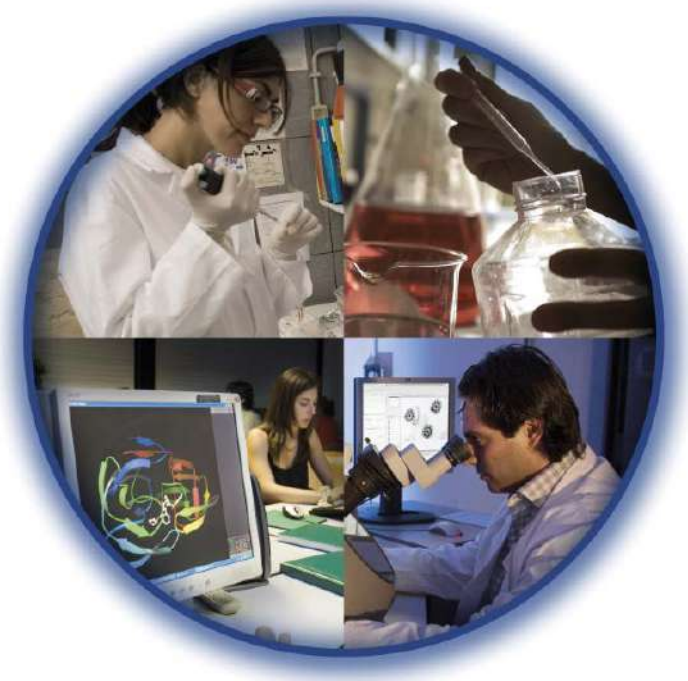
ISTITUTO PASTEUR ITALIA
FONDAZIONE CENCI BOLOGNETTI

2017 Annual Report



ISTITUTO PASTEUR ITALIA
FONDAZIONE CENCI BOLOGNETTI

Istituto Pasteur Italia – Fondazione Cenci Bolognetti
Viale Regina Elena 291 00161 Roma
pasteuritalia@istitutopasteur.it

CONTENTS

FOREWORD

The **Istituto Pasteur Italia - Fondazione Cenci Bolognetti**, the italian member of the Institute Pasteur International Network (33 institutes worldwide), is a private *non-profit* foundation established according to the terms of the bequest of princess Beatrice Cenci Bolognetti with the purpose to create a Center of Biomedical Research with the same mission and values of *Institut Pasteur* in Paris.

The **Istituto Pasteur Italia** research activity is committed to biomedicine, with particular references to infectious diseases, drug design, molecular medicine extended to frontiers therapy (e.g. cancer immunotherapy; therapy of genetic diseases; regenerative medicine). The **funding of research projects** is possible thanks to the income from the donated real estates and thanks also to donations from citizens. In 2017 the Institute has invested a total of 955.000 euros to fund **high level research projects in different areas** (microbiology, virology, molecular genetics, molecular recognition in proteins and nucleic acids, cellular and molecular immunology as well as biology of malaria) and to support brilliant young researchers with **fellowships** (to return to work in Italy or as a follow up of their PhD).

Istituto Pasteur invested in the Research Projects on I**mmunotherapies for Cancer and Infectious Diseases** carried on at **Laboratorio Pasteur Italia,** at its third year of establishment and directed Dr. John Hiscott. The Group supervised by Hiscott made important progresses on 1. The Characterization of antiviral and adjuvant properties of RIG-I agonists 2. The Metabolic regulation of the innate immune response in dengue virus infection 3. The individuation of Combination strategies in the development of oncolytic immunotherapy. The studies resulted in high quality publications (i.e. *Journal of Immunology and Trends in Immunology).*

13 “three-year Research projects” funded and carried out in affiliated laboratories of Departments of Sapienza University were also concluded in 2017. Such projects had been selected in 2014 following a call for applicants. In order to ensure **scientific rigor and excellence**, all the funded research projects were selected through an international peer review process. The researchers have focused on the study of therapies for the treatment of infectious diseases, metabolic diseases, genetic diseases, cancer and neuromuscular degenerative disorders.

Istituto Pasteur Italia also carried on still ongoing **collaborations with the International Network of Pasteur Institutes** (33 Institutes worldwide). This is possible thanks to funding of the Institut Pasteur of Paris for projetcs such as the Programmes Transversaux de Recherche. These Programs bring together researchers, engineers and technicians within the Paris campus and the International Network and work towards a shared discovery research goal, also to develop synergy and allow further collaborations.

The scientific excellence reached over 2017 is demonstrated by high quality publications in peer-reviewed scientific journals, for a cumulative impact factor: 533 . At the end of 2017, Istituto Pasteur Italia has launched a new call for applicants for 22 new studies to be carried out at Sapienza starting from 2018.

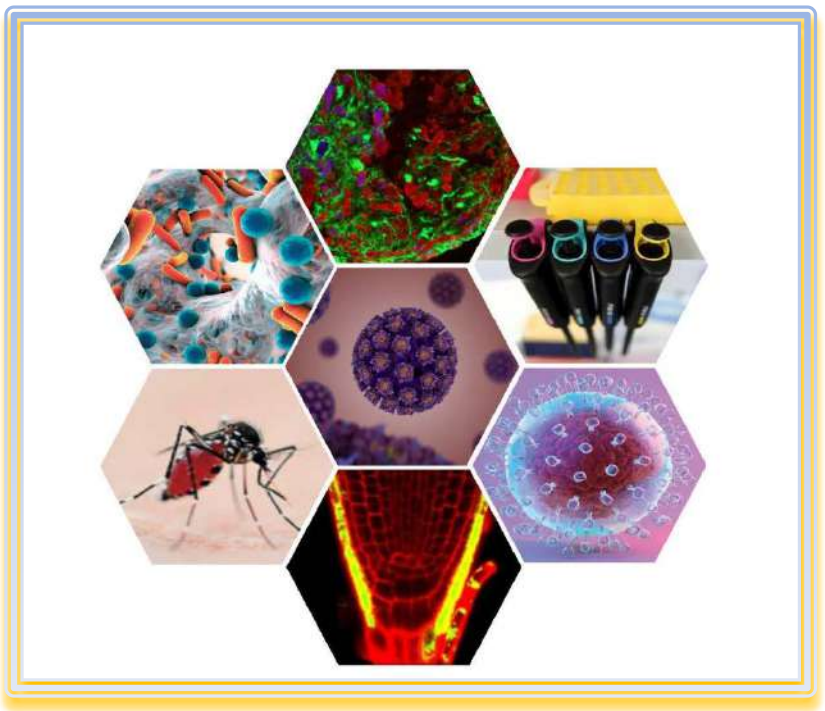
Last but not least, the Institute has as always also been active in promoting **educational programs** and **scientific communication.** First and foremost we hosted the **III international Course on Persistent Viral Infections and Immune Evasion** organized in collaboration with the Pasteur Institute of Paris. Moreover, the Institute has carried on a well-established **educational project** for secondary schools involving a book series coupling Science with Comics as well as meetings and practical activities with students, so they can learn to appreciate the importance of science.

This Annual Report documents the results obtained during the year 2017 thanks to the enthusiasm and the effort of the Italian "Pasteur" community.

Luigi Frati
President

Angela Santoni
Scientific Director

RESEARCH PROJECTS



Research carried out at

- Laboratorio Pasteur Italia
- Sapienza University (affiliated laboratories)

IMMUNOTHERAPIES FOR CANCER AND INFECTIOUS DISEASES

Laboratori Pasteur Italia
Director of Research: John Hiscott

IMMUNOTHERAPIES FOR CANCER AND INFECTIOUS DISEASES

JOHN HISCOTT

Istituto Pasteur Italia – Fondazione Cenci Bolognetti
@: john.hiscott@istitutopasteur.it

This research program focuses on the early events involved in the host response to RNA virus infection, with the long-term objective to utilize knowledge of the immune response against virus infection to develop novel immunotherapeutic approaches for the treatment of infectious diseases and cancer. Our goal is relevant for the translational development of novel antiviral and adjuvant compounds to augment immunity against diverse viral pathogens, including influenza, dengue, and chikungunya. This objective is also important for the development of oncolytic virus therapies for cancer, since defects in innate antiviral signaling in tumor cells contribute to the selective growth of replicating oncolytic viruses in cancer versus normal tissues. Below the main research themes of the laboratory are summarized.

