

RESEARCH REPORT 2021



FONDAZIONE
RICERCA BIOMEDICA
AVANZATA

V.I.M.M.



RESEARCH REPORT 2021

FONDAZIONE PER LA RICERCA BIOMEDICA AVANZATA ONLUS
VIMM - VENETO INSTITUTE OF MOLECULAR MEDICINE

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PADOVA AND ITS RESEARCH AREA

FRANCESCO PAGANO

President of Foundation for Advanced Biomedical Research

The idea to create the Veneto Institute of Molecular Medicine (VIMM) was prompted by the rapid and profound evolution of research in biology and medicine. This can be traced back to dramatic advancements in related disciplines, of which molecular biology has been the most important.

The most significant consequence of this evolution has been the possibility of rapidly transferring the results of basic research to the clinic. To facilitate this transfer - that is now commonly defined as “translational research” - it is essential to operate in a structure devoted to establish a close collaboration between basic and clinical research.

This permits the continuous interchange of ideas between the two components, and eventually fosters the development of research lines capable of piloting research towards clinical objectives in real time. A corollary of these ideas, that ultimately led to the creation of the VIMM, has been the awareness that the best way to maintain an optimal level of bedside assistance, and even to improve it along the lines of modern frontiers in medicine, is an active role for physicians in research.

Taking all these factors into account, and supported by the existence in Padova of an environment of high-level biological and clinical research such as the University-Hospital complex, we felt morally obliged to invest time and energy to bring into being a structure capable of responding to the modern requests of biomedical research.

We had the good fortune of working in the north-east of Italy and this has been the most important factor in giving substance to our hopes, since it gave us a dual advantage: the possibility of raising substantial resources from a private sector that is avant-garde in Italy and, most importantly, a sector that is mentally open to the social and economic aspects of innovative enterprises.

Based on all these factors, our initiative was crowned with the creation of the Foundation of Advanced Biomedical Research which has attracted contributions from industrialists and banks of the Veneto Region, from the City and the Province of Padova, and from the Regione Veneto. The Foundation operates in close collaboration with the University of Padova and the Hospital, an example of successful integration between the private and the public sector. The Foundation has brought to life the Venetian Institute of Molecular Medicine (VIMM), which is a structure of translational research with over 3000 square meters, where over 180 basic and clinical scientists currently operate in close day-to-day collaboration. Their interaction is fostered by meetings, seminars, and informal discussions which generate ideas for new research lines, for the better integration of those already in progress, while having in mind the final goal of applying results to the clinic: it is indeed an institute whose motto could well be “From bench to bed”.

PREFACE

GIANPIETRO SEMENZATO

VIMM Scientific Director



VIMM is the research institute of the Foundation for Advanced Biomedical Research, a non-profit organization headquartered in Padua, that promotes cutting-edge research in biomedicine. VIMM is a unique multidisciplinary research institute devoted to conducting, supporting, and encouraging applied research with a tangible impact on the progress of medicine and human health. The Institute is accomplishing its mission by coupling excellent basic and translational research and at the same time fostering the career of several young independent investigators. VIMM is strategically located in an academically and research rich area close to the Life Sciences complex of the Padua University School of Medicine and its University Hospital, thus encouraging collaborative relationships with many scientists involved in basic science and clinical activities.

VIMM is built upon a 25-year history of innovation and sustained excellence, with an active intellectual community that spans multiple departments and colleagues throughout the University of Padua. We are home to 20 tenured and research faculty, and more than 150 graduate and undergraduate students coming from 18 countries, hosted in fully equipped open space labs. Driven by commitment, curiosity and passion our scientists conduct forefront research thus making VIMM a leading Institute in the Italian biomedical science scenario with a prominent international standing in its core domains: mitochondrial dynamics in medicine, cardiovascular and muscular pathobiology, biology and immunology of cancer, neuroscience and neurodegeneration, metabolism and bioengineering. These research areas share underlying mechanisms consisting of molecular alterations that we plan to investigate with the ultimate goal of making these abnormalities druggable targets, according to the current concept of personalized medicine.

As scientific director of VIMM, I envision that tomorrow's challenges in terms of research and public health demand novel and unconventional approaches that dissolve the boundaries of classic disciplines defying today's traditional academic settings. The future of medicine lies in the development of new knowledge and technology as well as its rapid translation into patient care. With this as a background, faculty at VIMM works with a spirit of innovation and corporate identity on up-to-date problems set at the intersection of medicine, biology, physics, bioinformatics, and engineering.

This booklet includes the description of latest research activities of the different teams currently working in the Institute detailing their projects, achievements and future plans. In the brief scientific reports that follow you can have a glimpse of the quality of research carried out at VIMM by our scientists, internationally renowned and recognized leaders in their field.

The integration of public and private institutions was instrumental to support VIMM both for the establishment of the Institute and for keeping the research going all the past years. For this reason let me close by acknowledging the generous financial support provided by a number of funding agencies and private donors without which none of the projects and accomplishments herein reported would have been possible. On behalf of VIMM researchers I also want to thank the governance, the administrative and technical staff of Foundation for Advanced Biomedical Research for the availability, dedication and efficiency with which they carry out their daily tasks. Their contribution, in synergy with researchers, is central for the high-level scientific ranks achieved by VIMM.



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ANDREA ALIMONTI

SENESCENCE THERAPIES IN AGING AND CANCER UNIT



Biographical Sketch

- 2020 to date Full Professor of Experimental Oncology and Translational Cancer Medicine, ETH Zurich, *Switzerland*
- 2017 to date Full Professor of Oncology, Università della Svizzera Italiana, *Lugano, Switzerland*
- 2017 to date Full Professor of Pharmacology, University of Padua, *Italy*
- 2017 to date Group Leader, VIMM, *Padua, Italy*
- 2010 to date Group Leader, Institute of Oncology Research, *Bellinzona, Switzerland*
- 2007-2009 Postdoctoral Fellow, BIDMC-Harvard Medical School, *Boston, USA*
- 2004-2007 Postdoctoral, Memorial Sloan-Kettering Cancer Center, *New York, USA*



Group Members

- Monica Montopoli - *Professor, Associate Investigator*
- Silvia Bressan - *PhD Student*
- Liliana Contu - *Research Assistant*
- Federico Gianfanti - *PhD Student*
- Luisa Maraccani - *PhD Student*
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- Saman Sharifi - *PhD Student*
- Sara Zumerle - *Postdoctoral Fellow*
- Nicoletta D'Elia - *Administrative Assistant*

RESEARCH ACTIVITY

Fields of interest and ongoing research

Our laboratory mainly focuses on characterizing and subsequently manipulating pathways involved in the induction of senescence as a novel therapeutic approach for the treatment of PTEN-deficient prostate tumors.

Prostate cancer is the second commonest malignancy in men worldwide and the second commonest cause of male mortality. Patients with metastatic prostate cancer, specifically those with disease progression following primary androgen ablation therapy and addition or withdrawal of an anti-androgen, are generally considered to be refractory to hormonal therapy. The treatment of castration-resistant prostate cancer (CRPC) remains unsatisfactory. Unfortunately, chemotherapy can only marginally improve patient survival. Patients with hormone-refractory prostate cancer have, thus far, not been cured with either hormonal treatments or chemotherapy. It is hoped that the development of novel targeted therapies and immunotherapies will improve the outcome of patients with androgen refractory diseases. PTEN is one of the most frequently altered tumor suppressor genes in prostate tumors and PTEN loss is often associated to both chemo- and radiotherapy resistance in prostate cancer patients. Despite the high incidence of PTEN mutations or deletions, a treatment that targets prostate tumors harboring PTEN alterations still does not exist.

Our major objective is to identify novel treatment modalities for the therapy of PTEN deficient prostate cancer.

Cellular senescence is a stable cell growth arrest that occurs in tumor cells subjected to different stresses including treatment with chemo-/radiotherapy or targeted therapies. Several findings *in vivo* demonstrate that senescence limits tumor progression. Although arrested, senescent tumor cells remain metabolically active and secrete a variety of cytokines and inflammatory factors known as the senescence-associated secretory phenotype (SASP). Tumor cells' SASP can activate the tumor immune response and promote the clearance of senescent tumor cells, a phenomenon referred as senescence surveillance. However, we have recently found that tumor-infiltrating myeloid cells can hinder senescence surveillance by inhibiting the proliferation and function of T cells. In these tumors, remaining senescent tumor cells, through the SASP, can support tumor growth by increasing angiogenesis, cell migration, invasion and even metastasis. Thus, removal of senescent cells (senolytic therapy) has been proposed as a strategy to improve the efficacy of currently available treatments in tumors where immunosuppression hinders the clearance of senescent cells. Nowadays, there are few examples of effective senolytic compounds for cancer therapy. However, their efficacy is variable and it depends by the genetic background of the senescent tumor cells, being effective in certain type of senesce response but not in others.

Our first hypothesis is that eradication of senescent cells

by “senolytic” compounds, may improve the efficacy of clinically available chemotherapies. Our second hypothesis is that pro-senescence compounds that enhance “senescence surveillance” improve the efficacy of checkpoint inhibitors by boosting the tumor innate and adaptive immunity.

Future research plans

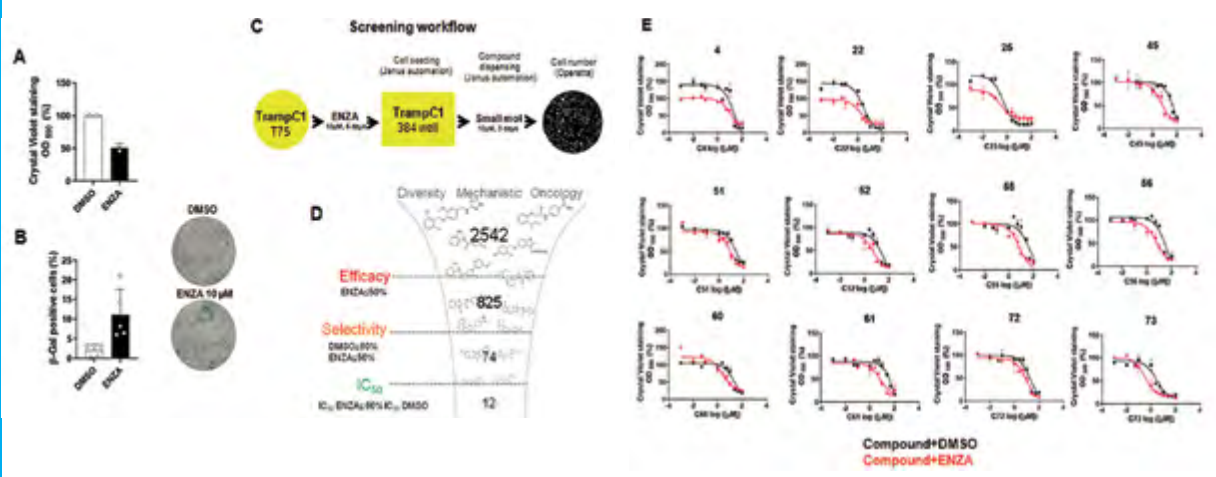
- 1) To identify pro-senescent compounds that trigger a positive anti-tumor immune response in PTEN null prostate tumors.
- 2) To identify small molecules that can selectively eliminate senescent-tumor cells.
- 3) To implement, in tandem, a pro-senescence approach followed by senolytic therapy.
- 4) To combine pro-senescent compounds with checkpoint inhibitors.

By screening 5000 small molecule inhibitors in PTEN null cells, we have identified a number of clinically available compounds that can induce senescence in tumor cells without affecting normal cells (**Fig. 1**).

We are now investigating the mechanism of action of the identified pro-senescence compounds and characterizing their SASPs to understand whether the treatment with these pro-senescence leads can reprogram the chemotherapy-induced SASP and stimulate an anti-tumoral immune response. We are running pre-clinical trials *in vivo* to assess their efficacy and their impact on the tumor immune response.

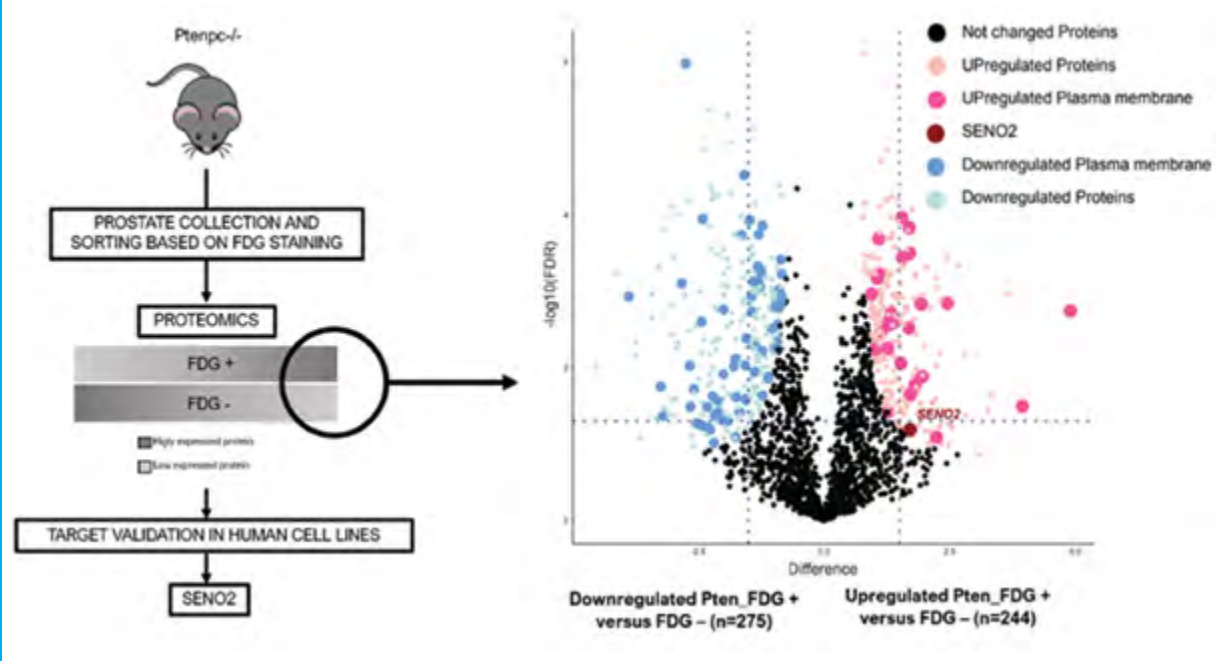
On the other hand, we are developing novel senolytic therapies for chemotherapy-treated prostate cancers: we are running a screening of small molecules to identify novel senolytic compounds that kill PTEN null senescent tumor cells, and we are developing a new drug delivery system to target senescent tumor cells. Preliminary data from a proteomic and bioinformatic analysis comparing senescent and non-senescent cell populations in the tumor allowed us to identify membrane proteins specifically upregulated in the senescent tumor cells (**Fig. 2**).

Figure 1



Screening of small molecules that could selectively kill ENZA-senescent PCa cells. A) Proliferation (crystal violet) and B) senescence (β-Gal) of TrampC1 cells treated for 6 days with Enzalutamide 10 μM. C) Screening workflow. D) Screening platform with selection criteria plus positive hits in each phase. E) Concentration-response for the 12 selected hits.

Figure 2



Proteomic analysis and identification of senolytic targets. Membrane proteins upregulated in the senescent population compared to the non-senescent one with a factor higher than 1.5 were considered as a possible target for further analysis.

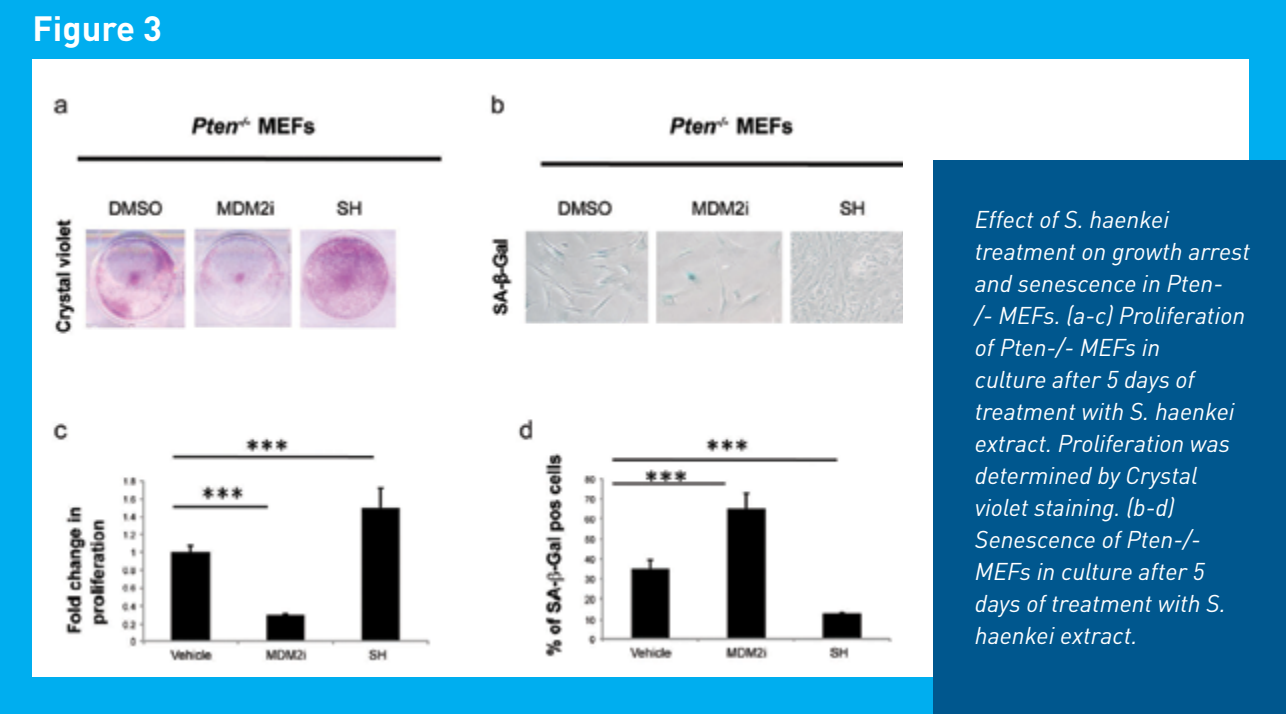
Thus, we are exploiting these targets to develop immunoliposomes that selectively target senescent tumor cells for senolysis.

Another field of interest of our research is the identification of gerosuppressant agents for the treatment of aging-related disorders.

Aging can be described as a progressive loss of physiological strength, leading to impaired body function and increased vulnerability to death, hence it can be considered as a primary risk factor for main aging-related disorders such as inflammatory diseases, cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases. Cellular senescence, genomic instability, telomere attrition, epigenetic alterations, and mitochondrial dysfunction can be mentioned as hallmarks of aging. Targeting these hallmarks could significantly improve health in the studied models.

With ageing, senescent cells accumulate in different tissues and can cause damages due to their secretome components. We aim to discover novel anti-senescence or senolytic agents via high-throughput screening of natural compounds libraries.

We have identified *Salvia haenkei*, a native plant of Bolivia, having anti-senescence and anti-aging activity (**Fig. 3**), and we are running the pre-clinical validation of *Salvia haenkei* extract as anti-aging agent.



Our results show that *Salvia haenkei* extract can ameliorate aging parameters, animals' life expectancy, tumors incidence, and bone and joint health in naturally aged mice.

Recently, we have also identified a natural compound having strong senolytic activity in different types of senescence models. We are now studying its chemical structure to investigate the active metabolites of this molecule which are leading to senescent cells death. Moreover, we are investigating its mechanism of action, and performing the *in vivo* studies to determine the toxicity and anti-aging effect of the compound alone and of the whole extract of the plant it comes from.

2020 and 2021 have been years dominated by the Covid-19 pandemic. Starting from the intriguing fact

that TMPRSS2, a human protease required by influenza and corona viruses to infect human healthy cells, is a known genetic and pharmacological target in prostate cancer, and that the androgen receptor (AR) regulates TMPRSS2 expression in non-prostatic tissues, including lung, our group has started investigating if AR antagonists could be used to block or decrease the severity of SARS-CoV-2 infection in male patients. In collaboration with the Veneto Tumour Registry, we have run a population-based study that has shown that cancer patients have an increased risk of SARS-CoV-2 infections compared to non-cancer patients. However, prostate cancer patients receiving androgen deprivation therapy (ADT) appear to be partially protected from SARS-CoV-2 infections (see the accompanying **Table**).

Table

	PCa in ADT	PCa non-ADT	OR (95% CI)	Other tumors	OR (95% CI)
Cancer patients in Veneto Region population	5273	37 161 ^a		84934 ^a	
Total no. of SARS-CoV-2-positive	4	114	4.05 (1.55–10.59) <i>P</i> = 0.0043	312	4.86 (1.88–12.56) <i>P</i> = 0.0011
Mild disease	3	83	3.93 (1.31–11.77) <i>P</i> = 0.0144	223	4.62 (1.56–13.69) <i>P</i> = 0.0057
Non-hospitalized	1	7		9	
Hospitalized	2	76		214	
Severe disease	1	31	4.40 (0.76–25.50) <i>P</i> = 0.0982	89	5.53 (0.97–31.58) <i>P</i> = 0.0544
Intensive care (ICU)	1	13		32	
Deceased	0	18		57	
Estimated total SARS-CoV-2-positive cases/100 000	76	307		367	

Epidemiological characteristics of prostate cancer patients with confirmed SARS-CoV-2 infection in Veneto, treated with or without androgen-deprivation therapy.

We have then extended our interest to explore the implications of hormonal regulation in Covid-19 in the female population. Our results have shown that female cancer patients have an increased risk of SARS-CoV-2 infection and develop more severe forms of Covid-19, in line with

the previous findings in men. However, ablation of estrogens in these patients reduced prevalence Covid-19 prevalence, suggesting that the use of selective estrogen receptor modulators (SERMs) in the treatment of Covid-19 patients may represent a possibility.

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MILENA BELLIN

STEM CELLS TO STUDY AND CORRECT CARDIAC DISEASES UNIT



Biographical Sketch

Since Oct 2021	Full Professor of Genetics, Dept. Biology, University of Padua, <i>Italy</i>
2019-2021	Assistant Professor, Dept. Biology, University of Padua, <i>Italy</i>
2019 to date	Group Leader, VIMM, <i>Padua, Italy</i>
2016 to date	Assistant Professor, Dept. Anatomy and Embryology, Leiden University Medical Center (LUMC), <i>The Netherlands</i>
2019-2020	Managing Editor, Stem Cell Reports
2020 to date	Member, International Society Stem Cell Research
2014-2016	Senior Researcher, LUMC, <i>The Netherlands</i>
2012-2014	Marie Curie Fellow, LUMC, <i>The Netherlands</i>
2008-2010	Postdoctoral Fellow, Cardiology Division, Technical University of Munich (TUM), <i>Germany</i>



Group Members

Daniele Ottaviani - *Postdoctoral Fellow*
Federica D'Ettorre - *Postgraduate Fellow*

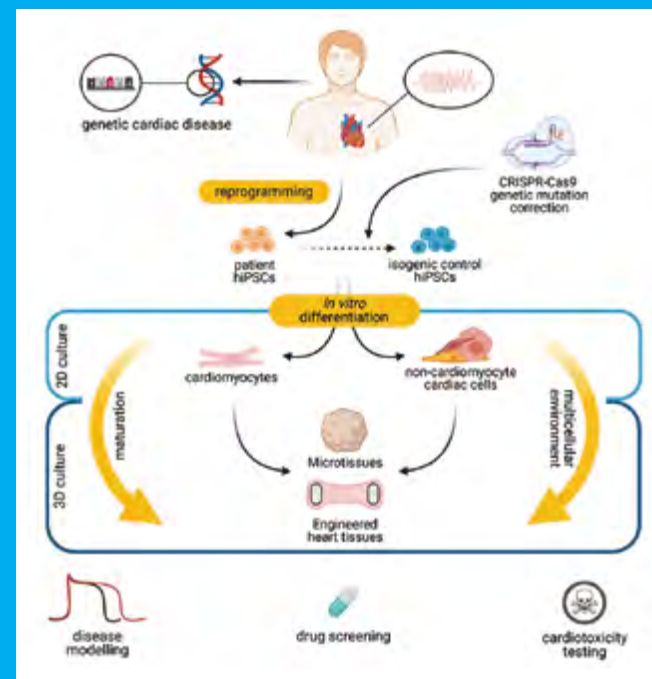
RESEARCH ACTIVITY

Fields of interest

Cardiovascular diseases are the leading cause of death globally, but predicting arrhythmia and heart failure, as well as understanding the underlying genetic and cellular mechanisms, are still a big challenge in medicine. Human induced pluripotent stem cells (hiPSC) are generated from somatic tissue of patients and capture their genetic background. hiPSC can assist the study of human disease and development of new treatments. Our research is focused on using hiPSCs from patients with inherited cardiac diseases, to understand the mo-

lecular mechanisms underlying the pathological condition with view to develop new possible treatments. We use the recent technology of CRISPR/Cas9 to precisely modify hiPSC genome at the mutation sites and create cell lines that are identical (isogenic) with the patient hiPSCs but corrected for the specific mutation. Using this strategy, we are able to dissect the mutation contribution to the disease, to identify the molecular mechanisms underlying the pathological changes, and to test the effect of drugs (**Fig. 1**).

Figure 1



Overview of Bellin's lab research.

The heart is a complex tissue composed by several cell types including the contractile part of the heart (cardiomyocytes), but also by the other cardiac cell types, like vascular and stromal cells. We have recently developed a three-dimensional (3D) *in vitro* cardiac system called "cardiac microtissue" or "mini-heart", composed by cardiomyocytes and the two main non-myocyte cells types, endothelial cells and cardiac fibroblasts, to more accurately reproduce the multicellular organization and the dynamic function of native heart (Fig. 2). These constructs will assist cardiovascular disease modelling and drug screening.

Figure 2

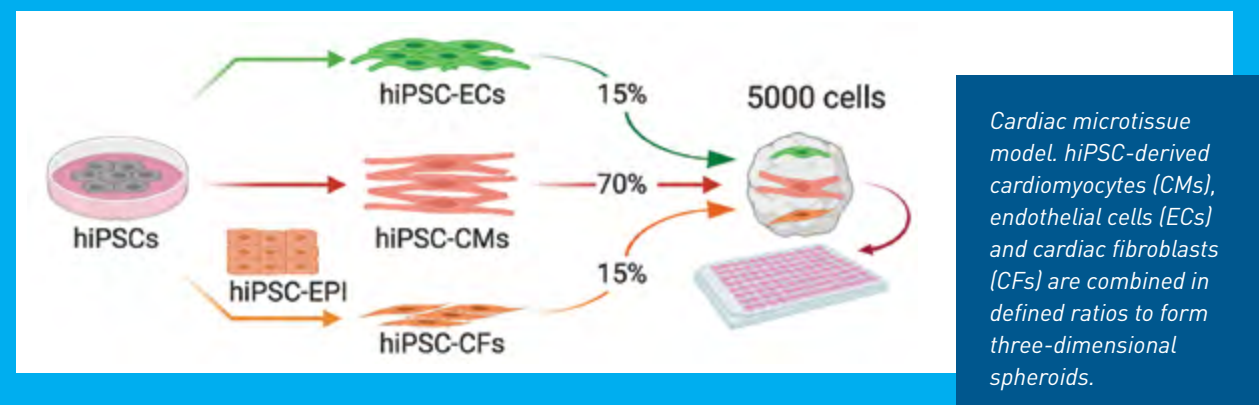
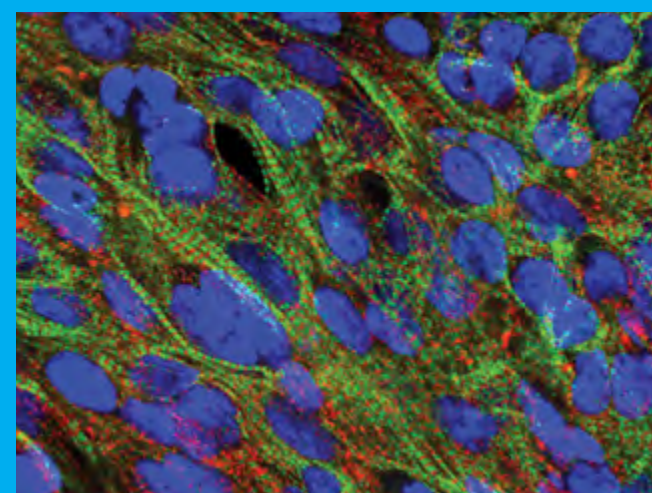


Figure 3



Sarcomeric structure within cardiac microtissue.

Arrhythmogenic cardiomyopathy is a rare disease which causes sudden death in young individuals. By combining different cell types carrying the mutation or cells in which the mutation was corrected by CRISPR/Cas, we form the 3D-multicellular microtissues. We analyse their gene expression signature by RNA-seq and look at their contraction properties. For example, we showed that when we included in microtissues stromal cells from patients with arrhythmogenic cardiomyopathy, this was sufficient to generate arrhythmia even though the cardiomyocytes were healthy (i.e. without the mutation). We are currently studying in more depth the mechanism.

Cardiomyocytes from hiPSCs are typically immature; another advantage of the cardiac microtissue constructs is the enhancement of myocyte maturation. Enhanced maturation was detected at the structural (Fig. 3), functional and gene expression level in the typically immature hiPSC-derived cells, which lays the foundations for studying cardiac diseases that are only evident postnatally. With this system we are now studying mutations in ion channels causing cardiac disease and subjected to developmentally regulated expression. Mitochondrial diseases are a group of genetic disorders characterized by defects in the respiratory chain. Friedreich's ataxia is one such condition that causes severe cardiomyopathy in more than half of the

patients. We use hiPSCs from patients and CRISPR/Cas gene correction to understand the link between energy defects and cardiomyopathy.

Future research plans

We plan 1) to use this model to study complex and multicellular diseases of the heart; 2) to study the molecular mechanisms involved not only in inherited disease, but also in more common processes linked to aging, such as fibrosis and arrhythmia; 3) to further develop this model by including other disease modulators, such as the immune system and the nervous system; 4) to explore therapeutic rationales based on small molecules and on gene therapy.

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BERT BLAAUW

EXERCISE-INDUCED PLASTICITY OF SKELETAL MUSCLE MASS AND FUNCTION UNIT



Biographical Sketch

- 2016 to date Associate Professor, Dept. Biomedical Sciences, University of Padua, *Italy*
- 2012 to date Group Leader, VIMM, Padua, *Italy*
- 2011-2016 Assistant Professor, Dept. Biomedical Sciences, University of Padua, *Italy*
- 2008-2011 Postdoctoral Fellow, VIMM, Padua, *Italy*
- 2005-2008 PhD, University of Padua, *Italy*
- 1997-2004 Degree in Applied Physics, University of Groningen, *The Netherlands*



Group Members

- Martina Baraldo - *Postdoctoral Fellow*
- Paula Bermudez - *Research Fellow*
- Stefano Ciciliot - *Postdoctoral Fellow*
- Georgia Dumitras - *Undergraduate Student*
- Alessia Geremia - *Undergraduate Student*
- Leonardo Nogara - *Postdoctoral Fellow*

RESEARCH ACTIVITY

Fields of interest

Adult skeletal muscle is an extremely plastic tissue, rapidly modifying its size and function in response to changes in demands. In the lab we are focusing our attention on the intracellular signaling pathways regulating increases in both mass and function of adult skeletal muscle. Considering the significant problems which arise

during aging, disuse and numerous other pathologies leading to muscle atrophy and weakness, together with the well-established beneficial effect of exercise, it is of fundamental importance to understand which pathways regulate muscle growth and how these can be linked to exercise.

Ongoing researches

We have currently three lines of research in the lab:

- Dissecting the signaling pathways and cellular processes critical for increasing adult muscle mass and force. Using various new transgenic mouse lines in combination with transcriptomics and proteomics analyses we aim to identify potential new therapeutic targets which can be used to stimulate increase in muscle mass and force.
- Determine why muscle function is impaired during cancer-dependent muscle wasting. We have observed that stimulation of specific intracellular signaling pathways can be sufficient to completely revert muscle wasting during cancer cachexia, without affecting tumor size. Furthermore, we have set up a research line evaluating functional properties in muscle fibers taken from patients with different levels of cachexia. Combining these functional analyses with different omics will allow us to identify biomarkers of reduced contractile performance in cancer patients.

- Identify the role of skeletal muscle in the systemic beneficial effects of exercise. Here we will address how muscle activity, as occurs during exercise, can affect other organs in the body. Again, using state-of-the-art transgenic mouse models we will determine which muscle-specific factors play a role in the homeostasis of other organs, and how these are modulated by activity.

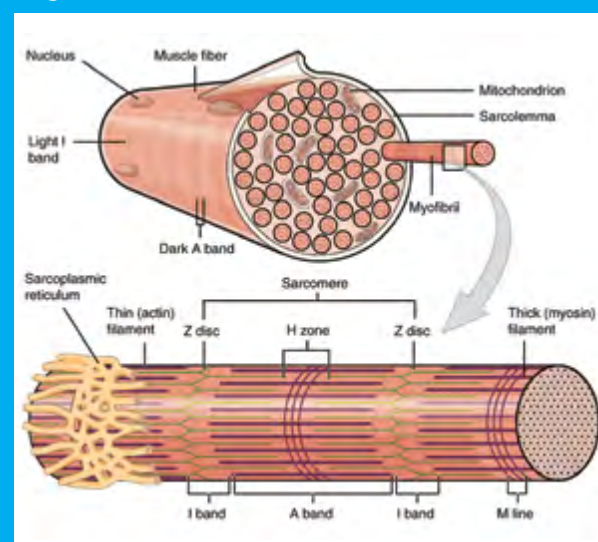
In all our projects we use a wide range of physiological and molecular biological tools to address these questions in an *in vivo* context: electroporation, *in vivo* and *ex vivo* muscle force measurements, muscle-specific transgenic and knockout models, ChIP-seq, human muscle biopsies, state-of-the-art microscopy, proteomics, transcriptomics. Many national and international collaborators provide the expertise and technical support for specific parts of the projects.

Future research plans

Mechanisms regulating adult muscle hypertrophy

To prevent skeletal muscle atrophy leading to muscle weakness, as observed in aging, neuromuscular diseases and other pathological conditions, it is essential to get a better understanding of the signaling pathways which regulate skeletal muscle mass and function. Using different newly generated transgenic mouse lines we aim to increase the understanding of the basic changes in protein translation and homeostasis which are critical for muscle growth. These new tools will allow us to better understand the answer to apparently simple questions, yet without an answer currently; 1) Which proteins are important for muscle growth/function after specific stimuli? 2) Where do they localize? 3) When are they made?

Figure 1



Basic structure of a skeletal muscle fiber highlights the extreme intracellular organization required to make a well-functioning muscle fiber.

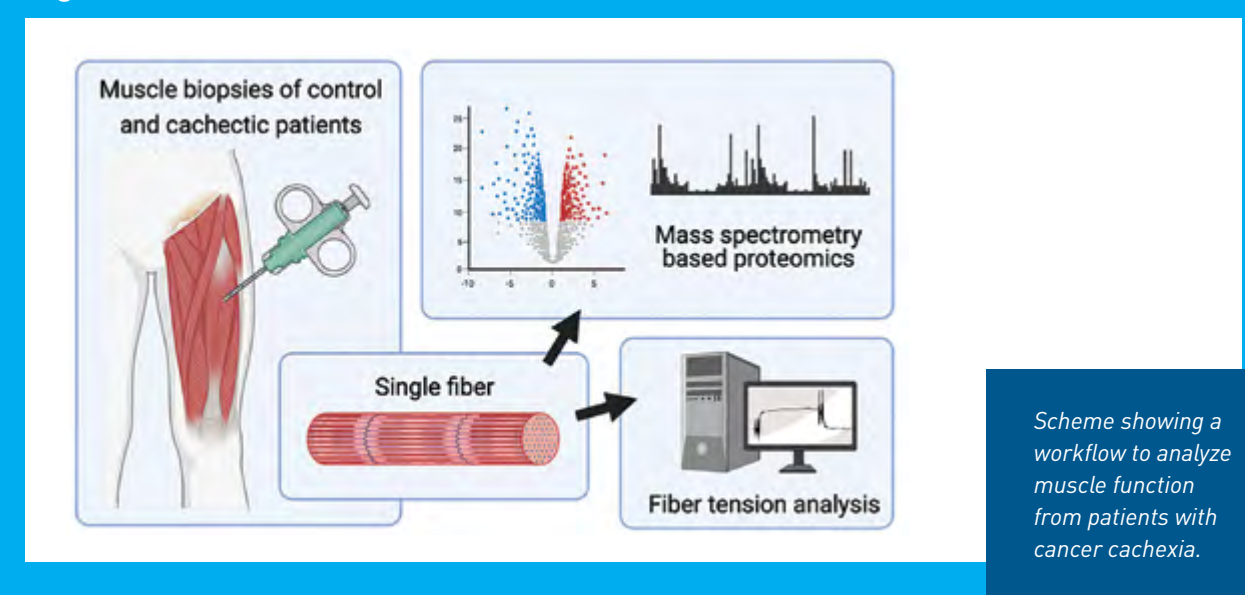
How does cancer affect muscle mass and function?

