Annual Meeting

GIBB

Italian Group of Biomembranes and Bioenergetics

June, 21-23, 2018
Comparto di San Geminiano
Via San Geminiano 11, Modena

Gruppo Italiano di Biomembrane e Bioenergetica
GIBB

UNIVERSITAS STUDIORUM MUTINENSIS ET REGIENSIS
1175
UNIMORE
UNIVERSITÀ DEGLI STUDI DI MODENA E REGGIO EMILIA

Contents

Gruppo Italiano di Biomembrane e Bioenergetica

The primary aim of the Italian Group of Biomembranes and Bioenergetics (GIBB) is to promote Bioenergetics, i.e. the discipline that studies energy transformation at the 'energy-conserving' membranes. This area of research is key to biology and physiology in all forms of life, and has major implications for our understanding of health and disease. GIBB stimulates research networking in Italy and abroad. One of its major goals is the training of young scientists, an attitude that is reflected in the unique format of its annual meeting, where the core of scientific program is represented by oral presentations by young scientists, while established Investigators chair sessions and promote discussion. From 2017 the meeting is open to the International Community.

GIBB was created in 1973 by a group of Italian Scientists coming from a variety of disciplines – Biological Chemistry, Biophysics, Pathology, Pharmacology, Physiology – with the idea of promoting Bioenergetics in the Country and to establish an interface with the growing International Bioenergetics Community. GIBB is actively involved in the promotion and organization of the European Bioenergetics Conferences (EBEC), a biannual series that was started in Urbino (Italy) in 1980.

GIBB is managed by an Executive Committee (Consiglio Direttivo) of eight members elected for a two-year term. The Executive Committee nominates the President, the Vice-President and the Secretary-Treasurer. Membership is open to scientists qualified in the field of Bioenergetics and Biomembranes. New applicants are presented by active members and admission is decided at the General Assembly, which gathers at the Annual Meeting.

GIBB Executive Board

Paolo Bernardi (Padova)

Alessandro Giuffrè (Roma)

Giovanna Lippe (Udine)

Vito De Pinto (Catania)

Francesco Francia (Bologna)

Cesare Indiveri (Cosenza)

Luigi Palmieri (Bari)

Claudia Piccoli (Foggia)

Organizing Committee

Marcello Pinti

Lara Gibellini

Anna De Gaetano

Elena Bianchini

Anita Neroni

Beatrice Lusenti

Venue

The Meeting will be held in **the Monastery of San Geminiano**, a beautiful ex monastery in the city centre of Modena, which currently hosts the Faculty of Law of the University of Modena and Reggio Emilia.

Transportation

Modena is 40 km far from Bologna; it is well connected to Italy and Europe thanks to the airport of Bologna – Guglielmo Marconi, served by the major airlines and low-cost carriers (www.bologna-airport.it), to the railway station, to the high speed train station of Reggio Emilia AV (20 km from Modena), and to the A1 and A22 motorways.

Accomodation

The official website of Modena Tourism Promotion (www.visitmodena.it) can provide all the information necessary for organizing your trip, including transport, accomodation, restaurants and more.

Welcome to Modena

Modena is a friendly city, with an interesting history and plenty of modern creations, where visitors can discover ancient flavours and music on the city theatres. The historical centre recalls the greatness of the House of Este, thanks to whom Modena became State Capital from the end of the sixteenth-century to 1859. The emblem of all this magnificence is Palazzo Ducale, designed by the architect Avanzini on which other architects also worked, including Vigarani and Soli. Since the Unification of Italy, this building has been home to the prestigious Military Academy.

The centre of Modena is based around the Via Emilia, as in the past the Roman city developed along the consular road, whose construction in 187 BC preceded foundation of the Modena colony by a few years (183 BC). Remains and monuments from the Roman city can be admired in the Museum of Archaeology, in the Estense Lapidary Museum and the Roman Epigraphic Museum, which houses the monumental altar of Vetilia Egloge.

An old Roman road can be walked along by visitors to the NoviArk archaeology park, after the latter was created following digs in the Novi Sad area during building of an underground carpark.

Modena has a good number of churches, full of masterpieces, from the oldest Santa Maria della Pomposa in the square of the same name that is the heart of Modena's nightlife, to Baroque churches such as San Biagio, Sant'Agostino, called the Pantheon of the Estensi, through to San Vincenzo where the Este family tombs are. The San Pietro abbey complex is particularly interesting, including the Abbey full of works of art like that of Begarelli and a Benedictine monastery with beautiful cloisters open to the general public and the old dispensary, stocked with products made following ancient recipes.

The Cathedral and the Ghirlandina

These masterpieces, together with Piazza Grande, are UNESCO World Heritage Sites and date back to medieval times. The Cathedral, one of the most beautiful and elegant from the European Romanesque period, is a book of stone where the architecture of Lanfranco and the sculpture of Wiligelmo communicate symbolic messages of faith and hope to citizens and the faithful.

The Piazza ends with a complex of civic buildings, constructed over the centuries which have merged to become a big single building, Palazzo Comunale, the address for several municipal offices. Inside are paintings, tapestries that give symbolic messages recalling civic and moral virtues.

A byword for the industrious city, Modena boasts a long list of ingenious entrepreneurs, among them the Panini brothers, trading card manufacturers now celebrated in the Museo della Figurina. Housed in Palazzo Santa Margherita, the museum is the only one of its kind, with a collection of some 500,000 collectible cards.

Modena is also synonymous with photography and contemporary art. Since October 2017, as well as the Museo della Figurina, FONDAZIONE MODENA ARTI VISIVE has also been in charge of the 2 locations of the city's art museum, Galleria Civica di Modena (Palazzo Santa Margherita and Palazzina dei Giardini), Fondazione Fotografia Modena, housed in a former tobacco factory known as MATA, and the city's higher-education institute specializing in the contemporary image (Scuola di Alta Formazione sull'immagine contemporanea).

Just a few minutes from the train station and the historical centre is the MEF, the Enzo Ferrari Museum, a modern building in the shape of a yellow aluminium car bonnet that houses the cars that have written the History of Ferrari and its founder, explained in videos and multimedia effects. It is also home to temporary exhibitions. This museum is linked to the Ferrari Museum in Maranello, a must-visit for car fanatics.

Program

Thursday 21st

14:00-15:30	Registration
15:30-16:00	Welcome Address (Authorities, GIBB President, Organizing Committee)
16:00-17:00	**Opening Plenary Lecture** Chair: Paolo Bernardi **Thorsten Friedrich**, University of Freiburg (Germany) *On the mechanism of respiratory complex I*
17:00	**Session I: ATP synthase and complexes of respiratory chain** Chair: Giovanna Lippe
17:00-17:20	Andrea Urbani (University of Padova) *Electrophysiological properties of channel formed by bovine FOF1 ATP synthase in planar lipid bilayer*
17:20-17:40	Chiara Galber (University of Padova) *Role of F-ATP synthase f subunit in dimer formation and PTP modulation*
17:40-18:00	Elisa Astro (University of Bologna) *Dissecting the effects of MT-ND1 and NDUFS3 suppression on respiratory Complex I assembly and supercomplex biogenesis*
18:00-18:20	Luisa Iommarini (University of Bologna) *Respiratory complex I ablation in aggressive cancers induces oncocytic indolence but triggers macrophage-mediated adaptive response*
18:20-18:40	Antonio Gnoni (University of Bari) *Functional and expression study identifying plasma membrane ecto-ATP synthase as a novel player in the early phase of Liver regeneration*
18:40-20:00	Welcome cocktail
20:00-21:00	Tour “A spasso con lo scienziato” in the historical centre of Modena

Program

Friday 22nd

9:00-10:00	**Plenary lecture** Chair: Luigi Palmieri **Paolo E. Porporato,** University of Torino *Mitochondrial metabolism in cancer: impact on metastasis and tumor progression*
10:00	**Session II: Specialized transporters and pores: structure and function I** Chair: Cesare Indiveri
10:00-10:20	Fabio Martinelli (University of Basilicata) *The ABCC6 expression is regulated by adenosine and ATP externally added to the HepG2 cells*
10:20-10:40	Anna Stocco (University of Padova) *The effects of PTP inhibition in Duchenne muscular dystrophy*
10:40-11:00	Christina Pfeiffer (Medical University of Vienna) *Role of MICS1, interaction partner of LETM1, in mitochondrial calcium homeostasis*
11:00-11:30	Coffee break
11:30	**Session III: Specialized transporters and pores: structure and function II** Chair: Vito De Pinto
11:30-11:50	Andrea Magrì *Channel activity of yeast VDAC2, the second mitochondrial porin isoform of Saccharomyces cerevisiae*
11:50-12:10	Lara Console, University of Calabria *Identification and functional characterization of an exosomal cargo protein: the plasma membrane carnitine transporter (OCTN2)*
12:10-12:30	Faustino Bisaccia (University of Basilicata) *The multidrug resistance inhibitor 8-(4-chlorophenyl)-5-methyl-1 8-[(2Z)-pent-2-en-1-2 yloxy]-8H-[1,2,4]oxadiazolo[3,4-c][1,4]thiazin-3-one diltiazem-like compound inhibits the ABCC6 transporter*

Program

12:30-12:50	Jessica Cosco (University of Calabria) *Characterization of the CAT2 transporter from Solanum lycopersicum (Tomato)*
13:00-14:30	Lunch
14:30-15:30	**Plenary lecture** Chair: Marcello Pinti **Claudio Procaccini,** IEOS - CNR Napoli *Integrating metabolism and immunity*
15:30-15:50	**Session IV: Bioenergetics metabolism and mitochondrial dysfunctions I** Chair: Francesco Francia
15:50-16:10	Carlos Sanchez-Martin, University of Padova *Targeting the oncogenic role of the chaperone TRAP1 in tumor cell mitochondria*
16:10-16:30	Elena Bianchini, University of Modena and Reggio Emilia *Impairment of T cell metabolism and phenotype in patients with primary progressive form of multiple sclerosis*
16:30-17:00	Coffee break
17:00-17:20	Marco Schiavone, University of Padova *A mitochondrial therapy for Duchenne muscular dystrophy*
17:20-17:40	Anna De Gaetano, University of Modena and Reggio Emilia *Phenotypic and molecular characterization of Lonp1wt/- mouse*
17:40-18:00	Roberto Costa, University of Padova *Mitochondrial ATP production is required for Wnt signaling modulation*
18:00-18:45	GIBB members annual meeting
20:30	Social Dinner Caffè Concerto, Modena