1. Characterization of antiviral and adjuvant properties of RIG-I agonists

The cytosolic innate sensor RIG-I senses 5'triphosphate containing RNA to initiate a potent antiviral immune response against RNA virus infection, activating both interferon and inflammatory responses, through TBK1/IRF3 and IKK/IκBα/NF-κB pathways, respectively. RIG-I activation can also trigger apoptosis, thus limiting viral replication and spread, independently of induction of the antiviral program. Previously, our group developed and characterized a sequence- and structure-optimized RIG-I agonist (M8) that stimulated a robust innate immune response against viral infection. Furthermore, it was shown that M8 acted as a potent vaccine adjuvant against influenza, leading to high antibody titers and Th1-shift in immune responses. During the past year, we tested M8 as a cancer immunotherapeutic by taking advantage of its dual ability to induce cell death and activate innate immunity. Our results demonstrate in different cancer cell models that stimulation of the RIG-I pathway by M8 induced immunogenic cell death and maturation of dendritic cell function.

In multiple cancer cell lines, M8 treatment activated RIG-I signaling, leading to interferon and inflammatory cytokine upregulation and cancer cell apoptosis, dependent on activation of NOXA, caspase 9/3 cleavage, and Poly ADP ribose polymerase cleavage.

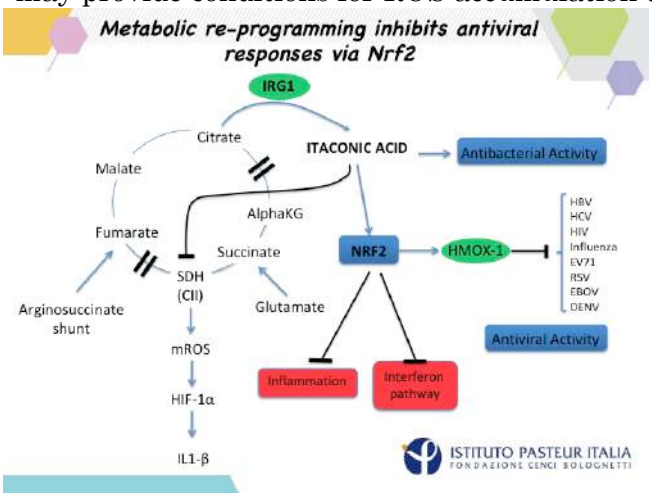
Furthermore, M8-induced inflammatory activation in tumor cells was sufficient to mature dendritic cells (DCs) and induced chemokine synthesis. Additionally, direct effects of M8 on DCs included upregulation of costimulatory molecules (CD80, CD86), strong induction of inflammatory chemokines CXCL9 and CXCL10, and upregulation of Th1 cytokine IL-12. Th1-biasing activity was further validated with *in vitro* stimulation assay of Ag specific T cells. Next we analyzed whether M8 treated cells showed the typical markers of immunogenic cell death by flow cytometric staining of calreticulin and HMGB1 release by ELISA. M8 treated cells showed high level expression of calreticulin on cell surface, as well release of HMGB1 at a level comparable to the immunogenic cell death inducer mitoxantrone. Altogether, these results highlight the potential of the RIG-I agonist M8 in cancer immunotherapy, and as a complementary strategy in combination with immune checkpoint inhibitors. This project is progressing with the collaboration of BioNTech/TRON (Mainz) who are formulating M8-nanoparticles for delivery, and with Bristol Myers Squibb who are testing M8 in combination with immune checkpoint inhibitors to evaluate synergistic anti-tumor efforts in a variety of tumor models.

We are also investigating the crosstalk between RIG-I signaling and activation of the DNA sensing cGAS-STING pathway. Both RIG and STING pathways stimulate immunogenic cells death and contribute significantly to tumor-specific cell death mechanisms, as highlighted in Zevini et al, 2017. The mechanisms of cross-talk between the innate cytosolic sensors RIG-I and STING and their relative contributions to antiviral immunity and myeloid differentiation are currently being investigated. Preliminary results from our laboratory indicate that activation of the RIG-I pathway also induces STING expression in an IRF3/NF-kB dependent manner, thus eliciting a functional amplification/cross-talk between both pathways. Our hypothesis is that STING activation is a critical component of the amplification of the host antiviral response and changes in STING expression may alter cellular function during myeloid differentiation.

2. Metabolic regulation of the innate immune response in dengue virus infection

Dengue virus (DENV) is a mosquito-borne virus that causes dramatic public health issues in more than 100 countries, with an estimate of 390 million people infected annualy. Given the elevated levels of oxidative stress markers in patients with severe dengue infection, we sought to analyze the relationship between reactive oxygen species (ROS) and DENV infection. Previously, we demonstrated a positive correlation between ROS production and viral replication in DENV-infected monocyte-derived dendritic cells (MDDCs). We now show that DENV-infected MDDCs are defective in activating the antioxidant defense system and demonstrate the transcription factor Nrf2, a master regulator of the cellular response to oxidative insults, actively translocated into the nucleus upon infection, without upregulating antioxidant gene transcription. The concomitant use of chemical NRF2 activators during infection improved DENV-induced NRF2 nuclear import, but not the expression of heme oxygenase, one of the most highly

upregulated NRF2 target genes. To understand the role of NRF2, we studied DENV infection in human lung epithelial A549 cells that contain a loss-of-function mutation in KEAP1, the natural inhibitor of NRF2. Although the antioxidant response was constitutively active in A549, DENV infection still caused ROS accumulation. Using WT, Keap1-knockout (Keap1 $^{-/-}$), and Nrf2-knockout (Nrf2 $^{-/-}$) A549 cells, DENV infection and replication were facilitated in Nrf2 $^{-/-}$ cells, whereas in Keap1 $^{-/-}$ cells, DENV infection was strongly inhibited. These results suggest that DENV infection manipulates the NRF2-dependent antioxidant response to modulate viral replication in MDDC and epithelial cells. The loss of the antioxidant gene expression during the DENV infection may provide conditions for ROS accumulation that aggravate DENV pathogenesis.



The link between the innate antiviral immune response and changes in Krebs cycle metabolism is also being investigated; it has recently been shown that the metabolic by-product itaconate induced Nrf2 expression and interfered with inflammatory and antiviral gene expression via the Nrf2 antioxidant pathway; treatment with itaconate or the chemical Nrf2 inducer sulforaphane repressed STING expression as well as other interferon stimulated genes-ISGs (see Figure 1). These preliminary studies link metabolic re-programming and Krebs cycle by products with the induction of the Nrf2 anti-oxidant pathway and inhibition of the antiviral response.

3. Combination strategies in the development of oncolytic immunotherapy

Our laboratory has for more than ten years explored a novel virus-based approach to immunotherapy of cancer, involving oncolytic viruses that replicate to high titers in tumor tissue, resulting in immune-mediated and virus-induced lysis. Although virotherapy with the prototype Vesicular Stomatitis Virus (VSV) is often effective against a variety of cancer cells, many primary tumor cells are resistant to VSV oncolysis. We were the first group to demonstrate that resistant tumor cells are sensitized to VSV-mediated killing in combination with epigenetic modulators -histone deacetylase inhibitors (HDI) - in a variety of resistant cancers. HDIs represented reversible chemical switches that dampen the innate antiviral response and improve the susceptibility of resistant cancer cells to VSV infection and spread. *(Nguyen et al, Proc. Natl. Acad. Sci. USA 2008; Samuel et al, Mol Ther. 2010; 2013; Shulak et al, J. Virol. 2014; Beljanski et al, Biol. Chem. 2015).* Despite significant progress in OV

immunotherapy, the heterogeneity of the clinical response to OV-based therapies, as well as the engagement of the adaptive immune response against viral rather than tumor antigens, represent significant obstacles to the large-scale clinical implementation of oncolytic virotherapy against multiple types of cancer. We have continued to investigate the role of autophagy in regulating synergism between the HDI vorinostat and oncolytic VSV in prostate cancer, using human PC3 and DU145 cells and a murine model bearing TRAMP-C2 mouse prostate cancer cells.