Cancer cachexia is a multi-organ syndrome which is characterized by a strong loss in body weight. It occurs in 50-80% of cancer patients and is due to a drastic loss in muscle and adipose tissue. As cancer cachexia leads to a decrease in physical performance and quality of life, and is associated with poor survival (accounting for more than 20% of cancer deaths), it is of major clinical relevance. Furthermore, cachectic patients show lower response rates to chemotherapy and a reduced tolerance to anticancer treatment. However, despite its clinical importance and the foreseen impact on patients, the pathophysiology of cachexia-associated muscle wasting

is still poorly understood preventing the development of specific therapies. In addition, cancer treatments aiming at inhibition of tumor growth are generally given systemically and can therefore also affect the size of other organs, like muscle and adipose tissue.

We are using transgenic mouse models to determine why muscle function is impaired, and how a rescue of only muscle wasting can improve overall well-being and survival of tumor-bearing animals. We are also analyzing muscle biopsies from cancer patients to try and identify markers of muscle dysfunction.

Figure 2



Determine how exercise can affect muscle plasticity and how this leads to systemic improvements

It is well known that exercise has numerous beneficial effects, both by improving muscle performance, but also for numerous other organs. It has become clear the last years that some of the systemic beneficial effects observed after exercise are strictly linked to the changes induced in skeletal muscle. We are currently working on trying to elucidate the signaling changes in skeletal muscle that occur after different types of exercise and

how these affect muscle function. On a more systemic level, we have seen that changes in key signaling pathways in skeletal muscle are critical for the maintenance of a healthy nerve-muscle interaction. We are now further expanding this observation and are trying to identify new markers involved in the crosstalk of skeletal muscle with other organs.

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MARIO BORTOLOZZI

CELLULAR NEUROPHYSIOLOGY AND SYSTEMS BIOLOGY UNIT



Biographical Sketch

2017 to date	Associate Professor, Dept. Physics and Astronomy, University of Padua, Italy
2014 to date	Group Leader, VIMM, Padua, Italy
2012-2013	Visiting scientist, Oxford University, UK
2010-2017	Assistant Professor, Dept. Physics and Astronomy, University of Padua, Italy
2008	PhD in Neurobiology, University of Padua, Italy
2004	Master degree in Physics, University of Padua, Italy



Group Members

Erva Bayraktar, Pharmacologist - *Postdoctoral Fellow*
Diego Pigozzi Lopez, Biochemist - *Postdoctoral Fellow*
Stefano Trevisan, Engineer - *Research Assistant*
Alessandra Sabatti, Physicist - *Master Degree Student*

RESEARCH ACTIVITY

Fields of interest and ongoing researches

Degenerative neuropathies: Charcot-Marie-Tooth X-linked (CMT1X) and Parkinson's disease.

Systems biology: numerical simulations of cellular pathways and data analysis software development.

French Telethon (AFM) project: Connexin 32 (Cx32) is a fundamental protein in the peripheral nervous system since its mutations cause the X-linked form of Charcot-Marie-Tooth disease (CMT1X), the second most common form of hereditary motor and sensory neuropathy and a

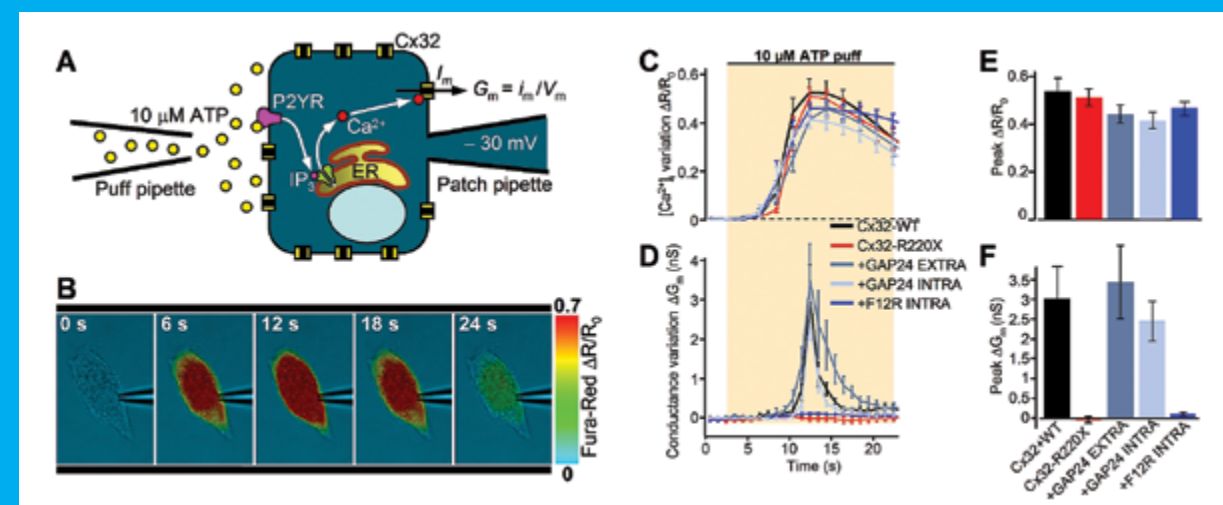
demyelinating disease. There is no effective therapy for CMT1X, but orthopaedic devices, physiotherapy, pain-killing drugs and even surgery can help individuals to cope with the disabling symptoms of the disease. Drawing on state-of-the-art technology and a combination of Physics and Biology expertise, this AFM project aims to provide crucial molecular information about the function of Cx32 in the peripheral nervous system and to explore novel pathways to the development of treatments that will benefit patients affected by CMT1X disease.

Research project of National Relevance (PRIN) project: Increasing evidence suggests that Parkinson's disease (PD) might be driven by lysosomal dysfunction with cellular waste-clearing. We aim to investigate this hypothesis using PD patient-derived brain organoids, a new three-dimensional tissue culture platform that have recently emerged to study neuronal and glial properties in physiological and pathological conditions. Altered structure and functionality of the organoids can be unveiled both at single cell and network level using a combination of advanced electrophysiological and fluorescence optical microscopy equipment available at VIMM. The multidisciplinary nature of this new research project is evident from the broad range of expertise of the groups and Italian centres involved: VIMM, University of Milan, Policlinico of Milan, Humanitas University.

Best results obtained

Recent work, carried out also by our group, suggests a new paradigm for the molecular pathogenesis of CMT1X as being no longer linked to Cx32 GJs but rather to Cx32 hemichannels, which could regulate the ATP-mediated paracrine signaling that is critical for the myelination process. Demonstrating this hypothesis would strongly focus the therapeutic strategy on a class of chemical and biological agents (peptides and antibodies) which can selectively bind mutant Cx32 hemichannels modulating their gain/loss of function. The known R220X mutation was selected as an animal model benchmark to investigate the pathogenesis of CMT1X and to develop a therapeutic strategy based on a mimetic peptide (GAP24) which was able to restore the inhibited function of R220X hemichannels expressed *in vitro* (Fig. 1).

Figure 1

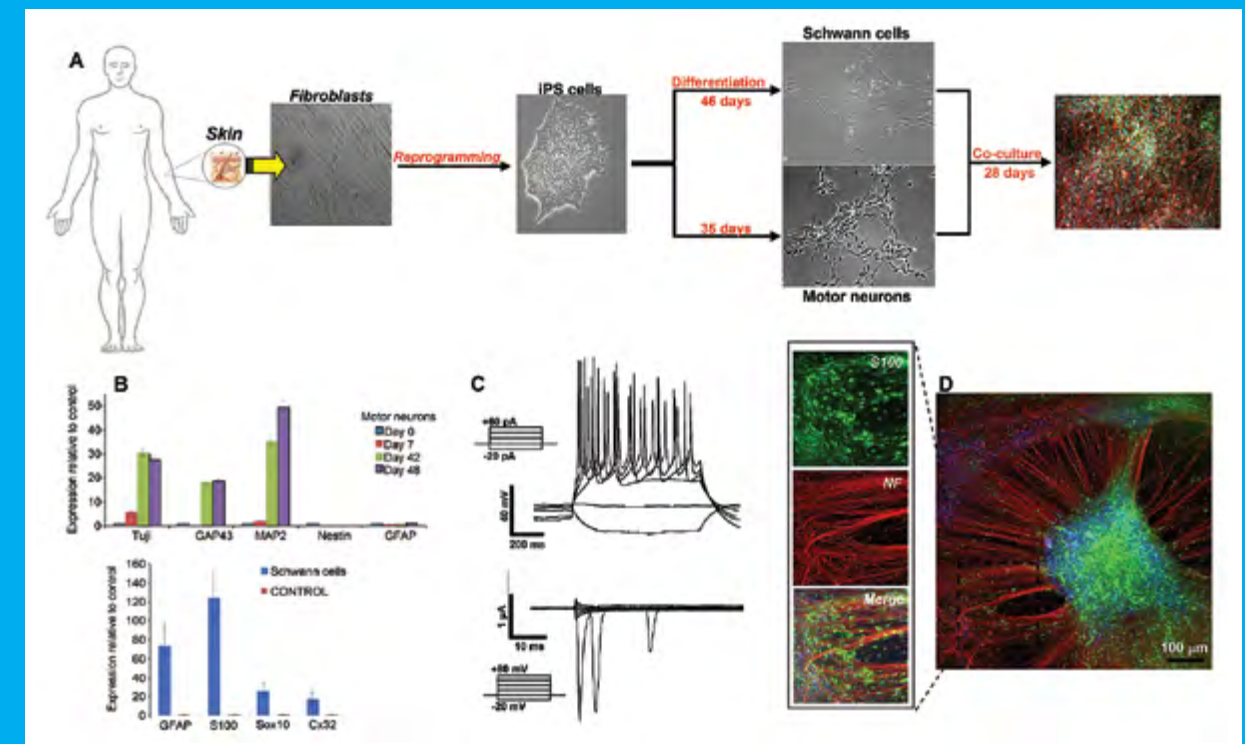


GAP24 peptide restores the cytosolic Ca^{2+} -dependent gating of human Cx32-R220X hemichannels. (A) Scheme of single patch clamp experiment to study the hemichannel gating by the cytosolic Ca^{2+} ([Ca^{2+}] [Bortolozzi, 2018]) in a single HeLa cell transfected with wild-type (WT) or mutant (R220X) Cx32. An IP₃-dependent [Ca^{2+}]_i transient is stimulated by an extracellular puff containing 10 μ M ATP. Opening and closure of Cx32 hemichannels is monitored by deriving the membrane conductance G_m from the membrane current i_m , having blocked contributions from other Ca^{2+} -activated channels. (B) Representative frame sequence of an experiment using fura-red dye to monitor [Ca^{2+}]_i (encoded as $\Delta R/R_0$) in a HeLa cell transfected with Cx32-WT. (C-D) Average [Ca^{2+}]_i transients and membrane conductance variations ΔG_m stimulated by ATP in Cx32-WT (black trace) or Cx32-R220X transfected cells without (red trace) or with application of GAP24 via extracellular incubation at 200 μ M (violet trace) or via patch pipette at 2 μ M (azure trace). The peptide F12R is a control (blue trace). (E-F) Comparison between the average peak $\Delta R/R_0$ values recorded in (C) together with their average peak ΔG_m values recorded in (D) [Carrer et al. 2018].

To study the molecular pathogenesis of CMT1X, we developed a human motor neuron/Schwann cell co-culture derived from induced pluripotent stem cells (iPSCs) that could be of potential interest for the

understanding of Cx32 expression and function, as well as for testing patient-specific therapeutic molecules (e.g. GAP24). The myelination process is, in general, the most challenging step to obtain a functional co-culture. (Fig. 2).

Figure 2



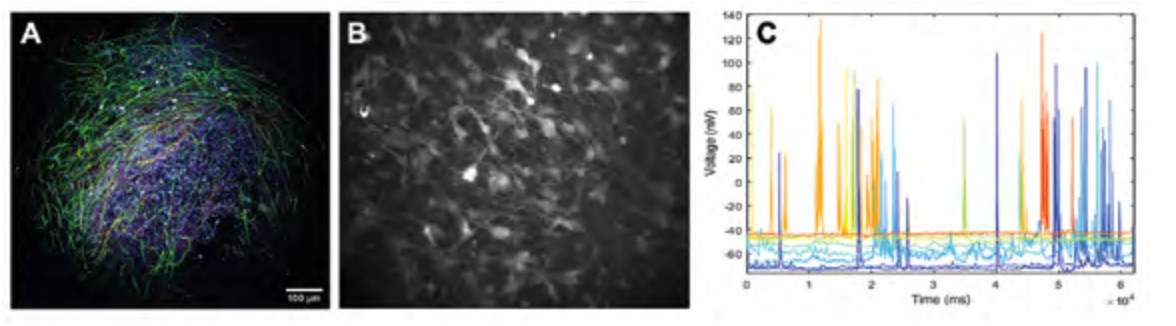
Derivation of a human motor neuron/Schwann cell co-culture from iPSCs. (A) We derived motor neurons and Schwann cells by reprogramming skin fibroblasts of a healthy subject into iPSCs. Then, iPSCs were differentiated by adapting selected differentiation protocols. Once obtained functional motor neurons and Schwann cells separately, we co-cultured them for several weeks to stimulate Schwann cell myelination. (B) Both motor neurons and Schwann cells were found to express specific markers (RT-PCR analysis). (C) Mature motor neurons were found to fire action potentials in patch-clamp experiments (top). Voltage-activated Na^+ channel currents were also recorded. (D) Immunofluorescence experiments performed by confocal microscopy in a motor neuron culture after 4 weeks application of a glial supportive solution revealed an endogenous population of Schwann cells which have lengthened on the neuronal processes, a step which is considered as the first one in the myelination process.

Future research plans

Our future plans are conceived to provide support to the groundbreaking hypothesis which links CMT1X pathogenesis to alteration of the purinergic signaling in myelinating Schwann cells. A combination of *in vitro* and *in vivo* experiments could pave the way for new therapeutic approaches.

As for the organoids project, this is a recent and new promising field of the lab (Fig. 3). We aim to utilize our *in vitro* model not only for investigating the molecular pathways involved in the development of PD but also for drug screening testing, i.e., for personalized medicine.

Figure 3



Midbrain organoids with PARKIN2 gene mutation exhibit altered Ca^{2+} activity. (A) Two-photon image of a 154 days old midbrain organoid stained for the glial marker GFAP (green). Nuclei were stained by DAPI (blue), whereas collagen (white) was label-free imaged by second harmonic generation at 395 nm. (B) A dissociated organoid carrying a pathological mutation of PARKIN2 gene was loaded by the Ca^{2+} indicator Fluo-4 to study the neuronal spontaneous Ca^{2+} activity. (C) Electrical activity recorded by patch-clamp at the same cells of (B) suggests an increased Ca^{2+} activity possibly due to kainate receptor hyperexcitability.

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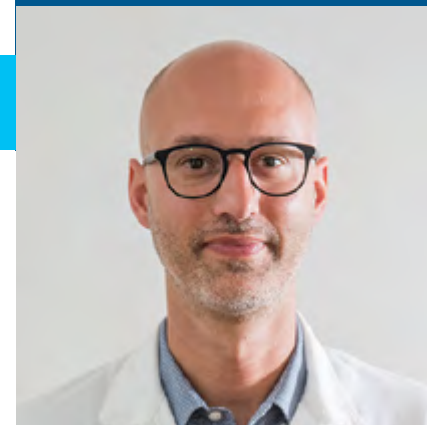
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ALESSANDRO CARRER

TUMOR METABOLISM UNIT



Biographical Sketch

2020 to date	MSCA Fellow, Dept. Biology, University of Padua, Italy
2019 to date	Group Leader, VIMM, Padua, Italy
2018-2019	Research Associate, Cancer Biology Department, University of Pennsylvania, Philadelphia, USA
2013-2017	Postdoctoral Fellow, Cancer Biology Department, University of Pennsylvania, Philadelphia, USA
2010-2012	Postdoctoral Fellow, Molecular Medicine Laboratory at ICGEB, Trieste, Italy
2005-2010	PhD student, Molecular Medicine laboratory at ICGEB, Trieste, Italy



Group Members

Marco Fantuz - Postdoctoral Fellow

Carlotta Paoli - PhD Student

Arianna Picco - Undergraduate student

Martina Spacci - Postgraduate Fellow

Thauan Serafini - Undergraduate Student

RESEARCH ACTIVITY

Rewiring of cellular metabolism in cancer cells: impact on epigenetic reprogramming

Fields of interest

Our lab is interested in characterizing the metabolic disturbances of malignant cells and understanding their impact on the epigenome and signaling pathways.

The main focus is pancreatic ductal adenocarcinoma (PDA), the most common form of pancreatic cancer. PDA is the deadliest among human cancers; therapeutic op-

tions are scant and ineffective. A better understanding of molecular events leading to disease initiation might provide significant benefits for public health by curtailing disease incidence and enhancing the opportunity to treat it early.

Nutrient utilization and cell metabolism are extensively rewired in PDA. Reprogramming of cellular metabolism is orchestrated by KRAS signaling, which has been shown to drive increased carbon flux into anabolic pathways, and also regulates mitochondrial function. My prior work demonstrated that KRAS-mediated metabolic rewiring plays a critical role in oncogenic transformation and disease initiation, by impacting the availability of metabolites that also function as co-substrates for chromatin-remodeling enzymes [i.e.: acetyl-CoA, which provides the acetyl group for histone acetylation reactions]. Targeting acetyl-CoA production suppresses PDA onset in experimental models of pancreatic carcinogenesis. At the same time, acetyl-CoA is also the building block for sterol synthesis and my work demonstrated that cholesterol production is also critical for PDA onset.

The research in my lab is funded by several non-profit international agencies and aims to 1) document mechanisms that lead to acetyl-CoA dysregulation in cancer, 2) define which genes are under the control of metabolic pathways and 3) ultimately characterize how metabolism contributes to cellular oncogenesis.

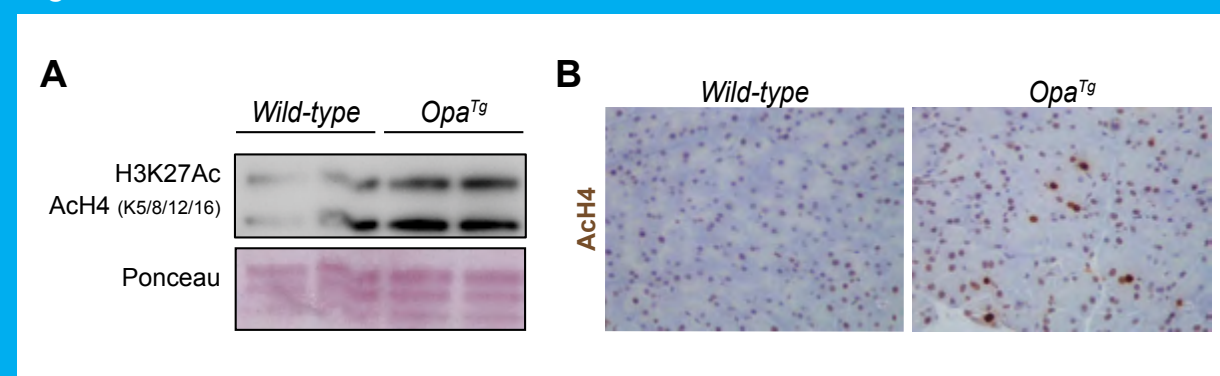
Ongoing investigations

- Mitochondrial activity (and/or structure) might be altered by KRAS signaling. Deploying transgenics

we can turn on the mutated form of KRAS (*Kras*^{G12D}) in pancreatic acinar cells, which are the cell of origin for PDA, upon administration with tamoxifen. This allows us to track early morphological and functional changes at the level of mitochondria and examine alterations in mitochondria-associated metabolites. We found that expression of mutant KRAS causes a marked tightening of mitochondrial cristae, the folding of inner mitochondrial membrane that allocates respiratory complexes. To understand whether altered mitochondrial ultrastructure might impact the epigenome, we interrogated pancreatic acinar cells harvested from mice overexpressing the major cristae biogenesis factor, OPA1. These cells show elevated histone acetylation levels (Fig. 1), corroborating the hypothesis that increased cristae density is linked to epigenetic reprogramming. Current studies are looking to document metabolic and epigenetic rewiring in OPA1-overexpressing acinar cells.

- Acetyl-CoA levels dictate chromatin opening at selected loci. We hypothesize that metabolic cues control the expression of specific genes during cellular oncogenesis. We are working to document a set of genes under mitochondrial control to further examine their role in carcinogenesis. Using lineage-restricted fluorescence reporters, we are able to track cells undergoing transformative events *in vivo* and recover them using FACS-mediated cell sorting. Chromatin accessibility is interrogated in recovered cells, that allows to identify actively transcribed genomic elements. The analysis compared oncogene-transformed cells with or without impairments in mitochondrial function to specifically uncover genes under metabolic control

Figure 1

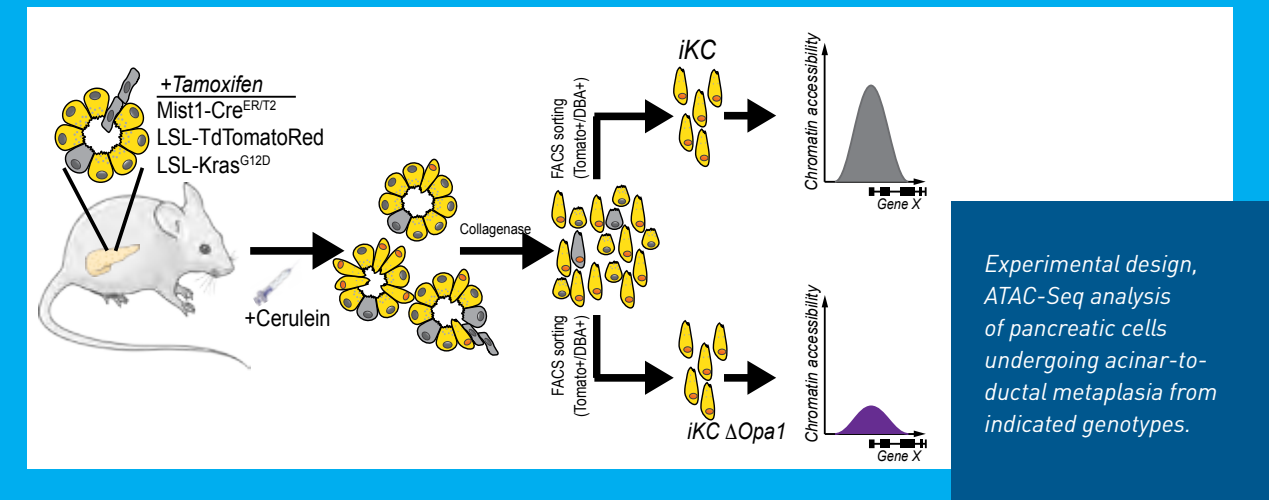


Histone acetylation was examined in *Opa1*-overexpressing mice [*Opa1*^{Tg}, Varanita et al. 2015]. In A, western blotting of histone extracted of isolated acinar cells showing indicated histone marks. In B, immunohistochemistry staining of mouse pancreata showing tetra-acetylated histone H4.

(Fig. 2). As metabolism is amenable to therapeutic targeting, we envisage the possibility to prevent tumor onset thanks to well-designed metabolic manipulations.

- Diet, smoking and history of pancreatitis are the major risk factors for PDA. Research in the lab is dedicated to understand how western diet and tobacco-related

Figure 2

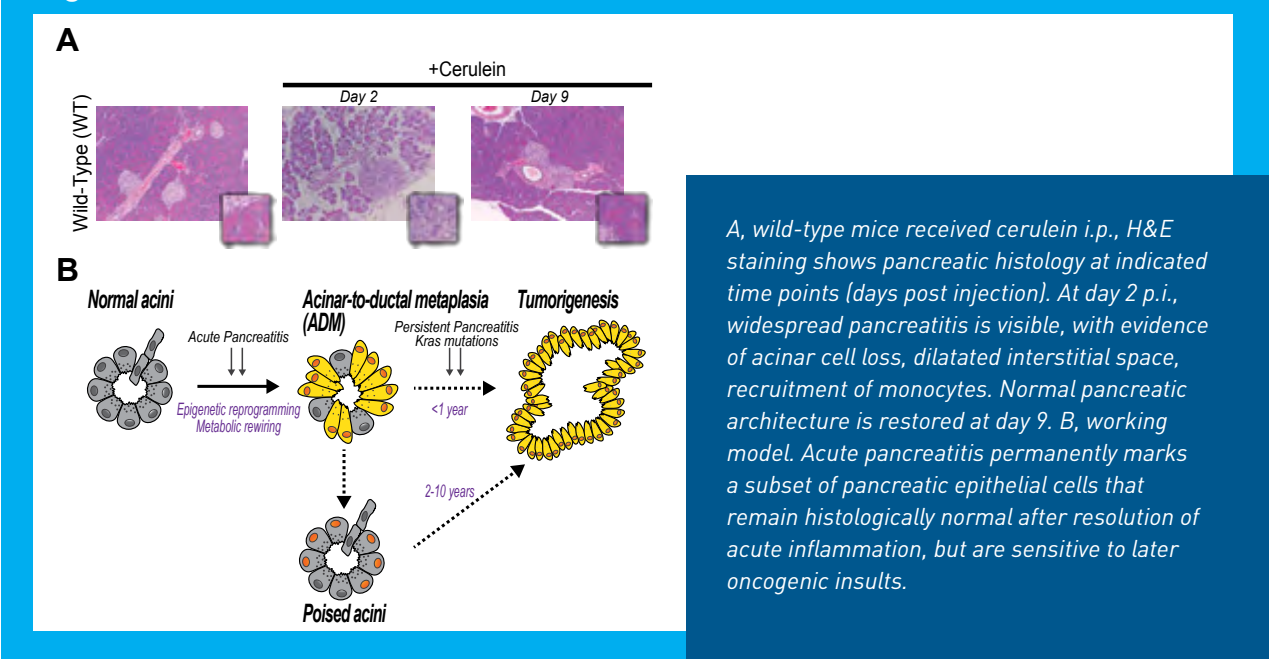


Experimental design, ATAC-Seq analysis of pancreatic cells undergoing acinar-to-ductal metaplasia from indicated genotypes.

compounds rewire pancreatic cell metabolism in a way that may support carcinogenesis. This could help designing improved guidelines for the monitoring of individuals at high risk of developing PDA, an incurable disease when diagnosed at advanced stages. Similarly, we hypothesize that early inflammatory events (acute pancreatitis) may permanently mark pancreatic ep-

ithelial cells, making them more vulnerable to late oncogenic insults. We are investigating the molecular and cellular remodeling of the pancreas that outlasts an acute inflammatory event. Preliminary data suggest the presence of an enduring mitochondrial reprogramming (Fig. 3).

Figure 3

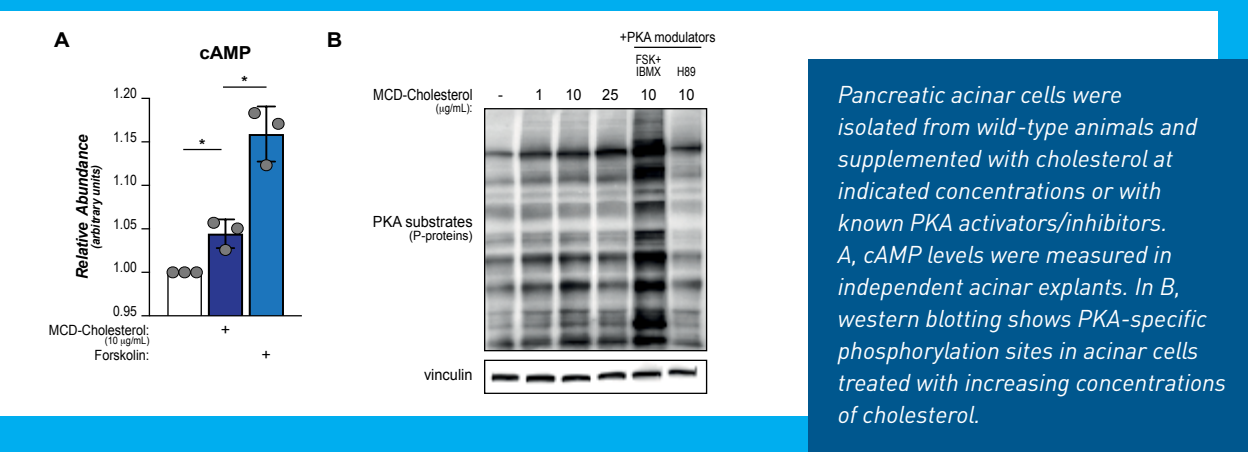


A, wild-type mice received cerulein i.p., H&E staining shows pancreatic histology at indicated time points (days post injection). At day 2 p.i., widespread pancreatitis is visible, with evidence of acinar cell loss, dilated interstitial space, recruitment of monocytes. Normal pancreatic architecture is restored at day 9. B, working model. Acute pancreatitis permanently marks a subset of pancreatic epithelial cells that remain histologically normal after resolution of acute inflammation, but are sensitive to later oncogenic insults.

- Cholesterol production and uptake is augmented in PDA, but the role is unclear. We demonstrated that cholesterol enhances PKA signaling in metaplastic aci-

ni (Fig. 4). As cholesterol is abundantly exported from malignant cells, we are now investigating the role of cholesterol in paracrine signaling to stromal cells.

Figure 4



• Levels of acetyl-CoA are dynamically regulated during B cell maturation. We found that the acetyl-CoA-producing enzyme ACLY is upregulated and hyperactivated (phosphorylated) in germinal center (GC)-B cells, which also

display elevated histone acetylation. We are now investigating the impact of ACLY ablation for B cell proliferation to understand whether metabolic inputs may play a role in B cell maturation and, possibly, lymphomagenesis.

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MAURIZIO CORBETTA

NETWORK AND COMPUTATIONAL NEUROSCIENCE UNIT



Biographical Sketch

2020	Emeritus Professor of Neurology, Washington University (WU), St. Louis, USA
2017	Founding Director Padova Neuroscience Center, University of Padua, Italy
2015	Professor and Chair of Neurology, University of Padua, Italy
2005	Norman J. Stupp Chair Professor of Neurology, WU, St. Louis, USA
2005	Professor of Neurology, Radiology, Neuroscience, WU, St. Louis, USA
1996	Assistant then Associate Professor of Neurology, WU, St. Louis, USA
1993	Residency Neurology, Barnes Hospital, WU, St. Louis, USA
1992	Internship Internal Medicine, Jewish Hospital, WU, St. Louis, USA
1986	Residency Neurology, University of Verona, Italy
1979	MD Summa cum laude, University of Pavia, Italy

Group Members

- Antonio Bisogno - Resident Neurology
- Miriam Celli - PhD Student
- Silvia Facchini - Postdoctoral Fellow
- Chiara Favaretto - Postdoctoral Fellow
- Mohammad Hadi Arabi - PhD Student
- YanJun Lu - PhD Student
- Lorenzo Pini - Postdoctoral Fellow
- Andrea Porsio - Resident Neurology
- Alessandro Salvalaggio - PhD Student
- Giulio Sansone - Resident Neurology
- Tommaso Volpi - PhD Student
- Andrea Zangrossi - Postdoctoral Fellow
- Zhang Lu - PhD Student

Human brain activity and its relationship with behavior

Fields of interest and ongoing researches

The brain is one of the last frontiers of science. The focus of our research is to understand how the structural and functional organization of the brain mediates cognitive functions and recovery of function after injury (stroke, tumors), with an eye toward novel strategies of repair and rehabilitation. We use a variety of methods to measure brain activity: functional magnetic resonance imaging (fMRI), diffusion MRI, electroencephalography/magnetoencephalography (EEG/MEG), positron emission tomography (PET), and transcranial magnetic stimulation (TMS). We measure behavior with neuropsychological assessments, but also dynamically with eye movement recordings and sensors. We also study patients with focal lesions from stroke and brain tumors to understand the effect of lesions on brain organization. A special kind of computational modeling called 'whole brain modeling' takes biological information from neuroimaging studies to simulate in silico patterns of brain activity and connectivity. We are applying these models to studies of healthy connectivity and patients with stroke.

The Network Neuroscience lab at VIMM, coordinated by Professor Maurizio Corbetta, is a multidisciplinary team composed of physicians, psychologists, engineers, and physicists.

The lab focuses on three main lines of research:

Cognitive functions and brain networks

Maurizio Corbetta has worked for years on the brain mechanisms of human attention, i.e., the ability to select relevant information while ignoring potentially distracting information. In collaboration with Gordon Shulman, he has developed the most cited anatomical model of human attention (>10,000 citations). They discovered that human attention is regulated by two cortico-cortical networks: the dorsal and ventral attention networks (Corbetta & Shulman, 2002). The dorsal network (DAN), related to mechanisms that control eye movements, interacts with sensory-motor regions to optimize responses in line with ongoing goals. The ventral network (VAN) specializes in detecting novel and important stimuli in the environment to redirect the focus of attention (Corbetta *et al.* 2008). These networks remain synchronized at rest in the absence of any task (Fox *et al.* 2005; Fox *et al.* 2006). In patients with brain

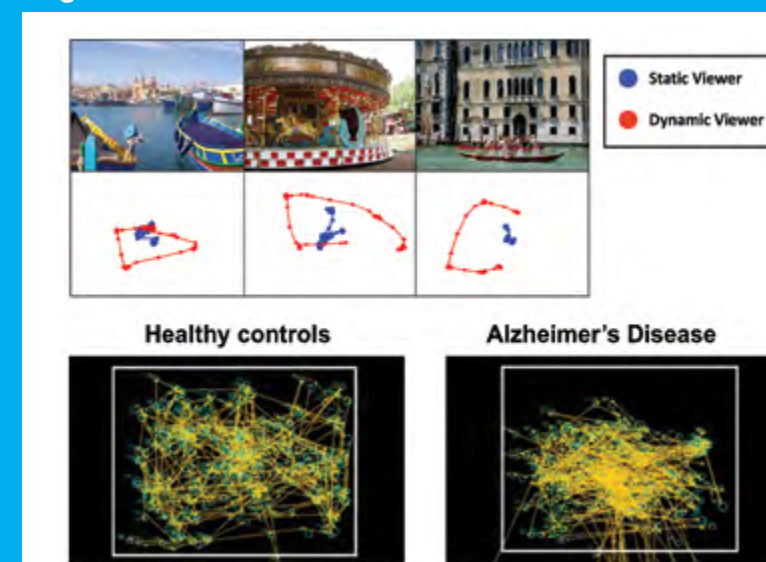
injuries, alterations in the synchronization of these networks drive the most common cognitive deficit after right hemisphere lesions: the so-called spatial neglect (Corbetta & Shulman, 2011). One method for studying visuospatial attention is recording eye movements during natural visual exploration. We are carrying out behavioral and EEG studies of eye movement exploration in healthy subjects and patients with stroke and neurodegenerative conditions (e.g., Alzheimer's dementia). One recent important result was to show that eye movements in healthy subjects watching hundreds of visual scenes can be summarized with a few characteristic features (e.g., fixation duration), which sensibly capture the individual variability of healthy observers (Zangrossi *et al.* 2021), and that appear altered in patients with Alzheimer's disease (Fig. 1). We are carrying out studies to assess the specificity and sensitivity of this biomarker, as well as using eye movements as a possible tool for rehabilitation.

Intrinsic brain activity and function

The coordinated activity among brain regions requires an interplay between patterns of intrinsic and sensory-driven activity. One of the most important discoveries in system neuroscience of the last two decades has been to understand that the brain is not a simple sensory-motor analyzer, but that most activity is 'intrinsic', i.e., present also in the absence of any task (rest). Our lab has contributed extensively to the discovery that intrinsic activity measured with BOLD-fMRI, a metabolic indicator of neural activity, is not random but coordinated across multiple regions forming so called 'networks' (Fox *et al.* 2005; Fox *et al.* 2006; Vincent *et al.* 2007). We have also described the electrophysiological counterparts of these networks (Mantini *et al.* 2006; de Pasquale *et al.* 2010), and their modification with learning (Lewis *et al.* 2009) and focal injuries (He *et al.* 2007). We have recently provided a theory of how this intrinsic activity is dynamic (Fig. 2) and contributes to cognition by simulating patterns of future activity that will be used in the most frequent behavioral conditions (Pezzulo, Zorzi, Corbetta, 2021).

We are currently conducting experiments to understand the metabolic cost of this intrinsic activity mea-

Figure 1



Top: different observers look with a different style. These features can be used to separate patients with Alzheimer disease (bottom) (Zangrossi *et al.* 2021; Zangrossi *et al.* in preparation).

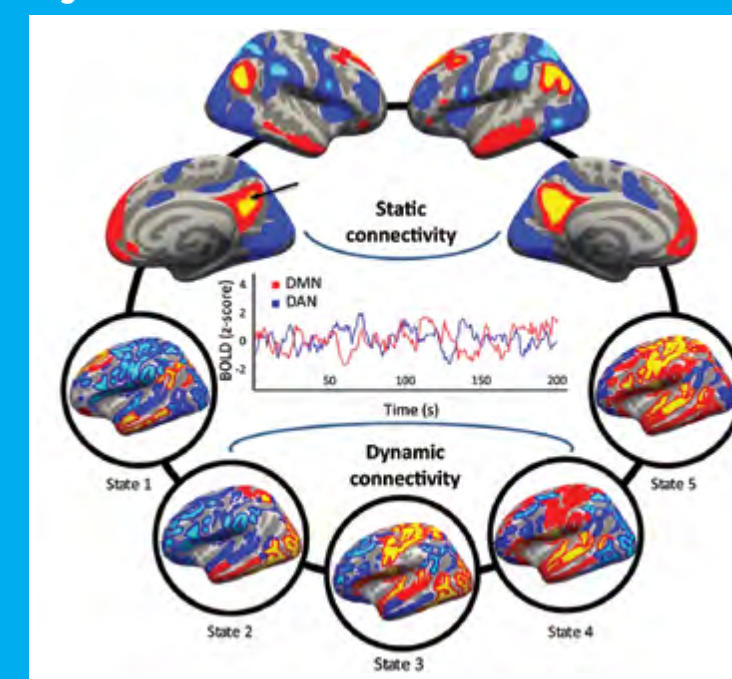
suring simultaneously glucose metabolism (the brain's food) and brain networks both metabolic and electrophysiological.

