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Venetian Institute of Molecular Medicine

16th Annual Retreat

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Dear Friends,

Welcome to the 17th Annual VIMM Retreat. I would like to use this occasion to thank and the President of the Advanced Biomedical Research Foundation Prof. Pagano and Vice-President Mrs Mistrello-Destro for the continuous support to the Institute and to our activities.

I am very glad to announce that this year we continue our tradition of distinguished guests with Prof. Dr. Ernst Bamberg, Director, Max Planck Institute of Biophysics, Gottingen, who will talk about how optogenetics became an essential tool, not only in neuroscience; and Dr. Rosalind Mott, Scientific Editor at Cell Metabolism, Boston, who will describe the publishing process at Cell Metabolism and tell us about her career as a Scientific Editor. I am particularly pleased to host them during our retreat

Like in the past years, three prizes generously funded by Mrs Manzin to honour the memory of her husband and father will be awarded to recognize the presentations of three PhD students or postdoctoral fellows working at our Institute. Along this line, your young investigators are asked to increase your participation by chairing the sessions and by actively discuss the data presented by your peers.

I am sure that the meeting will keep the high scientific level of the past and I wish you two fruitful days of scientific interaction!

Luca Scorrano

VIMM Scientific Director

March 2	
8.30	leave from VIMM - 9.15 arrival and check in
9.15-10.00	coffee break
10.00-12.05	**Metabolism: from basics to disease. Chair Lena Pernas**
10.00-10.25	**Martina Semenzato** (Group Scorrano) – *OPA1 oxidation is a crucial component of oxidative stress-mediated cellular damage.*
10.25-10.50	**Francesca Grisan** (Group Lefkimmiatis) – *PKA and EPAC: a crosstalk regulating migration?*
10.50-11.15	**Jingjing Chen** (Group Alimonti) – *Compartmentalized activities of the pyruvate dehydrogenase complex sustain lipogenesis in prostate cancer*
11.15-11.40	**Serena Tedesco** (Group Fadini) – *PPARγ reprograms bone marrow macrophages and counters diabetic stem cell mobilopathy*
11.40-12.05	**Federico Fabris** (Group Alberti/Realdon) – *Role of Insulin Resistance and Resident Microbiota in the Pathogenesis of Esophageal Adenocarcinoma*
12.05-13.30	Poster Session 1
13.30-14.30	lunch
14.30-15.45	**Signals in immune system function and malignancies. Chair Sara Zumerle**
14.30-14.55	**Andrielly Agnellini** (Group Molon) – *Cytokine nitration boosts myeloid suppressor cell commitment and functions in tumor bearing hosts*
14.55-15.20	**Giulia Calabretto** (Group Semenzato) – *Identification of a miR-146b-FasL axis in the development of neutropenia in T-LGL leukemia*
15.20-15.45	**Marilena Carrino** (Group Piazza) – *Protein kinase CK1α modulates prosurvival autophagy in Multiple Myeloma.*
15.45-16.40	**Biotechnology for molecular medicine. Chair Onelia Gagliano**
15.45-16.15	**Eleonora Dal Sasso** (Group Gerosa) – *Decellularized pericardial tissues as novel scaffolds for cardiovascular regenerative medicine*
16.15-16.40	**Anna Urciuolo** (Group Elvassore) – *Biomaterials: towards in vitro modelling and in vivo application*.
16.40-17.00	coffee break
17.00-18.30	**Plenary Invited Lectures**
17.00-17.45	**Prof. Dr. Ernst Bamberg, Director, Max Planck Institute of Biophysics, Gottingen (DE)** – *Rhodopsin-based Optogenetics: Basics, Applications, Chances*
17.45-18.30	**Dr. Rosalind Mott, Scientific Editor, Cell Metabolism, Boston (USA)** – *The World of Scientific Publishing*
18.30-18.45	**Sponsored talk.**
18.30-18.45	**Tecniplast** *Equipment technologies: From the static cage to the IVC cage (going) through the history of the Equipment to achieve the best Bio-Protection of Animals, Personnel and Environment*
18.45-20.45	Poster Session 2
21.00-	Dinner and music

March 3

10.00-11.15	**From molecular to systems Neuroscience Chair Andrea Maset**
10.00-10.25	**Saima Imran** (Group Bortolozzi) – *Development of an iPSC-based model for the study of peripheral neuropathies*
10.25-10.50	**Simona Francia** (Group Lodovichi) – *Odorant receptors at the axon termini act as axon guidance molecules activated by cues elaborated in the olfactory bulb.*
10.50-11.15	**Marta Bisio** (Group Corbetta) – *Wavelet analysis of resting state and evoked local field potentials in rodent barrel cortex.*
11.15-11.30	coffee break
11.30-12.45	**Molecular mechanisms of muscle pathophysiology. Chair Roberta Sartori**
11.30-11.55	**Martina Baraldo** (Group Blaauw) – *The role of Raptor in adult skeletal muscle*
11.55-12.20	**Anais Franco Romero** (Group Sandri) – *Identification of a novel FoxO-dependent regulator of muscle mass*
12.20-12.45	**Valentina Prando** (Group Zaglia) – *Circulating muscle-derived MiR-206 links skeletal muscle dysfunction-to-heart autonomic denervation*
12.45-13.45	**lunch**
14.00	**departure**

Lecture Abstracts

Optogenetics: basics, applications and chances.

E.Bamberg, T. Moser, V. Gordeliy

Department of Biophysical Chemistry, Max-Planck -Institute of Biophysics, Frankfurt/GER

Microbial Rhodopsins are widely used in these days as optogenetic tools in neuro and cell biology. We were able to show that rhodopsins from the unicellar alga *Chlamydomonas reinhardtii* with the 7 transmembrane helix motif act as light-gated ion channels, which we named channelrhodopsins(ChR1,ChR2). Together with the light driven Cl^- pump Halorhodopsin ChR2 is used for the non-invasive manipulation of excitable cells and living animals by light with high temporal resolution and more important with extremely high spatial resolution The basic functional mechanism and structural description of this unusual class of ion channels is given (electrophysiology, noise analysis ,flash photolysis and 2D crystallography and a high resolution structure of the WT and a mutant). New tools and their application with a biomedical perspective in the cochlea and the restoration of vision are presented .

The World of Scientific Publishing.

Rosalind Mott, PhD

Cell Press, Cambridge, MA

Scientific papers are the currency of biomedical research and are essential for the dissemination of new information. But what constitutes ‘a paper’ and how do scientists choose the right venue for publication? In this introduction to the World of Scientific Publication, I will discuss the seemingly boundless range of journals and publishers available to scientists and will present strategies to help you reach the right audience with your research. Other points of discussion will include the role of the editor, the peer review process, the path to publication and the evolving landscape of publishing in the digital era. In addition, I hope to give you a glimpse of my experience as an editor at Cell Press, detailing the pursuit of the career path and elaborating on how the scientific editor fits into the ecosystem of biomedical research.

Presentation Abstracts

Cytokine nitration boosts myeloid suppressor cell commitment and functions in tumor bearing hosts.

Andrielly H.R. Agnellini1,2, Andrea Predonzani1, , Bianca Cali 1,2, Giulia Ilaria Toffolo1 , Ilaria Marigo 1,2,3 and Barbara Molon1,2

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3 Veneto Institute of Oncology IOV—IRCCS, Padova, Italy.

Tumors are crowded lesions where cancer cells and host cells dynamically interplay by intricate cellular and molecular interactions. Among these, defined immune cell subsets significantly influence cancer fate by their active recruitment within locoregional tumor environment. Despite the relative profusion of each cell type, the predominant cytokine and chemokine milieu within the tumor microenvironment importantly tips the balance in favor of either anti-tumor immunity or tumor-induced immunosuppression. Moreover, Reactive Nitrogen Species (RNS) produced detrimentally influence homeostatic properties of several proteins at post-translational level. Indeed, the post-translation modifications (PTM) of proteins represent an important additional level of functional regulation that must be deeply investigated in cancer and other inflammatory diseases. Our data indicated that RNS impact on the molecular dynamics and functions of granulocyte-macrophage colony-stimulating factor (GM-CSF), which is an important regulator of inflammation chronic inflammatory diseases and cancer.

The role of Raptor in skeletal muscle mass.

Martina Baraldo1, Marco Sandri1,2, Bert Blaauw1,2

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2 Department of Biomedical Sciences, University of Padova, Padova

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. A longer deletion of Raptor, however, leads to a myopathic phenotype with central-core structures and a high number of small and large muscle fibers.

Wavelet analysis of resting state and evoked local field potentials in rodent barrel cortex.

Marta Bisio1,2,3, Alessandra Bertoldo1,3,4, Claudia Cecchetto5, Mufti Mahmud5, Stefano Vassanelli3,5 and Maurizio Corbetta1,2,3

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5 Department of Biomedical Sciences, Padova, Italy

The rodent barrel cortex is a widely used model of information processing in the somatosensory area, thanks to its precise and easily recognizable organization.
Indeed, the tactile somatosensory pathway from whisker to cortex provides a well-defined system for exploring the link between molecular mechanisms, synaptic circuits, and behavior.
The aim of this work was to analyze the frequency content of Local Field Potentials (LFPs) spontaneously arising under anesthesia both in absence of external stimuli and under mechanical whisker stimulation, in order to investigate which are the main features in the two studied experimental conditions.
The results reveal the existence of complex connectivity patterns at low frequency corresponding to the relevant signals' content, and the presence of a static pattern at higher frequencies. Furthermore, the immediate stimulus' effect is to synchronize the system which returns to the resting state after a few seconds.

Identification of a miR-146b-FasL axis in the development of neutropenia in T-LGL leukemia.

Giulia Calabretto1,2, Antonella Teramo1,2, Cristina Vicenzetto1,2, Vanessa Rebecca Gasparini1,2, Gregorio Barilà1,2, Matteo Leoncin1,2, Barbara Mariotti3, Marzia Rossato3, Monica Castellucci3, Flavia Bazzoni3, Monica Facco1,2, Renato Zambello1,2 and Gianpietro Semenzato1,2

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T Large Granular Lymphocytes (T-LGLs) leukemia is a chronic lymphoproliferation of clonal T-LGLs. Leukemic T-LGLs are characterized by a constitutively activated STAT3 pathway, that regulates FasL transcription, which is a molecule involved in neutropenia development. We aimed to investigate whether STAT3 could play its pathogenetic role through an altered expression of microRNAs. Therefore, we assessed the expression of 756 mature miRNAs on purified T-LGLs, identifying two clusters of patients: one characterized by neutropenia and higher STAT3 activation, another one characterized by normal absolute neutrophil count (ANC) and lower STAT3 activation. Remarkably, miR-146b, found down-regulated in patients with higher STAT3 activation, was the only one miRNA that correlated with ANC. Therefore, we investigated its role in neutropenia development, demonstrating that miR-146b downregulation increased HuR expression, which is a miR-146b target and a known mRNA stabilizer. HuR-mediated stabilization of FasL mRNA lead to increased FasL production and, consequently, to neutropenia development.

