSC), which are directly responsible for the generation of new myofibers, distinct interstitial stem cell populations play important roles during muscle regeneration. The complex interplay between these populations is considered essential to coordinate the regeneration process. Fibro Adipogenic Progenitors (FAPs) and mesoangioblasts (MABs) are subjects of increasing interest. FAPs are the source of fibrotic and fat tissue infiltrations in dystrophic patients while mesoangioblasts are multipotent stem cells, which have the potential to differentiate into many mesoderm cell types, including myocytes. High content screening offers the opportunity to identify compounds that reshape the differentiation trajectories of these multipotent stem cells. We have identified and partially characterized compounds that increase MABs myogenic potential or negatively modulate the propensity of FAPs to differentiate into adipose and fibrotic tissues. These can be considered as pre-drugs to be assayed for their ability to counteract the consequences of muscle myopathies in animal models.

1. *Yin H, et al. Satellite Cells and the Muscle Stem Cell Niche. Physiol Rev 2013;93:23–67. http://doi.org/ 10.1152/ physrev.00043.2011*
2. *Uezumi A, et al. Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle. Nat Cell Biol 2010;12:143–52. http://doi.org/10.1038/ ncb2014*
3. *Minasi MG, et al. The meso-angioblast: a multipotent, self-renewing cell that originates from the dorsal aorta and differentiates into most mesodermal tissues. Development 2002;129, 2773-83.*

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SESSION 3.
BIOPHYSICS AND E-C COUPLING IN THE PATHOPHYSIOLOGY OF NEUROMUSCULAR DISEASES

Assembly of Calcium Entry Units improves muscle resistance to fatigue

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Store-operated Ca^{2+} entry (SOCE) is a mechanism triggered by depletion of intracellular Ca2+ stores. In muscle, SOCE is important to limit fatigue during repetitive stimulation. We recently discovered that treadmill exercise (1h of incremental treadmill running from 5 m/min to 25 m/min) promotes formation of new junctions between sarcoplasmic reticulum and transverse-tubules, which contain STIM1 and Orai1, the main proteins mediating SOCE. We named these junctions Calcium Entry Units (CEUs). Interestingly, in mice lacking Calsequestrin-1 (CASQ1-null) CEUs are constitutively present. The goal of the present work is to demonstrate that the presence of CEUs improves muscle resistance to fatigue by increasing SOCE activity. Here, we used a high-frequency stimulation protocol (30 x 1s-60Hz pulses every 5 seconds) to compare fatigue resistance in EDL muscles from control WT, pre-exercised WT, and CASQ1-null mice in presence or absence of extracellular Ca^{2+}, or after addition of SOCE inhibitors (BTP-2, 2-APB and SKF 96365). Results of our experiments indicate that: a) in 2.5 mM Ca^{2+} external solution, EDL muscles from pre-exercised WT and CASQ1-null mice, exhibited a significantly increased capability to maintain contractile force during repetitive stimulation compared to control WT mice; b) when Ca^{2+} was removed from the external solution, the decay of contractile force was more pronounced in muscles containing a higher number of CEUs; c) identical results were obtained when SOCE inhibitors were added to the external solution. Our data suggest that CEUs provide a preferential pathway for Ca^{2+} entry during repetitive muscle activity, reducing in this way muscle fatigue.

1. *Feske S, et al. A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. Nature 2006;441:179-85.*
2. *Roos J, et al. STIM1, an essential and conserved component of store-operated Ca^{2+} channel function. J Cell Biol 2005;169:435-45.*
3. *Zhao X, et al. Enhanced resistance to fatigue and altered calcium handling properties of sarcalumenin knockout mice. Physiol Genomics 2005;23:72-8.*

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Role of STIM1 and Orai1 in the formation of Tubular Aggregates in ageing skeletal muscle fibers

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Tubular aggregates (TAs), ordered arrays of sarcoplasmic reticulum (SR) tubes, form in ageing fast twitch fibers of mice, preferentially in males. TAs are also the main morphological alteration in biopsies from patients affected by TA Myopathy (TAM). TAM has been linked to mutations in STIM1 and Orai1, the two main players in store-operated Ca^{2+} entry (SOCE), a mechanism that allows recovery of extracellular Ca^{2+} when the SR is depleted. We have previously shown that: i) tubes of TAs appear linked by small bridges, resembling aggregated STIM1 molecules; ii) TAs contains SERCA1 and CASQ1, two proteins involved in re-uptake and storage of Ca^{2+} in the SR. Here, we combined different experimental approaches - electron and confocal microscopy (EM and CM), western blots (WB), and force

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measurements (high-frequency stimulation in EDL muscles) - to unravel the role of STIM1 and Orai1 in the formation of TAs. Preliminary results collected in EDL muscles from mice of 4 and 24 months of age indicate that: i) ageing causes STIM1 to accumulate in TAs; ii) the expression levels of both STIM1 splicing variants increase with age (STIM1S = 0.44±0.03 vs 0.66±0.08 A.U.; STIM1L = 0.38±0.05 vs 0.56±0.05 A.U. respectively for young and aged mice); iii) EDL muscles from aged mice exhibit a decreased capability to maintain contractile force compared to young animals (relative force after 10 tetani: 61.6±3.0%, and 52.7±4.3% respectively for young and aged EDL muscles). Our findings may provide insights for the understanding of mechanisms leading to formation of TAs in ageing and disease.

1. *Boncompagni S, et al. Sequential stages in the age-dependent gradual formation and accumulation of tubular aggregates in fast twitch muscle fibers: SERCA and calsequestrin involvement. Age (Dordr) 2012;34:27-41.*
2. *Endo Y, et al. Dominant mutations in ORAI1 cause tubular aggregate myopathy with hypocalcemia via constitutive activation of store-operated Ca^{2+} channels. Hum Mol Genet 2015;24:637-48.*
3. *Böhm J, et al. Constitutive activation of the calcium sensor STIM1 causes tubular-aggregate myopathy. Am J Hum Genet 2013;92:271-8.*

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Aerobic training, a possible strategy to reduce oxidative stress and prevent hyperthermia?

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Calsequestrin 1 knockout (CASQ 1-null) mice suffer lethal episodes when exposed to both high environmental heat or halogenated anesthetics, a phenotype similar to malignant hyperthermia (MH) susceptibility in humans. We previously demonstrated that excessive oxidative stress plays a key role in lethal MH crises. The goal of the present work is to demonstrate that aerobic training can reduce oxidative stress and, hence, the mortality rate, of CASQ1-null mice during heat-stress. C57Bl/6 (controls) and CASQ1-null male mice had their individual maximal exercise capacity evaluated at 2 – 2.5 months of age before being subjected to aerobic training for 2 months (60% of maximal speed, 5x/week). At 4-4.5 months of age all mice were first re-evaluated: CASQ1-null mice displayed an improved exercise aerobic capacity, although no differences in strength were detected. Mice were then submitted to a heat stress protocol (41°C/1h): the mortality rate of trained CASQ1-null mice dramatically decreased (16.6%) when compared to the untrained group (85.6%). Measurements of hyperthermia revealed that aerobic training: a) was effective in reducing the increase in core temperature to levels lower that WT; and b) doubled the time to reach the maximum temperature (compared to untrained CASQ1-null mice). Finally, following training the lipid peroxidation in isolated sarcoplasmic reticulum (45%) and mitochondria (35%) membranes, which was elevated inuntrained CASQ1-null mice, returned to values close to those of wild types. In conclusion: 2 months of aerobic training a) reduced oxidative stress, b) lowered increase in core temperature, and c) prevented sudden death in CASQ1-null mice.

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1. *Michelucci, A, et al. Antioxidants protect Calsequestrin-1 knockout mice from halothane- and heat-induced sudden death. Anesthesiology 2015;123:603-17.*
2. *Paolini C, et al. Oxidative stress, mitochondrial damage, and cores in muscle from calsequestrin-1 knockout mice. Skeletal muscle 2015.5:10.*

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SESSION 4:
GENETIC AND EPIGENETIC ALTERATIONS IN MUSCLE DYSTROPHIES AND MYOPATHIES

Molecular characterization of DBE-T lncRNA driving FSH muscular dystrophy

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Only about 1% of the human genome encodes for our ~20000 proteins, which are similar in number and largely orthologous to those found in organisms of significant lower complexity. On the contrary, the proportion of non protein-coding DNA has increased with developmental complexity, reaching 98.5% in humans. Interestingly, this produces a vast pool of long non protein-coding RNAs (lncRNAs). Despite the growing interest on lncRNAs, they still remain poorly explored in terms of biological relevance, cellular function, mechanism of action and involvement in disease.

We contributed to this field with the discovery of DBE-T, the first activating lncRNA involved in a human genetic disease: facioscapulohumeral muscular dystrophy (FSHD). FSHD, one of the most common neuromuscular disorders, is a disease with a strong epigenetic component associated with a reduced copy number of the D4Z4 macrosatellite repeat. DBE-T is a chromatin-associated lncRNA that is produced preferentially in FSHD patients, where it acts as master regulator of the expression of FSHD candidate genes through the recruitment of the histone methyl transferase ASH1L.

Through a structure/function characterization, we have unveiled several DBE-T functional domains. In particular, we have mapped the minimal portion of DBE-T required for the interaction with ASH1L; and we identified the region of DBE-T that is needed to activate transcription. Interestingly, we noticed that ASH1L is dispensable for DBE-T-mediated transcriptional activation. Thus, additional transcriptional regulators might be recruited by this lncRNA and play a

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crucial role in FSHD. To assess DBE-T-transcriptional interactome, we conducted RNA-affinity purification coupled to mass spectrometry approaches in human cells. Our analyses retrieved several proteins that were specifically associated to the DBE-T domain required to promote transcription. Among them, we are currently focusing on those proteins that are known to be involved in chromatin regulation by using a reporter cellular-based assay. Our aim is to identify new molecular targets whose depletion impairs DBE-T-mediated transcriptional activation and potentially restores the physiological transcription levels of the FSHD locus in primary cells derived from FSHD patients.
Overall, our work will contribute to elucidate the mechanism of action of lncRNAs. In addition, it will allow the identification of pathways that could be exploited for therapeutic purposes in FSHD.

1. *Gerstein MB et al. Comparative analysis of the transcriptome across distant species. Nature 2014;512:445-48.*
2. *Tawil R et al. Facioscapulohumeral dystrophy: the path to consensus on pathophysiology. Skelet Muscle 201410;4:12.*
3. *Cabianca DS et al. A long ncRNA links copy number variation to a polycomb/trithorax epigenetic switch in FSHD muscular dystrophy. Cell 2012;11;149:81931*

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A novel role for Collagen VI at the neuromuscular junction

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Collagen VI (ColVI) is a major extracellular matrix component made of three genetically distinct α chains and abundantly deposited in the basement membrane of both skeletal muscles and peripheral nerves. Mutations in COL6A1, COL6A2 and COL6A3 genes are known to cause different forms of muscle diseases, including Bethlem myopathy, Ullrich congenital muscular dystrophy and myosclerosis myopathy. ColVI null (Col6a1–/–) mice display a myopathic phenotype characterized by latent mitochondrial dysfunction, spontaneous apoptosis, defective autophagy regulation and compromised muscle regeneration. We recently demonstrated that the absence of ColVI in peripheral nerves leads to hypermyelination, altered Remak bundles, sensory-motor functional deficits and decreased nerve conduction velocities, thus pointing at ColVI as a crucial molecule for peripheral nerve structure and function.
Given the muscle and nerve defects displayed by Col6a1 null mice, we decided to explore the role of ColVI in the neuromuscular junction (NMJ). Our unpublished studies revealed that ColVI is indeed deposited at the synapse. Immunofluorescence and immunoelectron microscopy indicated the α3 chain, rather than the alternative α4, α5 and α6 chains, as the main component of the α1α2αX ColVI assemblies deposited at the NMJ. Labeling of post-synaptic AChR clusters with α-bungarotoxin showed that Col6a1–/– muscles display abnormal NMJ morphology. Moreover our results revealed altered expression of synaptic genes and altered deposition of NMJ-enriched protein network, including some known ColVI binding partners with key functional and structural roles at the NMJ. Also, abnormal electrophysiological parameters in the NMJs of Col6a1–/– mice were observed. Lastly we were able to demonstrate that ColVI treatment in vitro, on C2C12 myotubes, is able to induce AchR clustering and to upregulate important NMJ genes, while its effect is prevented by the treatment with anti-ColVI antibody. These findings indicate a specific role for ColVI at the NMJ, and further studies will allow shedding new light on the contribution of the NMJ defects to the etiopathology of ColVI-related myopathies.

1. *Irwin et al, Mitochondrial dysfunction and apoptosis in myopathic mice with collagen VI deficiency: Nature Genetics 2003;35,367–71.*
2. *Grumati et al, Autophagy is defective in collagen VI muscular dystrophies, and its reactivation rescues myofiber degeneration. Nature Medicine 2010;16, 1313-20.*
3. *Chen et al, Collagen VI regulated peripheral nerve myelination and function. The FASEB Journal 2014;28:1145-56.*

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HDAC4 Is important for preserving skeletal muscle structure and function in muscular dystrophy

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Muscular dystrophies are lethal, genetic diseases characterized by progressive muscle degeneration and weakness. Histone deacetylase 4 (HDAC4) is a member of class II HDACs that controls skeletal muscle response to many stimuli (1,2); its expression is up-regulated in dystrophic muscles (3), suggesting a role for this protein in controlling important cellular aspects of the disease. Pan-HDAC inhibitors are currently in clinical trial for the treatment of muscular dystrophies (4); however, long-term use of pan-HDACi has been associated with numerous side effects. While the function of Class I HDACs in muscular dystrophy has been partially elucidated (5), little is known about the role of Class II HDACs. To investigate the role of HDAC4 in muscular dystrophy with a genetic approach, we crossed mice lacking HDAC4 in skeletal muscle (HDAC4mKO) with mdx mice, a mouse model of Duchenne muscular dystrophy. Muscular dystrophy progression has been analyzed over time, by histological and functional evaluation. The absence of HDAC4 in skeletal muscle anticipates and exacerbates muscle degeneration of mdx mice, worsening muscle functionality. Moreover, compromised muscle regeneration occurs in mdx;HDAC4mKO mice that may negatively influences muscular dystrophy progression. Indeed, satellite cells cultured with conditioned media from mdx;HDAC4mKO mice show significantly impaired myotube differentiation respect to satellite cells cultured with conditioned media from mdx mice. From our results, we conclude that HDAC4 is

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important for maintaining skeletal muscle integrity, regeneration and functionality in mdx mice.

