Health, disease and environmental research: biology, tools and applications

IBPM Annual Meeting

Rome, May 8th 2018
Aula Convegni CNR

Scientific committee
Nicoletta Corbi, Andrea Ilari, Pietro Laneve, Roberto Piergentili, Patrizia Somma

Organizing committee
Giulia Guarguaglini, Isabella Monosi, Barbara Ognibene, Roberto Piergentili

Segreteria IBPM
Phone: +39 06 4993 3874-3873
email: segreteria.ibpm@cnr.it

On-line Abstracts Book:
Roberto Piergentili
email: roberto.piergentili@cnr.it

Web Support:
Mario Incarnato
email: mario.incarnato@cnr.it

SUMMARY

PROGRAMME

09.00 - 09.20 ***Registration***

Session 1: Opening session in honor of Prof. Chiancone: 15 years of protein studies at IBPM

9.20 - 9.30 **Patrizia Lavia, Direttrice ff IBPM**
Welcome message from the IBPM Acting Director

9.30 - 9.45 **Tullio Pozzan, Direttore Dipartimento Scienze Biomediche (DSB)**
Greetings from the Director, CNR Department of Biomedical Sciences

9.45 - 10.15 **Maurizio Brunori, Vice Presidente Accademia Nazionale dei Lincei**
University-CNR cooperation in Structural Biology

10.15 - 10.45 **Alberto Boffi, Direttore Dipartimento Scienze Biochimiche - Sapienza**
Ferritins: ironsiders from science to technology

10.45 - 11.15 **Andrea Ilari, Primo Ricercatore IBPM**
Protein crystallography at IBPM: function discovery of cage-like proteins and sorcin

11.15 - 11.45 ***Coffee break***

Session 2. Innovative Resources, Tools and Technologies

11.45 - 12.25 **Veronica Morea, Loredana Le Pera, Allegra Via**
Bioinformatics@IBPM: atomic level, whole genome scale and the ELIXIR Infrastructure for Bioinformatics

12.25 - 12.45 **Giulia Guarguaglini**
Imaging protein interactions and modifications in space and time

12.45 - 14.15 ***Lunch break and poster session***

Session 3. Innovative Applications: Agrifood and the Environment

14.15 - 14.35 **Maura Cardarelli**
Control of male fertility in self-pollinating plants by alternative splicing

14.35 - 14.55 **Patrizia Brunetti**
Heavy metal and arsenic accumulation in plants: molecular mechanisms and applications in phytoremediation

Session 4. Innovative Design in Cancer Therapy

14.55 - 15.15 **Barbara Illi**
Novel diagnostic and prognostic perspectives for Glioblastoma Multiforme

15.15 - 15.35 **Angela Tramonti**
Inhibition of serine hydroxymethyltransferase, a promising target for future chemotherapy intervention

15.35 - 15.55 ***Coffee break***

Session 5. Perspectives in Molecular and Cellular Neurobiology

15.55 - 16.25 **Paola Fragapane**
The IBPM Neuronet Consortium: a network to investigate molecular mechanisms underlying neuro-pathologies

16.25 - 16.45 **Cecilia Mannironi**
Neuronal circular RNAs in normal and pathological conditions: the case of autism spectrum disorders

16.45 - 17.05 **Nicoletta Corbi**
Molecular mechanisms of memory persistence

17.05 - 17.25 **Pietro Laneve**
Integrated RNA circuitries in motor neuron differentiation and degeneration

17.25 - 17.40 ***Concluding remarks***

INDEX

Posters **page**

Late Abstracts **page**

FOREWORD

Patrizia Lavia, IBPM Acting Director

I would like to welcome all the participants to the IBPM meeting 2018 and I thank you very much for being here. I will give a very short foreword, as the programme of the meeting is very dense.

The IBPM annual meeting is an opportunity for us to discuss our research results and directions. This year IBPM enters its 15th year and we thought this would make an excellent occasion to assess the progress and novel directions that the Institute is taking in the broader perspective of the unprecedented developments we are witnessing in the life sciences. I am not going to even attempt to summarise those developments here in a few minutes. As you all know, these changes not only reflect powerful technological developments that enable global approaches, but also respond to the ever growing awareness that inter-disciplinarity and multi-disciplinarity are key to address complex biological processes that require conceptual and experimental tools at a "systems" level. The annual meeting this year also coincides with the birthday of Prof. Chiancone, who has actually driven the IBPM foundation as its first director and a prominent member of the former *comitato ordinatore* (organizing committee) of the CNR Life Sciences department, now part of the department of BioMedical Sciences.

This dual anniversary has stimulated us to trace back the research path followed at the IBPM in an effort to critically assess our directions and expand collaborations. I will just briefly recall the foresight that is at the origin of the IBPM foundation. As you know, the IBPM comes from the merging of three pioneering CNR Centers established at the University La Sapienza by the imagination and wisdom of three great personalities: Professors Alessandro Rossi-Fanelli, Giorgio Tecce and Giuseppe Montalenti. Each of the three Centers had the mission to advance a most challenging topic of their times: the Center of Molecular Biology focused on the structure of proteins, with the paradigmatic idea that in structure lies the function — which encapsulates what we now call structural biology; the Center of Nucleic Acids was a pioneering Center in Italy in studies of nucleic acids and the mechanisms that regulate genome expression and shape its organization; and the Center of Evolutionary Genetics developed the intuition of a mutual feedback between genetics, mutations and evolution. The three Centers were joined by researchers from the former CNR Institute of Biomedical Technologies committed to study complex cellular networks and their dysfunction in oncological, infectious and degenerative diseases. The complementarity of scientific approaches, experimental systems, and levels of investigation — from molecules to cells, to whole organisms and organism/environment interactions — was obvious, yet the strong scientific characterisation of the four founding units initially hindered a smooth blending, as it was difficult to accept that disciplinary borders had to be crossed or even cancelled. In the longer run, however, this was to become the strength and the uniqueness of IBPM. Prof. Chiancone has been a relentless and tenacious stimulus in pursuing that direction. Later, under Ida Ruberti's direction, a further leap was taken when the Chemistry Unit of the CNR Institute of Biomolecular Chemistry, also located at Sapienza, joined IBPM further expanding the range of conceptual and experimental approaches that make IBPM what it is today. We now see how effectively that foresight has contributed to forge IBPM. The continuous effort to integrate and merge is paying off: it has enabled us to develop an interdisciplinary view, possibly the most distinctive feature of IBPM today, overcome reductionism and advance scientific developments.

With these considerations in mind, we have decided this year to hold an open meeting and have invited colleagues from several Institutions; CNR Institutes with whom we share collaborative interests, not only in the Department of BioMedical Sciences but also from the Agri-Food, Environment, and Chemistry and Materials Sciences Departments; from Istituto Superiore di Sanità and the Rome Cancer Institute Regina Elena; and from three Universities of Rome, particularly Sapienza of course. I would like to mention, as a side note, that we are currently renewing the agreements (*convenzioni*) with our hosting departments at Sapienza and updating what we call "Joint Research Structures", in which researchers from both Institutions merge their common research interests.

The first session of the meeting will focus on research in biochemistry and its evolution at IBPM, from classical protein biochemistry and enzymology towards structural biology, bioinformatics, nanobiology, and their applications. The session is ideally dedicated to Prof. Chiancone and represents a paradigm of the evolution of IBPM research. In the next years we will focus on developments in parallel strategic areas. This year, we have asked Prof. Brunori to give an introductory lecture recalling the foundations and developments of the CNR Centre of Molecular Biology, then Alberto Boffi and Andrea Ilari will describe evolutions towards the latest developments in IBPM research in nanobiology and structural biology, respectively.

In the next session, the speakers will illustrate ongoing developments in imaging and bioinformatics studies, linking to infrastructures that make a significant asset of IBPM: the European infrastructure for bioinformatics Elixir, of which our colleague Allegra Via is the training coordinator, and the Microscopy and Imaging unit at IBPM which also serves as a Nikon reference center for Central and Southern Italy. It is recognized that research needs advanced infrastructure and equipment to meet the challenges coming from our growing appreciation of complexity in biology. The EU is therefore concentrating significant efforts on such infrastructure. In Italy the CNR is the driving organization in two projects in which IBPM participates: Elixir and the EuroBioImaging projects, for which the CNR has presented two applications for PON grants. The participation of IBPM in both National projects is an important scientific recognition of the capacity of the Institute to develop front line studies in both fields. This is again to be regarded as a collective, successful product of the interdisciplinarity we have developed over the years. Although PON grants are to be invested for 85% of their amount on infrastructure in less developed regions in Southern Italy, the PON projects, if awarded, will bring IBPM some resources at this time of harsh cuts in funding. Albeit strictly limited to equipment, they will enable some renewal and implementation; an important expected innovation in this regard will be represented by the establishment of a CNR facility for Cryo Electron Microscopy, which involves IBPM researchers and associates.

Part of the presentations that will follow in the next sessions have important potential applications. The value of applied research is enormous; in addition to the improvements it introduces in our lives, it also represents the side that the lay public sees of the research that we do. Let me shortly mention that only in the last three months, work produced at IBPM has been the object of four press releases, concerning: 1. the design of innovative vectors with muscular trophism of potential use in Duchenne Distrophy; 2. the structure of proteins implicated in neurodegenerative diseases and the expected advances from culturing patient-derived primary neurons; 3. the clarification of mechanisms that regulate the terminal steps of cell division and how their failure can generate genetic instability in the cellular offspring; 4. the characterisation of a novel splicing mechanism that regulates fertility in plants, with relevant implications for sustainable agriculture. In addition, a new study will link plant molecular biology to the

field of phytoremediation, with an expected remarkable impact on the environment and public health. It is important that the significance of this type of research is explained to the lay public and in this occasion I would like to thank the CNR press office for their excellent support. For time reasons, not all applicative studies can be presented here, but only part of them.

IBPM researchers participate in many international collaborations, in the form of joint research projects or of mobility schemes, between CNR and International partner organisms. Again, not all of these activities will be presented in this meeting for time reasons, but some of the presentations will offer a glimpse on International collaboration perspectives. In addition to the European Elixir/Excelerate project, work at the core of bilateral cooperation projects with Russia, Canada, and Argentina will be presented. These projects open up new venues and perspectives of collaboration in several fields.

Finally, the last session will present a set of studies that can be grossly grouped in the field of the neurosciences to launch a proposal for a collaborative network, called by its founders the "NeuroNet" consortium. NeuroNet intends to gather an effective critical mass of researchers, model systems, experimental tools and diversified scientific approaches to address some of the most challenging health issues of our times, i.e. cancer of the central nervous system and neurodegenerative diseases, but also some of the most intriguing and challenging molecular circuits underlying for example the making of memories, emotions and behaviors. NeuroNet is already effective within IBPM and is based on strong collaborative ties and joint project with researchers from related CNR Institutes. It is hoped that it can build up a broader National consortium.

As usual, the meeting programme presents proposals that spontaneously come from the speakers. The organizers have stimulated them to present their work to an open audience and have encouraged them to delineate not only their successful results but also what they foresee as possible perspectives and future research directions. That is particularly important in the case of our junior colleagues who have just joined IBPM. We hope to make the meeting a real occasion to progress collectively along a perspective of continuous development. In this occasion I like to stress that true development needs renewal and recruitment. The CNR has recently opened up some much needed recruitment, which has brought, and is due to bring in the course of 2018, three new researchers to IBPM. There are reasonable expectations that the recent Madia bill will further expand the recruitment of young researchers.

Finally I would like to mention the people without whom the meeting would not have been possible: Roberto Piergentili for his scientific and organizational coordination, as well as the other members of the scientific committee, Patrizia Somma, Andrea Ilari, Nicoletta Corbi and Pietro Laneve, and the members of the organizing committee Giulia Guarguaglini, Isabella Monosi and Barbara Ognibene.

ORAL PRESENTATIONS

O-1: Protein crystallography at IBPM: function discovery of cage-like proteins and sorcin

Andrea Ilari

Istituto di Biologia e Patologia molecolari del CNR c/o Dipartimento di Scienze Biochimiche, Sapienza P.le A. Moro 5, 00185 Roma, Italy.

Keywords: X-ray crystallography, Dps (DNA binding protein from starved cells), oxidative damage, sorcin (soluble resistance-related calcium binding protein), calcium homeostasis.

Macromolecular crystallography is a powerful tool to solve three-dimensional structure of biological macromolecules to understand their biological function. In this framework, the X-ray structures of Dps (DNA binding protein from starved cells) and sorcin (soluble resistance-related calcium binding protein), are paradigmatic examples. Dps are cage-like proteins belonging to the ferritin super family and are able to incorporate up to 500 iron ions thereby protecting DNA from oxidative damage. The *Listeria innocua* Dps structure allowed the identification of structural features necessary for its function namely: the negatively charged channels along the three-fold symmetry axes that serve for iron entry into the cavity; a negatively charged internal cavity for iron deposition and an unique ferroxidase center at the interface between two-fold symmetry related subunits. The structural analysis together with biochemical experiments demonstrated that Dps is able to oxidize iron by using hydrogen peroxide as oxidant agent thereby avoiding the formation of the highly toxic hydroxyl radical (1). Cage-like proteins as Dps and Ferritin have been used as bioreactors able to synthesize nanoparticles of different metals. Biomineralization using protein cavities is an important issue for the fabrication of biometamaterials under mild synthetic conditions. We have shown that silver nanoparticles (AgNPs) were produced with high yields within PfFt and to explain the molecular basis of silver incorporation we solved the X-ray crystal structure of Ag-containing PfFt (2). This structure revealed the presence of specific binding and nucleation sites of Ag(I) that are not conserved in other ferritin templates. The AgNP encapsulated by PfFt are endowed with a narrow size distribution and high stability in water solution, properties that make these AgNPs exploitable for a range of applications, in different fields as preparation of metamaterials, *in vivo* diagnosis and antimicrobial therapy. We used the silver nanoparticles obtained in PfFt as antileishmanial agents and demonstrated that silver released by AgNPs inhibit the Trypanothione Reductase an essential enzyme for Leishmania survival, thereby killing the parasite in both amastigote and promastigote stages (3).

Sorcin is an essential penta-EF hands calcium binding protein, able to confer multi-drug resistance phenotype to drug-sensitive cancer cells and to reduce Endoplasmic Reticulum stress and cell death. Sorcin silencing blocks cell cycle progression in mitosis and induces cell death by triggering apoptosis. Sorcin participates in the modulation of calcium

homeostasis and in calcium-dependent cell signalling in normal and cancer cells. We solved he X-ray structures of Sorcin in the apo (apoSor) and in calcium bound form (CaSor) to reveal the structural basis of Sorcin action; we demonstrated that calcium binding to the EF1-3 hands promotes a large conformational change, involving a movement of the long D-helix joining the EF1-EF2 sub-domain to EF3 and the opening of EF1(4). This movement promotes the exposure of surfaces which allow its interaction with molecular partners carrying the consensus binding motif identified by phage display experiments.

References

1. Ilari A, Stefanini S, Chiancone E, Tsernoglou D. The dodecameric ferritin from Listeria innocua contains a novel intersubunit iron-binding site. Nat Struct Biol. 2000 Jan;7(1):38-43. PubMed PMID: 10625425.

2. Kasyutich O, Ilari A, Fiorillo A, Tatchev D, Hoell A, Ceci P. Silver ion incorporation and nanoparticle formation inside the cavity of Pyrococcus furiosus ferritin: structural and size-distribution analyses. J Am Chem Soc. 2010 Mar 17;132(10):3621-7. doi: 10.1021/ja910918b.