Protein kinase CK1α modulates prosurvival autophagy in Multiple Myeloma.

Marilena Carrino1,2, Sabrina Manni1,2, Laura Quotti Tubi1,2, Sara Canovas Nunes1,2, Ketty Gianesin1,2, Anna Fregnani1,2, Elena Daniele1, and Francesco Piazza1,2.

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2 Department of Medicine (DIMED), Hematology Branch, University of Padova, Italy

Multiple Myeloma (MM) is an incurable cancer of plasma cells. The introduction of novel drugs, such as Bortezomib and Lenalidomide, has significantly improved the prognosis of patients; still, new therapeutic strategies are urgently needed. Several survival signaling pathways, including the ones related to autophagy, are regulated by CK1α, a kinase whose inactivation leads to MM cells apoptosis. In MM, the deregulation of autophagy is maladaptive; therefore, we studied the role of CK1α in autophagy in MM.
We found that chemical inhibition of CK1α induced apoptosis, cooperated with anti-MM drugs and increased FOXO3a dependent autophagic genes transcription; however, an impairment of the autophagic flux was observed in this condition. CK1α silencing still culminated in apoptosis, but the autophagic flux resulted correctly completed, even if autophagic genes seemed not to be altered.
Our results suggest that CK1α supports MM cells survival through the modulation of the prosurvival autophagic pathway.

Compartmentalized activities of the pyruvate dehydrogenase complex sustain lipogenesis in prostate cancer.

Jingjing Chen1,3, Ilaria Guccini1, Diletta Di Mitri1, Daniela Brina1, Ajinkya Revandkar1,3, Manuela Sarti1, Emiliano Pasquini1, Abdullah Alajati1, Sandra Pinton1, Marco Losa1, Gianluca Civenni1, Carlo V. Catapano1, Jacopo Sgrignani4, Andrea Cavalli4, Rocco D'Antuono5, John M. Asara6, Andrea Morandi7, Paola Chiarugi7, Sara Crotti8, Marco Agostini8,9, Monica Montopoli10, Ionica Masgras11, Andrea Rasola11, Ramon Garcia-Escudero12,13,14, Nicolas Delaleu15, Andrea Rinaldi1, Francesco Bertoni1, Johann de Bono16, Arkaitz Carracedo14,17,18,19 & Andrea Alimonti1,2,3

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6 Division of Signal Transduction, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, USA
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8 Nano-inspired Biomedicine Lab, Institute of Paediatric Research - Città della Speranza, Padova, Italy.
9 Surgical Clinic, Department of Surgical, Oncological and Gastroenterological Sciences, University of Padova, Padova, Italy.
10 Department Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Italy
11 CNR Institute of Neuroscience and Department of Biomedical Sciences, University of Padova, Padova, Italy
12 Molecular Oncology Unit, Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas, Madrid, Spain
13 Biomedical Research Institute I+12, University Hospital 12 de Octubre, Madrid, Spain
14 Centro de Investigación Biomédica en Red de Cáncer, Spain
15 Broegelmann Research Laboratory, Department of Clinical Science, University of Bergen, Bergen, Norway
16 Drug Development Unit, Division of Cancer Therapeutics and Division of Clinical Studies, The Royal Marsden NHS Foundation Trust and The Institute of Cancer Research, United Kingdom
17 CIC bioGUNE, Bizkaia Technology Park, Bizkaia, Spain
18 Ikerbasque, Basque Foundation for Science, Bilbao, Spain
19 Biochemistry and Molecular Biology Department, University of the Basque Country, Bilbao, Spain.

The mechanisms by which mitochondrial metabolism supports cancer anabolism are still unclear. Here, we unexpectedly find that genetic and pharmacological inactivation of Pyruvate Dehydrogenase A1 (PDHA1), a subunit of pyruvate dehydrogenase complex (PDC) inhibits prostate cancer development in different mouse and human xenograft tumour models by affecting lipid biosynthesis. Mechanistically, we show that in prostate cancer, PDC localizes in both mitochondria

and nucleus. While nuclear PDC controls the expression of Sterol regulatory element-binding transcription factor (SREBF) target genes by mediating histone acetylation, mitochondrial PDC provides cytosolic citrate for lipid synthesis in a coordinated effort to sustain anabolism. In line with these evidence, we find that PDHA1 and the PDC activator, Pyruvate dehydrogenase phosphatase 1 (PDP1), are frequently amplified and overexpressed at both gene and protein level in prostate tumours. Taken together, these findings demonstrate that both mitochondrial and nuclear PDC sustain prostate tumourigenesis by controlling lipid biosynthesis thereby pointing at this complex as a novel target for cancer therapy.

Decellularized pericardial tissues as novel scaffolds for cardiovascular regenerative medicine.

Eleonora Dal Sasso+,1, Sabra Zouhair+,1, Sugat Ratna Tuladhar+,1, Paola Aguiari1, Cátia Fidalgo1, Andrea Filippi2,3,4,5, Giulia Borile2,4,5, Andrea Bagno6, Sotiris Korossis7, Willem F. Wolkers8, Filippo Romanato2,4,5,9, Gino Gerosa*,1, Laura Iop*,1
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Xenogenic pericardia-based substitutes are used in cardiac surgery after chemical shielding, hampering their biocompatibility and long-lasting therapeutic effect. Adverse responses to these replacements might be prevented by tissue decellularization, ideally removing cells and preserving the original extracellular matrix (ECM).
Bovine and porcine pericardia were submitted to TRICOL decellularization (osmotic shock, detergents, and endonucleases). TRICOL resulted effective in cell removal, included DNA, as demonstrated histologically and biochemically. ECM architecture was preserved, as verified by immunofluorescence, scanning electron, and two-photon microscopy. Biochemical assessment revealed retention of intact collagen and elastin but glycosaminoglycan reduction. Unaltered protein secondary structure and thermal denaturation profile were confirmed by spectrometry and scanning calorimetry. Mechanical properties remained unvaried. ECM bioactivity was unaffected, sustaining the viability and proliferation of human mesenchymal stem cells and umbilical vein endothelial cells.
In conclusion, TRICOL decellularization preserves the ECM properties of bovine and porcine pericardia, rendering available more biocompatible scaffolds for clinical application.

Role of Insulin Resistance and Resident Microbiota in the Pathogenesis of Esophageal Adenocarcinoma.

Federico Fabris1, Diletta Arcidiacono1,2, Alice Zaramella1, Daniele Nucci1,2, Alfredo Alberti1,3, Stefano Realdon1,2

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Esophageal adenocarcinoma (EAC) represents the final step of a long-standing pathological process: gastroesophageal-reflux-disease (GERD)/Barrett's Esophagus (BE)/dysplasia. EAC incidence has dramatically increased in the last three decades and it seems to be related to obesity, insulin-resistance and type-2 diabetes. Obesity is also linked to chronic inflammation and intestinal dysbiosis.
The aim of this study was to evaluate the role of metabolic state and esophageal microbiota in EAC onset and progression.
Insulin and c-peptide levels were higher in EAC patients. High insulin-levels were linked to a higher activation of insulin-signalling mediated by IGF1R activation.
Streptococcus/Prevotella ratio results, used to define the severity of esophageal dysbios (ED), showed a progressive reduction from Barrett's esophagus to Non-Invasive-Neoplasia and Esophageal Adenocarcinoma [median value (IQ range): 2.7(1.1; 5.1); 1.3 (0.6; 2.0); 0.8 (0.5, 1.6), respectively].
From our data, worsening of ED and hyperinsulinemia may play a role in the transformation from BE to EAC.

Odorant receptors at the axon termini act as axon guidance molecules activated by cues elaborated in the olfactory bulb.

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3 Department of Biomedical Sciences, University of Padua;
4 Department of Visual Neuroscience, Sissa, Trieste;
5 Armenise Harvard CDA

It is known for more than 20 years that odorant receptors (OR) not only detect odors but also determine the convergence of sensory neurons to form glomeruli in specific locations in the olfactory bulb, giving rise to the sensory map. The expression of the OR at the axon terminal corroborates this role.
In previous work, we found that the OR at the axon terminal is coupled to local increase of cAMP and Ca2+. To identify the mechanism of activation of the axonal OR, we screened cues elaborated in the olfactory bulb by performing Ca2+ imaging on sensory neurons and HEK293T cells, expressing specific OR. We identified a ligand that is able to activate the axonal OR and to modulate axon turning behaviour.
Mice carrying a null mutation for the ligand, exhibit a deeply perturbed sensory map.

Identification of a novel FoxO-dependent regulator of muscle mass.

Anaïs Franco-Romero 1,2, Giulia Milan3, Vanina Romanello 1,2, Roberta Sartori2, Marco Sandri1,2

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2 Venetian Institute of Molecular Medicine, Padova, Italy
3 Biozentrum, University of Basel, Basel, Switzerland

An exacerbated activation of FoxO-family members leads to increased protein breakdown and muscle wasting. This occurs in diseases such as cancer-cachexia, AIDS, or denervation. FoxOs are required for the induction of several atrophy-related genes(atrogenes). However, the activation of the already identified atrogenes cannot sustain all the protein breakdown during atrophy.
We identified several new FoxOs-dependent genes by microarray analysis, here called RIKENs, whose functions were until now unrevealed. We showed that in particular RIKEN1 is up-regulated in fasting, disuse and cancer cachexia. Colocalization experiments showed a Riken1GFP-LC3cherry interaction in the autophagosomes suggesting a potential role in the autophagy-lysosome pathway. Importantly, Knocking-down RIKEN1 protected from fasting-induced atrophy and decreased LC3 puncta in FDB isolated fibers suggesting that RIKEN1 may have a role in the formation of autophagosomes.
Our findings contribute to the discovery of a novel Foxo-dependent mediator of muscle mass in order to develop new therapeutic approaches against muscle wasting.

PKA and EPAC: a crosstalk regulating metastatic migration?

Francesca Grisan1,2, Giulietta Di Benedetto1,2, Elisa Penna3, and Konstantinos Lefkimmiatis1,2

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The effects of cAMP on motility are debated, in fact, this messenger can both promote and inhibit cell movement. Two cAMP effectors, Protein Kinase A (PKA) and Exchange Protein Activated by cAMP (EPAC) are involved in cell motility, however their individual and combined effects in migration are not well established. We used the human colon adenocarcinoma cell line HT29 to test how PKA and EPAC regulate cell motility.

In wound-healing assays, inhibition of PKA drastically accelerated migration whilst inhibition of EPAC blocked it, suggesting a functional crosstalk between the two proteins. To investigate the signalling underlying this effect we used single cell imaging (FRET-based PKA sensors) and found that PKA-dependent phosphorylation is low in the cytosol and high at the endoplasmic reticulum and outer mitochondrial membrane.
Interestingly, cAMP increases lead to EPAC translocation to the mitochondria opening the possibility that coordinated PKA and EPAC signals regulate migration in HT29 cells.