Program

Saturday 23rd

9:00-10:00	**Plenary lecture** Chair: Alessandro Giuffrè **Pia Adelroth,** Stockholm University *Organisation and regulation of respiratory chains*
10:00	**Session V: Bioenergetics metabolism and mitochondrial dysfunctions II** Chair: Nazzareno Capitanio
10:00-10:20	Karim Zuhra, University of Rome *On the regulation of human hydrogen sulfide metabolism*
10:20-10:40	Claudio Laquatra, University of Padova *Zebrafish (Danio rerio) as a model to study the pathophysiological role of the mitochondrial chaperone TRAP1*
10:40-11:00	Francesco Antonio Tucci, University of Foggia *What time is it? Rhythmic Interplay between Circadian Clock and Mitochondrial Oxidative Metabolism*
11:00-11:20	Coffee break
11:20-11:40	Vito Porcelli, University of Bari *Investigation of pathogenetic mechanisms of AGC1 deficiency in neuron cell models*
11:40-12:00	Giuseppe Cannino, University of Padova *TRAP1 regulation in cancer metabolism: identification of new i nteractors*
12:00-12:20	Ciro Leonardo Pierri, University of Bari *Adenine nucleotide transporters: apoptosis master regulators, from neglected tropical diseases to space travels, trhough mitochondrial rare diseases and cancer*
12:20-13:00	Concluding remarks and awards

On the mechanism of respiratory complex I

Respiratory complex I couples the electron transfer from NADH to ubiquinone with the translocation of protons across the membrane. In humans, its dysfunction is associated with several neurodegenerative diseases. While the mitochondrial complex is made up of more than 40 different subunits, bacteria contain a structural minimal form of the complex that is used as a simple structural model. The complex has a two-part structure with a peripheral arm catalyzing electron transfer and a membrane arm involved in proton translocation. For electron transfer, the peripheral arm contains one non-covalently bound flavin mononucleotide and, depending on the species, up to ten iron-sulfur (Fe/S) clusters as cofactors. The X-ray structure of the complex showed that the NADH oxidation-site is connected with the quinone-reduction site by a chain of seven Fe/S-clusters. Fast enzyme kinetics revealed that this chain of Fe/S-clusters is used to regulate electron-tunneling rates within the complex. A possible role of a special binuclear, off-pathway cluster N1a in preventing the formation of reactive oxygen species (ROS) is discussed.

Thorsten Friedrich

Full Professor of Biochemistry
University of Freiburg, Germany

He received his Ph.D in Chemistry from Dusselforf University (Germany) in 1992. In 1994 was research fellow at the Johnson Foundation in Philadelphia, and from 1994 to 2000 at the Heinrich-Heine University in Dusselforf. Since 2012 he is Full Professor in Biochemistry at the Albert-Ludwigs-University, in Freiburg, Germany. His main research interests are focused on the energy converting enzymes of bacterial respiratory chains, mainly on the proton pumping NADH:ubiquinone oxidoreductase, respiratory complex I, and the *bd* oxidase.

Mitochondrial metabolism in cancer: impact on metastasis and tumor progression

Despite neglected for a long time, mitochondrial metabolism is emerging as an essential element in fostering cancer progression and metastasis. Particularly, low levels of mitochondrial ROS (mtROS) promote breast cancer metastasis by inducing Src and Pyk2. Comparatively, high levels of mtROS are a common pro-apoptotic signal. Because several antitumor drugs, including anthracyclines, can trigger mtROS production, we decided to evaluate the effects of antimetastatic drugs targeting mtROS on the doxorubicin-mediated cell death of breast cancer cells. Breast cancer cell lines were treated with different combinations of mtROS scavenger and doxorubicin, and cell death was and clonogenicity were assessed. No protective effects of mtROS scavengers on doxorubicin-induced cell death were detectable, suggesting that there is no contraindication to combine antimetastatic mtROS scavengers with a normal doxorubicin chemotherapy regimen. Interestingly, we further found that, compared to untreated cells, breast cancer cells treated with doxorubicin consumed more oxygen, resulting in increased ROS generation. This metabolic reprogramming promoted Pyk2 activation and SNAIL expression, along with morphological changes typical of an epithelial-to-mesenchymal transition, increased migration, and invasion. mtROS scavengers prevented these responses and reduced metastatic colonization of the lung. Altogether, this study provides a strong rationale for combining anthracycline-based chemotherapy with drugs lowering mtROS production in breast cancer.

Paolo Ettore Porporato

Assistant Professor of Experimental Biology

University of Torino, Italy

Paolo Porporato is a tenuer-track Assistant Professor at the Department of Molecular Biotechnologies at the University of Torino. Following a PhD thesis on the role of ghrelin in preventing skeletal muscle atrophy and a postdoctoral experience at Université catholique de Louvain in defining the metabolic determinants of tumour metastasis, he is currently working on defining the impact of metabolism on the progression of cancer cachexia.

Integrating metabolism and immunity

The field of immunometabolism has thrived over the last decade, revealing not only the major roles played by immune cells in metabolic homeostasis but also the impact of metabolic pathways on immune cell function. Epidemiological evidence and recent experimental data have suggested that the prevalence of autoimmune disorders in affluent countries has reached epidemic proportion. In this context, we have hypothesized the metabolic workload can modulate immunological tolerance, acting on regulatory T cells (CD4+CD25+FoxP3+ T cells).

In Multiple Sclerosis (MS), a chronic demyelinating disease affecting the central nervous system, a correlation between the occurrence of autoimmunity and the functional impairment in Treg cells has been reported. The transcription factor FoxP3 has a key role in the development and proper function of Treg cells. Recent studies have shown the link between metabolic programs and lymphocyte activation. We recently found that proliferation of Treg cells is impaired in subjects with relapsing-remitting multiple sclerosis (RRMS) and this is associated with decreased expression of the forkhead box P3 (FoxP3) 44- and 47-kDa splicing forms and overactivation of mTOR pathway. Moreover, we identified a dominant proteomic signature at the metabolic level that primarily impacted the highly-tuned balance between glucose and fatty-acid oxidation in human Treg and Tconv cells, changing during culture conditions. We next evaluated FoxP3 expression in natural and inducible regulatory T cells (iTreg) generated in vitro from CD4+CD25- (Tconv) cells in MS patients and healthy subjects, respectively. We evaluated whether and how metabolic changes may influence the FoxP3 expression and the activity of regulatory T cells in physiological and pathological conditions. We found an impairment of iTreg cells suppressive function in MS subjects, which associated with an altered expression of specific FoxP3 splicing variants; these phenomena were secondary to a reduced glycolytic metabolism. Our results unveil a novel key molecular mechanism linking the highly tuned balance between glucose and lipid metabolisms with the induction of specific FoxP3-splicing variants, pivotal for the generation and function of Treg cells, thus accounting for the control of peripheral immune tolerance.

Claudio Procaccini

CNR Napoli
Italy

After the degree in 2004 at the University of Naples "Federico II", in 2008 Dr Procaccini obtained his PhD in Molecular Pathology and Pathophysiology, at the same University under the supervision of Prof. Giuseppe Matarese. Then he was Visiting Scientist at the David Geffen School of Medicine at University of California Los Angeles (UCLA), under the supervision of Prof. Antonio La Cava. From 2016 he is Researcher in the Institute of Experimental Endocrinology and Oncology of the National Research Council (CNR-IEOS) in Naples.
He is member of several scientific societies (AINI, SIICA, SIPMET) and he has been awarded with the Prize Space Import-Export (in 2008 and 2011), "Piernicola Boccuni Prize" (2008) and with “Ricercatamente Award”, in 2015 as the best young researcher working in CNR.

Organisation and regulation of respiratory chains

The respiratory chain in aerobic organisms is composed of a number of membrane-bound protein complexes through which electrons are transferred to finally reach oxygen. In mitochondria, Complex III (cyt. bc1) links the two-electron oxidation of quinol (QH2) to the one-electron reduction of water-soluble cyt. c., which delivers electrons to Complex IV (cytochrome c oxidase, cyt. aa3), which catalyzes the reduction of O2 to H2O. The respiratory-chain enzymes in mitochondria are to a varying degree also parts of so-called supercomplexes composed of two or more individual complexes. In Saccharomyces cerevisiae, the composition and activity of these supercomplexes are regulated by the so-called Rcf proteins (respiratory complex factor) 1 and 2, the structure and functional role of which will be discussed. There are also an increasing number of reports of supercomplexes in bacteria, where the bc1-aa3 supercomplexes from Corynebacterium glutamicum and Mycobacterium smegmatis will be discussed. In these gram-positive bacteria, there are no soluble c cytochromes, and instead, the bc1 complex harbours an additional heme c bound in the c1 subunit which presumably mediates the electron transfer between the two complexes.

Pia Adelroth

Stockholm University
Sweden

Pia received her Ph. D. in Chemistry from Göteborg University, Sweden in 1998 with a thesis entitled ‘Pathways and mechanisms for proton transfer in cytochrome *c* oxidase’. In 1999-2001, she was a post-doctoral fellow at the University of California, San Diego, working on proton transfer reactions in the bacterial photosynthetic reaction center. Since 2002, she is employed at the University of Stockholm, where she became a full professor of biochemistry in 2012. Her research interests include structure-function relationships in the diverse heme-copper oxidase superfamily, regulation of the respiratory chain in mitochondria and biological proton transfer reactions.

Electrophysiological properties of channel formed by bovine FOF1 ATP synthase in planar lipid bilayer

Andrea Urbani[1], Valentina Giorgio[1,2], Andrea Carrer[1], Chimari Jiko[3], Christoph Gerle[4], Ildikò Szabò[5], Paolo Bernardi[1,2]

[1]Dept. of Biomedical Sciences, University of Padova, Padova (Italy); [2]Consiglio Nazionale delle Ricerche, Institute of Neuroscience (Italy); [3]Kyoto University, Kyoto (Japan); [4]Laboratory of Protein Crystallography, Institute for Protein Research, Osaka University, Osaka (Japan); [5]Department of Biology, University of Padova, Padova (Italy)

Permeability transition (PT) in mitochondria is triggered by Ca^{2+} and specific activators and finally leads to increased permeability to ions and solutes of the inner mitochondrial membrane (IMM). In the 1970s the idea was advanced that PT is mediated by a Ca^{2+}-regulated pore, named the Permeability Transition Pore (PTP). By means of patch clamp on mitoplasts the PTP was identified as a high-conductance channel, named Mitochondrial Mega Channel (MMC). Despite many years of study and the key role of PTP opening in cell death and several diseases, the molecular nature of the pore remains unclear. In 2013 Giorgio et al. provided evidence that dimers of mammalian F_OF_1 ATP synthase purified from native gels can form the PTP/MMC [1] but the exact molecular mechanism is still unclear. The present work aimed at further understanding whether mammalian F_OF_1 ATP synthase generates channel in in single channel recordings in planar lipid bilayer (PLB). Highly pure F_OF_1 ATP synthase was prepared from bovine hearts using the very mild, lipid-like detergent LMNG [2]. The resulting preparation was analyzed by clear native PAGE, SDS-PAGE, mass spectrometry, and negative stain electron microscopy and was shown: (i) to be intact and inclusive of all subunits; and (ii) to be active and highly sensitive to oligomycin (>95%). After incorporation into the PLB channel activity was assessed in the presence of different concentrations of Ca^{2+} and Bz-423, a compound that is able to sensitize the PTP to Ca^{2+}. Here we provide evidence of high conductance, Ca^{2+}-dependent mammalian F_OF_1 ATP synthase channel activity resembling the MMC-PTP key features, including inhibition by PTP specific compounds.