3. Baiocco P, Ilari A, Ceci P, Orsini S, Gramiccia M, Di Muccio T, Colotti G. Inhibitory Effect of Silver Nanoparticles on Trypanothione Reductase Activity and Leishmania infantum Proliferation. ACS Med Chem Lett. 2010 Dec 29;2(3):230-3. doi: 10.1021/ml1002629. Central PMCID: PMC4017975.

4. Ilari A, Fiorillo A, Poser E, Lalioti VS, Sundell GN, Ivarsson Y, Genovese I, Colotti G. Structural basis of Sorcin-mediated calcium-dependent signaltransduction. Sci Rep. 2015 Nov 18;5:16828. doi: 10.1038/srep16828.

O-2: Bioinformatics@IBPM: Atomic level, whole genome scale and the ELIXIR Infrastructure for Bioinformatics

Loredana Le Pera, Veronica Morea, Allegra Via

Institute of Molecular Biology and Pathology (IBPM) - CNR, Rome, Italy

Keywords: Bioinformatics; Biological macromolecules; Biological "Omics" data; ELIXIR; Training

The Biocomputing Lab at IBPM has a long standing tradition in the study of biological macromolecules. Relationships between sequence, three-dimensional (3D) structure and biochemical/biological function of homologous proteins and tRNA molecules are identified and exploited to: build 3D protein models at atomic level; assign biochemical and biological function to protein sequences and structures; design proteins and peptides with desired properties; unveil molecular mechanisms underlying biological phenotypes; and infer evolutionary relationships.

Recent applications include: construction of a 3D model of the GDP- and GTP-bound conformations of the RHES protein (Ras Homologue Enriched in the Striatum), which is involved in Huntington's disease; humanization of murine antibody EP1 against a specific breast-cancer antigen; prediction of the mode of binding of resveratrol analogs to tubulin; and design of ferritin variants able to incorporate diagnostic or therapeutic agents and deliver them selectively to target sites.

Future developments comprise the implementation of semi-automated procedures to aid in the antibody humanization and structure analysis procedures. While some aspects will always benefit from expert evaluation, automation of at least the most general steps is nowadays indispensable, given the amount of data to be analyzed. As an example, the number of 3D structures of biological macromolecules available from the Protein Data Bank has been growing exponentially for the past 20 years, and is rapidly approaching a total of 140,000.

In parallel, the increase in the volume of data produced by whole genome scale experiments has been even more dramatic (GenBank today contains more than 200 billion sequences) and it is expected to continue to grow at the same or higher speed.

In the past two years, the IBPM Biocomputing Lab has acquired new people endowed with expertise in the analysis of data resulting from '-omics' experiments, ranging from genomics (i.e. DNA-seq, whole genome/exome sequencing, genome assembly) and transcriptomics (RNA-seq) to epigenomics and protein-DNA interactions (i.e. ChIP-seq). Each type of data needs specific analysis procedures requiring the development of *ad hoc* computational strategies and pipelines.

We have implemented approaches and tools for the integration of experimental data produced in researchers' own labs with available public biological and medical dataset archives (such as Gene Ontology, TCGA, ENA, 1000 Genomes Project, etc.). As an example, by integrating proteomics data produced by IBPM and Sapienza University collaborators with public datasets, we identified interesting biological interaction partners

of the importin beta protein, a master regulator of mitosis that is overexpressed in many cancer types. These interactors are currently undergoing detailed experimental characterization.
We are also developing an automated procedure to identify and analyze known and putative point mutations from bacterial genomes that have been associated with antibiotic resistance.

The IBPM Biocomputing Lab is also strongly involved in the design, development and delivery of Bioinformatics training activities by coordinating the ELIXIR-IIB Training Platform and collaborating with several European countries.
ELIXIR is a European platform that unites leading life science organizations in managing and safeguarding the increasing volume of data being generated by publicly funded research. It coordinates, integrates and sustains bioinformatics resources across 21 member states and enables users in academia and industry to access services that are vital for their research.
The Italian node of ELIXIR (ELIXIR-IIB) is led by the CNR and involves 17 academic and technological institutions. ELIXIR-IIB services include storage and computing facilities, specialized datasets, standards for data deposition and exchange, tools for Bioinformatics data analysis and training activities, thus providing a solid infrastructure support to Italian research in the Life Sciences. In this context, the IBPM Biocomputing Lab has developed competences and research interests in learning processes, best teaching practices, educational psychology, formative and summative assessment, and cognitive sciences.
All the projects mentioned above have been or are being carried out in collaboration with researchers either within IBPM (groups of Ilari, Colotti, Degrassi, Ceci, Lavia) or external institutions (groups of Schininà, Giacomini, Vriend). Further detail is presented in the namesake poster.

O-3: Imaging protein interactions and modifications in space and time

Giulia Guarguaglini[1], Annalisa Verrico[1], Italia Anna Asteriti[1], Paola Rovella[1], Michela Damizia[1], Pietro Cirigliano[2], Patrizia Lavia[1]

[1] Institute of Molecular Biology and Pathology-CNR, Via degli Apuli, 4, 00185, Rome
[2] Nikon Instruments S.p.A., Campi Bisenzio, IT.

Keywords: imaging, spatio-temporal resolution, in situ proximity ligation assay (is-PLA), automated screening

In the last years microscopy has undergone an unprecedented development that has amplified its informative power and classifies it as a high content approach with applications at multiple levels in biological research. Protein-protein interaction (PPI) studies, and their implications in signalling and regulatory processes, can take highest advantage from the spatio-temporal dimension offered by innovative microscopy approaches. Among imaging methodologies developed to visualise PPIs, the *in situ* proximity ligation assay (is-PLA) has the unique feature of visualising PPIs between endogenous components, avoiding the use of tagged chimaeras and enabling investigations under physiological conditions.
We have developed applications of the is-PLA methodology to investigate protein complex composition, localisation and post-translational modifications with spatio-temporal resolution during the cell cycle.
In one set of applications we have developed an is-PLA automated workflow to validate interacting partners of the karyopherin Importin beta-1 identified in proteome-wide interactomic studies. The PLA method enabled a rapid validation of newly identified interactors and provided information on their changes in composition and localisation during nuclear envelope breakdown, mitotic progression and nuclear envelope reassembly at mitotic exit.
We provided an independent proof-of-concept that is-PLA can be used to identify PPI inhibitors in a screening of molecules with therapeutic potential capable to disrupt the oncogenic complex between Aurora-A and its activator TPX2.
Finally, we developed the is-PLA methodology at the intramolecular level to detect certain types of protein post-translational modifications, e.g. SUMOylation.
Overall, we show that is-PLA and *ad hoc* optimizations are valuable tools for the analysis of interactions at the single cell level, providing key spatio-temporal information to understand the dynamic interaction scenario in eukaryotic cells.

O-4: Control of male fertility in self-pollinating plants by alternative splicing

Maura Cardarelli

Istituto di Biologia e Patologia Molecolari, Consiglio Nazionale delle Ricerche, Sapienza Università di Roma

Keywords:

Male sterility is a most important trait in plant breeding programs and in the commercial production of hybrid seeds. Stamen development is governed by a complex network of different regulatory proteins and hormones. Mutations that impair any step of this development give rise to stamen defects and therefore causes male sterility.
In *Arabidopsis thaliana* the hormone auxin coordinates all three processes that occur during late stamen development: stamen filament elongation, anther dehiscence and pollen maturation. Auxin controls gene expression via different Auxin Response Factors (ARFs), many of which have different splice variants whose function has not been investigated. *ARF8* plays a main role in stamen development and *arf8* mutant flowers show reduced male fertility; in addition to the full-length transcript *ARF8.1*, a splice variant of unknown function named *ARF8.2* has been reported.
We identified a new flower-specific splice variant of *ARF8* that we denominated *ARF8.4*, which bears a stop codon similar to *ARF8.2* but presents an in-frame intron retention. We showed that *ARF8.4* has a different tissue-specificity than *ARF8.1* and *ARF8.2*. Through inducible expression of each variant in *arf8-7* flowers, we showed that *ARF8.4* fully complements the short-stamen phenotype of the mutant and directly regulates the expression of the positive regulator of stamen elongation *AUX/IAA19*. We also showed that *ARF8.4* and *ARF8.2* control the timing of anther dehiscence acting on different key genes involved in these process.
ARF8.4 is among the first examples of a splice variant that regulates a developmental process (Ghelli et al, 2018).
Whether male fertility is controlled by the response to auxin via alternative splicing also in other crops, and whether new splice variants can be used to obtain male sterile plants remains to be explored.

References

1. Ghelli R, Brunetti P, Napoli N, De Paolis A, Cecchetti V, Tsuge T, Serino G, Matsui M, Mele G, Rinaldi G, Palumbo GA, Barozzi F, Costantino P, Cardarelli M. A Newly Identified Flower-Specific Splice Variant of AUXIN RESPONSE FACTOR8 Regulates Stamen Elongation and Endothecium Lignification in Arabidopsis. Plant Cell. 2018 Mar;30(3):620-637. doi: 10.1105/tpc.17.00840.

O-5: Heavy metal and arsenic accumulation in plants: molecular mechanisms and applications in phytoremediation

Patrizia Brunetti

Consiglio Nazionale delle Ricerche, Istituto di Biologia e PatologiaMolecolari, Università Sapienza di Roma

Keywords: heavy metals, arsenic, model species, hyperaccumulators, phytoremediation

Cadmium and arsenic are widespread environmental contaminants with harmful effects on living cells. Plants can absorb from the soil Cd and As and the uptake of toxic elements by plants can be used to remove pollutants from contaminated sites. This technology called phytoremediation has mainly focused on a small group of plants, called hyperaccumulators, that are capable of growing on contaminated soils since they translocate toxic elements from the roots to the shoots and sequester them in their aerial tissues. A pre-requisite to optimize the use of hyperaccumulators is to understand various mechanisms operating in plants for their uptake, transport and hyperaccumulation.

The research activity in the lab is mainly focused on the main process of Cd and As detoxification: chelation of elements in the cytoplasm with phytochelatins (PCs) and sequestration of PC-Cd (or As) complexes into the vacuole. We demonstrated that overexpression of the Arabidopsis PC biosynthetic gene AtPCS1 in *Nicotiana tabacum* plants increases Cd tolerance (Pomponi et al., 2006). In contrast in Arabidopsis Cd tolerance depends on an optimal ratio between PC level and Cd (Brunetti et al., 2011). We have also demonstrated that in Arabidopsis, the ABC-type transporter AtABCC3 is involved in the compartimentalization of PC–Cd complexes into the vacuoles, and suggested that its activity is coordinated with that of two other transporters AtABCC1/AtABCC2 (Brunetti et al., 2015). As for Arsenic we showed that overexpression of AtPCS1 in tobacco plants increases As tolerance and accumulation (Zanella et al., 2016) and we are currently analysing the role of ABCC3 in As compartimentalization into the vacuole.

Very recently we have focused our activity on the fern *Pteris Vittata* with the aim of using this As-hyperaccumulator for experiments on contaminated soils. We are using a non invasive and rapid new technology, based on µXRF imaging to analyse As accumulation in different plant tissues (Capobianco et al., under revision).

References:

1. Brunetti et al. J Exp Bot. 2011 Nov; 62:5509-19
2. Brunetti et al. J Exp Bot. 2015; 66:3815-29.
3. Pomponi et al. Planta 2006; 223:180–190.
4. Zanella et al., Planta 2016; 243:605-22.

O-6: Novel diagnostic and prognostic perspectives for Glioblastoma Multiforme

Annarita Favia, Luisa Salvatori, Fiorella Scagnoli, Sergio Nasi, Barbara Illi

Institute of Molecular Biology and Pathology, National Research Council, Rome

Keywords: Glioblastoma multiforme; Stem cells; Arginine methylation; Myc; PRMT5/PRMT1

Glioblastoma Multiforme (GBM) still represents the most deadly and untreatable brain tumour in the adult. Despite a decade of advances in GBM molecular and genomic characterization, therapeutic options are disconcertingly very poor. Tumour recurrence and resistance to conventional treatments seem to depend on a pool of tumour initiating cells, holding the characteristics of embryonic and adult stem cells and, therefore, are named glioblastoma stem cells (GSCs). GSCs present a “Myc signature”, underpinning the essential role of c-myc oncogene in maintaining GSC stem properties. Protein Arginine (R) methylation is the most common post-translational methylation in mammalian cells, ruling a wide variety of cell functions. Protein Arginine Methyltransferase (PRMT) 1 and 5 are ubiquitously expressed, play fundamental roles in the onset and progression of tumour malignancies, including GBM, and di-methylate their substrates asymmetrically or symmetrically, respectively. We have found that i) Myc interacts with PRMT1 and PRMT5 in GSCs; ii) Myc is asymmetrically or symmetrically di-methylated by PRMT1 and PRMT5 respectively, in GSCs; iii) these modifications regulate Myc stability in an opposite fashion and mark specific cell phenotypes in GBM. Specifically, asymmetrically di-methylated Myc is associated with differentiated (less aggressive) GBM cells, whereas symmetrically di-methylated Myc is typical of a stem-like (more aggressive) phenotype, *in vitro*.
Our future perspective is to thoroughly pinpoint, by a multidisciplinary approach, the specific function of differentially di-methylated Myc species in glioblastoma cells, both *in vitro* and *in vivo*. The obtained results will possibly suggest asymmetrically and symmetrically di-methylated Myc species as novel GBM diagnostic/prognostic markers, for a better classification/stratification of patients. Moreover, the identification of either asymmetrically or symmetrically di-methylated Myc-dependent specific transcripts, will possibly suggest unpredictable, druggable, therapeutic molecular targets for glioblastoma treatment.

O-7: Inhibition of serine hydroxymethyltransferase, a promising target for future chemotherapy intervention

Angela Tramonti[1], Roberto Contestabile[2], Alessandro Paiardini[2], Alessio Paone[2], Francesca Cutruzzolà[2]

[1] Institute of Molecular Biology and Pathology, National Research Council
[2] Department of Biochemical Sciences, University of Rome "Sapienza"

Keywords: cancer metabolic reprogramming, one carbon metabolism, serine hydroxymethyltransferase, enzyme inhibition, enzyme kinetics

Serine hydroxymethyltransferase (SHMT) is a ubiquitous pyridoxal 5'-phosphate (PLP) dependent enzyme catalyzing the reversible transfer of Cβ of serine to tetrahydrofolate (THF), with formation of glycine and 5, 10-methylene-THF. This enzyme is a well-recognized target of cancer research since its activity is critical for purine and pyrimidine biosynthesis and because of its prominent role in the metabolic reprogramming of cancer cells. In the human genome, two genes encoding SHMT are found, respectively expressing a cytosolic (SHMT1) and a mitochondrial (SHMT2) isoform. Moreover, SHMT2 is also expressed as a cytosolic form (SHMT2α) that lacks the mitochondrial import sequence. It is well established that SHMT2 is overexpressed in most human cancer cell types, however also SHMT1 plays a relevant role in some tumours. Most literature on one-carbon fluxes showed that the SHMT1-catalysed reaction goes in the direction of serine synthesis, whereas the reaction catalysed by SHMT2 in the mitochondria goes in the opposite direction, which is that of serine degradation.
Our research group is involved in the biochemical characterization of this important enzyme. In particular, our investigations focus on the different structural, functional and inhibition properties of the two isoforms. For this purpose, the kinetic properties of SHMT1 and SHMT2 have been characterised and compared. Moreover, in the context of a funded AIRC project, we focused on the design and experimentation of specific SHMT1 and SHMT2 inhibitors. First of all, we tested 3-bromopyruvate, a potent anti-tumour agent believed to function by blocking energy metabolism, and we demonstrated that it differentially inactivates human SHMT1 and SHMT2. Moreover, we characterized the inhibition properties of a pyrazolopyran compound 2.12, which was previously reported to inhibit SHMT activity in lung cancer cell lines and to cause apoptosis. These studies provided precious indications for the future design of specific SHMT inhibitors that are able to discriminate between SHMT1 and SHMT2.