To address the role of specific HDACs to impinge viral infection and output, the capacity of different HDAC inhibitors to augment VSV replication and oncolysis was evaluated in human prostate cancer (PC-3) cells that display significant resistance to VSV□51-mediated cell killing. The effect of Tubastatin A (TBSA), an HDAC6 specific inhibitor, or RGFP109, a specific HDAC 1/3 inhibitor, or Resminostat (Resm), a well-known HDAC 1/3/6 inhibitor, were compared to the ability of Vorinostat (SAHA) to potentiate VSV infectivity, as quantified GFP positive cells. HDAC6 inhibition was sufficient to enhance viral infection, as demonstrated by an increase in the number of VSV-infected cells (11 to 62% with TBSA) but did not increase cell death in the VSV infected population. In contrast, combined treatment that included SAHA or Resminostat, increased cancer cell death to 60-80%. To assess the involvement of the intrinsic apoptotic pathway in mediating VSV-induced cell death, apoptotic gene expression was evaluated in cells treated with SAHA, RESM or TBSA and infected with VSV. BH3-only pro-apoptotic genes Puma and Noxa were upregulated in HDIs pretreated cells, while a strong impairment of anti-apoptotic Mcl1 gene expression was observed in SAHA and RESM-treated infected cells, but not in TBSA-treated infected cells.

SAHA treatment induced a strong cell cycle block in PC-3 cells, accompanied by a reduction in S-phase and accumulation in G2/M-phase. To address a possible role for the cell cycle arrest in the enhanced VSVΔ51 infectivity of PC-3 cells treated with SAHA, the effect of the different HDIs on cell proliferation was determined. Comparative analysis demonstrated that RESM reduced the percentage of S-phase cells and accumulated cells in G2/M-phase; furthermore, SAHA and RESM treatments led to upregulation of p21 and p16/INK4A cyclin dependent kinases at the protein level. Conversely, TBSA treatment did not exert the same effect, suggesting that simultaneous inhibition of HDAC 1/3/6 was responsible for cell cycle arrest. SAHA and RESM strongly activated IkB degradation, as well as enhanced autophagic flux as evidenced by turnover of p62 and increased lipidated LC3B II accumulation. Either SAHA or RESM pretreatment was able to induce the production of pro-inflammatory cytokines, such as IL6 and IL8, in VSV-infected cells. Taken together, these features are characteristic of a senescent-associated secretory (SASP) phenotype, suggesting that a SASP-like phenotype may be implicated in the increased susceptibility to VSV infection in SAHA and RESM-treated PC-3.

Using the highly metastatic Tramp-C2 cell model as a representative of aggressive prostate tumors, we analyzed efficacy of Vesicular Stomatitis Virus (VSV) with a special focus on immune activation in immunocompetent mice. VSV intratumoral injection led to infection of both cancer and immune cells (especially monocytes and macrophages) within tumor microenvironment, as assessed by flow cytometry at 24h after injection. Upon VSV treatment, rapid immune changes were observed in the spleens of treated mice with a marked increase of myeloid subpopulations and a decrease of NK and T cells, thus highlighting a potent reshaping of immune system upon VSV treatment. Moreover, injection of VSV at $500x10^6$ PFU was sufficient to block tumor growth of subcutaneously implanted Tramp-C2 tumors. Furthermore, the block in tumor progression was associated with a strong increase of T cell tumor infiltration, particularly cytotoxic CD8+ cells, thus indicating that VSV infection stimulated an antitumor immune response. Together, these results highlight the strong immunostimulatory properties of VSV in aggressive prostate cancer.

In part from above studies on DENV pathogenesis, we sought to evaluate the efficacy of small molecule regulators of the anti-oxidant response in oncolytic virotherapy, since manipulation of the anti-oxidant network via transcription factor Nrf2 augmented VSV replication and sensitized cancer cells to viral oncolysis. Activation of Nrf2 signaling by the antioxidant compound sulforaphane (SFN) enhanced VSV spread in OV-resistant prostate cancer cells and improved the therapeutic outcome in different murine syngeneic and xenograft tumor models. Furthermore, chemoresistant A549 lung cancer cells that display a constitutive dominant hyperactivation of Nrf2 signaling were highly susceptible to VSV oncolysis. Mechanistically, enhanced Nrf2 expression and signaling stimulated viral replication in cancer cells and disrupted the type I IFN response via increased autophagy. This study revealed a previously unappreciated role for Nrf2 in the regulation of the innate antiviral response that complements the therapeutic potential of VSV-directed oncolysis against multiple types of OV-resistant or chemoresistant cancer.

2017 PUBLICATIONS

Vijayan M, Xia C, Song YE, Studstill CJ, Johnson M, Baldwin M, **Hiscott J**, Kester M, Alexander S, Hahm B. Sphingosine 1-phosphate lyase enhances the activation of IKKε to promote the antiviral type I interferon response. J. Immunol. 99:677-687 (2017)

Olagnier D, Lababidi R, Bel Hadj S, Goulet ML, Chiang C, Sze A, Liu Y, Knatko E, Dinkova-Kostova A, Lin R, **Hiscott J.** Re-programming the oxidative stress response by manipulation of Nrf2 enhances viral oncolysis. Mol. Therapy 25:1900-1916 (2017).

Pelliccia S, Wu Y-H, Coluccia A, La Regina G, Tseng C-Km Famiglini V, **Hiscott J**, Lee J-C, Silvestri R. Inhibition of dengue virus multiplication by novel inhibitors of RNA-dependent RNA polymerase and protease activities. J Enzyme Inhib Med Chem. 32: 1091-1101 (2017).

Olagnier D, Chiang C, **Hiscott J**. Evaluation of innate immune gene expression following HDAC inhibitor treatment by high throughput qPCR and PhosFlow cytometry. Methods Mol Biol.1510:245-255 (2017).

Metcalf TU, Wilkinson PA, Cameron MJ, Ghneim K, Chiang C, Wertheimer AM, **Hiscott J**, Nikolich-Zugich J, Haddad EK. Human monocyte subsets are transcriptionally and functionally altered in ageing in response to pattern recognition receptor agonists. J Immunol. 199:1405-1417 (2017).

Zevini A, Olagnier D, **Hiscott J.** Cross-talk between cytosolic RIG-I and STING innate sensing pathways. Trends in Immunology 38:194-205 (2017).

Di Nicola M, Apetoh L, Bellone M, Colombo MP, Dotti G, Ferrone S, Muscolini M, **Hiscott J**, Anichini A, Pupa SM, Braud F, Del Vecchio M. Innovative therapy, monoclonal antibodies and beyond. Cytokine Growth Factor Rev. 38:1-9 (2017).

Lab Members

Luciano Castiello, PhD
Michela Muscolini PhD
Alessandra Zevini PhD
Matteo Ferrari MSc
Enrico Palermo MSc
Dominga Lovechia BSc
Fani Souvalidou BSc

International Student Exchanges

Shirley Mann (MSc Student, Leiden University Medical Center)
Giselle Rangel (PhD student, University of Madrid, Spain)
Apurwa Trivedi (MSc Student, Pierre & Marie Curie University, Paris France)

Collaborators

David Olagnier/Martin Jakobsen/Soren Paludan – Aarhus University
Bernadette van den Hoogen/Ron Fouchier - Erasmus University
Marjolein Kikkert/ Eric Snijder - Leiden University Medical Center
Frank van Kuppeveld - Utrecht University
Luke O Neill/Andrew Bowie - Trinity College Dublin
Romano Silvestri/Francesca Cutruzzola – Sapienza University
Marco Sgarbant/Angela Battistini/Enrico Proietti - ISS

RESEARCH PROJETCS CARRIED OUT IN AFFILIATED LABORATORIES AT SAPIENZA UNIVERSITY OF ROME (2015 – 2017)