Development of an iPSC-based model for the study of peripheral neuropathies.

Saima Imran1,2, Giulia Crispino1,2, Angela Pellizzon1, Alessandro Leparulo3, Peter Andrews4, and Mario Bortolozzi1,2

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3 Department of Biomedical Sciences, University of Padua, Italy
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Human induced pluripotent stem cell (hiPSC)-derived neurons have opened new opportunities to understand the development of several neurologic diseases, such as Charcot-Marie-Tooth disease (CMT), a degenerative and incurable peripheral neuropathy that may severely impair normal life activities. Our lab aims to investigate CMT caused by mutations of connexin 32 (Cx32) channels in several models, including a stem cell-derived system that mimics the peripheral nervous system. After obtaining successful differentiation of hiPSCs into motor neurons firing action potentials and Schwann cells expressing typical glial markers, we optimized the co-culture conditions to stimulate axonal myelination. Schwann cell elongation on neurites was observed as a first step of the myelination process. If successful, the co-culture model can be an important tool to advance research and personalized medicine of CMT and other human neurologic diseases.

Circulating muscle-derived MiR-206 links skeletal muscle dysfunction-to-heart autonomic denervation.

Valentina Prando1,2,*, Giulia Favaro1,2,*, Francesca Lo Verso1,*, Silvia Bertoli1,2, Vittoria di Mauro3, Michele Guescini4, Mattia Albiero1,5, Paola Pesce6, Daniele Catalucci3, Marco Mongillo1,2,7, Marco Sandri1,2,§ and Tania Zaglia1,2,8,§

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**, § = equal contribution*

Muscle-specific ablation of the autophagy-related protein, Atg7, leads to block of autophagy, sarcopenia and neuromuscular junction destabilization. Atg7 KO muscle fibers release, into the bloodstream, exosomes containing miR-206, which causes muscle denervation and, interestingly, can be detected in the myocardium.
Here, we aim to define the effects of miR-206 in heart homeostasis and determine its role in 'skeletal muscle-to-heart' communication.
Our data demonstrate that circulating exosomes containing miR-206 are taken up by the heart, leading to sympathetic dys-innervation, accompanied to increased arrhythmogenesis. In vitro assays demonstrate that exosomes-carried miR-206 targets both CMs and SNs, compromising cellular structure and function. In SNs, miR-206 leads to cell atrophy, irregular axonal distribution of the active neurotransmitter release sites, as well as reduction in axonal sprouting. These effects are likely attributed to miR-206-mediated downregulation of the NGF receptor, p75, as demonstrated by bioinformatics, luciferase assays, molecular and biochemical analyses in vitro and ex vivo.

OPA1 oxidation is a crucial component of oxidative stress-mediated cellular damage.

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5 Molecular Modeling Section, Dep. of Pharmaceutical Sciences, University of Padova, Padova, Italy
6 Dept. of Biomedical Sciences, University of Padova, Padova, Italy

Cardiac ischemia reperfusion injury (I/R) is mediated by several factors including production of reactive oxygen species (ROS), particularly during reperfusion. ROS act by causing mitochondrial dysfunction, but the exact molecular targets of ROS at the mitochondrial level are unknown. Here we report that during oxidative stress the mitochondria cristae-shaping protein Opa1 is inactivated by ROS accumulation, leading to cell death. Oxidative proteomics of hearts subjected to ischemia-reperfusion revealed a specific oxidation in the C-terminal domain of Opa1. In vitro, oxidative stress conditions recapitulate Opa1 oxidation at specific cysteine residues, leading to its oligomerization in inactive, high molecular weight complex. Genetics proved that inhibition of Opa1 oxidation ameliorates cristae structure and improves cell survival, independently from mitochondrial fusion. Our findings define a thiol-dependent process impacting on mitochondrial dynamics to hamper ROS mediated cell death.

PPARγ reprograms bone marrow macrophages and counters diabetic stem cell mobilopathy.

Serena Tedesco1, Elisa Pegoraro1, Mattia Albiero1, Gian Paolo Fadini1,2

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2 Department of Medicine, University of Padova, Padova, Italy

Diabetes reduces circulating stem cells (SCs) by affecting bone marrow (BM) structure and function in mice and humans. In turn, SCs pauperization leads to diabetic multiorgan pathology. An excess of pro-inflammatory BM-macrophages that secrete Oncostatin M (OSM) contributes to the impaired mobilization of SCs in diabetes, a condition termed diabetic SCs mobilopathy (1). Diabetic patients treated with a PPARγ agonist pioglitazone displayed a recovery of normal SCs mobilization. We hypothesized this is mediated by the ability of PPARγ to regulate macrophage activation. In vitro, pioglitazone modulated macrophage polarization and abated OSM expression in human and murine M1 macrophages. In vivo, administration of pioglitazone suppressed BM macrophages and OSM expression in models of type 1 and type 2 diabetes. In T1D mice, which display the most profound defect in SCs mobilization, treatment with pioglitazone rescued SCs mobilization. These data illustrate a role for PPARγ in regulating SCs kinetics and a therapeutic target to counter SCs defects in patients with diabetes.

References:
1. Albiero M, Poncina N, Ciciliot S, et al. Diabetes 2015;64(8):2957-68.

Biomaterials toward in vitro modelling and in vivo application.

Anna Urciuolo1,3, Ilaria Poli2, Mariasole Del Vecchio3, Nicola Elvassore1,2,3

1 Venetian Institute of Molecular Medicine, Padova, Italy
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The extracellular matrix represents the biologic scaffold that allow somatic and stem cells to generate tissues and finally multicellular organisms. Natural and synthetic materials have been extensively used to mimic such essential cue to develop artificial tissues, both in vitro and in vivo. Among the different approaches, bioprinting represents one of the most promising technology to reach this aim. By taking advantage from a cell-friendly photo-chemistry, we developed new materials that allow i) shape-defined surface functionalization for cell patterning culture or ii) polymerization of tunable hydrogels for 3D culture. Photo-chemistry was achieved by using not cytotoxic wavelengths with micrometric definition thanks to the implementation of two-photon microscopy. Hydrogels could also be polymerized in presence of cells, leading to 3D culture systems able to be remodelled by seeded cells. Moreover, the proof of concept of in vivo application of such chemistry was also demonstrated in alive murine animal models.

Poster Abstracts

Role of autophagy in the diabetic stem cell mobilopathy.

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Diabetic stem cell mobilopathy is a defect of hematopoietic stem cell (HSC) mobilisation from the bone marrow to peripheral blood that affect diabetic patients. Since mobilization of HSCs requires activation of autophagy and autophagy has been shown to be affected in several diabetic tissues, we hypothesized that impaired autophagy contributes to HSC mobilopathy in diabetes. Using GFP-LC3 transgenic mice as a tool to study autophagy, we found that the mobilizing cytokine G-CSF activates autophagy in murine Lin-Sca1+cKit+ (LKS) hematopoietic progenitor cells, an that an impairment in such activation parallels mobilization defects in a model of type 1 diabetes. Oral supplementation with the natural polyamine spermidine, a known autophagy activator, restored normal mobilization in diabetic animals. Ongoing studies will investigate the mechanism that hamper autophagy in diabetes HSCs. These preliminary data pinpoint a role for deregulated autophagy in diabetic stem cells mobilopathy and opens the way to new therapeutic opportunities.

Extracellular Vesicles derived from licensed Mesenchymal Stem Cells: a tunable approach to regulate angiogenesis.

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Angiogenesis is the process that leads to the formation of new blood vessels from a pre-existing vascular network, playing a key role in many physiological and pathological processes. Consequently, targeting angiogenesis represents a very interesting therapeutic approach.
We have already shown that mesenchymal stem cells stimulated with pro-inflammatory cytokines (st-MSCs) block angiogenesis through the release of soluble factors, thus inhibiting the immune response. Here, we demonstrate that extracellular vesicles derived from stimulated MSCs (EV st-MSC), but not from their unstimulated counterparts, inhibit angiogenesis, thus recapitulating MSC effect.
EV st-MSC express high levels of the ecto-5'-nucleotidase CD73, which mediates the inhibition of endothelial cell migration through the generation of adenosine. We further demonstrated that the adenosine-mediated inhibition of cell migration involves the production of ROS in endothelial cells at the migrating front, both in vitro and in vivo.
These results indicate that EVs derived from st-MSCs display anti-angiogenic properties and could be exploited for cell-free therapeutic strategies.

Perilipin2 controls lipid metabolism and muscle mass.

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Perilipin 2 is a highly conserved protein known for its role in the control of lipid accumulation within cytoplasm in several non-adipose tissues. However, the biological role of Plin2 is much more complex than previously thought. Indeed, Plin2 appears to contribute to cellular metabolism and its downregulation prevents or mitigates several diseases. In humans high levels of Plin2 are associated with loss of muscle mass and strength, but its role in skeletal muscle is still unclear. We performed gain and loss-of-function experiments in adult murine muscles finding that Plin2 inhibition is sufficient to induce a 30% increase in myofiber size. Moreover this hypertrophy is paralleled by a dramatic alteration of lipid content. In particular, we observed a strong decrease of lipid intermediates, such as ceramides, that contrast muscle anabolism. In summary, our data suggest that Plin2 is a major contributor of muscle lipid metabolism, affecting the maintenance of muscle mass.

The mitochondria and cristae shaping protein Opa1 promotes chromatin remodelling in fat browning to protect against obesity and metabolic disorders.

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Adipose tissue dysfunction is the major determinant of the development of insulin resistance with substantial impact on human metabolic and cardiovascular diseases. Here we find that obese subjects have decreased level of Opa1 revealing for the first time a link between human obesity and mitochondrial dynamics. In mice, Opa1 counteracts obesity and diabetes since controlled Opa1 overexpression reduces weight, improves glucose metabolism and insulin sensitivity, by reducing fat depots and promoting brownization of white adipose cells in vivo and in vitro. Conversely, adipocyte-specific Opa1 deletion triggers a lipodystrophic phenotype characterized by hyperglycemia, insulin resistance, brown adipose tissue whitening and hepatosteatosis. Mechanistically, Opa1 cell-autonomously regulates adipocyte browning by retrogradely coordinating a nuclear gene expression program. By integrating genomic and metabolomic data we identify the Opa1-dependent mitochondria-to-nucleus signalling that drives WAT browning via specific Jumonji chromatin-modifying demethylases, paving the way for developing new effective strategies to counteract obesity and its associated complications.

Block of autophagy in skeletal muscle leads to heart atrophy and contractile dysfunction via mir-206.