O-8: The NeuroNet consortium: a network to investigate molecular mechanisms underlying neuro-pathologies

Paola Fragapane, Elisa Caffarelli, Micaela Caserta, Barbara Illi, Cecilia Mannironi, Luisa Salvatori, Loredana Verdone

Institute of Molecular Biology and Pathology, National Research Council, Rome

Keywords:

Neurological disorders, affecting peripheral and central nervous systems, include a large range of diseases such as epilepsy, Alzheimer's disease and other dementias, cerebrovascular diseases including stroke, migraine and other headache disorders, multiple sclerosis, muscular dystrophy, Parkinson's disease, autism spectrum disease, Amyotrophic Lateral Sclerosis, neuroinfections, brain tumors, trauma- and malnutrition-related disorders.
The research interests of a significant number of IBPM researchers converge on a common theme: the study of the molecular mechanisms underlying normal and pathological neural physiology to identify novel drivers of neurological disorders.
To formalize the interactions among scientists exploring the molecular mechanisms underlying the neuronal physiology and pathology, we decided to establish the **NeuroNet** consortium, which provides the integration and sharing of multi-faceted competences, combined with the use of different *in vitro* and *in vivo* model systems and molecular approaches. Our final goal is the identification of specific macromolecules (DNA, RNA and proteins) that may represent novel diagnostic/prognostic biomarkers and potential targets for different neuro-pathologies.
In addition, the development of this research area will create the conditions to further deepen the knowledge on two biological conditions -stress and inflammation- that, being at the basis of a large number of diseases, are currently receiving increasing attention.

O-9: Neuronal circular RNAs in normal and pathological conditions: the case of autism spectrum disorders

Silvia Gasparini[1], Giorgia Del Vecchio[1], Arianna Rinaldi[1], Ivano Legnini[1], Valerio Licursi[2], Laura Ricceri[3], Maria Luisa Scattoni[3], Carlo Presutti[1], Cecilia Mannironi[4]

[1] Department of Biology and Biotechnology, Sapienza University of Rome
[2] Institute for system analysis and computer science "Antonio Ruberti", CNR, Rome
[3] National Institute of Health, Rome
[4] Institute of Molecular Biology and Pathology, CNR, Rome

Keywords: circRNAs, noncoding RNA, Autism Spectrum Disorder, BTBR, synapse

Circular RNAs (circRNAs) are a naturally occurring family of endogenous non-coding RNAs. (lncRNAs). They are unusually stable molecules with cell type and developmental stage-specific expression patterns. circRNAs highly abundant in the brain, where their expression is controlled during development and by synaptic plasticity. Although the function of most circRNAs is still elusive, their role in the regulation of synaptic functions has been recently proposed. Autism spectrum disorders (ASDs) are a common, highly heritable neuro-developmental condition, with a marked genetic heterogeneity. Although ASD is a clinically heterogeneous syndrome, it is defined by impairments in three core symptoms related to social interaction, language and range of interests. One of the main issues in the comprehension of ASD is how diverse genomic aberrations result in one clinical phenotype. In this regard, it has been recently proposed that ASD-associated mutations target few convergent molecular pathways implicated in the signaling network that controls synapse development and function. Moreover, regulatory RNAs such as microRNAs or lncRNAs have been implicated in ASD. In this work we aim to clarify the role of circRNAs in the etiology of ASD. By deep RNA sequencing, we analyzed circRNAs expression in the hippocampus of the ASD mouse model BTBR T+ Itpr3tf/J (BTBR), compared to C57BL/6J control mice. A number of circRNAs differentially expressed in the BTBR mice have been identified and relevant candidates have been selected. Interestingly, ASD-modulated circRNAs are exonic and they derive from host genes that code for synaptic proteins. We validated their circular structures by higher resistence to the RNAse R exonuclease treatment, compared to linear RNAs. Changes in the expression of selected circRNAs have been confirmed in individual animal samples and analyzed in different brain structures implicated in the disease. Gain of function and loss of function experiments are in progress to clarify of the effect of circRNAs modulation on synaptic functions. Ongoing experiments aimed to validate their potential biological functions will be discussed.

O-10: Molecular mechanisms of memory persistence

CNR:
Nicoletta Corbi[1], Annalisa Onori[1], Elisabetta Mattei[2], Cinzia Pisani[1], Francesca Gabanella[2], Maria Grazia Di Certo[2], Claudio Passananti[1]

[1] CNR-Institute of Molecular Biology and Pathology, Department of Molecular Medicine, Sapienza University, Rome, Italy.
[2] CNR-Cell Biology and Neurobiology Institute, Rome, Italy

CONICET-Buenos Aires
Ramiro Freudenthal, Leila Ameneiro, Agustina Robles, Candela Medina, Veronica de La Fuente, Arturo Romano

Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE), CONICET-Universidad de Buenos Aires, Buenos Aires, Argentina.

Keywords: long term memory, hippocampus, NOR, neuroepigenetics, RNA metabolism

Gene expression and RNA metabolism are key steps in formation of long-term memories. The role of the transcription factor NF-kappaB in memory was initially described by Romano and Freudenthal, both in invertebrates and vertebrates. There is now a significant body of evidence that supports a key role of NF-kappaB in synaptic plasticity/memory and more recently in synaptogenesis. NF-kappaB is present in neurons both at the soma and at the synapse. NF-kappaB functions could be evaluated by its local role in the synapse, as well as participating in synapse to nucleus communication. Che-1/AATF gene product, originally isolated by Passananti and Fanciulli as RNA polymerase II binding protein, has been demonstrated to directly bind the transcription factor NF-kappaB. This Che-1-NF-kappaB interaction prompted us to investigate a putative role of Che-1 in memory processes, focusing on the NF-kappaB dependent task “Novel Object Recognition” (NOR). We found that heterozygous Che-1 "KO" mice show deficiencies in persistent forms of memories. Our ongoing studies point to dissect the molecular mechanisms of memory persistence in which NF-kappaB and Che-1 are involved, focusing on: role on epigenetic regulation of gene transcription; nuclear and local role, looking at protein/protein and protein /RNA interaction.

References

1. Zalcman G, Corbi N, Di Certo MG, Mattei E, Federman N, Romano A. Heterozygous Che-1 KO mice show deficiencies in object recognition memory persistence. Neurosci Lett. 2016 Oct 6;632:169-74.

2. Salles A, Boccia M, Blake M, Corbi N, Passananti C, Baratti CM, Romano A, Freudenthal R. Hippocampal dynamics of synaptic NF-kappa B during inhibitory avoidance long-term memory consolidation in mice. Neuroscience. 2015 Apr 16; 291:70-80.

3. de la Fuente V, Federman N, Zalcman G, Salles A, Freudenthal R, Romano A. NF-κB transcription factor role in consolidation and reconsolidation of persistent memories. Front Mol Neurosci. 2015 Sep 9; 8:50.

4. Federman N, de la Fuente V, Zalcman G, Corbi N, Onori A, Passananti C, Romano A. Nuclear factor κB-dependent histone acetylation is specifically involved in persistent forms of memory.J Neurosci. 2013 Apr 24;33(17):7603-14.

5. Iezzi S, Fanciulli M. Discovering Che-1/AATF: a new attractive target for cancer therapy. Front Genet. 2015 Apr 10; 6:141.

6. Passananti C, Floridi A, Fanciulli M. Che-1/AATF, a multivalent adaptor connecting transcriptional regulation, checkpoint control, and apoptosis. Biochem Cell Biol. 2007 Aug; 85(4):477-83.

7. Fanciulli M, Bruno T, Di Padova M, De Angelis R, Iezzi S, Iacobini C, Floridi A, Passananti C. Identification of a novel partner of RNA polymerase II subunit 11, Che-1, which interacts with and affects the growth suppression function of Rb. FASEB J. 2000 May;14(7):904-12.

O-11: Integrated RNA circuitries in motor neuron differentiation and degeneration

Silvia Biscarini[1,2], Davide Capauto[1,2], Alessio Colantoni[2], Beatrice Salvatori[1], Andrea Carvelli[1,2], Giovanna Peruzzi[1], Tiziana Santini[1], Elisa Caffarelli[3], Irene Bozzoni[1,2,3], Pietro Laneve[3]

[1] Center for Life NanoScience@Sapienza, Istituto Italiano di Tecnologia, Rome
[2] Department of Biology and Biotechnology, Sapienza University of Rome
[3] Institute of Molecular Biology and Pathology of CNR, Rome

Keywords: MN transcriptome, non-coding RNAs, FUS RNA binding protein, post-transcriptional control, Amyotrophic Lateral Sclerosis

BACKGROUND. Motor neurons (MNs) mediate the connections between nervous system (NS) and muscles, and their specific loss underlies severe neuromuscular disorders, such as Amyotrophic Lateral Sclerosis (ALS). RNA dysmetabolism is considered to contribute significantly to ALS pathogenesis, based on the identity of several ALS causative genes. One example is represented by the multifunctional RNA binding protein FUS, which participates to a large degree in RNA processing and whose mutations account for familiar and sporadic cases of ALS. FUS mutations affect gene expression both directly, by gain- and loss-of-function mechanisms, and indirectly, through the crosstalk between different classes of FUS-dependent RNAs.

METHODS. To get insights into the complex MN-specific physiopathological RNA circuitries, we combined an efficient stem cell-based MN differentiation/purification system with Next Generation Sequencing. This allowed us to characterize the whole mouse MN coding and noncoding transcriptome. Moreover, the availability of stem cell lines carrying the murine equivalent (P517L) of a severe human ALS-FUS mutation enabled us to investigate how aberrant FUS impinges on MN RNA landscape.

RESULTS. By integrating bioinformatics and molecular analyses, we highlighted the inverse correlation between deregulated microRNAs and their target mRNAs in FUS^{P517L} MNs. We characterized a regulative axis comprising specific brain-expressed microRNAs and a crucial subunit of glutamate receptors implicated in synaptic transmission and MN degeneration. We also focused on linear and circular long non-coding RNAs (lncRNAs), recognized as critical actors in NS development, function and dysfunction. We identified lncRNAs enriched in MNs, conserved in human, deregulated by FUS mutation and possibly involved in MN differentiation.

CONCLUSIONS AND PERSPECTIVES. Our comprehensive description of the MN transcriptome triggers the identification of RNA molecules that control MN physiological and pathological gene networks. We plan to disentangle the MN regulative circuitries by applying convergent biochemical, molecular and cellular approaches, both *in vitro* and *in*

vivo. Prediction and validation of RNA interactors, visualization of subcellular localization/dynamics of the identified species followed by gene editing and functional analysis will allow us to further clarify the RNA-dependent mechanisms underlying MN differentiation, activity or degeneration.

REFERENCES

1. Chia et al., (2018) Lancet Neurol, 17:94-102.
2. Errichelli et al., (2017) Nat Commun, 8:14741.
3. Biscarini et al., (2018) Stem Cell Res, 27:172-179.
4. Capauto et al., (2018) Mol Neurobiol, [Epub ahead of print].

POSTERS

P1: Investigation of the Aurora-A/TPX2 complex in cell division and cancer

Italia A. Asteriti[1], Francesco D. Naso[1], Valentina Sterbini[1], Elena Crecca[1], Federica Polverino[1], Alessandro Rosa[2], Gianni Colotti[1], Alessandro Paiardini[3], Giulia Guarguaglini[1]

[1] Institute of Molecular Biology and Pathology-CNR, Via degli Apuli, 4, 00185, Rome
[2] Department of Biology and Biotechnology, Sapienza University of Rome
[3] Department of Biochemical Sciences, Sapienza University of Rome.

Keywords: Aurora-A, TPX2, mitosis, cancer, protein-protein interaction inhibitors, chromosomal instability

The Aurora-A kinase controls several mitotic events, and hence chromosome stability. The microtubule binding protein TPX2 regulates Aurora-A activity, stability and localization to the mitotic spindle. Aurora-A and TPX2 are frequently co-overexpressed in human cancers and we proposed that the Aurora-A/TPX2 complex may act as a whole oncogenic unit. ATP-competitive inhibitors of Aurora-A kinase activity are being investigated for therapeutic purposes, yet incomplete selectivity remains a major issue.
To investigate the effects of overexpression of the complex in non-transformed human cells we generated hTERT-RPE-1 cell lines expressing Aurora-A, TPX2 or the whole complex; a deleted version of TPX2 lacking the Aurora-A-interaction region (TPX2Δ43) enables us to discriminate effects that actually depend on complex formation. We observe:
(i) mitotic defects and chromosome mis-segregation events, exacerbated by whole complex overexpression and attenuated with the TPX2Δ43 deletion mutant;
(ii) specific defects at the mitosis-to-interphase transition induced by excess TPX2 *per se*, in an Aurora-A-independent manner.
In parallel, as an innovative approach for Aurora-A inhibition, we search for protein-protein interaction inhibitors that can disrupt the Aurora-A/TPX2 interaction. A virtual screening of small molecules led to the identification of 25 potential inhibitors; *in vitro* experiments confirmed that 4 hits bind Aurora-A in the low micromolar range and compete for TPX2 binding. 2 of those compounds also yield lowered Aurora-A activity and spindle pole defects in cultured osteosarcoma cells. As a follow up we have performed a secondary screening to improve affinity and solubility of compounds; cellular assays with hits selected from in vitro assays are ongoing. The identified protein-protein interaction inhibitors of the Aurora-A/TPX2 complex might represent lead compounds for further development towards pioneering anti-cancer drugs and provide the proof-of-concept for a new exploitable strategy to target mitotic kinases.

P2: Early onset of brain insulin resistance in Down syndrome: a bridge towards the development of Alzheimer pathology

Eugenio Barone[1], Cesira Foppoli[2], Antonella Tramutola[1], Andrea Arena[1], Chiara Lanzillotta[1], Fabio Di Domenico[1], Marzia Perluigi[1]

[1] Department of Biochemical Sciences "A. Rossi-Fanelli", Sapienza University of Rome
[2] Institute of Molecular Biology and Pathology, National Research Council, Rome, Italy

Keywords: Alzheimer disease, Brain insulin resistance, Down Syndrome, Insulin receptor substrate 1, Insulin signaling

Background: A dysregulation of the insulin signaling with reduced neuronal survival and plasticity mechanisms are fundamental abnormalities of Alzheimer Disease (AD) brain. Indeed, brain insulin resistance, condition in which an inadequate response by target cells causes the inability of insulin to increase glucose uptake and utilization, is associated with poor cognitive performance (1) and is driven by the uncoupling of insulin receptor (IR) from its direct substrate (IRS1) (2).
AD pathology is present in individuals with Down Syndrome (DS), most of which develop AD-like dementia by the age of 40s. The high incidence of AD symptoms in DS is thought to be due to the extra copy of Chr21, which encodes some of the genes already demonstrated to be involved in AD pathology, such as APP, however, the precise mechanisms of the AD neuropathology onset in DS remain to be elucidated. Also glucose uptake is impaired in DS: in the adolescence insulin resistance appears (3), without any clinical manifestation and many years before the development of diabetes mellitus, whose incidence is significantly high in DS and may be associated with an increased risk of developing AD (4). For these reasons, DS may provides a model to study the AD pathogenesis and progression.