1. *Choi MC. et al. HDAC4 promotes Pax7-dependent satellite cell activation and muscle regeneration. EMBO Rep 2014;15:1175-83.*
2. *Moresi V. et al. Myogenin and class II HDACs control neurogenic muscle atrophy by inducing E3 ubiquitin ligases. Cell 2010;143:35-45.*
3. *Cohen TJ. et al. The histone deacetylase HDAC4 connects neural activity to muscle transcriptional reprogramming. J Biol Chem 2007;282:33752-9.*
4. *Consalvi S. et al. Preclinical studies in the mdx mouse model of duchenne muscular dystrophy with the histone deacetylase inhibitor givinostat. Mol Med 2013;19:79-87.*
5. *Colussi C. et al. HDAC2 blockade by nitric oxide and histone deacetylase inhibitors reveals a common target in Duchenne muscular dystrophy treatment. Proc Natl Acad Sci 2008;105:19183-7.*

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The first epigenetic regulator of muscle stem cells biology and myoblast fusion

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Aging and degenerative pathologies are coupled with a decline of tissue regeneration potential. For example, muscular dystrophies, sarcopenia or muscle cachexia are often associated with a default in regeneration by muscle stem (satellite) cells. In healthy muscle, satellite cells reside in a quiescent state to ensure a pool of muscle stem cells available to repair damaged myofibers throughout our lifetimes. In response to muscle damage or disease, satellite cells activate and proliferate to generate sufficient muscle progenitor cells that fuse to form new myofibers, while a small number of the activated satellite cells will return to quiescence to repopulate the satellite cell niche. Though several studies have identified factors important for the transition between the quiescent and activated satellite cell states and for the regulation of myoblast fusion, we continue to have a poor understanding of the epigenetic mechanisms that regulate this important cell fate transitions. In this study, we investigated a novel role for a histone methyltransferase (HMT) that is involved in several diseases including FSHD muscular dystrophy, autism and cancer. For the first time, we found that its expression is regulated during muscle development and regeneration, being maximal in quiescent satellite cells. Intriguingly, during physiological muscle differentiation or during regeneration following muscle damage, this HMT shows a peak of expression when myoblasts fusion occurs. Moreover, gain- and loss-of-function experiments support an evolutionary conserved requirement in myoblast fusion in human and mouse. Accordingly, KO mice display muscle hypoplasia (small and underdeveloped skeletal muscles) due to a myoblast fusion defect. Expression profiling and chromatin immuno-precipitation experiments indicate that the protein in study activates the expression of several genes required for myoblast fusion. Cell therapy is considered one of the most promising treatments for muscle diseases. Nevertheless, so far limited clinical benefit has been reported and cellular transplantation is still facing a number of limitations. Therefore, one of the fundamental goals of modern regenerative medicine is the understanding of the molecular mechanisms by which stem cells undergo cell fate decisions, especially terminal differentiation. Our results promote a histone methyltransferase as the first epigenetic regulator of myoblast fusion and suggest a role in maintenance muscle stem cell quiescence that could be targeted to improve cell therapy for muscle diseases.

1. *Demonbreun AR, et al. Membrane fusion in muscle development and repair. Semin Cell Dev Biol 2015;45:48-56.*
2. *Kim JH, et al. Mechanisms of myoblast fusion during muscle development. Curr Opin Genet Dev 2015;32:162-70.*
3. *Millay DP, et al. Myomaker is a membrane activator of myoblast fusion and muscle formation. Nature 2013;499(7458):301-5.*

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Targeting the Achilles heel of DUX4 to challenge FSHD muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common myopathies, but its pathogenesis is not fully understood. One of the leading player in the disease is DUX4, a transcription factor normally repressed in somatic tissues, while aberrantly expressed in FSHD muscle.[1] DUX4 overexpression leads to the formation of atrophic myotubes, oxidative stress and apoptosis.[2,3] Nevertheless, the molecular mechanism responsible for DUX4-induced toxicity is not known and, as a result, no therapeutic option is currently available for FSHD patients. To get insights into the mechanism of DUX4 activity, we generated a model cellular system mimicking DUX4 ectopic expression occurring in FSHD muscle cells. These are inducible human cell lines expressing either the full-length DUX4 (iDUX4-fl) or the non-toxic splice isoform of DUX4 (iDUX4-s). As expected, they showed robust DUX4 expression upon induction. Moreover, the ectopically expressed DUX4-fl displayed nuclear localization, transcriptional induction of known target genes and pro-apoptotic functions similar to FSHD muscle cells. Treatment with siRNAs against DUX4 fully rescued DUX4-induced toxicity. On the contrary, the ectopic DUX4-s failed to activate target genes and to induce apoptosis, despite its nuclear localization. Thus, the ability of DUX4 to trigger apoptosis is strictly dependent on its

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proficiency to activate gene expression. On the contrary, the ectopic DUX4-s failed to activate target genes and to induce apoptosis, despite its nuclear localization. Thus, the ability of DUX4 to trigger apoptosis is strictly dependent on its proficiency to activate gene expression. We also plan to perform affinity purification coupled to mass spectrometry analyses to identify proteins selectively interacting with DUX4-fl in the nucleus. In parallel, we will perform high content genome-wide siRNA and CRISPR/Cas9 loss of function screenings to identify factors required for DUX4-induced toxicity. Promising hits with a known role in transcription and apoptosis will be further validated. Targeting these new identified factors would offer a long awaited therapeutic opportunity for FSHD.

1. *Sacconi S, et al. Facioscapulohumeral muscular dystrophy. Biochim Biophys Acta. 2015;1852:607-14.*
2. *Kowaljow V, et al. The DUX4 gene at the FSHD1A locus encodes a pro-apoptotic protein. Neuromuscul Disord 2007;17:611-23.*
3. *Vanderplanck C, et al. The FSHD atrophic myotube phenotype is caused by DUX4 expression. PLoS One. 2011. 6(10):e26820.*

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Premature muscular senescence as a possible determinant of Emery Dreifuss Muscular Dystrophy

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The term of Laminopathies includes a large variety of diseases genetically determined by mutation of Lamin A/C with different clinical symptoms including skeletal muscle dystrophy and premature senescence. Among these disorders, Emery Muscular Dystrophy EDMD affects 1/100.000 people in the world and it is characterized by progressive muscle waste, atrophy and cardiac defects, which often leads to death. Lamin A/C is a nuclear scaffold protein involved in maintenance of nuclear structure and directly regulates many cellular processes including gene expression. Recently we described a functional cross talk between Lamin A/C and key epigenetic repressors involved in the maintenance of cell identity, the Polycomb group of proteins (PcG). We have shown that Lamin A/C is evolutionarily required for PcG proteins nuclear compartmentalization and that Lamin A/C knock-down leads to PcG bodies disassembly and PcG protein dispersion. This causes detachment from chromatin and defects in PcG-mediated higher order structures, thereby leading to impaired PcG repressive functions. In the muscles impairment of Lamin A/C-PcG interplay determines a premature expression of PcG-regulated muscular genes and an anticipated onset of muscle differentiation. In line with these findings in the EDMD mouse model, the Lamin Delta 8-11, we observed an alteration in the muscular stem cell niche, that mirrors a defect in regeneration properties. In parallel, mutant mice exhibit an accumulation of DNA damage and Phospho P38, two markers of muscular senescence. We speculate that EDMD mice exhibit a premature muscular senescence, probably ascribed to a PcG dysfunctioning, which might ultimately leads to the impaired muscle growth and Lamin A/C-dependent muscular dystrophy.

1. *Helbling-Leclerc A, et al. Emery-Dreifuss muscular dystrophy. Eur J Hum Genet 2002;10:157-61.*
2. *Dubinska-Magiera M, et al. Muscle development, regeneration and laminopathies: how lamins or lamin-associated proteins can contribute to muscle development, regeneration and disease. Cell Mol Life Sci 2013;70:2713-41.*
3. *Cesarini E, et al. Lamin A/C sustains PcG protein architecture, maintaining transcriptional repression at target genes. J Cell Biol 2015;211:533-51.*

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Characterization of the molecular mechanism responsible for DBE-T lncRNA tissue-specific expression and chromatin association in FSHD muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is one of the most common neuromuscular diseases. The major form of the disease is linked to a reduction in copy number of the D4Z4 macrosatellite repeat array. Our group recently identified DBE-T, a chromatin-associated long non-coding RNA that is selectively produced by FSHD patients from a region immediately proximal to the D4Z4 array. DBE-T functions in cis by directly recruiting the Trithorax group protein ASH1L to the FSHD locus, driving chromatin remodelling and the aberrant expression of FSHD candidate genes. One of the most intriguing and yet unexplained aspects of FSHD is that, although the D4Z4 deletion is present in all the cells of the body, the disease is restricted only to skeletal muscle. Since DBE-T is required for the activation of all FSHD candidate genes, its characterization could elucidate the FSHD pathogenesis and provide therapeutic opportunities. To address whether DBE-T and FSHD candidate gene expression is selective to muscles, we are conducting expression profiling of human embryonic stem cells (hESCs) and inducible pluripotent stem cells (hiPSCs) derived from control and FSHD patients and differentiated in the skeletal muscle and other lineages. Preliminary results confirm the concordant and FSHD restricted expression of DBE-T and FSHD candidate genes. Intriguingly, for the first time we discovered that their expression is already occurring in undifferentiated FSHD-derived hESCs or hiPSCs, at levels actually much higher than muscle cells. Moreover, we are differentiating hiPSCs into somatic cell types to address whether the molecular signature of FSHD is present also in non-muscular cells from FSHD

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patients. To elucidate the mechanism responsible for DBE-T chromatin tethering, we have considered two possibilities: DBE-T binding to a chromatin associated protein or DBE-T direct binding to DNA. Our data indicate that ASH1L is not required for DBE-T chromatin association. Instead, we found that DBE-T is capable to form a sequence specific antiparallel RNA-DNA:DNA triple helix through a polypurine stretch. Characterization of DBE-T expression pattern and elucidation of the mechanism underlying DBE-T chromatin tethering are fundamental to develop therapeutic approaches to treat FSHD.

1. *Cabianca DS, Gabellini D. FSHD: copy number variations on the theme of muscular dystrophy. J Cell Biol 2010;191:1049-60.*
2. *Cabianca DS, et al. A long ncRNA links copy number variation to a polycomb/trithorax epigenetic switch in FSHD muscular dystrophy. Cell 2012;149:819-31.*
3. *Caron L, et al. Human Pluripotent Stem Cell Model of Facioscapulohumeral Muscular Dystrophy-Affected Skeletal Muscles. Stem Cells Transl Med 2016;5:1145-61. doi: 10.5966/sctm.2015-0224.*

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Role of Histone H3 lysine 9 methyltransferases during Duchenne Muscular Dystrophy progression

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Lysines Methyltransferases (KMTs) have recently raised increased interest as potential targets of therapeutic value thanks to the possibility to revert aberrant epigenetic states associated with human diseases. KMTs catalyzing mono-and di-methylation of lysine 9 on histone 3 (H3K9me1/2) are typically involved in gene repression and heterochromatin formation. In the context of muscle differentiation, the H3K9 KMTs G9a and GLP are emerging as critical epigenetic modulators able to maintain the repression of muscle-specific genes in embryonic precursors and in myoblasts, therefore preventing their premature differentiation.[1,2] Our preliminary data suggest that H3K9 KMTs are also involved in the epigenetic control of lineage choice of a population of muscle-resident mesenchymal stem cells, called fibro-adipogenic progenitors (FAPs). FAPs play key roles in Duchenne Muscular Dystrophy (DMD) by both supporting the myogenic differentiation of muscle stem cells in the regenerating phase or by contributing to fibrosis and fat deposition in advanced stages of disease.[3,4] However, the molecular regulation governing their lineage determination is largely unknown. We show here that pharmacological inhibition of G9a/GLP, by the use of its specific inhibitor (UNC0642), induce a FAPs' lineage switch. Indeed, FAPs isolated from young dystrophic (mdx) mice, cultured ex vivo in the presence of UNC0642 unmask a myogenic potential, as suggested by the appearance of MyoD positive cells and increased expression of myogenic genes. This is paralleled by an impaired adipogenic differentiation, as confirmed by a decreased number of FAPs-derived adipocytes, upon UNC0642 treatment. In sum, our preliminary evidence suggest that the H3K9 KMTs G9a/GLP might be involved in maintaining silent the capacity of FAPs to give rise to myogenic cells and indicate these proteins as possible pharmacological targets for therapeutic approaches aimed to promote regeneration, and to prevent fibro-adipogenic degeneration, of dystrophic muscles.