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Rationale. Block of autophagy in skeletal-muscle causes sarcopenia, denervation and reduced contractile performance. Autophagy impairment also leads to increased secretion, by skeletal muscle fibers, of exosomes containing the muscle-specific micro-RNA, miR-206, which interestingly appears also in the myocardium.
Purpose: To determine the effects of miR-206 on cardiac structure and performance. Results: Our in vitro and ex vivo assays demonstrate that exosome-carried miR-206 is taken-up by cardiomyocytes, compromising cellular structure and function. Indeed, miR-206 leads to cell atrophy, sarcomeric dysarrangement and ultrastructural abnormalities. In addition, miR-206 overexpression affects signaling pathways downstream to □□-adrenoceptors and alters intracellular Ca2+ dynamics.
Conclusions and Perspectives: Our findings identify miR-206 as a key mediator of the 'skeletal muscle-to-heart' communication. We are now searching for novel molecular targets of miR-206 in cardiomyocytes. The results of this study have the potential to define the mechanisms underlying the development of cardiomyopathies in muscular and neuro-muscular disorders.

Evaluation of resting state and evoked electrophysiological signals in rodent barrel cortex.

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The rodent barrel cortex is a widely used model of information processing in the somatosensory area, thanks to its precise and easily recognizable organization.
Indeed, the tactile somatosensory pathway from whisker to cortex provides a well-defined system for exploring the link between molecular mechanisms, synaptic circuits, and behavior.
The aim of this work was to analyze the frequency content of Local Field Potentials (LFPs) spontaneously arising under anesthesia both in absence of external stimuli and under mechanical whisker stimulation, in order to investigate which are the main features in the two studied experimental conditions.
The results reveal the existence of complex connectivity patterns at low frequency corresponding to the relevant signals' content, and the presence of a static pattern at higher frequencies. Furthermore, the immediate stimulus' effect is to synchronize the system which returns to the resting state after a few seconds.

Generation and phenotyping of a novel knock-in (KI) mouse model of Arrhythmogenic Cardiomyopathy (AC).

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AC is a familial cardiac disease, mainly caused by mutations in desmosomal genes, at risk of sudden death in the athletes. AC hearts display cardiomyocyte death and fibro-fatty tissue replacement, which lead to heart dysfunction and stress-related arrhythmias. Current AC murine models overexpress disease-mutations in cardiomyocytes and only partially recapitulate the human AC phenotype.
Therefore, AC pathogenesis is poorly understood and no therapies are available. Knock-in mice would represent the best preclinical model for a comprehensive study of the AC disease mechanisms. By using the Crispr-Cas9 technology, we generated a novel AC KI mouse, harbouring the point mutation Ser311Arg in Desmoplakin, which we have identified in a large part of the Italian AC population. Hearts from KI mice will be analyzed at structural and functional levels. We expect our mice to recapitulate the features of the human disease and to represent a valuable tool to understand AC pathogenesis.

CXCL12/SDF-1 alternative signalling pathways modulate neutrophil nuclear deformability.

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Neutrophil migration is fundamental for immune responses. Upon inflammation, neutrophils leave the bone marrow and migrate towards inflamed tissue following chemotactic gradients. Although the cytoplasm can quickly change consistence and form to allow cells to penetrate the endothelium, the deformation of the nucleus, the largest and stiffest cellular organelle, seems to be an awkward process. Since immune cell migration is driven by chemokines, it is likely that chemokines may also modulate nuclear deformability. The homeostatic chemokine CXCL12 has been demonstrated to regulate neutrophil homing and chemotaxis by binding its canonical receptor CXCR4. Exploiting live cell imaging and original micro-fabricated devices, we investigated the signaling pathways connecting chemokine signals to nuclear biomechanical properties modifications. Here we show that CXCL12 induces neutrophil nuclear deformation and sustains transendothelial migration towards inflammatory stimuli. Intriguingly, we found that atypical receptors and unexpected protein kinases are involved in the modulation of neutrophil nuclear deformability.

Role of the Mitochondrial Calcium Uniporter (MCU) in cardiomyocyte adaptation to hypertrophic stimuli.

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Cardiac hypertrophy (HCM) is associated with high incidence of heart failure (HF), an end-stage disease. Due to the incomplete understanding of HCM pathogenesis, current therapies are unsuccessful in preventing the HCM-to-HF transition. Alterations in Ca2+ homeostasis are linked to HCM contractile and metabolic abnormalities, but the responsible mechanisms are unknown. Mitochondrial Ca2+ is the primary regulator of metabolic energy supply in muscles, and the Ca2+ uniporter complex (MCU) has a key role in myocardial adaptation to stressors, associated to β-adrenergic receptor (β-AR) activation. Interestingly, we observed that MCU protein level is differently modulated in compensated and maladaptive HCM, in both murine and human hearts. To understand the role of MCU in HCM, we genetically modulated its expression level both in vitro and in vivo, and subjected isolated cardiomyocytes and adult mice to chronic β-AR stimulation and pressure overload, respectively. In both models, MCU has an important role in sustaining hypertrophy, and its protein level is determinant for adaptive response to pro-hypertrophic factors. These data lay the grounds for possible targeting of MCU in the treatment of heart failure.

The mitochondrial fission factor Dynamin Related Protein 1 limits VEGF/VEGFR2 endocytic trafficking and angiogenesis.

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Because of the mostly glycolytic nature of endothelial cell metabolism, the role of mitochondria and mitochondrial shape in angiogenesis, the new blood vessel formation from existing vasculature, has not been studied. Here we show that the mitochondrial fission factor Dynamin related protein 1 (Drp1) unexpectedly limits endosomal VEGFR2 signaling and hence angiogenesis. Drp1 levels were reduced when Human Umbilical Vein Endothelial Cells (HUVECs) were activated, and angiogenesis was accordingly stimulated in HUVECs where DRP1 was silenced. In vivo, constitutive and inducible Drp1 ablation in endothelial cells increased early stage postnatal retina vascular sprouting. Mechanistically, upon VEGF stimulation Drp1 interacted with the internalized VEGFR2 and its early endosome partner Rab5 at the endosomal VEGFR2 signaling platform. Drp1 deletion unleashed VEGFR2 activation and its downstream signaling, indicating that the VEGFR2-Rab5-Drp1 interaction limits VEGFR2 mediated angiogenesis. Our data reveal an unexpected extramitochondrial function of Drp1 in endothelial cells, where it localizes also at the endosomes to constrain the endosomal VEGFR2 signaling platform.

An interplay between gut microbiota and p66Shc affects obesity-associated insulin resistance.

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P66Shc promotes adipogenesis and p66Shc-/- mice accumulate less body weight than wild type (Wt) mice. As the metabolic consequences of the leaner phenotype of p66Shc-/- mice is debated, we hypothesized that gut microbiota may be involved. P66Shc deletion significantly modified the composition of gut microbiota and their modification after a HFD. This was associated with changes in gene expression of Il1b and Reg3g in the gut and in systemic TMAO and BCAA levels, despite no difference in intestinal structure and permeability. Depleting gut microbiota at the end of HFD rendered both strains more glucose tolerant, but improved insulin sensitivity only in p66Shc-/- mice. Microbiota-depleted Wt mice co-housed with microbiota-competent p66Shc-/- mice became significantly more insulin resistant than Wt mice co-housed with Wt mice, despite no difference in weight gain. These findings reconcile previous inconsistent observations on the metabolic phenotype of p66Shc-/- mice and illustrate the complex microbiome-host-genotype interplay under metabolic stress.

Self-control and the human brain.

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We analyzed data of 1206 healthy subjects from the Human Connectome Project to investigate the differences in brain structure and functional connectivity (FC) associated with self-control and time preference for rewards (i.e., choosing either smaller immediate rewards or larger delayed rewards). We found that stronger self-control is associated with larger gray matter volume.
Furthermore, stronger and more flexible behaviors are associated with stronger FC between reward/emotion related regions of limbic system (e.g. amygdala, caudate) and control regions (prefrontal regions, anterior cingulate cortex). The evolutionarily older impulsive system, which comprises limbic and paralimbic regions, values immediate rewards. The more recently evolved control system is important for the inhibition/regulation of the impulsive system and the associated valuation of delayed rewards. This finding suggests that stronger functional coupling between cortical and subcortical regions underlies a more efficient regulatory influence.

Protein kinase CK1α sustains Mantle Cell Lymphoma survival.

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Mantle cell lymphoma (MCL) is a B-cell neoplasm that accounts for around 5-10% of all lymphomas, which urgently needs new therapeutic strategies. The Ser/Thr protein kinase CK1α has been found pivotal for multiple myeloma and Diffuse Large B cell Lymphoma. It is essential in the regulation of several survival signalling cascades (such as Wnt/βcatenin, NF-κB, AKT, the p53-driven response) which are important for MCL cell growth.
Nevertheless the role of CK1 in MCL pathogenesis and in associated survival signalling has never been studied. We found that CK1α was highly expressed in purified primary MCL B cells and cell lines, compared with healthy controls. Inhibiting CK1α in MM cells with the compound D4476 or RNA interference caused apoptosis and cell cycle arrest. Moreover, CK1α inhibition caused a strong impairment of NF-κB and AKT survival signalling pathways. Therefore, CK1α is a growth-propelling kinase in MCL representing a new possible molecular therapeutic target.

New culture protocol promotes in vitro maturation of neonatal cardiomyocytes.

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Isolated cardiomyocytes, obtained from either neonatal or adult hearts, are the preferred cellular model used in molecular cardiology. While cells isolated from adult hearts faithfully replicate the cardiomyocyte phenotype of the intact myocardium, they can only be maintained in culture for a short time (24-48 hours), and they are difficult to manipulate genetically. These limitations dictated the success of neonatal cardiomyocytes, which have commonly been used in heart research, despite having an immature phenotype which reflects on structural and metabolic differences with the adult cells. Our aim is to implement a cell culture protocol promoting the maturation of neonatal cardiomyocytes towards a more developed phenotype. In our preliminary experiments, by refining the culture media, we were able to obtain cardiomyocytes with a morphology suggestive of enhanced maturation. Currently, we are assessing cell structure and second messenger dynamics (Ca2+ and cAMP) in response to contractile activity and hormonal (e.g. catecholamines) stimulation.

Multimodal mapping of MEG and fMRI signals at rest and during a visuospatial attention task.

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We compared fMRI with MEG-derived band-limited power (BLP) correlation on a group of 16 healthy subjects, during a visual-attentional task and rest . Aims are: (i) measuring frequency specific task-related FC modulations in MEG, (ii) comparing fMRI- and MEG-FC modulations (task-rest). Dorsal attention (DAN) and occipital visual (VIS) networks were considered.
We found that MEG BLP-FC decreased during task, especially in alpha and beta bands. This decrement involved both VIS, coherently with fMRI, and VIS-DAN FC, which was stronger in fMRI. Some specific DAN-VIS connections were increased in task as compared to rest in gamma consistently with increased observed with fMRI.
These findings indicate that task-rest fMRI FC modulations have variable relationships with corresponding MEG-BLP FC modulations.
Directly comparing fMRI and MEG correlation matrices, their correlation resulted higher during task than rest, however, DAN-VIS FC in fMRI and MEG were negatively correlated in alpha, but positively correlated in gamma band.