[1] V. Giorgio et al., Dimers of mitochondrial ATP synthase form the permeability transition pore, PNAS 110(15) (2013) 5887-9

[2] S. Maeda et al., Two-dimensional crystallization of intact F-ATP synthase isolated from bovine heart mitochondria, Acta Crystallographica Section F, Structural Biology and Crystallization Communications 69 (2013) 1368–1370

Andrea Urbani

Department of Biomedical Sciences
University of Padova, Italy

Role of F-ATP synthase f subunit in dimer formation and PTP modulation

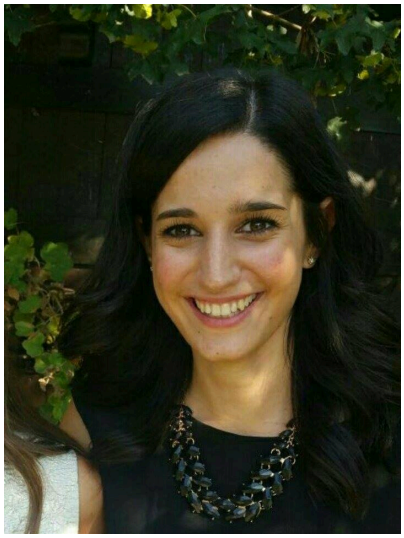
Chiara Galber[1], Giovanni Minervini[1], Giuseppe Cannino[1], Andrea Carrer[1], Silvio Tosatto[1], Giovanna Lippe[2], Valentina Giorgio[1], Paolo Bernardi[1]

[1]Dept. of Biomedical Sciences and CNR Neurosceince Institute, University of Padova, Padova (Italy); [2]Dept. of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Udine (Italy)

The mitochondrial F-ATP synthase is a large multisubunit complex of 600 kDa organized into a catalytic part (F_1) and a membranous moiety (Fo) linked by central and peripheral stalks. Recently, it has been demonstrated that purified dimers of F-ATP synthase added to a lipid bilayer form channels matching features of the permeability transition pore (PTP) [1]. The PTP is a mitochondrial mega-channel which induces cell death through the sudden membrane permeabilization to solutes and collapse of membrane potential. Although many candidates have been proposed as components of the PTP, there is now the evidence that the F-ATP synthase is its major constituent [2], even if the subunits directly involved in PTP formation and regulation remain undefined. We have proposed that channel formation takes place in F-ATP synthase dimers at the interface between two monomers. Structural data of the enzyme suggest an important role of f subunit in dimer stabilization in *Yarrowia lipolytica* and *Saccharomyces cerevisiae*. Based on bioinformatics analysis we have focused on f subunit to characterize its role as a possible pore-forming site. Electrophysiological studies are under investigation. We have used the CRISRP-Cas9 and the shRNA interference technologies to generate human cells lacking or with a decreased level of subunit f, respectively. We will report our progress at characterizing cell viability, mitochondrial morphology, F-ATP synthase catalysis and PTP features in these models.

1. V. Giorgio, S. von Stockum, M. Antoniel, A. Fabbro, F. Fogolari, M. Forte, G.D. Glick, V. Petronilli, M. Zoratti, I. Szabó, G. Lippe, P. Bernardi, Dimers of mitochondrial ATP synthase form the permeability transition pore, Proc Natl Acad Sci USA, 110 (2013) 5887-92

2. P. Bernardi, A. Rasola, M. Forte, G. Lippe, The Mitochondrial Permeability Transition Pore: Channel Formation by F-ATP Synthase, Integration in Signal Transduction, and Role in Pathophysiology, Physiol. Rev. 95 (2015) 1111-1155.



Chiara Galber

Department of Biomedical Sciences and CNR Neurosceince Institute
University of Padova, Italy

Dissecting the effects of MT-ND1 and NDUFS3 suppression on respiratory Complex I assembly and supercomplex biogenesis

Elisa Astro[1], Luisa Iommarini[1], Erika Fernandez-Vizarra[2], Ivana Kurelac[3], Laura B. Amato[3], Giuseppe Gasparre[3], Massimo Zeviani[2]*, Anna M. Porcelli[1]*

[1]Dept. of Pharmacy and Biotechnology (FABIT), University of Bologna, Bologna (Italy); [2]Mitochondrial Biology Unit (MBU), Medical Research Council, Cambridge (UK); [3]Dept. of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna (Italy); *Co-last author

Complex I (CI) or NADH-ubiquinone oxidoreductase is the largest multiprotein complex of the OXPHOS system, composed of 45 subunits, encoded by either nuclear or mitochondrial DNA [1]. CI can associate with CIII and CIV, forming “supercomplexes” (SCs), but the assembly pathways of either CI or SCs are still controversial [2]. In this study we investigated CI and SCs biogenesis in cell models lacking two CI structural subunits, namely the mtDNA-encoded ND1 and the nDNA-encoded NDUFS3. A residual amount of functional CI was found within SCs in both models and it interacted preferentially with pre-$CIII_2$ (missing the catalytic Rieske Fe-S subunit) rather than the fully-assembled $CIII_2$, suggesting that CI and $CIII_2$ interact with each other before their assembly is completed. The absence of NDUFS3 did not compromise neither $CIII_2$ nor CIV steady state levels, whereas a reduction of the assembly/stability of these two respiratory complexes was observed in the lack of full-length ND1. Moreover, a quantitative differential proteomic approach allowed us to identify several putative CI interactors, with a possible role as assembly factors in the early stages of CI biogenesis. Since most of the identified proteins are involved in metabolic pathways, we hypothesize a correlation between such pathways and CI biogenesis.

[1] J. Hirst, Mitochondrial Complex I, Annu. Rev. Biochem. 82 (2013) 551–575.

[2] T. Lobo-Jarne, C. Ugalde, Respiratory chain supercomplexes: Structures, function and biogenesis, Semin. Cell Dev. Biol. 76 (2018) 179–190.

Elisa Astro

Department of Pharmacy and Biotechnology (FABIT)
University of Bologna, Italy

Respiratory complex I ablation in aggressive cancers induces oncocytic indolence but triggers macrophage-mediated adaptive response

Luisa Iommarini[1], Ivana Kurelac[2], Renaud Vatrinet[1,2], Laura B. Amato[2], Lara Gibellini[3], Andrea Cossarizza[3], Manuela Sollazzo[1], Daniela Grifoni[1], Giuseppe Gasparre[2], Anna Maria Porcelli[1]

[1]Dept. of Pharmacy and Biotechnology (FABIT), University of Bologna, Bologna (Italy); [2]Dept. of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna (Italy); [3]Dep of Medical and Surgical Sciences for Children and Adults, University of Modena and Reggio Emilia, Modena (Italy)

Converting aggressive cancers in benign oncocytomas has been suggested as a potential anti-cancer strategy. Since lack of respiratory complex I (CI) is a hallmark of oncocytomas, we successfully use genetic ablation of this enzyme to induce indolence in two types of aggressive cancers, and show this is reversed by allowing the stabilization of Hypoxia Inducible Factor 1 alpha (HIF1a). We further show that on the long run CI-deficient tumors re-adapt to their inability to respond to hypoxia, concordantly with the persistence of human oncocytomas. Due to the low toxicity of metformin and its potential use in a wide-spectrum of human solid neoplasms, it is of great interest to identify adaptive mechanisms activated upon CI inhibition to design combinatorial therapies. We indeed demonstrate that CI-deficient tumors survive by triggering non cell-autonomous mechanism of angiogenesis, independent of HIF1a. Such adaptive response is mediated by tumor associated macrophages (TAMs), whose blockage by the sue of clodronate improves the effect of CI targeting. Based on these results, we propose CI inhibitors in cancer therapy should be used in combination with TAM targeting agents to guarantee a synergistic effect.

Luisa Iommarini

Department of Pharmacy and Biotechnology (FABIT)
University of Bologna, Italy

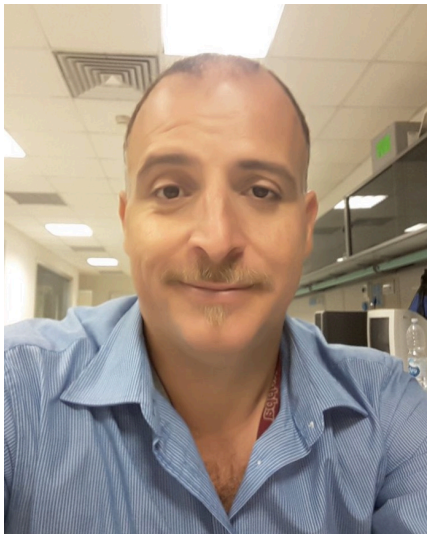
Functional and expression study identifying plasma membrane ecto-ATP synthase as a novel player in the early phase of Liver regeneration

Federica Taurino[1], Caterina Giannoccaro[1], Salvatore Scacco[1], Anna Maria Sardanelli[1], Alessandro Cavallo[2], Elisa De Luca[3], Salvatore Santacroce[4], Sergio Papa[5], Franco Zanotti[1], Antonio Gnoni[1]

[1]Dept. of 1Dept. of Basic Medical Sciences, Neurosciences and Sensor Organs, University of Bari "Aldo Moro", Bari (Italy); [2]Dept. of Biological and Environmental Sciences and Technologies, University of Salento, Lecce (Italy); [3]Italian Institute of Technology, Lecce (Italy); [4]Department of Emergency and Organ Transplants, University of Bari "Aldo Moro", Bari (Italy); [5]Inst. of Biomembranes and Bioenergetics, National Research Council, Bari (Italy)

ATP synthase, canonically mitochondrially located, is reported to be ectopically expressed on the plasma membrane outer face of several cell types [1]. We analysed, for the first time, the expression and catalytic activities of the ecto- and mitochondrial ATP synthase during liver regeneration. Liver regeneration was induced in rats by two-thirds partial hepatectomy. The protein level and the ATP synthase and/or hydrolase activities of the hepatocyte ecto- and mitochondrial ATP synthase were analysed on freshly isolated hepatocytes and mitochondria from control, sham-operated and partial hepatectomized rats. During the priming phase of liver regeneration, 3 h after partial hepatectomy, liver mitochondria showed a marked lowering of the ATP synthase protein level that was reflected in the impairment of both ATP synthesis and hydrolysis. The ecto-ATP synthase level, in 3 h partial hepatectomized hepatocytes, was decreased similarly to the level of the mitochondrial ATP synthase, associated with a lowering of the ecto-ATP hydrolase activity coupled to proton influx. Noteworthy, the ecto-ATP synthase activity coupled to proton efflux was completely inhibited in 3 h partial hepatectomized hepatocytes, even in the presence of a marked intracellular acidification that would sustain it as in control and sham-operated hepatocytes. At the end of the liver regeneration, 7 days after partial hepatectomy, the level and the catalytic activities of the ecto- and mitochondrial ATP synthase reached the control and values. The specific modulation of hepatocyte ecto-ATP synthase catalytic activities during liver regeneration priming phase may modulate the extracellular ADP/ATP levels and/or proton influx/efflux trafficking, making hepatocyte ecto-ATP synthase a candidate for a novel player in the liver regeneration process.