1. *Ohno H, et al. EHMT1 controls brown adipose cell fate and thermogenesis through the PRDM16 complex. Nature 2013;504:163-7.*
2. *Ling BM, et al. Lysine methyltransferase G9a methylates the transcription factor MyoD and regulates skeletal muscle differentiation. Proc Natl Acad Sci U S A. 2012;109:841-6.*
3. *Mozzetta C, et al. Fibroadipogenic progenitors mediate the ability of HDAC inhibitors to promote regeneration in dystrophic muscles of young, but not old Mdx mice. EMBO Mol Med 2013;5;626-39.*
4. *Joe AW, et al. Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. Nat cell biol 2010;12;153-63.*

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Exosome-mediated communication between fibro-adipogenic progenitors and satellite cells in the pathogenesis and treatment of DMD

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Duchenne Muscular Dystrophy (DMD) is a hereditary fatal disorder caused by mutations of the dystrophin gene, implicated in the maintenance of myofibers integrity. A key feature in DMD is the initial compensatory response of degenerating muscles through a reactive regeneration, that tends to counterbalance muscle loss. A pharmacological therapy based on the histonic deacetilase inhibitors (HDACi) have been recently demonstrated to enhance Fibro/Adipogenic Progenitors (FAPs) ability to influence the satellite cells (MuSCs) regenerative potential that promotes endogen muscle regeneration and limits the fibro/adipogenic deposition in young dystrophic mouse (yMDX). The muscular regeneration mechanism of support through the communication of these two cellular populations, it is still unknown. Recently, intercellular communication via exosomes was discovered being able to transfer genetic information in physiological and pathological processes. An increasing trend of exosomes secretion by FAPs was observed between wild type mice, yMDX not treated and treated with HDACi, (-/+ HDACi) suggesting their possible role during the disease. We show exosomes ability to pass information from yMDX FAPs to MuSCs, influencing MuSCs differentiating potential, which is amplified with HDACi+ FAPs exosomes. Inhibition of FAPs exosomes release showed a loss of FAPs differentiating influence.
We observed that exosomes content is modulated by HDACi, in detail exosome myogenic microRNAs are increased after HDACi treatment. Furthermore, HDACi FAPs exosomes transplant in MDX mice mimics HDACi regenerative process. All this data suggest the relevance of exosomes in DMD and their possible application as a treatment, cohesive or not, with HDACis.

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1. *Dalkilic I1, Kunkel LM. Muscular dystrophies: genes to pathogenesis. Curr Opin Genet Dev 2003;13:231-8.*
2. *Mozzetta C, et al. Fibroadipogenic progenitors mediate the ability of HDAC inhibitors to promote regeneration in dystrophic muscles of young, but not old Mdx mice.EMBO Mol Med 2013:626-39. doi: 10.1002/emmm.201202096. Epub 2013 Mar 18*
3. *Lee Y, et al. Exosomes and microvesicles: extracellular vesicles for genetic information transfer and gene therapy. Hum Mol Genet 2012;21:R125-34. Epub 2012 Aug 7. Review*

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Deep phenotyping of POMK (SGK196) related muscular dystrophy patients with novel mutations reveals congenital mirror movements

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Muscular dystrophies due to hypoglycosylation of α-dystroglycan, called dystroglycanopathies (DGpathies), may also lead to neuronal migration defects. The associated neurological and movement phenotypes have not been fully functionally understood. In our study of DGpathies, we have identified novel mutations in POMK leading to a remarkable movement disorder. The genetic analysis was performed using whole exome sequencing with the NimbleGen V2 Kit, yielding an x80 coverage. We have identified two patients. The first case is a 12-year-old boy with severe limb-girdle weakness with dysmetria and dyskinesia who had never achieved independent ambulation. Brain-MRI showed cerebellar hypoplasia and cysts. MR spectroscopy showed an increase of N-acetyl-aspartate (NAA) in the white matter, whereas other metabolites were reduced. Exon sequencing of the patient revealed a homozygous splice site mutation in POMK c.283-3delC. Second case is a 17-year-old right-handed boy with LGMD. Past medical history revealed mirror movements in the upper limbs, starting from early infancy. On examination, he had learning difficulties, short stature, calf pseudohypertrophy and proximal muscle weakness (4+/5) and showed Gowers sign, pes cavus deformity and mirror movements in the upper limbs. Structural MRI revealed cerebellar cortical disorganization with micro cysts and brainstem hypoplasia, functional MRI studies demonstrated bilateral symmetrical activation in the precentral and postcentral gyrus during both ipsilateral and contralateral hand-clenching tasks. Remarkably, we found a novel homozygous POMK mutation c. 401T>G, p.V134G. To date, congenital mirror movements were previously reported in only one DGpathy patient with LARGE mutations. Our results indicate that α-dystroglycan hypoglycosylation can lead to axonal guidance defects.

1. *Wright KM, Lyon KA, Leung H, Leahy DJ, Ma L, Ginty DD. Dystroglycan organizes axon guidance cue localization and axonal pathfinding. Neuron. 2012 Dec 6;76(5):931-44. PubMed PMID: 23217742. Pubmed Central PMCID: 3526105.*

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SESSION 5:
METABOLIC ALTERATIONS AND MUSCLE DISEASES

Muscle-specific Plin2 down-regulation affects accumulation of ectopic lipid metabolites including ceramides

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Aging is characterized by dramatic changes in body composition, leading to a decline in muscle mass and quality, and thus to sarcopenia. The mechanisms underlying sarcopenia are not completely understood, however a role for high accumulation of ectopic lipid metabolites, causing lipotoxicity, has been proposed. Fat accumulates within lipid droplets (LDs), surrounded by perilipins (Plins). In skeletal muscle one of the most abundant is Plin2, known for its role in lipid storage and considered as marker of lipid accumulation. Recently we found that Plin2 expression in humans increases with age, is inversely associated with muscle mass and strength. We analyzed muscle samples from mice undergone denervation-induced atrophy, and we found a higher expression of Plin2 and atrogenes in denervated muscle with respect to the non-denervated side. Moreover, muscle-specific in vivo silencing experiments showed a higher cross-sectional area of Plin2 down-regulated fibres. It is not clear whether such a manipulation of Plin2 affects the intracellular accumulation of lipid metabolites. We therefore measured lipid species by TLC technique in Tibialis muscle from mice with either denervation, or Plin2 down-regulation. Results indicate that denervation and Plin2 down-regulation induce a dramatic change of intramuscular lipid metabolites and in particular of ceramides. As a whole, these data suggest that Plin2 modulation plays a role in intracellular lipid accumulation likely affecting sarcopenia. Therefore a modification of Plin2 could be a key factor to reduce muscle atrophy and therapeutic approaches to sarcopenia associate with lipotoxicity.

1. *Buch A. et al. Muscle function and fat content in relation to sarcopenia, obesity and frailty of old age - An overview. Exp Gerontol 2016;76:25-32.*
2. *Conte M. et al. Increased Plin2 expression in human skeletal muscle is associated with sarcopenia and muscle weakness. PLoS One 2013; 8: e73709.*
3. *Conte M. et al. Differential expression of perilipin 2 and 5 in human skeletal muscle during aging and their association with atrophy-related genes. Biogerontology 2015; 16: 329-40*

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Investigating the cell origin and heterogeneity of embryonal Rhabdomyosarcoma

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With an incidence of 4.5 cases per million adolescents, the rhabdomyosarcoma (RMS) is the most common type of soft tissue sarcoma. It develops in different tissues, most commonly in the head and neck, in the extremities and in the genitourinary tract. According to its histological and pathological characteristics, RMS can be classified in two major subtypes, embryonal (eRMS) and alveolar (aRMS), which seem to share the same initiating cell type(s), even if this point is still debated. In fact, some evidence supports the notion that skeletal muscle progenitors, such as satellite cells, could give rise to RMS even though alternative theories point to mesenchymal stem cells or even progenitors of the adipocyte lineage, as possible tumor-initiating cells. The clinical differences between the two RMS types result from different molecular genetic mechanisms of origin. To study the eRMS, which is our main focus, we adopted the KrasG12D/+Trp53Fl/Fl conditional mouse model to induce cell transformation, by in vivo or in vitro infecting cells with an Adenovirus vector expressing the CRE recombinase that leads to the constitutive activation of the oncogene KRAS along with the inactivation of the P53 tumor suppressor gene. Since our goal is to identify which cell population(s) can give rise to eRMS, we triggered embryonal RMS formation by infecting purified muscle mononuclear cell populations with the CRE recombinase adenovirus which activates expression of Kras(G12D) and inactivates the p53 gene. Both satellites and FAPS are transformed in vitro by this approach and induce the formation eRMS like tumors when grafted into nude mice. We are in the process of characterizing the changes in the cell populations from the tumor mass, at different stages of development, by flow cytometry techniques.

1. *Ognjanovic SA. et al. Trends in childhood rhabdomyosarcoma incidence and survivale in the United States, 1975-2005. Cancer 2009;115:4218-26.*
2. *Saab R, et al. (). Myogenesis and rhabdomyosarcoma: the jekyll and hyde of skeletal muscle. Curr Top Dev Bio 2011;94:197-234.*
3. *Kirsch DG, et al. A spatially and temporally restricted mouse model of soft tissue sarcoma. Nat Med 2007;13:992-7.*

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Polyglutamine expansion and overexpression of androgen receptor cause muscle atrophy through distinct mechanisms *in vivo*

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Spinal and bulbar muscular atrophy (SBMA, Kennedy's disease) is an X-linked adult neuromuscular disease caused by expansion of a polyglutamine (polyQ) tract in the gene coding for the androgen receptor (AR). The main clinical features of SBMA are loss of motor neurons in the brainstem and spinal cord, together with proximal limb and bulbar muscle weakness and atrophy. PolyQ expansion causes disease mainly through a gain of function mechanism. To uncover the mechanism by which expansion of polyglutamine in AR causes muscle pathology, we generated transgenic mice with ubiquitous expression of either normal (AR24Q) or mutant (AR100Q) human androgen receptor. The comparison between the AR24Q mice and the AR100Q mice allow us to discriminate between the consequences of the overexpression of normal AR and the effects produced by the presence of the polyQ. We found that the overexpression of AR24Q is sufficient to induce muscle atrophy, but it does not cause motor dysfunction. On the other hand, expression of expanded polyQ AR leads to muscle atrophy, motor impairment, muscle force decrease and mitochondria dysfunction. We also found that the AR24Q lines express higher levels of human AR than the polyQ lines but this does not result in aggregation. Strikingly, accumulation of AR100Q in forms of 2%SDS-resistant aggregates was detected solely in skeletal muscle. Our new transgenic mouse models recapitulate the main features of SBMA and suggest that the toxic gain of function underlying SBMA results from impaired motor neuron-muscle communication.

1. *La Spada AR, Wilson EM , Lubahn DB, Harding AE. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 1991;352:77-79.*
2. *Kennedy WR , Alter M , Sung JH. Progressive proximal spinal and bulbar muscular atrophy of late onset A sex-linked recessive trait. Neurology 1968;18:671-80.*
3. *Pennuto M, Basso M. In vitro and in vivo modeling of spinal and bulbar muscular atrophy. J Mol Neurosci 2016;58:365-73.*

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SESSION 6: SIGNALLING IN MUSCLE GROWTH, HOMEOSTASIS AND DISEASES

Skeletal muscle deterioration in dilated cardiomyopathy: molecular mechanisms and effect of prolonged endurance training in a mice model

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Dilated cardiomyopathy (DCM) is a progressive disease that results in death. A clinical hallmark is the exercise intolerance (EI). To clarify the molecular mechanisms underlying EI, we used Tgαq*44h transgenic mice with slow development of DCM. We performed functional and molecular analysis on soleus and gastrocnemius (GS) muscles before and after 2 months of free wheel running, at different stages of disease (6-12-14 months). Tg mice showed a reduced in vivo performance concomitant to the onset of the disease that persisted in the late stage of DCM. Slow and fast muscles were differently affected by DCM. The basal PGC1α, DRP1 and AMPK levels decreased in GS of Tg mice at all stages of the disease suggesting mitochondrial dynamics and energy state impairment. Unlike GS, soleus did not show any energy alteration and only in the late stage of the disease (14 months) a DRP1 decrease was observed. Furthermore, a downregulation of antioxidant defenses (SOD and catalase) and an increased protein oxidation index were found only in GS of DCM mice. At 12 months (when mice develop the disease), a downregulation of atrogin1 and MuRF1 (ubiquitin proteasome system markers) was observed in both muscles of cardiopathic mice, suggesting a deficit in basal degradation process with possible alteration of proteins quality control. After two months of free wheel running, an improvement of the in vivo performance was observed in cardiopathic mice. The functional recovery was associated with upregulation of several factors whose baseline levels were found lower in muscles of Tg mice.