In separate studies, we are modeling these signals to understand how activity in one region relates to activity in another region, for instance how a metabolic signal relates to a neurophysiological signal at rest or during an attention task (Favaretto *et al.* 2021).

Brain mechanisms after injury

Focal injuries like strokes or tumor alter the function of brain networks and these alterations explain neurological impairment and relate to recovery of function. Our lab has been at the forefront of this research showing the neurological impairment is correlated across patients, and that lesions cause relatively simple albeit distributed changes in brain connectivity and networks (He

Figure 2

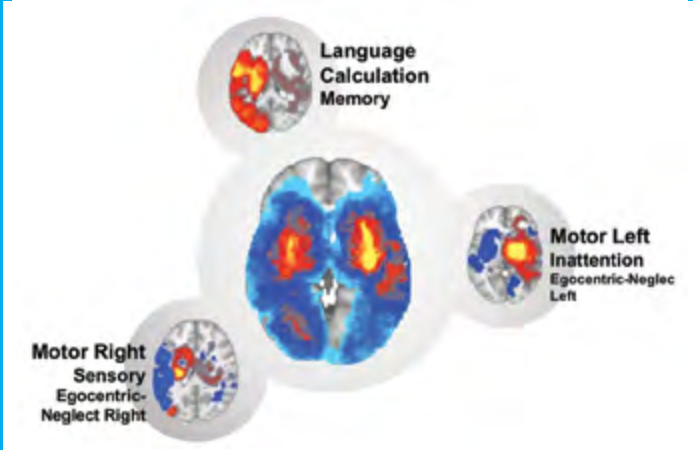


Top: correlation between brain regions with positive (orange) or negative synchronization (blue). Inset: temporal patterns of activity recorded with fMRI from two networks (DMN, DAN). Bottom: dynamically changing states of connectivity that anticipate common behaviorally relevant patterns (Pezzulo *et al.* 2021).

et al. 2007; Siegel et al. 2016; Corbetta et al. 2015; Bisogno et al. 2021). We are also using these signals as a predictor of behavioral impairment and potentially the degree of recovery (Salvalaggio et al. 2020; Bisogno et al. 2021; **Fig. 3**).

We are currently conducting studies that integrate structural, metabolic, and neurophysiological alterations in stroke toward developing whole brain models that can guide at the level of individual subject novel interventions.

Figure 3



Center: the most common site of injury in >250 patients with stroke randomly selected. These patients manifest a low dimensional stereotypical pattern of impairment that divides in three domains: 1. Language-calculation-memory; 2. Deficits of attention and left motor function; 3. Deficits of right motor function and attention. The corresponding maps show the regions that statistically are more damaged in patients with the most severe deficits in each domain (Bisogno et al. 2021).

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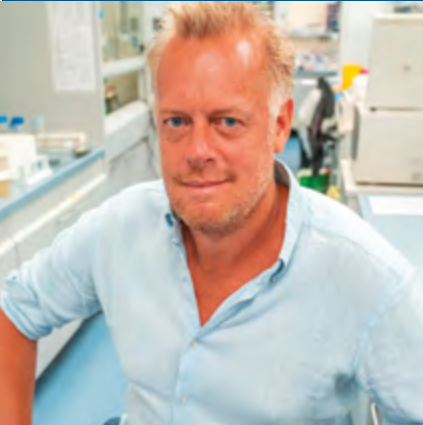
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NICOLA ELVASSORE
STEM CELL ENGINEERING UNIT



Biographical Sketch

2020 to date	Full Professor, Dept. Industrial Engineering, University of Padua, Italy
2017-2019	Associate Professor, Dept. Industrial Engineering, University of Padua, Italy
2015-2021	Distinguished Professor-in-Residency, SIAIS - ShanghaiTech University, China
2015 to date	Honorary Professor, UCL GOS Institute of Child Health, Univ. College London, UK
2007 to date	Group Leader, VIMM, Padua, Italy
2005-2007	Fulbright visiting Scientist and Research Affiliate, Harvard-MIT HST, USA
2002-2019	Assistant Dept. Industrial Engineering, University of Padua, Italy
1999	Visiting Researcher, University of California, Berkeley, USA
1999	PhD in Engineering, University of Padua, Italy



Group Members

- Monica Giomo - Professor
- Silvia Angiolillo - PhD Student
- Pietro Bellet - Research Associate
- Luca Brandolino - Research Associate
- Elisa Cesare - PhD Student
- Onelia Gagliano - Postdoctoral Fellow
- Cecilia Laterza - Postdoctoral Fellow
- Carmela Ribecco - PhD Student
- Serena Pinci - Research Associate
- Hannah Stuart - Postdoctoral Fellow

RESEARCH ACTIVITY

Fields of interest

The BioERA lab is an interdisciplinary environment composed by people with biology, biotechnology, material science, chemical and biomedical engineering backgrounds that work together to develop micro-engineer-

ing tools and bio-materials to study stem cell biology, especially in cell reprogramming, stem cell 3D self-organization and differentiation. In the recent years, the technological advancements to-

wards 3D culture systems have revolutionized the cell biology landscape and their potential to recapitulate *in vitro* the human biology become an invaluable tool able to fill the gap between animal models and human subjects. In this context BioERA lab efforts are focused on developing reliable *in vitro* models based on human artificial tissues and organoids for investigating molecular and biological mechanisms in a high-throughput fashion. In particular we designed microengineering technology, including microfluidic devices, hydrogel culture system and 3D bioprinting approaches. These tools offer unprecedented possibilities to dissect the dynamic reciprocity between stem cells and their environment both in space and time.

Ongoing researches

Our research activities are mainly focused on 4 major topics:

In vitro human organoids

The role of 3D environment in stem cell identity, organogenesis and homeostasis is an established concept that brought life scientists and engineers to develop new strategies for cell culture systems able to mimic *in vivo* conditions.

To reach this goal, we combine innovative biomaterials,

micro-fabrication techniques, human induced pluripotent stem cell engineering, including differentiation and self-organization capability of stem cells and organoid technology. Organoids have the intrinsic ability to self-organise into 3D structures that are highly similar, and in some cases, histologically indistinguishable from actual human organs (**Fig. 1**).

Moreover, by using such *in vitro* culture we aim at elucidate the intricate crosstalk that exist between three-dimensional structure and differentiation, as well as function of tissues, which are on the basis of physiological and physio-pathological processes. In this context we are also developing sophisticated microfluidic-based system to create gradients able to guide human organoid's growth and differentiation.

In vivo 3D bioprinting

In vivo bioprinting approaches represent a ground-breaking advancement in tissue engineering field, since they give the possibility of fabricating hydrogel-based structures *in situ* into alive animals. We recently developed photo-sensitive polymers that allow *in vitro* and *in vivo* fabrication of customized 3D hydrogels by two-photon mediated crosslinking. By this technique, we can both fabricate complex 3D hydrogel structures in the presence of living cells or inside a pre-existing hydrogel structures for controlling human organoid's behaviour

at different time points during 3D culture. After injection of these polymers *in vivo*, we can fabricate 3D hydrogels inside tissues of living mice, including in the brain across meninges to boost endogenous regeneration or to guide stem cell integration.

Patient-specific disease modelling

We developed robust and efficient techniques to reprogram adult somatic cells back to their pluripotent state, and to program them towards a variety of differentiated tissue-specific cells, such as neurons. We are taking advantage of this technology to model patient-specific biology (e.g. Alzheimer's disease) in a fast and cost-effective manner. In addition, we also adapted the microfluidic-based reprogramming protocol to generate naïve iPSCs, which represent the ground state of pluripotency, have a broad unmethylated genome and are the only possible cell type that can be used for *in vitro* modelling of certain epigenetic-based diseases (i.e. Fragile X Syndrome), which cannot be modelled with standard primed iPSCs. Therefore we can obtain patient-specific stem cells that can be differentiated into the disease-affected cell type and provide *in vitro* models of various pathologies recapitulating the genotypic influence on the pathological phenotype for personalized medicine.

Study of the effect of rhythmic oscillatory factors on cell behavior

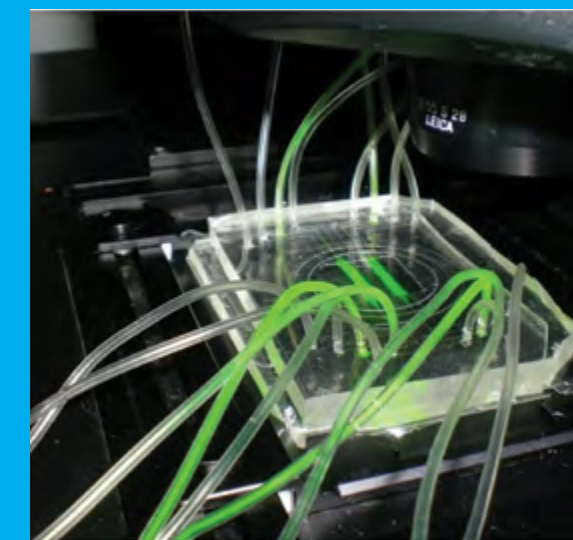
In mammals, circadian rhythms function to coordinate a diverse panel of physiological processes with environmental conditions such as food and light. The circadian clock consists of endogenous self-sustained molecular oscillations of specific proteins, that are synchronized to the environment by extrinsic factors. We developed experimental strategies to create an highly physiological *in vitro* model that allows to study the influence of frequency-encoded metabolic signals, resembling the day-life routine using microfluidic platform (**Fig. 2**). With specific focus on liver, we study the circadian system in regulating metabolism and energy homeostasis and other mechanisms of hepatic circadian clock to gain better understanding of liver physiology and associated diseases.

Best results obtained

Reprogramming and generation of hiPSC – Start-up funded Onyel Biotech

In recent publications, we reported that the efficiency of reprogramming human somatic cells to hiPSCs and

Figure 2



Microfluidic platform for live imaging.

their differentiation in specific lineage can be dramatically improved in microfluidic environment. The precise control of the cellular micro-environment allows to define accurately the concentration of soluble endogenous and exogenous extracellular components with a strong positive impact in both the differentiation and reprogramming process. Very recently, through the use of microfluidics, we reported an extremely relevant result in this research field, generating naïve iPSCs (niPSCs) directly from fewer than 1,000 primary human somatic cells, without requiring stable genetic manipulation.

These extraordinary results are contained in several patents, which allowed to build up the Start-up Onyel Biotech.

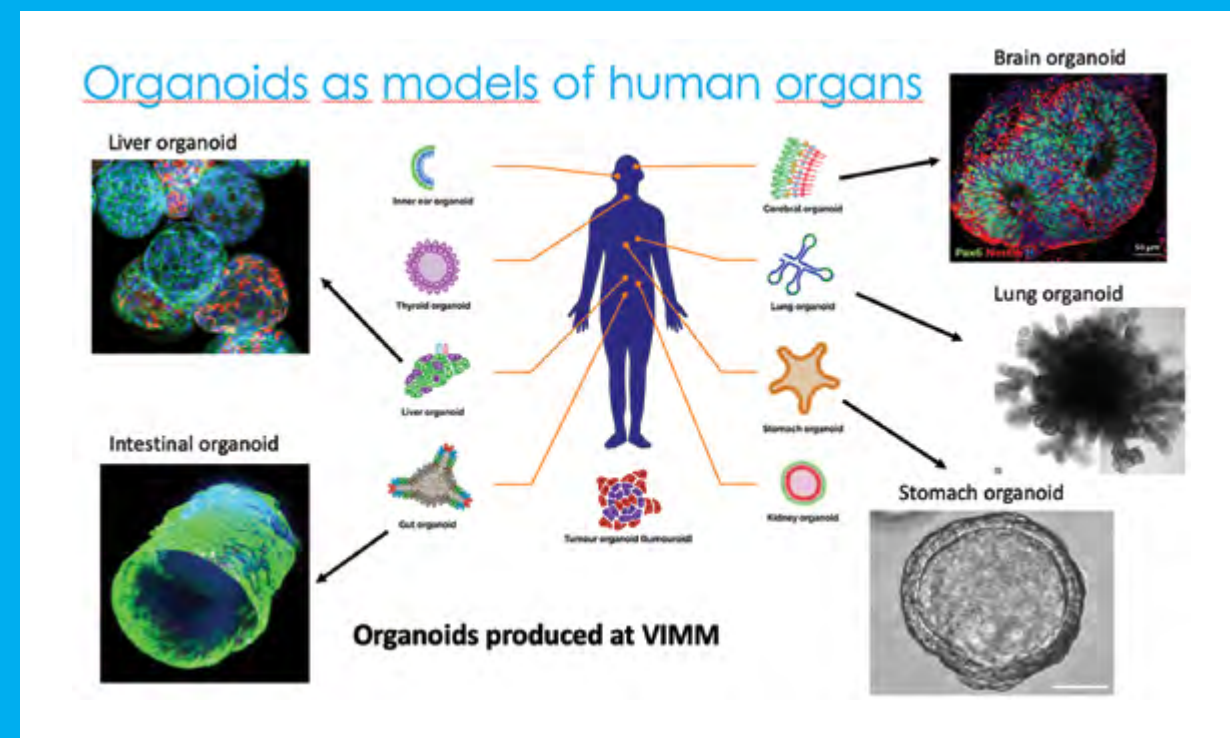
Organoids from hiPSC primed and naïve

We moved from 2D to 3D differentiation of human iPSCs and we developed efficient methods to create organoids from human pluripotent stem cells both in primed and naïve state, which have never been reported before. We focused on neural ectodermal lineage, and we successfully generated brain organoids from naïve iPSCs with specific regional identity.

In vivo bioprinting

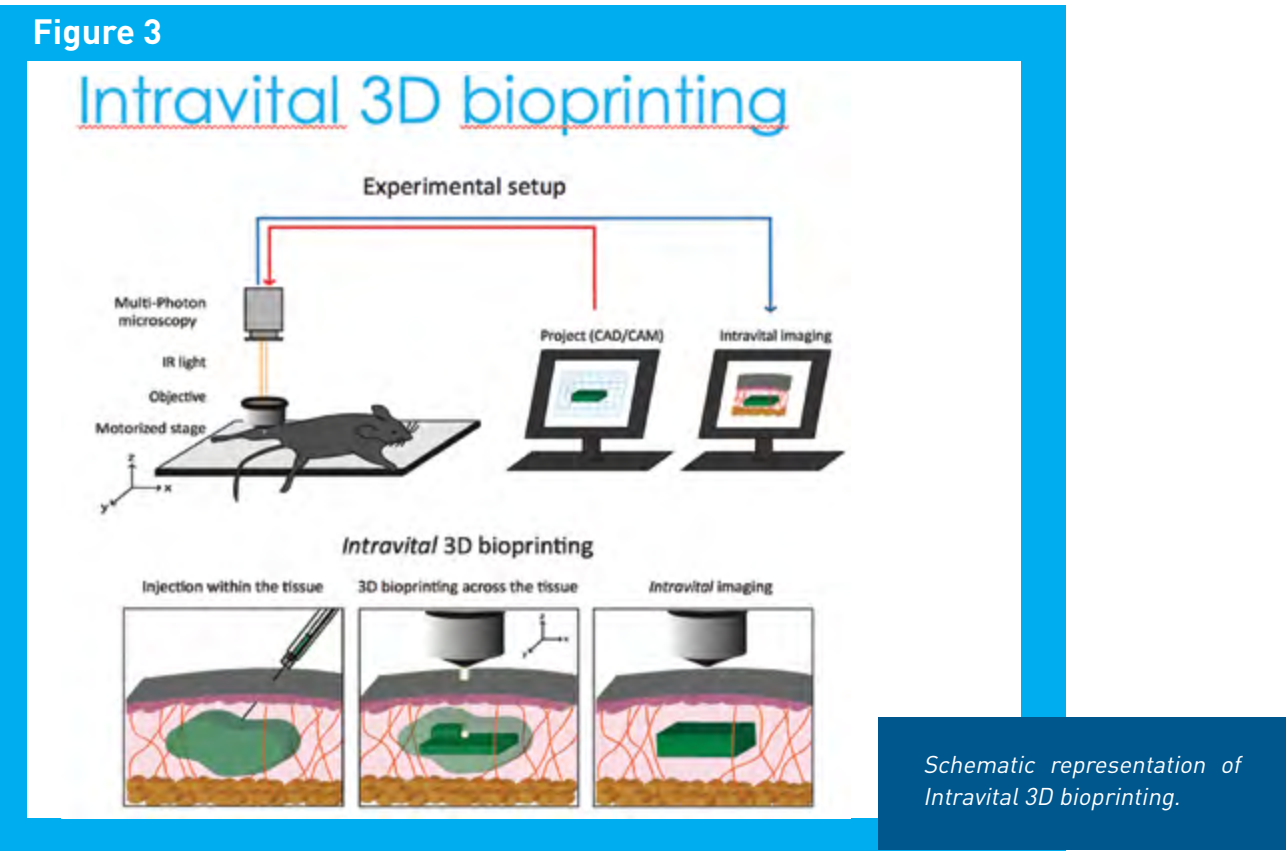
We developed a photosensitive biopolymer published in Nature Biomedical engineering that can be photo-crosslinked *in vitro* or, after a simple injection inside the biological tissues, *in vivo* into a 3D object by using NIR laser light. We showed that the 3D bioprinting can be used *in vitro* to generate instructive geometries for organoid culture or *in vivo* to fabricate *in situ* 3D con-

Figure 1



Organoids platform for disease study.

structs across and inside tissues in a live organism, such as dermis across epidermis, the skeletal muscle across epimysium or the brain across meninges. We named this technique intravital 3D bioprinting (**Fig. 3**).

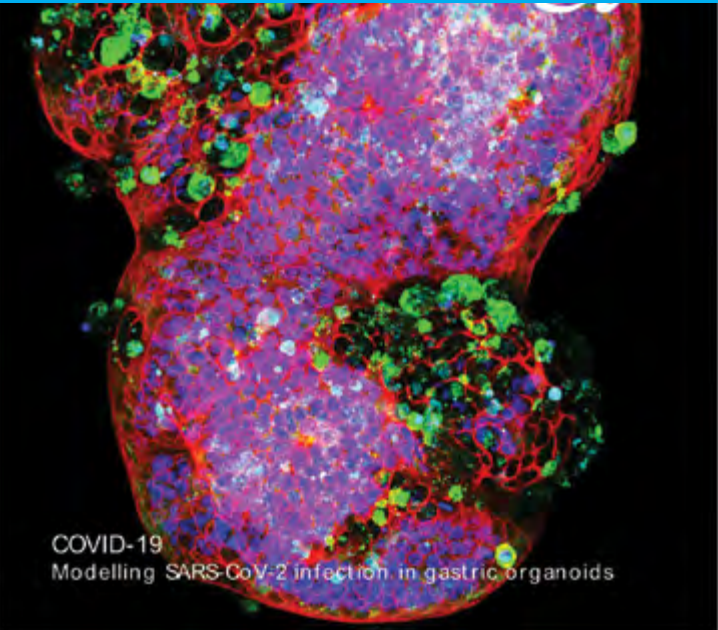


Future research plans

Based on our recent achievements, BioERA future research will be focused on:

1. *In vivo bioprinting in the brain*: we will use the 3D *in vivo* bioprinting technology to precisely guide the functional reconnection of human brain organoids with mouse cortical tissue.
2. *Engineering organoids*: taking advantage of the photo-sensitive polymer developed in BioERA lab, together with the possibility to create custom-designed microfluidic chips and microwells, we will engineering the culture microenvironment to guide human organoid growth, patterning and differentiation.
3. *Large-scale patient specific organoids generation*: starting from the high-efficient microfluidic cell reprogramming protocol we will develop an high-throughput patient-specific generation of organoids belonging to all the three germ layers in a time and cost effective manner (less than one month from patient's primary cells to organoids).
4. *Human Organoid-based platform for Infection disease studies*: we are developing a human organoid-based platform for efficiently evaluate the infection capability of viruses (including SARS-CoV-2 and pre-pandemic viruses) and screen for antiviral drugs (an example in **Fig. 4**).

Figure 4



Modelling SARS-CoV-2 infection in human gastric organoids. In green SARS-CoV-2 Nucleocapsid Antibody.

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GIAN PAOLO FADINI

EXPERIMENTAL DIABETOLOGY UNIT



Biographical Sketch

- 2020 Minkowski Prize, European Association for the Study of Diabetes
- 2016 to date Associate Professor of Endocrinology, University of Padua, Italy
- 2015 to date Group Leader, VIMM, Padua, Italy
- 2010-2016 Assistant Professor of Endocrinology, University of Padua, Italy
- 2010 to date Associate Medical Director, Division of Metabolic Diseases, University Hospital of Padua, Italy
- 2009 Rising Star of the European Association for the Study of Diabetes



Group Members

- Mattia Albiero - Assistant Professor
- Benedetta Maria Bonora - Assistant Professor
- Ludovica Migliozi - PhD Student
- Francesco Ivan Amendolagine - PhD Student

RESEARCH ACTIVITY

Fields of interest

Diabetes is progressively growing worldwide with epidemic diffusion. Its prevalence reaches up to 10% of the general population in Western countries, and it is growing even faster in the developing world. Importantly, diabetes leads to severe multi-organ complications and is associated with a shortened life expectancy. Diabetes develops as a consequence of a complex interplay between genes and environment. The metabolic alterations observed in diabetes promote the development of chronic complications within several

organs, including the arteries, heart, kidneys, nerves and eyes. In turn, these are responsible for the high burden of morbidity and mortality in diabetic patients. In the Laboratory of Experimental Diabetology, research activities are focused on the cellular and molecular mechanisms underlying the development of diabetes and its long-term complications, as well as the therapeutic strategies to prevent and treat these conditions. In the Lab, expertise from multiple disciplines converge to increase the impact of the work,

including clinical medicine and basic science. The specific commitment of this Lab is to integrate basic science with clinical medicine, creating a unique environment, ideal to provide significant advancements in the field, through approaches of “translational medicine”. The group comprises a set of medical doctors, research fellows, as well as lab personnel with extensive experience in molecular and cellular biology, in silico studies, tissue analysis, and animal models of disease. Our major goal is to study how the bone marrow and stem cells are affected by diabetes and contribute to the development of metabolic abnormalities and chronic complications.

Ongoing research and best results obtained

Therapeutic modulation of stem and progenitor cells in diabetes to interrupt the pathologic processes leading to complications

We have extensively characterized alterations of bone marrow-derived stem/progenitor cells *in vitro* as well as *in vivo* in animals and in humans with di-

abetes. Thanks to the contribution of our lab, endothelial progenitor cells (EPCs) are now considered a determinant of vascular health and their alterations in the setting of diabetes a driver of complications. We have established a connection between the number and function of EPCs to the development of long-term macro- and microvascular complications. These studies have allowed to define a new model that emphasizes the crucial role of the progressive shortage of bone-marrow derived stem cells, a condition similar to that observed in the ageing process. We have also identified pharmacologic approaches able to restore the stem cell population, which are being transferred to the clinical setting (Fig. 1).

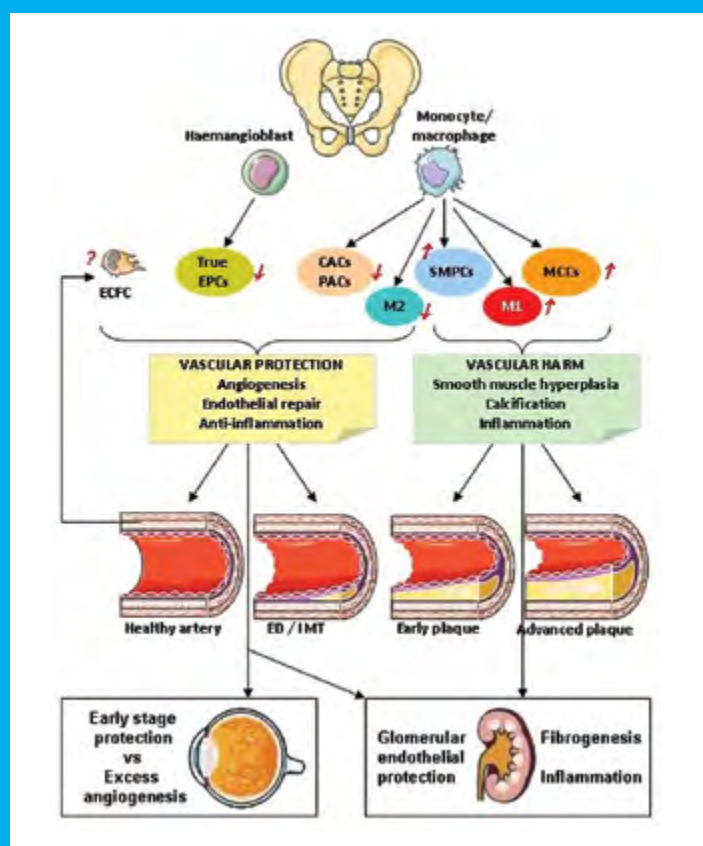
The diabetic bone marrow as a link to distant end-organ complications

We have moved from the analysis of definite progenitor cell phenotypes and their characteristics to the study of bone marrow alterations induced by diabetes. We have clarified that diabetes is characterized by a dysfunction of the stem cell niche, which caus-

es a significant impairment in stem cell mobilization from the bone marrow into peripheral blood. Besides its importance in the haematology field, this new notion has also strong implications for the development and progression of diabetic complications, because bone marrow derived cells contribute to the homeostasis of non-hematopoietic organs. More specifically, we found that diabetes-induced impairment in stem cell mobilization after ischemia and G-CSF depend, at least in part, on a tissue specific dysregulation of DPP-4 activity. In mice, we also found that

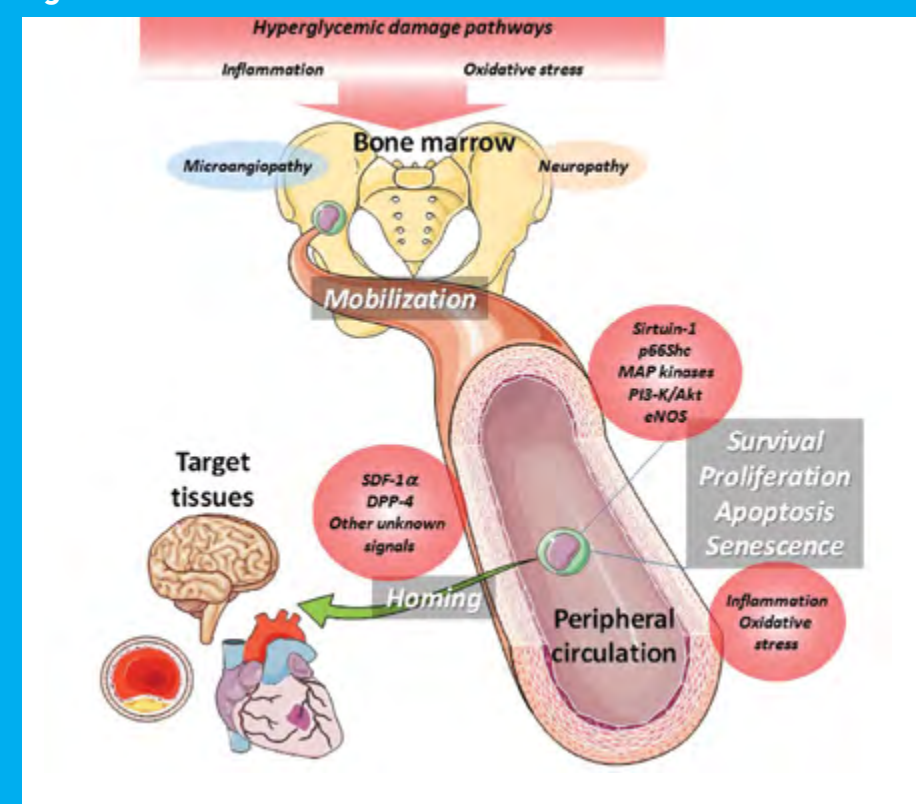
diabetes induced sympathetic and sensory denervation of the bone marrow, which is critical for the induction of diabetic stem cell mobilopathy. In parallel to bone marrow sympathectomy, we found that inflammation drives myelopoiesis and differentiation of intramarrow macrophages that, when presence in excess such as in diabetes, retain stem cells in the niche by producing oncostatin M (OSM). We have established effective therapeutic strategies to counter BM niche dysfunction by various pharmacologic interventions (Fig. 2).

Figure 1



Origin and function of circulating (progenitor) cell phenotypes in diabetic complications. Modified from Fadini 2014.

Figure 2



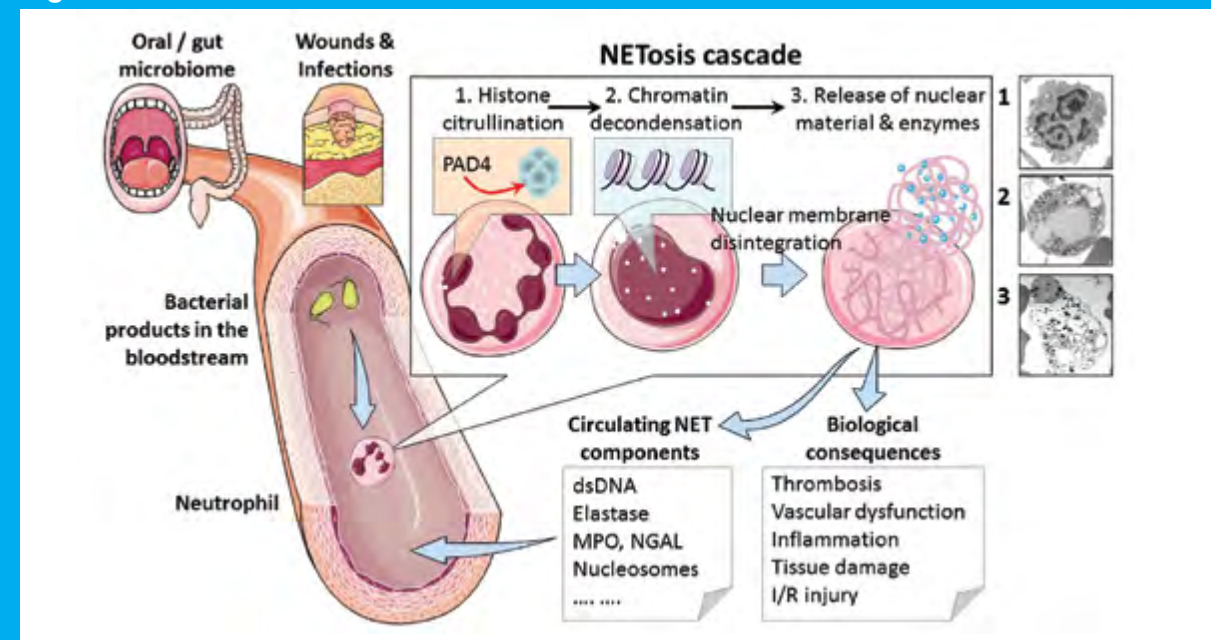
The mechanisms of EPC alterations in diabetes. Modified from Menegazzo et al. 2012.

NETosis as a link between the environment, metabolism and diabetic complications

Diabetes and the metabolic syndrome are characterized by activation of the innate immune system. As the lab is committed to the study of circulating cells in diabetes, we have recently moved to analyse the role played by neutrophils. Upon challenge with microbes and inflammato-

ry triggers, neutrophils undergo histone citrullination by protein arginine deiminase-4 (PAD4), release enzymes and nuclear material, forming neutrophils extracellular traps (NETs) and thereby dying by NETosis (Fig. 3).

Figure 3



An overview of the pathophysiology of NETosis.
Modified from Fadini et al. 2016.

We have found for the first time that hyperglycemia increase release of NETs and circulating markers of NETosis. This finding provides a link among neutrophils, inflammation and tissue damage in diabetes. Therefore, we examined the effect of NETosis on the healing of diabetic foot ulcers (DFU). Using proteomics, we found that NET components were enriched in non-healing human DFU. In an independent validation cohort, a high concentration of neutrophil elastase in the wound was associated with infection and a subsequent worsening of the ulcer. NET components were elevated in the blood of patients with DFU. Circulating elastase and proteinase-3 were associated with infection, and serum elastase predicted delayed healing. Neutrophils isolated from the blood of DFU patients showed an increased spontaneous NETosis but an impaired inducible NETosis. In mice, skin PAD4 activity was increased by diabetes, and FACS detection of histone citrullination, together with intravital microscopy showed that NETosis occurred in the bed of excisional wounds. PAD4 inhibition by Cl-amidine reduced netting neutrophils and rescued wound healing in diabetic mice. Cumulatively, these data suggest that NETosis delays DFU healing in mice and humans.

Future research plans

Study of the inter-relationships between bone marrow niche dysfunction and structural changes induced by diabetes in the bone marrow including adipocytes, the microvasculature and inflammatory cells.

Exploring potential therapeutic strategies in diabetes to restore vascular repair processes mediated by bone marrow-derived cells. To this end, we focus on pharmaceutical compounds that currently used in clinical practice or will become available in the years to come. Both studies *in vitro* and *in vivo* will be developed to assess the potential of these approaches to reverse diabetes-associated anomalies in progenitor cell levels and function.

We are exploring the roles of myeloid calcifying cells (MCCs) in atherosclerotic calcifications of diabetic patients *in vivo*, also in relation to other potential mechanisms of vascular calcification induced by soluble mediators and cell-related processes.

We are characterizing in detail the multiple alterations seen in neutrophils from patients and animals with diabetes, testing how NETosis is induced by diabetes and whether it is regulated by the gut microbiome, a potent mediator of inflammatory and metabolic signals.

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KOSTANTINOS LEFKIMMIATIS

MOLECULAR SIGNALING MECHANISMS IN HEALTH AND DISEASE UNIT



Biographical Sketch

- 2019 to date Associate Professor, Dept. Molecular Medicine, University of Pavia, *Italy*
- 2017 to date Group Leader, VIMM, *Padua, Italy*
- 2017-2019 Researcher, National Research Council (CNR), *Padua, Italy*
- 2013-2016 Intermediate Fellow, University of Oxford, *UK*
- 2010-2012 Instructor, Harvard Medical School, *Boston, USA*
- 2006-2010 Postdoctoral Fellow, Harvard Medical School, *Boston, USA*
- 2006 PhD (Genetics and molecular evolution), University of Bari, *Italy*
- 2001 Master's (Molecular biology), University of Bari, *Italy*



Group Members

- Giulietta Di Benedetto - *Researcher CNR*
- Filippo Conca - *Undergraduate Student*
- Liliana F. Iannucci - *Postdoctoral Fellow*
- Nicoletta C. Surdo - *Researcher CNR*

RESEARCH ACTIVITY

Fields of interest

The Lefkimmatis group is interested in the molecular mechanisms that underlie human pathologies with particular attention to cardiovascular disease and cancer. We use state of the art molecular tools that allow the measurement and manipulation of the main signaling cascades (cyclic AMP and Calcium [Ca²⁺]) in living cells. Using these tools, we are able to study how cells communicate and elaborate information.

Ongoing researches

We are currently working on three overarching questions:

How kinase activity is regulated within the cells and what is the physiological importance of these regulatory events?

We have developed ad hoc Fluorescence Resonance Energy Transfer (FRET) tools and devised a set of protocols that allow us to follow the kinase activity in different cellular compartments in real time in living cells. By comparing the responses of different kinases in response to

specific stimuli we aim to generate a signaling activity map for each kinase. By comparing the activity maps of a specific kinase in health and diseased cells we will be able to understand the contribution of each kinase in the insurgence and progression of the pathology.

How cAMP signals are perceived and interpreted by the nucleus?

While the nucleus is a highly compartmentalized cellular organelle our knowledge on how this domain perceives the increases of cAMP remains superficial. We have generated tools for following the behavior of the two main cAMP effectors (Protein Kinase A (PKA) and EPAC1) in the nucleus of living cells. Our preliminary results suggest that the nuclear moieties of these proteins behave differently than their cytosolic counterparts. We are now studying which are the functions that each effector exerts in the nucleus and how these proteins coordinate their action in this compartment.

What is the involvement of protein kinase A (PKA) in non-apoptotic programmed cell death?