R. Mangiullo, et al., Structural and functional characterization of F(o)F(1)-ATP synthase on the extracellular surface of rat hepatocytes. BBA1777(2008) 1326-35.



Antonio Gnoni

Department of Basic Medical Sciences, Neurosciences and Sensor Organs
University of Bari, Italy

The ABCC6 expression is regulated by adenosine and ATP externally added to the HepG2 cells

Fabio Martinelli[1], Maria C. Pace[1], Angela Ostuni[1], Faustino Bisaccia[1]

[1]Dept. of Sciences, University of Basilicata, Potenza (Italy)

The ABCC6 protein is an ATP dependent transporter mainly found in the basolateral plasma membrane of hepatic and kidney cells. Mutations in ABCC6 gene were associated to the Pseudoxanthoma elasticum (PXE), a disease characterized by the mineralization of connective tissues [1]. Has been reported that the over-expression of ABCC6 in HEK293 cells results in the cellular efflux of ATP and other nucleoside triphosphates [2]; in the extracellular space, ATP is converted by an ecto-nucleosidase in PPi (inhibitor of mineralization) and AMP, the latter is in turn transformed into adenosine and phosphate, then, ABCC6 is involved in the production of adenosine. Our previous studies showed that in ABCC6 knockdown HepG2 cells the expression of some genes, related with the calcification processes, is dysregulated [3]. In this study, experiments have been designed in order to verify if ABCC6, besides supplying the pyrophosphate necessary to prevent the mineralization of soft tissues, also plays a role in the purinergic system activation. In the liver, purinergic signaling has been shown to regulate key cellular functions. For this purpose, the ABCC6 transport activity was inhibited with Probenecid, a drug used for gout, and the expression of ABCC6 and NT5E was analyzed with real-time PCR and western blotting. The results of this study showed that both proteins are downregulated in the presence of Probenecid and upregulated in the presence of adenosine or ATP.

1. K. Moitra et al., ABCC6 and Pseudoxanthoma Elasticum: the face of a rare disease from genetics to advocacy, Int. J. Mol. Sci. (2017), 18(7):1488.

2. R.S. Jansen et al., ABCC6-mediated ATP secretion by the liver is the main source of the mineralization inhibitor inorganic pyrophosphate in the systemic circulation-brief report, Arterioscler. Thromb. Vasc.Biol. (2014), 34(9):1985-9.

3. R. Miglionico et al., Dysregulation of gene expression in ABCC6 knockdown HepG2 cells, Cell. Mol. Biol. Lett. (2014), 19(4):517-526.

Fabio Martinelli

Department of Sciences
University of Basilicata, Italy

The effects of PTP inhibition in Duchenne muscular dystrophy

Anna Stocco[1], Marco Schiavone[1], Alessandra Zulian[1], Justina Sileikyte[2], Michael Forte[2], Paolo Bernardi[1]

[1]Dept. of Biomedical Sciences and CNR Neuroscience Institute, University of Padova, Padova (Italy); [2]Vollum Institute, Oregon Health and Science University, Portland, OR (USA)

Duchenne muscular dystrophy (DMD) is a severe X-linked muscle disease caused by mutations in the gene encoding for dystrophin. Lack of dystrophin leads to muscle weakness, low resistance to stress, impairment of muscle regeneration and eventually fiber demise. The main trigger of these alterations is probably the increase of Ca^{2+} levels in the sarcoplasma of the affected fibers. Increased Ca^{2+} levels in DMD muscle fibers lead to mitochondrial dysfunction, which contributes to the vicious cycle of Ca^{2+} deregulation that eventually triggers fiber death [1]. One of the potential mechanisms involved in DMD pathogenesis is opening of the mitochondrial permeability transition pore (PTP), a high-conductance channel located in the inner membrane, whose activity is positively regulated by cyclophilin (CyP) D and high matrix Ca^{2+} levels. The PTP is desensitized by cyclosporin A and its non-immunosuppressive derivatives NIM811 and Alisporivir, which can inhibit CyPD binding. Alisporivir allowed recovery of respiration, rescue of muscle ultrastructure and improvement of survival in the *sapje* zebrafish model of DMD [2]. However, Alisporivir is not specific for CyPD and thus inhibits all cyclophilins. To further identify specific PTP inhibitors, a high-throughput screening has been performed, which led to identification of novel compounds that do not target CyPD [3]. Following optimization, compound MF1 showed promising results *in vivo* using the *sapje* zebrafish model, such as recovery of muscle structure and motor impairments. Given the results with Alisporivir and MF1, a combined treatment could be done. Since Alisporivir inhibits all CyPs, is also reasonable to observe if this inhibition causes side effects. So, we plan to obtain a genetic knockdown of the CyPA, CyPB and CyPD isoforms both in the wild type and the *sapje* zebrafish model. As preliminary data, in the wild type zebrafish, by CyPB knockdown, some embryos exhibit developmental delay and phenotypic abnormalities.

[1] K. Wrogemann et al. Lancet (1976) 672–674.
[2] M. Schiavone et al. Pharmacological Research 125 (2017) 122–131.
[3] J. Šileikytė et al. Biochim Biophys Acta 1857(8) 1197–1202

Anna Stocco

Department of Biomedical Sciences and CNR Neuroscience Institute
University of Padova, Italy

Role of MICS1, interaction partner of LETM1, in mitochondrial calcium homeostasis

Christina Pfeiffer[1], Shane Austin[1], Maria Francesca Scalise[2], Cesare Indiveri[2], Karin Nowikovsky[1]

[1]Dept. of Internal Medicine I and Comprehensive Cancer Center, Medical University of Vienna, Vienna (Austria); [2]Unit of Biochemistry & Molecular Biotechnology, Università della Calabria, Italy

The vital role of LETM1 in maintaining the mitochondrial osmotic homeostasis by regulating K+-H+ exchange was challenged by a proposed function as a mitochondrial Ca2+-H+ exchanger. In order to clarify the debated function of this single span membrane protein, we performed a proteomic - based screening for interaction partners with a potential role in mitochondrial K+ or Ca2+ exchange against H+. We have identified several potential novel LETM1 interaction partners. Validation assays confirmed the oligomerisation of LETM1 with a novel mitochondrial protein belonging to the highly conserved protein family TMBIM with regulatory functions in intracellular Ca2+ homeostasis. We are presenting data on TMBIM5, also known as MICS1, and its role in the control of transmitochondrial Ca2+ exchange.

Christina Pfeiffer

Department of Internal Medicine I and Comprehensive Cancer Center
Medical University of Vienna,
Austria

Channel activity of yeast VDAC2, the second mitochondrial porin isoform of Saccharomyces cerevisiae

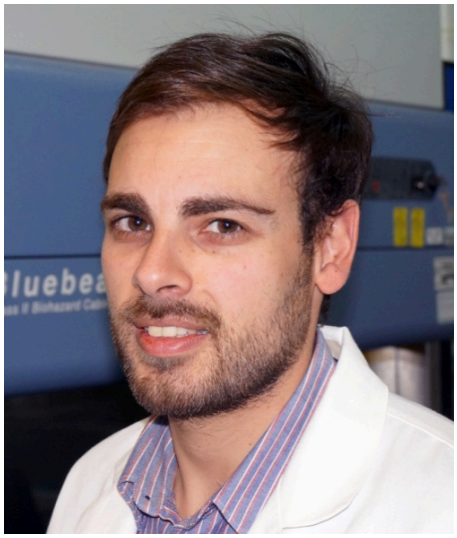
Andrea Magrì[1,2], Maria Carmela Di Rosa[1], Stefano Conti Nibali[2], Simona Reina[1,2], Vito De Pinto[1]

[1]Dept. Of Biomedical and Biotechnological Sciences, University of Catania, Catania (Italy); [2]Department of Biological, Geological and Environmental Sciences, Section of Molecular Biology, University of Catania, Catania (Italy)

The permeability of Mitochondrial Outer Membrane in all eukaryotes is conferred by the presence of Voltage-Dependent Anion Channel (VDAC) proteins, a conserved group of β-barrel porins allowing mainly ATP/ADP and ions exchange between cytosol and mitochondria. The budding yeast Saccharomyces cerevisiae has two genes encoding for two distinct VDAC proteins (yVDAC). The yVDAC1 is the main porin and is particular important during respiration: cells lacking VDAC1 (Δpor1), indeed, show a strong impairment of yeast growth in non-fermentable condition. The yVDAC2 is less known and one of the most elusive member of VDAC family. Since its discovery, yVDAC2 function was controversial: if on one side the lacking of yVDAC2 has no consequence for the yeast growth, questioning the channel formation, on the other side the overexpression of yVDAC2 restores yeast growth in Δpor1 cells [1], supporting the channel activity. In order to definitely understand yVDAC2 function, the corresponding encoding sequence was overexpressed in Δpor1 cells and the protein was purified from mitochondria under native conditions. Reconstitution experiment, performed at the Planar Lipid Bilayer system, have clearly shown that yVDAC2 forms voltage-dependence channels with a conductance of about 3.6 nS in 1M KCl [2]. Moreover, the application of a KCl gradient between two sides of the membrane resulted in shifts in the current/voltage curve slope, allowed for identification of up to three different states with different calculated parameters of ionic selectivity for yVDAC2 [2]. Interestingly, two of them appear to be high-conductance states but with opposite selectivity. In conclusion, our results give the definitive message that yVDAC2 is another member of the VDAC family.