Drp1-mediated mitochondrial shape controls calcium homeostasis and muscle mass.

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Mitochondrial quality control is essential in complex and highly structured cells such as neurons, cardiac and skeletal muscles. In skeletal muscle mitochondrial network is fragmented during catabolic and inflammatory conditions. However, whether mitochondrial fission is per se essential for muscle homeostasis is unclear. Here we show that skeletal muscle specific loss of the pro-fission dynamin related protein (DRP) 1 induces muscle wasting, weakness and causes a global fasting-like condition. Constitutive as well as inducible Drp1 ablation in the skeletal muscle reduced growth and lifespan. Drp1 deficient mitochondria were morphologically and functionally abnormal, leading to protein synthesis inhibition, activation of ubiquitin-proteasome system, inhibition of autophagy and induction of Unfolded Protein Response. The change of mitochondrial shape altered normal calcium homeostasis causing important weakness and myofiber death. Our findings reveal that the shape of mitochondria is critical for several fundamental biological processes that control nuclear programs and calcium handling in adult fibers.

The circadian clock development during hepatic differentiation of human pluripotent stem cells.

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Circadian rhythms are daily cycles of physiology and behaviors that are driven by an endogenous oscillator with a period of approximately 24h. This system is fundamental to achieve temporal homeostasis with the environment at the molecular level. During development, the environmental conditions surrounding and affecting the embryo, such as the availability of nutrients or the concentration of metabolites, change throughout the day. The embryo does not yet possess a mature functional clock able to anticipate these variations; the development of such mechanism is accomplished during gestation.
It is clear that in mammal circadian rhythmicity develops gradually during ontogenesis, but the mechanisms involved at cellular level during differentiation of embryonic stem cells are still unknown. The characterization of the circadian system and its complex network of interactions with relevant physiologic and pathologic pathways is in continuous evolution. Up to now, the knowledge of clock ontology and development and its relevance during cellular differentiation is restricted to a very few articles.
This work represents the first attempt of investigating the cell-autonomous circadian clock onset in human pluripotent stem cell during hepatic differentiation. Human pluripotent stem cells differentiated in microfluidic environment achieved sustained expression of hepatic markers and of most of circadian genes. Only after the stage of definitive endoderm circadian rhythmicity of 24h cycles start to be observed in most of circadian genes. The circadian onset seems to be correlated with the daily variation of metabolic signals in which the microfluidic cell culture is exposed.

Mfn1 is essential to oocyte formation in mice whereas Mfn2 knockout partially rescues development of double-knockout oocytes.

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Mitochondria play a fundamental role during oocyte development, distinguishing from other cells by their increased number, small size and round shape. We investigated the role of mitochondrial dynamics in oocytes by condition knockout (cKO) of Mfn1, Mfn2 or Mfn1+Mfn2. Mfn1-cKO females were infertile due to arrest of folliculogenesis. In addition, Mfn1-cKO oocytes contained abnormal mitochondria along with lower levels of mtDNA, ΔΨm and ATP. On the other hand, Mfn2-cKO females were fertile and their progeny viable. Surprisingly, although Mfn1+2-cKO females were infertile, folliculogenesis was not arrested and their oocytes grew similarly to Mfn2-cKO ones. Mfn1+2-cKO oocytes contained damaged mitochondria along with intermediate levels of mtDNA and ΔΨm, but normal levels of ATP. In conclusion, this work provides evidence that oocytes rely on Mfn1 to develop normally, whereas Mfn2 ablation in Mfn1+2-cKO oocytes partially reverses the impact of Mfn1 deficiency.

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Biological applications of a multiphoton-multicolor and NIR STED microscope.

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Two-photon microscopy has proven an excellent technique for in vivo fluorescence imaging of deep structures and tissues due to low absorption and scattering of infrared light. The combination of two pulsed laser beams in the 750-1400 nm wavelength region introduces additional imaging capabilities, such as simultaneous excitation of three fluorophores at the same time (multiphoton-multicolor) and sub-diffraction resolution of cellular structures (STED) labeled by fluorescent dyes. Fluorescent proteins are also utilized with super resolution, but their imaging in the near infrared region (NIR) remains challenging with respect to the visible proteins. Our tests suggest that the depletion process is the bottle neck of the NIR STED, so further investigation with new hardware and optical configuration is required. The new microscope setup will then include a high power Katana laser, as well as the possibility to combine 1P and 2P optogenetics experiments in collaboration with the new Padova Neuroscience Center.

The mitochondria shaping protein OPA1 controls angiogenesis by regulating NFκB signalling.

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Mitochondria not only synthesize most of the cellular ATP, but they are also centrally placed in intermediate metabolism Ca2+ signaling, redox homeostasis and apoptosis. The multifunctional inner mitochondrial membrane mitochondrial fusion protein Optic Atrophy 1 (OPA-1) is placed at the crossroad of fusion, cristae biogenesis, metabolism, apoptosis and regulation of cardiomyocyte differentiation, yet the role of mitochondrial dynamics in angiogenesis, the physiological process through which new blood vessels form from pre-existing ones, has not been addressed. Here we show that Opa1 is a crucial component of the angiogenetic program. Upon endothelial cells angiogenic stimulation, mitochondria elongate and OPA-1 level increase. Genetic Opa1 ablation signals retrogradely from mitochondria to the nucleus to modify angiogenic genes expression and therefore inhibit all features of angiogenesis. Conditional Opa1 ablation substantiates its role in mouse and zebrafish angiogenesis and in lymphangiogenesis mediated tumor metastatization. Thus, Opa1-dependent mitochondrial dynamics is a targetable component of angiogenesis.

Exploring the role of mitochondrial dynamics in developmental and psychiatric disorders.

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The pathological mechanisms of schizophrenia (SZ) are unknown. It has been suggested oligodendrocytic differentiation is disrupted in SZ. In this study, we explored whether mitochondrial dynamics is involved in differentiation of oligodendrocyte. We found an increase in expression level of mitochondrial mass during differentiation of human oligodendrocytic cell line (MO3.13). In addition, we also found that the expression of the one of isoforms of PGC-1□ was dramatically increased during the oligodendrocytic differentiation. Intriguingly, chromosome 4p15-p16 region including PPARGC1A is associate with schizophrenia (Christoforou A et al., 2007). Now we are trying to knockdown of the PGC-1□ isoform in the cell line and investigate the function of the isoform during the oligodendrocytic differentiation. These findings provide a starting point for exploring the involvement of mitochondrial dynamics in the pathological mechanisms of SZ.

Modelling Fragile X Syndrome with iPSCs.

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Fragile X syndrome (FXS) is the major monogenetic cause for autism and mental retardation and is caused by a trinucleotide repeat expansion, methylation and silencing of Fragile X Mental Retardation 1 (FMR1) gene promoter. The molecular mechanism and timing leading to FMR1 silencing and protein loss are still unknown.
The generation of naïve induced pluripotent stem cells (iPSCs) showing a broader unmethylated genome (including in FMR1) opened a new hope for disease modeling of FXS.
In this project we aim at understanding the molecular mechanism of FXS taking place during the early phase of neural development, using FXS-patient specific naïve iPSCs and micro-technologies.
While collecting the patients' samples, we set up protocols for neural differentiation and mRNA based direct conversion of iPSCs into neurons. We are now optimizing these protocols using microfluidics, in order to create a scalable platform instrumental for our studies on FXS molecular mechanisms.

Chemokine control of nuclear plasticity in metastatic cells.

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Metastasis represents a multi-step process involving tumor cell migration, and finally growth at the new target organ. Remarkably, the CXCL12/CXCR4/CXCR7 axis plays a crucial role in cancer progression and consolidated evidence clearly demonstrated that the CXCR4 and CXCR7 receptors tailor tumor metastasis in several types of cancers. Nonetheless, the molecular mechanisms triggered by the CXCL12/CXCR4/CXCR7 axis in this context are not completely defined. Since nuclear deformability plays a key role in determining the cell ability to migrate, signals controlling tumor cell migration, as chemokines, might directly control nuclear plasticity and function. Hence, on the basis of our preliminary clues we aim at investigating an unexpected role of chemokine signaling in the control of tumor cell dissemination by affecting nuclear deformability and stiffness. The understanding of alterations in nuclear biomechanical properties of metastatic cancer cells will allow the identification of new targets, which can be pharmacologically exploited to control cancer spreading.

Inhibition of the Mitochondrial Potassium Channel Kv1.3 Selectively Kills Chronic Lymphocytic Leukemia B Cells.

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The high expression of the potassium channel Kv1.3 in both plasma membrane (PM) and inner mitochondrial membrane might promote tumor cell proliferation. We selectively targeted the mitochondrial Kv1.3 using the PAP-1 psoralen derivatives (PAPTP and PCARBTP) demonstrating that they didn't affect normal B cell viability, while induced 50% apoptosis in B cells from Chronic Lymphocytic Leukemia (CLL) patients, even in absence of multi-drug resistance pumps inhibitors (MDRi) or in presence of mesenchymal stromal cells. Furthermore, we found that effector memory T cells (TEM) from CLL patients and healthy subjects are refractory to PAP-1 derivatives, probably as a conseguence of their lower basal ROS production with respect to CLL B cells. These results point out the Kv1.3 inhibitors as a new potential tool to selectively kill leukemic cells.

Altered migration of inhibitory interneurons in a mouse model of intellectual disability.

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Among the X-linked intellectual disability genes, OPHN1 encodes for a Rho-GTPase activating protein involved in several developmental processes.
Inhibitory interneurons are fine regulators of network activity and exert a prominent role in neuronal plasticity, mostly in the postnatal period. Proper migration of inhibitory interneurons to their final destination is therefore essential to ensure proper inhibitory circuitry.
Whether and how OPHN1 mutation impacts on the development of inhibitory interneurons and in particular on their migration, remains largely obscure. Combining quantitative morphological analysis and in vivo time-lapse imaging we dissected the role of OPHN1 in the migration of neuronal precursors. We found that distribution, morphology and motility of neuroblasts were deeply perturbed in OPHN1-/y mice. Furthermore, GABA signalling, known to modulate cell migration, appeared to be subverted in OPHN1-/y mice, resulting in hampered migration. Pharmacological modulation of intracellular Cl- concentration that affects GABA response polarity, largely rescued the progression of neuroblasts.

Investigating the role of Opa1 in controlling adipocyte size.