1. *Garnier A, et al. Depressed mitochondrial transcription factors and oxidative capacity in rat failing cardiac and skeletal muscles. J Physiol 2003;551:491-501.*
2. *Mende U, et al. Dilated cardiomyopathy in two transgenic mouse lines expressing activated G protein alpha(q): lack of correlation between phospholipase C activation and the phenotype. J Mol Cell Cardiol 2001;33:1477-91.*
3. *Poole DC, et al. Muscle oxygen transport and utilization in heart failure: implications for exercise (in)tolerance. Am J Physiol Heart Circ Physiol 2012;302:H1050-63.*

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Biomechanics of the Octopus arm muscular hydrostat: involvement of mTOR pathway during arm exercise

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The Octopus vulgaris arm is a highly flexible structure with virtually unlimited degrees of freedom. The arm's extraordinary motor capabilities are achieved despite the absence of a rigid skeleton and a composition of mainly incompressible muscle tissues.[1,2] In this study we aim at elucidating the structure and biophysical properties that contribute to the special biomechanics of the octopus arm musculature. A new hypothesis has been proposed whereby in the octopus the muscle and collagen tissue close interactions contribute to the response of the arm to stretch, contraction and to create stiffening. To confirm this we performed two series of investigation: (I) Analysis of the architectural organization of muscle and connective tissue elements within each arm muscle type; (II) Study of the passive and active components in both isometric and isotonic activation of the arm musculature with a Dual-Mode Lever Arm System on in-vitro preparations. We found that each muscle type might differently contribute to arm contraction and stiffening and that the existence of passive elastic forces, modulated by the level of the arm activation, might act as storing energy compartments in each muscle type. Moreover, in order to decipher the molecular bases of the arm force production we investigated exercise induced variation of mTOR signaling in the arm. mTOR complex represents an interesting family of molecules involved in sensing of cellular nutrition and energy status.[3] Taken together these studies open to further investigations onto the biomechanics and molecular biology of hydrostatic muscles.

1. *Kier W, Stella MP. The arrangement and function of octopus arm musculature and connective tissue. J.Morph. 2007;268:831–43.*
2. *Fossati SM, Benfenati F, Zullo L. Morphological characterization of the Octopus arm. Vie et Milieu/Life & Environment 2011;61:191-5.*
3. *Betz C1, Hall MN. Where is mTOR and what is it doing there? J Cell Biol 2013;203:563-74.*

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Beclin 1 is required for skeletal muscle homeostasis and its deficiency leads to muscular dystrophy

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Autophagy is a cellular catabolic process whose integrity is essential for tissue maintenance and homeostasis, with crucial importance in skeletal muscles.[1] Beclin 1 is a core component of the phospatidylynositol-3-kinase/Vps34 complexes guiding autophagosome nucleation and other membrane trafficking processes in mammals.[2] It is involved in protein aggregate clearance supporting the cytoprotective function of autophagy, thus being implicated in multiple human diseases.[2] However, Beclin 1 role in skeletal muscle functions and determination is currently poorly understood. We generated muscle-specific Beclin 1 knockout mice, in which Cre recombinase is under the control of the myosin light chain 1 fast promoter. Beclin 1 muscle-specific knockout mice display reduced body weight, compared to their littermate controls. Cre-induced Beclin 1 deletion in skeletal muscle lead to dystrophic morphological features in mice. In particular, transgenic mice progressively develop a myopathy sharing several similarities with the human centronuclear myopathies.[3] Beclin 1 deficient muscles display a large number of centronucleated fibers, increased presence of interstitial mononucleated cells and fibrosis, getting worse with age. In addition, Beclin 1 knockout muscles underwent to chronic degeneration and regeneration cycles, finally resulting in inflammation, muscle atrophy, and

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a decrease in muscle function. Consistently with a disruption of the Beclin 1-dependent activation of autophagy, mutant skeletal muscle accumulate autophagic and lysosomal proteins in the myofibers. Although further investigations are needed to confirm a possible implication of Beclin 1 in human idiopathic myopathies, our study postulate a crucial importance for Beclin 1 protein in the regulation of skeletal muscle homeostasis, likely due to its autophagy-regulating function.

1. *Sandri M, et al. Misregulation of autophagy and protein degradation systems in myopathies and muscular dystrophies. J Cell Sci 2013;126(Pt 23):5325-33. doi: 10.1242/jcs.114041.*
2. *Funderburk SF, et al. The Beclin 1-VPS34 complex--at the crossroads of autophagy and beyond. Trends Cell Biol 2010;20:355-62. doi: 10.1016/j.tcb.2010.03.002. Epub 2010 Mar 29.*
3. *Jungbluth H, Gautel M. Pathogenic mechanisms in centronuclear myopathies Front Aging Neurosci, 2014;6:339. doi: 10.3389/fnagi.2014.00339. eCollection 2014.*

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TFEB controls glucose homeostasis and energy balance during exercise

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The Transcription Factor EB (TFEB) is an essential component of lysosomal biogenesis, autophagy and the adaptive response to food deprivation.[1] We have recently reported that physical exercise promotes TFEB activity, through contraction- dependent calcium influx activation of calcineurin, which dephosphorylates TFEB and induces its translocation from the cytoplasm to the nucleus.[2] To address the physiological function of TFEB in skeletal muscle we have now used muscle-specific gain- and loss-of-function approaches to define the specific role of TFEB in the control of muscle fuel metabolism. TFEB regulates glucose uptake and glycogen content by controlling glucose transporters, glycolytic enzymes and enzymes involved in pathways related to glucose homeostasis. Moreover, TFEB induces the expression of genes involved in mitochondrial biogenesis, fatty acid oxidation (FAO) and oxidative phosphorylation (OXPHOS) independently of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α) expression. Thus, TFEB mediates metabolic adaptations that are important for energy production and exercise capacity. These findings identify TFEB as a critical mediator of the beneficial effects of exercise on metabolism.

1. *Ballabio, A. (). The awesome lysosome. EMBO Mol Med, 2016;8:73-6. doi:10.15252/emmm.201505966.*
2. *Medina DL, et al. (). Lysosomal calcium signalling regulates autophagy through calcineurin and TFEB. Nat Cell Biol 2015;17:288-99. doi:10.1038/ncb3114.*

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The role of raptor in skeletal muscle hypertrophy

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Mammalian target of rapamycin (mTOR) plays a central role in cell growth. mTOR assembles into two distinct complexes, namely the rapamycin-sensitive complex mTORC1 and the rapamycin-insensitive complex mTORC2. One of the key members of the mTORC1 complex is Raptor, which recruits mTOR substrates S6K1 and 4EBP1. Mice lacking Raptor only in skeletal muscle from birth show a pronounced myopathy. However, treating adult mice with the specific mTORC1 inhibitor rapamycin does not lead to a myopathic phenotype. Here we want to examine the role of Raptor and mTORC1 using a model in which we can delete Raptor in muscles of adult mice. One month after Raptor deletion, muscle weight and basic histology are unchanged. We are currently examining the role of Raptor both in muscle atrophy and hypertrophy. From preliminary experiments it seems that Raptor might not be required for Akt-induced hypertrophy

1. *Marabita M, et al. S6K1 Is Required for Increasing Skeletal Muscle Force during Hypertrophy. Cell Rep 2016;17:501-13.*
2. *Blaauw B, et al. Mechanisms modulating skeletal muscle phenotype. Compr Physiol 2013;3:1645-87.*

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SESSION 7:
MUSCLE WASTING, SARCOPENIA AND CACHEXIA

The receptor RAGE: a potential molecular target in cancer cachexia

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Cachexia is a highly debilitating syndrome affecting more than a half patients with advanced cancer. The major clinical feature of cachexia is severe muscle wasting leading to pronounced weight loss, impaired quality of life, reduced response to anti-cancer therapy and poor outcome. Although several molecules have been implicated in cancer-induced muscle wasting, cachexia remains an untreated and poorly understood process. RAGE (Receptor for Advanced Glycation End-products) and its physiological ligands, S100B and HMGB1, are involved in muscle regeneration, inflammation, and tumor growth, all of which represent key processes in cachexia. We found that RAGE, S100B and HMGB1 counteract atrophy induced by TNFα±IFNγ in myotubes in vitro and in muscles in vivo by interfering with the ubiquitin-proteasome system. However, excess RAGE ligands lead to myotube atrophy, and we detected high amounts of RAGE, S100B and HMGB1 in cachectic muscles, and elevated S100B levels in the serum of cachectic mice. Interestingly, LLC (Lewis Lung carcinoma) cells and cachectic muscles, which re-express RAGE, release high

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amounts of S100B and HMGB1. Finally, LLC-bearing RAGE-null mice show delayed tumor appearance, reduced loss of muscle mass and atrogenes expression, and dramatic increase of survival rate compared with LLC-bearing WT mice. Thus, RAGE, S100B and HMGB1, which have a role in restoring muscle homeostasis in physiological conditions [1,2], appear to concur to muscle wasting in cancer conditions due to increased RAGE expression/activity and expression levels of S100B and HMGB1. Thus, RAGE, S100B and/or HMGB1 might represent molecular targets in therapeutic strategies to prevent or counteract muscle atrophy in cancer patients. **Equally contributed to the present work.*

1. *Riuzzi F. et al. HMGB1–RAGE regulates muscle satellite cell homeostasis through p38-MAPK- and myogenin-dependent repression of Pax7 transcription. J Cell Sci 2012;125:1440-54.*
2. *Riuzzi F. et al. S100B engages RAGE or bFGF/FGFR1 in myoblasts depending on its own concentration and myoblast density. Implications for muscle regeneration. PLoS ONE 2012;7: e28700.*

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Oxidative stress and exercise training in experimental cancer cachexia

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Cachexia is a multifactorial syndrome that occurs in 50 to 80% of cancer patients. It is becoming evident that oxidative stress is also involved in the pathogenesis of cachexia (1). Some years ago, moderate physical training has been proposed as a component of cachexia treatment (2, 3) and, in physiological conditions, has been demonstrated to induce the overexpression of anti-oxidant enzymes (4). The present study, developed using an experimental model of cancer cachexia, has been aimed at evaluating: i) the involvement of oxidative stress in the pathogenesis of skeletal muscle atrophy; ii) the effects of moderate exercise training on muscle wasting, with particular focus to the oxidative balance. Exercise appeared to protect tumor-bearing mice from reduced food intake, body weight loss as well as muscle mass and function loss. Moreover, exercise decreased the levels of carbonylated proteins compared to sedentary tumor-bearing mice. Regarding proteins involved in the antioxidant defense, exercise appeared to increase G6PD activity (while not significantly) and the levels of catalase. The Cu/Zn SOD content is increased in tumor bearing-mice without differences between sedentary and exercised groups. In conclusion, oxidative stress does not seem to be a main factor in the pathogenesis of cancer-induced muscle wasting, at least in the experimental model used in the present study, probably because, despite being altered, muscle metabolism is still able to compensate. However, this observation does not mean that impinging on the redox balance is useless to correct muscle loss

1. *Puig-Vilanova E, et al. Oxidative stress, redox signaling pathways, and autophagy in cachectic muscles of male patients with advanced COPD and lung cancer. Free Radic Biol Med 2015;79:91–108.*
2. *Penna F, et al. Combined approach to counteract experimental cancer cachexia: eicosapentaenoic acid and training exercise. J Cachexia Sarcopenia Muscle. () 2011;2: 95-104.*
3. *Penna F, et al. Novel investigational drugs mimicking exercise for the treatment of cachexiaExpert Opin Investig Drugs 2016;25(1):63-72.*
4. *Gomez-Cabrera MC, et al. Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. Free Radic Biol Med 2008;44:126-31.*

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Protective effects of unacylated ghrelin in aging mice

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Sarcopenia is a multifactorial syndrome defined as the irreversible loss of skeletal muscle mass and functionality in aged individuals that results in frailty, mobility disorders, loss of independence, and high risk of mortality. The loss of skeletal muscle mass and function is accompanied by accumulation of fat in interstitial space, increase of fibrosis, and progressive loss of neuromuscular junction. Muscle atrophy and impaired regeneration are other very important characteristics of sarcopenia. The underlying mechanisms and etiology of sarcopenia remain poorly defined, but include hormonal changes, decrease physical activity, chronic inflammation, insulin resistance and nutritional deficiency.

Acylated and unacylated ghrelin (AG and UnAG, respectively) are circulating peptide hormones generated by the ghrelin gene mainly in the stomach in consequence of fasting or caloric restriction. AG, through binding to growth hormone secretagogue receptor type 1a (GHSR-1a), induces strong release of GH, stimulates food intake, adiposity and positive energy balance. Acylation of ghrelin is essential for its binding to GHSR-1a, since the unacylated form does not activate this receptor and is devoid of any GH-releasing activity. However, both peptides act directly on skeletal muscle where they protect against atrophy, caused by denervation and fasting, and promote skeletal muscle differentiation.[1,2] Moreover, UnAG promotes muscle regeneration after hindlimb ischemia. Since AG plasmatic levels decrease with age, we hypothesize that AG/UnAG may play a protective role in sarcopenia. We exploited Myh6/Ghrl transgenic mice, characterized by constitutively high UnAG circulating level, to investigate the effects of UnAG on sarcopenia establishment. We compared 24-months old WT and Myh6/Ghrl transgenic mice and evaluated functional and morphological features of their muscles. The preliminary results show that old mice with high levels of UnAG show a decrease in body fat accumulation and feature a stronger grip, suggesting that this

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hormone helps maintaining muscle strength and functionality during aging. This study is supported by Fondazione Cariplo.

1. *Porporato PE, et al. Acylated and unacylated ghrelin impair skeletal muscle atrophy in mice. J Clin Invest 2013;123:611-22.*
2. *Filigheddu N, et al. Ghrelin and des-acyl ghrelin promote differentiation and fusion of C2C12 skeletal muscle cells. Mol Biol Cell 2007;18:986-94.*

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Muscle overexpression of microRNAs: impact on cancer-induced muscle wasting

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Cancer cachexia is a wasting syndrome mainly characterized by progressive loss of skeletal muscle mass, impairment of myogenesis and metabolic abnormalities. MicroRNAs (miRs) are endogenous, small RNAs that regulate gene expression by paring with the 3'-UTRs of mRNAs, inhibiting protein translation. They play essential roles in development, cell proliferation, cell differentiation, apoptosis and metabolism. In the skeletal muscle of mice bearing the C26 carcinoma we have observed a downregulation of some myomiRs (miR-1, miR-133, and miR-206) and of one non muscle-specific miR (miR-30a). In vivo overexpression (tibialis muscle) of these myomiRs did not have major consequences on C26-induced muscle wasting. By contrast, overexpression of miR-30a produced a reduction of muscle mass in both cachectic and control animals. While showing that miR-30a overexpression is not useful for preventing muscle wasting in tumor-bearing mice, these results also suggest this miR may be relevant to muscle homeostasis.