1. A. Magrì, M.C. Di Rosa, et al. BBA Bioenergetics 1857 (2016) 789-798.

2. C. Guardiani, A. Magrì, A. et al. BBA Bioenergetic 1959 (2018) 270–279.



Andrea Magrì

Department of Biomedical and Biotechnological Sciences
University of Catania, Italy

Identification and functional characterization of an exosomal cargo protein: the plasma membrane carnitine transporter (OCTN2)

L. Console[1], M. Scalise[1], A. Tonazzi[2], N. Giangregorio[2], C. Indiveri[1].

[1]Dept. DiBEST (Biologia, Ecologia, Scienze della Terra) Unit of Biochemistry and Molecular Biotechnology, University of Calabria (Italy); [2]IBIOM Institute of Biomembrane, Bioenergetics and Molecular Biotechnologies, Consiglio Nazionale Delle Ricerche (CNR), Bari (Italy)

Several studies showed that eukaryotic cells release small vesicles, among which exosomes. Initially, they were considered to be platelet “dust” or cellular debris but it is becoming clear that exosomes play important roles in cell-to-cell communication and are involved in the immune response, angiogenesis, inflammation, cell death and cancer progression. Several recent studies focus on characterization of cargos of exosomes derived from body fluids as well as from medium of cell cultures. Large-scale proteomic analyses show the presence of peptides deriving from several solute transporters (SLCs), which are fundamental for cell physiology and are linked to many human pathologies. However, no or few data on the function of exosomal SLCs is available, so far. We have revealed the presence in exosomes of a transporter which has a crucial role in cell homeostasis: SLC22A5 also known as OCTN2 (Organic Cation Transporter Novel 2). It is a sodium dependent carnitine transporter and plays a crucial role in the cellular uptake of L-carnitine which is an essential co-factor for the mitochondrial beta oxidation pathway. OCTN2 was detected by Western Blot analysis in both HEK293 and urinary derived exosomes. To investigate the functional properties of the exosomal OCTN2, proteins extracted from vesicles were reconstituted in proteolipsomes and the transport function was measured as uptake of 3H-carnitine. Data showed that exosomal OCTN2 is fully functional. Furthermore, the pro-inflammatory cytokine, INFγ, increases the level of OCTN2 in exosomes. The data suggest that OCTN2 maintains its native functional properties also in exosomes and that its presence in this exocellular location may be related to inflammatory states. The fact that OCTN2 is present in urinary exosomes might represent the starting point to discover a novel, non-invasive candidate biomarker for inflammation related pathologies.

Lara Console

DiBEST (Biologia, Ecologia, Scienze della Terra) Unit of Biochemistry and Molecular Biotechnology
University of Calabria

The multidrug resistance inhibitor 8-(4-chlorophenyl)-5-methyl-1 8-[(2Z)-pent-2-en-1-2 yloxy]-8H-[1,2,4]oxadiazolo[3,4-c][1,4]thiazin-3-one diltiazem-like compound inhibits the ABCC6 transporter

A. Ostuni[1], F. Cuviello[1], D. Bisaccia[1], B. Cosimelli[2], D. Spinelli[3], F. Bisaccia[1]

[1]Dept. of Sciences, University of Basilicata, Potenza (Italy); [2]Dept. of Pharmacy, University of Naples Federico II, Napolii(Italy); [3]Dept. of Chemistry "G. Ciamician", Alma Mater Studiorum University of Bologna, Bologna (Italy)

Proteins mostly involved in multidrug resistance (MDR), one of the major causes of cancer treatment failure, are members of the ATP-binding cassette (ABC) transporters superfamily [1]. With the aim to reduce MDR, several molecules were synthesized and tested for their ability to inhibit ATP-binding cassette transporters activity and between them the 8-(4-chlorophenyl)-5-methyl-8-[(2Z)-pent-2-en-1-yloxy]-8H-[1,2,4]oxadiazolo[3,4-c][1,4]thiazin-3-one namely 2c, related to the myocardial-calcium-channel-modulator diltiazem [2]. Our study showed that 2c compound, at the concentration suggested to prevent drug resistance, inhibits the activity of ABCC6 transporter, a member of the ABCC subfamily associated to low resistance to some chemotherapeutic agents [3]. As 2c also inhibits esterase activity and histone acetylation we believe that these biological activities must be taken into account as possible side effects.

1. Zhang YK et al. Multidrug Resistance Proteins (MRPs) and Cancer Therapy. The AAPS Journal (2015) 17: 802-812.

2. Rosano C et al. ABCB1 structural models, molecular docking, and synthesis of new oxadiazolothiazin-3-one inhibitors. ACS Med Chem Lett. (2013), 4: 694–698.

3. Belinsky MG, et al. Characterization 302 of the drug resistance and transport properties of multidrug resistance protein 6 (MRP6, ABCC6). Cancer Res 2002;304 62:6172–6177.

Faustino Bisaccia

Department of Sciences
University of Basilicata, Italy

Characterization of the CAT2 transporter from Solanum lycopersicum (Tomato).

J. Cosco[1], T. Regina[1], M. Scalise[1], M. Galluccio[1], C. Indiveri[1].

[1]Dept. DiBEST (Biologia, Ecologia, Scienze della Terra) Unit of Biochemistry and Molecular Biotechnology, University of Calabria (Italy)

The vacuole is the largest organelle in plant cells and it is essential for life. Although the vacuole has always been associated with turgor regulation and storage of water and metabolites, it is now clear that this organelle is also involved in pH homeostasis, cell energetics and signal transduction. Among metabolites exchanged in vacuoles, amino acids are particularly represented since they play both metabolic and signaling functions. In particular Arginine is crucial for plant cells, being a precursor for nitric oxide and polyamine. We have very recently expressed the CAT2 transporter from *Solanum lycopersicum* in E.coli. The recombinant protein has been reconstituted in the artificial membrane of proteoliposomes and the transporter has been functionally characterized. The ability for CAT2 to transport cationic amino acids has been demonstrated measuring the uptake of several radiolabeled amino acids in proteoliposomes. Arginine, Lysine and the non-proteogenic amino acid Ornithine are the most efficiently transported. Arginine transport is stimulated by the presence of cholesterol in the membrane. Since the physiological pH of vacuoles is acidic inside, the transport activity has been recorded as a function of the pH gradient. Optimal transport was observed in the presence of intraliposomal pH 5.5 and external pH 8.0-8.5. The possible mechanism of pH control has been predicted on the basis of the homology model of CAT2 obtained using the 3D structure of the ApcT transporter from *Geobacillus Kaustophilus* as a template. Glu 142 has been hypothesized of being involved in proton binding. Since the existence of a non-neuronal cholinergic system has also been ascertained in plants, the possible role of CAT2 in Acetylcholine transport in the vacuoles has been investigated. Acetylcholine transport mediated by CAT2 has been revealed. This data suggests that CAT2 can promote the Acetylcholine storage into the vacuole.

Jessica Cosco

DiBEST (Biologia, Ecologia, Scienze della Terra) Unit of Biochemistry and Molecular Biotechnology
University of Calabria

Targeting the oncogenic role of the chaperone TRAP1 in tumor cell mitochondria

Carlos Sanchez-Martin[1], Mariarosaria Ferraro[2], Elisabetta Moroni[3], Ionica Masgras[1], Marina Serra[4], Amedeo Columbano[4], Giorgio Colombo[2], Andrea Rasola[1]

[1]Dept. of Biomedical Sciences, University of Padova, Padova (Italy); I[2]stituto di Chimica del Riconoscimento Molecolare, Milano (Italy); [3]IRCCS Multimedica, Milano (Italy); [4]Dept. of Biomedical Sciences, University of Cagliari, Cagliari (Italy)

We have previously shown that the mitochondrial chaperone TRAP1, a conserved member of the Hsp90 family, is involved in the metabolic rewiring of tumor cells [1] by down-regulating the oxidative phosphorylation through inhibition of succinate dehydrogenase (SDH), the complex II of the respiratory chain. In this way, TRAP1 prompts tumor growth through a succinate-dependent stabilization of the transcription factor HIF1α [2]. Moreover, SDH inhibition has an antioxidant effect that shields neoplastic cells from oxidative insults [3]. We have also found that TRAP1 is involved in a mitochondrial signaling cascade following deregulated activation of the Ras/ERK kinase pathway. In mitochondria, active ERK1/2 phosphorylates TRAP1 and this enhances TRAP1-dependent inhibition of SDH activity and contributes to neoplastic growth [4]. These findings suggest that TRAP1 could be a good target to develop innovative therapeutic strategies for cancer treatment. In this context, we have exploited computational approaches based on the characterization of TRAP1 internal dynamics in order to identify potential regions that can be targeted by selective TRAP1 inhibitors in an allosteric way. Based on this approach, we have tested a set of putative TRAP1 allosteric inhibitors, finding that all these molecules inhibit TRAP1 activity in a highly selective way. At a cellular level, these lead compounds completely reverse the inhibition of the SDH activity exerted by TRAP1 in both murine and human malignant peripheral nerve sheath tumors, while TRAP1 knockout cells are completely insensitive to the effects of these molecules. Our studies provide evidence that novel computational approaches can be used to design highly selective TRAP1 inhibitors. These molecules are useful tools for the comprehension of the role played by TRAP1 in the regulation of signaling and metabolic pathways in tumor cell mitochondria and can be further developed in order to find novel antineoplastic strategies.

1. I. Masgras et al. Frontiers in Oncology 7 (2017) 58.
2. M. Sciacovelli et al. Cell Metabolism 17 (2013) 988-99.
3. G. Guzzo et al. Oncotarget 5 (2014) 11897-908.
4. I. Masgras et al. Cell Reports 18 (2017) 659-672.

Carlos Sanchez-Martin

Department of Biomedical Sciences
University of Padova, Italy

Impairment of T cell metabolism and phenotype in patients with primary progressive form of multiple sclerosis

Elena Bianchini[1], Sara De Biasi[2], Milena Nasi[1], Lara Gibellini[3], Simone Pecorini[4], Domenico Lo Tartaro[3], Anna De Gaetano[2], Anna Maria Simone[5], Diana Ferraro[5], Francesca Vitetta[5], Patrizia Sola[5], Andrea Cossarizza[3], Marcello Pinti[2]

[1]Dept. of Surgery, Medicine, Dentistry and Morphological Sciences, University of Modena and Reggio Emilia, Modena (Italy); [2]Dept. of Life Sciences, University of Modena and Reggio Emilia, Modena (Italy); [3]Dept. of Medical and Surgical Sciences, University of Modena and Reggio Emilia, Modena (Italy); [4]Dept. of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena (Italy).; [5]Neurology Unit, Dept. of Biomedical, Metabolic and Neural Sciences, Nuovo Ospedale Civile Sant'Agostino Estense, University of Modena and Reggio Emilia, Modena (Italy)

Cellular metabolism and its dynamic reprogramming are essential for T cell function. Inappropriate remodeling underlies aberrant immune responses and T cells from autoimmune models exhibit altered metabolic profiles. Multiple sclerosis is an immune-mediated disease triggered by autoreactive T cells. We focused on its progressive forms, investigating the metabolic, mitochondrial (mt) and phenotypic features of T cells, to understand if there were peculiarities that could account for the heterogeneous clinical courses. We enrolled 50 patients, 29 with secondary progressive (SP) and 21 with primary progressive (PP) form, and 20 controls (CTR). First, we evaluated T cell metabolism and mt function. Basal and maximal respiration of CD4+ naïve T (TN) cells from SP patients were higher than PP patients and CTR. Mitochondria of PP patients had lower mass and membrane potential than those from the other groups. Second, we analyzed the expression of key metabolic transcription factors, and the activation of mTOR by GLUT1 expression and pS6 phosphorylation. PP patients had an upregulation of glycolysis-activating genes and a downregulation of quiescence-promoting genes, an upregulation of GLUT1 in CD4+ T cells, and increased CD4+ and CD8+ effector memory T (TEM) cells with phospho-pS6. Finally, to investigate if these metabolic differences reflect modifications in T cell phenotype, we characterized activation, differentiation and proliferation. PP patients had higher levels of CD4+ TEM cells and CD8+ effector memory T cells re-expressing CD45RA, and lower levels of CD8+ TN cells than SP patients. All CD4+ T cell subsets and CD8+ TN cells from PP patients had low proliferative potential. Thus, a dysregulation of T cell metabolism exists in PP patients, who appear more prone to activate glycolysis; this could favour a more rapid shift toward effector cells, and a faster disease progression