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White adipose tissue is specialized in the storage and release of fat, the balance of which is critical to maintain healthy energy homeostasis. In addition to its lipid-storing capacity, WAT has been described as an important endocrine organ controlling the systemic handling of energy substrates.
Excessive lipid load causes adipocyte stress, which in turn accounts for many adverse effects of obesity; also conditions of absence or scarcity of WAT such as lipodystrophy are associated with severe metabolic complications. Mitochondrial dysfunction has been first associated to impaired glucose tolerance 40 years ago, and it's still under debate whether mitochondrial dysfunction is the cause or a consequence of insulin resistance and type 2 diabetes. In particular, the role of the fusogenic protein Opa1 remains to be elucidated.
Controlled Opa1 overexpression favors adipocyte plasticity, resulting in overall improved glucose metabolism and insulin sensitivity. Adipocyte-specific deletion of Opa1 triggers a lipodystrophic phenotype, with adipocytes unable to adapt to metabolic challenges to increase lipid storage. Moreover, RNAseq analysis performed on Wt and Opa1tg pre-adipocytes and mature adipocytes reveals changes in pathways associated with control of cell size. We therefore hypothesize that Opa1 impacts on signaling pathays that are responsible for regulating cell size.

Modelling liver disease in Alpha1-Antitrypsin Deficiency with human pluripotent stem cells.

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Alpha1-Antitrypsin Deficiency (ATD) is an inherited metabolic disorder, which causes hepatic fibrosis, cirrhosis and hepatocellular carcinoma. A point mutation in the gene SERPINA1 encodes the mutant protein ATZ, which accumulates as toxic polymers within hepatocytes. However, a certain percentage of affected individuals tolerates this hepato-toxic accumulation, escaping liver disease.
We aim at developing an ATD in vitro model using patient-specific induced pluripotent stem cells (iPSCs) to recapitulate key-pathogenic events causing liver damage.
We developed a microfluidic-based protocol for the differentiation of a cohort of patient-specific iPSCs into functional hepatocytes, which show accumulation of mutant ATZ polymers within the endoplasmic reticulum.
We also developed a stress-response assay in order to exacerbate the ATZ accumulation through a 4-days heat-shock treatment, achieving an increase of polymers accumulation and gene expression alteration, compared to healthy controls.
The final goal is to discriminate among patients, predicting their risk of developing liver disease.

Theoretical study of mutant connexin 32 hemichannels by Molecular Dynamics simulation.

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Connexins are a family of transmembrane proteins that assemble into hexameric channels, called connexons or hemichannels. Single hemichannels control the plasma membrane permeability to paracrine signaling molecules, while two adjacent hemichannels belonging to neighbouring cells can dock to form a gap-junction channel, which creates a direct path for intercellular communication. In this work we tested our realistic three-dimensional model of connexin 32 (Cx32) connexon for a relatively long time (500 ns), both in wild type (WT) and pathological configurations carrying selected point mutations (H73l, V63I or V181M). These mutations are known to cause Charcot-Marie-Tooth (CMT) disease, a hereditary neuropathy characterized by progressive muscular atrophy and peripheral axon degeneration. The current work aimed to set up a theoretical tool for the analysis and interpretation of in vitro imaging and electrophysiological results as well as to provide predictions about possible structural and functional alterations induced by the mutations.

MCUb in muscle regeneration: a role of macrophage phagocytosis.

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Muscle regeneration is a complex mechanism occurring after trauma and/or in chronic conditions and relies both on inflammation and programs of gene expression by satellite cells, a population of myogenic precursor cells (MPCs) that reside between the myofibers in quiescent state. Macrophages plays a crucial role in regeneration, removing apoptotic and necrotic cells and secreting cytokines that regulate MPCs proliferation, differentiation and tissue remodelling. We observed that during regeneration, there is an up-regulation of MCUb, the dominant negative form of the Mitochondrial Calcium Uniporter (MCU), that parallels the pro- to anti-inflammatory switch of macrophages: MCUb is indeed finely tuned in macrophages and plays a pivotal role in phagocytosis. Macrophages lacking MCUb present several impairments in their physiological functions, and the heaviest is phagocytosis. We hypothesized that this defective phagocytosis in macrophages, may be responsible of impaired muscle regeneration we observed in MCUb KO mice.

Gemini/DOPE - based Lipoplexes as efficient nanocarriers of MFN1 and OPA1 proteins in in vitro and in vivo experiments.

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Mitochondria form a highly dynamic network of organelles that constantly fuse and divide. Balances between fusion and fission are very important for normal cellular function. In mammalian cells, there are three main proteins involved in the mitochondrial fusion: Mfn1 and Mfn2 (OMM) and OPA1 (IMM). Deletion of any of them in MEFs, produce mitochondrial fragmentation, leading mitochondrial diseases (MD), to which there is no cure at present. In this work, lipoplexes have been conceived as efficient therapeutic agents against MD. Lipoplexes, consisting on a lipid/DNA complex, are composed by Gemini/DOPE liposomes and a plasmid DNA coding for Mfn1 or OPA1 protein. These lipoplexes are able to transport and efficiently delivery plasmid DNA into the cytoplasm inducing the recovering of normal mitochondrial phenotype in MFN1-KO and OPA1-KO MEFs. They not only show a good viability and high TE in in vitro experiments but also show an efficient bioaccumulation and transfection in in vivo experiments without any toxicity.

Mitofusin 2, mutated in Charcot-Marie-Tooth type IIa, is alternatively spliced in endoplasmic reticulum-specific variants controlling organelle morphology, Ca2+ content and tethering to mitochondria.

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Mitofusin 2, mutated in Charcot-Marie-Tooth type 2A (CMTIIa) neuropathy, is a large mitochondrial GTPase that besides its involvement in mitochondrial fusion, is crucial to shape the endoplasmic reticulum (ER) and to tether the two organelles. Here we report the existence of different splice variants of mitofusin 2 expressed in different human tissues. Splice variants lacking part (Variant 1) or all (Variant 2) of the GTPase domain are localized in ER and Variant 2 is enriched at the interface between ER and mitochondria. The mitochondrial targeting of full length Mfn2 requires the integrity of coiled-coils 1 and 2, while the transmembrane domain alone is sufficient to target the Mfn2 variants to the ER. Re-expression of ER-specific Mfn2 variants in Mfn2-/- cells rescues ER morphology, corrects ER-mitochondrial tethering and normalizes ER Ca2+ levels, improve mitochondria Ca2+ uptake and Ca2+-depend ATP production, without rescuing mitochondrial morphology. The discovery of ER-specific mitofusin 2 variants reveal the existence of entirely extramitochondrial MFN2 functions that are likely to contribute to the pathogenesis of CMTIIa.

Exploring the role of mitochondrial dynamics in tracheal epithelial cells.

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Somatic stem cells adjust their self-renewal and lineage commitment by regulating various signaling pathways. Although biological signals controlled by mitochondrial dynamics have been identified, whether mitochondria morphology can regulate states of airway stem cells remains unclear. We focus on the basal cells as tracheal epithelial stem cells. During homeostatic turnover and cell replenishment after injury, tracheal basal cells undergo long-term self-renewal and give rise to differentiated cells, such as ciliated and secretory Club cells. Here, we show TEM images of cristae structure in each tracheal epithelial cell and techniques to assess the states of basal cells in dynamin-like GTPase optic atrophy 1 (OPA1) mutants.

Exploring the roles of mitochondrial dynamics in melanocytes and melanoma.

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Melanocytes produce pigment Melanin, which gives color to skin and hair. It has been studied that hair graying such as aging symptoms is caused by defective self-maintenance of melanocyte stem (MSC) cells. In fact, the Bcl2 deficiency causes selective apoptosis in the MSC and accelerates hair graying.
Anti-apoptotic protein of Bcl2 suppresses cytochrome c release from mitochondrial cristae. These reports suggest that mitochondria play important roles for melanocyte/MSC survival. Mitochondrial dynamics play critical roles in maintaining functional mitochondria and they are regulated by ubiquitously expressed Dynamin-related GTPases such as Optic atrophy (Opa1). Until now, the anti-apoptotic role of Opa1 has not known in the melanocyte and melanoma. Remarkably, We found the early gray hair syndromes in the melanocyte-specific Opa1 KO (Opa1Δmel/Δmel) mouse. Furthermore, Opa1Δmel/Δmel have not induced hyperpigmentation when we crossed with a melanoma induced model mouse (melanocyte-specific NRAS gene driven mouse: TyrNRASQ61K/+). These data suggested that the Opa1 may function for melanocyte and would be potential clinical therapeutic targets in the melanoma.

The role of the myokine FGF21 in skeletal muscle homeostasis.

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Skeletal muscle is a plastic tissue that responds to changes in exercise and nutrition by secreting myokines. These muscle-derived factors have autocrine, paracrine and endocrine effects, explaining how muscles regulate metabolic homeostasis in other tissues. We have recently proposed an interplay between the myokine Fgf21 and the mitochondrial quality control pathways that greatly contributes to a systemic pro-senescence metabolic shift. However, whether Fgf21 is beneficial or detrimental for human health is still not clear, in part because the contribution of autocrine-derived FGF21 to muscle homeostasis has not been resolved. Here, we investigate the role of FGF21 in regulating skeletal muscle mass during starvation.

Large-scale brain communication networks: a metabolic perspective.

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Previous studies locally confirmed that the majority of brain glucose demand is devoted to the spontaneous brain activity.
Leveraging on the spatio-temporal organisation principles that characterize the brain spontaneous activity, we aim to understand role of communication structure over the metabolic consumption applying graph theory modelling methods.
We investigated the relationship between the network organisation principles of human functional brain networks, described by their resting state functional connectivity (FC), and the cortical glucose metabolism, simultaneously measured by position emission imaging (PET) with 18F-FDG tracer in a group of 21 healthy subjects.
The connectivity strength of each network node was significantly associated with the local glucose uptake, corroborating the principle that an increased connectivity level involves a higher glucose demand.
Moreover, brain areas with high glucose consumption seem to follow a different energy optimisation mechanism indicating a potentially different efficiency. As this result could also be observed over known macro-scale resting state networks, the communication performance could be advocated as main factor for the brain energy demand, hierarchically shaping the connectivity structure of macro-scale networks as well as single brain areas.

Development of a new chemical inhibitor of OPA1 activity to increase cancer cells sensitivity toward apoptosis.

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Mitochondria participate as signaling units in numerous cancer-related processes. Optic Atrophy 1 (OPA1) is a large dynamin-related GTPase protein of the inner mitochondrial membrane that independently promotes mitochondrial fusion and regulates apoptosis via cristae remodeling process. Interestingly, OPA1 is overexpressed in different type of cancers and it correlates with a poor prognosis and an increased chemotherapy resistance. Conversely, OPA1 downregulation improves drug sensitivity, and mutations that abolish OPA1 catalytic activity impair its antiapoptotic function. In this work, inhibitors of OPA1 are explored as a tool, to be employed in combination with chemotherapy, to sensitize resistant tumor cells toward apoptosis induction. A GTPase colorimetric high-throughput screening identified MYLS22 as a potent and selective OPA1 inhibitor among a library of drug-like small molecules. OPA1-inhibitor interactions were characterized with kinetics assays and by NMR. Further experiments confirmed its non-mitochondriotoxicity. Apoptosis of treated cells and mitochondrial functionality were evaluated through real time membrane potential, respiration and cytochrome c release measurements.