1. *Fearon K, et al., Definition and classification of cancer cachexia: an international consensus. Lancet Oncol 2011;12:489-95.*
2. *Lee CT, et al., Evolutionary conservation of microRNA regulatory circuits: an examination of microRNA gene complexity and conserved microRNA-target interactions through metazoan phylogeny. DNA and Cell Biology 2007;26:209–18.*

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The metabolic reprogramming agent Trimetazidine as an 'exercise mimetic' in cachectic C26-bearing mice

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Cachexia is characterized by muscle depletion and exercise intolerance caused by an imbalance between protein synthesis and degradation rates and by impaired myogenesis. Myofiber metabolic efficiency is crucial so as to assure optimal muscle function. Some drugs are able to reprogram cell metabolism and, in some cases, to enhance metabolic efficiency. Based on these premises, we chose to investigate the ability of the metabolic modulator trimetazidine (TMZ) to counteract skeletal muscle dysfunctions and wasting occurring in cancer cachexia. For this purpose we used mice bearing the C26 colon carcinoma as a model of cancer cachexia. Mice received 5mg/kg TMZ once a day for 12 days. A forelimb grip strength test was performed and tibialis anterior and gastrocnemius muscles were excised for analysis. Ex-vivo measurement of skeletal muscle contractile properties was also performed. Our data showed that TMZ induces some effects typically achieved through by exercise, among which is an increase in fast-to slow myofiber phenotype shift, PGC1α up-regulation, oxidative metabolism enhancement, mitochondrial biogenesis and grip strength increase. TMZ also partially restores the myofiber cross-sectional area (CSA) in C26-bearing mice while modulation of autophagy and apoptosis were excluded as mediators of TMZ effects. In conclusion, our data show that TMZ acts like an "exercise mimetic" and is able to enhance some mechanisms of adaptation to stress in cancer cachexia. This makes the modulation of the metabolism, and in particular TMZ, a suitable candidate for a therapeutic rehabilitative protocol design, particularly considering that TMZ has already been approved for the clinical use. *Equally contributed to the present work.

1. *Jaswal JS1, et al. Targeting fatty acid and carbohydrate oxidation - a novel therapeutic intervention in the ischemic and failing heart. Biochim Biophys Acta 2011;1813:1333-50. doi: 10.1016/j.bbamcr.2011.01.015.*
2. *Ferraro E et al. The metabolic modulator trimetazidine triggers autophagy and counteracts*

stress-induced atrophy in skeletal muscle myotubes. FEBS J 2013;280:5094-108.
3. *Ferraro E et al. Improvement of skeletal muscle performance in ageing by the metabolic modulator Trimetazidine. Journal of Cachexia, Sarcopenia and Muscle. 2016 Jan 5. doi: 10.1002/jcsm.12097.*

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Effects of vitamin D and vitamin D binding protein in muscle wasting

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Cachexia, a common complication of cancer, is characterized by several metabolic alterations and a massive loss of skeletal muscle mass. Occurring in up to 80% of cancer patients, cachexia has a dismal prognosis, reducing the efficacy of therapeutic interventions, and being often the direct cause of death. 25OH-vitamin D (25OHVD) blood levels have been correlated with the incidence and evolution of some cancers suggesting that vitamin D (VD) can play a

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role in improving patients' prognosis. In addition, the complex and highly orchestrated VD system, besides its well-known role in bone health, is also important in the maintenance of muscle homeostasis and functionality,[1] and this led to the hypothesis that VD could be used as anti-cachectic treatment. However, VD supplementation failed in clinical trials to either enhance cancer patients' survival or prevent muscle wasting.[2] Several factors could be advocated for this debacle, including pharmacokinetic reasons. The 90-95% of 25OHVD in the blood is bound to its carrier VD binding protein (DBP) that could act as a scavenger, thus reducing the free, bioavailable 25OHVD to the target tissues. Our preliminary results demonstrate that indeed, in an in vitro model of cancer cachexia, DBP abolishes the protective effect of 25OHVD. In addition, DBP has a direct effect on the induction of atrophy in C2C12 myotubes.

1. *Polly P, Tan TC. The role of vitamin D in skeletal and cardiac muscle function. Front Physiol 2014;5:145. doi: 10.3389/fphys.2014.00145. eCollection 2014.*
2. *Mochamat et al. A systematic review on the role of vitamins, minerals, proteins, and other supplements for the treatment of cachexia in cancer: a European Palliative Care Research Centre cachexia project. J Cachexia Sarcopenia Muscle. 2016. doi:10.1002/jcsm.12127.*

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Muscle physiological adaptations to voluntary running in cancer cachexia

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The muscular system, consisting of grouping of muscles that contract to produce movements, represents up to 50% of the body mass of a healthy subject. Skeletal muscle tissue has a remarkable potential to alter its phenotype, by the reversal of contractile characteristics in fast- and slow-twitch muscles, during exercise training. Cancer cachexia is a chronic degenerative syndrome characterized by the loss of body weight and skeletal muscle mass. The progressive muscle wasting results in impaired muscle function, fatigue and eventually decreased quality of life of the patients. So far, no nutritional or pharmacological therapies have been established to successfully counteract cancer-related muscle wasting. Exercise, however, has been proposed as a promising intervention strategy to be included in the multimodal approach to treat cancer-related muscle wasting. The known beneficial effects of physical activity in cachectic muscles include the ability to counteract muscle atrophy and chronic inflammation and to improve muscle function. However, a complete picture of muscle physiological modulations upon physical activity occurring in both healthy and cachectic conditions is still missing. Using sedentary and voluntary exercise conditions, we describe the most evident muscle adaptations occurring in healthy and C26-bearing cachectic mice, providing extensive molecular and histological analyses of MYHC composition, distribution of oxidative versus glycolytic fibers and capillary density. Overall, our results show that voluntary running and cancer cachexia stimulate the fast-twitch oxidative phenotype and muscle oxidative activity. These insights will be useful to develop and optimize exercise intervention strategies to treat cancer-related muscle wasting.

1. *Argiles JM, et al. Cancer cachexia: understanding the molecular basis. Nat Rev Cancer 2014;14:754-62.*
2. *Gould DW, et al. Cancer cachexia prevention via physical exercise: molecular mechanisms. J Cachexia Sarcopenia Muscle 2013;4:111-24.*
3. *Ciciliot S, et al. Muscle type and fiber type specificity in muscle wasting. Int J Biochem Cell Biol 2013;45:2191-9.*

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Activation of the SDF1/CXCR4 pathway retards muscle atrophy during cancer cachexia

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Cancer cachexia is a life-threatening syndrome that affects most patients with advanced cancers and causes severe body weight loss, with rapid depletion of skeletal muscle. No treatment is available. We analyzed microarray datasets to identify a subset of genes whose expression is specifically altered in cachectic muscles of Yoshida hepatoma-bearing rodents, but not in those with diabetes, disuse, uremia or fasting. Ingenuity Pathways Analysis indicated that three genes belonging to the CXCR4 pathway were downregulated only in muscles atrophying because of cancer: SDF1, PAK1 and ADCY7. In Rectus Abdominis muscle of cancer patients, the expression of SDF1 and CXCR4 were inversely correlated with that of two ubiquitin ligases induced in muscle wasting, atrogin-1 and MuRF1, suggesting a possible clinical relevance of this pathway. The expression of all main SDF1 isoforms (α, β and γ) declined also in Tibialis Anterior from cachectic mice bearing murine colon adenocarcinoma or human renal cancer and drugs with anti-cachexia properties restored their expression. Overexpressing genes of this pathway (i.e. SDF1 or CXCR4) in cachectic muscles increased the fiber area by 20%, protecting them from wasting. Similarly, atrophying myotubes treated with either SDF1α or SDF1β had increased total protein content, resulting from reduced degradation of overall long-lived proteins. However, inhibiting CXCR4 signaling with the antagonist AMD3100 did not affect protein homeostasis in atrophying myotubes, whereas normal myotubes treated with AMD3100 showed reduced diameters and lower total protein content. Overall, these findings support the idea that activating the CXCR4 pathway in muscle suppresses the deleterious wasting associated with cancer.

1. *Martinelli et al., Activation of the SDF1/CXCR4 pathway retards muscle atrophy during cancer cachexia. Oncogene 2016;35:6212-22.*

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2. *Pretto et al., Sunitinib prevents cachexia and prolongs survival of mice bearing renal cancer by restraining STAT3 and MuRF-1 activation in muscle Oncotarget 2015;6:3043-54.*

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Cellular determinants during denervation-induced muscle atrophy

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The loss of function of the motor neuron, in case of diseases (such as ALS, PLS, PMA and CMT) and in mouse models (denervation mouse, SOD1 mouse and SCI mouse), causes myofibers atrophy. Denervation leads to the induction of catabolic machinery that influences the equilibrium between protein synthesis and degradation, driving the muscle fibers towards atrophy, when proteins degradation rates exceed protein synthesis.[1] The major catabolic pathways activated during these events are the proteasome and the autophagic machinery. IL-6 is an inflammatory cytokine, associated with regeneration and cachexia, able to activate STAT3, a transcriptional factor involved in the promotion of myogenic lineage progression. IL-6 is also released by fibro/adipogenic progenitors (FAPs), a population of cell involved in both muscle regeneration and fats accumulation and fibrosis,[2] in particular during pathologic atrophy, like denervation. In our model, we observed an activation of IL6-STAT pathway induced by FAPs involvement. Our work is focused on the role played by FAPs during denervation atrophy and their interaction with other cell populations. Our aim is to understand if STAT3 could be a target for new pharmacological approaches, which have the purpose to counteract the atrophy process induced by the loss of trophic fluxes that normally exert an important role in the maintenance of muscle homeostasis.

1. *Sandri M. Protein breakdown in muscle wasting: role of autophagy-lysosome and ubiquitin-proteasome. Int J Biochem Cell Biol 2013;45:2121-9. doi: 10.1016/j.biocel.2013.04.023.*
2. *Joe AW, Yi L, Natarajan A, Le Grand F, So L, Wang J, Rudnicki MA, Rossi FM. Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. Nat Cell Biol 2010;12:153-63. doi: 10.1038/ncb2015.*
3. *Haddad F, Zaldivar F, CooperDM , Adam GR. IL-6-induced skeletal muscle atrophy. J Appl Physiol 2005;98:911–7. First published November 12, 2004; doi:10.1152/japplphysiol.01026.2004.*

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POSTER SESSIONS

PKCε as a novel promoter of satellite cell differentiation

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Satellite cells are muscle resident stem cells and represent principal players for muscle regeneration. PKCε is a protein kinase involved in many cellular processes like proliferation and differentiation. In this study we investigated the involvement of PKCε during satellite cell differentiation *in vitro* and *in vivo*. In particular, we functionally modulated PKCε in murine skeletal myoblast (C2C12 cell line), in murine satellite cells and in a model of muscle regeneration *in vivo*. We found that PKCε was up-regulated during skeletal muscle differentiation and translocated to the nucleus where it promoted Myogenin and Mrf4 accumulation. Moreover, in a cardiotoxin model of muscle injury, PKCε accumulated in regenerating, centrally-nucleated myofibers and its specific inhibition impaired the expression of two crucial markers of muscle differentiation, like MyoD and Myogenin. Our conclusion is that PKCε is positively involved in satellite cell myogenic differentiation. Future work will focus on the signalling pathway of this phenomenon

1. *Queirolo V, et al. PKCε is a regulator of hypertrophic differentiation of chondrocytes in osteoarthritis. Osteoarthritis Cartilage. 2016 Aug;24(8):1451-60. doi: 10.1016/j.joca.2016.04.003.*
2. *Galli D, et al. PKCε is a negative regulator of PVAT-derived vessel formation. Exp Cell Res 2015;330(2):277-86. doi: 10.1016/j.yexcr.2014.11.011.*
3. *Di Marcantonio D, et al. PKCε as a novel promoter of skeletal muscle differentiation and regeneration. Exp Cell Res 2015;339:10-9. doi: 10.1016/j.yexcr. 2015.09.017.*

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The effects of hypobaric hypoxia on female skeletal muscle regeneration

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High altitude exposure together with physical exercise provides oxygen supply restriction inducing human skeletal

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muscle adaptation via hypoxia inducible factor (HIF) activation that initiates transcription of HIF-responsive genes. Skeletal muscle remodelling is due to satellite cells (SCs), adult stem cells, that once activated are able to proliferate (myoblasts), differentiate (myotubes) and fuse with existing fibers or to form new ones. The aim of this study was to determine whether satellite cell pool of young female subjects is affected by the oxidative imbalance that might be caused by hypobaric hypoxia and physical exercise as during a 14-day trekking expedition. We collected biopsies from Vastus Lateralis muscle for both single fiber analysis and SC isolation.[1-3] The samples collected before (PRE-Hypoxia) and after (POST-Hypoxia) the trekking in the Himalayas were compared. SCs were investigated for oxidative stress markers. We found that POST-Hypoxia myoblasts obtained by two out of six volunteers showed high superoxide anion production, lipid peroxidation and impaired dismutase/catalase activities. Also mitochondrial potential variation was affected, as mitochondria are the cellular oxygen sensors, manage the reactive oxygen species production and detoxification. In addition, we studied the transcription profile of HIF, myogenic transcription factors (Pax7, MyoD, myogenin) and miRNAs (miR-1, miR-133, miR-206) that we found to be different for oxidized and non oxidized cells. The present study supports the phenomenon of hypobaric-hypoxia-induced oxidative stress and its role in the impairment of the regenerative capacity of SC derived from the Vastus Lateralis muscle of young adult female subjects.[4]