Elena Bianchini

Department of Surgery, Medicine, Dentistry and Morphological Sciences
University of Modena and Reggio Emilia, Italy

A mitochondrial therapy for Duchenne muscular dystrophy

Marco Schiavone[1], Alessandra Zulian[1], Anna Stocco[1], Valeria Petronilli[1], Justina Sileikyte[2], Michael Forte[2], Francesco Argenton[3], Luciano Merlini[4], Patrizia Sabatelli[5], Paolo Bernardi[1]

[1]Dept. of Biomedical Sciences and CNR Neuroscience Institute, University of Padova, Padova (Italy); [2]Vollum Institute, Oregon Health and Science University, Portland, OR (USA); [3]Dept. of Biology, University of Padova, Padova (Italy); [4]Dept. of Biomedical and Neuromotor Sciences, University of Bologna, Bologna (Italy); [5]CNR Institute of Molecular Genetics and Laboratory of Musculoskeletal Cell Biology, Istituto Ortopedico Rizzoli-IRCCS, Bologna (Italy)

Duchenne muscular dystrophy (DMD) is a life-threatening X-linked muscle disease caused by mutations in the dystrophin gene. It is characterized by progressive degeneration of muscle fibers and an effective, or generally applicable therapy is lacking. Studies in animal models (hamster, chicken and mouse) demonstrated the presence of mitochondrial dysfunction during the disease pathogenesis. Opening of the mitochondrial Permeability Transition Pore (PTP, a mitochondrial high-conductance channel), due to an increase of calcium concentration in both sarcoplasm and mitochondria, was observed to be one of the main mechanisms involved in DMD pathogenesis [1]. Since the *mdx*$^{-/-}$ mouse (the best-characterized mouse model of the disease) displays a rather mild dystrophic phenotype, we took advantage of the severe *sapje* zebrafish mutant, which lacks dystrophin and shows ultrastructural muscle defects close to those of DMD patients [2]. Our aim is to explore *in vivo* a possible mitochondrial therapy targeting the PTP with non-immunosuppressive derivatives of cyclosporine A and new PTP inhibitors developed by our research group. As previously observed in muscle biopsies from DMD patients, we show that *sapje* zebrafish (i) display a dramatic disruption of muscle structure, (ii) a strong decrease of respiratory reserve capacity and (iii) are prone to mitochondrial dysfunction due to opening of the PTP, whose features in zebrafish are the same as those of mammals. Treatment with the FDA-approved cyclophilin inhibitor Alisporivir - a cyclosporin A derivative that desensitizes the PTP but does not inhibit calcineurin - and the new PTP inhibitor MF1 led to a striking recovery of muscle structure, motor impairments and respiratory function [3]. Improvement of sapje zebrafish survival was observed as well. As Alisporivir has an excellent safety profile, it could be used in combination with MF1 for treatment of DMD.

1. J. Reutenauer et al. British J Pharmacol 155 (2008) 574-584

2. D. Bassett et al. Clinical Experimental Pharmacology and Physiology 31(2004) 537-540

3. M. Schiavone et al. Pharmacological Research 125 (2017) 122-131

Marco Schiavone

Department of Biomedical Sciences and CNR Neuroscience Institute
University of Padova, Italy

Phenotypic and molecular characterization of Lonp1$^{wt/-}$ mouse.

Anna De Gaetano[1]; Lara Gibellini[2]; Elena Bianchini[3]; Sara De Biasi[1]; Simone Pecorini[4]; Domenico Lo Tartaro[2]; Anita Neroni[2]; Beatrice Lusenti[2]; Milena Nasi[3]; Andrea Cossarizza[2]; Marcello Pinti[1]

[1]Dept. of Life Sciences, University of Modena and Reggio Emilia, Modena (Italy); [2]Dept. of Medical and Surgical Sciences for Children and Adults, University of Modena and Reggio Emilia, Modena (Italy); [3]Dept. of Surgery, Medicine, Dentistry and Morphological Sciences, University of Modena and Reggio Emilia, Modena (Italy); [3]Dept. of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena (Italy)

LONP1 protease is a nuclear genome encoded protein located in the mitochondrial matrix belonging to the ATPase associated with diverse cellular activities (AAA+) superfamily. Its main function is to degrade misfolded, oxidized and damaged proteins but also it actively participates in mitochondrial function and mtDNA maintenance and functionality. Mutations in *LONP1* gene are linked to CODAS (cerebral, ocular, dental, auricular, and skeletal anomalies) syndrome, a rare recessive disorder. *In vitro*, in colon carcinoma cells, LONP1 downregulation affects mitochondrial proteome including proteins involved in stress response, ribosome assembly, mitochondrial and mtDNA architecture and energetic metabolism. These modifications deeply impair cellular respiration and mitochondrial functionality and morphology. To evaluate the effects of LONP1 downregulation *in vivo*, we generated and characterized a heterozygous *Lonp1*$^{wt/-}$ mouse model. *Lonp1*$^{wt/-}$ ♂ x *Lonp1*$^{wt/-}$♀ mating produces no *Lonp1*$^{-/-}$ mice, confirming the embryonic lethality of *Lonp1* inactivation in mouse, previously shown in another mouse model. *Lonp1*$^{wt/-}$ reveals no altered phenotype or development, and morphometric study of organs and bones shows no impairment. Quantification of multi-tissue expression of proteins related to mitochondrial functionality and of mtDNA levels, histological and fertility analysis reveal no significant difference between heterozygous and wild-type mouse. *Lonp1*$^{wt/-}$ express the same amount of LONP1 protein compared with *Lonp1*$^{wt/wt}$, suggesting an unknown mechanism of expression compensation by the intact allele. Comparing the number of offspring, a significant difference is found between *Lonp1*$^{wt/wt}$ ♂ x *Lonp1*$^{wt/wt}$ ♀ and *Lonp1*$^{wt/wt}$ ♂ x *Lonp1*$^{wt/-}$ ♀ mating. Further studies regarding mitochondrial respiration and Oxidative Phosphorylation on Mouse Embryonic Fibroblasts derived from *Lonp1*$^{wt/-}$ mice are ongoing.

Anna De Gaetano

Department of LifeSciences
University of Modena and Reggio Emilia, Italy

Mitochondrial ATP production is required for Wnt signaling modulation

Roberto Costa[1], Roberta Peruzzo[1], Magdalena Bachmann[1], Giulia Santinon[2], Mattia Vicario[3], Giulia Dalla Montà[1], Andrea Mattarei[4], Ruben Quintana Cabrera[1], Enrico Moro[2], Luca Scorrano[1], Massimo Zeviani[5], Mario Zoratti[3,6], Cristina Paradisi[7], Francesco Argenton[1], Marisa Brini[1], Tito Calì[3], Sirio Dupont[2], Ildikò Szabò[1,6], Luigi Leanza[1].

[1]Dept. Biology, University of Padova, Italy; [2]Dept. Molecular Medicine, University of Padova, Italy; [3]Dept. of Biomedical Sciences, University of Padova, Italy; [4]Dept. of Pharmaceutical and Pharmacological Sciences, University of Padova, Italy; [5]MRC Mitochondrial Biology Unit, Cambridge, UK; [6]CNR Institute of Neuroscience, Padova, Italy; [7]Dept. of Chemical Sciences, University of Padova, Italy.

Mitochondria are central organelles for cellular metabolism and are involved controlling both cell proliferation and death. The way(s) how mitochondrial function/dysfunction affects downstream signaling pathways in the context of proliferation is still poorly defined. While Wnt signalling, crucial for cellular proliferation and differentiation and often upregulated in cancer, is known to influence mitochondrial function, the possibility that mitochondrial function affects Wnt signaling has not been explored so far. We show that sub-lethal concentrations of different pharmacological compounds all able to decrease mitochondrial ATP production down-regulate canonical Wnt signaling in different cancer cell lines. The same pharmacological treatments led to reduced Wnt signaling *in vivo*, in *Danio rerio* (zebrafish) reporter lines, while leaving other important signaling pathways such as Sonic hedgehog (Shh) unaffected, indicating specificity of the mitochondria-Wnt signaling axis. In accordance, fibroblasts from patients harbouring a genetic mutation leading to impaired function of the respiratory chain complex III, displayed reduced Wnt signaling with respect to healthy cells. The new paradigm proposed here further underlines the importance of mitochondrial fitness and suggests that chemotherapeutics causing mitochondrial dysfunction may have an additional benefit.

Roberto Costa

Department of Biology
University of Padova, Italy

On the regulation of human hydrogen sulfide metabolism

Karim Zuhra[1,2], Francesca Malagrinò[1,2], Ludovica Mascolo[1], Daniela Mastronicola[1,2], João B. Vicente[3], Elena Forte[1], Alessandro Giuffrè[2]

[1]Dept. of Biochemical Sciences, Sapienza University of Rome, Rome (Italy); [2]CNR Institute of Molecular Biology and Pathology, Rome, Italy; [3]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras (Portugal)

Hydrogen sulfide (H2S), currently viewed as the third 'gasotransmitter' in addition to nitric oxide (NO) and carbon monoxide (CO), has been recognized to play a regulatory role in cell bioenergetics. While inhibiting complex IV in the mitochondrial respiratory chain at higher levels, at lower concentrations H2S acts as a respiratory substrate, stimulating ATP synthesis. H2S is synthesized by cystathionine β-synthase (CBS), cystathionine γ-lyase and 3-mercaptopyruvate sulfurtransferase, and is oxidatively catabolized in the mitochondrion where H2S-derived electrons are transferred to coenzyme Q by sulfide quinone reductase (SQR). Several metabolic, neurodegenerative and oncologic diseases are associated to dysregulation of H2S metabolism. Hence, the importance of understanding how H2S bioavailability is regulated under (patho)physiological conditions. Relevant for the crosstalk between the three gasotransmitters, we showed that both CO and NO negatively modulate CBS with high efficacy [1], particularly in the presence of its allosteric activator S-adenosyl-L-methionine [2], and that a CBS variant responsible of a rare genetic disease (classical homocystinuria) displays unusually high propensity to inhibition by CO, suggesting a novel pathogenic mechanism [3]. Here, by investigating the effect of hypoxia on H2S metabolism in SW480 colon cancer cells, we report that, compared to control cells, hypoxia-treated cells display mitochondria with reduced mass, but higher SQR content, overall accounting for a lower mitochondrial sulfide-detoxifying activity. These data suggest that under hypoxic conditions the lower sulphide-detoxifying activity may contribute to ensure higher protective H2S levels, while SQR enrichment of mitochondria may protect cellular bioenergetics preventing toxic accumulation of H2S.