Mitochondrial dynamics regulate cellular cholesterol levels.

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The trafficking of LDL-derived cholesterol to mitochondria is required for mitochondrial membrane integrity and the biosynthesis of steroids and bile salts. Whether mitochondrial function regulates levels of intracellular cholesterol is an open question. Here, we show that the loss of mitochondrial fusion proteins MFN1 and MFN2 results in a global transcriptional downregulation of the cholesterol biosynthesis. Decreasing cellular levels of cholesterol by removing exogenously supplied LDL and inhibiting lysosomal cholesterol efflux substantially reverses the observed downregulation. Conversely, the inhibition of cholesterol esterification, a cellular strategy to cope with higher cholesterol levels, exacerbates this repression in cells deficient in mitochondrial fusion. Together, these results cells suggest that cells deficient in mitochondrial fusion accumulate higher levels of intracellular cholesterol and are thus more sensitive to LDL-derived cholesterol. Indeed, lipidomic analyses reveal increased free cholesterol levels in mitochondrial fusion-deficient cells, supporting a model in which mitochondria are integral for homeostasis of cellular cholesterol levels.

Erythropoiesis has a new player: protein kinase CK2.

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Here, we used hematopoietic specific conditional knockout mice for protein kinase CK2 regulatory subunit. Since CK2 knockout led to birth lethality, the study was carried out during gestation. The lack of CK2β caused depletion of hematopoietic cells in particular of the erythroid compartment. CK2β resulted to be important for erythroid maturation and red cell viability. CK2β KO showed a different impact on cell cycle depending on the stage of erythroid maturation. RNA seq analysis revealed the upregulation of Tp53 associated genes and p21; down-modulation of STAT5, c-kit and genes associated to PI3K/AKT pathway. GATA-1, the key transcription factor for definitive erythropoiesis, was reduced in KO mice with a negative impact on its down-stream genes. We used also an in vitro model of erythroid differentiation based on G1E-ER cells, an estrogen inducible GATA-1 null erythroblast cell line; the combined treatment of β-estradiol and blockade of CK2 confirmed the negative effect on differentiation and WB data suggested a possible role of the kinase in the regulation of AKT, GATA-1 and STAT5 protein stability.

Targeting HSP70/HSF1 axis in chronic lymphocytic leukemia.

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We recently found that the Heat Shock Protein of 70kDa (HSP70) is overexpressed in neoplastic B cells from Chronic Lymphocytic Leukemia (CLL). HSF1 (heat shock factor 1) is the primary responsible for HSP70 transcription being the major regulator of its expression. Considering the pro-survival role played by HSP70 and HSF1, we studied and targeted HSP70/HSF1 axis in CLL. We observed that these proteins were correlated to poor prognosis. Moreover, we found that in a most CLL patients HSF1 is constitutively phosphorylated at activatory Ser326, thus being positively regulated. We previously analyzed HSP70 and HSF1 inhibition in leukemic cells using Zafirlukast and Fisetin, their respective inhibitors, demonstrating a dose-dependent B cell apoptosis. We decided to test also new drugs as Honokiol, Pterostilbene and Triacetyl Resveratrol to inhibit HSF1 activity. We found that also these treatments led to cell apoptosis, indicating the HSF1/HSP70 axis as a potential therapeutic target in CLL.

Dissecting the dual role of ATAD3A:
the link between nucleoid stability and cristae ultrastructure.

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The regulation of mitochondrial dynamics and ultrastructure is crucial for cell function and viability. A well-characterized regulator of such processes is the inner mitochondrial membrane GTPaseOPA1, which forms high molecular weight (HMW) complexes that maintain cristae ultrastructure and thus restrict cyt c to the cristae lumen. Our proteomic analysis of OPA1-HMW complexes has enabled the identification of proteins that potentially interact with OPA1 during apoptotic cristae remodeling. Among these, ATAD3A stands out as one of the most promising OPA1 interactors, data confirmed by coimmunoprecipitation experiments. Our studies demonstrate that ATAD3A regulates cristae biogenesis and stability. The formation or disruption of ATAD3 oligomers results in narrow or swollen cristae, respectively. We also demonstrate that ATAD3A oligomers are not required for cristae biogenesis, but instead are likely involved in nucleoid and mtDNA stability. Indeed, inhibiting ATAD3 oligomerization results in the specific disruption of protein complexes that we have identified to depend on the presence of mtDNA and of which ATAD3A is a component.
Our studies support that ATAD3A plays a key role in cristae formation and in mtDNA stability, functions that we propose to depend on the ATPase and coiled-coil domain, respectively.

BMP pathway counteracts muscle wasting and denervation in cancer cachexia.

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Most patients with solid cancers exhibit features of cachexia, a syndrome characterized by significant loss of skeletal muscle mass and strength. As the underlying mechanisms of this multifactorial syndrome are incompletely defined, effective therapeutics have yet to be developed. We observed diminished Bone Morphogenic Protein (BMP) signaling in muscles of cachectic mice and human cancer patients. Cancer triggers in muscle the expression of the BMP inhibitor Noggin, which blocks the actions of BMPs on muscle fibers and motor nerves, causing muscle wasting, neuromuscular junction (NMJ)'s disruption and denervation. Increasing BMP signaling in the muscles of tumor-bearing animals prevents muscle wasting and preserves NMJ function. We identify perturbed BMP signaling and denervation of muscle fibers as important pathogenetic mechanisms of muscle wasting associated with cachexia, and present modulation of BMP-mediated signaling as an attractive approach for the development of interventions to address the loss of functional musculature in cancer patients.

Neuropeptide Y (NPY) promotes Cardiac Mesenchymal Stromal cell (C-MSC) adipogenic differentiation in Arrhythmogenic Cardiomyopathy (AC).

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AC is a genetic cardiac disease, mainly caused by mutations in desmosomal proteins, at risk of sudden death in athletes. The disease displaces cardiomyocyte death and fibro-fatty myocardial replacement. AC pathogenesis is poorly understood and no effective therapies are available. Recently, it has been demonstrated that C-MSCs are the adipogenic source in AC, but the molecular mechanisms mediating their transdifferentiation are still unknown.
Our hypothesis is that cardiac sympathetic neurons have a direct role in AC cardiac remodeling.
Molecular and biochemical assays demonstrated that the sympathetic neurotransmitter, NPY, promotes adipogenesis in AC, but not in control C-MSCs, isolated from human heart biopsies. AC C-MSCs express increased levels of NPY receptors, compared to controls. Live imaging experiments revealed alterations in intracellular Ca2+ dynamics in cultured AC C-MSCs, both during acute and chronic NPY stimulation.
Our data suggest that NPY may be determinant for the fibro-fatty remodeling of the AC myocardium.

PAPTP Leads to Neoplastic Cell Apoptosis in the Eμ-TCL1 Chronic Lymphocytic Leukemia Mouse Model.

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The potassium channel Kv1.3 is highly expressed in the mitochondria of patients with CLL. We demonstrated that direct inhibition of Kv1.3 using mitochondria-targeted inhibitors alters mitochondrial function and leads to ROS mediated death of even chemoresistant cells. The inhibitor PAPTP killed 98% of ex vivo primary CLL cells while sparing healthy B cells. With evaluated PAPTP effectiveness and toxicity in the Eμ-TCL1 CLL murine model, that is characterized by a high expression of TCL1 protein in B cells leading to a CLL-like disease.
After therapy, we observed an improvement of treated mice in term of appearance with respect to controls, a decrease in total lymphocyte percentage and a reduction of pathological B cells in spleen and bone marrow of treated mice. The high selectivity of PAPTP and its capability to induce apoptosis in CLL B cells also in the Eμ-TCL1 mouse model may suggest the use of this inhibitor for designing new therapeutic strategies.

4D Morphometric and Kinetic Analysis of Mitochondrial transformation.

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Mitochondria are essential organelles that provide cellular ATP by oxidative phosphorylation, and regulate housekeeping processes. They undergo dynamic morphological changing and make a complex network in eukaryotic cells. The dynamics is linked to their functional versatility so deeply that it is important to elucidate the networks and mechanisms by which they change their shape. Motor proteins (kinesin, dynein and myosin) move along the cytoskeleton with using the energy of ATP and can be linked to mitochondria via adapter proteins on the mitochondrial membrane. Mitochondria use force from the motor proteins for movement and transformation. However, dynamic model how motor proteins control and regulate the mitochondrial morphology has not established. To clear the mechanism, we performed morphometric and kinetic analysis for 4D images (x, y, z and time) of mitochondria in living cells, and try to transform and control mitocondrial shape artificially.

Early signaling events involved in muscle remodeling after exercise.

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Muscle plasticity after exercise is achieved by different molecular mechanisms that regulate gene transcription by impinging on chromatin structure and transcription factors. Which are the downstream signalling pathways involved in exercise-induced muscle remodelling is not defined yet. Here, we performed an unbiased quantitative phosphoproteomic approach in vivo to determine the early signalling changes occurring after high-intensity exercise. In preliminary analyses, we identified multiple histone modifications which are strongly increased after exercise and potentially linked to increased gene transcription. Furthermore, we identify a completely new phosphorylation of MRTF-B, a transcriptional co-activator of SRF-dependent gene transcription, which is critical for its nuclear localization. From the results obtained so far, we are proposing a model in which histone modifications and nuclear translocation of MRTF-B act in concert in the activation of SRF-dependent gene transcription, a well-known mediator of exercise-induced muscle remodelling

Monoamine oxidase-dependent oxidative stress promotes NLRP3 inflammasome activation in macrophages.

Reserved unpublished data present.

OPA1 controls muscle mass, metabolism and epithelial senescence.

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An healthy mitochondrial network is essential for post-mitotic tissues as muscle. Mitochondria-shaping machinery is downregulated in sarcopenia and is maintained with lifelong exercise. OPA1 is a pro-fusion protein that plays an important role in mitochondrial dynamics. To address it's role in skeletal muscle we generated tissues specific ko mice. Ablation of OPA1 in adulthood results in a profound atrophy that is initially mediated by mitochondrial ROS that activate a FoXOs dependent program. ROS promote also ER-stress that increased FGF21 induction. Treatment with antioxidants blunted the program and restored a normal muscle mass. In mice are present important metabolic changes, precocious senescence and degeneration of multiple organs and this phenotype is reverted by FGF21 ko. Our recent findings underline a new central role of inflammation in this scenario.

Unravelling the roles of Fission Protein 1: a forgotten mitochondrial fission factor with pleiotropic functions.