Persistent activity of PKA has been connected to cardiac death, however the type of death through which cardiac myocytes perish is not fully understood. Using *in vi-*

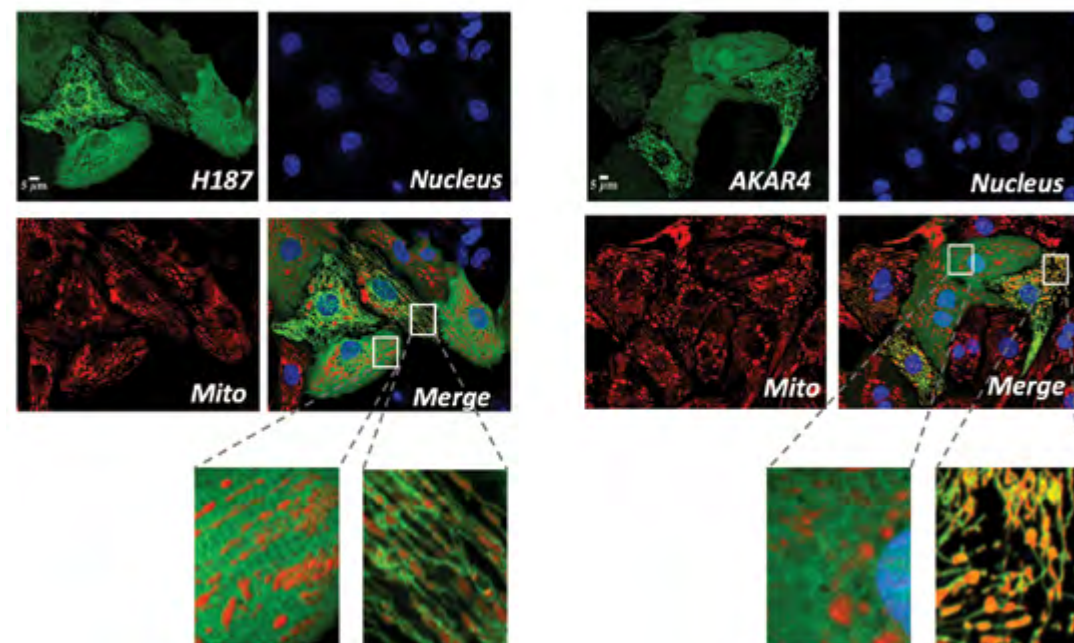
tro and *ex vivo* assays we identified necroptosis (a novel form of non-apoptotic cell death) as the primary type of cardiomyocyte death in response to PKA activation. We are further exploring the molecular connection between these two pathways as well as possible pharmacological treatments for blocking necroptotic death in the heart.

Best results obtained

The primary interest of the Lefkimmiatis group has been the identification of the modalities through which cAMP achieves its functional pleiotropy. We initially focused on the mitochondria, where we found that the mitochondrial matrix does not have detectable PKA activity (Lefkimmiatis *et al.* 2013). This study proved to be a seminal discovery that significantly increased our understanding on how mitochondrial function can be regulated by localized cAMP/PKA signaling events. In addition, our findings raised a series of new questions in the field, on the function and role of the cAMP/PKA axis in sub-mitochondrial domains.

Focusing on these new queries in a subsequent study we developed and validated a set of novel tools for studying mitochondrial signaling more in detail (Fig. 1).

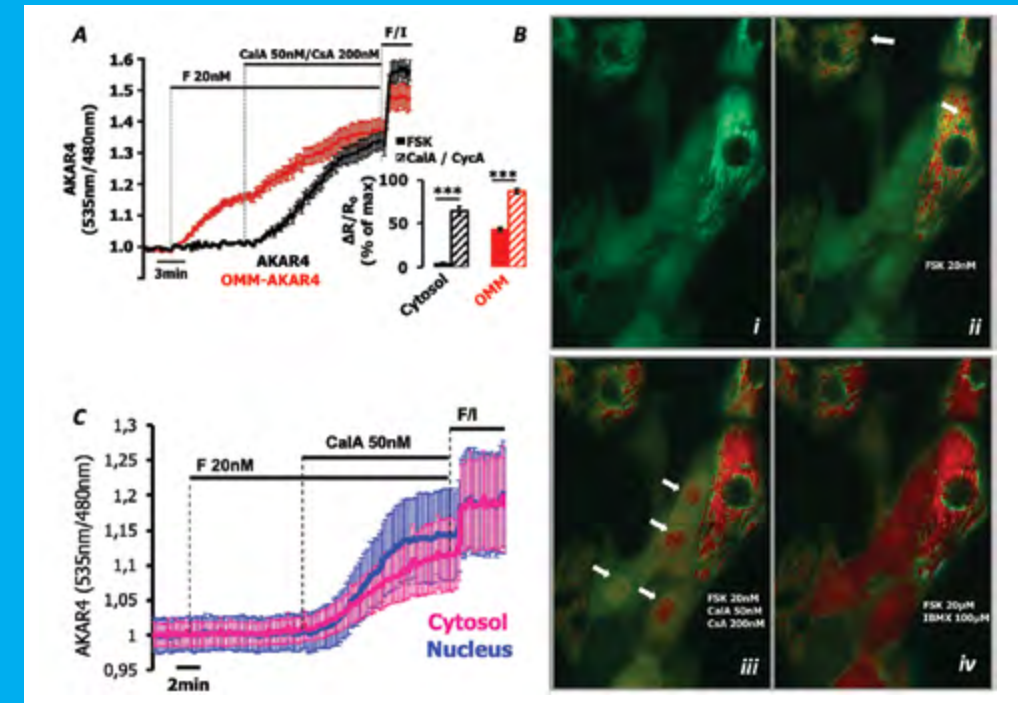
Figure 1



Confocal photomicrographs of cardiac ventricular myocytes (NRVMs) in co-culture.

A. NRVMs in co-culture, expressing the cytosolic cAMP sensor H187 or its OMM variant OMM-H187.
B. NRVMs in co-culture, expressing the soluble PKA activity sensor AKAR4 or its OMM variant OMM-AKAR4.

Figure 2



A. Cardiac myocytes (CMs) expressing PKA FRET sensors at the mitochondria (OMM-AKAR4) or nucleus and cytosol (AKAR4) challenged with cAMP increasing agents (Fsk) followed by phosphatase inhibitors Calyculin A (CalA) and Cyclosporin A (CsA).

B. Snapshots of mixed populations of CMs expressing AKAR4 or OMM-AKAR4 and treated with the protocol described in (A) Scale: Cold shades (low PKA activity) hot shades (high PKA-activity).

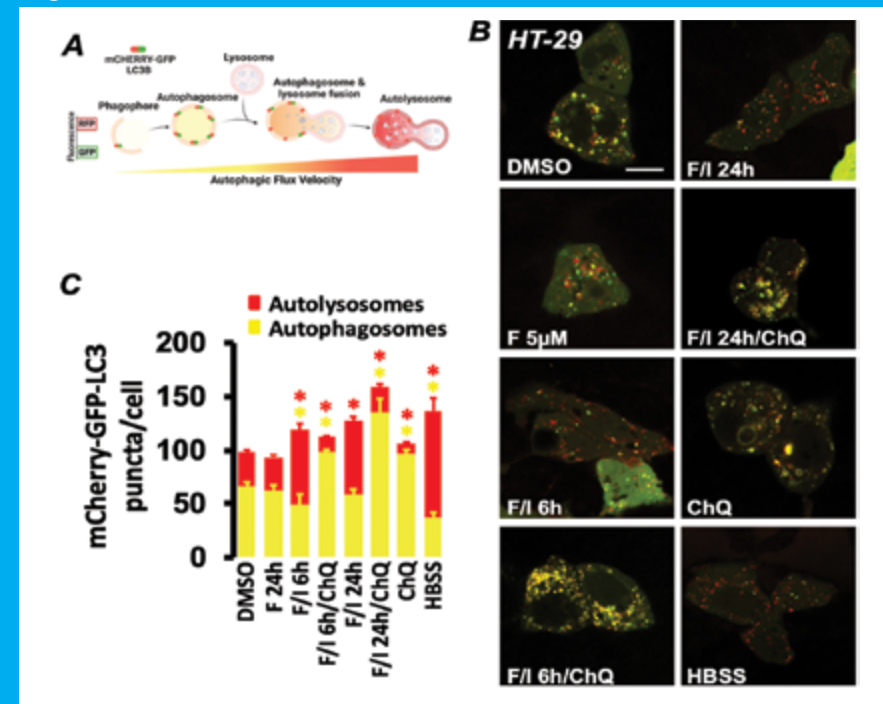
C. AKAR4 responses in the nuclear (blue) and cytosolic (purple) trace.

By using these constructs in primary cardiac myocytes we demonstrated that PKA at the outer mitochondrial membrane is significantly higher than the bulk cytosol and nucleus (Burdyga *et al.* PNAS 2018). This study challenged the dogma that regarded cAMP degradation as the sole mechanism ensuring the spatiotemporal regulation of PKA, by identifying phosphatases as significant players in the formation of distinct functional PKA microdomains, independently of the messenger itself. But what is the functional significance of these “privileged” PKA activity rich sites?

To address this question, we compared a colon adenocarcinoma cell line to a widely used ovary carcinoma model. We found striking differences in the signaling of these two lines, and we were able to connect the autophagic responses of these cells to the presence of cAMP/PKA microdomains. Indeed, we discovered that phosphatases are sufficient to generate distinct PKA microdomains that are indispensable for the cAMP-induced autophagic responses of these cells (Grisan *et al.* Cell Death Differ. 2021) (Fig. 3).

A striking finding during these studies was the extreme control exercised by the phosphatases on nuclear PKA (Fig. 2). Indeed, we consistently found that nuclear PKA activity was contrasted by phosphatases much more efficiently than that of PKA in other compartments. However, while in primary normal cardiac myocytes nuclear PKA would overcome the phosphatases in response to elevated cAMP signals (Burdyga *et al.* PNAS 2018), that is not the case in human colon adenocarcinoma cells, where nuclear PKA is unable to “break” the block by phosphatases even in the presence of extremely high (saturating) concentrations of messenger (cAMP). These findings point to the importance of phosphatases in compartmentalized signaling and provide a valid alternative mechanism for the generation of cAMP/PKA microdomains. In addition, this work brought to the realization that the nuclear cAMP cascade and its functions in pathology are not singularly regulated by PKA but other effectors acting with previously unknown modalities are in place.

Figure 3



Cyclical AMP elevating agents increase the autophagic flux in HT-29.

A. Schematic representation of how the sensor mCherry-GFP-LC3 works (created with BioRender.com).
B. Confocal photomicrographs of HT-29 cells expressing mCherry-GFP-LC3. Treatment with 5 μ M forskolin (F) did not alter neither the number or the balance between autophagosomes (mCherry+/GFP+) and autolysosomes (mCherry+/GFP-). Treatment with F and IBMX (F/I) increased the number of autolysosomes and decreased the number of autophagosomes similarly to starvation (HBSS). Treatment with ChQ alone or in combination to F/I drastically decreased the number of autolysosomes and increased the number of autophagosomes.
C. Summary of the effects on mCherry-GFP-LC3 puncta number and type of each treatment. (* $p < 0.01$), (Scale bar 20 μ m).

Future research plans

Our overarching future plan is to breach the gap between our basic studies and clinical applications:

How kinase activity is regulated within the cells and what is the physiological importance of these regulatory events?

We plan to compare the activity maps of specific kinases in health and diseased cells with the goal to identify the contribution of each kinase in the insurgence and progression of the pathology. The final goal will be to develop and identify drugs able to interfere with the pathological kinase signals.

How cAMP signals are perceived and interpreted by the nucleus?

We will continue to develop more refined tools for studying the nuclear cAMP cascade aiming in understanding the precise nuclear processes where this pathway is involved. Our final aim will be to identify the pathophysio-

logical effects driven by the nuclear cAMP axis and how are connected to human disease.

What is the involvement of protein kinase A (PKA) in non-apoptotic programmed cell death?

Once established the connection between persistent PKA signals and necroptosis we will test FDA-approved inhibitors of the latter as potential cardioprotective drugs. This will be done by employing a high throughput approach. Briefly, we have generated cell lines that express under an inducible promoter the main components of the necroptotic pathway, as well as mutant versions on the sites we have identified to interact with the cAMP / PKA axis. Using these cell lines, we will test FDA-approved drug libraries looking for compounds capable to block the cell death induced by the cAMP elevations. In preliminary studies we have already identified one such compound which will be used in a proof of principle set of experiments to further demonstrate the possibility to interfere with cardiac myocyte death.

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CLAUDIA LODOVICHI

NEURONAL CIRCUITS FORMATION AND FUNCTION UNIT



Biographical Sketch

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- 2018 to date Scientist affiliated to the Padova Neuroscience Center
- 2009-2019 Researcher, IN-CNR, *Padua, Italy*
- 2006 to date Group Leader, VIMM (Armenise-Harvard Foundation Career Development Awardee), *Padua, Italy*
- 2003-2005 Post-doc, J. A. Gogos's laboratory, Columbia University, *New York, USA*
- 1999-2003 Post-doc, L.C. Katz's laboratory, Duke University, *Durham, USA*
- 1999 PhD Neuroscience, Sant'Anna School of Advanced Studies, *Pisa, Italy*
- 1995 MD Medical School, Sant'Anna School of Advanced Studies, *Pisa, Italy*



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- Marco Albanesi - *PhD Student*
- Marco Brondi - *Postdoctoral Fellow*
- Martina Canova - *Undergraduate Student*
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- Andrea Maset - *Postdoctoral Fellow*
- Siddhartha Pandit - *Undergraduate Student*

RESEARCH ACTIVITY

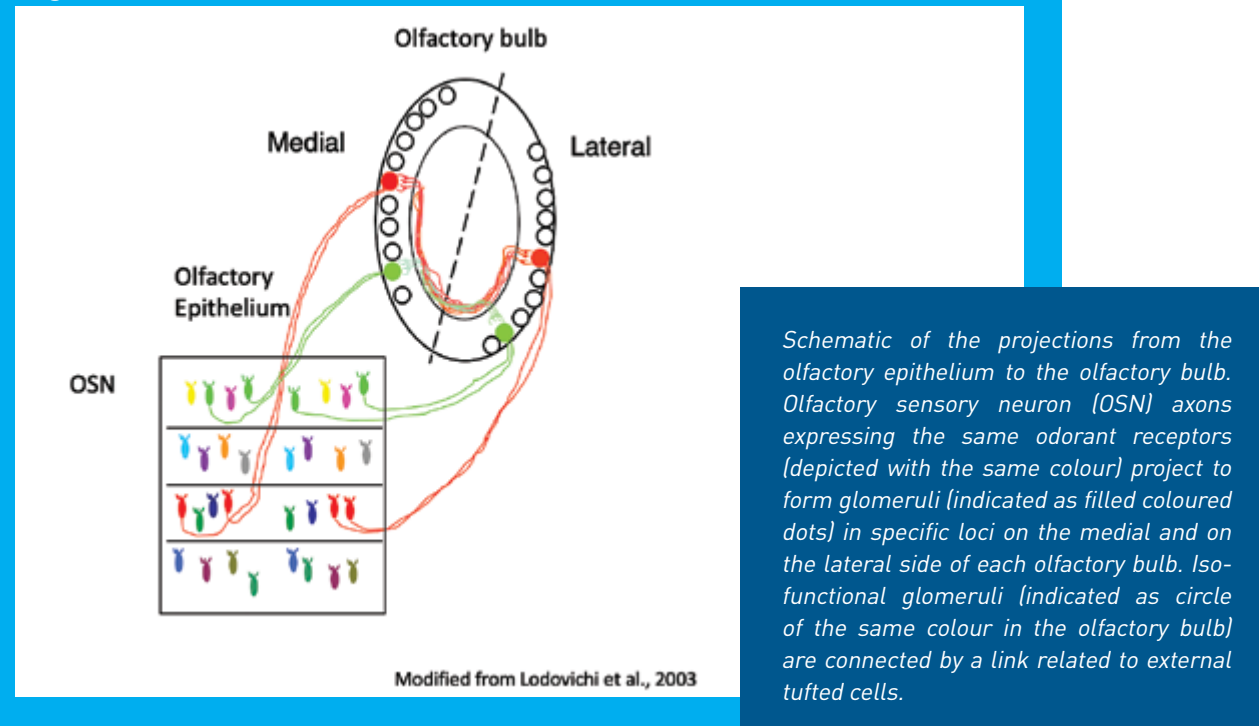
Fields of interest

The goal of our research is to understand how neurons wire together to form specific neuronal circuits and how these circuits process neuronal information, to support distinct behaviors. We address these questions mostly in the olfactory system and connected areas. Here, I provide a profile of our research.

We studied neuronal circuits formation in the olfactory bulb, the first olfactory brain area. Combining functional imaging and quantitative anatomy, we found that the

identity of the odorant receptors determines not only the convergence of like-axons in specific loci but also functional circuitry within the olfactory bulb (Belluscio Lodovichi 2002). We then unraveled that iso-functional glomeruli within each olfactory bulb are specifically and reciprocally connected by a link related to external tufted cells. This intrabulbar link supports a second level of topography (Fig. 1) (Lodovichi 2003).

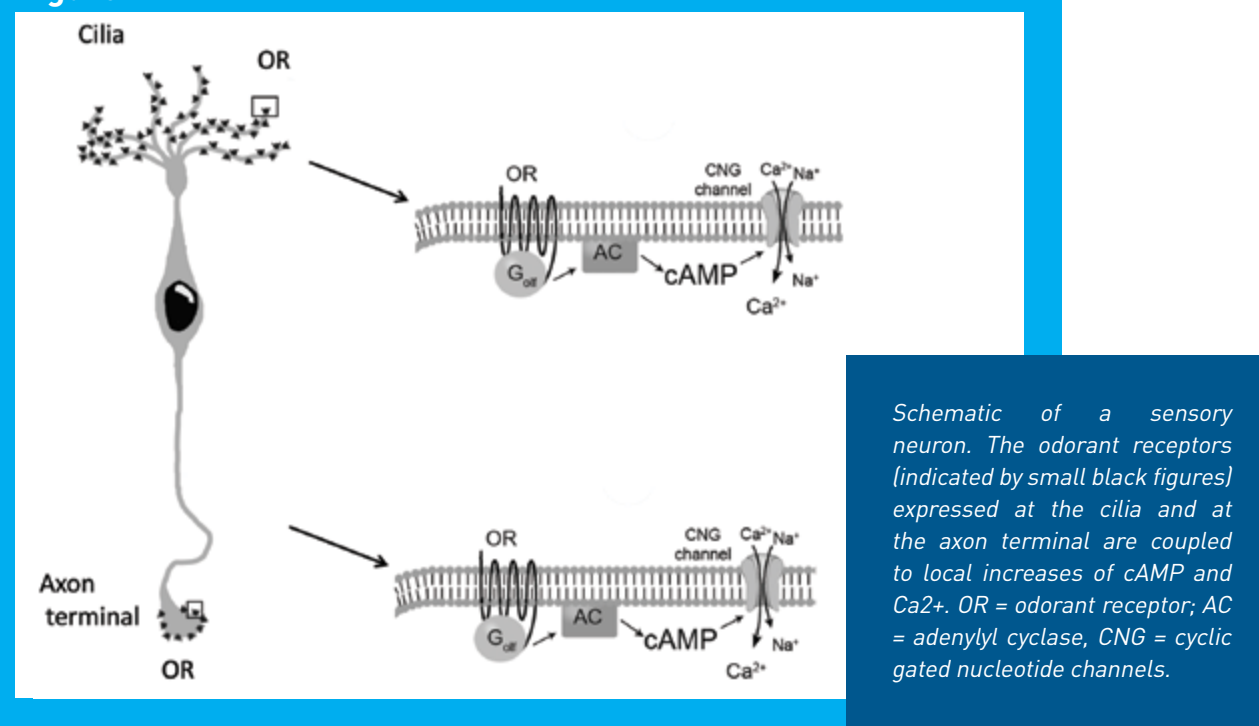
Figure 1



A unique feature in the olfactory system is the dual role of the odorant receptors. They detect odors but they also direct sensory axons to their targets in the brain. How the same molecules can accomplish such two different roles remained elusive for a long time. We demonstrated that the odorant receptors expressed at the axon terminal of sensory neurons are functional and coupled to local increases of cAMP and Ca^{2+} (Fig. 2) (Maritan 2009, Pietrobon 2011, Lodovichi Belluscio 2012). In addition,

we unveiled their mechanism of activation. Through an unbiased screen of cues elaborated in the olfactory bulb, we identified the first putative ligand of a subset of axonal receptors and we demonstrated that in mice carrying a null mutation for this molecule the sensory map was deeply disrupted. Our findings solved a long-standing riddle in the field and demonstrated the key role of the axonal receptors in the formation of the olfactory map (Zamparo 2019, Lodovichi 2021).

Figure 2



The development of a sensory map is regulated by the complex interplay between molecules and neuronal activity. We focused on the role of afferent spontaneous activity in the formation of the sensory map. We found that spontaneous afferent neuronal activity does not instruct the formation of neuronal wiring but plays a critical role in the refinement and maintenance of specific connections in the olfactory bulb (Lorenzon 2015).

Another distinct feature of the olfactory system is its high level of plasticity. The major population of GABAergic inhibitory interneurons of the olfactory bulb, the granule cells, constantly regenerate. These interneurons are thought to play a critical role in postnatal developmental plasticity, a key process for normal brain development, in all mammals including human infants. We thought therefore to study whether this critical process could be affected in mouse models of neurodevelopmental disorder. To this end we studied postnatal neurogenesis in a validated mouse model of intellectual disability- autism spectrum disorder associated to mutation of Oligophrenin1 (OPHN1), i.e. OPHN1 KO mice. We found that the number, the morphological and synaptic properties of the postnatally generated inhibitory GABAergic interneurons were deeply altered, in OPHN1 KO mice (Redolfi 2016). To dissect the mechanisms underlying these defects, we studied the migration of the neuronal precursors by means of two photon imaging. We found that mutant cells exhibited altered Cl^- homeostasis, related to an early maturation of the mutant interneurons, that prevent the proper migration of these cells. Upon the identification of the mechanism underlying the altered migration in OPHN1 KO mice, we devised specific pharmacological approaches and we rescued the migration of the newly generated cells (Maset 2021). Our results open a different perspective in the field of neurodevelopmental disorders. In most cases, the persistence of the

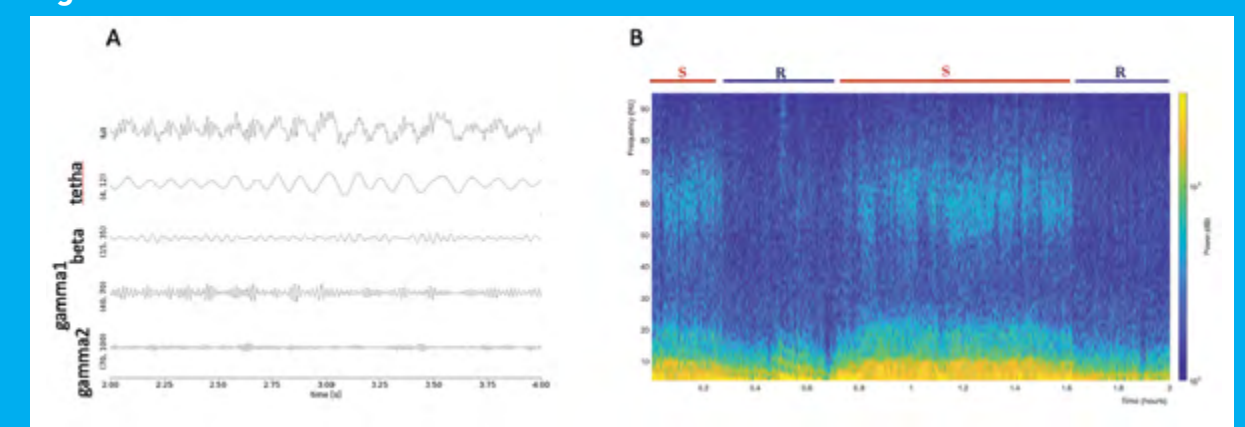
immature phenotype of interneurons into adult hood, and not an early maturation, exerts a critical role in the etiopathogenesis of neurological disorders. Our findings unraveled that alterations of GABAergic interneurons and GABA responses can exhibit a wider range, hence the need to rigorously study the functional properties of these neurons to understand the distinct mechanisms underpinning each disorder and design tailored cures.

Investigations of neurodevelopmental disorders posit the problem of understanding how the cellular effects of the mutation impact neuronal network activity. In order to achieve this goal is necessary to discriminate cells carrying the mutation from cells which are healthy. To unravel this cellular mosaic, we contributed to the generation a genetically encoded sensor which expresses fluorescent proteins of different colors in different cellular environments, making possible to distinguish between "healthy and sick cells" (Trovato 2020).

Having identified alterations of the inhibitory circuits in OPHN1 KO mice, we are studying the impact of OPHN1 mutation at system level. We aim to understand how correlated activity among neurons, also known as oscillations, are affected in OPHN1 mice. Oscillations are a signature of normal brain function and support sensory and cognitive functions. A growing body of evidence indicates that aberrant oscillations are early signs in neurodevelopmental and neurodegenerative disorders.

The focus of our current and future research is to characterize neuronal network activity at resting state and in response to sensory stimuli, during behavioral tasks, in physiological conditions and understand how these processes are altered in animal models of diseases. We address these questions combining several experimental approaches, including electrophysiological recordings *in vivo* (LFP and EEG, see Fig. 3), functional two photon

Figure 3



Electrophysiological recordings of oscillations in the olfactory bulb. A. Top, raw trace of local field potential recordings and bottom, filtered traces, in the different frequency bands. B. spectrogram of local field potential recording in the olfactory bulb, during sniffing- explorative (S) behaviour and during resting (R) behaviour.

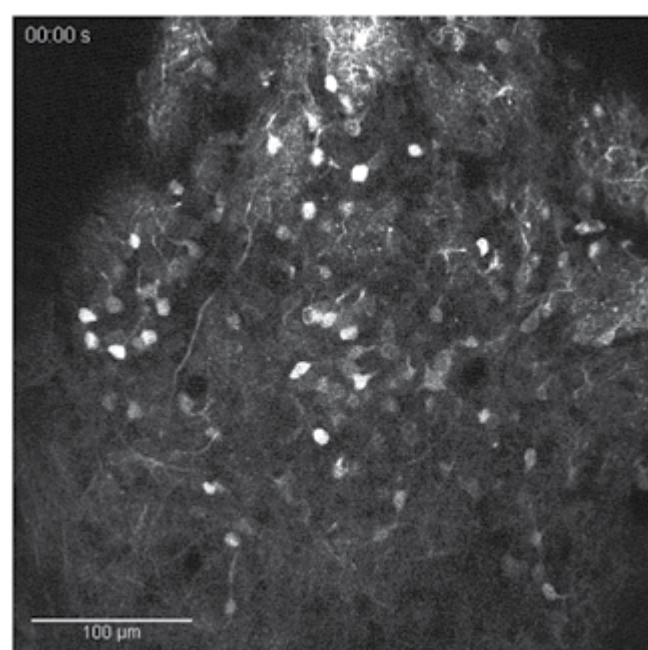
imaging *in vivo* (**Fig. 4**), optogenetics and behaviour, in the olfactory and connected brain areas. Since even an easy sensory task requires the involvement of several brain areas, imaging whole-brain activity is critical to achieve a deep understanding of these processes. To this end, larvae of zebrafish represent an amenable model organism. Their transparency along with their small size allow to record neuronal activity across the brain. These advantages make zebrafish a suitable model organism for systems neuroscience approaches. Therefore, in parallel to the studies in mice, we perform investigations in zebrafish larvae recording whole-brain neuronal activity by means of two photon imaging, at basal and in response to sensory stimuli, during behavioral tasks.

In addition, we work also on neurodegenerative diseases. It is well known that in Parkinson's disease olfactory deficits precede the onset of motor impairments by

years. Although more than 90% of patients present olfactory impairments, the neuronal mechanisms underlying olfactory deficits are still unknown. Our goal is to characterize alterations in neuronal activity in olfactory areas first affected by Parkinson's pathology aiming at identifying biomarkers for early diagnosis and design effective therapeutic interventions to prevent the progression of the disease.

The aim of our present and future research is to characterize neuronal activity at baseline and in response to sensory stimuli during behavioral tasks to understand how neuronal information is encoded in physiological conditions and analyze how these patterns are altered in animal models of diseases. Understanding how the brain works in physiological conditions is the essential pre-requisite to fix its alterations during neurological disorders.

Figure 4



Example of a stop-frame of functional 2 photon imaging of cells expressing a genetically encoded sensor for Ca²⁺ (GCaMP6) in the olfactory bulb.

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DENIS MARTINVALET

CYTOTOXIC LYMPHOCYTES, OXIDATIVE STRESS AND CANCER UNIT



Biographical Sketch

- 2021 to date Associate Professor, Dept. Biomedical Sciences, University of Padua, *Italy*
- 2018 to date Group Leader, VIMM, *Padua, Italy*
- 2018-2021 Assistant Professor, Dept. Biomedical Sciences, University of Padua, *Italy*
- 2017 to date Privat-Docent, Faculty of Medicine, University of Geneva, *Switzerland*
- 2017-2018 Visiting fellow Collar' Lab University of Geneva, *Switzerland*
- 2009-2016 Group Leader, Dept. Cell Physiology and Metabolism, University of Geneva, *Switzerland*
- 2006-2009 Instructor, Immune Disease Institute, Harvard Medical School, *Boston, USA*
- 2001-2006 Research Fellow, Immune Disease Institute, Harvard Medical School, *Boston, USA*
- 2000-2001 Research Fellow, Manitoba Institute of Cell Biology, *Winnipeg, USA*



Group Members

- Achille Homère Tchamptda Dondjang - *Research Fellow*
- Marianna Gasparrini - *Master Trainee*
- Stefania Lionello - *PhD Student*
- Nunzia Miglionico - *Bachelor Trainee*
- Giulia Palomba - *Bachelor Trainee*

RESEARCH ACTIVITY

Fields of interest

Denis Martinvalet PhD, is an Associate Professor of general pathology and immunology at the department of Biomedical Sciences of the University of Padua and a Principal Investigator at the Veneto Institute of Molecular Medicine. He is best known for his work on

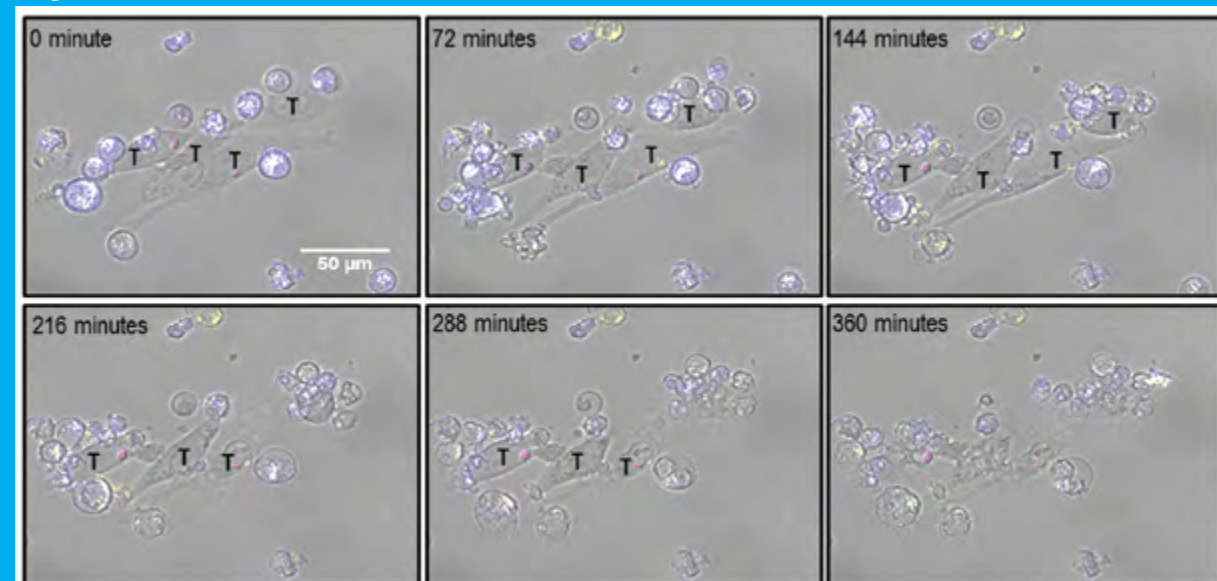
the mechanism of cytotoxic lymphocyte-mediated cell death. Now his research focus on the understanding of the dynamic of interaction between the cytotoxic lymphocytes and their target cancer cells in a model of glioblastoma multiforme, a very aggressive primary

brain tumor for which there is currently no cure.

Reactive oxygen species (ROS) result from the partial reduction of oxygen. This large family of reactive compounds include both radical species and non-radical species. Interestingly, ROS are very pleiotropic compounds. They are involved in both physiological processes such as inflammation, vasoconstriction, signal transduction, cell migration, differentiation, prolifera-

tion and pathological conditions as they are common determinants of various forms of cell death, e.g. apoptosis, necrosis/necroptosis, ferroptosis, pyroptosis and autophagic cell death. The molecular understanding of their biogenesis and their mode of action during cell death is not fully understood. In the context of lymphocyte-mediated killing, we found an essential role for mitochondrial ROS.

Figure 1



Experiment was recorded by live cell imaging in atmosphere control chamber using Leica TCS SP5 confocal microscope. Images represent acquisition every 72 minutes. Over the time, the target cells shrink indicating that they undergo apoptosis cell death following the lethal hit delivered by the CLs.

Natural killer (NK) cells and cytotoxic T lymphocytes (CTL) are innate and the adaptive cytotoxic lymphocytes (CLs) dedicated to the elimination of stressed cells such as cancer cells or pathogen-infected cells. We have found that cytotoxic lymphocyte-mediated cell death requires target cell cytosolic delivery of the effector proteases granzymes to induce ROS-dependent cell death. We found that both granzyme A and B (GA and GB) enter the target cells mitochondria to cleave NDUFS3, NDUFB1, NDUFS2 and NDUFS1 subunits of the NADH: ubiquinone oxidoreductase complex I of the electron transport chain (ETC) (Martinvalet *et al.* 2005, Martinvalet *et al.* 2008 and Jacquemin *et al.* 2015). Cleavage of complex I subunits triggers a rapid and robust mitocentric ROS production, loss in complex I, II, and III activity, disorganization of the respiratory chain, impaired mitochondrial respiration and loss of the mitochondrial cristae junction (Jacquemin *et al.* 2015). Unexpectedly, Granzymes and caspase 3

enter the mitochondria independently from TOM40 complex, the organelle known entry gate, and use instead SAM50 channel as a translocase (Chiusolo *et al.* 2017). SAM50 is the core channel of the mitochondrial sorting and assembly machinery involved in the insertion of *de novo* β -barrel proteins into the mitochondrial outer membrane. Preventing granzymes and caspase 3 from accessing the mitochondria matrix thus significantly reducing their cytotoxicity, suggesting that their mitochondrial entry is a critical step for ROS-dependent cell death. More importantly, these results also unravel an unanticipated novel SAM50-dependent mitochondrial anterograde signaling. Using a model of glioblastoma multiforme, a highly heterogeneous and aggressive primary brain tumor, we found that this novel anterograde signaling seems hyper activated in the cancer cells compared to primary astrocytes used as control. One aim in the lab is to continue to characterize the function of this

novel SAM50-dependent anterograde signaling both for the cancer cell maintenance and for the outcome of anti-tumoral immune response.

We have also found that in the glioblastoma cells, mitochondrial-ER interorganelle communications are profoundly dysregulated. We showed that mitochondrial-ER contacts (MERCs) not only control glioma cell stemness but also the surface expression of some glycans, the later regulates the glioma cell susceptibility to CLs. Indeed, we have showed that glioma stem-like cells have fragmented mitochondria and altered MERCs which are associated with reduced sialylated glycans surface expression resulting in an increased recognition and susceptibility to CLs (Bassoy *et al.* 2017). Interestingly, both the glioma stem-like cells and glioma differentiated cells were capable of producing to a similar extent a wide spectrum of glycans. Therefore, these results indicate that the differential surface glycan expression observed between these two differentiation stages of these cancer cells, is likely due to a defect in the glycans export at the cell surface rather than alteration in their

biosynthesis. Glioma stem-like cells are not able to bring some glycans in the form of glycolipids at their surface, probably due to a defect in lipid biosynthesis or bioavailability because of the defect in their MERCs (Bassoy *et al.* 2016; Bassoy *et al.* 2017). Another aspect of our work is to investigate the role of interorganellar contact in the biology of cancer stem cells and their susceptibility to cytotoxic lymphocytes and chemotherapies. Lastly, since cancer cells have a hypoxic and oxidized microenvironment, we are also investigating the role of the ROS in the remodeling and reprogramming of the tumor ecosystem.

Future research plans

Recently, we have also initiated a new project aiming at understanding the mechanism of dysregulation of cytokine production by the SARS-CoV-2. We focus our attention on the different virus encoded non-structural proteins, to characterize novel aspect of the host-virus interaction regulating the innate immune responses.

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Biographical Sketch

2015 to date	Associate Professor, Dept. Biomedical Sciences, University of Padua, <i>Italy</i>
2009 to date	Group Leader, VIMM, <i>Padua, Italy</i>
2007-2015	Assistant Professor, University of Padua, <i>Italy</i>
2006-2008	Research Fellow, Dept. Physiology and Cellular Biophysics, Columbia University, <i>New York, USA</i>
2005-2006	Clinical Fellow, Cardiology, Imperial College, <i>London, UK</i>
2005	PhD, Molecular and Cellular Biology, University of Padua, <i>Italy</i>
2001	MD, University of Padua, <i>Italy</i>



Group Members

- Lolita Dokschocka (co-supervised with Dr. Zaglia) - *Postdoctoral Fellow*
- Marco Ronfini - *PhD Student*
- Nicola Moro - *PhD Student*

RESEARCH ACTIVITY

Fields of interest

The lab is devoted to the understanding of the physiology and pathophysiology of the cardiac sympathetic nervous system, and the mechanisms whereby its dysfunction is involved in the development of cardiovascular diseases including arrhythmias and myocardial hypertrophy. Our overall aim is to define, at the molecular, cellular and *in vivo* levels the mechanisms underlying the control operated by cardiac sympathetic neurons on heart tissue

homeostasis, through specific interaction and signaling with the different myocardial cell types. In addition, we will transfer our findings to the study of disease models of inherited and acquired arrhythmogenesis, as well as of familial hypertrophic diseases (e.g. familial hypertrophic cardiomyopathy).