DECIPHERING THE IDENTITY OF TORQUE TENO VIRUS (TTV) AS MARKER AND POTENTIAL DETERMINANT OF IMMUNITY

GUIDO ANTONELLI
RESEARCH AREA: PATHOGENETIC MECHANISMS OF INFECTIOUS-RELATED DISEASES

Department of Molecular Medicine
@: guido.antonelli@uniroma1.it

Torque Teno virus (TTV), a member of the Alphatorquevirus genus within the newly established family Anelloviridae, is the virus more represented into the human virome. Although TTV is considered ubiquitous and appears to establish persistent infections, no clear association between the virus and any human disease has been proved. Plasma TTV loads may vary extensively in diseased individuals. In specific categories (i.e. patients with inflammatory or neoplastic disorders, transplant recipients, and HIV-infected individuals), there is a tendency to carry very high TTV burdens. TTV load may be strictly related to the immunological status of the host; then, the study of TTV viremia kinetics in (solid organ and HSC) transplanted patients and its use in their management may become an interesting emerging field. Our current understanding of the biological and/or molecular mechanisms through which TTV interacts with the immune system is however rather limited. In particular, it is nearly completely unknown how innate immunity determinants may affect the dynamics of TTV and the reactivation of other viruses, such as cytomegalovirus (CMV), which represents an important cause of morbidity and mortality in transplanted patients.
This project was performed in collaboration with the Department of Experimental and Clinical Medicine of Florence University, the Virology Unit of Pisa University Hospital and the Microbiology and Virology Unit of Turin.
First, we were able to measure several TTV produced microRNAs in plasma exosomes, the small vesicles exploited by the cells to intercommunicate, in patients HBV, HCV or HIV-infected, in transplanted patients and in healthy controls. We proved, for the first time, that microRNA production is common among many TTV strains and that their expression levels present wide individual variability. TTV microRNAs expression and TTV viremia were not always correlated; exosomes of healthy subjects possessed higher miRNA-t3b levels than diseased patients whereas the latter showed higher miRNA-t1b and miRNA-t3a levels. The differences in TTV microRNAs in exosomes encourages further investigation to understand its potential role in the expansion of anelloviruses upon immunosuppression.
Secondly, we aimed to verify whether TTV viremia could represent a surrogate marker of immune function and whether the dynamics of TTV load could influence the recovery of immune response other than being a marker that reflects the state of the immune

system. Then, in the framework of the project, a total of 300 adult patients who received kidney or liver transplant and completed a follow-up of at least one year were enrolled. Thirty healthy blood donors served as a control group. Peripheral blood serum samples were obtained from patients just before transplantation and every 10 days within the first 3 months and then at 4, 5, 6, and 12 months post-transplant. Presence and load of TTV genome were determined in a single step TaqMan-PCR assay. In healthy donors, monthly measurement of TTV loads in plasma showed essentially stable values, whereas patients undergoing transplantation exhibited marked fluctuations in TTV viremia kinetics. Before transplantation 258/300 patients (92%) were plasma TTV positive and mean TTV levels were 3.9 and 4.2 log DNA copies/ml of plasma in patients receiving kidney or liver transplant, respectively. The first significant increase (about 1 Log) of mean plasma TTV load occurred at month 1. Then, TTV viremia progressively increased of approximately 3 Log at day 90 and stabilized at levels of approximately 2-3 Logs higher than those at the baseline for the remaining 9 months of observation. Maintenance immunosuppression consisted of tacrolimus or cyclosporine A (CsA) alone, and photopheresis plus CsA; stratifying TTV viremia according to the immunosuppressive treatments, CsA alone was associated with statistically significant higher median TTV loads compared to tacrolimus. Immunosuppressants are likely one of the main important determinants of these changes, since TTV replication occurs mostly in T lymphocytes. The extent of lymphocyte depletion early after induction of immunosuppression has indeed an effect on short-term kinetics but it does not affect long-term kinetics of TTV viremia. On the contrary, maintenance immunosuppression is the main determinant of long-term TTV viremia.
In a subgroup of 235 patients, pre-transplantation TTV load was higher in patients with CMV reactivation compared to those in which CMV reactivation did not occur, suggesting that TTV pre-transplant levels could have a correlation with the reactivation of CMV. From these data, we could estimate a threshold (cutoff value) above which the probability to experience a CMV reactivation is high at 3.45 log TTV DNA copies/ml, thus corroborating the usefulness of TTV load detection to predict opportunistic infections or reactivations.
Results from this project allowed us to strengthen the possible use of TTV load as marker of a tailor-making maintenance of immune suppression and a marker of immune function with the potential to predict opportunistic infections in transplanted patients.

Publications

Maggi F, Focosi D, Statzu M, Bianco G, Costa C, Macera L, Spezia P, Medici C, Albert E, Navarro D, Scagnolari C, Pistello M, Cavallo R, Antonelli G. Early Post-transplant Torquetenovirus viremia predicts cytomegalovirus reactivations in solid organ transplant recipients. (submitted)

Scagnolari C, Turriziani O, Monteleone K, Pierangeli A, Antonelli G. Consolidation of molecular testing in clinical virology. *Expert Rev Anti Infect Ther*. 2017 Apr;15(4):387-400. doi: 10.1080/14787210.2017.1271711.

Focosi D, Antonelli G, Pistello M, Maggi F. Torquetenovirus: the human virome from bench to bedside. *Clin Microbiol Infect* 2016, 22: 589-93. doi: 10.1016/j.cmi.2016.04.007.

Vignolini T, Macera L, Antonelli G, Pistello M, Maggi F, Giannecchini S. Investigation on torquetenovirus (TTV) microRNA transcriptome *in vivo*. *Virus Res* 2016, 217: 18-22. doi: 10.1016/j.virusres.2016.03.003.

Antonelli G, Cutler S. Evolution of the Koch postulates: towards a 21st-century understanding of microbial infection. *Clin Microbiol Infect*. 2016 Jul;22(7):583-4. doi: 10.1016/j.cmi.2016.03.030.

Antonelli G, Scagnolari C, Moschella F, Proietti E. Twenty-five years of type I interferon-based treatment: a critical analysis of its therapeutic use. *Cytokine Growth Factor Rev* 2015, 26: 121-31. doi: 10.1016/j.cytogfr.2014.12.006.

Antonelli G, Spagnoli GC. Why do infections cause cancer? *Clin Microbiol Infect* 2015, 21: 967-8. doi: 10.1016/j.cmi.2015.07.008.

D'Ettorre G, Ceccarelli G, Andreotti M, Selvaggi C, Giustini N, Serafino S, Schietroma I, Nunnari G, Antonelli G, Vullo V, Scagnolari C. Analysis of Th17 and Tc17 frequencies and antiviral defenses in gut-associated lymphoid tissue of chronic HIV-1 positive patients. *Mediators Inflamm* 2015, 2015:395484. doi: 10.1155/2015/395484.

Research Group

Giuseppe Gentile, **Carolina Scagnolari**, **Alessandra Pierangeli**, Researchers; **Maura Statzu**, PhD student.

Collaborations

Fabrizio Maggi, Dipartimento di Ricerca Traslazionale, Università di Pisa; **Rossana Cavallo**, Unità di Microbiologia e Virologia, "Città della Salute e della Scienza di Torino"; **Simone Giannecchini**, Dipartimento di Medicina Sperimentale e Clinica, Università di Firenze; **Mauro Pistello**, Dipartimento di Ricerca Traslazionale, Università di Pisa.

ALLOSTERIC CONTROL IN THE SYNTHESIS AND SENSING OF CYCLIC-DI-GMP, A MASTER REGULATOR OF BACTERIAL GROWTH AND PHYSIOLOGY

FRANCESCA CUTRUZZOLÀ
RESEARCH AREA: MOLECULAR INTERACTIONS

Department of Biochemical Sciences "A. Rossi Fanelli"
@: francesca.cutruzzola@uniroma1.it