1) Vicente et al. (2014) J. Biol. Chem. 289, 8579-87
2) Vicente et al. (2016) J. Biol. Chem. 291, 572-581
3) Vicente et al. (2017) Oxid. Med. Cell. Longev. 2017, 8940321

Karim Zuhra

Department of Biochemical Sciences, Sapienza University of Rome, Italy

Zebrafish (Danio rerio) as a model to study the pathophysiological role of the mitochondrial chaperone TRAP1

Claudio Laquatra[1], Ionica Masgras[1], Marco Schiavone[1], Alessandra Zulian[1], Giovanni Minervini[1], Silvio Tosatto[1], Francesco Argenton[2], Andrea Rasola[1]

[1]Dept. of Biomedical Sciences and CNR Neurosceince Institute, University of Padova, Padova (Italy); [2]Dept. of Biology, University of Padova, Padova, Italy

Expression of the Hsp90-family chaperone TRAP1 is restricted to mitochondria and increased in most tumor types. We have previously shown that TRAP1 elicits the stabilization of the transcriptional factors HIF1α, thus generating a pseudohypoxic state that supports neoplastic growth. Following oncogenic activation of the Ras/ERK pathway TRAP1 is phosphorylated by ERK1/2, leading to inhibition of succinate dehydrogenase (SDH), the complex II of respiratory chain, and to the ensuing accumulation of the oncometabolite succinate, which eventually stabilizes HIF1 α. However, little is known about the timing and importance of TRAP1 induction during the process of neoplastic onset and growth, as well as on the role of TRAP1 in the physiology of non-transformed cells. To answer these questions, we decided to exploit the Zebrafish model, whose bioenergetics features are still poorly investigated. We found that TRAP1 is highly expressed during the early stages of Zebrafish embryo development, but its levels are dramatically decreased at 96 hpf. Notably, an increase in TRAP1 mRNA and protein level was detected after HIF1α stabilization, causing a reduction in SDH activity, and an *in silico* analysis of Zebrafish TRAP1 promotor showed the presence of hypoxic responsive elements motifs. Moreover, TRAP1 is highly expressed in a Zebrafish model of pancreatic adenocarcinoma induced by $KRas^{G12D}$ expression in Ptf1a positive cells where it causes a strong decrease of SDH activity, while it is absent in normal pancreas.

Altogether, these data suggest the existence of a positive feedback loop between HIF1 α and TRAP1, in which HIF1α stabilization induces TRAP1 expression acting at the transcriptional level, and in turn TRAP1, through SDH inhibition, leads to HIF1 α stabilization. This regulatory mechanism could play an important role for the adaptation of cells to fluctuating level of oxygen both in embryogenesis and during the process of neoplastic transformation.

Claudio Laquatra

Department of Biomedical Sciences and CNR Neurosceince Institute
University of Padova, Italy

What time is it? Rhythmic Interplay between Circadian Clock and Mitochondrial Oxidative Metabolism

Francesco Antonio Tucci; Rosella Scrima; Olga Cela; Consiglia Pacelli; Claudia Piccoli; Gianluigi Mazzoccoli; Nazzareno Capitanio.

Department of Clinical and Experimental Medicine, University of Foggia, Foggia (Italy)

Mounting evidences are disclosing the tight correlation between circadian rhythms and cell metabolism to align bioenergetic demand to environmental variants. In the last few years we focused on the interplay between the clock gene machinery and the mitochondrial physiology. Using well-established in-vitro-synchronized cultured cells, we demonstrated a BMAL1-dependent ultradian oscillation of the mitochondrial respiratory activity [1] as well as of the glycolytic activity. This translated in a rhythmic change of the cellular energy charge. The rhythmic respiratory activity was associated with: i) oscillation in cellular NAD content; ii) clock-genes-dependent expression of NAMPT, NMNAT3 and Sirtuins 1/3; iii) reversible acetylation of a single subunit of the mitochondrial respiratory chain Complex I; iv) reversible phosphorylation state of the pyruvate dehydrogenase (PDH). In this context the mitochondrial-endoplasmic reticulum (ER) calcium homeostasis appears to be involved as inhibition of either of the mitochondrial calcium uniport, the ER Ca2+-channel(s), the cyclic ADP-ribose (cADPR) synthesis resulted in alteration of the rhythmic respiratory activity. Notably, pharmacological inhibition of the mitochondrial OxPhos system resulted in dramatic deregulation of the clock-gene expression in synchronized cells and a similar result was attained with mtDNA depleted (Rho0) cells [2].

All together our findings provide novel levels of complexity in the interlocked feedback loop controlling the interplay between cellular bioenergetics and the molecular clockwork.

1. O. Cela, et al., Clock genes-dependent acetylation of complex I sets rhythmic activity of mitochondrial OxPhos, Biochimica Et Biophysica Acta (BBA) - Molecular Cell Research. 1863 (2016) 596–606.
2. 2. R. Scrima, et al., Clock-genes and mitochondrial respiratory activity: Evidence of a reciprocal interplay, Biochimica Et Biophysica Acta (BBA) - Bioenergetics. 1857 (2016) 1344–1351. do

Francesco Antonio Tucci

Department of Clinical and Experimental Medicine, University of Foggia,
Foggia

Investigation of pathogenetic mechanisms of AGC1 deficiency in neuron cell models

Vito Porcelli[1], Emanuela Profilo[1], Luis E. Pena-Altamira[2], Carlotta Giorgi[3], Sabrina Petralla[2], Simona Nicole Barile[1], Alberto Danese[3], Mariangela Corricelli[3], Alessandra Castegna[1], Luigi Sbano[3], Luigi Viggiano[4], Giulia Giannuzzi[1], Gennaro Agrimi[1], Carlo Viscomi[5], Massimo Zeviani[5], Luigi Palmieri[1,6], Paolo Pinton[3], Barbara Monti[2], Ferdinando Palmieri[1,6], Francesco Massimo Lasorsa[6].

[1]Dept. of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Bari (Italy); [2]Dept. of Pharmacy and Biotechnologies, University of Bologna, Bologna (Italy); [3]Dept. of Morphology, Surgery and Experimental Medicine, Sec. of Pathology, Oncology and Experimental Biology, Lab. for Technologies of Advanced Therapies,University of Ferrara, Ferrara (Italy); [4]Department of Biology, University of Bari, Bari (Italy); [5]MRC-Mitochondrial Biology Unit, Cambridge,(UK.); [6]Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies IBIOM CNR – Bari (Italy)

Mutations in the SLC25A12 gene cause AGC1 deficiency (OMIM 612949), an early infantile encephalopathy associated with severe neuromuscular delay, hypomyelination, epilepsy and reduction of brain N-acetyl-aspartate (NAA), the precursor of the myelin synthesis in CNS [1]. The SLC25A12 gene encodes for the aspartate/glutamate carrier AGC1 which is mainly expressed in brain, heart and muscle, the carrier catalyzes a Ca2+-regulated entry of glutamate into mitochondria in exchange for aspartate and is a component of the mitochondrial malate-aspartate NADH shuttle (MAS) that is essential for the correct oxidation of glucose in neurons.

In order to elucidate the pathogenesis of AGC1 deficiency, we down-regulated the gene encoding AGC1 by RNAi in the mouse brain-derived Neuro2A cells. Undifferentiated Neuro2A cells with down-regulated AGC1 showed a significant proliferation deficit associated with reduced mitochondrial respiration, and were unable to synthesize NAA properly. In the presence of high glutamine oxidation, cells with reduced AGC1 restored cell proliferation, although oxidative stress increased and NAA synthesis deficit persisted. Our data suggest that the cellular energetic deficit due to AGC1 impairment is associated with inappropriate aspartate levels to support neuronal proliferation when glutamine is not used as metabolic substrate, and we propose that delayed myelination in AGC1 deficiency patients could be attributable, at least in part, to neuronal loss combined with lack of NAA synthesis occurring during the nervous system development [2].

[1] R. Wibom et al. N Engl J Med. 361 (2009) 489-95
[2] E. Profilo et al. Biochim Biophys Acta 1863 (2017) 1422-35.

Vito Porcelli

Department of Biosciences, Biotechnologies and Biopharmaceutics
University of Bari, Italy

TRAP1 regulation in cancer metabolism: identification of new interactors

Giuseppe Cannino[1], Ionica Masgras[1], Marco Gaspari[1], Paolo Bernardi[1], Andrea Rasola[1]

[1]Dept. of Biomedical Sciences, University of Padova, Padova (Italy); [2]Dept. of Clinical and Experimental Medicine, Magna Grecia University of Catanzaro, Catanzaro (Italy)

The metabolic rewiring of cancer cells is a multifaceted process, and how mitochondria contribute to it remains poorly defined. Recently we demonstrated that the mitochondrial chaperone TRAP1 binds and inhibits succinate dehydrogenase. The consequent succinate accumulation causes HIF1α stabilization even under normal oxygen tension, setting a pseudohypoxic phenotype instrumental in prompting tumor growth. In addition, ERK-dependent phosphorylation enhances this oncogenic effect of TRAP1. In analogy with its cognate chaperone Hsp90, it is possible that TRAP1 has multiple interacting partners endowed with relevant functions in the oncogenic process. In order to identify novel TRAP1 interactors, we used a mass spectrometry analysis on TRAP1 immunoprecipitated by a human glioblastoma cell model; cells where TRAP1 was knocked-out with a CRISPR/Cas9 approach were used as a negative control. Among the potential TRAP1 partners fished out by MS, we found proteins involved in OXPHOS, tricarboxylic acid cycle and glutaminolysis. Interestingly, we found some subunits of the ATP synthase, the central enzymatic complex in the process of energy conservation. Our preliminary data indicate that the absence of TRAP1 markedly inhibits the activity of ATP synthase dimers.

Giuseppe Cannino

Department of Biomedical Sciences
University of Padova, Italy

Adenine nucleotide transporters: apoptosis master regulators, from neglected tropical diseases to space travels, through mitochondrial rare diseases and cancer.