Objectives: This work aims at gaining new insight into the molecular mechanisms of AD neuropathology in DS, investigating on the age-dependent link between trisomy 21 and aberrant insulin signaling. Considering that DS and AD share many common pathological hallmarks and that ageing is the major risk factor for dementia, we aim to understand the metabolic side of neurodegeneration, i.e. brain insulin resistance, in DS and how it could contribute to the early onset of AD pathology.

Methods: Studies were performed using a transgenic animal model of DS, the Ts65Dn mice, and its corresponding Wt strain. Ts65Dn mice are trisomic for a segment of murine chromosome 16 orthologous to the region of human chromosome 21 that contains much of the genetic material responsible for the DS phenotype and exhibit developmental delay and abnormal behaviors that appear to be analogous to mental retardation in DS patients (5). The analyses were performed in the pre-frontal cortex of Wt and Ts65Dn mice (1, 3, 9 and 18 month-old) and in that of human DS brains, with or without AD. We analyzed expression levels and activation of IR and IRS1 together with its main

downstream targets, specific regulators of the insulin signaling (PTEN and Sirt1) and also changes of APP processing/amyloid-beta production.

Results: We observed increased IR activation together with IRS1 inhibition along with the aberrant activation of either Akt or MAPK in the presence of reduced activity of PTEN and Sirt1 in Ts65Dn mice compared with Wt. These differences among transgenic and control mice were already evident at 1 month of life, thus suggesting that an uncoupling of the insulin signaling occurs early in DS. This picture persists with aging and seems to be independent of amyloid-beta levels. Intriguingly, similar observations were collected in post-mortem brains of DS subjects, with or without AD pathology.

Discussion and conclusion: Our data indicate that markers of brain insulin resistance rise very early in DS and we suggest that this alteration of insulin signaling may contribute to the cognitive impairment that ultimately results in AD.
AD is now recognized to be not solely the end-product of aberrantly processed, misfolded, and aggregated oligomeric amyloid-beta peptides and hyperphosphorylated tau. Other factors, including impairments in energy metabolism, increased oxidative stress, inflammation and insulin resistance in the brain play a role in AD pathology. Recent evidence of a tight correlation between neurodegenerative and metabolic diseases has emerged and the novel idea is that brain insulin resistance represents a key molecular alteration leading to cognitive decline.
In conclusion, an understanding of the link between insulin signaling dysregulation and AD neuropathology might offer novel approaches to modulating the onset and progression of the disorder.

References:

1. De Felice FG; *J Clin Invest*. 2013;123(2):531-9.
2. de la Monte SM, Wands JR; *J Alzheimers Dis*. 2005;7(1):45-61.
3. Fonseca CT et al.; *BMC Endocr Disord*. 2005; 17;5:6.
4. Baglietto-Vargas D et al, *Neurosci Biobehav Rev*. 2016;64:272-87.
5. Holtzman DM et al; *Proc Natl Acad Sci U S A.* 1996;93(23):13333-8.

P3: Bioinformatics@IBPM: atomic level, whole genome scale and the ELIXIR Infrastructure for Bioinformatics

Miriam Carbo[1,2], David Sasah Staid[1,2], Gerda Užubalytė[2], Loredana Le Pera[1,3], Veronica Morea[1], Allegra Via[1]

[1] CNR - Institute of Molecular Biology and Pathology (IBPM)
[2] Department of Biochemical Sciences "A. Rossi Fanelli", Sapienza University, Rome, Italy
[3] CNR - Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies (IBIOM)

Keywords: Bioinformatics; Biological macromolecules; Biological "Omics" data; ELIXIR; Training

ELIXIR (https://www.elixireurope.org/) is an intergovernmental organisation that brings together life science resources from across Europe. These resources include databases, software tools, training materials, cloud storage and supercomputers. The goal of ELIXIR is to coordinate these resources so that they form a single infrastructure. It is the European platform that unites leading life science organizations in managing and safeguarding biological data generated by research.
The ELIXIR Italian Node (ELIXIR-IIB: http://elixir-italy.org/) is led by CNR. The ELIXIR-IIB training platform is coordinated by the IBPM Bioinformatics group.

Collaborations:

- Andrea Ilari, Gianni Colotti, Francesca Degrassi, Pierpaolo Ceci, Patrizia Lavia (IBPM)
- Patrizio Giacomini (IRE-IFO, Rome)
- Gert Vriend (CMBI, Nijmegen, NL)
- Maria Eugenia Schininà (Sapienza University – Department of Biochemical Sciences "A. Rossi Fanelli")

Abstract: see O2, page 16.

P4: The Molecular side of Quadrato Motor Training (QMT)

Micaela Caserta[1], Sabrina Venditti[2], Valerio Vetriani[2], Patrizio Paoletti[3], Tal Dotan Ben-Soussan[3], Loredana Verdone[1]

[1] Institute of Molecular Biology and Pathology, CNR, c/o Sapienza University of Rome
[2] Dept. of Biology e Biotechnology Charles Darwin, Sapienza University of Rome
[3] Research Institute for Neuroscience, Education and Didactics, Patrizio Paoletti Foundation

Keywords: Quadrato Motor Training, proNGF, proBDNF, neuroplasticity, well-being

Neurotrophins are closely related to stress and well-being. Initially synthesized as precursor proteins, they can influence both developing and mature neural circuits, utilizing distinct receptors to mediate divergent neuronal actions, such as neuronal differentiation, synaptogenesis, and synaptic plasticity. Neuroplasticity implies remodeling of neuronal structures, which in turn involves molecular modifications. Maintaining neuroplasticity is an important goal, which can be stimulated through training, by activating molecular mechanisms such as, for example, regulation of growth factors.

The *Quadrato Motor Training* (QMT) is a specifically-structured movement meditation in response to verbal commands, which was found to improve neuronal synchronization (increase alpha, 8-12 Hz) and to increase creativity, reflectivity, attention, as well as neuroplasticity. In comparison to other practices, the QMT has the advantage of being a training of relatively short duration and that can be practiced in limited spaces. These unique aspects render the QMT a technique warranted for scientific exploration, with the future aim of implementing this technique in various health promoting and educational setups.

The aim of the current study is to examine the link between structural and neurotrophic changes following the QMT, providing novel insights regarding the possible underlying neural and molecular mechanisms.

P5: The nucleoporin RANBP2 regulates SUMOylation and function of kinetochore proteins

Michela Damizia[1], Paola Rovella[1], Jessica Bartoli,[1] Annalisa Verrico[1], Erica Di Cesare[1,2], Andrea Musacchio[3], Alessandro Rosa[4], Eugenia Schininà[5], Patrizia Lavia[1]

[1] Istituto di Biologia e Patologia Molecolari (IBPM), CNR, Roma
[2] Department of Pathology, Stony Brook University, Stony Brook, NY, USA
[3] Max-Plank Institute of Molecular Physiology, Dortmund, Germany
[4] Dipartimento di Biologia e Biotecnologie "Charles Darwin", Università La Sapienza, Roma
[5] Dipartimento di Scienze Biochimiche "A. Rossi Fanelli", Università La Sapienza, Roma

Keywords: SUMO, RANBP2, Aurora-B, PLA, mitosis

SUMOylation is a post-translational modification that modulates proteins interaction and localization. Growing evidence implicate protein SUMOylation in control of critical processes during mitosis. Particularly, many kinetochore proteins are potential targets of SUMOylation in vitro reactions. In addition, several de-SUMOylating enzymes reside at kinetochores, suggesting that dynamic cycles of SUMOylation and de-SUMOylation actually take place in mitotic cells to regulate kinetochore function. RANBP2 is a large nucleoporin with SUMO ligase and SUMO stabilizing activity. After mitotic onset and disassembly of nuclear pore complexes, RANBP2 activity is required in several mitotic steps.

To begin to identify RANBP2-guided SUMOylation processes and understand their importance during mitosis, we have developed interactomic studies in mitotic cell populations (see Carbo's poster) and intramolecular proximity ligation assays (PLA) methods. We found that:

a) a subtle mechanism, operated by the nuclear transport receptors Importin Beta and CRM1, regulates the localization of RANBP2 to kinetochores, in space and time;

b) kinetochore-associated RANBP2 in turn regulates Topoisomerase II-alpha SUMOylation and kinetochore accumulation, and hence decatenation of sister kinetochores during segregation;

c) RANBP2 is also required for SUMOylation of the mitotic kinase Aurora-B, which acts in the correction of erroneous kinetochore-microtubule interactions and is therefore essential for balanced chromosome segregation. Indeed, expression of a SUMO-null Aurora-B mutant results in uncorrected chromosome segregation errors. RANBP2 depletion causes a decrease of Aurora-B activity at kinetochores, placing RANBP2 in this control pathway.

Together these results reveal a key role of RANBP2 in local SUMOylation of key mitotic regulators at kinetochores. The failure to ensure spatial and temporal control of this post-translational modification impairs several independent, yet converging, pathways in mitotic control, and can originate genetic instability in daughter cells.

P6: The histone acetyltransferase inhibitor CPTH6 impairs tumor angiogenesis

Marta Di Martile[1], Marianna Desideri[1], Simonetta Buglioni[1], Carla Azzurra Amoreo[1], Donatella Del Bufalo[1], Daniela Trisciuoglio[1,2]

[1] Department of Research, Advanced Diagnostics and Technological Innovation, Regina Elena National Cancer Institute, Rome, Italy
[2] Institute of Molecular Biology and Pathology, National Research Council, Rome, Italy

Keywords:

Protein acetylationis typically catalyzed by enzymes with histone acetyltransferase (HATs) or histone deacetylase (HDACs) activity. To date, it has been identified the involvement of protein acetylation in different tumorigenic signaling events, including angiogenesis. The biological process of neo-vasculature formation from pre-existing blood vessels is widely considered to be an essential process to sustain tumor growth as well as to provide a route for tumor cell metastatization. In this context, the role exerted by HDACs in tumor angiogenesis is well known, whilst the role of HATs is largely unknown. Among molecules with HAT inhibitory activity, the thiazole derivative CPTH6 has been characterized by our group for its antitumor activity in different tumor models, including non-small cell lung cancer (NSCLC). In this study, we assessed the effect of CPTH6 on angiogenesis-related properties of both endothelial and NSCLC cells. The human umbilical vein endothelial cell (HUVEC) and H1299 NSCLC cell lines were used. HUVEC and H1299 morphogenesis was analyzed by plating cells on matrigel and evaluating their ability to organize capillary-like structures. The effect of CPTH6 on protein acetylation was assessed by WB analysis. Transwell supports were employed to evaluate HUVEC migration and invasion. Human Angiogenesis Antibody Array was used to test the conditioned media derived from CPTH6-treated H1299 cells. C57/BL6 and nude mice were used to perform matrigel plug assay and to evaluate tumor growth, respectively. IHC analysis of CD31 was employed to evaluate the number of intra-tumor vessels in tumor xenografts. The HAT inhibitor CPTH6 affected some endothelial cell functions *in vitro*. In particular, CPTH6 impaired HUVEC invasion, migration and differentiation abilities at doses that did not alter proliferation. Although CPTH6 did not affect histone H3 acetylation, it slightly reduced α-tubulin acetylation in HUVEC. In addition, CPTH6 decreased the neovascularization *in vivo*, as evidenced by the impairment of the VEGF-induced vascularization of matrigel plugs. Interestingly, CPTH6 affected also the angiogenesis-related properties of cancer cells. In particular, this compound reduced the ability of H1299 to organize capillary like structures and, conditioned media derived from CPTH6-treated H1299 cells, impaired HUVEC morphogenesis. Accordingly, CPTH6 reduced the secretion of some pro-angiogenic factors (VEGF, EGF, ANG, TIE-2, TNF-α) and, at the same time, increased the release of anti-angiogenic ones (Endostatin, PLG). Finally, in H1299-tumor xenografts, CPTH6 decreased significantly the number of intra-tumor vessels, even though it did not impair tumor growth. Overall, this study adds information to the role of HATs in tumor angiogenesis, and proposes HAT inhibition as an attractive target for antiangiogenic therapy of NSCLC.

P7: Nucleophosmin interactions with protein partners as a target for the treatment of Acute Myeloid Leukemia

Adele Di Matteo[1], Antonella De Cola[2], Pier Antonio Furbetta[2], Serena Rocchio[1,3], Daniele Santorelli[2], Gianni Colotti[1], Maurizio Brunori[3], Carlo Travaglini-Allocatelli[3], Vincenzo De Laurenzi[2], Luca Federici[2]

[1] Institute of Molecular Biology and Pathology, National Research Council, Rome
[2] Department of Medical, Oral and Biotechnological Sciences Ce.S.I. Center for Studies on Ageing University "G. d'Annunzio", Chieti
[3] Department of Biochemical Sciences, “Sapienza” University of Rome

Keywords: Nucleophosmin, nucleolus, nucant, protein-protein interactions, Acute Myeloide leukemia

Nucleophosmin (NPM1) is a nucleus-cytoplasm shuttling protein implicated in processes such as ribogenesis, centrosome duplication, cell cycle control and response to stress. It is one of the main “hub” proteins in nucleoli and, as such, it interacts with a plethora of other proteins including the tumor suppressors p14arf and Fbw7γ, the main E3-ubiquitin ligase of c-MYC [1].
NPM1 is the most frequently altered protein in Acute Myeloid Leukemia (AML). Mutations are located in the NPM1 C-terminal domain and lead to the stable and aberrant delocalization of the protein in the blasts’ cytoplasm [2]. In doing so, both p14arf and Fbw7γ are also delocalized and consequently degraded thus impairing a critical p14arf-HDM2-p53 tumor suppressor axis and leading to c-MYC stabilization [3].
By combining NMR, molecular dynamics, fluorescence spectroscopy and site-directed mutagenesis, we analysed the interaction of NPM1 with p14arf and Fbw7γ at the molecular level and identified critical residues in both NPM1 and protein partners [3,4]. Moreover, the ability of N6L, a positively charged pseudopeptide, to disrupt NPM1 protein-protein associations was tested [5]. The effect of N6L on AML cell lines bearing NPM1 mutations or not was also analysed. Targeting NPM1 interactions, in combination with conventional chemotherapy, may be a novel route for the treatment of AML.

References:

1. Di Matteo A., Franceschini M., Chiarella S., Rocchio S., Travaglini-Allocatelli C., Federici L. (2016). Molecules that target nucleophosmin for cancer treatment: an update. *Oncotarget,* 2016, 7(28):44821-44840.

2. Chiarella S., De Cola A., Scaglione G.L., Carletti E., Graziano V., Barcaroli D., Lo Sterzo C., Di Matteo A., Di Ilio C., Falini B., Arcovito A., De Laurenzi V., Federici L. (2013). Nucleophosmin mutations alter its nucleolar localization by impairing G-quadruplex binding at ribosomal DNA. *Nucleic Acids Res*, 41:3228-39.