1. *Pietrangelo T, et al.Tiny percutaneous needle biopsy: An efficient method for studying cellular and molecular aspects of skeletal muscle in humans. Int J Mol Med 2011;27:361-7. doi: 10.3892/ijmm.2010.582. Epub 2010 Dec 14.*
2. *Pietrangelo T, et al. Low Intensity Exercise Training Improves Skeletal Muscle Regeneration Potential. Front Physiol 2015;6:399. doi: 10.3389/fphys.2015.00399. eCollection 2015.*
3. *Fulle S. et al. Age-dependent imbalance of the antioxidative system in human satellite cells. Exp Gerontol 2005;40:189-97. Epub 2004 Dec 15.*
4. *Mancinelli R, et al. The Regenerative Potential of Female Skeletal Muscle upon Hypobaric Hypoxic Exposure. Front Physiol 2016;7:303. doi: 10.3389/fphys. 2016.00303.*

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Chronic exposure to H2O2 alters the excitability of human and mouse myotubes

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Skeletal muscle regeneration and repair processes by Satellite Cells (SCs), muscle-specific stem cells, is impaired by ageing. The pool of SCs in aged animals (including humans) is reduced as compared to young individuals, but this feature alone does not account fully for impairment severity. Functional impairment of SCs by enhanced oxidative stress may contribute to reduce their regenerative potential. However, the functional properties of SCs differentiating under a condition of chronic oxidative stress remain poorly characterized. We investigated this point using human myotubes and the mouse C2C12 cell line. Since day 0 of differentiation we repeatedly treated cells with H2O2 at different concentrations to mimic a chronic oxidative stress and we studied both the formation and the function of myotubes. In particular, we performed electrophysiological recordings and Ca^{2+} imaging experiments to study active and passive electrical properties of the myotubes. Acute treatment resulted in a strong membrane hyperpolarization, possibly indicating an initial "defensive" behaviour. Cytoplasmic Ca^{2+} transients were also altered. By contrast, chronically-treated cells were depolarized in comparison to control cells and showed an increased excitability. Both features alter calcium homeostasis, which plays a critical role during skeletal muscle regeneration and repair processes *in vivo*.

1. *Barbieri E, Sestili P. Reactive Oxygen Species in skeletal muscle signaling. Journal of Signal Transduction 2011 Dec 5;2012.*
2. *Luin E, et al. Effects of H_2O_2 on electrical membrane properties of skeletal myotubes. Free Radic Biol Med 2011;50:337-44.*
3. *Liu J-H, , et al. Acceleration of human myoblast fusion by depolarization: graded Ca^{2+} signals involved. Development 2003;130:3437-46.*

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Human Elastin-Like Polypeptides as biomimetic materials for in vitro myogenesis

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Skeletal muscle cells and their precursors detect both the biochemical composition of the surrounding extracellular matrix (ECM), and its physical properties such as stiffness and topography. Human elastin-like polypeptides (HELPs) are recombinant biomimetic polymers mimicking the properties of the native matrix protein; they have been employed to support the adhesion of several cell types, among which myoblasts. We recently synthesized a novel polypeptide, HELPc, by fusing the elastin-like backbone to a 41aa domain of type IV collagen, containing two RGD motives. We employed this peptide as adhesion substrate for C2C12 myoblasts and compared its effects to those induced by two other polypeptides of the HELP series. Myoblast adhered to all HELPs, and assumed a cytoarchitecture strictly dependent on the polypeptide sequence. Adhesion to HELPs stimulated, at a different extent, cell differentiation, Myosin Heavy Chain expression and fusion into multinucleated myotubes. The different substrates altered the cells Ca2+ handling capacity and the maturation of excitation-contraction coupling machinery. ECM stiffness is now recognized a critical factor for myoblast development: accordingly, we employed HELPc-based hydrogels at

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different monomer density as adhesion substrates for C2C12 cells. Depending on support rigidity, adhesion to hydrogels dictated cell morphology, spreading, focal adhesions formation and cytoskeleton organization. Intriguingly, it greatly stimulated cell proliferation, particularly in low serum-medium, while resulted partially inhibitory of myogenic differentiation. Overall, our findings indicate that the properties of HELP polypeptides can be exploited for dissecting the causal links underlying the different steps of myogenesis and for designing novel substrates for skeletal muscle regeneration.

1. *Bandiera A, et al. Comparison of thermal behavior of two recombinantly expressed human elastin-like polypeptides for cell culture applications. Biomacromolecules 2010;11,3256-65. doi: 10.1021/bm 100644m. Epub 2010 Nov 2.*
2. *D'Andrea P, et al. In vitro myogenesis induced by human recombinant elastin-like proteins. Biomaterials 2015; 67,240-253. doi: 10.1016/j.biomaterials.2015.07.041. Epub 2015 Jul 23*
3. *D'Andrea P, et al. J App Biomat Func Mat 2016;doi: 10.5301/jabfm.5000331.*

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mIGF-1 overexpression in dystrophic skeletal muscles: modulating tissue microenvironment to improve stem cell therapy for DMD

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Duchenne muscular dystrophy (DMD) is a degenerative disease characterized by muscle wasting, weakness and loss of walking capability in the first decade of life. Loss of functional dystrophin protein results in an increased muscle fibers sensitivity to mechanical damage, leading to degeneration and necrosis.[1] While dystrophin deficiency is the primary defect, secondary mechanisms are important features of pathology.[2] Chronic inflammation and excessive ROS (reactive oxygen species) production exacerbate the disease, making dystrophic muscle incapable to sustain an efficient regeneration process and affecting muscle niche.[2,3] In this pathological context the hostile microenvironment could represent a limiting factor for stem cells activity and survival.[4] Thus the modulation of specific factors could render the dystrophic niche more hospitable for stem cell-mediated therapy in DMD patients. In particular, the overexpression of mIGF-1 (muscular Insulin-like growth factor-1), which is able to ameliorate the dystrophic phenotype and improves muscle strength,5 could be useful to sustain both resident and transplanted stem cells survival in a mdx mouse model. In this work we show how mIGF-1 overexpression in mdx mice (mdx/mIGF-1 mice) modulates important pathological mechanisms, such as inflammatory response, positively affecting muscle environment. Moreover, basing on these results we have developed a combined therapy in which a stem cell approach, using nLacZ labeled mesoangioblasts (MABs), is associated with the modulation of the dystrophic microenvironment by mIGF-1. Our results highlight the needs of a less compromised milieu to ameliorate the efficacy of a promising therapeutic approach to treat DMD.

1. *Grounds MD, et al. Towards developing standard operating procedures for pre-clinical testing in the mdx mouse model of Duchenne muscular dystrophy. Neurobiol Dis 2008;31:1-19. doi: 10.1016/j.nbd. 2008.03.008*
2. *Porter JD, et al. A chronic inflammatory response dominates the skeletal muscle molecular signature in dystrophin-deficient mdx mice. Hum Mol Genet 2002;11:263-72. doi: 10.1093/hmg/11.3.263*
3. *Renjini R, et al. Oxidative damage in muscular dystrophy correlates with the severity of the pathology: role of glutathione metabolism. Neurochem Res 2012;37:885-98. doi: 10.1007/s11064-011-0683-z*
4. *Barton ER, et al. Muscle-specific expression of insulin-like growth factor I counters muscle decline in mdx mice. J Cell Biol 2002;157:137-48. doi: 10.1083/jcb. 200108071*
5. *Pelosi L, et al. MicroRNAs modulated by local mIGF-1 expression in mdx dystrophic mice. Front Aging Neurosci 2015;7:69. doi: 10.3389/fnagi.2015.00069*

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Protein supplementation rescues myotube damages after antineoplastic drug treatment

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Autophagy is a physiological mechanism, responsible for cell homeostasis, aimed to remove damaged organelles or unfolded proteins and avoid their accumulation in the cytoplasm. Autophagic flux impairment seems to be involved in several diseases, including muscular dystrophies and myopathies.[1] Moreover, chemotherapeutic drugs have been reported to trigger autophagy.[2] We previously demonstrated that C2C12 myotubes treated with Etoposide (Eto), a chemotherapeutic drug known to induce cell-death and oxidative stress, undergo an abnormal autophagic activation, nuclear disorganization and cytoplasmic shrinkage.[3] Protein supplementation stimulated by glutamine has been described as protective from the degradative effect of proinflammatory cytokines implicated in many degenerative processes.[4] In this study, differentiated C2C12 cells have been exposed to Eto after a previous exposure to glutamine. Cytofluorimetric and morphological analyses revealed that Eto treatment induces damages to the lysosomal compartment, causing the accumulation of autophagic vacuoles, and a reduction of myotube area. Interestingly, glutamine pre-treatment is able to preserve myotube size and prevent the autophagic impairment, partially restoring the normal lysosomal activity. These findings suggest that glutamine supplementation could prevent the Eto-induced abnormal autophagic activation and

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hamper the atrophic pathway in differentiated C2C12 cells. Given the increased attention and controversies on glutamine supplementation, further studies are necessary to evaluate if glutamine supplementation can attenuate muscle atrophy in tumor-bearing mice.

1. *Sandri M, Coletto L, Grumati P, Bonaldo P. Misregulation of autophagy and protein degradation systems in myopathies and muscular dystrophies. J Cell Sci 2013;126:5325-33.*
2. *Fanzani A, et al. Cisplatin triggers atrophy of skeletal C2C12 myotubes via impairment of Akt signalling pathway and subsequent increment activity of proteasome and autophagy systems. Toxicol Appl Pharmacol 2011;250:312-21.*
3. *Salucci S, et al. The peculiar apoptotic behavior of skeletal muscle cells. Histol Histopathol 2013;28:1073-87.*
4. *Bonetto A, et al. Glutamine prevents myostatin hyperexpression and protein hypercatabolism induced in C2C12 myotubes by tumor necrosis factor-α. Amino Acids 2011;40:585-94.*

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Mitochondria association to Ca^{2+} release units is controlled by muscle activity

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At the most basic level, skeletal muscle contraction requires Ca^{2+} and ATP and, thus, is under direct control of two major intracellular organelles: Ca^{2+} release unit (CRU) and mitochondria. CRUs are the sites of excitation-contraction (EC) coupling, the process responsible for triggering Ca^{2+} release from the sarcoplasmic reticulum (SR) in response to propagating action potentials in the transverse-tubule membrane. Mitochondria are the powerhouse of the cell, being responsible for aerobic production of ATP. CRUs and mitochondria in adult skeletal muscle fibers are functionally and structurally coupled: a) entry of Ca^{2+} into the mitochondrial matrix is able to stimulate the respiratory chain; b) mitochondria and CRUs are structurally linked to one another by small stands, or tethers. Here we tested the following hypothesis: muscle activity improves/maintains the correct association of mitochondria to CRUs, which is challenged by ageing and inactivity. Using electron and confocal microscopy, we studied: a) EDL muscle fibers from 2 year old mice trained for 1 year on the treadmill; and b) EDL muscles denervated by crash of sciatic nerve. Our quantitative analysis shows that exercise (in old mice) and re-innervation (in transiently denervated rats) either maintains or improves the association between the two organelles (up to control levels) that was partially lost during ageing and inactivity. As Ca^{2+} uptake into mitochondria and efficient ATP production likely depend on the correct association between the two organelles, the functional implication of maintained/rescued association between mitochondria and CRUs is potentially large.

1. *Boncompagni S, et al. Progressive disorganization of the excitation contraction coupling apparatus in ageing human skeletal muscle as revealed by electron microscopy: a possible role in the decline of muscle performance. J Gerontol Biol Sci 2006;61:995-1008.*
2. *Boncompagni S, et al. Mitochondria are linked to calcium stores in striated muscle by developmentally regulated tethering structures. Mol Biol Cell 2009;20:1058-67.*
3. *Pietrangelo L, et al. Age-dependent uncoupling of mitochondria from Ca^{2+} release units in skeletal muscle. Oncotarget 2015;6:35358-71.*

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Estrogens protect Calsequestrin-1 knockout mice from lethal hyperthermic episodes

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Mice lacking Calsequestrin-1 (CASQ1-null) exhibit lethal hyperthermic episodes, resembling human Malignant Hyperthermia (MH), when exposed to halothane and environmental heat. Similarly to what reported in humans, incidence of mortality in CASQ1-null animals is far greater in males than females: the reason for this gender difference is still unclear. Excessive production of oxidative species of oxygen and nitrogen (ROS/RNS) is a key event leading to death of CASQ1-null mice during MH crises. Here we treated for 1 month male and female CASQ1-null mice respectively with Premarin (conjugated equine estrogens) and Leuprolide (GnRH analaog), both administered subcutaneously (respectively 40 and 100 ng/g bw/day). Premarin treatment protects CASQ1-null male mice from lMH episodes significantly reducing the mortality rate: halothane, from 79 to 33%; heat from 86 to 20%. Conversely, Leuprolide treatment increased the incidence of halothane- and heat-induced deaths in females: respectively, from 18 to 73% and from 24% to 82%. In addition, during heat challenge and in-vitro contracture test (IVCT by caffeine and temperature): a) in males, Premarin reduced the rise in core temperature and the sensitivity of EDL muscles to IVCT; b) in females, Leuprolide induced hyperthermia and increased responsiveness of EDL muscles during IVCT. Finally, we investigated the effect of Premarin and Leuprolide on the expression levels of SOD1, SOD2 and 3-NT and GSH/GSSG ratio: Premarin reduced oxidative stress in males, while Leuprolide had the opposite effect in females. In conclusion, our results demonstrate that hormones affect susceptibility of CASQ1-null mice to MH, likely by controlling levels of oxidative stress.