Giuseppe Punzi, Veronica Stendardo, Francesco Colella, Immacolata G Palazzo, Giovanna Pisilli, Antonella Recchia, Nicola Gambacorta, Raffaella Piazzolla, Paola Quatraro, Lucia Trisolini, Luna Laera, Anna De Grassi, Ciro Leonardo Pierri*

[1]Dept. of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari (Italy)

The mitochondrial ADP/ATP carriers (AACs) specifically translocate the ATP synthesized within mitochondria to the cytosol in exchange for the cytosolic ADP, playing a key role in energy production, in promoting cell viability and regulating mitochondrial permeability transition pore opening [1, 2]. In Homo sapiens four genes code for AACs with different tissue distribution and expression patterns. Since AACs are dysregulated in several cancer types and involved in mitochondrial dysfunction observed in several rare diseases, the employment of AAC inhibitors or agonists might be crucial for inducing or preventing mitochondrial-mediated apoptosis in human cells [1,2 and ref therein]. In this regard carboxyatractyloside (CATR) and bongkrekic acid (BKA) are known to be powerful and highly selective AAC inhibitors. CATR shows pro-apoptotic features whereas BKA shows anti-apoptotic features. Recently, we estimated for the first time their affinity for the human recombinant AAC2 by in vitro transport assays. We found that the inhibition constants of CATR and BKA are 4 nM and 2.0 mM, respectively [1]. Given the crucial role played by AAC in triggering or preventing mitochondrial apoptosis we searched for new AAC modulators by performing a docking-based virtual screening of an in-house developed chemical library and we identified 2 highly selective inhibitors, namely suramin and chebulinic acid, showing inhibition constants equal to 0.3 mM and 2.1 mM, respectively. We also demonstrated that chebulinic acid and suramin are “highly selective” AAC2 inhibitors, since they poorly inhibit other human mitochondrial carriers (namely ORC1, APC1 and AGC1) [1]. The newly identified AAC inhibitors among CATR analogs will be assayed for their ability in triggering mitochondrial apoptosis in cancer cell-lines, whereas the newly proposed AAC modulators identified among BKA analogs will allow to test them for preventing mitochondrial apoptosis in neuro/muscular degenerative diseases showing an excess of apoptosis. Notably, preventing mitochondrial apoptosis could also be a winning approach for limiting the muscular/bone loss and/or neurological disorders observed in astronauts living for long periods in microgravity conditions or after prolonged exposure to cosmic rays.

Finally, considering that mitochondrial apoptosis is the only apoptotic pathway shared by several protista, fungi, plants and mammalia, it becomes intriguing to identify new apoptosis modulators acting through the species-specific AACs. It was indeed ascertained that AACs are present in several fungi and protista pathogens like candida albicans, botrytis cinerea and aspergillus clavatus, from fungi; and leishmania infantum, trypanosome brucei, plasmodium falciparum and toxoplasma gondii from protista. AACs from the cited species show specific structural features that will allow to draw new species-specific AAC inhibitors starting from the characterized pro-apoptotic human AAC inhibitors (i.e. CATR, suramin, chebulinic acid).

Ciro Leonardo Pierri

Department of Biosciences, Biotechnologies and Biopharmaceutics
University of Bari, Italy

List of participants

Pia Adelroth
Department of Biochemistry and Biophysics
Stockholm University
pia.adelroth@dbb.su.se

Elisa Astro
Department of Pharmacy and Biotechnology
University of Bologna, Bologna, Italy
elisa.astro2@unibo.it

Simona Nicola Barile
Department of Biosciences, Biotechnologies and Biopharmaceutics
University of Bari, Bari, Italy
simona.barile@uniba.it

Paolo Bernardi
Department of Biomedical Sciences
University of Padua, Padua, Italy
bernardi@bio.unipd.it

Elena Bianchini
Department of Surgery, Medicine, Dentistry and Morphological Sciences
University of Modena and Reggio Emilia, Modena, Italy
elena.bianchini.88@gmail.com

Faustino Bisaccia
Department of Sciences
University of Basilicata, Potenza, Italy
faustino.bisaccia@unibas.it

Giuseppe Cannino
Department of Biomedical Sciences
University of Padua, Padua, Italy

Nazzareno Capitanio
Department of Clinical and Experimental Medicine
University of Foggia, Foggia, Italy
nazzareno.capitanio@unifg.it

Andrea Carrer
Department of Biomedical Sciences
University of Padova, Italy
Andrea.carrer@phd.unipd.it

Lara Console
Department of Biology, Ecology and Earth Sciences
University of Calabria, Arcavacata di Rende, Rende (CS), Italy
consolelara@gmail.com

Jessica Cosco
Department of Biology, Ecology and Earth Sciences
University of Calabria, Arcavacata di Rende, Rende (CS), Italy
jessicacosco@libero.it

Andrea Cossarizza
Department of Medical and Surgical Sciences for Children and Adults
University of Modena and Reggio Emilia, Modena, Italy
andrea.cossarizza@unimore.it

Roberto Costa
Department of Biology
University of Padua, Padua, Italy
roberto.costa@unipd.it

Sara De Biasi
Department of Life Sciences
University of Modena and Reggio Emilia, Modena, Italy
debiasisara@yahoo.it

Anna De Gaetano
Department of Life Sciences
University of Modena and Reggio Emilia, Modena, Italy
anna.degaetano@unimore.it

Vito De Pinto
vdpdbiofa@unict.it
Department of Biotechnology and Biomedical Sciences
University of Catania, Catania (Italy)

Domenico De Rasmo
Istituto di Biomembrane, Bioenergetica e Biotecnologie Molecolari (IBIOM)
CNR, Bari, Italy
d.derasmo@ibiom.cnr.it

Elena Forte
Department of Biomedical Sciences
Sapienza University of Rome, Rome, Italy
elena.forte@uniroma1.it

Francesco Francia
Department of Pharmacy and Biotechnology
University of Bologna, Bologna, Italy
francesco.francia@unibo.it

Thorsten Friedrich
Institut für Biochemie
Universität Freiburg, Freiburg, Germany
friedrich@bio.chemie.uni-freiburg.de

Chiara Galber
Department of Biomedical Sciences
University of Padua, Padua, Italy
chiara.galber@phd.unipd.it

Anna Maria Ghelli
Department of Pharmacy and Biotechnology
University of Bologna, Bologna, Italy
annamaria.ghelli@unibo.it

Lara Gibellini
Department of Medical and Surgical Sciences for Children and Adults
University of Modena and Reggio Emilia, Modena, Italy
lara.gibellini@unimore.it

Valentina Giorgio
Department of Biomedical Sciences and CNR-Neuroscience Institute
University of Padua, Padua, Italy
vgiorgio@bio.unipd.it

Alessandro Giuffrè
CNR Institute of Molecular Biology and Pathology
Rome, Italy
alessandro.giuffre@uniroma1.it

Antonio Gnoni
Department of Basic Medical Sciences, Neurosciences and Sense Organs
University of Bari, Bari, Italy
gnoniantonio@gmail.com

Cesare Indiveri
Department of Biology, Ecology and Earth Sciences
University of Calabria, Arcavacata di Rende, Rende (CS), Italy
cesare.indiveri@unical.it

Luisa Iommarini
Department of Pharmacy and Biotechnology
University of Bologna, Bologna, Italy
iommarini.luisa@gmail.com

Claudio Laquatra
Department of Biomedical Sciences
University of Padua, Padua, Italy
claudiolaquatra@gmail.com

Giovanna Lippe
Department of Agricultural, Food, Environmental and Animal Sciences
University of Udine, Udine, Italy
giovanna.lippe@uniud.it

Domenico Lo Tartaro
Department of Medical and Surgical Sciences of children and adults
University of Modena and Reggio Emilia, Modena, Italy
domenicolotartaro@gmail.com

Beatrice Lusenti
Department of Medical and Surgical Sciences for Children and Adults
University of Modena and Reggio Emilia, Modena, Italy
beatrice.lusenti@gmail.com

Andrea Magrì
Department of Biological, Geological and Environmental Sciences
University of Catania, Catania, Italy
andrea.magri@unict.it

Fabio Martinelli
Department of Sciences
University of Basilicata, Potenza, Italy
fabiomartinelli@alice.it

Milena Nasi
Department of Surgery, Medicine, Dentistry and Morphological Sciences
University of Modena and Reggio Emilia, Modena, Italy
milena.nasi@unimore.it

Anita Neroni
Department of Medical and Surgical Sciences for Children and Adults
University of Modena and Reggio Emilia, Modena, Italy
anitaneroni@hotmail.it

Salvatore Nesci
Department of Veterinary Medical Sciences
University of Bologna
salvatore.nesci@unibo.it

Karin Nowikovsky
Department of Internal Medicine I
Medical University Vienna, Vienna, Austria
karin.nowikovsky@meduniwien.ac.at

Luigi Palmieri
Department of Biosciences, Biotechnologies and Biopharmaceutics
University of Bari, Bari, Italy
luigi.palmieri@uniba.it

Simone Pecorini
Department of Medical and Surgical Sciences for Children and Adults
University of Modena and Reggio Emilia, Modena, Italy
simone.pecorini@gmail.com

Christina Pfeiffer
Department of Internal Medicine I and Comprehensive Cancer Center
Medical University of Vienna
ChristinaPfeiffer@gmx.at

Claudia Piccoli
Department of Clinical and Experimental Medicine
University of Foggia, Foggia, Italy
Claudia.piccoli@unifg.it

Ciro Leonardo Pierri
Department of Biosciences, Biotechnologies and Biopharmaceutics
University of Bari, Bari, Italy
ciro.pierri@uniba.it

Marcello Pinti
Department of Life Sciences
University of Modena and Reggio Emilia, Modena, Italy
marcello.pinti@unimore.it

Vito Porcelli
Department of Biosciences, Biotechnologies and Biopharmaceutics
University of Bari, Bari, Italy
vito.porcelli@uniba.it

Paolo Ettore Porporato
Molecular Biotechnology Center - MBC
Dept. of Molecular Biotechnology and Health Sciences
University of Turin, Turin, Italy
paolo.porporato@unito.it

Claudio Procaccini
Isntitute of Endocrinology and Experimental Oncology
CNR Naples, Naples, Italy
claudio.procaccini@cnr.it

Andrea Rasola
Department of Biomedical Sciences
University of Padua, Padua, Italy
andrea.rasola@unipd.it

Michela Rugolo
Department of Pharmacy and Biotechnology
University of Bologna, Bologna, Italy
michela.rugolo@unibo.it

Carlos Sanchez-Martin
Department of Biomedical Sciences
University of Padua, Padua, Italy
carlossanmarti@gmail.com

Marco Schiavone
Department of Biomedical Sciences
University of Padua, Padua, Italy
marco.schiavone@unipd.it

Anna Signorile
Department of Basic Medical Sciences, Neurosciences and Sense Organs
University of Bari, Bari, Italy
Anna.signorile@uniba.it

Anna Stocco
Department of Biomedical Sciences
University of Padua, Padua, Italy
stocco.anna.7491@gmail.com

Annamaria Tonazzi
Institute ob Biomembranes, Bioenergetics and Molecular Biotechnologies
CNR Bari, Bari, Italy
a.tonazzi@ibiom.cnr.it

Francesco Antonio Tucci
Department of Clinical and Experimental Medicine,
University of Foggia, Foggia (Italy)
fran.tucci@gmail.com

Andrea Urbani
Department of Biomedical Sciences
University of Padua, Padua, Italy
andrea.urbani@unipd.it

Marco Zancani
Department of Agricultural, Food, Environmental and Animal Sciences
University of Udine, Udine, Italy
marco.zancani@uniud.it

Karim Zuhra
Department of Biochemical Sciences
Sapienza University of Rome, Rome, Italy
karim.zuhra@uniroma1.it

Thank you