3. Di Matteo A., Franceschini M., Paiardini A., Grottesi A., Chiarella S., Rocchio S., Di Natale C., Marasco D., Vitagliano L., Travaglini-Allocatelli C., Federici L. (2017). Structural investigation of nucleophosmin interaction with the tumor suppressor Fbw7γ. *Oncogenesis* 18;6(9):e379.

4. Luchinat E., Chiarella S., Franceschini M., Di Matteo A., Brunori M., Banci L., Federici L.. (2018). Identification of a novel nucleophosmin-interaction motif in the tumor suppressor p14arf. *FEBS J.* doi: 10.1111/febs.14373.

5. De Cola A., Franceschini M., Di Matteo A., Colotti G., Celani R., Clemente E., Ippoliti R., Cimini A.M., Dhez A. C., Vallée B., Raineri F, Cascone I., Destouches D., De Laurenzi V., Courty J. and Federici L. (2018). N6L pseudopeptide interferes with nucleophosmin protein-protein interactions and sensitizes leukemic cells to chemotherapy. *Cancer Lett.* 412:272-282.

P8: The presence of glutamate residues on the PAS sequence of the stimuli-sensitive nano-ferritin improves *in vivo* biodistribution and mitoxantrone encapsulation homogeneity

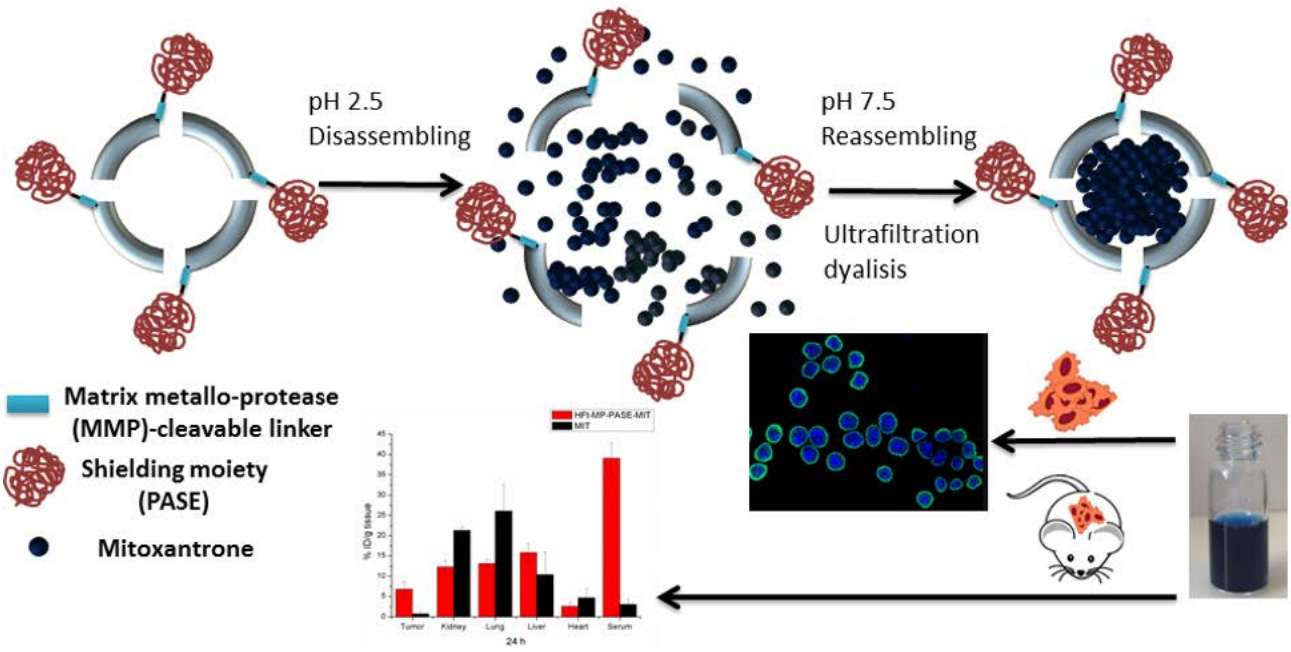
Elisabetta Falvo[1], Francesca Malagrinò[1,2], Alessandro Arcovito[3], Francesco Fazi[4], Gianni Colotti[1], Patrizio Di Micco[2], Martina Pitea[2], Giuseppe Cipolla[2], Alessandro Giuffrè[1], Giulio Fracasso[5], Pierpaolo Ceci[1]

[1] IBPM CNR, Institute of Molecular Biology and Pathology, Italian National Research Council, Rome, Italy
[2] Department of Biochemical Sciences, Sapienza University, Rome, Italy
[3] Institute of Biochemistry and Clinical Biochemistry, Catholic University of Sacred Heart, 00168 Rome, Italy
[4] Department of Anatomical, Histological, Forensic & Orthopedic Sciences, "Sapienza" University, 00161 Rome, Italy
[5] Department of Medicine, University of Verona, 37134 Verona, Italy

Keywords: Protein-cage nanocarrier, pasylated ferritin, mitoxantrone, drug-encapsulation, drug-delivery, cancer

A genetically engineered human ferritin heavy chain (HFt)-based construct has been recently shown by our group to efficiently entrap and deliver doxorubicin to cancer cells. This construct, named HFt-MP-PAS, contained a tumor-selective sequence (MP) responsive to proteolytic cleavage by tumor proteases (MMPs), located between each HFt subunit and an outer shielding polypeptide sequence rich in proline (P), serine (S) and alanine (A) residues (PAS). HFt-MP-PAS displayed excellent therapeutic efficacy in xenogenic pancreatic and head and neck cancer models *in vivo*, leading to a significant increase in overall animal survivals. Here we report a new construct obtained by the genetic insertion of two glutamate residues in the PAS sequence of HFt-MP-PAS. Such new construct, named HFt-MP-PASE, is characterized by improved performances as drug biodistribution in a xenogenic pancreatic cancer model *in vivo*.
Moreover, HFt-MP-PASE efficiently encapsulates the anti-cancer drug mitoxantrone (MIT), and the resulting MIT-loaded nanoparticles proved to be more soluble and monodispersed than the HFt-MP-PAS counterparts. Importantly, *in vitro* MIT-loaded HFt-MP-PASE kills several cancer cell lines of different origin (colon, breast, sarcoma and pancreas) at least as efficiently as the free drug. Finally, our MIT loaded protein nanocages allowed *in vivo* an impressive incrementing of the drug accumulation in the tumor with respect to the free drug.

Acknowledgments: This research was supported by MIUR of Italy (flagship Project "Nanomax" to P.C. and PNR-CNR Aging Program 2012–2014 to A.G.) and Associazione Italiana per la Ricerca sul Cancro - AIRC (I.G. Grant 16776 to P.C.). G.F. gratefully acknowledges Fondazione Cariverona, Verona Nanomedicine Initiative and Italian Minister of Health RF-2010-2305526 for supporting this work.



Graphical abstract

P9: Characterization of extracellular vesicles isolated from cancer cells positive for Human Papilloma Virus

Gianna Fiorucci[1,3], Marco Iuliano[2], Giorgio Mangino[2], Maria Vincenza Chiantore[3], Massimo Tommasino[4], Giovanna Romeo[2]

[1] Institute of Molecular Biology and Pathology, CNR, Rome, Italy
[2] Department of Medico-Surgical Sciences and Biotechnologies, Sapienza University of Rome, Latina, Italy
[3] Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy
[4] Infections and Cancer Biology Group, International Agency for Research on Cancer, Lyon, France

Keywords: Human Papillomavirus, oncoproteins, extracellular vesicles, microRNAs, tumor microenvironment

Human Papillomaviruses (HPVs) are small DNA viruses that can be classified on the basis of their correlation with the development of malignant or benign lesions: mucosal high-risk (HR)-HPVs are the causative agents of squamous cervical carcinoma, whereas low-risk HPVs mainly causes condylomas. The HR-HPV16 persistent infection can lead to cell transformation by accumulation of DNA damages and activation of pro-tumoral genes, since HPV16 E6 and E7 oncoproteins target and inhibit p53 and pRb of the host cell. In addition, the integration of the virus in the host cell genome that cause genetic rearrangements, the activation of proto-oncogenes and other events, can generate genomic instability and increase the risk of neoplastic transformation. However, the infection itself is not sufficient for the transformation of the infected cells.
Extracellular vesicles (EVs), including microvesicles and exosomes, are small membrane vesicles derived from the cellular membrane or from multivesicular bodies and are secreted into the extracellular space by many cell types. EVs convey a collection of bioactive molecules: membrane-derived receptors, proteins, nucleic acids, lipids, carbohydrates and genetic material including mRNA and microRNAs (miRNAs). Emerging evidence demonstrates a role of EVs in a variety of fundamental physiological as well as pathological processes.
We have studied whether the HPV-induced tumors are able to exploit EVs (cargo, dissemination and internalization from different types of recipient cells) to amplify tumor microenvironment (TME) action.
EVs derived from two HPV16 positive cell lines (i.e. CaSki, SiHa) and by human foreskin keratinocytes transduced by E6 and E7 from mucosal HPV16 (K16) have been purified by sequential centrifugation. EVs have been analyzed for their cargo composition by Real Time RT-PCR and for their distribution (i.e. amount and sizes) in the cellular supernatant by cytometric analyses.

Size distribution analysis of EVs indicates the presence of discrete populations. Every cell type shows his own populations of EVs. A different RNA profile characterizes each EV size population, suggesting a specific RNA uptake mechanism.
The analysis of the expression levels of E6 and E7 has shown that they are higher into EVs compared to related producer cells. By studying miRNAs in K16 cells, silenced or not for E6 and E7, we observed a different expression of specific miRNAs, indicating a role of the HPV16 oncoproteins in the EV cargo.
Overall these data could propose an active loading into EVs, according to an EV size-dependent fashion, and suggest a complex mechanism of TME regulation.

P10: Hydrogen sulfide catabolism in cancer: effect of hypoxia

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

[1] Department of Biochemical Sciences, Sapienza University of 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

Keywords: Hydrogen sulfide, catabolism, sulfide:quinone oxidoreductase, hypoxia, colon cancer

Hydrogen sulfide (H_2S) is a gaseous signalling molecule involved in important pathophysiological processes, favouring neo-angiogenesis and stimulating energy metabolism in cancer cells. H_2S is synthesised by three different enzymes and catabolized by a mitochondrial sulfide-oxidizing pathway. Here, we investigated the effect of hypoxia, a common factor of tumoral microenvironment, on H_2S catabolism in colon cancer. The model cells SW480 were exposed to either normoxic (20% O_2) or hypoxic (1% O_2) conditions for 24 hours and their ability to metabolize H_2S at the mitochondrial level was assayed by high resolution respirometry. Exposure to hypoxia resulted in reduced mitochondrial mass and overall lower H_2S-metabolizing activity, intriguingly associated with enhanced expression of the H_2S-consuming mitochondrial enzyme sulfide:quinone oxidoreductase (SQR). These hypoxia-induced metabolic changes may represent an adaptive mechanism to ensure higher H_2S levels with pro-survival effects on cancer cells.

P11: Looking for Inhibitors of Human H_2S-synthesizing Enzymes by Orthogonal Methods

Karim Zuhra[1,2,3], Pedro M.F. Sousa[4], Francesca Malagrinò[1,2], Giulia Paulini[1,2], Ana Rita Lemos[4], Zenta Kalme[5], Imants Bisenieks[5], Egils Bisenieks[5], Brigita Vigante[5], Gunars Duburs[5], Tiago M. Bandeiras[3,4], Luciano Saso[6], Alessandro Giuffrè[2], João B. Vicente[3]

[1] Department of Biochemical Sciences, Sapienza University of 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
[4] Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
[5] Latvian Institute of Organic Synthesis, Riga, Latvia
[6] Department of Physiology and Pharmacology "Vittorio Erspamer", Sapienza University of Rome, Italy

Keywords: Hydrogen sulfide, cystathionine β-synthase, cystathionine γ-lyase, 3-mercaptopyruvate sulfurtransferase, inhibitors

Hydrogen sulfide (H_2S), a key signalling molecule in human physiology, is produced by the human enzymes cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (MST). Several lines of evidence suggest a correlation between increased H_2S production and human diseases, such as several cancer types and amyotrophic lateral sclerosis. Identifying selective and potent inhibitors may therefore be beneficial for future therapeutic interventions. Here, recombinant CBS, CSE and MST were purified, and 31 pyridine derivatives were synthesized and screened for their ability to bind and inhibit these enzymes. Using a combination of orthogonal techniques, such as differential scanning fluorimetry (DSF), surface plasmon resonance (SPR), circular dichroism (CD) spectropolarimetry, and activity assays based on fluorimetric and colorimetric H_2S detection, two compounds sharing the same molecular scaffold were found to weakly inhibit both CBS and CSE. Here, while presenting a robust methodological platform for screening putative inhibitors of the three human H_2S-synthesizing enzymes, we highlight the importance of employing complementary methodologies to identify artifacts in the drug discovery process.

P12: Merosin-deficient Congenital Muscular Dystrophy type 1A (MDC1A): an innovative therapeutic strategy based on Zinc Finger Artificial Transcription Factors (ZF-ATFs)

Annalisa Onori[1], Cinzia Pisani[1], Maria Grazia Di Certo[2], Georgios Strimpakos[2], Francesca Gabanella[2], Irene Carrozzo[1], Elisabetta Mattei[2], Yaffa Beck[3], Nicoletta Corbi[1], Claudio Passananti[1]

[1] Istituto di Biologia e Patologia Molecolari, CNR – Roma
[2] Istituto di Biologia Cellulare e Neurobiologia, CNR – Roma
[3] Zingenix Ltd. - Tel Aviv - Israel

Keywords: Congenital Muscular Dystrophy Type1A, Lama2, Lama1, ZF-ATF, AAV viral vectors

Congenital Muscular Dystrophy Type1A (MDC1A) is an autosomal recessive disease caused by mutations in the *LAMA2* gene that encodes the extracellular protein laminin-α2 (Lama2). Laminin-α2 is required for the formation of heterotrimeric laminin-211 and laminin-221, which are the major constituents of skeletal muscle basal lamina. Mutations that result in complete loss of lama2 function result in severe neuromuscular dysfunction, whereas mutations that result in partial loss of function are associated with less severe disease. There are currently no effective treatments or cures for MDC1A. Laminin-111, a form of laminin found in embryonic skeletal muscle, can substitute for the loss of laminin-211/221 and prevent muscle disease progression. Supported by the predicted ability of the embryonic laminin alpha 1 isoform (Lama1) to functionally replace Lama2 function, we propose to up-regulate/reactivate Lama1 gene in MDC1A congenital disorder using zinc finger artificial transcription factors (ZF-ATFs). To construct novel ZF-ATF genes, we compared human and mouse Lama1 gene promoters focusing on two highly conserved regions containing stretches with 100% of human/mouse homology. The human Zif268 natural transcription factor was used as gene backbone to produce seven novel Lama1-ZF-ATF genes (LZifs) by introducing only few amino acid substitutions. All LZif genes, were individually cloned in the adeno-associated virus vector pAAV, giving rice to pAAV-LZif-1 to 7. The battery of the pAAV-LZif-1 to 7, renamed: "DO, RE, MI, FA, SOL, LA, SI" were tested to select the best performing genes. The selection criteria were based on their ability to specifically bind Lama1 promoter in the context of native chromatin infrastructure and to re-activate/promote transcription. We are analysing the effects of the selected “mi” and “si” genes in different cell lines, including MDC1A patient-derived myoblasts.
Moreover, we delivered by AAV the LZifs constructs “mi” and “si” in wt mice and we detected a strong and muscle-specific expression of both genes, thanks to the use of AAV-serotype 8. These experiments precede the studies in the appropriate MDC1A mouse animal model.