1. *Campbell KP, et al. Purification and characterization of calsequestrin from canine cardiac sarcoplasmic reticulum and identification of the 53,000 dalton glycoprotein. J Biol Chem 1983;258:1197-204.*
2. *Dainese M, et al. Anesthetic- and heat-induced sudden death in calsequestrin-1-knockout mice. FASEB J 2009;23:1710-20.*
3. *Michelucci A, et al. Antioxidants protect calsequestrin-1 knockout mice from halothane- and heat-induced sudden death. Anesthesiology 2015;123:603-17.*

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Ageing causes ultra-structural modification to calcium release units and mitochondria in cardio-myocytes

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Ageing is associated to a dramatic increase in the incidence of heart failure, even if the existence of a real age-related cardiomyopathy remains controversial. As effective contraction and relaxation of cardiomyocytes depends on Ca^{2+} supply to myofibrils (handled by calcium release units - CRUs- or dyads) and efficient production of ATP (provided by mitochondria), here we used structural (electron and confocal microscopy, EM and CM) and biochemical (western blots, WB) approaches to investigate the effect of ageing on CRUs and mitochondria, comparing hearts from 4 and ≥24 months old mice. EM and CM indicate that CRUs and mitochondria undergo structural damage and spatial re-organization with increasing age. Indeed, CRUs may be miss-oriented (longitudinal) or miss-placed (found at the A band of the sarcomere), while mitochondria are often damaged and grouped in an abnormal fashion between myofibrils. Quantitative analysis of CRUs indicates that, with age: a) dyads become shorter (362±314nm vs 254±240nm); and b) the number of CRUs/50μm2 decreases (5.1±0.3 vs 3.9±0.2). Changes in morphology of dyads may correlate with the reduced expression of proteins involved in maintaining stability of dyads and transverse-tubules (a component of CRUs): junctophilin-2 (A.U.=1.2±0.04 vs 0.8±0.03); and caveolin-1 (A.U.=1.1±0.04 vs 0.9±0.04).
Finally, we measured i) the percentage of mitochondria presenting structural alterations, and ii) the relative cell volume occupied by empty-cytoplasmic space and determined that both values were significantly increased by age: respectively i) 3.5% vs 16.5%; ii) 2.2±3.1 vs 9.7±5.7. In conclusion, our results provide possible additional explanations for the cardiac dysfunction associated to ageing.
*These authors contributed equally to this work.

1. *Chopra N1, et al. Ablation of triadin causes loss of cardiac Ca^{2+} release units, impaired excitation-contraction coupling, and cardiac arrhythmias. Proc Natl Acad Sci U S A 2009;106:7636-41.*
2. *Loud AV. A method for the quantitative estimation of cytoplasmic structures. J Cell Biol 1962;15:481-7.*
3. *Protasi F, et al. Formation and maturation of the calcium release apparatus in developing and adult avian myocardium. Dev Biol 1996;173:265-78.*

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Role of Histone H3 Lysine 9 (H3K9) methyltransferases G9a and GLP in the epigenetic regulation of Fibroadipogenic progenitors (FAPs) differentiation during Duchenne Muscular Dystrophy (DMD) progression

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Duchenne muscular dystrophy (DMD) is a severe X-linked neuromuscular degenerative disorder that leads to progressive muscle weakness. This is due to loss of muscle tissue that culminates with its replacement with fat and fibrotic infiltrates, in coincidence with the final stages of disease. Despite recent progresses in genome editing approaches have demonstrated the possibility to correct the genetic defect in vivo, the cure for DMD is still a big challenge. Therefore, pharmacological therapies aimed to counteract the fibro-adipogenic degeneration and to promote the compensatory regeneration that is typical of the early stages of disease hold great promise to slow-down DMD progression. Fibroadipogenic progenitors, FAPs, have been shown to be responsible of fat and fibrotic tissue deposition in degenerating dystrophic muscles, while also contributing to muscle regeneration at early stages of the disease.[1,2] As such, understanding the molecular basis of FAP's differentiation might reveal possible pharmacological targets to manipulate their phenotypical plasticity in vivo, with the ultimate goal to promote muscle regeneration and concomitantly block fibro-adipogenic degeneration. Our results and data from the literature suggest that methylation of Lysine 9 of histone H3 (H3K9) by specific methyltransferases (KMTs), is one of the epigenetic pathway involved in the control of FAPs' alternative fates. In particular, among the different H3K9 KMTs, the mono- and di- methyltransferases G9a and GLP are of particular relevance in controlling the repression of muscle-specific genes in myogenic precursors,[3,4] and likely in FAPs. In fact, our preliminary data show that the in vitro inhibition of H3K9 KMTs in FAPs from mdx mice (the DMD murine model) induces myogenic differentiation at expenses of their adipogenic potential. We show here that FAPs isolated from injured wild type mice treated in vivo with G9a/GLP specific inhibitors display increased expression of myogenic markers and de- regulation of fibroadipogenic genes. Taken together, our results suggest that H3K9 KMTs inhibitors could promote the myogenic potential of muscle progenitor cells and might become a potential new therapeutic approach in the treatment of DMD.

1. *Mozzetta C, et al. Fibroadipogenic progenitors mediate the ability of HDAC inhibitors to promote regeneration in dystrophic muscles of young, but not old Mdx mice. EMBO Mol Med 2013;5: 626-639.*
2. *Joe AW, et al. Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. Nature cell biology 2010;12:153-63.*
3. *Ling BM, et al. Lysine methyltransferase G9a methylates the transcription factor MyoD and regulates skeletal muscle differentiation. Proc Natl Acad Sci U.S.A. 2012;109:841-6.*
4. *Mozzetta C, et al. Sound of silence: the properties and functions of repressive Lys methyltransferases. Nat Rev Mol Cell Biol 2015;16:499-513.*

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Molecular identification and functional characterization of mitochondrial transporter SVCT2 in C2C12 skeletal muscle cells

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We initially confirmed[1] the mitochondrial expression of functional high affinity SVCT2 in undifferentiated C2C12 cells.[1,2] Their differentiation to myotubes, while not affecting the overall expression of the transporter and the cellular uptake of ascorbic acid (AA), seriously complicated the isolation of "pure" mitochondria. None of the different methodologies employed enabled us to safely determine the SVCT2 contribution to the mitochondrial uptake of the vitamin. We therefore employed an approach entirely based on confocal microscopy and obtained results suggesting a correlation between the degree of differentiation and the loss of SVCT2 expression. We next moved to mitochondria directly taken from the murine tibialis anterior muscle. We succeeded in obtaining rather pure mitochondrial fractions, as assessed by both WB and TEM methodologies, in which however the SVCT2 signal was rather poor. At this stage, our conclusion is that the expression of mitochondrial SVCT2 cannot be dismissed as a simple cell culture effect, since it can be negatively modulated by the differentiation process elicited still under in vitro conditions. A second conclusion is on the very poor expression of SVCT2 in the mouse skeletal muscle, which did not allow us to test the possibility of the expression of a high affinity transport of AA in a subcellular compartment that, under specific conditions, extensively produce reactive oxygen species.[3]

1. *Azzolini C, et al. Sodium-dependent transport of ascorbic acid in U937 cell mitochondria. IUBMB Life 2013;65:149-53.*
2. *Fiorani M, et al. The mitochondrial transporter of ascorbic acid functions with high affinity in the presence of low millimolar concentrations of sodium and in the absence of calcium and magnesium. Biochim Biophys Acta 2015;1848:1393-401.*
3. *Mason SA, et al. Muscle redox signalling pathways in exercise. Role of antioxidants. Free Radic Biol Med 2016;98:29-45.*

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Lnc-31, a newly identified long non-coding RNA, promotes murine myoblasts proliferation

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Myogenesis is a complex process regulated by myogenic protein factors as well as non coding RNAs, microRNAs (miRNAs) and long non coding RNAs (lncRNAs). Transcriptome analysis performed during in vitro murine myoblast differentiation allowed the identification of new lncRNAs differentially expressed along myogenesis. Among them we focused on lnc-31 that originates from the same RNA precursor of miR-31. Lnc-31 is expressed in proliferating myoblast and plays a relevant function in the maintenance of proliferation condition. Notably, despite the poor sequence conservation with the human counterpart, lnc-31 function is conserved also in this species. In order to molecular dissect the mode of action of lnc-31 we characterized its interactors such as proteins, mRNAs as well as non coding RNAs. We found that lnc-31 binds miR-31, miR-24 and miR-152 even though luciferase assays revealed that only mir-31 can bind to lnc-31 directly. We also found that lnc-31 interacts with the 5'UTR of Rock1 mRNA and with the RNA/DNA binding protein Ybx1. Knock-down and overexpression experiments showed that, in proliferating condition both lnc-31 and Ybx1 are required for controlling Rock1 expression at post-trascriptional level, possibly facilitating its translation. Rock1 has been described as negative regulator of myogenesis by preventing the exit of myoblast from the cell cycle, therefore, our data allow us to suggest that the binding between lnc-31 and Ybx1 may affect myoblast proliferation by controlling Rock1 levels; upon differentiation, when lnc-31 is down-regulated, Rock1 protein levels decrease, allowing myoblasts to enter into the myogenic differentiation program.

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A new function for an old kinase: the role of CK2 in myogenic differentiation

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Protein kinase CK2 is a ubiquitous and highly conserved Ser/Thr kinase, endowed with constitutive activity. It is usually present as a tetrameric holoenzyme composed of two catalytic subunits (α and/or α') and two regulatory β subunits, which stabilize the kinase and modulate its substrate specificity (1). CK2 phosphorylates a large number of protein substrates thus being implicated in multiple cellular processes (2) and several observations also point to its involvement in cell differentiation processes (such as osteoblast, osteoclast and adipocyte differentiation).

CK2 has been also implicated in the regulation of skeletal muscle differentiation where it has been shown to regulate the entry of muscle cell into the process by modulating the activity of key myogenic transcription factors (such as Pax3, Pax7 and members of the MRF family). In addition, CK2 phosphorylates the muscle-specific receptor tyrosine kinase MuSK, which mediates the acetylcholine receptor clustering at the plasma membrane, thus participating to the organization of the neuromuscular junction in mature myotubes. Despite all these findings, little is known about the contribution of CK2 activity during the progression of the myogenic program and at the different stages of the muscle differentiation process.

Here, for the first time, we aim to unveil the role of CK2 during the process of skeletal muscle formation both in cell culture systems and in vivo. In particular, we will use pharmacological (3) and genetic approaches to explore the contribution of CK2 to myotube differentiation in both

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C2C12 and primary muscle derived stem cells in vitro, and to mouse muscle regeneration and zebrafish development in vivo. Furthermore, we will establish knock out cellular models to study the relative contribution of each CK2 subunit.*These authors contributed equally to this work.

1. *Pinna LA. The raison d'être of constitutively active protein kinases: the lesson of CK2. Acc Chem Res 2003;36:378-84. PubMed PMID: 12809523.*
2. *Meggio F, Pinna LA. One-thousand-and-one substrates of protein kinase CK2? FASEB J 2003;17:349-68. Review. PubMed PMID: 12631575.*
3. *Zanin S, et al. Effects of the CK2 inhibitors CX-4945 and CX-5011 on drug-resistant cells. PLoS One. 2012;7:e49193. PubMed PMID: 23145120.*

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High content screening identifies azathioprine as an inhibitor of adipogenic differentiation in FAPs – Disrupting muscle cell differentiation trajectories by small molecules

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The muscle regenerative program is mainly supported by Muscle Satellite Cells (MuSCs), quiescent adult stem cells that, once activated, efficiently differentiate into myotubes repairing damaged myofibers. Additional cell types residing in the interstitial space also participate in muscle regeneration by establishing a complex crosstalk culminating in muscle recovery. Understanding and learning to control the regeneration machinery could help to establish strategies to reverse the consequences of pathologies leading to muscle wasting. By performing a high content screening of the ~1200 FDA approved drugs in the Prestwick Chemical Library (http://www.prestwickchemical.com), we have identified azathioprine as a molecule that negatively modulates adipogenic differentiation in heterogeneous cultures of muscle mononuclear cells derived from C57BL/6J mice. We have characterized the effect of the drug on different purified progenitor cells and we have identified the FAPs as the progenitor cells whose differentiation is affected by azathioprine. Fibro-Adipogenic Precursors (FAPs) are the main cell population involved in fibrous and fatty degeneration in dystrophic muscles. Targeting these cells using small molecules may represent a strategy finalized to ameliorate the dystrophic condition

1. *Uezumi, A., Fukada, S.-I., Yamamoto, N., Takeda, S. & Tsuchida, K. Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle. Nat Cell Biol 2010;12:143–52.*
2. *Joe, AWB. et al. Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. Nat Cell Biol 2010;12:153–63.*
3. *Uezumi, A. et al. Fibrosis and adipogenesis originate from a common mesenchymal progenitor in skeletal muscle. Journal of Cell Science 2011;124:3654–64.*

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Discovering new "perturbagens" of osteogenic differentiation of muscle progenitor cells