Bacterial biofilm represents the main cause of chronic infections in developed countries; biofilm formation requires a dramatic re-shaping of the cellular metabolism and physiology, controlled by the global regulator 3', 5'-cyclic diguanylic acid (c-di-GMP). The biomedical relevance of c-di-GMP signalling is huge, given that the enzymes responsible for its homeostasis, namely diguanylate cyclases- (DGCs) and phosphodiesterases (PDEs), are found only in bacteria. For this reason, studying and targeting c-di-GMP biosynthesis represents an attractive goal to treat biofilms. We have contributed to this field by characterizing selected DGCs and PDEs relevant to biofilm formation in the human pathogen *Pseudomonas aeruginosa*. Moreover, we have identified small molecule compounds able to inhibit DGCs.
The relevant results of this project can be briefly summarized into three major outcomes:
- *identification of novel c-di-GMP-derived signals*: structural biology and biophysiscal analysis of selected HD-GYP proteins reveals that the active site of these proteins is unexpectedly diversified. In particular, we found that the re-shaping of the metal binding site of PA4781 HD-GYP is allosterically controlled and that this protein preferentially binds the pGpG linear dinucleotide (rather than c-di-GMP), which in turn could be considered a genuine signalling molecule (Rinaldo et al., 2015).
- *identification of novel compounds able to specifically target DGCs or PDEs*. We identified novel compounds targeting the DGCs through two different and complementary approaches i.e. rational design (in collaboration with Camerino's group) and virtual screening (Fernicola et al., 2015 a,b). In parallel, we have developed (in collaboration with the Roma Tre's group) a cell-based assay for *in vivo* inhibitors screening (Pawar et al., 2016). Given the impact of our research, we have been invited to describe the workflow required for these studies (Rinaldo et al., 2017).
- *allostery connects nutrients, energy and c-di-GMP*. We recently demonstrated that PA0575 protein, a complex transmembrane transducer harbouring both putative DGC and PDE domains and other uncharacterized domain, is a one-component system whose PDE activity is allosterically controlled by integrating multiple metabolic and environmental factors including GTP and aminoacids (Mantoni et al. 2018, *manuscript under evaluation*). The fact that nutrients and central metabolism crosstalks with c-di-GMP metabolism is now emerging as a hallmark of biofilm formation. We propose that

the analysis of the metabolic reprogramming underlying the environment-cell fate axis provides a novel and original perspective to study biological processes such as biofilm formation in bacteria (Cutruzzolà and Frankenberg-Dinkel, 2016; Rinaldo et al., 2018).

In line with the aim of this project, all these data confirm that regulation of c-di-GMP metabolism is mainly due to protein control *via* multiple levels of allosteric regulation; the fine sensing of the environmental conditions is therefore crucial for the bacterium to determine the cellular fate (including biofilm formation).

Publications

Rinaldo S, Giardina G, Mantoni F, Paone A, Cutruzzolà F. Beyond nitrogen metabolism: nitric oxide, cyclic-di-GMP and bacterial biofilms. FEMS Microbiol Lett. 2018 doi: 10.1093/femsle/fny029. [Epub ahead of print].

Rinaldo S, Giardina G, Mantoni F, Paiardini A, Paone A, Cutruzzolà F. Discovering Selective Diguanylate Cyclase Inhibitors: From PleD to Discrimination of the Active Site of Cyclic-di-GMP Phosphodiesterases. Methods Mol Biol. 2017;1657:431-453. doi: 10.1007/978-1-4939-7240-1_32. PubMed PMID: 28889312.

Cutruzzolà F, Frankenberg-Dinkel N. Origin and Impact of Nitric Oxide in Pseudomonas aeruginosa Biofilms. J Bacteriol. 2016;198:55-65. DOI: 10.1128/JB.00371-15

Pawar SV, Messina M, Rinaldo S, Cutruzzolà F, Kaever V, Rampioni G, Leoni L. Novel genetic tools to tackle c-di-GMP-dependent signalling in Pseudomonas aeruginosa. J Appl Microbiol. 2016; 120:205-17. doi: 10.1111/jam.12984.

Rinaldo S, Paiardini A, Stelitano V, Brunotti P, Cervoni L, Fernicola S, Protano C, Vitali M, Cutruzzolà F, Giardina G. Structural basis of functional diversification of the HD-GYP domain revealed by the Pseudomonas aeruginosa PA4781 protein, which displays an unselective bimetallic binding site. *J Bacteriol*. 2015; 197:1525-35. doi: 10.1128/JB.02606-14.* *selected for spotlight and cover image.*

Fernicola S, Torquati I, Paiardini A, Giardina G, Rampioni G, Messina M, Leoni L, Del Bello F, Petrelli R, Rinaldo S, Cappellacci L, Cutruzzolà F. Synthesis of Triazole-Linked Analogues of c-di-GMP and Their Interactions with Diguanylate Cyclase. *J Med Chem*. 2015a; 58:8269-84. doi:10.1021/acs.jmedchem.5b01184.

Fernicola S, Paiardini A, Giardina G, Rampioni G, Leoni L, Cutruzzolà F, Rinaldo S. In Silico Discovery and In Vitro Validation of Catechol-Containing Sulfonohydrazide Compounds as Potent Inhibitors of the Diguanylate Cyclase PleD. *J Bacteriol*. 2015b **198**:147-56. doi: 10.1128/JB.00742-15. * *selected for cover image.*

Research Group

Prof. Francesca Cutruzzolà PI
Alessandro Paiardini Researcher
Serena Rinaldo Researcher (TD)
Giorgio Giardina Researcher (TD)
Paolo Brunotti PhD student
Federico Mantoni, PhD Student

Collaborations

Livia Leoni, **Giordano Rampioni**, Università di Roma Tre
Loredana Cappellacci, Università di Camerino.

IDENTIFICATION OF NOVEL HEDGEHOG/GLI PATHWAY ANTAGONISTS IN BRAIN TUMORS TREATMENT

LUCIA DI MARCOTULLIO
RESEARCH AREA: GENETICS, BIOLOGY AND PATHOPHYSIOLOGY OF EUKARYOTES

Department of Molecular Medicine
@: lucia.dimarcotullio@uniroma1.it

The Hedgehog (Hh) signaling has emerged in recent years as an attractive target for anticancer therapy because its aberrant activation is implicated in several cancers. Major progress has been made in the development of Smoothened (Smo) antagonists, although they have shown several limitations due to downstream Smo pathway activation or the occurrence of drug-resistant Smo mutations. Recently, particular interest has been elicited by the identification of molecules able to hit glioma-associated oncogene (Gli) factors, the final effectors of the Hh pathway, which provide a valid tool to overcome anti-Smo resistance.

The main goal of this project was to identify novel small molecules able impairing Hh-dependent tumors growth and to elucidate the molecular mechanisms that control Hh pathway in brain tumors, such as medulloblastoma (MB). MB is the most frequent pediatric brain tumor, arising from aberrant cerebellar precursors development, a process mainly controlled by Hh pathway. Drugs, such as Vismodegib (GDC-0449/Erivedge) and others Smo antagonists, recently developed, have shown promising results in MB and Hh-dependent Basal Cell Carcinomas (BCC). However, despite an initial clinical response, a number of drug-resistant Smo mutations were observed in patients. Further, some clinical trials have failed so far due to poor pharmacokinetics, low selectivity on cancer stem cells (CSCs), and the presence of bystander co-regulatory mechanisms of the Hh pathway. Indeed, resistance to anti-Smo inhibition can be mediated also by hyperactivation of the powerful downstream Gli factors due to Gli2 amplification or upregulation of Gli via a non-canonical Hh signaling activation.

Identification of novel Smo antagonists. With the aim to identify new Smo antagonists able to overcome these limits, we performed a docking-based virtual screening of an library of natural compounds and their derivatives, developed in house, towards the crystallographic structure of Smo bound to cyclopamine, the first identified Smo antagonist. Subsequently, by means of molecular and cell biology validation, through assay based on Hh activity, we identified a compound named Chalcone 12, and compound 2d and 2t as the most effective Hh inhibitors within the test set. These biomolecules bind to Smo and they are not sensitive to previously described drug-

resistant Smo mutations. Our compounds suppress the expression of endogenous Hh target genes in Ptch1$^{-/-}$, a cell model in which the constitutive activation of Hh signaling is consequence of the genetic ablations of the upstream Ptch1-negative regulators. Of note, our compounds show anti-oncogenic properties promoting growth arrest of Hh-driven tumor cells *in vitro* and *in vivo*, including MB and BCC, as well as the clonogenic self-renewal ability of MBSCs, by reducing the expression of Hh pathway and stemness target genes *(Infante, et al, Cell Death and Disease 2016; Alfonsi et al, Journal of Medicinal Chemistry 2017).* The molecules described above, represent new interesting Smo inhibitors opening new venues for the discovery of anticancer compounds targeting the Hh pathway and overcoming drug resistance.