P13: Synthesis and antioxidant activity of tyrosol derivatives present in the residues of olive oil processing

Alessandra Ricelli[1], Giuliana Righi[1], Veronica Bonfantini[2], Ludovica Primitivo[1], Martina De Angelis[1], Luciano Saso[3], Sandra Incerpi[4], Fabio Gionfra[4], Paolo Bovicelli[1]

[1] Institute of Molecular Biology and Pathology-CNR, Rome
[2] Dept. of Chemistry, "Sapienza" Univ., Rome
[3] Dept. of Physiology and Pharmacology V. Erspamer, "Sapienza" Univ., Rome; Dept. of Sciences, Roma Tre Univ., Rome

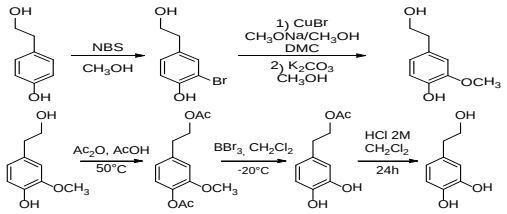
Keywords: Waste water, tyrosol derivatives, antioxidant activity, THP-1 cells

Introduction

Olive oil contains a huge concentration of polyphenols, but also waste water of olive oil processing (WW) contain a significant amount of these chemical compounds (e.g. tyrosol). The polyphenols in WW are pollutants, so their disposal is regulated by law. Tyrosol has a mild antioxidant action, but some of its derivatives, such as hydroxytyrosol and homovanillic alcohol, can have a much higher antioxidant activity. We developed a strategy to synthesize the phenethyl alcohol hydroxytyrosol (HT) and homovanillic alcohol (HA) from tyrosol. HT and HA were assayed for their ability to control the generation of reactive oxygen species (ROS) in human leukemic monocytes (THP-1 cells).

Experimental methodology

- Tyrosol is converted in HA, in turn, HA is converted in HT through a sequence of bromuration-methoxylation-demethylation steps [2] (Scheme 1).



Scheme 1. Conversion of tyrosol in HA and in HT

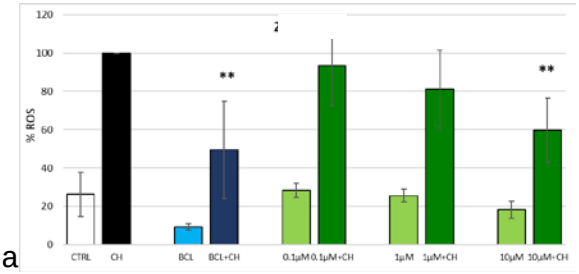
- ROS determination was carried out by the evaluation of the oxidized form of dichlorofluorescein diacetylate ($DCFH_2$-DA) in THP-1 cells stimulated with cumene hydroperoxyde (CH) and treated with increasing concentrations (0.01mM, 0.1mM, 1mM, and 10mM) of HA and HT. $DCFH_2$-DA was detected by fluorimetric reading (λex 498, λem 530).

Results and discussion

OA and HT have been proven to significantly prevent the formation of ROS in leukemic monocytes cells THP-1 (Fig. 1a, 1b).

The inhibiting effect is dose-related and is more evident in HT than in HA. This could be due to an higher reactivity of HT compared to HA.

a



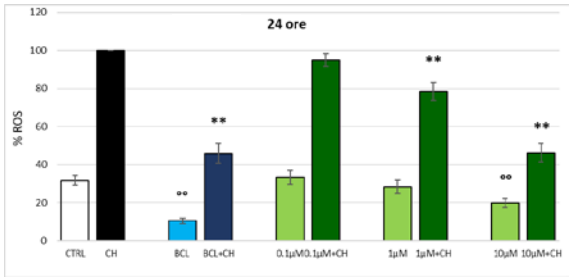


Fig. 1 a, b Effect of HA (a) and HT (b) treatment on ROS generation in THP-1 cells stimulated with (CH) and incubated at 37°C for 24 hours. Data analyzed by ANOVA test.

P14: Stereocontrolled synthesis of pyrrolidine iminosugars

Giuliana Righi[1], Martina De Angelis[2], Ludovica Primitivo[2], Paolo Bovicelli[1]

[1] CNR-IBPM Dipartimento di Chimica, Sapienza Università di Roma
[2] Dipartimento di Chimica, Sapienza Università di Roma

Keywords: iminosugar; polyhydroxylated pyrrolidines; 1,4-dideoxy-1,4-imino-D-galactitol; 1,4-dideoxy-1,4-imino-D-glucitol; asymmetric dihydroxylation

Polyhydroxylated pyrrolidine and piperidine alkaloids, often called azasugars or iminosugars, are known as specific and competitive inhibitor of glycosidase and glycosyltransferase. Thanks to these properties they can be used for therapeutic applications, in particular in the treatment of some disease as diabetes, inflammation, cancer, viral and bacterial infections.

The first pyrrolidine iminosugar (2R,5R)-bis(dihydroxymethyl)-(3R,4R)-dihydroxypyrrolidine (DMDP) [1] was isolated in 1976 from *Derris elliptica* leaves, a legume present in Southeast Asia and southwest Pacific islands. It had already showed good activity as antiviral and glicosidase I inhibitor.

Afterwards *Stemona tuberosa*, a Chinese flowering plant from *Stemonaceae* family, was already famous in antiquities for its therapeutic effects. It was included in the 50 fundamental herbs of traditional Chinese medicine; from this plant was isolated DGDP (2,5-dideoxy-2,5-imino-D-glucitol).

Since then, lots of compounds structurally similar, were found in many plants and microorganisms (DAB-1, LAB-1, CYB-3) and identified for their interesting therapeutic properties.

Instead, the iminosugar 1,4-dideoxy-1,4-imino-D-galactitol, plays a weakly role in α-glycosidase inhibition [2] and is the first known inhibitor of *E. coli* K12 UDP-Gal mutase and of the galactan micobacterial biosynthesis. Its inhibitory actions are higly specific and they could represent a new therapeutic strategy for the treatment of micobacterial infection as leprosy and tuberculosis [3] (Fig. 1).

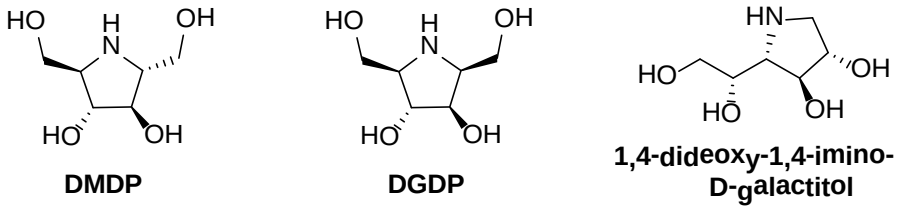
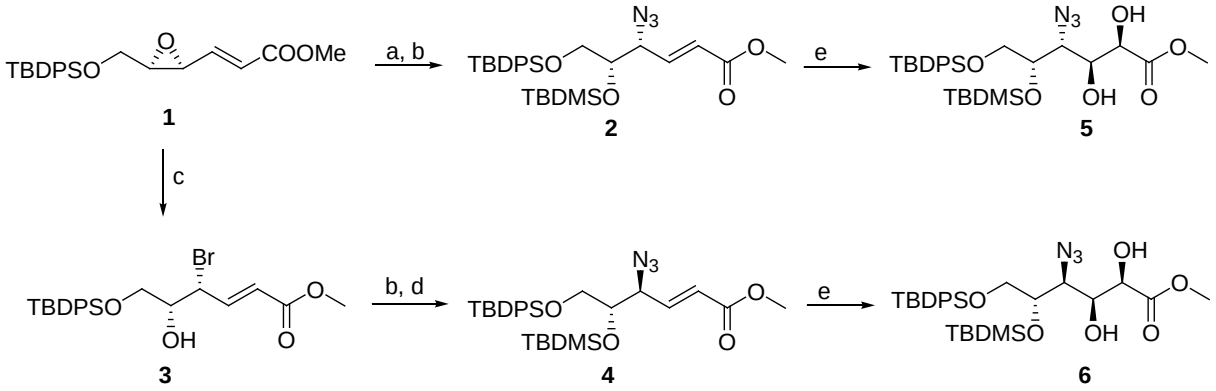
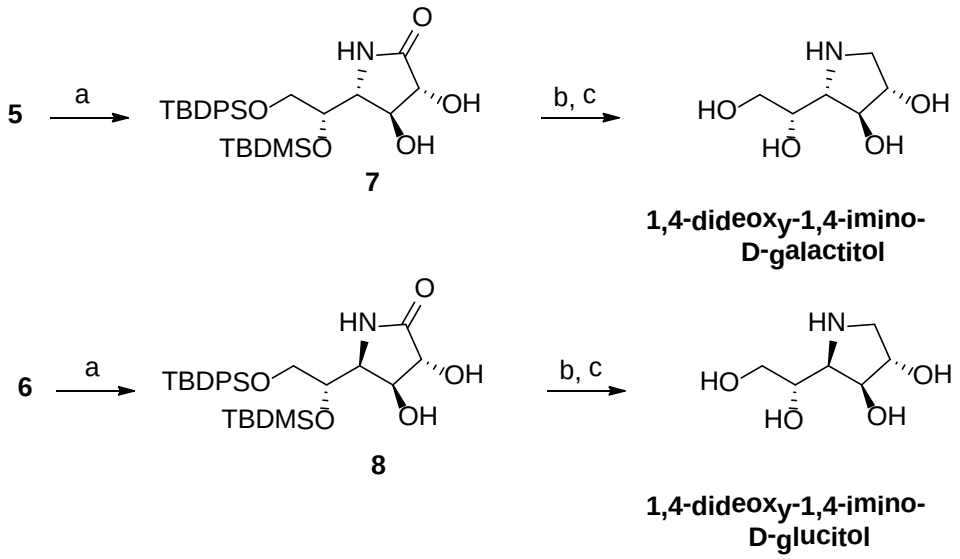


Figure 1 Pyrrolidine iminosugars structures

A synthetic strategy has been developed.using the results obtained from our precedent studies concerning the asymmetric dihydroxylation reaction on optically active azido alchols **2** and **4** [4]. This synthetic way has allowed us to obtain the 1,4-dideoxy-1,4-immino-D-galactitol [5] and its diastereisomer 1,4-dideoxy-1,4-imino-D-glucitol [6] starting from the same precursor, the epoxyde **1**, easily achievable from commercially *cis*-but-2-en-1,4-diol (Scheme 1).



Scheme 1. a) $TMSN_3$, BF_3, CH_2Cl_2, rt, >90%; b) TBSOTf, 2,6-lutidine, CH_2Cl_2, r.t., 74%; c) LiBr, Amb.15, acetone, r.t., >90%); d) NaN_3, dry DMF, r.t., >90%; e) OsO_4, NMO, $(DHQ)_2PHAL$, H_2O/acetone, r.t., >90%.



Scheme 2. a) PPh_3, THF, r.t., 85%; b) BH_3-SMe_2, THF, r.t., 68%; c) HCl (37%), MeOH, 70 °C, 92%.

A suitable planning of the synthetic steps has made possibile to prepare the azido vinyl alchols **2** and **4**, through regio and stereocontrolled opening of epoxide ring **1.** Afterwards, **2** and **4**, separately subjected to asymmetric dihydroxylation reaction, have given key intermediates **5** and **6**.

Finally compounds **5** and **6**, for treatment with PPh_3, have given directly five-membered heterocyclic rings **7** and **8**. The synthetic process ends with a reduction of amidic function and final deprotection, thereby achieving desired iminosugars with only ten steps and with an overall yield of 22%.

References:

1. Wrodnigg TM. *Monatshefte für Chemie*, **2002**, 133, 393-426.
2. Sadhu PS, Santoshi A, Rao VJ. *Bull. Korean Chem. Soc.* **2012**, 33, 3554-3558.
3. Pham Huu DP, Gizaw Y, BeMiller JN, Petruš L. *Tetrahedron* **2003**, 59, 9413-9417.
4. Righi G, Bovicelli P, Tirotta I, Sappino C, Mandic' E. *Chirality* **2016**, 28, 387-393.
5. Lundt I, Madsen, R. Synthesis **1993**, 720-724.
6. Fleet G W J, Son JC. *Tetrahedron Lett.* **1988**, 44, 2637-2640.

P15: Nitric oxide induces DNA damage and a reversible shift from symmetric to asymmetric division of Glioblastoma stem cells

Luisa Salvatori[1], Rosaria Anna Fontanella[2], Cinzia Fionda[3], Helena Stabile[3], Annarita Favia[1], Fiorella Scagnoli[1], Sergio Nasi[1], Barbara Illi[1]

[1] CNR, Institute of Molecular Biology and Pathology, Rome, Italy
[2] Dept of Anatomy, Histology, Forensic Medicine and Orthopedics, Sapienza University of Rome, Rome, Italy
[3] Dept of Molecular Medicine, Sapienza University of Rome, Rome, Italy

Keywords: glioblastoma multiforme, glioblastoma stem cells, nitric oxide, differentiation, DNA damage

Glioblastoma multiforme (GBM) is the most frequent primary malignant brain tumor in adults and presents a very aggressive course being associated with high mortality and recurrence. According to the tumor stem cell concept, tumorigenicity, as well as resistance to conventional therapy, may reside in the small subset of Glioblastoma stem cells (GSC). Therefore, depletion of GSC could have profound therapeutic implications.
Nitric oxide (NO) is a small diffusible molecule involved in a variety of signaling pathways, and cellular responses are differentially regulated by specific NO levels. At high concentrations NO acts as a potent anti-cancer agent which negatively regulates proliferation, induces apoptosis, radio- and chemosensitization in malignant glioma cells and increases permeability of the blood brain barrier. Therefore, exogenous NO administration may provide a new promising adjuvant treatment for GBM. Currently, very scarce information are available about the role of NO in brain cancer stem cells. In the attempt to identify novel strategies to target GSC, we evaluated the effects of NO on patient-derived GSC.
Our preliminary results showed that 100 µM of the NO donor diethylenetriamine/nitric oxide adduct (DETA/NO) progressively inhibited GSC self-renewal ability and cell proliferation, within 7 days. Interestingly, at longer times of treatment, tested over a time course up to 21 days, the number of viable cells did not change, whereas the mortality showed a slight increase. These findings led us to hypothesize that NO is able to induce a shift from symmetric to asymmetric GSC division, which is partial within 7 days of treatment and becomes total at longer times. Therefore, starting from 7 days the number of proliferating GSC remains unchanged whereas a portion of differentiated, not proliferating, cells progressively appears and in a short time dies in these unfavourable culture conditions. This is consistent with the observation that the levels of PCNA, which is involved in DNA replication, as well as of stemness markers SOX2 and Nestin, decreased after 7 days of NO treatment. The strong decline of cell proliferation was linked to the inhibition of cell-cycle progression, whose predominant feature is a S-phase arrest which reaches a maximum after 3 days of NO exposure and is reduced but still present after 7 days. During S phase, replication can cease in response to DNA damage. Actually, NO induces the activation of the sensor of DNA damage H2AX histone and the

expression of the effector protein RAD51 which has a central role in DNA repair. On the contrary, cell cycle arrest does not involve a p21-dependent mechanism. Interestingly, after 7 days of NO exposure a portion of the S phase-arrested cells can recover cycling progression after DNA repair, as confirmed by the level of γH2AX which returns similar to controls. However, the level of PCNA, lower than in control GSC, suggests the presence of a mixed population of cells, namely proliferating GSC and survived cells that are committed to differentiation and do not replicate. NO induced growth inhibition was not irreversible and GSC may quickly recover the symmetric proliferative capacity after treatment suspension. Interestingly, NO seems also able to reduce the proliferative ability of GSC upon differentiation conditions. Taken together, our findings suggest that although NO administration does not completely deplete GSC it may control the expansion of the stem cell pool in the tumor while promoting GSC differentiation. NO could also reduce GSC tumorigenic potential. Therefore, prolonged NO exposure could delay the appearance of GBM relapses and improve patient outcomes.