The main research projects and accomplishments of the group in the last three years are listed below.

Ongoing researches

Physiology and Biophysics of intercellular communication between sympathetic neurons and heart

Cardiac sympathetic neurons (SN) finely tune the rate and strength of heart contractions to match the blood demand, both at rest and during acute stresses, through the release of noradrenaline. Functional alterations in the neurogenic control of heart are common in several cardiac disorders (HF, HCM), and have been implicated in decreased cardiac performance as well as in triggering arrhythmias and sudden cardiac death (SCD). Although the general physiology of cardiovascular effects of the sympathetic nervous system has been subject of investigation for many decades, the molecular physiology and biophysics of neuro-cardiac coupling, i.e. the mechanisms whereby sympathetic neurons communicate to target cardiac cells, is largely unexplored. Using FRET-based imaging of cAMP in co-cultures and optogenetics *in vivo*, we investigated the dynamics of neuro-cardiac signaling. We demonstrated that SNs and cardiomyocytes interact at specific sites both in the human and rodent heart, and in co-cultures. Accordingly, neuronal activation elicited intracellular cAMP increases only in directly contacted myocytes, and cell-cell coupling utilized a junctional extracellular signaling domain with elevated noradrenaline concentration. Optogenetic activation of cardiac SNs innervating the sino-atrial node resulted in the instantaneous chronotropic effect, which shortened the heartbeat interval with single beat precision. Our *in vitro* and *in vivo* data demonstrated the novel concept that the control of cardiac function by SNs, occurs in “synaptic” fashion, underlain by specific intercellular junctional-site. These findings are relevant for the fine understanding of cardiac physiology and impact on cardiovascular pharmacology (e.g. the widely-used beta-adrenoceptor blocking drugs), and pathophysiology. (Prando *et al.* J. Physiol. 2018; Zaglia *et al.* Methods Protoc. 2019).

Effect of direct neuro-effector coupling on myocardial geometry

We have previously shown that cardiac sympathetic neurons provide trophic input to cardiomyocytes, which depends on neurogenic signaling influencing intracellular protein synthesis and degradation machineries, UPS and ALS (Zaglia *et al.* Cardiovasc Res. 2013), through stimulation of β_2 -ARs. Our findings on the dynamics of intercellular communication between neurons and cardiac cells demonstrated that neuro-cardiac signaling occurs directly and locally. Consistently, in co-cultures, cardiomyocyte size reflects the degree of direct innervation by the neuronal processes. To verify whether this concept applied to the intact heart, we reconstructed in 3-D

the sympathetic neuron network in the cardiac walls in the heart, using multiphoton microscopy. We identified transmural heterogeneity in sympathetic innervation, whereby the sub-epicardial cardiomyocytes establish more frequent neuronal interaction sites than the sub-endocardial ones. In keeping with our previous demonstration that communication between sympathetic neurons and cardiomyocytes occurs through direct and localized interaction at neuro-cardiac junctions, sub-epicardial cardiomyocytes have larger cross-sectional area than the less-innervated sub-endocardial ones.

This work which corroborates our previous findings is the natural extension of the study of neuro-effector dynamics in the acute setting, to the structural effects of innervation in the long term. In addition to the implications of our findings in cardiovascular pharmacology and patho-physiology, this data is the first to demonstrate the effect of a neuronal network on the structurally shaping of the otherwise homogeneous myocardium. This opens new points of view in cardiac physiology and pathologies associated to heart dysinnervation (e.g. HF) or denervation (e.g. heart transplantation) (Pianca *et al.* J. Physiol. 2019).

Role of neuro-cardiac junctions in cardiomyocyte-dependent neuronal trophism

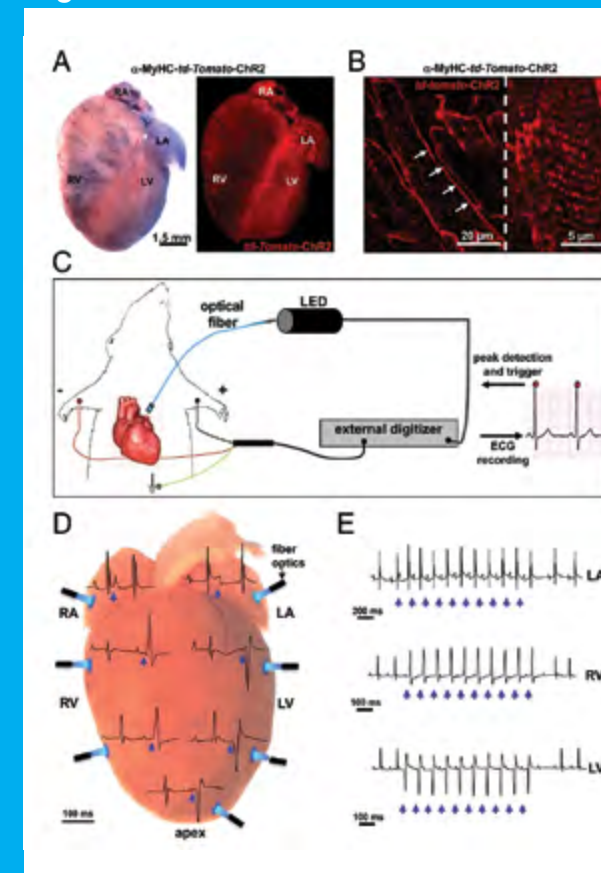
Neurotrophins released by cardiac cells control SN viability and myocardial distribution, which are impaired in heart diseases with reduced (e.g. heart failure) or heterogeneous (e.g. arrhythmias) sympathetic stimulation. We previously demonstrated (see above) that SNs interact directly with cardiomyocytes (CMs) at neuro-cardiac junctions (NCJ), and such structured contact sites allow neurons to efficiently activate β -adrenoceptors on the myocyte membrane.

Given that sympathetic neurons require continuous neurotrophic stimulation by specific growth factors (i.e. NGF), we extended the work on neuro-cardiac communication with the aim to test whether NCJs are implicated in retrograde (myocyte to neuron) neurotrophic signaling. Our current results support that the NCJ is essential for intercellular neurotrophin signaling required to maintain correct sympathetic innervation of the heart.

Best results that have been obtained

1) We have developed, in the first study by an Italian group, protocols to apply cardiac optogenetics to the study of heart physiology. The method allowed achieve, for the first time, selective control of the conduction system cells in the beating heart, and to determine the relative roles of conduction system cells vs. working myocardium in the generation of arrhythmic beats, in normal and ischemic hearts (Zaglia *et al.* PNAS 2015) (Fig. 1).

Figure 1

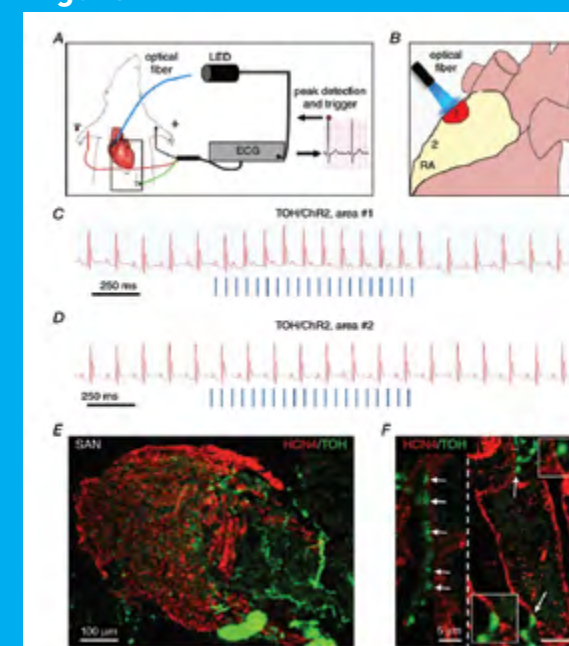


Cardiac optogenetics allows noninvasive investigation of heart electrophysiology *in vivo*. **A.** Bright field (left) and fluorescence (right) images of α -MyHC-tdTomato-ChR2 heart. The Right image of tdTomato fluorescence shows expression of ChR2 in both the atria and the ventricles. LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle. (Scale bar, 1.5 mm.) **B.** Confocal image of ventricular cryosections from α -MyHC-tdTomato-ChR2 transgenic hearts showing ChR2 localization at the level of cardiomyocyte sarcolemma (white arrows) and t-tubuli. (Scale bar, 20 μ m; high magnification, 5 μ m.) **C.** Representation of the setup used for epicardial photostimulation in open-chest anesthetized mice. LED stimulation occurred through fiber optics delivering time-controlled pulses of 470 nm blue light. The LED was connected to the ECG apparatus, allowing synchronization of the LED stimulation with a specific time point of the ECG recording. **D and E.** Representative ECG traces of ectopic beats originated by epicardial light stimulation of different regions of the myocardium from α -MyHC-ChR2 mice ($n = 10$ mice). Blue arrows in D and E indicate light pulses. (from Zaglia *et al.* 2015).

2) We have applied optogenetics to the study of neuro-effector coupling in the heart, which allowed demonstrate the existence of specific structured contacts at the base of neurogenic regulation of heart by sympathetic neu-

rons. This multidisciplinary study, which ranked “best paper” in the cardiology section of the Journal of Physiology in 2019, represented a pioneering study in the understanding of neuro-cardiac biophysics (Fig. 2).

Figure 2

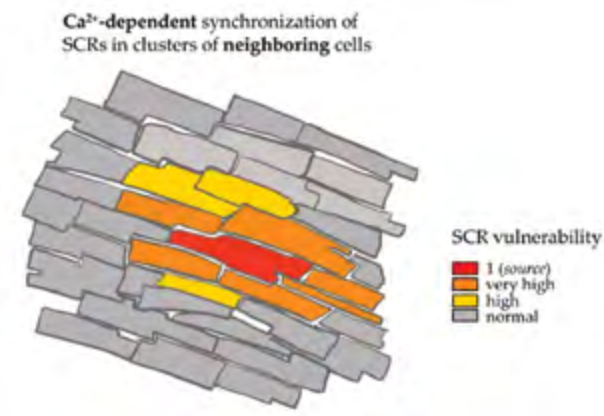


Optogenetic control of cardiac SNs. **A.** Schematic illustration of the neuronal optogenetic set up used for right atrium illumination in open-chest anaesthetized mice. **B.** Representation of the different photostimulated atrial regions (areas#1-2). **C.** Representative ECG trace of the optogenetic experiment, showing positive chronotropic response upon photoactivation (blue lines) of the right atrial area#1 in TOH/ChR2 mice. **D.** Representative trace showing unchanged HR upon illumination of area#2. Behaviour of experiments [C and D] was observed in $n = 15$ mice. **E and F.** Area#1 was analysed with double IF with SAN (HCN4) and SN (TOH) markers. The magnified image (F) highlights the close interaction between SN varicosities and SAN myocytes (left) and an example of multiple neuronal processes interacting with the same myocyte (right). (from Prando *et al.* 2018).

3) Additionally, aiming at the investigation and therapeutic targeting of arrhythmia mechanisms, we have addressed Ca2+ homeostasis using multiphoton microscopy in intact myocardial tissue from models of catecholaminergic polymorphic ventricular tachycardia (CPVT), linked to mutation

in Ryanodine Receptor 2 (RyR2^{R2474S}) [Borile *et al.* JBO 2014]. We determined the alterations of Ca2+ homeostasis at the single cell level, and addressed how the single cell dysfunction can be spread to the neighbouring ones in the myocardium to generate effective arrhythmia triggers (Fig. 3).

Figure 3



Factors potentially contributing to synchronization of diastolic depolarization in the myocardium. A “source-sink-source” model, showing the effect of a SCR occurring in one myocardial excitable cell (red labelled “source” cell) on vulnerability of surrounding CM to develop, in turn, diastolic “SR leak” and SCR. This positive feedback loop may, thus, “regenerate” a current source, enlarging the area of CMs depolarizing synchronously. (from Borile *et al.* 2021).

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Biographical Sketch

2018 to date	Deputy Director & Group Leader, VIMM, Padua, Italy
2017 to date	Associate Professor, Dept. Biomedical Sciences, University of Padua, Italy
2013-2017	Assistant & Associate Professor, CIBIO, University of Trento, Italy
2009-2014	Group Leader, Italian Institute of Technology, Genoa, Italy
2008-2009	Staff Scientist, Dept. Neurology, Pennsylvania Univ. (Mentor: Dr J.P. Taylor, MD, PhD), Philadelphia, USA
2005-2008	Visiting Postdoctoral Fellow, NIH-NINDS (Mentor: Kenneth Fischbeck, MD)
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1996-1997	Intern, Biology, University of Rome “La Sapienza”, Italy

Group Members

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- Elisa Bregolin - Master Student
- Federica Lia - PhD Student
- Caterina Marchioretti - PhD Student
- Roberta Marchioretti - PhD Student
- Emanuela Zuccaro - PhD, Marie Curie fellow and Stars Unipd Investigator

RESEARCH ACTIVITY

Fields of interest

Androgen receptor (AR) belongs to the family of nuclear hormone receptors, which also includes oestrogen, glucocorticoid, progesterone, and mineralocorticoid receptors. In its inactive state, AR resides in the cytosol in association with heat shock proteins (HSPs). Upon

binding to its natural ligands, testosterone (T) and the more potent derivative dihydrotestosterone (DHT), AR dissociates from HSPs, dimerizes and translocates to nucleus, where it binds to specific sequences on the DNA known as androgen-responsive elements (ARE).

Upon interaction with transcription co-factors (co-activators and co-repressors) and the basal transcription complex machinery, AR regulates the expression of the androgen-responsive genes. In addition, AR undergoes several post-translational modifications (PTMs), such as phosphorylation, methylation, acetylation and ubiquitination. AR is a ubiquitous protein. Partial or complete AR loss-of-function (LOF) mutations lead to different degrees of androgen insensitivity syndrome, whereas gain-of-function (GOF) mutations result in two clinically distinct age-related diseases, prostate cancer (PC) and spinobulbar muscular atrophy (SBMA).

We have previously established that 1) Androgen-induced AR nuclear translocation, DNA binding and interaction with transcription co-factors are all necessary steps towards neurodegeneration in SBMA; 2) Signaling pathways culminating in direct modification of AR affect its response to androgens, thereby resulting in major changes in protein structure, function, and toxicity; and 3) Peripheral tissues, such as skeletal muscle, play a key role in SBMA, indicating that AR expression in these tissues is important for disease pathogenesis. These findings imply that the toxic GOF underlying SBMA involves modification of the native function of AR, suggesting converging mechanisms with PC. Moreover, the observation that PTMs of AR

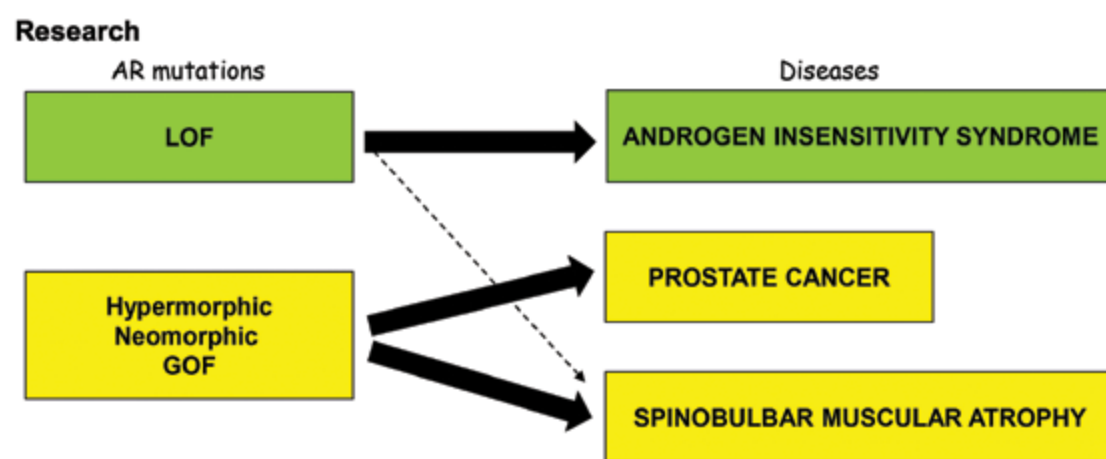
affect its structure-folding, native function and toxicity offers the opportunity to identify agents that enhance PTMs suppressing toxicity and test these compounds for therapeutic purposes. Based on our results obtained in mice, two agents have been moved to phase II clinical trials for SBMA. We are currently exploring the molecular details of AR dysfunction and deregulation of androgen-responsive gene expression. Moreover, we are investigating what is the role of AR in peripheral tissues in health and disease.

Ongoing researches and future plans

What is the molecular relationship between AR structure/function and disease?

Mutations in the AR gene can cause clinically distinct types of disease (**Fig. 1**). PC is a fatal disease and the second leading cause of death in USA and Europe. SBMA is a rare neuromuscular disease linked to expansions of the trinucleotide tandem repeat CAG encoding a polyglutamine (polyQ) tract in the first exon of the AR gene. While PC and SBMA diverge in their end-state, culminating in cell proliferation and cell death, respectively, these two diseases interestingly share several features. The primary events of both PC and SBMA are indeed strikingly androgen-dependent.

Figure 1



Mutations in the AR cause different diseases. Loss of function (LOF) mutations cause androgen insensitivity syndrome. Gain of function (GOF) mutations can be hypermorphic if they enhance the native function of the disease-related proteins and neomorphic if they confer aberrant properties to the mutant protein. GOF mutations can either cause prostate cancer or spinobulbar muscular atrophy.

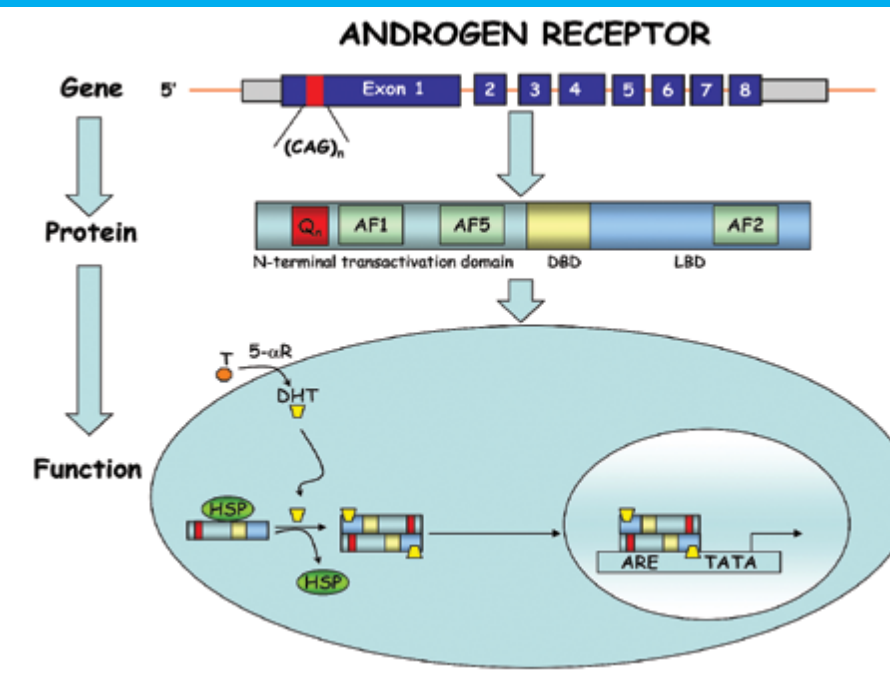
Moreover, these disorders are age-related, with most of cases manifesting after the fourth/fifth decade of life. We have previously shown that phosphorylation of polyQ-AR by Akt blocks androgen binding and that this phosphorylation is regulated by the protein arginine methyltransferase 6 (PRMT6). We are currently investigating the role of other PTMs in health and disease.

What is the mechanism through which AR integrates androgen signaling with extracellular signaling and cell metabolism?

AR is the main effector of androgen signaling. AR is a transcription factor activated by androgens (**Fig. 2**). Androgen binding results in nuclear translocation

and binding to DNA at the level of androgen-responsive elements. AR recruits transcription co-factors to regulate (activate and repress) the expression of androgen-responsive genes. AR integrates the androgen signaling with that of extracellular signaling pathways initiated by binding of specific ligands to their receptors. AR is indeed highly phosphorylated, and phosphorylation by kinases, such as PKA and Akt, modifies AR stability, function and toxicity. In light of these results, we are now investigating the molecular logics of changes in gene expression resulting from activation of AR by androgens and growth factors and neurotrophin signaling. Moreover, we are investigating how cell metabolism affects AR function.

Figure 2



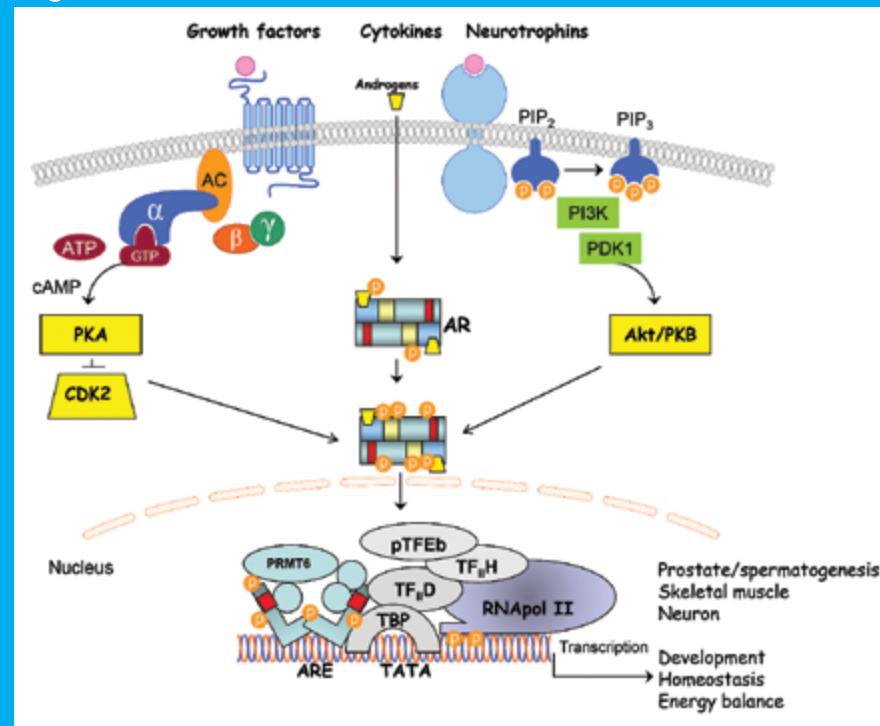
From gene to protein function. The AR gene is composed of eight exons. A CAG tandem repeat is present in exon 1. AR is composed of three domains, the N-terminal transactivation domain that contains two activating function (AF) surfaces, AF-1 and AF-2, the DNA-binding domain (DBD), and the ligand-binding domain (LBD), which contains AF-2. In the absence of its natural ligands, testosterone (T) and its more potent derivative dihydrotestosterone (DHT), AR resides in the cytosol in association with heat shock proteins (HSPs). Upon ligand binding AR dissociates from HSPs, dimerizes and translocates to nucleus, where it binds to androgen-responsive elements (ARE) and regulates gene expression. GOF mutations responsible for PC and SBMA result in dysregulation of gene expression.

What is the pathophysiological role of AR in neurons and peripheral tissues?

AR is expressed in all tissues, with particularly high expression levels in skeletal and cardiac muscle, liver, adrenal glands, and testis. Moreover, AR is expressed in neurons, and within the central nervous system it is expressed at high levels in motor neurons. AR integrates androgen signaling and inputs from extracellular pathways to drive gene expression in a tissue- and time-specific manner (**Fig. 3**). Alpha motor neurons (α MN) selectively degenerate in neuromuscular diseases, including amyotrophic lateral sclerosis, and SBMA. Distinct classes of α MNs (slow-fatigue resistant SFR-MNs, fast-fatigue resistant FFR-MNs, and fast-fatiguable FF-MNs) degenerate at different rate, with the FF-MNs degenerating first in aging and age-related diseases. The molecular mechanisms underlying this selective vulnerability of FF-MNs are poorly known. Building on our expertise

in studying the molecular development of distinct neuronal classes, and leveraging on new technologies that we pioneer to purify and molecularly profile human and mouse MNs, we will identify the molecular landscapes of MNs in physiological and pathological conditions, informing on the logics that shape the selective susceptibility of α MNs to motor neuron disorder. Concerning peripheral tissues, IGF-1 overexpression selectively in the skeletal muscles of SBMA mice leads to increased polyQ-AR phosphorylation and reduced motor dysfunction, improved muscle and spinal cord pathology, and extended lifespan. These results provide proof-of-principle that intervention in muscle has remarkable effects on disease manifestations and indicate that muscle is a primary target of mutant AR toxicity. The molecular details of the role of AR in neurons and peripheral tissues remain to be elucidated.

Figure 3



AR integrates androgen signaling and extracellular information by regulating gene expression. Signaling pathways initiated by extracellular molecules culminate in the post-translational modification of AR. Active AR translocates to nucleus, binds to chromatin, recruits transcription co-factors and interacts with the basal transcription machinery to regulate gene expression in response to androgen signaling and growth factors/neurotrophin-activated signal transduction pathways.

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FRANCESCO PIAZZA

MYELOMA AND LYMPHOMA PATHOBIOLOGY UNIT



Biographical Sketch

- 2018 to date** Associate Professor of Hematology, University of Padua, *Italy*
- 2012 to date** Group Leader, VIMM, *Padua, Italy*
- 2008-2018** Assistant Professor of Hematology, University of Padua, *Italy*
- 2006** “Guido Berlucchi” Prize for Cancer Research
- 2004** “Brian D. Novis Junior Award” International Myeloma Foundation, *USA*
- 2004-2007** Research Associate, University of Padua, *Italy*
- 1999-2003** Post-doctoral Fellow, Human Genetics and Pathology Departments, Memorial Sloan-Kettering Cancer Center, *New York, USA*
- 2000** Board in Medical Oncology, University of Padua, *Italy*
- 1995** MD degree, University of Padua, *Italy*



Group Members

- Sabrina Manni - *Assistant Professor*
- Anna Fregnani - *PhD Student*
- Laura Quotti Tubi - *Postdoctoral Fellow*
- Jessica Ceccato - *Postdoctoral Fellow*
- Giovanni Marchesoni - *Undergraduate Student*
- Maria Pesavento - *Undergraduate Student*
- Lara Saggin - *Undergraduate Student*
- Greta Scapinello - *PhD Student*
- Zaira Spinello - *Postdoctoral Fellow*

RESEARCH ACTIVITY

Fields of interest

Our research is aimed at pursuing two main goals: 1) to unravel novel mechanisms involved in normal blood cell development; 2) to contribute insights into the understanding of blood cancer pathogenesis. We investigate the processes underlying normal and oncogenic hema-

topoiesis, with the goal to understand the pathogenesis of multiple myeloma (MM) and non-Hodgkin Lymphomas (NHL) and, possibly, to identify novel therapeutic targets. Specifically, we use different model systems (conditional knockout mice/knockdown cells, biopsies and primary

samples from patients, tumor cell lines) to define the role of two protein kinases (CK1 α and CK2) in normal and pathological blood cell development, and their functional relationship with B cell receptor (BCR) signaling as well as with cellular stress response. These kinases share common critical targets in hematopoietic development and could represent central players in both normal and malignant blood cell biology. Our approach aims at obtaining parallel information on the role of these signaling molecules in physiological and oncogenic processes to define if they could be rational therapeutic targets in blood cancers. We plan to demonstrate that CK1 and CK2 are important modulators BCR dependent signaling pathways in NHL and MM and are fundamental to sustain the response to endoplasmic reticulum (ER)/proteotoxic stress and clonal expansion/sensitivity to currently employed drugs.

Ongoing research and best results obtained

Role of Protein Kinases CK1 α and CK2 in hema-to-lymphopoiesis

We address the role of CK1 α and CK2 during normal hemo-lymphopoietic cell development *in vivo* in the mouse, through the generation of conditional KO mice. We have generated mice with a floxed CK1 α allele (CK1 $\alpha^{flox/flox}$) and we have obtained mice with a floxed CK2 β allele (CK2 $\beta^{flox/flox}$) from the group led by Prof. Cochet (University of Lyon, France). CK1 $\alpha^{flox/flox}$ mice are crossed with Mb1-CRE mice (Prof. Michael Reth, Max Planck Institute of Immunobiology, University of Freiburg, Germany) to delete the gene in B cells. CK2 $\beta^{flox/flox}$ mice have been crossed with Vav1-CRE, CD19-CRE and Mb1-CRE mice to achieve the deletion of CK2 β in the hematopoietic compartment and in B cells.

CK1 α in normal B cell development. CK1 $\alpha^{flox/flox}$ Mb1-CRE mice display a dramatic impairment in B cell development and show an arrest at the proB stage in the bone marrow (BM). Remarkably, CK1 $\alpha^{wt/flox}$ Mb1-CRE heterozygous mice display a phenotype characterized by a reduction of mature B cells in the peripheral lymphoid organs, accumulation of transitional T1 and T2 cells and hypogammaglobulinemia. Also, CK1 α haploinsufficiency renders B cells defective in the signal transduction downstream from the B cell receptor (Fig. 1).

CK2 regulates B-lymphopoiesis. CK2 $\beta^{flox/flox}$ CD19-CRE mice have marked deficiencies in peripheral B cell development. Mice are hypogammaglobulinemic, defective in immunoglobulin class-switch and develop less antibody-producing cells. They display a reduced fre-

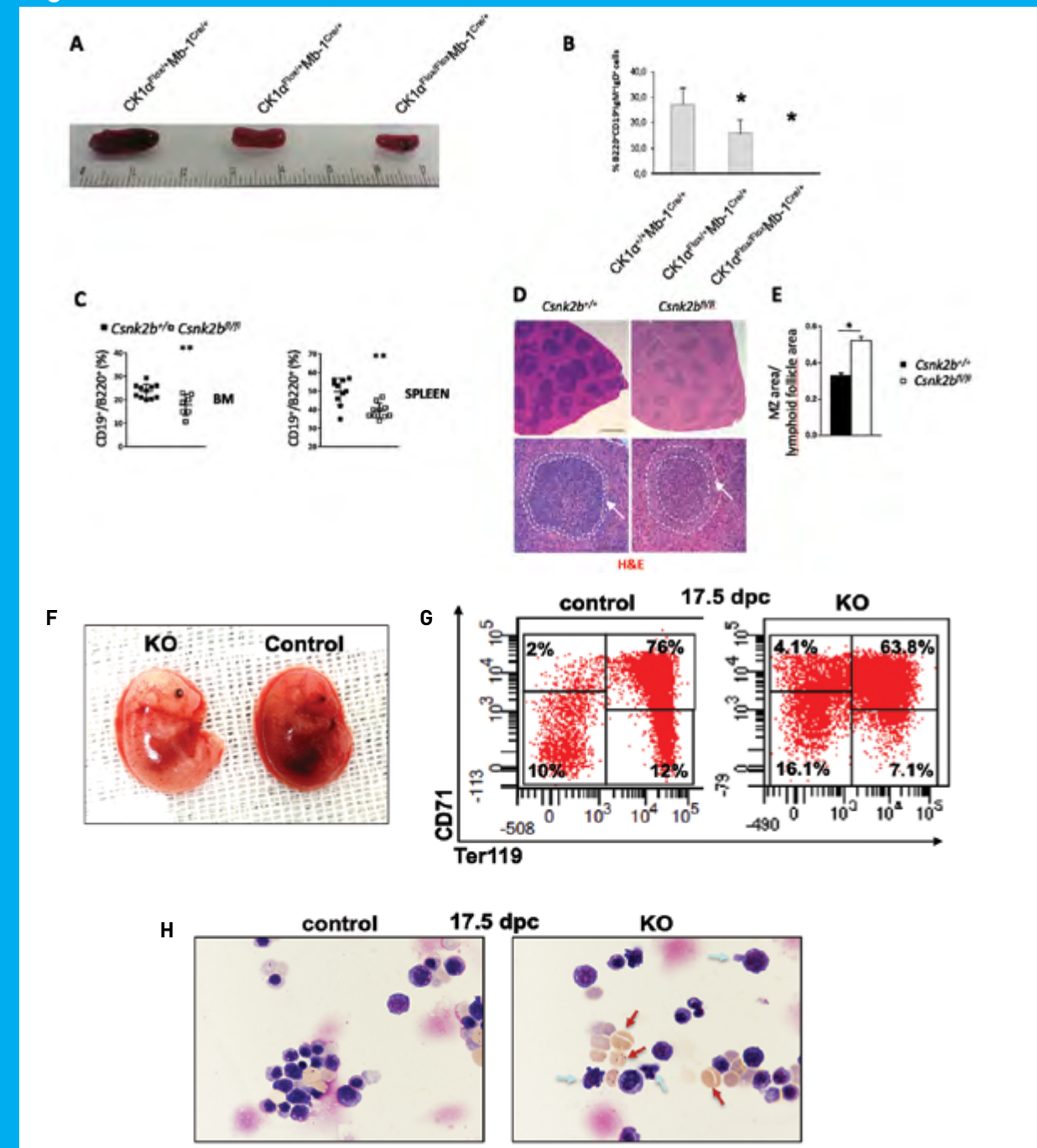
quency of follicular B cells and increased numbers of marginal zone B cells. Upon immunization, a germinal center reaction is initiated, however, it progresses aberrantly, since dark zone/light zone B cells are unbalanced, and dark zone B cells accumulate with impairment in immunoglobulin class-switch. These mice show clear signaling defects affecting critical signaling pathways downstream B cell receptor, in particular deficiencies in the PI3K/AKT and NF- κ B signaling cascades. We have also discovered profound transcriptional changes by RNA sequencing in spleen B lymphocytes.

We have also thoroughly analyzed the CK2 $\beta^{flox/flox}$ Vav1-CRE mice, in which CK2 β is deleted during hematopoiesis. The analysis showed that CK2 β is essential for definitive erythropoiesis, as CK2 $\beta^{flox/flox}$ Vav1-CRE mice die *in utero* starting from 12.5-13.5 dpc and cannot reach the term of pregnancy. Mice are anemic, and a dramatic reduction of late-stage erythroblasts is seen in the fetal liver. We discovered that CK2 is essential for early erythroid progenitor and terminal maturation, since CK2 $\beta^{flox/flox}$ Vav1-CRE mice have reduced levels of pro-erythroblasts, accumulation of late basophilic erythroblasts and less orthochromatic erythroblasts.