Identification of novel Gli inhibitors. Gli1 aberrant expression has been largely associated to the insurgence and the maintenance of Hh-dependent tumors. Importantly, inactivation of Gli1 determines inhibition of tumor progression and cancer cells proliferation, and Gli1 has been found active in some case of Smo antagonism or Hh upstream inactivation. However, only a few Gli-inhibitors have been identified, most likely because of the lack of structural details related to Gli activity. By a mixed computational and experimental structure-based study we clarify the structural requirements of the Gli1/DNA complex and identify Glabrescione B (GlaB) as the first small molecule binding to Gli1 zinc finger and impairing Gli1 activity by interfering with its interaction with DNA. Remarkably, as a consequence of its robust inhibitory effect on Gli1 activity, GlaB inhibited the growth of Hh-dependent tumor cells *in vitro* and *in vivo* as well as the self-renewal ability and clonogenicity of tumor-derived stem cells. These evidences confirmed the anti-tumor activity of GlaB that arises as a good candidate drug for pre-clinical studies in the treatment of Hh-dependent tumors, highlighting the potential translational in a clinic field of our research.

Publications

Alfonsi R, et al. Design, Palladium-Catalyzed Synthesis, and Biological Investigation of 2-Substituted 3-Aroylquinolin-4(1H)-ones as Inhibitors of the Hedgehog Signaling Pathway. *J Med Chem*. 2017 60:1469-1477. doi: 10.1021/acs.jmedchem.6b01135.

Coni S, et al. Selective targeting of HDAC1/2 elicits anticancer effects through Gli1 acetylation in preclinical models of SHH Medulloblastoma. *Sci Rep*. 2017 7:44079. doi: 10.1038/srep44079.

De Mori R, et al. Hypomorphic Recessive Variants in SUFU Impair the Sonic Hedgehog Pathway and Cause Joubert Syndrome with Cranio-facial and Skeletal Defects. Am J Hum Genet. 2017 101:552-563. doi: 10.1016/j.ajhg.2017.08.017.

Quaranta R, et al. Maml1 acts cooperatively with Gli proteins to regulate sonic hedgehog signaling pathway. Cell Death Dis. 2017 8:e2942. doi: 10.1038/cddis.2017.326.

Miele E, et al. ß-arrestin1-mediated acetylation of Gli1 regulates Hedgehog/Gli signaling and modulates self-renewal of SHH medulloblastoma cancer stem cells. BMC Cancer.2017 17:488. doi:10.1186/s12885-017-3477-0.

Po A, et al. Noncanonical GLI1 signaling promotes stemness features and in vivo growth in lung adenocarcinoma. *Oncogene*. 2017 Apr 3. doi: 10.1038/onc.2017.91.

Infante P, et al. Inhibition of Hedgehog-dependent tumors and cancer stem cells by a newly identified naturally occurring chemotype. Cell Death Dis 2016, 7:e2376. doi: 10.1038/cddis.2016.195.

Filocamo G, et al. MK-4101 a potent inhibitor of the hedgehog pathway is highly active against medulloblastoma and basal cell carcinoma. Mol Cancer Ther 2016, 15:1177-1189. doi: 10.1158/1535-7163.MCT-15-0371.

Raducu M, et al. SCF (Fbxl17) ubiquitylation of Sufu regulates Hedgehog signaling and medulloblastoma development. EMBO J. 2016, 35:1400-1416. doi: 10.15252/embj.201593374.

Di Magno L, et al. The energy sensor AMPK regulates Hedgehog signaling in human cells through a unique Gli1 metabolic checkpoint. Oncotarget 2016, 7:9538-9549. doi: 10.18632/oncotarget.7070.

Comba A, et al. Nuclear Factor of Activated T Cells-dependent Down-regulation of the Transcription Factor Glioma-associated Protein 1 (GLI1) Underlies the Growth Inhibitory Properties of Arachidonic Acid. J Biol Chem. 2016, 291:1933-1947. doi: 10.1074/jbc.M115.691972.

D'Amico D, et al. Non-canonical Hedgehog/AMPK-Mediated Control of Polyamine Metabolism Supports Neuronal and Medulloblastoma Cell Growth. *Dev Cell* 2015, 36: 21-35. doi:10.1016/j.devcel.2015.09.008.

Infante P, et al. Targeting GLI factors to inhibit the Hedgehog pathway. *Trends Pharmacol Sci.* 2015 36: 547-558. doi:10.1016/j.tips.2015.05.006.

Infante P, et al. Gli1/DNA interaction is a druggable target for Hedgehog-dependent tumors. *EMBO J.* 2015, 34: 200-217. doi:10.15252/embj.201489213.

La Regina G, et al. New Indole Tubulin Assembly Inhibitors Cause Stabler Arrest of Mitotic Progression Enhanced Stimulation of Natural Killer Cell Cytotoxic activity, and

repression of hedgehog-Dependent Cancer. *J Med Chem*. 2015, 58:5789-5807. doi: 10.1021/acs.jmedchem.5b00310.

Infante P, et al. Insights into Gli Factors Ubiquitylation Methods. *Methods Mol Biol.* 2015;1322:131-46. doi: 10.1007/978-1-4939-2772-2_12.

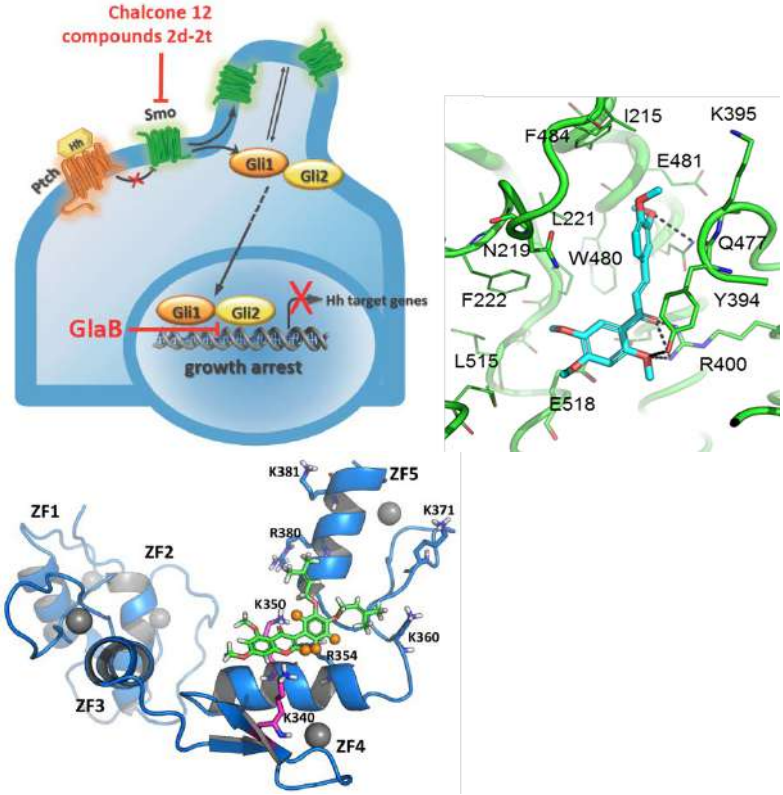


Figure. (**a**) Mechanism of Hh inhibition by Smo or Gli antagonists. (**b**) Docking-based binding conformation of chalcone 12 (blue stick) within the antagonist site of Smo (green cartoon). (**c**) The predicted binding mode of GlaB (green sticks) to Gli1ZF (blue cartoon). GlaB inhibits Gli1/DNA interaction by its ability to bind Gli1 zinc-finger domain.

Research Group

Romina Alfonsi, Flavia Bernardi, Francesca Bufalieri, Sonia Coni, **Roberta Faedda**, post-doctoral fellow.

Collaborators

Bruno Botta, Sapienza Università di Roma; **Paola Infante, Mattia Mori,** Center for Life Nanosciences, IIT, Roma.