P16: The kinase HIPK2 regulates spastin protein: implications in Hereditary Spastic Paraplegia (HSP)

Francesca Sardina[1], Davide Valente[1,2], Manuela Ferrara[1], Alessandra Pisciottani[1], Silvia Soddu[2], Cinzia Rinaldo[1,2]

[1] Institute of Molecular Biology and Pathology-CNR, Rome, Italy
[2] Regina Elena National Cancer Institute, Rome, Italy

Keywords: HIPK2; spastin; microtubules dynamic; neurodegeneration; HSP

The alteration of axonal-transport is an early and causal event in many neurodegenerative diseases (ND). Among the mechanisms contributing to axonal-transport defects, the loss of microtubules dynamism is one of the key mechanisms. Spastin is a microtubule severing protein involved in cytokinesis and in axonal-transport. Mutations in SPG4 gene encoding spastin, occur in 40% of HSP, an autosomal dominant ND. Recently, it has been shown that to increase spastin levels rescues the pathological phenotypes in HSP-patient-derived cells suggesting that intervening to modulate spastin levels may be a valid therapeutic strategy in ND characterized by spastin misregulation. However, spastin regulation is largely unknown.
We found that spastin is regulated by the kinase HIPK2 in neural compartment. HIPK2 depletion leads to spastin downregulation in a proteasome-dependent manner and impairs axonal-transport. Wild-type-HIPK2 overexpression, but not kinase-defective-HIPK2, increases spastin levels and rescues axonal transport defects in spastin-deficient motorneurons. Mechanistically, we showed that HIPK2 phosphorylates spastin at S268. This phosphorylation stabilizes spastin and prevents its polyubiquitination and proteasome degradation. These results, in addition to expanding our understanding of the HIPK2/spastin axis in neural compartment, might provide the basis for the development of a new therapeutic approach to treat HSP.

P17: The novel Resveratrol derivative 3,4,4'-trimethoxystilbene induces cancer cell death by targeting gamma-tubulin

Gianandrea Traversi[1], David Staid[2], Mario Fiore[2], Zulema Percario[1], Roberto Antonioletti[2], Veronica Morea[2], Francesca Degrassi[2], Renata Cozzi[1]

[1] Dipartimento di Scienze, Università "Roma TRE", Viale G. Marconi 446, 00146 Roma
[2] Istituto di Biologia e Patologia Molecolari CNR, Via degli Apuli, 4, 00185 Roma

Keywords: resveratrol analogues, tubulin polymerization, centrosome fragmentation, cancer cell growth, gamma tubulin

Stilbene is a structural scaffold abundantly present in nature and stilbene-based compounds have been widely reported for their biological activity. In the recent years, resveratrol and its natural stilbene-containing derivatives have been extensively investigated for their chemotherapeutic activity against a wide variety of cancers. The synthetic manipulation of the stilbene scaffold has led to new analogues with improved anticancer activity and better bioavailability, depending on the type and position of substituents on the stilbene skeleton.

In the present study we investigated the anticancer activity of a novel trimethoxystilbene (3,4,4'-trimethoxylstilbene) where two methoxyl groups are adjacent on the benzene ring (ortho configuration) and compared its activity to 3,5,4'-trimethoxylstilbene, where the two methoxyl groups are separated (meta configuration). Here, we provide evidence that the presence of the two methoxyl groups in the ortho configuration renders 3,4,4'-TMS more efficient in inhibiting cell proliferation and producing apoptotic death in colorectal cancer cells. Confocal microscopy of α and γ tubulin staining show that the novel compound strongly depolymerizes the mitotic spindle and produces centrosome fragmentation. Computer assisted docking studies show that both molecules potentially interact with γ tubulin, with a slightly better score for 3,4,4' TMS.

These findings confirm that the methylation of resveratrol leads to profound changes in the mode of action. Ortho configuration results more potent in killing tumor cells possibly through a stronger interaction with gamma tubulin. This finding should be taken into consideration when conducting structure optimization studies.

LATE ABSTRACTS

A1: HAT3 and ATHB4, members of the HD-ZIPII family, act downstream of SPATULA and HECATE, bHLH transcription factors, to coordinate auxin distribution and symmetry transition in the *Arabidopsis* gynoecium

Monica Carabelli[1], Laila Moubayidin[2], Luana Turchi[1], Lars Østergaard[2], Ida Ruberti[1]

[1] Institute of Molecular Biology and Pathology, National Research Council, P.le A. Moro 5, 00185 Rome, Italy
[2] John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK

Keywords: *Arabidopsis* gynoecium; bilateral and radial symmetry; plant development; b-HLH and Hd-Zip II transcription factor

The gynoecium develops initially as a bilaterally symmetric structure due to its origin as two fused leaves, but immediately prior to formation of the style, the distal end undergoes a symmetry transition, from bilateral to radial. It was previously shown that this bilateral-to-radial symmetry transition requires dynamic auxin redistribution mediated by two bHLH transcription factors, INDEHISCENT (IND) and SPATULA (SPT). We have recently shown that in the regulation of this process, SPT also interacts with members of the Homeodomain-Leucin zipper (HD-ZIP) II family, such as HOMEOBOX ARABIDOPSIS THALIANA 3 (HAT3) and ARABIDOPSIS THALIANA HOMEOBOX 4 (ATHB4). Moreover, SPT controls the balance of two antagonistic plant hormones, cytokinin and auxin, at the gynoecium apex by interacting with other bHLH proteins, HECATE1 (HEC1), HEC2, and HEC3. These interactions lead to a build-up of precisely located auxin maxima while restricting sensitivity to cytokinin. By means of multiple genetic combinations, expression pattern analysis, pharmacological treatments, and tissue-specific complementation experiments, our data show that SPT and HECs synergistically control the expression of the shade-avoidance effectors HAT3 and ATHB4 in white light conditions to sustain the formation of a coherent auxin ring, with radial symmetry, at the gynoecium apex. Our findings show a fine regulation of light response genes mediated by different key organ regulators that ultimately balances between auxin and cytokinin to allow correct symmetry establishment and transition.

A2: A *Drosophila* model for Congenital Disorder of Glycosylation type 2e.

Anna Frappaolo[1], Stefano Sechi[1], Roberta Fraschini[2], Angela Karimpour Ghahnavieh[1], François Foulquier[3], Michael Tiemeyer[4], Maria Grazia Giansanti[1]

[1] Consiglio nazionale delle Ricerche, Istituto di Biologia e Patologia Molecolari, Università Sapienza di Roma
[2] Dipartimento di Biotecnologie e bioscienze, Università degli studi di Milano Bicocca
[3] Université des Sciences et Technologies del Lille 1
[4] Complex Carbohydrate Research Center, University of Georgia, Athens, GA 30602, USA

Keywords: Congenital Disorders of Glycosylation, Golgi trafficking, glycosylation, *Drosophila*, neurological impairment

Congenital Disorders of Glycosylation (CDG) comprise a family of human diseases caused by mutations in genes required for synthesis of glycoconjugates. More than 100 distinct forms of CDGs have been identified and more than 80% of these diseases display severe neurological and neuromuscular impairment. CDGs comprise two large groups. Type I CDGs affect the synthesis of the dolichol-linked oligosaccharide, the major precursor of N-linked glycoproteins, and its transfer to acceptor proteins. Type II CDG (CDG-II) diseases disrupt either the processing of N-linked glycans, the biosynthesis of O-linked oligosaccharides or the addition of glycans to lipids. The COG complex mediates tethering of vesicles carrying glycosylation enzymes across the Golgi cisternae. It has been proposed that the COG complex orchestrates retrograde Golgi traffic acting as a protein interaction hub. However a full elucidation of the functional interplay between the COG proteins and the vesicle trafficking machinery is lacking. Mutations affecting human COG1, COG2, COG4-COG8 cause monogenic forms of inherited, autosomal recessive, CDGs-II. Typical clinical manifestations of COG-CDGs include psychomotor delay, epileptic seizures, general hypotonia and myopathy and failure to thrive. Most of the published studies, focused on clinical and biochemical characteristics of COG-CDG, documented the N-linked and O-linked glycosylation defects associated with this disease. Yet it is unknown how these abnormalities cause the neuromuscular aspects of CDGs and there is no effective therapy for this disorder.
Animal models can help to study the correlation between COG-dependent glycosylation defects and malfunction at the neuromuscular junction. We have generated a *Drosophila* COG7-CDG model which closely parallels the pathological characteristics of COG7-CDG patients including pronounced neuromotor defects associated with altered N-glycome profiles. The *Drosophila* Cog7-CDG disease model that we developed, with its powerful genetic tools, together with the COG-CDG patients' cells, will offer unique opportunities to study the molecular mechanisms underlying COG-CDGs and will suggest novel therapeutic strategies.

A3: Leucocyte Telomere Length (LTL) as a biomarker in Huntington's Disease

Daniela Scarabino[1], Liana Veneziano[2], Martina Peconi[3], Marina Frontali[2], Rosa Maria Corbo[1,3], Elide Mantuano[2]

[1] CNR Institute of Molecular Biology and Pathology, Rome, Italy
[2] CNR Institute of Translational Medicine, Rome, Italy
[3] Department of Biology and Biotechnology, Sapienza University, Rome, Italy.

Keywords: Huntington's disease (HD), neurodegeneration, CAG repeat expansion, leucocyte telomere length, telomere erosion

Huntington's disease (HD) is an autosomal dominant, fully penetrant, neurodegenerative disease caused by an expanded CAG repeat in the first exon of the HTT gene. The onset of symptoms most commonly occurs at midlife and inversely correlates with the CAG repeat expansion. However, age of clinical onset, progression rate, and severity of symptoms can vary between individuals (1). Leukocyte telomere length (LTL) has been widely investigated in neurodegenerative diseases such as Alzheimer's and Parkinson diseases (2,3), but very few data on LTL in HD have been reported (4). In the present preliminary study, we investigated the relationship between LTL and HD development, including pre-manifest and manifest HD patients.

LTL (T/S ratio) was measured in a sample of manifest HD patients (HD) or pre-manifest HD (pre-HD) and compared with LTL of age-matched controls. Significant LTL differences among controls, pre-manifest HD and manifest HD subjects were observed ($p<0.0001$), with mean LTL values in the following order: manifest HD < pre-manifest HD < controls (Fig. 1). After adjusting LTL for age, the differences in LTL across the three groups remained highly significant ($p<0.0001$) as shown in Fig. 2.

Current data indicate that shortened LTL are observed in HD patients as found in other neurodegenerative disorders. The analysis of LTL in premanifest-HD patients (never examined to date) suggests that a progressive telomere erosion may occur in the pre-manifest stage. Present data seem to indicate that LTL could have some of the characteristics of the ideal biomarker of HD progression (5). It can be obtained by blood sampling and, in our experience, it was readily quantifiable and highly reproducible. LTL showed in premanifest HD subjects a very significant linear relationship with the estimated years to HD clinical onset and could provide a prediction of the time at clinical diagnosis with good probability levels.

References:

1. Gusella JF, MacDonald ME, Lee JM. Genetic modifiers of Huntington's disease. Mov Disord. 2014; 29:1359-65.

2. Boccardi V, Pelini L, Ercolani S, Ruggiero C, Mecocci P. From cellular senescence to Alzheimer's disease, The role of telomere shortening. Ageing Res. Rev. 2015; 22:1-8.

3. Scarabino D, Broggio E, Gambina G, Corbo RM. Leukocyte telomere length in mild cognitive impairment and Alzheimer's disease patients. Exp Gerontol. 2017; 98:143-147.

4. Kota LN, Bharath S, Purushottam M, Moily NS, Sivakumar PT, Varghese M, Pal PK, Jain S. Reduced telomere length in neurodegenerative disorders may suggest shared biology. J Neuropsychiatry Clin Neurosci. 2015; 27:e92-6.

5. Weir DW, Sturrock A, Leavitt BR. Development of biomarkers for Huntington's disease. Lancet Neurol. 2011; 10:573-90.

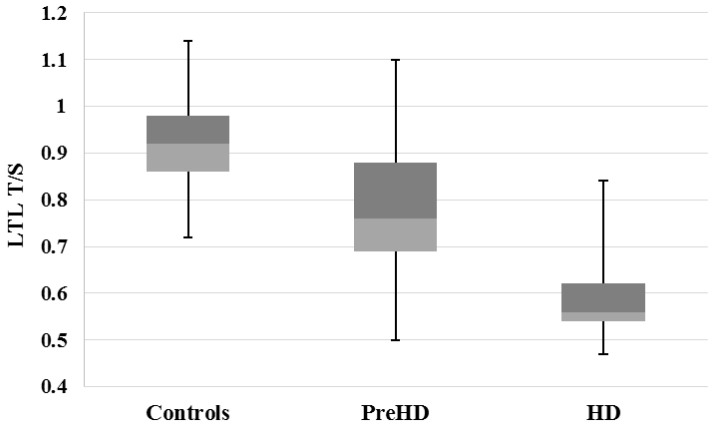


Fig. 1 Mean LTL (T/S ratio) in controls, pre-manifest HD and HD patients.

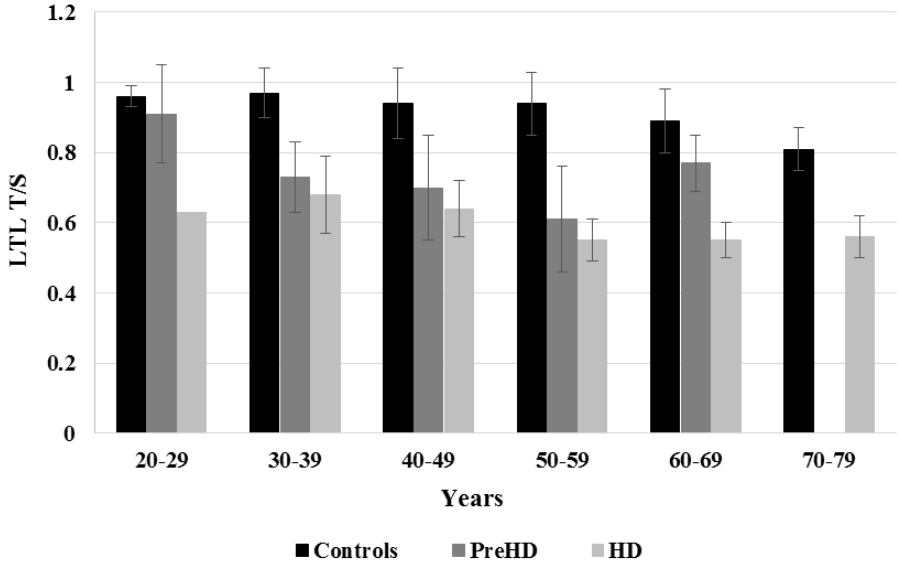


Fig. 2 LTL (T/S ratio) in controls, premanifest HD subjects and HD patients by age class (mean ±SD).