Role of Protein Kinases CK1 α and CK2 in multiple myeloma and non-Hodgkin lymphomas

MM and B-NHL are incurable B cell derived malignancies, which cause a significant morbidity. We are studying the potential of targeting CK1 α and CK2 as a novel therapy for MM and B-NHL. In tight collaborations with the Division of Pathology of the University of Padua, we found higher expression of these kinases in tissue samples from MM and NHL patients potentially correlated with clinical-biological features of these diseases. We demonstrated that protein kinases CK1 α and CK2 play an oncogenic function in MM, in Mantle Cell Lymphomas (MCL) and Diffuse Large B Cell Lymphomas (DLBCL). These two kinases sustain stress signaling, such as the ER stress/proteotoxic stress and Unfolded Protein Response (UPR) for CK2 and the autophagic process for CK1 α . CK2 inhibitors block MM growth in cell lines and *in vivo* in a mouse xenotransplant model and synergize with Hsp90 inhibitors and proteasome inhibitors (MM and MCL) (Fig. 2). Moreover, CK2 is highly expressed in DLBCL and in other germinal center-derived non-Hodgkin lymphomas. Inactivation of CK2 caused DLBCL and MCL cell death and a dramatic impairment in signaling pathways essential for lymphoma cell growth, such as the AKT, MAP/ERK and NF- κ B pathways. B-cell receptor signal transduction was defective in CK2-inhibited/silenced lymphoma cells, especially in the AKT

Figure 1

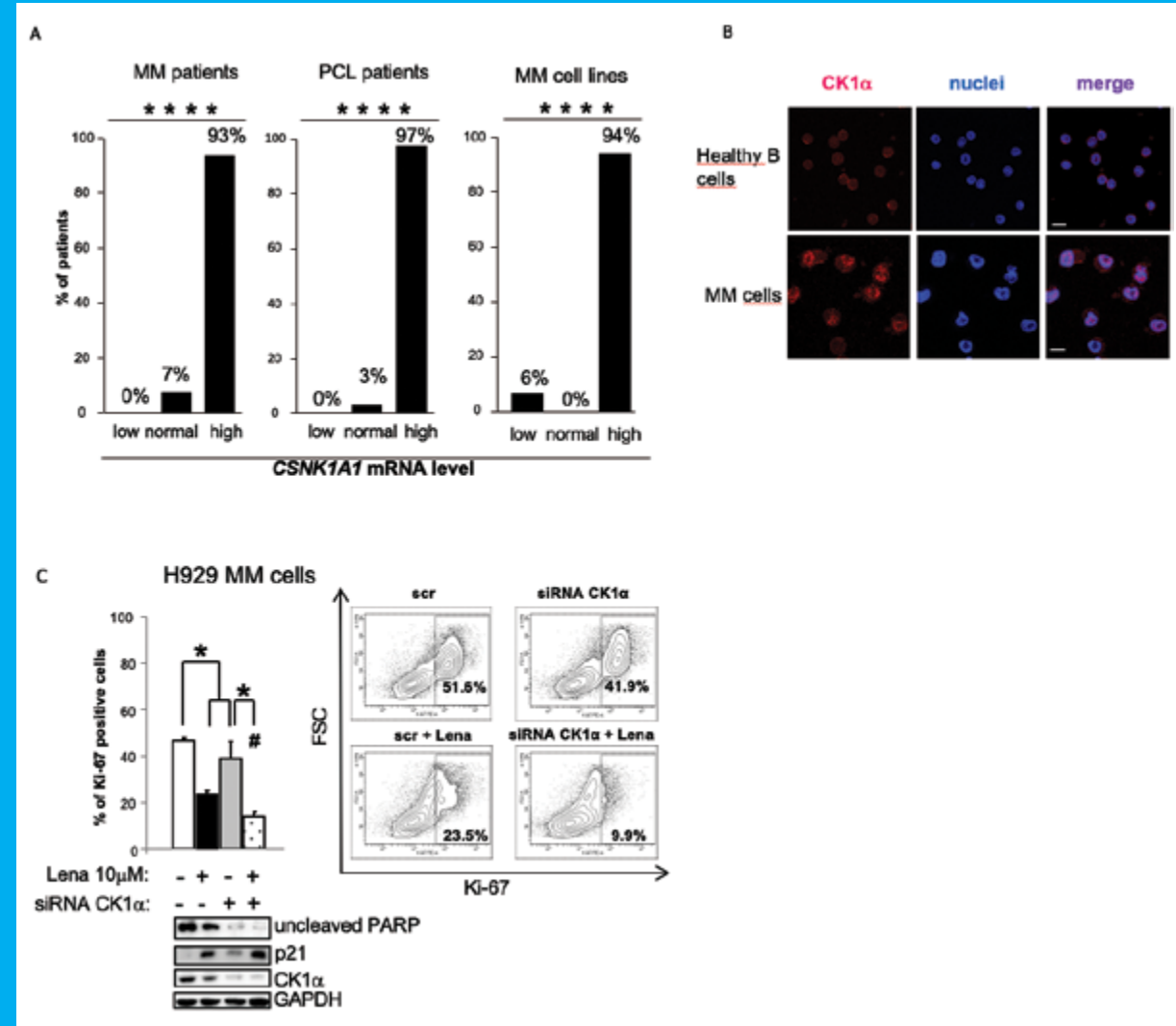


Phenotypic characterization of mice deficient for CK1 α and CK2 β in hemolymphopoiesis. A. Macroscopic analysis of spleens taken from 8 weeks old conditional CK1 α KO (CK1 $\alpha^{flox/flox}$ Mb-1 $^{Cre/+}$), heterozygous (CK1 $\alpha^{flox/+}$ Mb-1 $^{Cre/+}$), and control mice (CK1 $\alpha^{+/+}$ Mb-1 $^{Cre/+}$). Conditional KO mice exhibit hypoplastic spleen. **B.** Mature B cells (B220 $^{+}$ CD19 $^{+}$ IgM $^{+}$ IgD $^{+}$) were dramatically reduced in the bone marrow of CK1 α KO mice. * indicates $p < 0.05$; ** $p < 0.001$ compared to controls. **C.** Scatter plots representing the percentage of CD19 $^{+}$ B220 $^{+}$ cells in BM and spleen of Csnk2b $^{+/+}$ and Csnk2b $^{fl/fl}$ CD19-Cre mice, with each symbol representing a mouse. Statistical significance was determined by Student's t test (**, $p < .01$). **D.** Spleen sections from Csnk2b $^{+/+}$ and Csnk2b $^{fl/fl}$ CD19-Cre mice were stained with H&E. Bar, 500 μ m upper panels; 100 μ m lower panels. Arrows indicate the MZ. **E.** Ratio between MZ and lymphoid follicle areas in the spleen of Csnk2b $^{+/+}$ and Csnk2b $^{fl/fl}$ CD19-Cre mice was calculated using the Leica DMD108 Digital Microimaging Device and Software. **F.** CK2 β loss causes anemia in embryos at 14.5 days of conception; **G.** Flow cytometry analysis of erythroid maturation phases using CD71 [transferrin receptor] and Ter119 [glycophorin A associated protein] markers. **H.** Morphological analysis of purified Ter119 $^{+}$ erythroid cells through cytopsin and May-Grunwald Giemsa staining.

and NF- κ B cascades. Remarkably, CK2 inhibitor CX-4945 synergistically empowered the clinically employed Bruton's Tyrosine Kinase inhibitor Ibrutinib cytotoxicity. Regarding CK1 α we have demonstrated that it is highly expressed in MM and in MCL cells compared to healthy samples. Its inhibition or genetic knock down downregulated the activation and expression of critical survival signaling such as β -catenin, AKT, NF- κ B and surprising-

ly BTK causing MM and MCL cell death, synergistically empowering MM employed (bortezomib, lenalidomide) drugs or BCR inhibitors (Ibrutinib or Duvelisib) cytotoxicity. Therefore, our preclinical data demonstrated that CK1 α and CK2 could be promising molecular targets for the treatment of MM, MCL, DLBCL in association with novel agents, to overcome resistance and to prevent refractoriness.

Figure 2



CK1 α is highly expressed in multiple myeloma cells and its gene silencing boosted lenalidomide cytotoxicity. **A.** CSNK1A1 mRNA expression analysis on 129 specimens of multiple myeloma (MM) plasma cells (left panel), 36 plasma cell leukemia (PCL) patients PCs (middle panel), 18 MM cell lines, (right panel) compared to CSNK1A1 mRNA expression level of 4 healthy controls PCs. Samples were stratified according to CSNK1A1 mRNA expression as follow: normal (value = mean of controls $\pm$ 2SD), low (value < mean of controls - 2SD) and high (value > mean of controls + 2SD). **** = $p < 0.0001$ according to the Chi square test for trend. **B.** Immunofluorescence analysis of CK1 α expression and localization in healthy B cells and in a MM cell line. **C.** Lenalidomide cooperates with CK1 α silencing in inducing apoptosis and cell cycle arrest. Ki-67 positive H929 cells after transfection with CK1 α siRNAs and siGLO green indicator and treated for 3 days with Lenalidomide (Lena). The right panel shows a representative Contour Plot. Bottom panel: expression of PARP and p21 by WB. GAPDH was used as loading control.

3D in-vitro co-culture model for drugs screening in DL-BCL

In collaboration with Prof. F. Vianello at DIMED, to well investigate the BM involvement in refractory DLBCL cases, we developed a three-dimensional *in vitro* model that mimics the *in vivo* microenvironment. We used decellularized femoral bone fragments as a scaffold for BM-derived mesenchymal stromal cells (MSCs). Neoplastic cells were seeded into MSCs created niches. This model allowed to mimic a BM microenvironment in which tumor cells adhered, grew, and were protected from drug induced apoptosis.

Our study aims to realize patient-specific models that will be helpful for personalized therapies (**Fig. 3**).

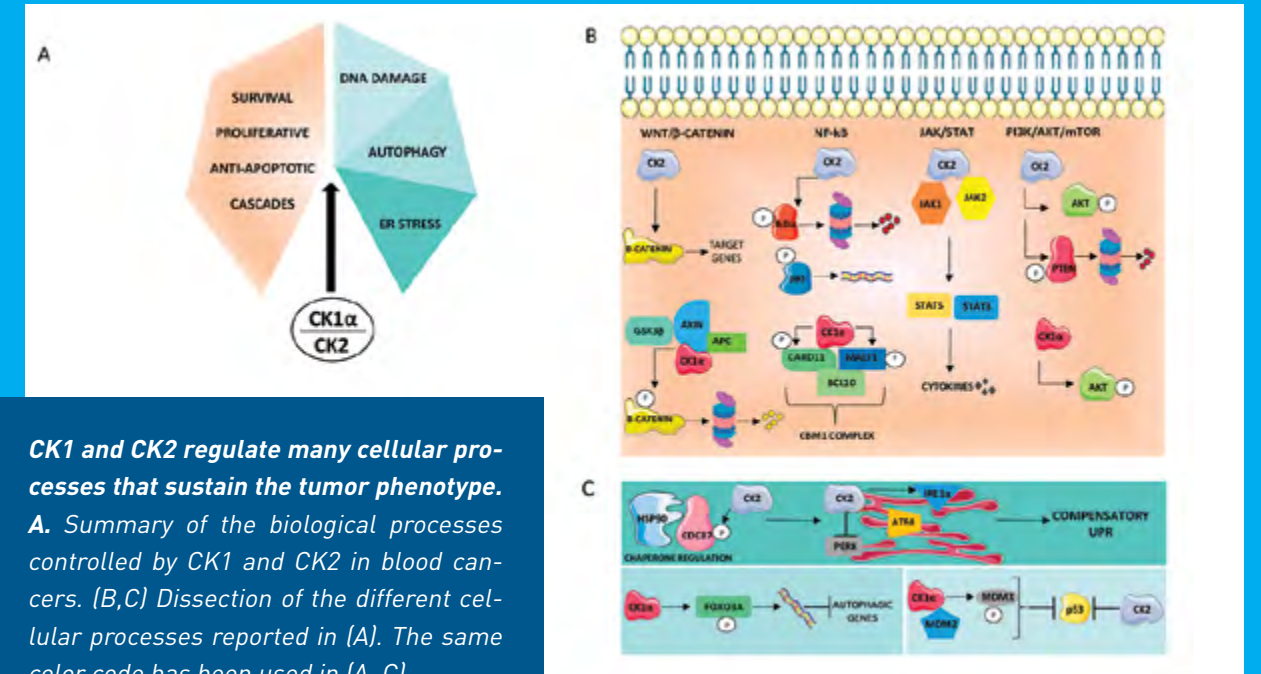
Future research plans

Identification of CK1 α and of CK2 regulated pathways in hematopoiesis, lymphomagenesis and in the B-cell compartment

We will exploit our unique mouse models to identify the pathways regulated by CK1 α and CK2 during normal and malignant hemo-lymphopoiesis. In particular:

High-throughput phosphoproteomics analysis on hematopoietic cells and B-lymphocytes derived from CK1 or CK2 wild type and conditional KO. We aim at identifying signaling events important for the development, maturation, lineage commitment and effector functions of blood cells.

Figure 3



CK1 and CK2 regulate many cellular processes that sustain the tumor phenotype.

A. Summary of the biological processes controlled by CK1 and CK2 in blood cancers. **(B,C)** Dissection of the different cellular processes reported in **(A)**. The same color code has been used in **(A-C)**.

B. Role of CK2 and CK1 on the Wnt/ β -catenin, NF- κ B, JAK/STAT, and PI3K/AKT/m-TOR signaling pathways, which sustain cell survival and proliferation, counteracting apoptosis. Considering the Wnt/ β -catenin pathway, CK2 sustains β -catenin dependent gene expression, while CK1 is responsible for the phosphorylation of β -catenin on Ser45, priming it for its subsequent proteasomal degradation. Focusing on NF- κ B, CK2 directly phosphorylates both I κ B (on Ser283, Ser289, and Tyr291) and p53 (on Ser529), promoting the transcription of the NF- κ B target genes. CK1 phosphorylates CARD11 (on Ser608) and MALT1 (on Ser562), regulating the CBM1 complex and NF- κ B activation. In the JAK/STAT pathway, CK2 constitutively associates with JAK1 and JAK2, favoring STAT5 and STAT3 activation. Considering the PI3K/AKT/m-TOR cascade, CK2 directly phosphorylates AKT (on Ser129) and PTEN proteins (on Ser370, Thr366, and Ser385). Phosphorylated PTEN is degraded by a proteasomal mechanism, indirectly regulating AKT activation. CK1 sustains AKT expression and modulates the level of phosphorylated Ser 473 AKT. **C.** Role of CK1 and CK2 in ER stress, autophagy, and DNA damage response. In ER stress (upper panel), CK2 phosphorylates Cdc37 on Ser13, increasing its association with Hsp90. CK2 influences the homeostatic molecules regulating UPR, in particular the PERK and IRE1 branches, promoting survival. Considering autophagy, (bottom left panel), CK1 phosphorylates FOXO3A (on Ser318 and Ser321), inhibiting autophagic gene transcription. In the DNA damage response (bottom right panel), the interaction of CK1 with the p53 regulators MDM2 and MDMX impairs p53 function. CK2 negatively regulates p53 expression and activation.

In vivo lymphoma models. We will generate mouse models of lymphoma to investigate the contribution of these two kinases to malignant transformation of B cells. We will cross CK1 α and CK2-deficient mice in B cells with Eu-Myc mice transgenic mice to achieve models B cell tumors. In parallel we will analyze the effect of CK1 α or CK2 knockdown *in vivo* in xenograft mouse models of MCL or MM.

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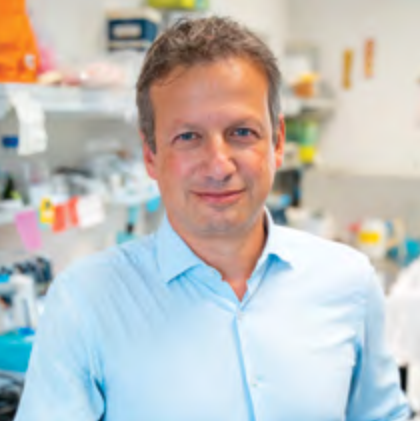
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MARCO SANDRI

CELL SIGNALING IN PROTEIN AND MITOCHONDRIAL TURNOVER UNIT



Biographical Sketch

2019 to date	Head of the Dept. Biomedical Sciences, University of Padua, <i>Italy</i>
2018 and 2020	ERC LS3 panel member
2014 to date	Full Professor of Pathology, University of Padua, <i>Italy</i>
2013-2019	Head of the Myology Center, University of Padua, <i>Italy</i>
2013-2015	Principal Investigator, TIGEM, <i>Naples</i>
2011	ERC Consolidator Research Grant
2011 to date	Adjunct Professor, McGill University, <i>Canada</i>
2006 to date	Group Leader, VIMM, <i>Padua, Italy</i>
2005-2015	Assistant and Associate Telethon Scientist, DTI, VIMM, <i>Padua, Italy</i>
2002-2005	Postdoctoral Fellow in A.L. Goldberg lab, Harvard Medical School, <i>Boston, USA</i>
2001	Specialist in Pathology, University of Padua, <i>Italy</i>
1996	MD degree, University of Padua, <i>Italy</i>



Group Members

- Vanina Romanello - Assistant Professor
- Andrea Armani - Postdoctoral Fellow
- Ludovica Carducci - Master Student
- Martina Esposito - Master Student
- Niccolò Faedda - Master Student
- Anaïs Franco Romero - Postdoctoral Fellow
- Camilla Pezzini - PhD Student
- Roberta Sartori - Postdoctoral Fellow
- Marco Scalabrin - PhD Student
- Davide Steffan - PhD Student
- Caterina Tezze - Postdoctoral Fellow
- Giulia Trani - Postdoctoral Fellow

RESEARCH ACTIVITY

Fields of interest

We are interested in understanding the signaling pathways that control protein synthesis and degradation, organelle biogenesis, autophagy, longevity, and the transcriptional regulation of the genes involved in these processes. Moreover, we aim to identify how skeletal muscles, liver, white adipose tissue, brain, and heart communicate with each

other in physiological and pathological conditions such as during tumor growth. To reach these goals, we use a multidisciplinary approach including genetics, *in vivo* imaging, physiology, molecular biology, and biochemistry at the single cell as well as tissue level.

Ongoing researches

In my lab, we have four main fields of research that are:

- 1) discover uncharacterized genes that control protein turnover and ageing;
- 2) understand how cancer cells induce cachexia, a process characterized by massive and uncontrolled body weight loss;
- 3) dissect the role of physical activity, mitochondrial dynamics (biogenesis and fragmentation) in energy balance, muscle mass regulation, and healthy ageing;
- 4) identify the signaling pathways and transcription factors that control protein breakdown and protein synthesis.

Best results obtained

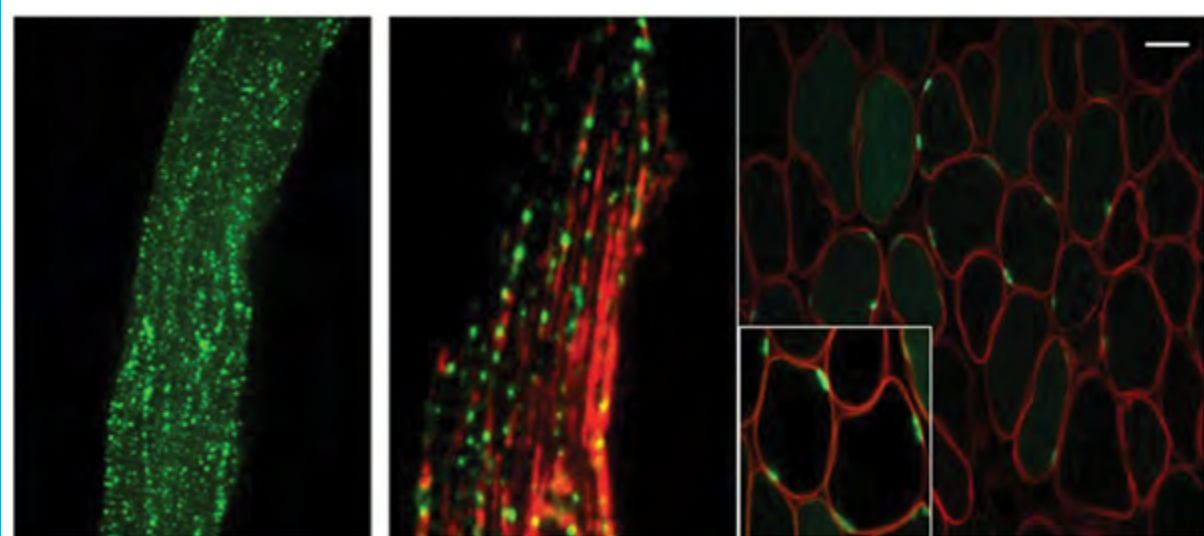
1) I was the first one to show that protein breakdown is controlled by transcriptional-dependent programs. My lab identified that FoxOs transcription factors regulate the expression of rate-limiting enzymes in ubiquitin-proteasome and autophagy-lysosome systems. Later, many different labs confirmed our findings in many other tissues such as the heart, kidney, brain, liver (Sandri *et al.* Cell. 2004; Mammucari *et al.* Cell Metab. 2007; Milan *et al.* Nat Commun. 2015).

- 2) My lab opened the field to the concept that autophagy dysregulation is an important pathogenetic mechanism in inherited muscle diseases (Masiero *et al.* Cell Metab. 2009; Grumati *et al.* Nat Med. 2010).
- 3) My lab pioneered the field of metabolic regulation of muscle mass. My lab was the first to image the mitochondrial network in atrophying muscles in living mice and to prove the importance of the mitochondrial fission and fusion machinery in muscle mass regulation (Sandri *et al.* Proc Natl Acad Sci USA 2006; Romanello *et al.* EMBO J. 2010; Tezze *et al.* Cell Metab. 2017; Favaro *et al.* Nat Commun. 2019).
- 4) My lab identified novel genes (Fbxo30/MUSA1, Fbxo21/SMART) that we showed to be ubiquitin ligases and criti-

cal players in protein breakdown and muscle loss. Moreover, we showed that Bone and Morphogenetic Proteins (BMPs) regulate MUSA1 expression, play critical anabolic and anti-catabolic functions, and dominate over Myostatin signaling to control muscle mass in cachexia (Sartori *et al.* Nat Genet. 2013; Milan *et al.* Nat Commun. 2015; Sartori *et al.* Sci Transl Med. 2021).

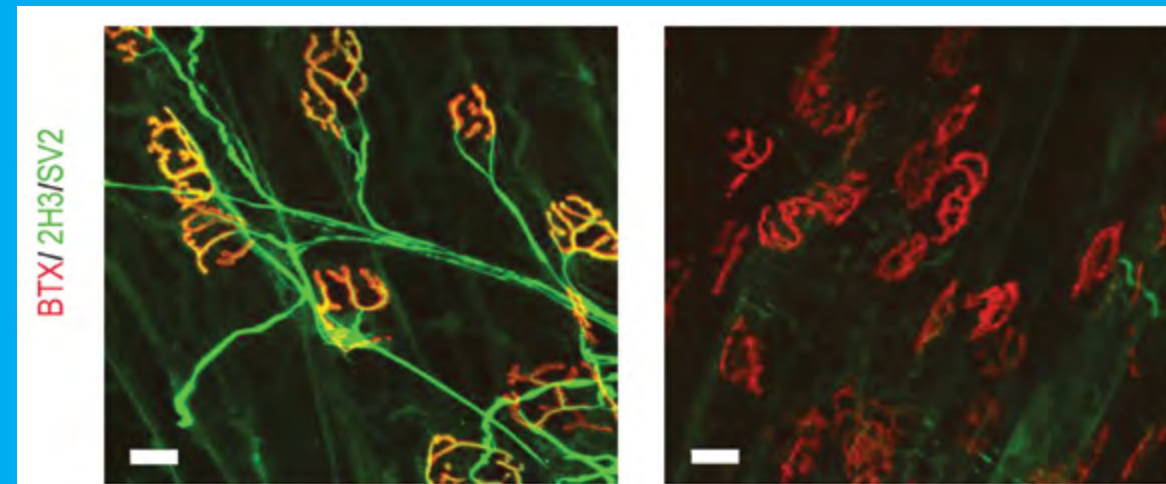
- 5) My lab showed for the first time that mitochondrial dysfunction triggered by inhibition of either mitochondrial fusion or mitophagy in adult muscles can reverberate to the whole body triggering premature senescence in multiple tissues and cell types, including spinal motor neurons (Tezze *et al.* Cell Metab. 2017; Carnio *et al.* Cell Reports 2014; García-Prat *et al.* Nature 2016).

Figure 1



Left Panel: Confocal microscopy analysis shows that autophagosome vesicles (green) are induced in isolated muscle fibers during starvation and are distributed throughout the fibers. Middle Panel: Confocal microscopy analysis shows that FoxO3 induces autophagosome vesicles (green) in isolated muscle fibers, and some engulf mitochondria (red). Single fibers from the FDB muscle were transfected with LC3-GFP (an autophagosome marker) mitoRed (mitochondrial marker), and FoxO3 and examined by confocal microscopy. Co-transfection of skeletal muscles with FoxO3 and LC3-GFP was found to induce a large number of green fluorescent dots in myofibers and concomitantly reduce the number and size of mitochondria (red). Furthermore, some mitochondria are inside the autophagosome (yellow stain in merge picture), suggesting that FoxO3 induces a reduction in mitochondria via mitophagy. Right Panel: Adult skeletal muscles transfected by electroporation with TFEB-GFP and stained for dystrophin (red). Exercise-induced a nuclear localization of TFEB that activates transcriptional programs for lysosomal and mitochondrial biogenesis to activate metabolic flexibility.

Figure 2



Muscle nerve synapses (neuromuscular junction) are lost when autophagy and/or mitochondrial function is altered. Neuromuscular junctions (NMJ) are visualized in muscle tissue by labeling the Acetylcholine Receptors, the post-synaptic component (green), and by immunostaining, for the neurofilament/synaptic vesicle to label the pre-synaptic site of NMJ and the axons (green). Images are from EDL muscles of controls (Left panel) and tumor-bearing mice (Right panel).

Future Research Plans

For each of the above-mentioned research lines, we have a plan on how to proceed.

Discover uncharacterized genes that control protein turnover and ageing

We are characterizing two unknown genes that are conserved through species till humans. The first one is involved in ageing and autophagy while the second one has exercise-dependent gene regulation. To dissect their function, we are generating by CRISPR/Cas9 technology knockout mice, zebrafish, worms, and cell lines. The characterization of the phenotypes of these animals and cells in association with molecular, biochemical, mor-

phological, and functional analyses will dissect the molecular insights of their function and their relevance in physiology and pathology.

Understand how cancer cells induce cachexia, a process characterized by massive and uncontrolled body weight loss

We are using the single-cell RNA seq and ATAC seq to establish in muscle and cancer cells the transcriptomic profiles characteristic of cachexia. This approach will be associated with proteomic analyses to identify the secretome and factors that trigger protein breakdown in muscles and white adipose tissue loss.

Dissect the role of mitochondrial dynamics (biogenesis and fragmentation) in energy balance, muscle mass regulation, and healthy ageing

We will further characterize the role of mitochondrial dynamics and mitophagy in ageing. We will use our inducible muscle-specific knockout for mitochondrial fusion (OPA1) and fission (DRP1) genes to identify the secretome that controls cellular senescence and sarcopenia onset. We have crossed these mice with transgenic mice expressing specifically in muscle a mutant methionyl-tRNA synthase to label the proteins metabolically. Proteomic analyses on blood serum will be performed to identify the muscle-derived proteins in the different knockout mice. Gain and loss of function experiments on

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the most interesting candidates will be performed, and the effects on ageing and senescence markers will be monitored.

Identify the signaling pathways and transcription factors that control protein breakdown and protein synthesis

We have shown that BMP pathway control protein synthesis and breakdown. These actions are mediated by the SMAD1/5/8 transcription factors. To identify the genes involved in protein turnover and modulated by Smads, we will perform Chip seq and single nucleus transcriptomic on muscles overexpressing the different Smad transcription factors. Gain and loss of function experiments will be performed on the most interesting candidate genes.

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MASSIMO SANTORO
ANGIOGENESIS AND CANCER METABOLISM UNIT



Biographical Sketch

2020 to date	Group Leader, VIMM, Padua, Italy
2017 to date	Full Professor, Dept. Biology, University of Padua, Italy
2016-2017	Full Professor, Dept. Biotechnology and Health, University of Turin, Italy
2013-2016	Associate Professor and Group Leader at Molecular Biotechnology Center, University of Turin, Italy
2013-2017	Group Leader, VIB, Belgium
2013-2017	Full Professor, Dept. Oncology, Katholieke Universiteit Leuven, Belgium
2008-2014	Assistant Professor and Group Leader at Molecular Biotechnology Center, University of Turin, Italy



Group Members

- Liasian Asrabaeva - Postdoctoral Fellow
- Matteo Astone - Postdoctoral Fellow
- Michael Donadon - PhD Student
- Nicola Facchinello - Postdoctoral Fellow
- Roxana Oberkersh - Postdoctoral Fellow
- Marco Ravazzolo - PhD Student
- Giovanni Tosi - Postdoctoral Fellow

RESEARCH ACTIVITY

Fields of interest

Our goal is to expand the current knowledge of how metabolism regulates endothelial redox homeostasis and vice-versa in healthy and diseased conditions. To accomplish this aim we have taken advantage of the innovative genetic and imaging technology as well as new molecular and biochemical approaches in vertebrate models,

including zebrafish and mouse. We are expanding the current vision of endothelial biology by identifying novel metabolic and redox signaling mechanisms and used them to develop novel therapies to treat cancer progression and vascular-related pathologies.

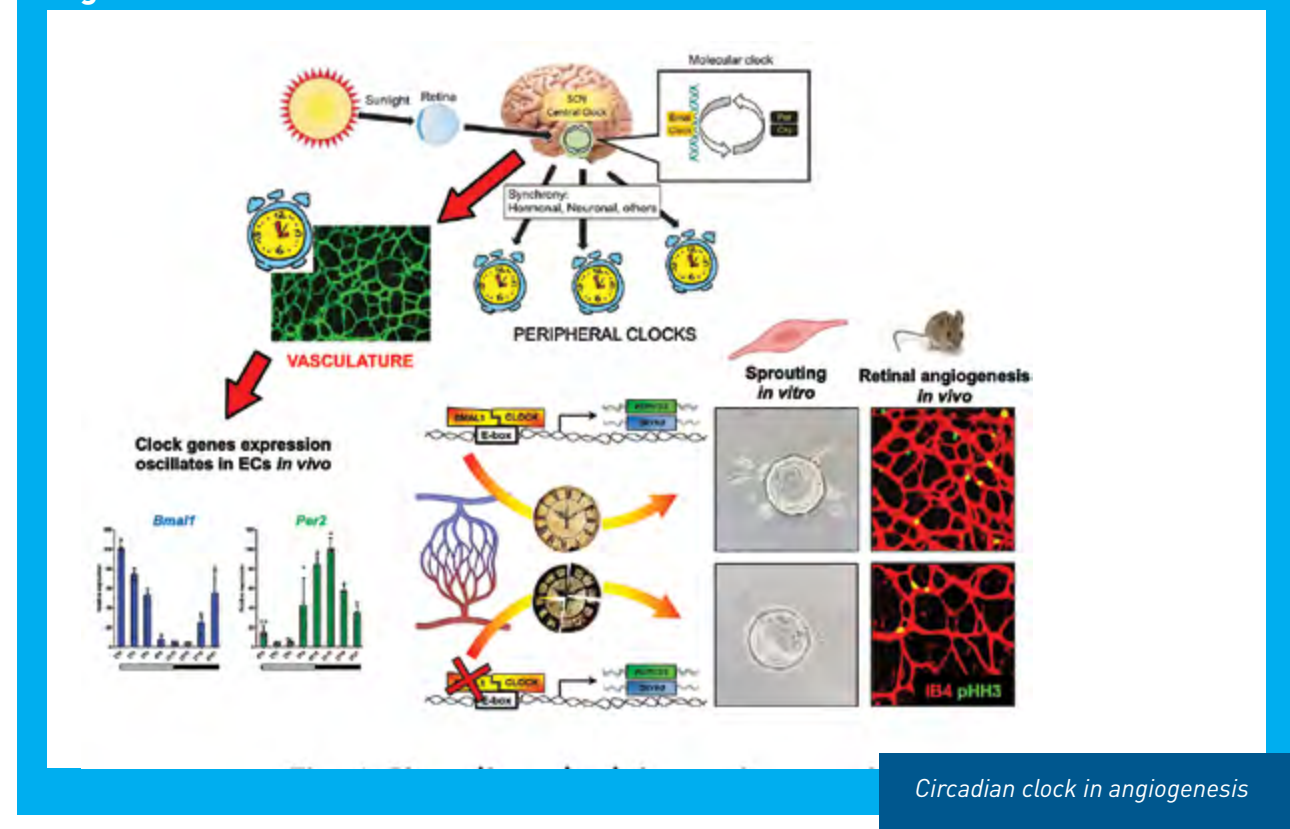
Ongoing researches and future research plans

Role of circadian clock and light in angiogenesis

Mechanisms underlying circadian- and light-regulated physiological processes remain largely unknown. In mammals and other vertebrates, the circadian clock plays a central role in the regulation of physiological functions of almost all tissues and organs. It is understood that some crucial genes involved in the regulation of cell growth, differentiation, and metabolism are tightly regulated by the clock genes. Recent findings indicate that also the UBIAD1 gene is regulated by circadian clocks. Due to the key importance of the circadian clock, certain transcription factors, such as Bmal1 and Clock, and transcription coregulators Period2 and Cryptochrome, or their homologs specialized for circadian regulation of targeted genes, are widely present in vertebrates, including zebrafish. Despite the vast knowledge of circadian biology and angiogenesis, the role of the circadian clock in angiogenesis and vascular patterning remains poorly investigated. Here we plan to define mechanistic insight on the role of the circadian clock and light in regulation of developmental angiogenesis. We show that

disruption of the circadian clock by constant exposure to light or genetic manipulation of key genes such as Bmal1 and per2 in zebrafish leads to impaired developmental angiogenesis. A bmal1-specific CRISPR/Cas9 zebrafish mutant show block of sprouting angiogenesis without causing obvious nonvascular phenotypes. Using a DLL4 promoter-reporter system consisting of various deleted promoter mutants, we can show that Bmal1 directly binds to and activates the DLL4 promoter via E-boxes. To further explore how Bmal1 may control vascular development and function via Notch we have already crossed conditional allele of Bmal1 with Ve-Cadherin-CreERT2 mice. We are currently characterizing these mice for developmental angiogenesis (retina angiogenesis) and pathological angiogenesis (tumor xenografts). Additionally, we are also performing ChIPSeq experiments in HUVEC with Bmal1 to identify novel Bmal1 target genes in ECs that can explain the angiogenic phenotype *in vivo*. Such findings may open up new therapeutic approaches in pathological angiogenesis and reasonably extended to other types of vascular-related conditions by targeting the circadian clock (**Fig. 1**).

Figure 1

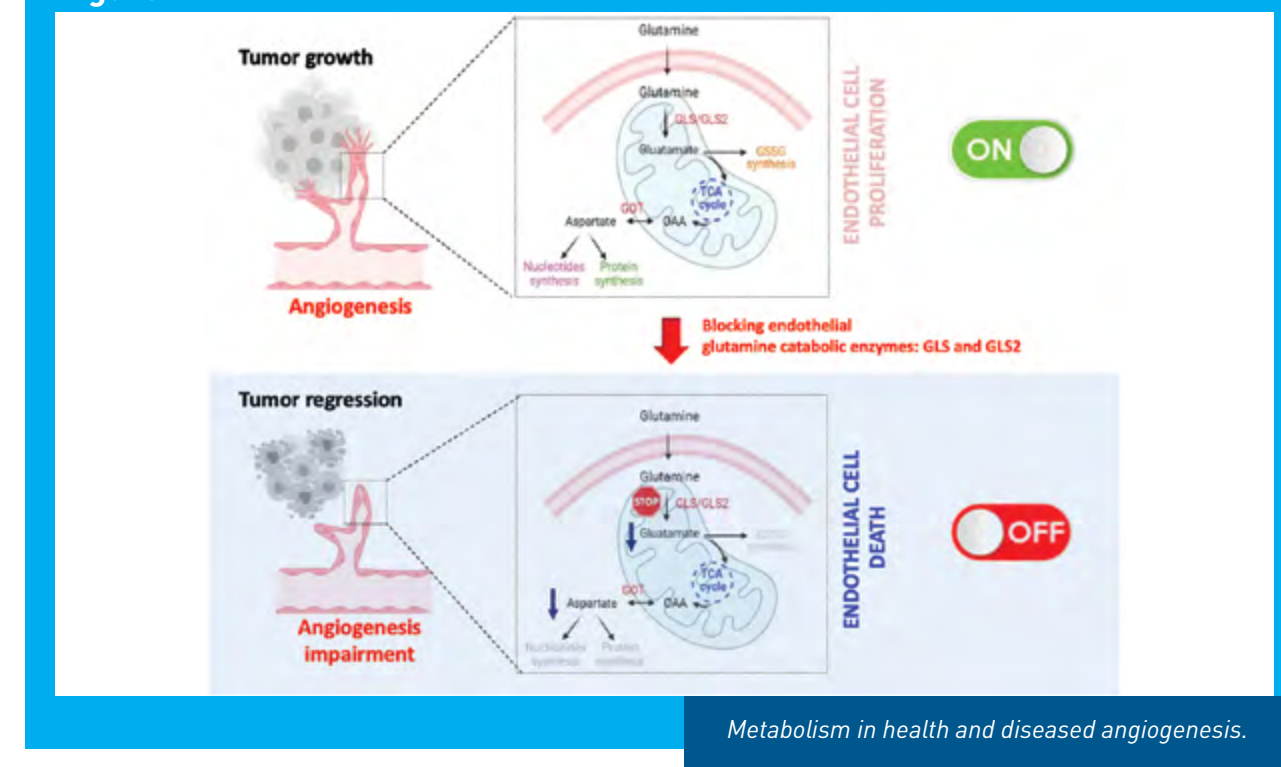


Metabolic control of angiogenesis

Laminar shear stress (LSS), ROS signaling and cell metabolism are known to play independently play crucial roles in controlling endothelial cells (EC) function and homeostasis. Interestingly, studies have demonstrated that LSS can regulate ROS production and signaling. On the other hand, metabolic pathways can regulate angiogenesis, such as glycolysis and others. Among the most important but yet uncharacterized metabolic pathway in ECs we have the oxidative pentose phosphate pathway (oxPPP). oxPPP is composed of two major enzymes, G6PD and PGD, that are able to control oxidative stress protection and nucleic acid synthesis in different cell types. However, the role of oxPPP in angiogenesis is still unclear. Also whether, LSS influence oxPPP or whether ROS can target this metabolic pathway is unknown. Interestingly, LSS and oxPPP acts via the regulation of ROS synthesis and homeostasis. ROS play a key role in cell signaling through cysteine oxidative post-translational modification (Ox-PTM) of selective target proteins. Despite the described role of the PPP and LSS in EC function is documented, no study has yet determined the relation between both LSS, ROS and oxPPP in the control of EC function. Particularly, nothing is known about how these events are connected and whether they might control epigenetic modification and gene expression. This project aims to establish a LSS^{ROS}oxPPP signaling that may be involved in the control of vascular development and mural cells function by epigenetic events. We have recently performed a redox proteomic screen (OxICAT) to

identify sensitive redox cysteine and to target enzymes during LSS. We identified 1524 peptides and 978 proteins exhibiting cysteine Ox-PTM during LSS including those involved in cytoskeleton, translation or cell cycle protein. We also found that LSS-mediated ROS production can oxidase the rate-limiting enzyme of the oxPPP, PGD, on a specific cysteine residue (C289A). The effect of this Ox-PTM on PGD activity as well as what its molecular and biological function is unknown. We have some preliminary results in zebrafish from testing endothelial specific embryos for PGD and G6PD (conditional CRISPR/Cas9 technology) that found the deletion of oxPPP in ECs *in vivo* can lead to an impairment of vascular mural cells recruitment. To further explore how EC may control vascular development and function via blood flow and cell metabolism adaptation through Ox-PTM we have already generated an endothelial-specific PGD mutant mice (EC-PGD^{-/-}) and a conditional KI mouse (cKI) harbouring a cys289Ala mutation. These conditional allele mice have been crossed with Ve-Cadherin-CreERT2 mice. We are currently verifying the inactivation of PGD specifically in EC by performing gene expression on EC isolated from WT and EC-PGD^{-/-} tissue (e.g. heart, aorta, kidney) and by performing co-immunostaining on the aortic ring (utilizing PGD and PECAM-1 or α-SMA antibodies, specific, respectively, for EC and smooth muscle cells). This deletion approach is able to confirming that the critical role of PGD in endothelial development and function and will open the way to investigating epigenetic events driven by LSS, ROS and metabolism (**Fig. 2**).