ROLE OF ATP-DEPENDENT CHROMATIN REMODELING COMPLEXES IN MIDBODY FORMATION AND CYTOKINESIS

PATRIZIO DIMITRI
RESEARCH AREA: GENETICS, BIOLOGY AND PATHOPHYSIOLOGY OF EUKARYOTES

Dipartimento di Biologia e Biotecnologie "Charles Darwin"
@: patrizio.dimitri@uniroma1.it

The overall aim this project was to investigate the role(s) of chromatin remodeling complexes during cytokinesis in human cultured cells. In particular, we planned to: 1) Study the recruitment of subunits of SRCAP and P400 chromatin complexes to the midbody; 2) Charcaterize how their depletion impacts cytokinesis, and hence the transmission of the genetic identity to daughter cells; 3) Identify interactors of remodelers that affect these processes.
First, we have carried out high-resolution immunofluorescence experiments in HeLa cells and found that 14 components of SRCAP and P400 ATP-dependent chromatin remodeling complexes (CFDP1, SRCAP, BAF53a, Myc-Arp6, GAS 41, Pontin, Reptin, p18, YL1, GAS41, MRG15, Tip60, P400 and H2A.Z) localize to the midbody. The midbody associations found in fixed HeLa cell preparations have been validated by IF and Western blotting experiments on isolated midbodies (Fig. 1). Interestingly, some of these proteins have been also found at the spindle in both metaphase and/or anaphase, or at the centrosomes. Since the main function of the SRCAP and P400 complexes is to govern H2AZ deposition into chromatin, such a bulky association with the miotitc apparatus is not obvious. To deepen our understanding of the role(s) played by SRCAP and P400 complexes in cell division, we have performed RNAi-mediated knock down experiments in HeLa cells. The results show that depletion of most subunits affects different aspects of cell division. The effects detected include aberrant spindle morphology, chromosome segregation defects and multinucleation. Interestingly, depletion of some subunits also results in prevention of cell cleavage, which produces a significant fraction of long intercellular bridges connecting daugther cells. A crucial question is how chromatin remodelers are recruited to the mitotic apparatus. To address this question, we have performed a series of co-immunoprecipitation assays. The results obtained thus far suggest that Aurora-B and other midbody players play a role in the relocation of a number of SRCAP and P400 subunits from chromatin to the mitotic apparatus. Overall, our results reveal the existence of a massive and evolutionary conserved phenomenon, whereby SRCAP and P400 chromatin remodeling complexes, in addition to their role in chromatin organization and function, are important regulators of mitotic apparatus and are required for efficient cell division. Moreover, our results

highlight a previously undiscovered scenario in which chromatin remodeling, cell cycle and tumorigenesis may be closely interlinked.

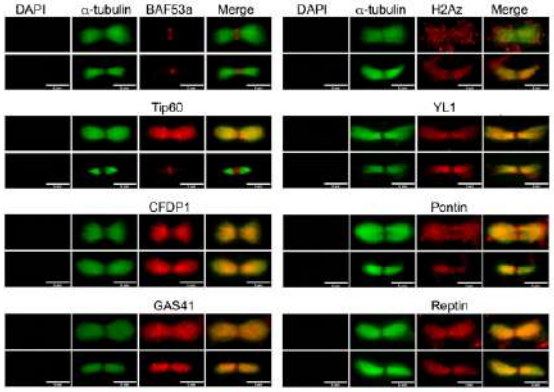


Figure 1. Examples of immunolocalization of components of SRCAP and P400 complexes on isolated midbodies

Publications

G. Messina, M. T. Atterrato, L. Fanti, E. Giordano and P. Dimitri (2016) Expression of human *Cfdp1* gene in *Drosophila* reveals new insights into the function of the evolutionarily conserved BCNT protein family. *Scientific Reports*. 6:25511. doi: 10.1038/srep25511.

G. Messina, M. T Atterrato and P. Dimitri (2016) When chromatin organization floats astray: the *SRCAP* gene and the Floating Harbor syndrome. *J. Med. Genet. 53(12):793-797.* doi:10.1136/jmedgenet-2016-103842.

G. Messina, M. T. Atterrato, L. Piacentini, A. Losada and P. Dimitri (2017) The human Cranio Facial Development Protein 1 (Cfdp1) gene encodes a protein required for the maintenance of higher-order chromatin organization. *Scientific Reports* 7, 45022; doi:10.1038/srep45022 .

E. Celauro, S. Carra, A. Rodriguez, F. Cotelli and P. Dimitri (2017) Functional analysis of the *Cfdp1* gene in zebrafish provides evidence for its crucial role in craniofacial development and osteogenesis. *Exp Cell Res.* 361(2):236-245. doi: 10.1016/j.yexcr.2017.10.022.

Hoskins RA, Carlson JW, Wan KH, Park S, Mendez I, Galle SE, Booth BW, Pfeiffer BD, George RA, Svirskas R, Krzywinski M, Schein J, Accardo MC, Damia E, Messina G, Méndez-Lago M, de Pablos B, Demakova OV, Andreyeva EN, Boldyreva LV, Marra M, Carvalho AB, Dimitri P, Villasante A, Zhimulev IF, Rubin GM, Karpen GH, Celniker SE. The Release 6 *Drosophila melanogaster* reference genome. *Genome Res* 2015, 25: 445-58. doi: 10.1101/gr.185579.114.

Messina G, Celauro E, Atterrato MT, Giordano E, Iwashita S, Dimitri P. The Bucentaur (BCNT) protein family: a long-neglected class of essential proteins required for chromatin/chromosome organization and function. *Chromosoma* 2015, 124: 153-62. doi: 10.1007/s00412-014-0503-8.

Research Group

1. Dr. **Giovanni Messina,** post-doc fellows, Sapienza University, Roma, Italy
2. Dr. **Emanuele Celauro**, PhD student, Sapienza University, Roma, Italy
3. **Maria Teresa Atterrato**, undergraduate student, Sapienza University, Roma, Italy
4. **Yuri Prozzillo**, undergraduate student, Sapienza University, Roma, Italy
5. **Manuela Leo**, graduate student, Sapienza University, Roma, Italy

Collaborations

Dr. **Patrizia Lavia**, Principal investigator, IBPM, CNR, Roma, Italy

RNASE H INHIBITORS TO DEFEAT HIV

ROBERTO DI SANTO
RESEARCH AREA: NOVEL THERAPEUTIC INTERVENTIONS

Department of Medicinal Chemistry and Technologies
@: roberto.disanto@uniroma1.it

The introduction of HAART (Highly Active Anti-Retroviral Therapy) for treating acquired immunodeficiency syndrome (AIDS) allowed reaching remarkable milestones, turning AIDS into a chronic disease. However, HAART still has several drawbacks, underscoring the demand of new antiretroviral agents with higher genetic barrier. Development of compounds able to inhibit essential but unexplored targets is mostly attractive to reduce selection of drug resistant strains, as reverse transcriptase (RT)-associated ribonuclease H (RNase H) function. Altough RNase H is a well-validated drug target, no RNase H inhibitors (RHIs) reached clinical approval so far. Since the discovery of structural homologies between IN and RH, due to pivotal role of Mg^{2+} cofactors, compounds capable to sequester these cations should inhibit both enzymes, like diketo acid (DKA) derivatives, that proved to inhibit them both. Our group reported a diketo acid derivative previously identified as IN inhibitor, as potent RHI.

In order to identify more efficient and specific RHIs, we synthesized a new series of pyrrolyl DKAs as dual IN/RH inhibitors aiming at defining Structure Activity Relationships. The newly synthesized compounds proved to be effective dual inhibitors, with low micromolar-nanomolar enzymatic potencies, micromolar-submicromolar antiviral activities and low cytotoxicity. We defined a trend according to which although acid group confers a better inhibitory activity on IN, ester function is amenable for RH inhibition, so the ester function is necessary for dual activity. Moreover, we found that pyrrole can be functionalized by introducing mono or difluorine substituted benzyl groups on nitrogen atom and phenyl ring in 4 position. Regarding DKA chain, the shortening of diketohexenoic into a diketobutanoic branch is comparable effective strategy for dual inhibiton. Furthermore, thanks to our molecular modeling coupled with site-directed mutagenesis studies, we found that the presence of 4-phenyl ring on the pyrrole, although improves the inhibitory potency, may lead to a decrease of activity against raltegravir resistant mutants. We applied the same approach on quinolinone scaffold, obtaining new quinolinonyl DKA derivatives as dual inhibitors. We studied the effect of the substituent linked on the N of the quinolinone, founding also in this case that mono or difluorine substituted benzyl rings are essential for dual inhibition. We also confirmed the aforementioned trend that the ester function is required for dual activity. Recently, we performed computational investigation of binding modes, Mg-complexation and mutagenesis studies on 4 RHI scaffolds, showing a Mg-independent mode of action

involving multiple interactions within RH domain. In a further effort to find new anti-HIV agents, we designed and synthesized a series of *N*-aryl-naphthylamines as effective inhibitors of the essential IN/human LEDGF factor interaction and a series of cinnamoyl derivatives as p300 HAT inhibitors, due to the pivotal acetylation of IN in viral integration. Noteworthy, we analysed multi-drug resistant (MR) RTs crucial in conferring resistance to nucleoside RT inhibitors. We tested if our best RHIs could inhibit the MR-RTs, founding that all MR-RTs exhibited similar sensitivity toward RHIs, indicating that the development and application of RHIs could be a potent tool against multi-drug resistance phenomenon.