A4: The roles of the oncoprotein GOLPH3 in cytokinesis and cell proliferation

Stefano Sechi[1], Anna Frappaolo[1], Angela Karimpour Ghahnavieh[1], Agnese Berducci[1], Roberta Fraschini[2], Michael Tiemeyer[3], Luisa Capalbo[4], Pier Paolo D'Avino[4], Maria Grazia Giansanti[1]

[1] Consiglio nazionale delle Ricerche, Istituto di Biologia e Patologia Molecolari, Università Sapienza di Roma
[2] Dipartimento di Biotecnologie e bioscienze, Università degli studi di Milano Bicocca
[3] Complex Carbohydrate Research Center, University of Georgia, Athens, GA 30602, USA
[4] Department of Pathology, University of Cambridge, UK

Keywords: cytokinesis, GOLPH3, tumorigenesis, Golgi trafficking, glycosylation

Golgi phosphoprotein 3 (GOLPH3), has been characterized as a Phosphatidylinositol 4-Phosphate [PI(4)P] effector at the Golgi, required for vesicle trafficking and Golgi glycosylation. Encoded by a gene on chromosome 5p13, GOLPH3 is frequently amplified in several solid tumor types including breast cancer, melanoma and lung cancer. Moreover GOLPH3 overexpression is correlated with poor prognosis in multiple cancer types. It has been suggested that upregulation of GOLPH3 may promote tumorigenesis by enhancing activity of the mammalian target of rapamycin (mTOR). However the molecular pathways through which GOLPH3 acts in malignant transformation require further investigation.

We believe that dissecting the signaling pathways that involve GOLPH3 in cytokinesis and cell proliferation, will clarify the molecular mechanisms underlying its oncogenic activity. We are investigating the molecular mechanisms underlying GOLPH3 function in cytokinesis and GOLPH3 cell cycle-dependent phosphorylation changes. To gain further insight into GOLPH3 function in vesicle trafficking, we are also analyzing the molecular interactions of GOLPH3 with regulators of endocytic/secretory pathways. Because the oncogenic activity of GOLPH3 might be linked to altered Golgi glycosylation we are using *Drosophila* to analyze the GOLPH3-dependent glycosylation effects in the whole organism by applying multi-dimensional ion trap mass spectrometry. Finally we are using the sophisticated genetic tools offered by the *Drosophila* model system to explore the relationship of GOLPH3 with the TOR signaling and how this may influence cell growth and proliferation.

A5: Identification of a novel molecular network involving the PSORS1 locus gene CCHCR1 and its relevance in *psoriasis vulgaris*

Cinzia Pisani[1], Francesca Gabanella[2], Annalisa Onori[1], Maria Grazia Di Certo[2], Francesca Di Rosa[1], Nicoletta Corbi[1], Claudio Passananti[1]

[1] CNR-Institute of Molecular Biology and Pathology, Department of Molecular Medicine, Sapienza University, Rome, Italy
[2] CNR-Cell Biology and Neurobiology Institute, Rome, Italy.

Keywords: *psoriasis vulgaris*, CCHCR1, HLA-C, Psors1, Hax1, Psors4

Psoriasis is a chronic immune-mediated skin disease inherited as a complex genetic trait. The region of the MHC on chromosome 6 that encompasses HLA-C, corneodesmosin (CDSN) and CCHCR1 (coiled-coil alpha-helical rod protein 1) is named PSORS1, and strongly correlates with psoriasis. Three psoriasis-associated susceptibility alleles HLA-C*w0602, CCHCR1*WWCC and CDSN*5 have been identified within this PSORS1 region, but strong linkage disequilibrium has made it difficult to distinguish their individual genetic effects. Among them, the risk-allele CCHCR1 *WWCC is reported to be expressed up to 60% of psoriatic patients; however little is known about the pathogenic role of CCHCR1 common gene product and related polymorphisms in psoriatic inflammation. Our previous studies on CCHCR1 protein suggest its involvement in vesicular docking/trafficking and its interaction with other molecules with a potentially pathogenic role in psoriasis. In this context, we are studying the patho-physiological role of CCHCR1 common gene and related-polymorphisms. In particular, using the anti-CCHCR1 rabbit polyclonal antibody, produced in our laboratory, we are studying CCHCR1 protein/protein and protein/mRNA interactions. We characterized the novel and un-published CCHCR1 interaction with the RNA binding protein Hax1. Mutations in Hax1 gene result in autosomal recessive severe congenital neutropenia, also known as Kostmann disease. Hax1 maps inside the PSORS4 locus on chromosome 1q21.3, it is up-regulated in psoriasis and is involved in RNA metabolism. We have identified novel transcripts and proteins interacting with both CCHCR1 and Hax1 and that could be potentially involved in the pathogenesis of psoriasis, unveiling a previously unrecognized molecular network.
Our ongoing plan is to evaluate whether CCHCR1-mediated molecular interactions are different in the presence of the psoriasis associated CCHCR1*WWCC allele, and what is the functional impact of the newly identified molecular network in psoriatic keratinocytes. To this end, a comparison of gene expression profiles between psoriatic keratinocytes carrying the risk CCHCR1*WWCC and common alleles will be performed.

References:

1. Tervaniemi MH1,2, Katayama S3,4, Skoog T3, Siitonen HA1,2, Vuola J5, Nuutila K6, Sormunen R7, Johnsson A8, Linnarsson S8, Suomela S9, Kankuri E6, Kere J1,2,3,4, Elomaa O1,2. NOD-like receptor signaling and inflammasome-related pathways are highlighted in psoriatic epidermis. Sci Rep. 2016 Mar 15;6:22745. doi: 10.1038/srep22745.

2. Asumalahti K, Veal C, Laitinen T, Suomela S, Allen M, Elomaa O, Moser M, de Cid R, Ripatti S, Vorechovsky I, et al. (2002). Coding haplotype analysis supports HCR as the putative susceptibility gene for psoriasis at the MHC PSORS1 locus. *Hum Mo .Genet.* 11, 589-597.

3. Corbi N, Bruno T, De Angelis R, Di Padova M, Libri V, Di Certo MG, Spinardi L, Floridi A, Fanciulli M, Passananti C (2005). RNA Polymerase II subunit 3 is retained in the cytoplasm by its interaction with HCR, the psoriasis vulgaris candidate gene product. *J Cell Sci*. 118, 4253-4260.

4. Ling YH, Wong CC, Li KW, Chan KM, Boukamp P, Liu WK (2014). CCHCR1 interacts with EDC4, suggesting its localization in P-bodies. *Exp Cell Res*. Sep 10;327(1)

5. Mirmohammadsadegh A, Tartler U, Michel G, Baer A, Walz M, Wolf R, Ruzicka T, Hengge UR (2003). HAX-1, identified by differential display reverse transcription polymerase chain reaction, is overexpressed in lesional psoriasis. *J Invest Dermatol*. 120,1045-1051.

6. Suomela S, Kainu K, Onkamo P, Tiala I, Himberg J, Koskinen L, Snellman E, Karvonen SL, Karvonen J, Uurasmaa T, Reunala T, Kivikäs K, Jansén CT, Holopainen P, Elomaa O, Kere J, Saarialho-Kere U (2007). Clinical associations of the risk alleles of HLA-Cw6 and CCHCR1*WWCC in psoriasis. *Acta Derm Venereol.* 87,127-34.

7. Tiala I, Wakkinen J, Suomela S, Puolakkainen P, Tammi R, Forsberg S, Rollman O, Kainu K, Rozell B, Kere J, Saarialho-Kere U, Elomaa O (2008). The PSORS1 locus gene CCHCR1 affects keratinocyte proliferation in transgenic mice. *Hum Mol Genet.* 17, 1043-1051.

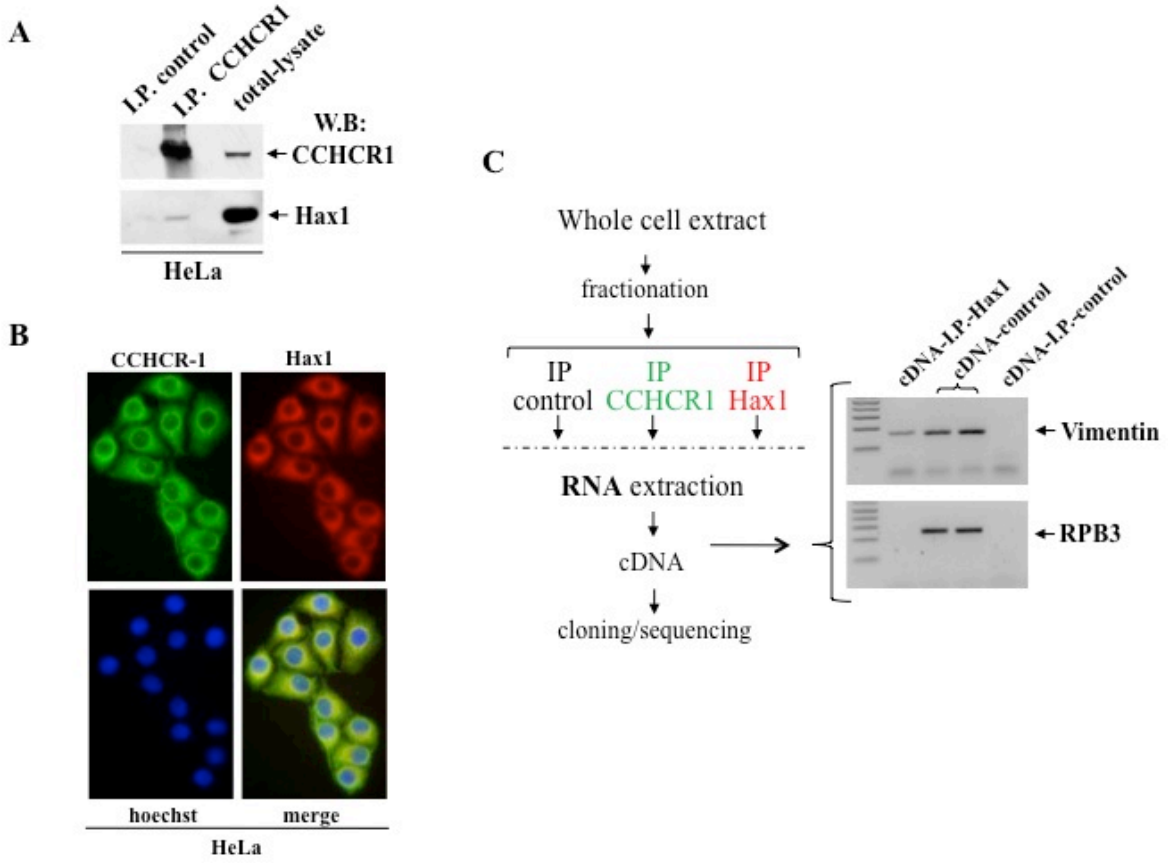


Figure legend.

CCHCR1 and Hax1 protein and mRNA interactions. ***A:*** *Whole extract from HeLa cells was immuno-precipitated with rabbit polyclonal anti-CCHCR1 antibody and with a control unrelated antibody. Co-immuno-precipitation was analyzed by Western blot using anti-Hax1 monoclonal antibody.* ***B:*** *Dual-label indirect immunofluorescence performed in HeLa cells with the anti-CCHCR1 rabbit polyclonal antibody and the anti-Hax1 monoclonal antibody to visualise the immunolocalisation of endogenous CCHCR1 (green) and Hax1 (red). Extensive co-localization (yellow) between CCHCR1 and Hax1 is visualised by the merged-color image. Nuclei were stained with Hoechst (blue).* ***C:*** *Schematic representation of RIP assay. HeLa cells extract, enriched in the appropriate cell fraction, is immuno-precipitated using either anti-CCHCR1 rabbit polyclonal or anti-Hax1 rabbit polyclonal. Unrelated antibody/protein G-agarose beads was used as a control (IP Control). On the right, semi-quantitative RT-PCR analysis of RIP assay output. Vimentin and RPB3 transcripts were used as RIP first validation.*

A6: The Omomyc case: a model strategy for inhibiting master transcription factors involved in human diseases

Fiorella Scagnoli, Mauro Savino, Annarita Favia, Barbara Illi, Sergio Nasi @ IBPM CNR
Silvia Galardi, Silvia Ciafrè @ Tor Vergata University
Maria Parizia Mongiardi, Andrea Levi @ IBCN CNR
Giulio Pavesi @ University of Milano

Keywords:

Omomyc is a small protein derived from MYC – a transcription factor deregulated in over 50% of human cancers. It effectively interferes with MYC oncogenic functions, preventing growth and triggering regression of a variety of tumors in model systems. The development of agents able to directly target MYC - like Omomyc - is our chief goal, and may represent a great leap forward.
To this end, we investigate the mechanism of Omomyc oncosuppressive function at the epigenomic level by employing doxycycline-inducible Omomyc expression in cells from human glioblastoma – the most frequent and lethal brain tumor – and in particular the glioblastoma stem cells, with the following results:

- MYC and Omomyc associate to the methylosome, a protein complex including the MEP50 cofactor and the arginine methylase PRMT5, which has multiple roles in development, cancer, and neurodegenerative diseases. Both MYC and Omomyc, alone, affect PRMT5 methylase activity with a potential impact on global gene expression, since specific arginine methylations in histones and Sm ribonucleoproteins regulate both transcription and splicing. We find that both MYC and Omomyc also control transcript termination by influencing, through PRMT5, RNA Polymerase II arginine methylation and recruitment of SMN.
- Omomyc inhibits the glycolytic reprogramming, which contributes to the tumor capacity to grow in a hypoxic environment. This occurs because Omomyc alters the transcriptional response to hypoxia – mediated by HIF transcription factors – finely controlling the expression of a HIF1-regulated gene subset.
- Glioblastoma stem cells are resistant to therapies and responsible for tumor regeneration. Their stemness features are quickly inhibited by Omomyc, which also hinders tumor formation in xenografts by sustaining differentiation and acting on microenvironment and angiogenesis. Omomyc dimers directly bind to the genome – preferentially at sites occupied by MYC, which is largely replaced - and interact with effector complexes of MYC activity. This causes a deep resetting of gene expression control nodes, which inhibits the master TF of stemness, supports the activity of differentiation and oncosuppression effectors, and tunes up a number of non-coding RNAs directed at molecules implicated in tumor growth and invasiveness.

Our work may lead to the development of Omomyc itself or molecules targeted by Omomyc as anti-cancer agents. It also suggests that similar, structure based strategies may provide alternative therapeutic systems directed at other transcription factors implicated in human diseases.

Acknowledgements: *Thanks to Luisa Salvatori for encouraging words.*

UNDER THE PATRONAGE OF

Consiglio Nazionale delle Ricerche

SPONSORS

ZEISS

We make it visible.

Nikon

SIAL

Bio-Fab research

Servizi di Supporto alla Ricerca