Figure 2



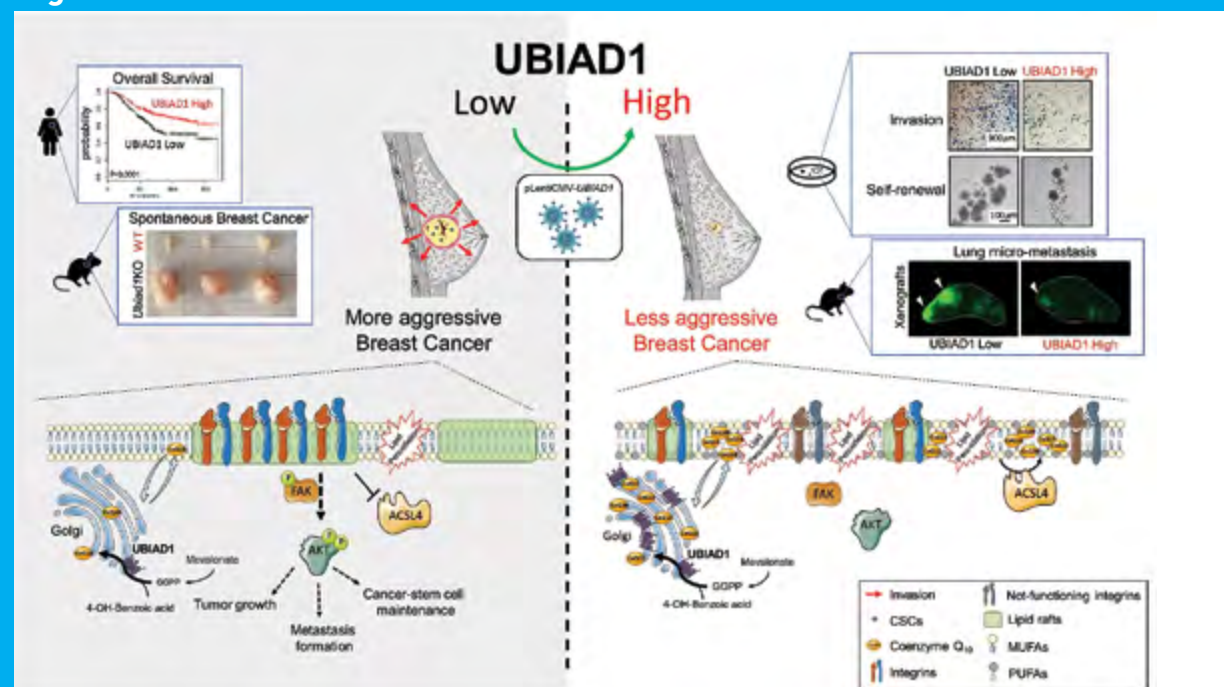
A critical function for UBIAD1 and oxidative stress in Breast tumor progression

UBIAD1 was identified by our laboratory as a non-mitochondrial prenyltransferase that synthesizes CoQ10 in the Golgi membrane compartment. By doing so we demonstrated that UBIAD1 is a critical antioxidant enzyme for the cardiovascular system during its development. Although the biochemical role of UBIAD1 is known, its function in cancer has not yet been investigated. It is now becoming clear that oxidative stress inhibits distant metastasis by melanoma cells while antioxidant promotes melanoma progression. Here we address the role of UBIAD1 in regulating tumor progression by regulating oxidative stress. By testing expression of UBIAD1 on different types of human cancers we found that UBIAD1 protein, but not RNA, is upregulated in almost 50% of colon and breast cancer samples, but not in their normal counterparts. More importantly, our analyses also show that UBIAD1 levels is strongly increase in 75% of metastatic breast cancer. To further analyze the role of UBIAD1 in breast cancer initiation and progression we have generated a knock-out mouse for UBIAD1 and

cross it with different mice breast cancer models (e.g. erbB2-Neu, PyMT-MMTV). We found that UBIAD1 loss protect from tumor dissemination and lung metastasis in these breast cancer models. Similar results were obtained when in xenograft tumor models (B16 melanoma cells and E0071 breast carcinoma cells silenced or over-expressing UBIAD1) (Fig. 3).

These preliminary results support a critical role for Ubiad1 in maintenance of tumor cell antioxidant defenses during tumor initiation and dissemination. We are currently investigating a novel molecular mechanism responsible for the stabilization of UBIAD1 by oxidative stress in cancer progression (redoxome approach in breast cancer tissues). If true, this idea will provide ground for new therapies against tumor metastasis: as most breast cancer patients die due to the metastasis and not the primary tumor, we believe that targeting Ubiad1 or CoQ10 synthesis would be valuable for the survival of these patients. Furthermore, the highly metastatic, triple negative breast cancers do not respond effectively to the available treatments indicating that anti UBIAD1 inhibition could be a life-saving therapy for these patients.

Figure 3



UBIAD1 and antioxidant in breast and melanoma progression.

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LUCA SCORRANO

MITOCHONDRIAL DYNAMICS IN CELL LIFE AND DEATH UNIT



Biographical Sketch

2019	Member, Academia Europaea
2014-2020	Scientific Director, VIMM, Padua, Italy
2013 to date	Full Professor of Biochemistry (chiara fama), Dept. Biology, University of Padua, Italy
2013	European Society for Clinical Investigation Award
2011	EMBO Member
2007-2013	Full Professor, Dept. Cell Physiology and Metabolism, University of Geneva Medical School, Switzerland
2006	Eppendorf Young Investigator Award
2003-2007	Assistant Telethon Scientist, Dulbecco-Telethon Institute, Italy
2000-2003	Postdoctoral Fellow, Harvard Medical School, Boston, USA
2000	PhD, University of Padua, Italy
1996	MD, University of Padua, Italy



Group Members

- Lukas Alan - Postdoctoral Fellow
- Tiago Branco - Postdoctoral Fellow
- Beatrice Calciolari - Research Assistant
- Mauricio Cardenas - Postdoctoral Fellow
- Antigoni Diokmetzidou - Postdoctoral Fellow
- Andre Djalalvandi - PhD Student
- Alice Lacombe - Postdoctoral Fellow
- Jonathan Lambert - Postdoctoral Fellow
- Aurora Maracani - PhD Student
- Ana Mendonca - Postdoctoral Fellow
- Masafumi Noguchi - Postdoctoral Fellow
- Akiko Omori - Postdoctoral Fellow
- Anna Pellattiero - Postdoctoral Fellow
- Erwan Rivière - PhD Student
- Anke Seydel - Postdoctoral Fellow
- Satoko Shinjo - Postdoctoral Fellow
- Keisuke Takeda - Postdoctoral Fellow
- Federica Vinelli - Research Assistant
- Margherita Zamberlan - PhD Student
- Kate Wang - Postdoctoral Fellow

Fields of interest

Mitochondria are crucial organelles in cell life and death: they synthesize most of cellular ATP, integrate signaling pathways and amplify cell death. This versatility is mirrored by their dynamic morphology, ultrastructure and juxtaposition to the endoplasmic reticulum, all controlled by a set of “mitochondria-shaping proteins”. We are interested in understanding the mitochondrial form-function relationship in health and disease. We use an integrated approach of genetics, advanced imaging, biochemistry, physiology and electron tomography to unravel the role of mitochondrial dynamics in signaling and in complex cellular responses.

Ongoing research and future research plans

What are the molecular mechanisms of mitochondrial structure regulation?

Using a multipronged proteomic and genetic approach we unveiled that Opa1 is epistatic to the MICOS multiprotein complex in cristae shape regulation in mam-

mals. We are now exploiting our proteomic catalogue of inner mitochondrial membrane protein complexes modified during cristae remodeling to characterize the function of novel regulators of mitochondrial structure. Moreover, we are generating and characterizing conditional animal models of mitochondrial fission to investigate their function in cell biology and in heart and blood disease (Fig. 1).

What is the role of Opa1-regulated mitochondrial ultrastructure in tissue damage?

Using a variety of genetic models, our lab demonstrated that the Opa1-controlled cristae remodeling pathway is an essential component of heart, brain, muscle dysfunction and thus placed Opa1 as a targetable component to counteract stroke, neurodegeneration, heart ischemia-reperfusion and muscle mass loss. We are now interested in understanding how we can epigenetically increase Opa1 levels to correct mitochondrial dysfunction in primary mitochondrial disorders as well as in acquired conditions such as muscle mass loss and heart damage. Furthermore, because of the crucial role

of Opa1 in neurodegeneration, we are investigating the relationship between mitochondria and neurodegeneration in the context of optic atrophies, a genetic model for the much more common conditions of glaucoma.

What is the role of mitochondrial shape and of autophagy in heart diseases?

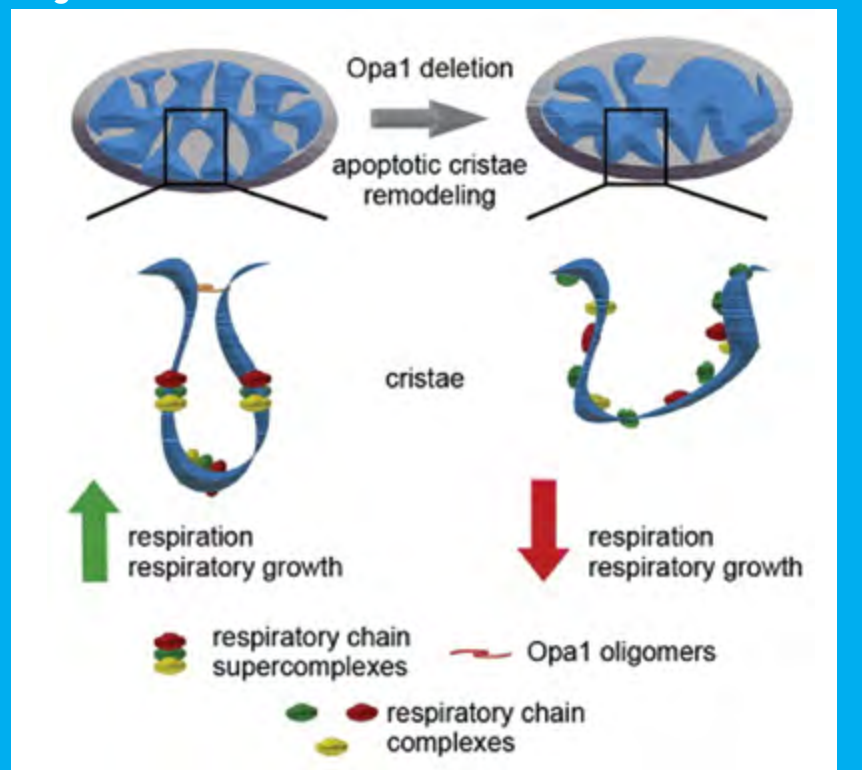
Stemming from our discovery that mitochondrial shape is a determinant of autophagy, we developed an interest on the interplay between mitochondria and autophagy especially in the heart, where we identified the role of mitochondrial fusion to sustain cardiomyocyte development. By using a combination of specific mouse models and of biochemistry, metabolomics and cell biology we are studying how druggable regulators of autophagy

and mitophagy recruit mitochondrial shape in the cardiomyocyte to exert their cardioprotective effects and we are defining how Opa1 defect contribute to cardiomyopathy and to ischemia-reperfusion damage.

Is mitochondrial remodeling important in metabolism?

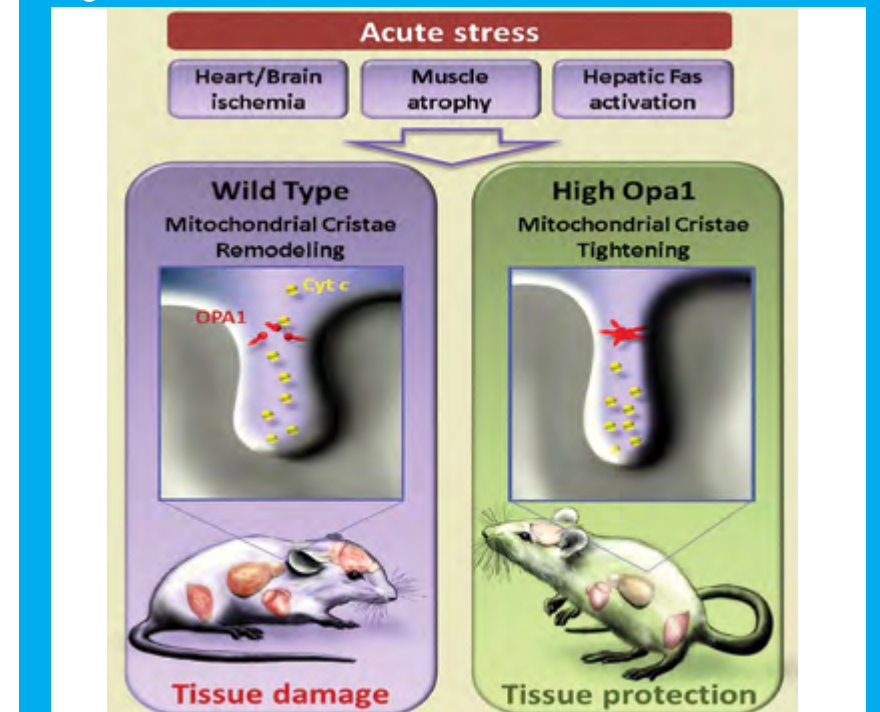
Our discovery that cristae shape defines efficiency of mitochondrial respiration and of mitochondria-dependent cell growth inspired us to study the role of mitochondrial remodeling in the context of metabolism, diabetes and obesity. By combining genetics, biochemistry, whole body physiology we are interested defining the role of mitochondrial shape in adipose tissue differentiation, expansion, plasticity and maintenance (Fig. 2).

Figure 1



A cartoon on the role of cristae shape in defining mitochondrial respiratory efficiency. Genetic and apoptotic maneuvers remodel mitochondrial cristae and reduce respiratory chain supercomplexes, eventually curtailing efficiency of respiration [Cogliati et al. 2013].

Figure 2



The Opa1 dependent cristae remodeling pathway defines the response of tissues to damage in vivo. Mild ubiquitous Opa1 overexpression protects heart, muscle, liver, brain from ischemic, apoptotic or atrophic damage, by reducing mitochondrial cytochrome c release and by increasing mitochondrial respiration [Varanita et al. 2015].

What is the role of mitochondrial shape in cancer?

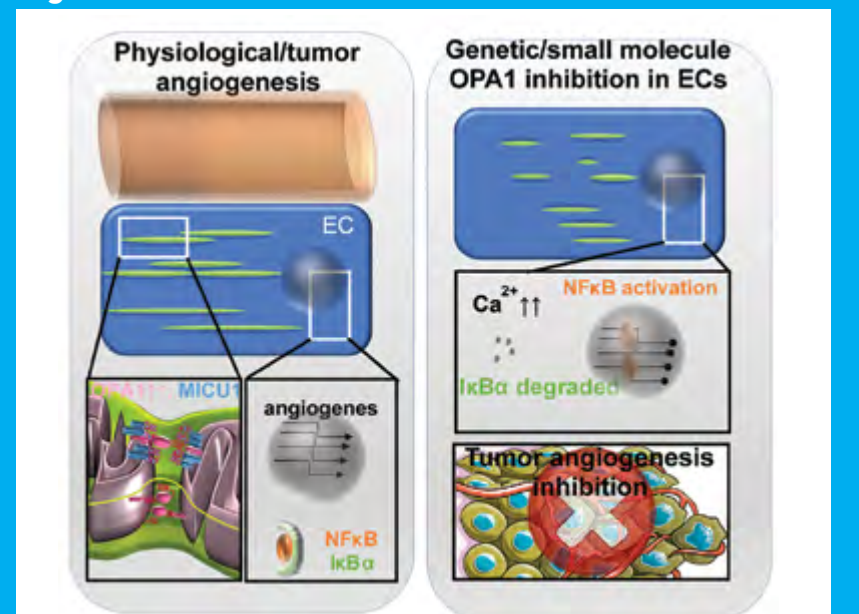
Since our original discovery that cristae remodeling is essential for the complete release of cytochrome c, we developed a program to investigate the role of Opa1 in cancer. We generated mice where Opa1 was overexpressed in the context of the well-known lymphoma driver Emu-Myc and found that the mild Opa1 overexpression increases severity of the disease and decreases survival, by favoring multisystemic spreading of lymphoma with severe cancer infiltrates in multiple organs. Because our genomic and epigenomic data indicate that Opa1 is up-regulated in melanoma cells characterized by high invasiveness potential and this up-regulation was confirmed in primary tumor samples, we generated mice lacking Opa1 in melanocytes and found that these mice display a pigmentation defect, substantiating a crucial role for Opa1 in melano-

cyte survival, similar to that of Bcl-2. Finally, we are exploring the essential role of endothelial Opa1 and Drp1 in cancer angiogenesis and metastasis, in lung cancer and developing first in class Opa1 inhibitors, by capitalizing on in house chemical biology screenings.

What are the molecular mechanisms and physiological consequences of ER-mitochondria tethering?

Since our discovery of the ER-mitochondria tethering function of Mfn2 we have been deepening our knowledge of the structural components of the ER-mitochondria interface. We are now analyzing the hits from a genome wide, FRET sensor based screening for ER-mitochondria tethers and compartments and we are characterizing the molecular mechanism of ER-mitochondria tethering by Mfn2 (**Fig. 3**).

Figure 3



Endothelial Opa1 is required for physiological and tumor angiogenesis and its inhibition curtails tumor growth. During angiogenesis, Opa1 is induced to control cytoplasmic Ca²⁺ fluxes and to keep the transcription factor NFkB at bay. If Opa1 is inhibited, Ca²⁺ rises, NFkB is unleashed and angiogenesis is impaired. This feature can be harnessed therapeutically using Opa1 inhibitors that block tumor angiogenesis and cancer growth.

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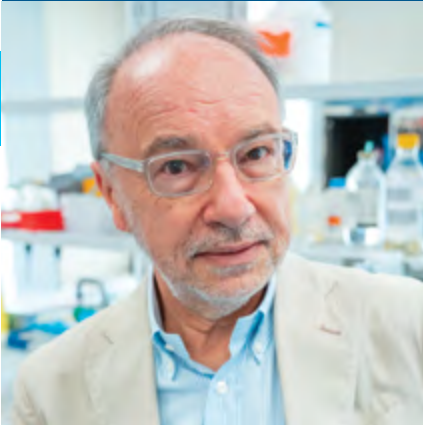
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GIANPIETRO SEMENZATO

HEMATOLOGICAL MALIGNANCY UNIT



Biographical Sketch

2021 to date	Emeritus Professor, University of Padua, <i>Italy</i>
2020 to date	Scientific Director, VIMM, <i>Padua, Italy</i>
2016 to date	Chairman, Veneto Region Hematological Network, <i>Italy</i>
2013-2019	Member, Padua University School of Medicine Executive Committee, <i>Padua, Italy</i>
2000-2020	Full Professor of Hematology
2009-2011	Chairman, Dept. Clinical & Experimental Medicine, University of Padua, <i>Italy</i>
2008-2011	President, Italian Society of Immunology & Clinical Immunology, <i>Italy</i>
2002-2020	Chief, Hematology & Clinical Immunology Branch, Padua University Hospital, <i>Italy</i>
1995-2001	Chief, Clinical Immunology Branch, Padua University Hospital, <i>Italy</i>



Group Members

- Monica Facco - *Professor*
- Renato Zambello - *Professor*
- Livio Trentin - *Professor*
- Gregorio Barilà - *Postdoctoral Fellow*
- Giulia Calabretto - *Postdoctoral Fellow*
- Federica Frezzato - *Postdoctoral Fellow*
- Vanessa Rebecca Gasparini - *Postdoctoral Fellow*
- Filippo Severin - *Postdoctoral Fellow*
- Serena Pizzo - *PhD Student*
- Edoardo Ruggeri - *PhD Student*
- Antonella Teramo - *Postdoctoral Fellow*
- Andrea Visentin - *Research Assistant*
- Cristina Vicenzetto - *Postdoctoral Fellow*

Investigation of regulatory mechanisms involved in the pathogenesis of chronic leukemias toward targeted therapy

Fields of interest

The normal hematopoietic cell biology is strictly driven by interactions with microenvironment, involving signaling molecules that finely tune their activation, survival, proliferation and differentiation. Although many studies are currently available on normal hematopoietic cell physiology, the knowledge on aberrant expression or activity of signaling molecules in the malignant counterpart still requires investigation. Our Unit focuses on two different chronic lymphoproliferative disorders:

- 1. Large Granular Lymphocytes Leukemia (LGLL)
- 2. B-cell Chronic Lymphocytic Leukemia (CLL)

Large Granular Lymphocytes Leukemia

LGLL is a rare disease, characterized by the clonal expansion of cytotoxic lymphocytes belonging to T or NK lineage. The disease encompasses different subsets: CD8+ T-LGLL, CD4+ T-LGLL, Tγδ LGLL and chronic lymphoproliferative disorders of NK cells (CLPD-NK). The main clinical manifestation is neutropenia, but the disease is clinically heterogeneous, ranging from indolent to aggressive and chemo-refractory disorder. Current treatment is based on immunosuppressive drugs, with a low overall response rate and several side effects. Thus, the lack of targeted and effective therapies still represents an unmet clinical need for LGLL patients.

B-cell Chronic Lymphocytic Leukemia

CLL is characterized by the accumulation of mature clonal CD19+/CD5+/CD200+ B lymphocytes in peripheral blood, bone marrow and lymphoid tissues. Structural and functional features of the B-cell receptor (BCR) signaling and pro-survival signals from the microenvironment are associated with the pathogenesis, progres-

sion and prognosis of this disease. Although research is making huge progress and the therapeutic armamentarium available today has significantly changed the management of CLL patients thus achieving extremely high efficacy, CLL is still an incurable disease. Hence, the need to find new strategies for the development of innovative targeted therapies for effective treatments.

Best results obtained

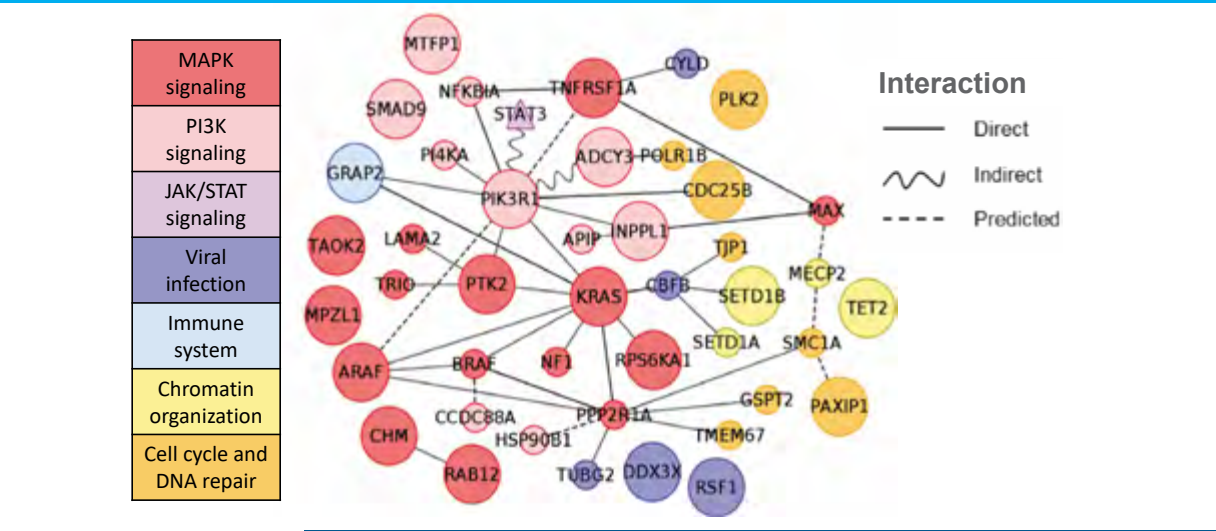
Biological and clinical impacts of STAT mutation in LGLL

The main distinctive genetic lesions in LGL disorders are somatic activating mutations on STAT family proteins, involved in the regulation of cell survival and proliferation. We demonstrated a peculiar association between *STAT3/5b* mutations and the immunophenotype of the leukemic clone; we also proved the adverse impact of *STAT3* mutations in LGLL patients' clinical course, being associated with neutropenia, anemia, treatment requirement and reduced overall survival. The role of *STAT5b* mutations, instead, is still controversial.

Characterization of the genomic landscape of CLPD-NK patients

A high-throughput whole exome sequencing (WES) highlighted a heavy mutational burden on leukemic NK cells. Bioinformatic analyses showed that somatic mutations prominently hit genes wired to cancer proliferation, survival and migration pathways (Fig. 1). Interestingly, new discovered genetic lesions in CLPD-NK, e.g. *TET2* and *DDX3X*, evidenced similarities with aggressive diseases of NK cells (ANKL).

Figure 1



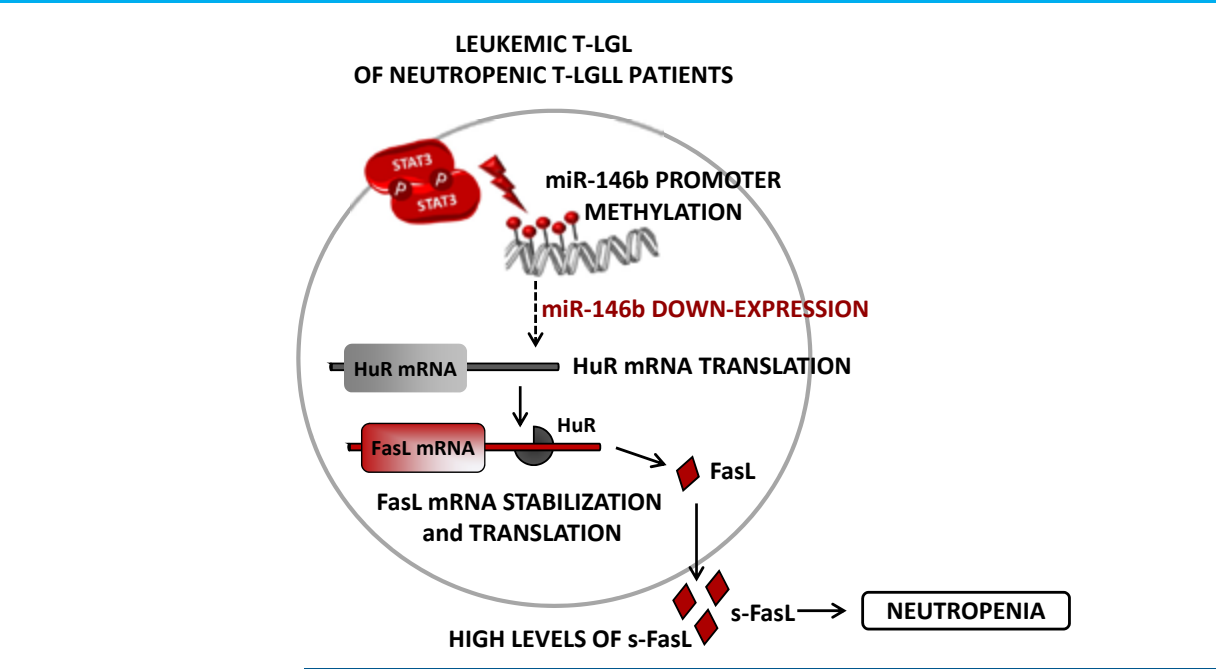
Pathways and functions of genes somatically mutated in CLPD-NK. KEGG (Kyoto Encyclopedia of Genes and Genomes database) and Reactome pathway-derived network of somatic mutations in CLPD-NK showing direct and indirect relations between genes and gene products annotated in pathways topologies (node size is proportional to the variant allele frequency, node colors indicate different groups of pathways and functions), according to the caption in the left insert.

The involvement of STAT3/miR-146b/HuR axis in LGLL neutropenia development

A high-throughput miRNome analysis was performed on both T-LGL from patients and cytotoxic T lymphocytes from healthy subjects. Our results demonstrated that miR-146b was down-modulated in T-LGLs of neutrope-

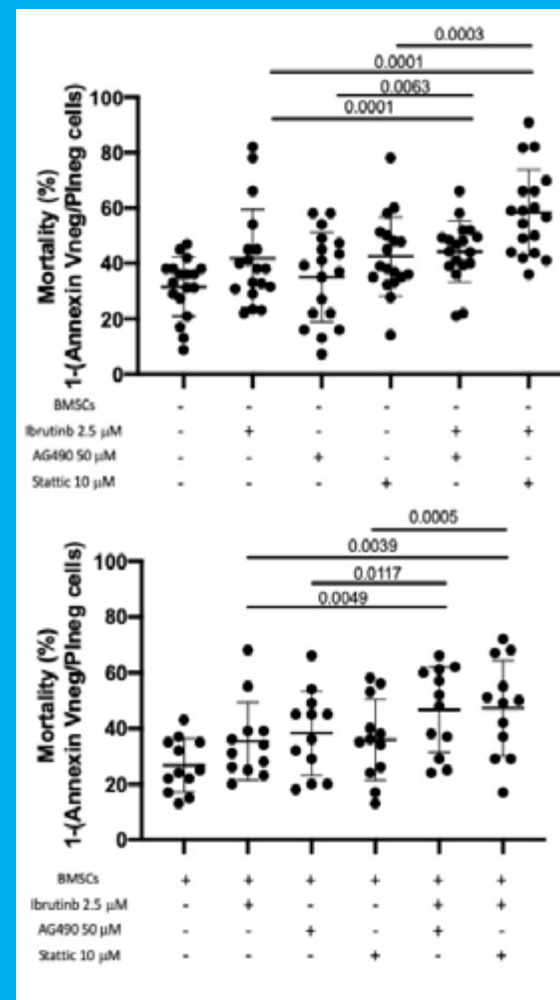
nic patients, due to a STAT3-mediated hypermethylation of its promoter. The defective expression of miR-146b leads to increased levels of soluble Fas Ligand (FasL) in sera of patients, which, in turn, triggers neutrophil apoptosis (Fig. 2).

Figure 2



Pathogenetic mechanisms of neutropenia development in T-LGL patients. MicroRNA-146b down-regulation results in increased FasL expression by leukemic T-LGL, leading to neutrophil death.

Figure 3



Combined effect of ibrutinib with JAK2/STAT3 inhibitors. Mortality of leukemic cells cultured in medium alone (top) or in presence of BMSCs (bottom) and treated with ibrutinib, AG490, and Stattic, as single agent or in combination. Mortality was calculated as 1-Annexin V^{neg}/PI^{neg} cells.

HSP70/HSF1 axis is a druggable target in CLL

Considering the role of the heat shock protein of 70kDa (HSP70) in cancer, we studied this protein and its regulator, the heat shock factor 1 (HSF1), in CLL. Both molecules were found to be overexpressed and their increase correlated with poor prognosis CLL. Their inhibition was proven to be effective in inducing a dose-dependent *in vitro* apoptosis of CLL cells. Since HSF1 and, in turn, HSP70 are finely regulated by kinases of RAS pathways, and taking advantage from a pro-

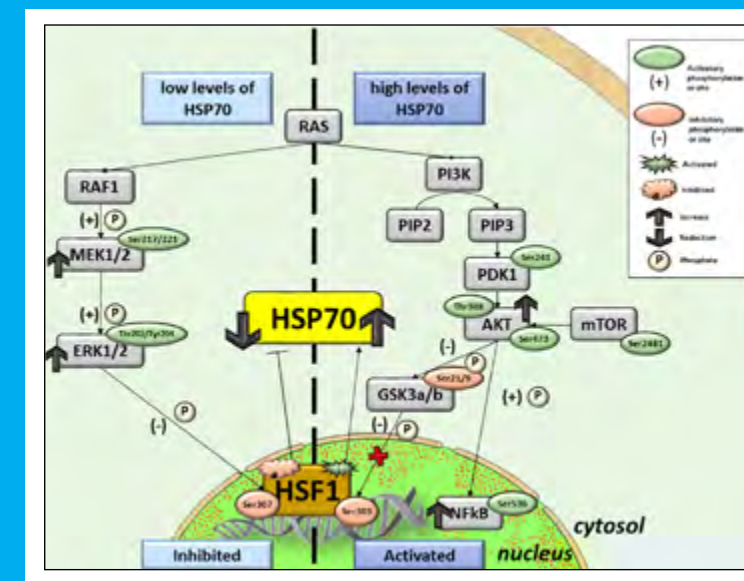
Importance of activated JAK2/STAT3 pathway in CLL

Since microenvironment promotes proliferation and drug resistance in CLL, we investigated the microenvironment-cell pathway JAK2/STAT3, its modulation by bone marrow mesenchymal stromal cells (BMSCs) and its targeting with AG490 or Stattic (JAK2 and STAT3 inhibitors, respectively). We found that treatment with AG490 and Stattic triggered CLL cell apoptosis also in presence of BMSCs.

The drug ibrutinib is very active in CLL; despite this, it is rarely able to clear leukemic cells in the marrow niche. We demonstrated that the combination of ibrutinib and JAK/STAT inhibitors increases ibrutinib-mediated leukemic cell death, bypassing pro-survival stimuli from BMSCs. We proved that JAK2/STAT3 signaling plays a role in the regulation of CLL-BMSC interactions and its inhibition enhances ibrutinib effect against the marrow niche (Fig. 3).

teomic study performed in CLL, we found that patients with high levels of HSP70 also expressed high Akt-S473, thus activating HSF1. Inhibition of PI3K, upstream AKT, reduced HSF1 and HSP70 levels. By contrast, HSP70-low patients displayed high activation of MEK1/2 and ERK1/2, that negatively regulate HSF1 (Fig. 4). These data demonstrate that the HSP70 expression is regulated by HSF1 activity through RAS pathways and suggest the HSP70/HSF1 interplay as an interesting target for antileukemic therapies.

Figure 4



Explanation of HSP70 expression by HSF1 via PI3K/AKT pathway. The model explains HSP70 expression by HSF1 via RAS-dependent pathways. (Right) In HSP70^{high} patients, we found an increased phosphorylation of Akt-S473 that is reflected in a high amount of GSK3a/b phosphorylated at inhibitory S21/9. In this way, GSK3a/b is no more able to inhibit HSF1 that, in turn, upregulates HSP70. (Left) In HSP70^{low} patients we found an upregulation of the RAS-RAF-MEK1/2-ERK1/2 pathway; activated ERK at T202/Y204 can phosphorylate HSF1 at inhibitory S307 thus preventing HSP70 transcription.

Ongoing activities and future research plans

In LGLL, we are currently investigating on:

Tumoral microenvironment

We are phenotypically and molecularly characterizing monocytes to evaluate whether they communicate with leukemic cells and through which mechanisms. Moreover, we are planning to investigate at a singular cell level the patients' bone marrow that is deemed the putative lymphoproliferation initiating site.

Molecular dissection of CD8+ and CD4+ T-LGLL

We recently obtained high-depth RNA-sequencing data of different subsets of T-LGLL patients and healthy donors. Our results will inform on gene expression dysregulation in T-LGLL, including circular and long non-coding RNAs, and on peculiarities of the most symptomatic patients.

Evaluation of new therapeutic options

We are investigating the potential effect of new therapeutic agents able to inhibit through different mechanisms STAT3 signaling. Moreover, we plan to restore mir-146b expression in leukemic LGLs taking advantage of targeted lipoplexes as an innovative biotechnological delivery system.

Differential diagnosis of CD4+ T-LGLL and CD4+ T-PLL

T-cell Prolymphocytic Leukemia (T-PLL) is an aggressive disease characterized by the clonal expansion of CD4+ lymphocytes and the same *STAT5b* mutations found in CD4+ T-LGLL, despite the indolent course of this lat-

ter. Thus, we are planning a transcriptomic profiling of T-PLL and CD4+ T-LGLL both to investigate the correlation between *STAT5b* mutations and clinical outcomes and to strengthen the differential diagnosis of CD4+ aberrant expansions.

In CLL, we will investigate:

Focal Adhesion Kinase (FAK) as new therapeutic target in CLL

Functional features of CLL cells are shaped by interactions with the microenvironment. FAK, a kinase whose activation promotes adhesion/motility, survival and proliferation, is a critical mediator of those signals. Data on FAK in CLL are absolutely poor. We hypothesize a role for FAK in CLL cell survival and its involvement in CLL pathogenesis and maintenance, at the crossroads of BCR, chemokine and integrin pathways.

Drug-resistance in CLL: the development of a cell line resistant to ibrutinib

The BTK inhibitor ibrutinib has significantly changed the management of patients with CLL. Despite this, relapses occur and outcomes after ibrutinib failure are dismal due to the lack of effective agents. Besides BTK and PLC γ mutations, mechanisms of ibrutinib-resistance remain to be clarified. Hence, the need to develop a model with a CLL cell line resistant to ibrutinib where applying studies to discover molecules/mechanisms involved in drug-resistance.