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Mitochondrial fission regulates a myriad of cellular functions, including mitochondrial clearance, and its ultimate physiological importance is underscored in devastating human disorders that arise from mutations in mitochondrial fission proteins. The machinery driving mitochondrial fission consists of the master regulator Dynamin-related protein 1 (Drp1) and its outer mitochondrial membrane adaptors, namely Fission Protein 1 (FIS1). Despite being the first proposed Drp1 receptor, FIS1 role in recruiting and regulating Drp1-mediated mitochondrial fission is highly controversial. To elucidate FIS1 role in mitochondrial dynamics, we generated a FIS1 hypomorphic mouse model, in which FIS1 levels are constitutively downregulated. FIS1 hypomorphic mice die at weaning age, due to a pleiotropic phenotype of defective growth, muscular atrophy, disseminated haemorrhages and reduced erythropoiesis and platelet formation. Using a conditionally ablation system to deplete FIS1 from megakaryocyte-erythrocytes precursor cells or from specific tissues will help us pinpoint FIS1 essential roles in vivo.

Distinct pluripotency states in human stem cells are characterized by peculiar extracellular matrix organization and intra-colony cell heterogeneity.

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Pluripotency is a transient state evolving during embryo development in vivo, while in vitro two distinct pluripotency states can be isolated: a more immature state (naïve), similar to pre-implantation embryo, and a more mature state, (primed), resembling the post-implantation embryo. Those states are characterized by different metabolic and gene expression profiles, involving different extrinsic signaling pathways.
Microfluidic devices are a useful tool to study cell milieu because they provide confined environment that promotes exogenous factor accumulation and paracrine signaling.
By analyzing primed and naive embryonic and induced pluripotent stem cells, we observe state-specific extracellular matrix (ECM) deposition that correlates with the 3D organization of the colonies. Moreover, we report intra-colony cell heterogeneity in matrix deposition linked to state-specific pluripotency markers expression. Starting from these data, by coupling CRISPR-KO lines for ECM components and antibody-based ECM-integrin interaction inhibition, we aim at elucidating ECM role in pluripotency.

PPM1K, novel insights in connecting metabolism and autophagy in the heart.

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Metabolic homeostasis is an integral part of cardiac function and mitochondria are the main sources of metabolites and energy in this highly specialized tissue. Indeed, metabolic remodeling is a hallmark of heart failure, and although there are recent insights on the impact of fatty acids1 and carbohydrates2 in it, aminoacids metabolism has not yet been studied extensively in this context. Additionally, maintaining a healthy pull of mitochondria in the cell by autophagic degradation of the dysfunctional ones is crucial to cardiomyocyte viability. Data from our lab suggest that there is a bidirectional regulation between PPM1K, a mitochondrial matrix protein phosphatase involved in branched chain aminoacid (BCAA) catabolism3,4 and autophagy. Interestingly, PPM1K is highly expressed in cardiac tissue besides low BCAA catabolism levels, and its ablation leads to cardiac impairment5,6. Thus, we are testing the hypothesis that the PPM1K-dependent regulation of autophagy impacts in cardiac physiology.

Crosstalk between T lymphocytes and monocytes in Large Granular Lymphocyte Leukemia.

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Pro-inflammatory stimuli have been demonstrated to mediate T large granular lymphocytes (LGL) proliferation in T-LGL Leukemia (T-LGLL). In terms of pro-inflammatory cytokines, higher amount of IL-6 and CCL5 have been found in patients' plasma than controls, but no data on the relationship between these cytokines have been reported yet. We identified LGLs as the main source of CCL5 and patients' monocytes were demonstrated to express high amount of IL-6 and CCR5, one of CCL5 receptors. In vitro stimulation of T-LGLs with IL-6 induced an increased CCL5 production, which in turn stimulated monocyte to transcribe IL-6. Interestingly, LGL patients were divided in two groups: one characterized by higher level of CCR5 and IL-6 in monocytes' patients and one by amount consistent with controls. These findings suggests that, in a subset of patients, T-LGLs might fuel the production of pro-inflammatory cytokines important for their survival.

Optogenetic modulation of the adrenergic component of motor nerves to understand the mechanisms of muscle atrophy and neurodegeneration in Amyotrophic Lateral Sclerosis (ALS).

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ALS is a neuromuscular disorder, characterized by motor neuron (MN) degeneration, muscle atrophy and weakness, leading to death within four years after diagnosis. ALS pathogenesis is unclear and no treatments are available.
Our hypothesis is that sympathetic neurons (SNs) innervating skeletal muscles, as intrinsic components of the motor nerves, modulate myocyte β2-ARs through local release of NE, leading to repression of muscle proteolysis and enhanced neurotrophin synthesis, thus contributing to muscle and MN viability.
Here, we combined sympathetic neuron optogenetics with muscle cAMP imaging in living mice, to assess neuro-effector coupling between the SNs of the sciatic nerve and the hindlimb myocytes. We thus demonstrated, for the first time in vivo, the role of the adrenergic component of the motor nerve in the direct control of muscle cAMP. Consistently, the effect of photostimulation was blunted by the β-blocker, propranolol.
Chronic optogenetics-based SN stimulation will assess whether neuronal activity influences muscle trophism.

Micropatterning technology applied in ESCs fate decision.

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We wish to introduce a robust micro-technology applied in embryonic stem cell culture and also cell differentiation. With this technology cells are confined to different disk-shaped, submillimeter colonies ranger from 62.5 m to 1000 m[1]. As cell fates are defined relative to the boundary with fixed length scale, our disk-shaped micropatterns are designed with the hope to reveal the influence of geometric confinement[2]. We have already developed the basic protocol to form 26 different micropatterns and it was observed that colonies of different sizes gave rise to different proportions of cell fates decision.

[1] Warmflash, A., et al., A method to recapitulate early embryonic spatial patterning in human embryonic stem cells. Nature methods, 2014. 11(8): p. 847-854.
[2] Deglincerti, A., et al., Self-organization of human embryonic stem cells on micropatterns. Nat Protoc, 2016. 11(11): p. 2223-2232.

The mitochondrial shaping protein Optic Atrophy 1 (OPA1) controls the proangiogenic potential of breast cancer cells.

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Breast cancer is the most frequently diagnosed cancer in females worldwide (one out of eight); over the last two decades, the incidence has considerably increased. Highly metastatic, these tumor cells secrete cytokines in order to induce angiogenesis, the formation of news blood vessels form pre-existing ones. Once blood vessels are formed, tumor cells start to migrate and reach the circulation to form metastases. While it has been shown that the multifunctional inner mitochondrial membrane mitochondrial fusion protein Optic Atrophy 1 (OPA-1) is placed at the crossroad of fusion, cristae biogenesis, metabolism, apoptosis, regulation of cardiomyocyte differentiation and migration lymphocyte tumoral cells, the role of Opa1 in the ability of tumoral cells to induce angiogenesis has not been addressed. Here we show that Opa1 is a crucial component of the angiogenic program. Genetic tumoral Opa1 ablation signals retrogradely from mitochondria to nucleus to modify angiogenic genes expression and therefore inhibit the proangiogenic potential of tumor cells. Thus, Opa1-dependent mitochondrial dynamics is a targetable component of tumor development and angiogenesis.

Predicting dementia in Parkinson Disease from MRI: a preliminary study.

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The scientific literature on Parkinson Disease (PD) shows that 24% to 70% of patients can develop dementia. In the present study we aim to predict PD-related dementia 2 years before its onset from MRI data. We analyzed MRI scans of 144 PD patients from the Parkinson's Progression Markers Initiative dataset (62 developed dementia 2 years after and 62 did not develop it). A Support Vector Machine (SVM) classifier was trained on a set of smoothed, Gray-Matter segmented MRI scans, focusing on specific ROIs (temporal, frontal, parietal and occipital lobes and basal ganglia), through a searchlight approach. The algorithm was trained on half participants and tested on the other half. Results showed the highest accuracies in temporal and occipital lobes (max. accuracy 80-82%; ps<.001). Our preliminary findings suggest that in the future multivariate analysis of GM volume could be a potential tool for early-diagnosis of dementia in PD patients.

Impact of a moderate caloric and protein restriction in patients with Barret's esophagus: microbiota composition and insulin signal analysis.

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An increasing amount of evidence suggests central obesity as a risk factor for Barrett's Esophagus (BE) development (pre-cancerous lesion) and esophageal adenocarcinoma. Central obesity is associated with insulin-resistance and hyperinsulinemia. Obesity is also related to resident microbiota modification.
The aim of this study was to evaluate the metabolic status of overweight BE patients included in a lifestyle-diet interventional program. The impact of the intervention was assessed through anthropometric parameters and quantification of serum analytes related to insulin-resistance. The intervention promoted weight loss and improved insulin-resistance in 50% patients that concluded the study as confirmed by decreased insulin levels and Akt esophageal activation.
Moreover, microbiota analysis showed a significant improvement. Streptococcus increased in 31.6% patients; Prevotella and Veillonella decreased in 52.6% and 21.0% patients, respectively; Actinomyces and Rothia decreased in 26.5% patients. Changes were observed in patients who decreased their insulin secretion, likewise their anthropometric parameters.

Novel preservation methodologies for decellularized cardiovascular scaffolds.

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Development of long-term preservation methods for cardiovascular grafts is of major importance to prolonging their shelf-life. The objective of this study was to evaluate the suitability of slow-freezing-rate cryopreservation, vitrification, and freeze-drying, for preservation of decellularized bovine pericardial (DBP) scaffolds. Following decellularization, DBP samples were subjected to either slow-freezing-rate cryopreservation, vitrification or freeze-drying. The impact of the preservation methods on the structural integrity of the scaffolds was assessed using histology and uniaxial tensile testing. FTIR was used to study the overall protein secondary structure and DSC was used to determine thermal protein denaturation profiles. Uniaxial tensile testing studies indicated that vitrification and freeze-drying did not modify the biomechanical behaviour of the scaffolds. Histology indicated no differences in the gross histoarchitecture after any of the preservation procedures, and also proteins were found to be stable. It is suggested that freeze-drying could replace currently used cryopreservation approaches for preservation of decellularized scaffolds.

Macrophages release ATP and propagate calcium signals to support phagocytosis.

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Extracellular ATP is a signalling molecule exploited by the immune cells for both autocrine regulation and paracrine communication. By performing live calcium imaging experiments we show that triggered mouse macrophages are able to propagate calcium signals to resting bystander cells by releasing ATP. ATP-based intercellular communication is mediated by P2X4 and P2X7 receptors, and is a feature of pro-inflammatory macrophages. In terms of functional significance, ATP signaling is required for efficient phagocytosis of pathogen-derived molecules, and represents a novel target for the immunosuppressive activity of regulatory T cells, which degrade extracellular ATP and inhibit phagocytosis. These results highlight a new mechanism of cell-to-cell communication regulating innate immunity.

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