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Skeletal muscle is a complex ad organized tissue with high regenerative capacity. Satellite cells are adult stem cells that play a leading role in muscle differentiation and regeneration after damage. This process can fail in pathological conditions, as in Duchenne Muscle Dystrophy (DMD). In this case aberrant trans-differentiation of muscle resident stem cells can occur. Aside from fibrotic and fat infiltrations, old mdx mice can show heterotopic ossification of skeletal muscles. The cellular origin of this phenomenon is not well understood but mesenchymal stem cells, pericytes and mesoangioblasts are good candidates. In fact, these cell populations are capable of osteogenic differentiation in vitro and in vivo when stimulated by Bone Morphogenic Protein 2 (BMP2).[1] BMP2 signaling is the main pathway controlling osteogenesis in physiological conditions but the contribution of alternative pathways is still unclear.[2] High-content screening is a powerful approach for the selection and characterization of small molecules able to perturb differentiation decisions.[3] In one such screening we selected the antiviral drug Idoxuridine (IdU) as an inducer of osteogenic differentiation of mesoangioblasts. We are currently aiming at characterizing the mechanism of action of IdU. Preliminary data suggest that IdU acts by a BMP2 independent mechanism, opening the possibility of alternative pathways controlling osteogenesis. By this approach we aim at understanding the molecular alterations occurring in heterotropic ossidification and possibly to control it. * These authors contribute equally to this Abstract

1. *Minasi MG, Riminucci M, De Angelis L, Borello U, Berarducci B, Innocenzi A, Caprioli A, Sirabella D, Baiocchi M, De Maria R, et al. The meso-angioblast: a multipotent, self-renewing cell that originates from the dorsal aorta and differentiates into most mesodermal tissues. Development 2002;129:2773-83.*
2. *Chen G, Deng C, Li Y-P. TGF-β and BMP signaling in osteoblast differentiation and bone formation. Int J Biol Sci 2012;8:272–88.*
3. *Fraietta I, Gasparri F. The development of high-content screening (HCS) technology and its importance to drug discovery. Expert opinion on drug discovery 2016;11:501-14.*

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Protective role of IGF-1 against Sarcopenia

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Sarcopenia is the age-related loss of muscle mass, strength and functionality.[1] The causes of sarcopenia are unknown; current hypotheses indicate that it may be the result of several factors, including activation of inflammatory and catabolic pathways, decline in neuromuscular function, hormonal changes and a withdrawal of anabolic factors, like IGF-1.[2] We demonstrated that the overexpression of two IGF-1 isoforms IGF-1, IGF-1EA and IGF-1EB, was able to counter the decrease in muscle mass, CSA and strength, in 24 months age old mice. We observed also a protective effect of IGF-1 on the integrity and the functionality of the neuromuscular junction (NMJ). Analysis of bungarotoxin stained NMJ by confocal microscopy, showed the presence, in both IGf-1 transgenic mice, of less degenerated structures compared to wt mice. Morover IGF-1 mice showed a reduced gene expression of the gamma subunit of the acetylcholine receptor, associated to denervation events. IGF1 isoforms induced a modulation of the expression of markers involved in the regulation of atrophic pathways: in particular a down-regulation of myogenin, an induction of PGC-1α, finally an up-regulation of miR-486, which is able to antagonize the atrophy phenomena mediated by FoxO1.[3]

1. *Kamel HK. Sarcopenia and aging. Nutr Rev 2003;61:157-67.*
2. *Rolland Y, et al. Sarcopenia: its assessment, etiology, pathogenesis, consequences and future perspectives. J Nutr Health Aging 2008;12:433-50.*
3. *Hitachi K, et al. Myostatin signaling regulates Akt activity via the regulation of miR-486 expression. Int J Biochem Cell Biol 2014;47:93–103.. doi: 10.1016/j.biocel.2013.12.003. Epub 2013 Dec 14.*

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Looking at the role of disordered E-tails of IGF-1

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Intrinsically disordered proteins (IDPs) encompass signalling and regulatory functions and altered expression of IDPs is associated with many diseases and imbalance in signalling pathways, transcriptional regulation, and splicing. Interest in insulin-like growth factor (IGF) – 1 isoforms on muscle homeostasis, regeneration, differentiation, and diseases has increased significantly. Inclusion or exclusion of exon 5 into the IGF-1 mRNA gives rise to three transcripts, IGF-1Ea, IGF-1Eb and IGF-1Ec, which yield three different C-terminal extensions called Ea, Eb and Ec peptides. Protein-coding sequences of exon 5 showed low rate of synonymous mutations and contain disorder-promoting amino acids, suggesting a regulatory role for these domains (Annibalini et al. 2016). To setup the analysis, the supernatants of HEK293 cells transfected with the specific IGF-1 isoform constructs as described in (De Santi et al. 2016) were studied by limited proteolysis combined with mass spectrometry (MS) using a Q-TOF microTM MS/MS (Micromass, Manchester, UK). Preliminary data showed that the C-terminal region of IGF-1Ea has lower resistance to trypsin digestion compared to the mature IGF-1 demonstrating IDRs in the Ea peptide. MS analyses to the detection of IGF-1E isoforms allowed us to identify both mature IGF-1 and IGF-1Ea isoform in transfected HEK293 cell culture supernatant. Analytical methods to correctly detect and quantify the IGF-1 isoforms are not currently available. In fact, the current existing methods rely on the use of antibodies that primarily recognize the mature IGF-1 peptide, thereby underestimate the isoforms. This finding could provide evidence allowing the detection and identification of the "E-tails" of IGF-1 and targeting these regulatory elements may represent a new strategy to control IGF-1 bioavailability in physio-pathological conditions.

1. *Annibalini G, et al. MIR retroposon exonization promotes evolutionary variability and generates species-specific expression of IGF-1 splice variants BBAGRM 2016;1859:757-68.*
2. *De Santi M, et al. Cell Oncol (Dordr) 2016;39:149-59.*

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The novel DMD experimental model, *mdx/Ager*$^{-/-}$ mouse reveals a role of RAGE in inflammatory processes in dystrophic muscles

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Duchenne muscular dystrophy (DMD) is a lethal X-linked disease characterized by progressive muscle degeneration and chronic inflammation. RAGE (Receptor for Advanced Glycation End-products) is a multiligand receptor of immunoglobulin superfamily involved in inflammation and myogenesis.[1] RAGE is absent in healthy adult muscle tissue but it is expressed in regenerating myofibers, dystrophic muscles and activated immune cells.[2,3] The double mutant *mdx*/*Ager*$^{-/-}$ mice, lacking dystrophin and RAGE, show significantly reduced numbers of necrotic myofibers, a shift towards higher values of cross-sectional areas (CSA) of regenerating myofibers, and reduced recruitment of activated macrophages (MΦ) in muscle tissue, compared with *mdx* mice. Interestingly, the MΦ (F4/80+/CD11b+) population found in muscles of 5 week-old *mdx/Ager*$^{-/-}$ mice was mostly composed by anti-inflammatory M2a (CD163-/Cd206+) and regenerative M2c (CD163+/Cd206+) MΦ, and showed dramatic reduction in M1 pro-inflammatory MΦ in comparison with age-matched *mdx* mice, as assessed by FACS analysis. Moreover, peritoneal MΦ from *Ager*$^{-/-}$ mice express lower levels of TNFα, IFNγ, IL-6, IL-12a and IL-12b compared with WT mice, when stimulated with the pro-inflammatory factors, LPS/IFNγ in vitro. Our results suggest that RAGE expressed on MΦ has a major role in sustaining the inflammatory process in dystrophic muscles. Thus, the inhibition of RAGE expression/activity in muscles of DMD patients might represent a therapeutic tool to reduce inflammation and rescue muscle morphology. *Contributed equally to this work .

1. *Sorci G, et al. Amphoterin stimulates myogenesis and counteracts the antimyogenic factors basic fibroblast growth factor and S100B via RAGE binding. Mol Cell Biol 2004;24:4880.*
2. *Riuzzi F, et al. HMGB1–RAGE regulates muscle satellite cell homeostasis through p38-MAPK- and myogenin-*

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dependent repression of Pax7 transcription. J Cell Sci 2012;125:1440-54.
3. *Haslbeck KM, et al. The RAGE pathway in inflammatory myopathies and limb girdle muscular dystrophy. Acta Neuropathol 2005;110:247-254.*

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A MICU1 splice variant confers high sensitivity to the Ca2+ uptake machinery of skeletal muscle

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Skeletal muscle is a dynamic organ, characterized by an incredible ability to rapidly increase its rate of energy consumption to sustain activity. The control of oxidative phosphorylation by Ca^{2+} is particularly crucial in skeletal muscle, being the latter one of the most ATP consuming organs of the body. We found that skeletal muscle mitochondria express a unique MCU complex, the channel responsible for Ca2+ entry into mitochondria, containing an alternative splice isoform of the positive regulator of MCU, MICU1, that we named MICU1.1, characterized by the addition of a micro-exon that is sufficient to greatly modify the properties of the MCU. Indeed, MICU1.1 binds calcium one order magnitude more efficiently than MICU1 and, when heterodimerized with the gatekeeper of the channel, MICU2, activates MCU current at lower calcium concentrations, resulting in a huge entry of Ca^{2+} into mitochondria. In skeletal muscle, MICU1.1 is required for sustained mitochondrial Ca^{2+} uptake and ATP production. These results highlight a novel mechanism of the molecular plasticity of the MCU Ca^{2+} uptake machinery that allows skeletal muscle mitochondria to be highly responsive to sarcoplasmic [Ca^{2+}] responses.

1. *Vecellio Reane D, et al. A MICU1 Splice Variant Confers High Sensitivity to the Mitochondrial Ca^{2+} Uptake Machinery of Skeletal Muscle. Mol Cell 2016;64:760-73.*
2. *Patron M, et al. MICU1 and MICU2 finely tune the mitochondrial Ca^{2+} uniporter by exerting opposite effects on MCU activity. Mol Cell 2014;53:726-37.*
3. *De Stefani D, et al. A 40 kDa protein of the inner membrane is the mitochondrial calcium uniporter. Nature 2011;476(7360):336–40.*

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Manipulating the muscle environment to improve the muscle repair and the outcome of cell mediated therapies in muscular dystrophy

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DMD is a genetic disease caused by lack of dystrophin and characterized by muscle wasting, chronic inflammation and progressive decrease of muscle regeneration capacity. The ability of satellite cells (MuSCs) to repair the injured tissue declines with age in the mdx mice, the mouse model of DMD. The continuous cycles of degeneration and regeneration and the hostile microenvironment may affect the MuSCs function and exhaust their regenerative capacity. We previously showed that lack of PKCθ in mdx mice improves muscle maintenance, regeneration and performance, preventing massive inflammation and muscle wasting. Indeed, PKCΘ is highly expressed in both immune cells and skeletal muscle. PKCΘ plays a unique role in T cell activation, and represents an attractive molecular target for the treatment of immune disorders. We show here that the lack of PKCΘ in mdx modifies the environment in order to preserve regenerative ability of MuSCs during the different ages. Moreover, lack of PKCΘ in mdx improves survival and the ability of transplanted stem cells to generate new muscle fibers and correct the genetic defect of the recipient. The muscle environment is composed of extracellular matrix (ECM) and local cell populations. Among them, it was shown that the fibroadipogenic progenitors (FAPs), a muscle interstitial cells, contribute to muscle regeneration but also to fibroadipogenic degeneration generating myofibroblasts and adipose cells. The characterization of FAPs activity isolated from mdxΘ -/- muscle suggests that the improved muscle regeneration observed depends, at least in part, to improved FAPs activity. Indeed, the reduced inflammatory environment in mdxΘ -/- prevents FAPs conversion into fibro-adipocytes and increases their pro-regenerative activity. These results may contribute to the identification of new targets for cell therapies and intervention aiming to shift the balance between muscle regeneration and fibroadipogenic degeneration in DMD.

1. *Madaro L, et al, PKC theta ablation improves healing in a mouse model of muscular dystrophy. Mol Biol Cell 2011;22:1409-19.*
2. *Brezar V, et al. PKC-Theta in Regulatory and Effector T-cell Functions. Front Immunol 2015 Oct 13;6:530.*
3. *Joe AW, et al. Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. Nat Cell Biol 2010;12:153-63.*

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Skeletal muscle interstitial cells are modulated during aging

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Sarcopenia is the age-related loss of muscle mass, strength and function. Although satellite cells are the critical stem cells in regenerative myogenesis, little is known about the contribution of muscle interstitial cells (MICs) to skeletal

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muscle degeneration and fibrotic deposition, hallmarks of aging. In order to elucidate the role of MICs, we isolated the non-satellite fraction (CD56-) from human muscle biopsies of young and elderly subjects. Firstly, we assessed the proliferation rate of these cells and we found that the elderly CD56- subpopulation shows a reduction in the proliferative potential and undergoes senescence earlier than the young counterpart. Then, we identified the CD56-/ALP+ cells as the most abundant population inside our CD56- cell fraction. Interestingly, elderly CD56-/ALP+ express pericyte markers and display a dramatic impairment in the myogenic differentiation capability. In addition, we looked for the presence of putative adipogenic stem cell subsets in CD56- cell fraction. Using flow cytometry we isolated CD56-/CD15+ cells and observed that the number of those cells is significantly higher in the old samples compared to the young ones. This also correlates with an up-regulation of PDGFRα gene expression and with a consequent increase of PDGFRα+ cells in the CD56-/CD15+ cell population. Taken together our results suggest that the CD56- cell fraction is modulated in skeletal muscles during aging, possibly involved in the deposition of fibroadipogenic tissue and could represent a feasible target for future treatments aimed to reduce the fibrotic tissue present in the aged muscle.

1. *Pannérec A, Formicola L, Besson V, Marazzi G, Sassoon DA. Development. 2013;140:2879-91.*
2. *Di Filippo ES, Mancinelli R, Pietrangelo T, La Rovere RM, Quattrocelli M, Sampaolesi M, Fulle S. Biochem Biophys Res Commun 2016;473:462-70.*

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