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TANIA ZAGLIA

CELL SIGNALING IN CARDIOMYOPATHIES UNIT



Biographical Sketch

2020 to date	Assistant Professor, Dept. Biomedical Sciences, University of Padua, Italy
2017 to date	Group Leader, VIMM, Padua, Italy
2017-2020	Assistant Professor, Dept. Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padua, Italy
2013-2016	Telethon Junior Researcher, VIMM, Padua, Italy
2011-2013	Research Fellowship, VIMM, Padua, Italy
2007-2011	Postdoctoral Fellow, Dept. Biomedical Sciences, University of Padua, Italy
2007	PhD Cardiovascular Sciences, University of Padua, Italy
2003	Biological Science Degree, University of Padua, Italy



Group Members

- Anna Di Bona - Postdoctoral Fellow
- Lolita Dokshokova (co-supervised with Prof. Mongillo) - Postdoctoral Fellow
- Antonio Mazzaro - Master Student
- Nikolsa Palma - Master Student
- Arianna Scalco - PhD Student
- Serena Tiberio - Research Fellow

RESEARCH ACTIVITY

Fields of interest and ongoing researches

- Define and target the disease mechanisms in Arrhythmic Cardiomyopathy;
- Understand the role of proteotoxic damage in the pathogenesis of Familial Hypertrophic Cardiomyopathy;
- Define the role of the adrenergic component of the motor nerve in the pathogenesis of Amyotrophic Lateral Sclerosis.

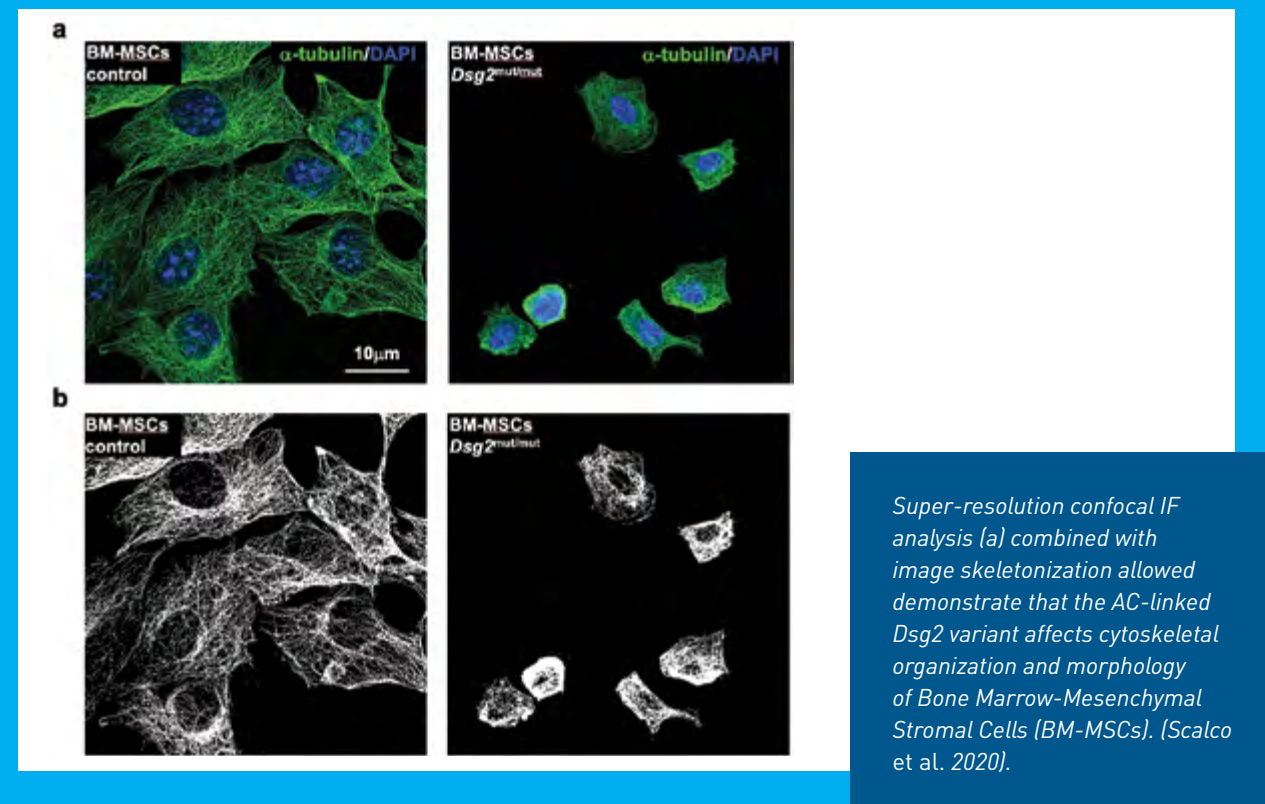
Disease mechanisms in Arrhythmogenic Cardiomyopathy

Arrhythmogenic Cardiomyopathy (AC) is a familial cardiac disease, accounting for most cases of stress-dependent arrhythmic sudden death, predominantly in the young and athletes. In the 50% of genetically diagnosed cases, AC is caused by mutations in genes encoding desmosomal proteins, which, in cardiomyocytes (CMs), make desmosomes. The hallmarks of the AC myocardi-

um are the progressive loss of CMs, tissue inflammation, and the replacement of the contractile myocardium with fibro-fatty lesions which, on the one hand compromise contractile performance, leading sometimes to heart failure, on the other generate the substrate for life-threatening arrhythmias, triggered during physical or emotional stresses. AC is incurable as its pathogenesis is still obscure. At the time being, research mainly approached AC as a disease of desmosomes, and as such of CMs. As a consequence, the majority of AC preclinical models were based on the overexpression

or downregulation of mutated genes selectively in CMs. However, we showed that desmosomal proteins are expressed, although not organized into desmosomes, by several cardiac and extra-cardiac cell types (Scalco *et al.* JCM 2020), which thus all harbour AC-linked mutations and may participate to the disease pathogenesis. On these bases, the guiding hypothesis of our research is that AC, although dramatically manifesting with the sudden heart short-circuit, develops with the contribution of multiple cell types and organs i.e. brain (Agrimi *et al.* JCM 2020) and bone-marrow (Scalco *et al.* JCM 2020)

Figure 1

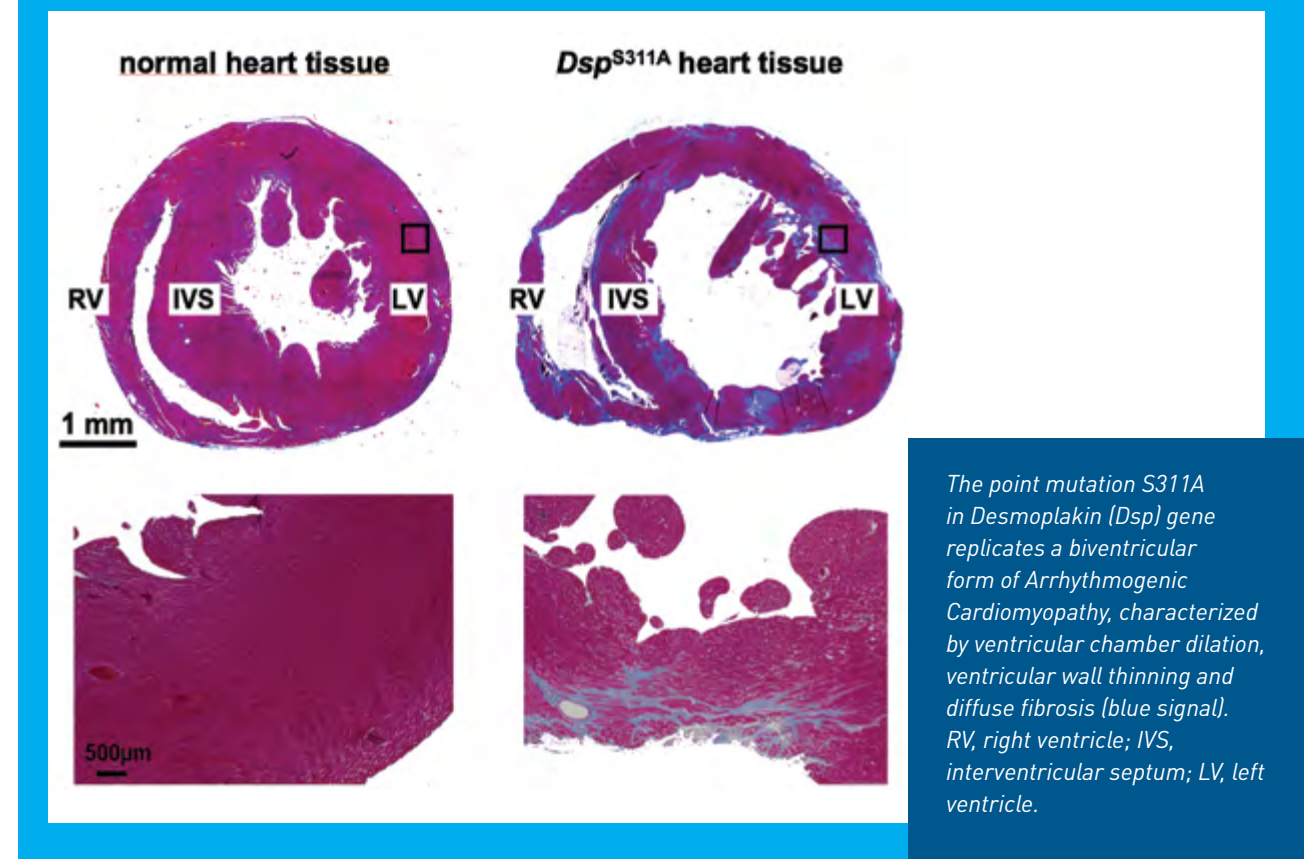


(Fig. 1). In other words, to shed new light on the pathogenetic mechanisms underlying AC, we are looking at this 'killer in disguise' as 'a multicellular and multiorgan disease'. Following this concept, we recently generated a novel knock-in preclinical model of AC, carrying the point mutation (Ser311Ala) in Desmoplakin gene (*Dsp*^{S311A}), which has been found mutated in a large fraction of the Italian AC population. This novel model well replicates both human genetics and the clinical phenotype of a biventricular form of AC (Fig. 2) and will be used for a comprehensive study of AC pathogenesis and mechanistic therapeutic interventions, using FDA-approved drugs.

We are aware that the view of AC as a multicellular dis-

ease may increase the complexity of its pathogenesis, apparently decreasing the chances of a specific therapy. However, our novel hypothesis is that sympathetic neurons (SNs) simultaneously operate a higher order control on several cellular types, including intrinsic cardiac cells (i.e. CMs, fibroblasts, resident inflammatory cells) and cells recruited to the site of injury (e.g. circulating stromal cells and inflammatory cells), all of which express druggable sympathetic neurotransmitter receptors. If our research confirms the strong insinuation that SNs may underlie the erratic behavior of all such cell types in the AC heart, it indicates that interfering with SN signals might open a novel therapy able to counteract the disease.

Figure 2

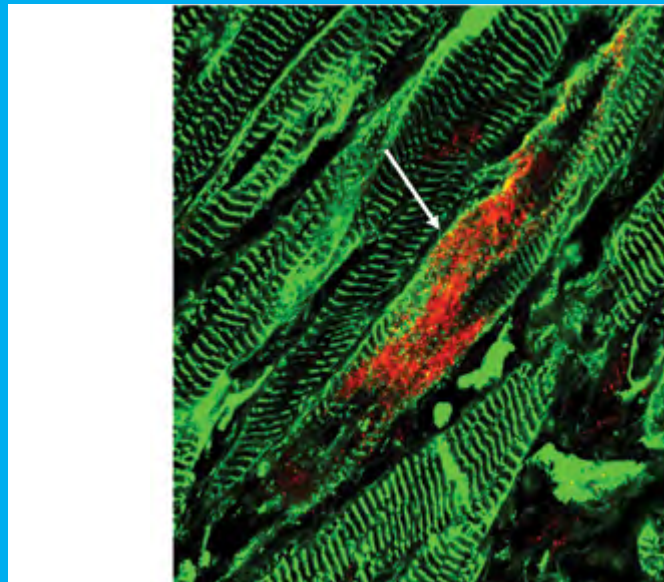


Disease mechanisms in Familial Hypertrophic Cardiomyopathy

Familial Hypertrophic Cardiomyopathy (FHC) was the first inherited heart disease to be characterized at the molecular level, with the demonstration that it is mainly caused by mutations in genes encoding sarcomeric proteins. The most common causes of FHC are mutations in the *MYBPC3* gene, which leads to a truncated Myosin-binding protein C. FHC also arises from mutations in other sarcomeric proteins, including Myosin Heavy Chain, cardiac Troponin I, Tropomyosin. To date, disease-causing mutations are identified in only about two-thirds of FHC patients, indicating that additional FHC genes need to be discovered. The disease causes enlargement in the heart size and manifests with symptoms ranging from weakness and exercise intolerance, to severe heart failure and arrhythmias, generally worsening with age. Notably, regardless of the underlying genotype, FHC hearts share a similar myocardial phenotype which includes intracellular protein aggregation and impairment in the main systems the cell uses to remove old and damaged proteins, that is to say, the Ubiquitin Proteasome System (UPS) and the Autophagy/Lysosome System (ALS), both essential in the control of protein homeostasis in the heart. During ageing, a condition associated to re-

duced UPS/ALS activity, the UPS- and ALS-dependent clearance of mutated proteins further decreases, and this is in line with the progressive worsening of the disease with time observed in FHC patients. Taken together, current literature and our original evidence suggest that the pathogenesis of FHC includes both the primary sarcomeric defect and the secondary impairment of UPS and ALS. The molecular mechanisms of proteotoxic damage, and the extent by which it participates to the progression of FHC remain to be determined, and these represent the aims of our current research. In addition, our preliminary data shows that pharmacological or genetic manipulation of protein quality control improves cardiac function in proteotoxic cardiomyopathy models. Although it is currently unknown whether reducing the oligomerization of misfolded proteins will actually ameliorate FHC, our data supports that protein quality control mechanisms may be important therapeutic targets. Thus, we aim to enhance protein quality control to reduce the workload of the proteolytic systems, as a novel therapeutic strategy in FHC treatment. If successful, our research has the potential to answer fundamental questions on FHC mechanism and identify novel therapies to treat such common and devastating cardiac disorder (Fig. 3).

Figure 3



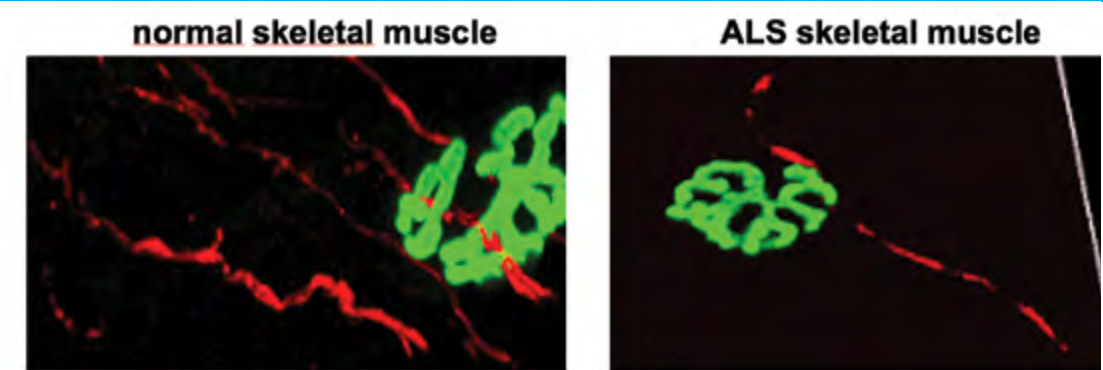
Anomalous accumulation of damaged proteins (red signal) causes sarcomere disarrangement (green signal) and proteotoxic cell death in the heart.

Role of sympathetic neurons in the pathogenesis of Amyotrophic Lateral Sclerosis

Amyotrophic Lateral Sclerosis (ALS) is a neuromuscular disorder characterized by motor neuron degeneration, hallmarked by muscle weakness, paralysis and, ultimately, respiratory failure. The disease has a complex and still nebulous pathogenesis and there is increasing consensus that multiple mechanisms of injury converge as the disease progresses. Thus, a comprehensive understanding of the disease mechanisms is urgently needed for the identification of mechanism-driven therapeutic interventions. Originally defined as 'a motor neuron disease', recent evidence supports that ALS is 'multicellular', involving at least glial cells, astrocytes and skeletal myocytes. Abnormalities in autonomic neurons, including altered sympathetic activity, have also been recognized in ALS patients, and may have a causal role in both life-worsening, as well as life-threatening manifestations, i.e. arrhythmias. Sympathetic neurons (SNs) may thus be additional

and underappreciated disease targets in ALS. Noradrenaline-releasing SNs innervate almost all tissues, including skeletal muscles, which are highly compromised in ALS. We recently showed that SNs co-innervate muscles with motor neurons, interacting with myocytes at the Neuromuscular Junction, where noradrenaline-sensing β_2 -adrenoceptors are found (Straka *et al.* Int J Mol Sci 2018). Notably, we observed that muscle SNs are degenerated in SOD1^{G93A} ALS muscles, already at early disease stages (Fig. 4). Thus, in our laboratory we are testing the hypothesis that SNs may be additional cells participating to ALS and may be modifiers of the disease manifestations (for dysautonomia dependent effects), and/or progression (for the direct involvement in the pathogenic mechanism). Our current research aims to improve knowledge on ALS pathogenesis, determine whether SN modulation may be looked after for additional therapeutic approaches, and identify biomarkers (e.g. SN function) for patient risk stratification.

Figure 4



Multiphoton image analysis allowed 3-D reconstruction of blocks of skeletal muscle, co-stained with FITC-conjugated a-bungarotoxin (green signal), to detect Neuromuscular Junction, and anti-tyrosine hydroxylase (TH, red signal), to identify sympathetic neurons (SNs). ALS muscles show reduced SN density and alterations in SN morphology.

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MASSIMO ZEVIANI

MITOCHONDRIAL MEDICINE UNIT



Biographical Sketch

2020 to date	Full Professor of Neurology, University of Padua, Italy
2020 to date	Group Leader, VIMM, Padua, Italy
2013-2019	Director, MRC Mitochondrial Biology Unit; Prof., UniCam, Cambridge, UK
2001-2012	Director, UOC Mol. Neurogenetics, IRCCS "C. Besta", Milan, Italy
1997-2001	Director, UOC Biochem. & Genetics, IRCCS "C. Besta", Milan, Italy
1996-1997	Director, UOC Mol. Medicine, IRCCS "Bambino Gesù", Roma, Italy
1995-1996	Scientist, INSERM 393, Paris, France
1990-1995	Neurologist, UOC Biochem. & Genetics, IRCCS "C. Besta", Milan, Italy
	Specialist, Endocrinology and Neurology; PhD, Genetics



Group Members

- Carlo Viscomi - Professor
- Valeria Balmaceda - Postdoctoral Fellow
- Michele Brischigliaro - Postdoctoral Fellow
- Raffaele Cerutti - PhD Student
- Samantha Corrà - PhD Fellow
- Giacomo Giacchin - Master Student
- Sara Volta - Med. Genetics Student

RESEARCH ACTIVITY

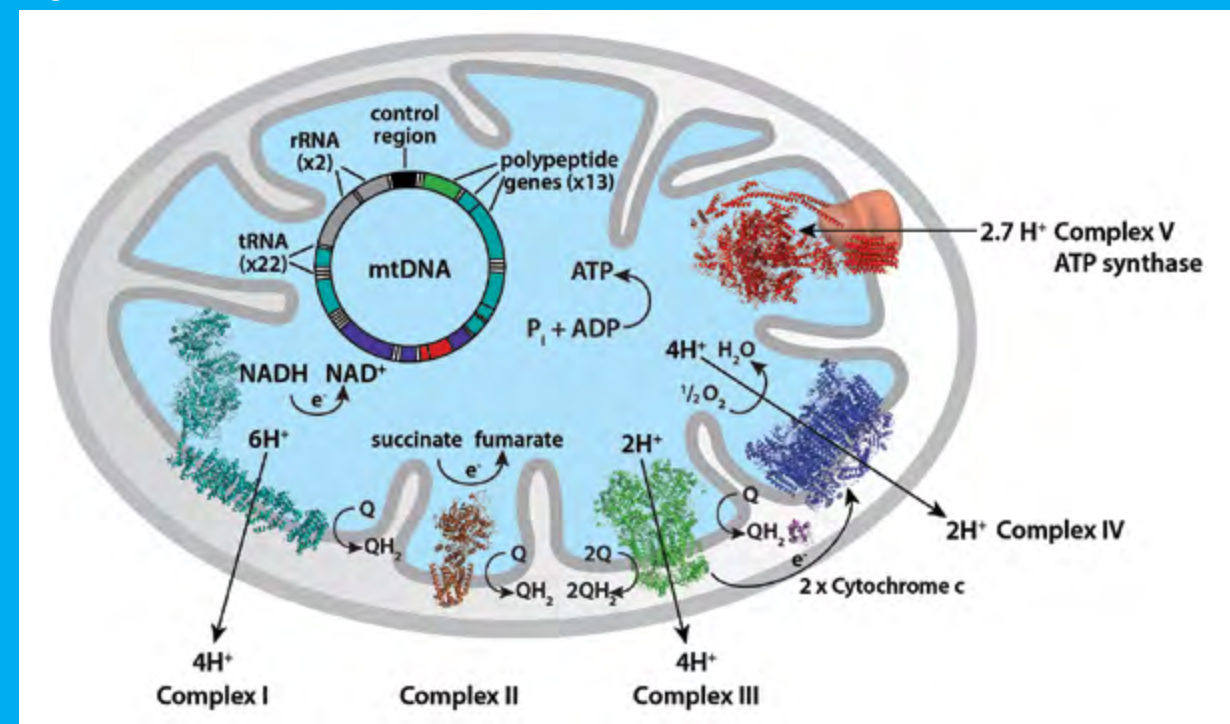
Fields of interest

Mitochondrial biology and medicine (Fig. 1).

Ongoing researches

- 1) identification of new mitodisease genes
- 2) investigation on mitochondrial pathophysiology, through *in vivo* and *in vitro* techniques
- 3) development of gene- and drug-based therapies.

Figure 1



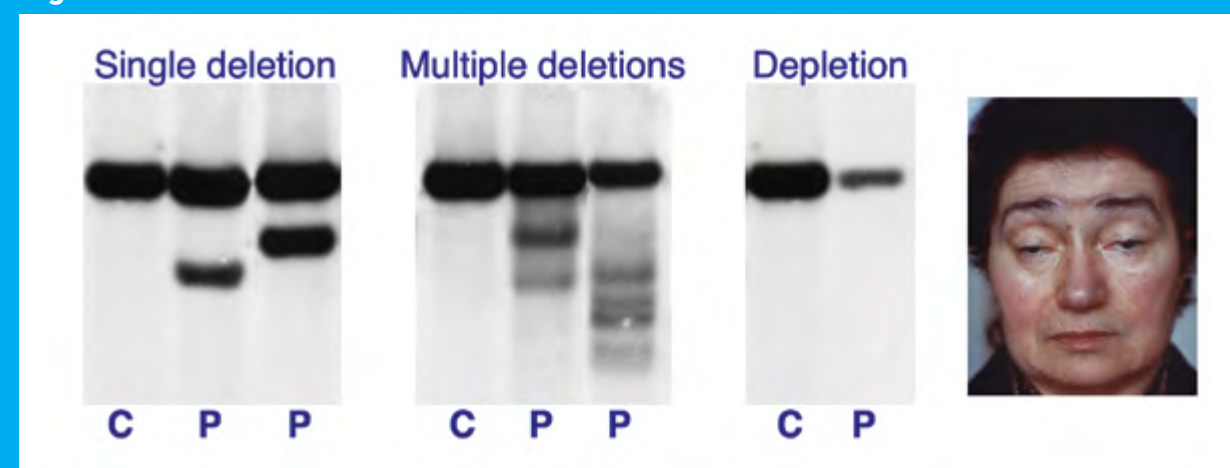
Scheme of mitochondria showing the arrangement of the respiratory chain and oligomycin-sensitive ATP-synthase.

Best results obtained

Identified numerous mutant genes in human OXPHOS defects: Kearns-Sayre s. and sporadic PEO (Zeviani *et al.* Neurology 1988; Moraes *et al.* NEJM 1989; Schon *et al.* Science 1989), mendelian inherited mtDNA multiple

deletions (Zeviani *et al.* Nature 1989), (Fig. 2) cardiomyopathy due to mutant mtDNA (Gellera *et al.* Lancet 1994). Identified Surf1, the first COX assembly factor mutated in Leigh's syndrome (Tiranti *et al.* Am J Hum Genet 1998), ANT1, the first mutant gene associated with ad-

Figure 2



Panel "Single deletion": Southern blotting of linearized muscle mtDNA showing a control, C, and two single mtDNA deletion specimens, P, P. Panel "Multiple deletions": Southern blotting of linearized muscle mtDNA showing a control, C, and two multiple deletions specimens, P, P. Panel "Depletion": Southern blotting of linearized muscle mtDNA showing a control, C, and a specimen with decreased mtDNA amount. Portrait shows severe bilateral eyelid ptosis in an ad PEO case with multiple mtDNA deletions.

PEO with multiple mtDNA deletions [Kaukonen *et al.* Science 2000], the first mutation in the 18kDa subunit of complex I [Petrizzella *et al.* Hum Mol Gen. 2000] as well as Twinkle, the mtDNA helicase [Spelbrink *et al.* Nat Genet. 2001] again in adPEO, EFTu and EFG1 in mtDNA-translation defects [Valente *et al.* Am J Hum Genet. 2007], ETHE1 in ethylmalonic encephalopathy [Tiranti *et al.* Am J Hum Genet. 2004], Mpv17 in hepatocerebral mtDNA depletion syndrome [Spinazzola *et al.* Nat Genet. 2006], FASTKD2 in COX deficiency [Ghezzi *et al.* Am J Hum Genet. 2008], SDHAF1, the first assembly factor of complex II in SDH deficiency [Ghezzi *et al.* Nat Genet. 2009], RNaseH1 associated in arPEO with multiple mtDNA deletions, FBXL4, an E3 ligase of the outer mitochondria, TTC19, a stabilizer of the Fe-S Rieske protein of complex III, TIM50, the "lid" protein of the TIM23 mitotranslocator system, APOPT1, a new COX assembly factor, and many others. This work has also been essential in the elucidation of previously unknown proteins, thus contributing to fundamental mitoscience. Participated in the identification of SMN1, responsible of SMA [Melki *et al.* Science 1994; Lefebvre *et al.* Cell 1995] and paraplegin, responsible of SPG7 [Casari *et al.* Cell 1998]. Several animal models were created to investigate pathogenesis and develop rational therapies. An ETHE1 KO

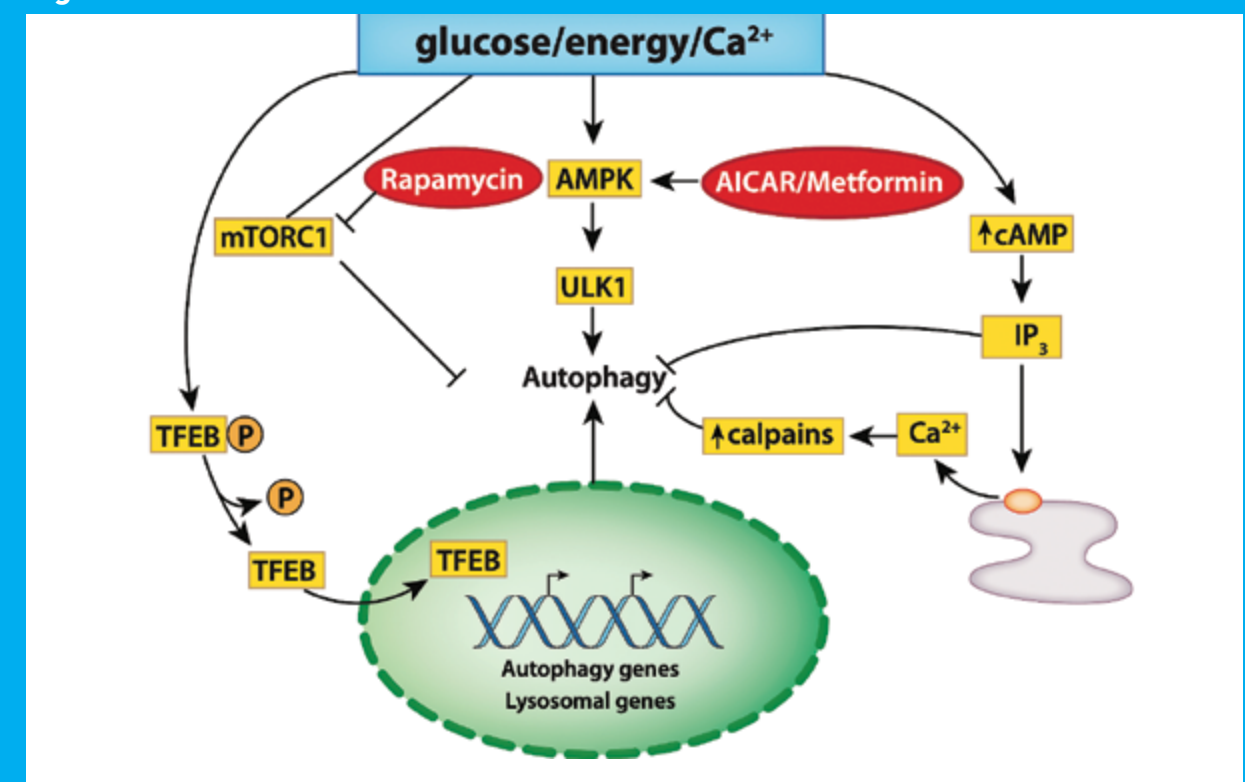
mouse unveiled the molecular defect of Ethylmalonic Encephalopathy, and its functional role [Tiranti *et al.* Nat Genet. 2009], allowing to propose the first effective drug therapy for this disease [Viscomi *et al.* Nat Med. 2010].

Drug-based stimulation of the PGC1alpha-dependent mitochondrial pathway was ameliorated mitochondrial pathic mouse models [Viscomi *et al.* Cell Metab. 2011; Cerutti *et al.* Cell 2013]. Also, muscle and brain mitochondrial failure improved by increasing mitophagy through inhibition of mTORC1 by rapamycin, or by correcting disarrayed mitocristae by moderate OPA1 overexpression. AAV-mediated gene therapy approaches were effective to treat mitodiseases due to accumulation of toxic compounds [Di Meo *et al.* EMBO Mol Med. 2012; Torres-Torronteras *et al.* Mol Ther. 2014], and other mitofailures due to defects in nuclear [Bottani *et al.* Mol Ther. 2014, Di Meo *et al.* Gene Ther. 2017, Pinheiro *et al.* Mol Ther. 2020], or mtDNA genes [Gammage *et al.* Nat Med. 2018].

Future research plans

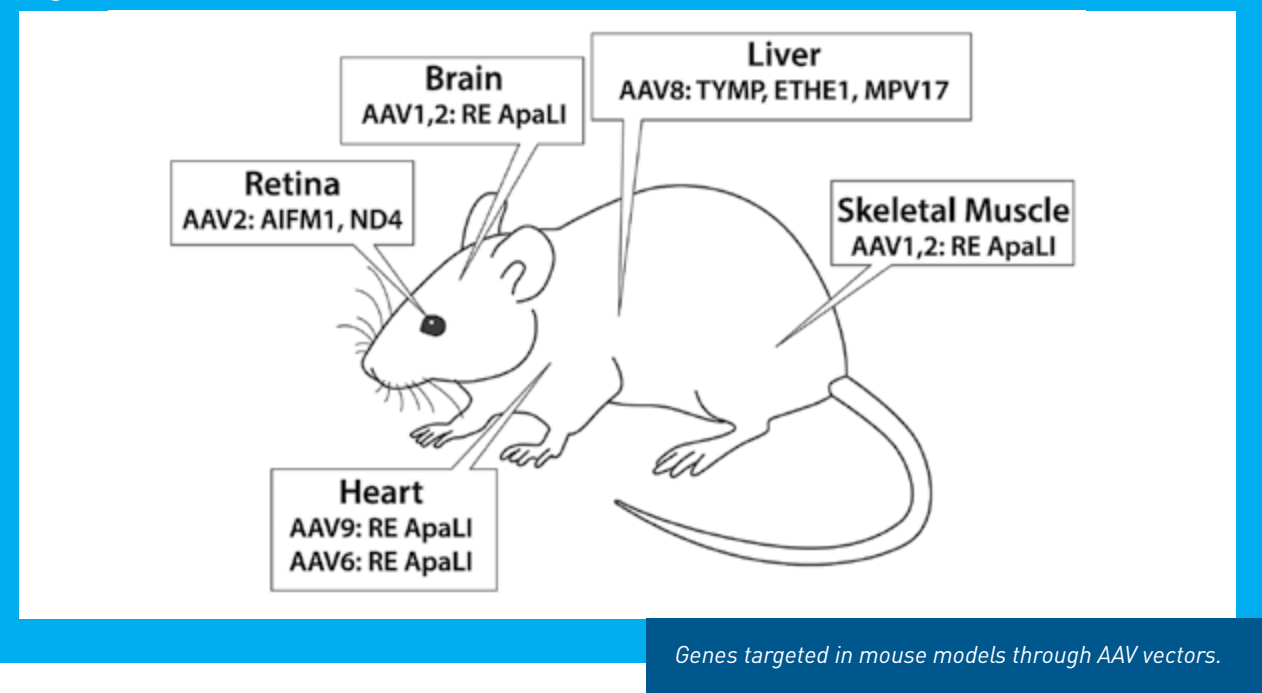
50% of pediatric mitodiseases remains undiagnosed. Many disease-genes are still poorly characterized. No effective therapy is currently available for mitodisorders. We will address these crucial issues in the coming years.

Figure 3



Metabolic pathways targeted by mitochondriogenic drugs (AICAR, Metformin) or TORC1 inhibitors (Rapamycin).

Figure 4



SELECTED PUBLICATIONS

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The following list shows the awardees since 2014 (year of publication of the last VIMM Research Report).

2014

Federico Ceriani

(Mammano Group)

Quantitative estimate of biochemical and electrical intercellular coupling in the developing cochlea

Giovanni Giuseppe Giobbe

(Elvassore Group)

Integrated multi-stage tissue on a chip generation from human pluripotent stem cells

Nicola Pianca

(Mongillo Group)

Cardiac sympathetic innervation determines regional heterogeneity in morphology and function of cardiomyocytes

2015

Elisa Mandato

(Piazza Group)

Protein kinase Csnk2 in healthy and neoplastic B cells

Andrea Predonzani

(Molon Group)

Focusing nitric oxide post-translational modification in cancer microenvironment: an emerging master player

Stefano Giulitti

(Elvassore Group)

High-throughput reprogramming through endogenous signals modulation

2016

Antonio Campo

(Mongillo Group)

Targeting the mechanisms of mitochondrial Ca²⁺ uptake in heart diseases

Roberta Angioni

(Viola Group)

Mouse Mesenchymal stem cells inhibit high endothelial cell activation and lymphocyte homing to lymph nodes by releasing TIMP-1

Mauro Corrado

(Scorrano Group)

Macroautophagy inhibition maintains fragmented mitochondria to foster T cell receptor-dependent apoptosis

2017

Andrea Armani

(Sandri Group)

Transcription factor EB controls metabolic flexibility during exercise

Leonardo Nogara

(Blaauw Group)

Early signaling events involved in muscle remodeling

Dijana Samardzic

(Scorrano Group)

The mitochondria shaping protein Opa1 defines severity of Myc-driven lymphoma

2018

Anaïs Franco Romero

(Sandri Group)

Identification of a novel FoxO-dependent regulator of muscle mass

Simona Francia

(Lodovichi Group)

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

Marilena Carrino

(Piazza Group)

Protein kinase CK1 α modulates prosurvival autophagy in Multiple Myeloma

2019

Lorenza Tsansizi

(Scorrano Group)

Genetic inactivation of USP8 in the heart, a deubiquitinase hyperactive in Cushing's syndrome adenomas, causes mitochondrial dysfunction and cardiomyopathy

Andrea Maset

(Lodovichi Group)

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

Sara Zumerle

(Alimonti Group)

Development of senolytic therapies for the treatment of ageing and prostate cancer

2020

Nicola Moro

(Mongillo Group)

Increasing mitochondrial Ca²⁺ uptake capacity improves heart adaptation to pressure overload

Nicoletta Surdo

(Lefkimmiatis Group)

Reversible phosphorylation decouples RIPK3 from necroptosis

Andrea Zangrossi

(Corbetta Group)

When style matters: low dimensionality in visual exploration dynamics and gaze fingerprint

2021

Samantha Corrà

(Zeviani Group)

Strategies to fighting mitochondrial diseases

Laura Quotti Tubi

(Piazza Group)

CK2 β regulatory subunit is crucial for normal stem cell biology and sustains erythropoiesis on multiple levels

Martina Spacci

(Carrer Group)

Cholesterol activates cyclic AMP signaling in metaplastic acinar cells





Giustina Mistrello Destro (*Vice President*) and Francesco Pagano (*President*)



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Progetto grafico e impaginazione: Maria Cuervo

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Finito di stampare nel mese di settembre 2021 per conto da Grafica Veneta S.p.A. di Trebaseleghe (PD)

Printed in Italy

RESEARCH REPORT 2021



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