In the view of our abovementioned results, our next step will rely in designing higher potent and selective compounds as RHIs.

Publications

G. Cuzzucoli Crucitti, M. Métifiot, L. Pescatori, A. Messore, V. N. Madia, G. Pupo, F. Saccoliti, L. Scipione, S. Tortorella, F. Esposito, A. Corona, M. Cadeddu, C. Marchand, Y. Pommier, E. Tramontano, R. Costi, R. Di Santo; Structure-activity relationship of pyrrolyl diketo acid derivatives as dual inhibitors of HIV-1 integrase and reverse transcriptase ribonuclease H domain. *J. Med. Chem.* 2015, 58, 1915-1928, N. doi: 10.1021/jm501799k.

L. Pescatori, M. Métifiot, S. Chung, T. Masoaka, G. Cuzzucoli Crucitti, A. Messore, G. Pupo, V. N. Madia, F. Saccoliti, L. Scipione, S. Tortorella, F. S. Di Leva, S. Cosconati, L. Marinelli, E. Novellino, S. F. J. Le Grice, Y. Pommier, C. Marchand, R. Costi, R. Di Santo; N-Substituted quinolinonyl diketo acid derivative as HIV integrase strand transfer inhibitors and their activity against RNase H function of reverse transcriptase. *J. Med. Chem.* 2015, 58, 4610-4623, N. doi: 10.1021/acs.jmedchem.5b00159.

G. Cuzzucoli Crucitti, L. Pescatori, A. Messore, V. N. Madia, G. Pupo, F. Saccoliti, L. Scipione, S. Tortorella, F. S. Di Leva, S. Cosconati, E. Novellino, Z. Debyser, F. Christ, R. Costi, R. Di Santo; Discovery of N-aryl-naphthylamines as in vitro inhibitors of the interaction between HIV integrase and the cofactor LEDGF/p75. *Eur. J. Med. Chem.* 2015, 101, 288-294, N. doi: 10.1016/j.ejmech.2015.06.036.

Schneider, A. Corona, I. Spöring, M. Jordan, B. Buchholz, E. Maccioni, R. Di Santo, J. Bodem, E. Tramontano, B. M. Wöhrl; Biochemical characterization of a multi-drug resistant HIV-1 subtype AG reverse transcriptase: antagonism of AZT discrimination and excision pathways and sensitivity to RNase H inhibitors. *Nucleic Acids Res.* 2016, 44, 2310-2322, N. doi: 10.1093/nar/gkw060.

Corona, F. S. Di Leva, G. Rigogliuso, L. Pescatori, V. N. Madia, F. Subra, O. Delelis, F. Esposito, M. Cadeddu, R. Costi, S. Cosconati, E. Novellino, R. Di Santo, E. Tramontano; New insights into the interaction between pyrrolyl diketoacids and HIV-1 integrase

active site and comparison with RNase H. *Antivir. Res.* 2016, 134, 236-243, N. doi: 10.1016/j.antiviral.2016.09.008.
V. N. Madia, R. Benedetti, M. L. Barreca, L. Ngo, L. Pescatori, A. Messore, G. Pupo, F. Saccoliti, S. Valente, A. Mai, L. Scipione,Y. G. Zheng, C. Tintori, M. Botta, V. Cecchetti, L. Altucci, R. Di Santo, R. Costi. Structure-Activity Relationships on Cinnamoyl Derivatives as Inhibitors of p300 Histone Acetyltransferase. *ChemMedChem* 2017, 12, 1359-1368. N. doi: 10.1002/cmdc.201700040.

V. Poongavanam, A. Corona, C. Steinmann, L. Scipione, N. Grandi, F. Pandolfi, R. Di Santo, R. Costi, F. Esposito, E. Tramontano, J. Kongsted. Structure-guided approach identifies a novel class of HIV-1 ribonuclease H inhibitors: binding mode insights through magnesium complexation and site-directed mutagenesis studies. *MedChemCom* 2018, in press. N. doi: 10.1039/c7md00600d.

Research Group

Roberta Costi, professor; **Valentina Noemi Madia**, post-doc fellow; **Francesco Saccoliti**, **Valeria Tudino**, PhD students.

Collaborations

Stuart Le Grice, National Institute of Health, Frederick, USA; **Enzo Tramontano**, Università di Cagliari; **Sandro Cosconati**, DiSTABiF, Seconda Università di Napoli; **Birgitta M. Wöhrl**, Universität Bayreuth, Bayreuth, Germany.

CICRADIAN RHYTHMS AND STRESS: FUNCTIONAL ROLE OF *PERIOD* GENE

LAURA FANTI
RESEARCH AREA: GENETICS, BIOLOGY AND PATHOPHYSIOLOGY OF EUKARYOTES

Department of Biology and Biotechnology "Charles Darwin"
@: laura.fanti@uniroma1.it

The stress-responsive mechanisms are conserved during evolution and involve changes in gene expression and signal transduction pathways. Among the genes involved in stress response are the heat-shock coding genes. These genes were discovered in *Drosophila* following the observation that third instar larvae polytene chromosomes subject to high temperature show specific puffs (Ritossa 1962;1964). Later, it was shown that the heat-shock genes are present in all organisms and are expressed following both biotic and abiotic stressors (Sørensen et al. 2003). Heat-shock proteins have multiple functions: they protect the genomes subject to environmental changes by helping proteins assume and maintain the right conformation (Buchner 2002; Borges and Ramos 2005); heat-shock proteins also repress general gene transcription during the stress; finally they may induce an increase in genetic variability in stressed populations causing the activation of transposable elements and consequently the induction of morphological abnormalities through insertional mutagenesis (Specchia et al., 2010; Piacentini et al. 2014; Fanti, Piacentini et al. 2017). Indeed, functional alterations of HSP90 affect the silencing pathway mediated by Piwi-interacting RNA (piRNA; a small, germ line-specific RNA), a specific mechanism for repetitive-element and transposon silencing. According to this point of view, the environmental changes can induce a stress response essential for survival and reproduction.

Our project proposed to test a kind of stress induced by the dysfunction of the biological clock. To this end, we planed to investigate the mechanisms of stress and the related effects produced by mutations in *period* (*per*), a conserved master gene involved in the biological clock in *Drosophila melanogaster*. We know that most of the processes of living organisms are controlled by a biological clock; therefore, the malfunctioning of this endogenous clock causes life-span shortening, stress and disease.

Since stress affects the biogenesis of piRNA, which functions to repress transposons, we expected to find more transposon transcripts in the gonads and brains of per^{01} mutants. We tested several different transposable elements and many of them were overtranscribed in *period* null mutant respect to the control. These results strongly suggest that the dysfunction of biological rhythms is perceived as a stress.

Another goal of our project was to conduct RNAseq experiments on a *per01* mutant respect to the wild-type stock, to identify the possible direct targets of PER. Among them, some genes involved in aging, life-span and genome stability were de-regulated in

per^{01} mutants. We performed experiments in different light conditions to test the possible involvement of *period* gene in these processes and we obtained positive results.

Publications

Laura Fanti, Lucia Piacentini, Ugo Cappucci, Assunta M. Casale, and Sergio Pimpinelli. Canalization by Selection of de Novo Induced Mutations. Genetics 2017, vol. 206 no. 4 1995-2006 doi: https://doi.org/10.1534/genetics.117.201079. Highlighted Article

Research Group

Lucia Piacentini, researcher
Assunta Maria Casale, research fellow,
Ugo Cappucci, research fellow,
Marcella Marchetti, technical graduate

Collaborations

Maria Pia Bozzetti, Dip. Sci. Tec. Biol. Amb, Univ. del Salento
Ezio Rosato, Dept. og Genetics and Genome Biology University of Leicester, UK
Rodolfo Negri, Dip. di Biologia e Biotecnologie "Charles Darwin"
Rodolfo Costa, Dip. di Biologia, Univ. di Padova

FUNCTIONAL INTERACTIONS BETWEEN THE MRN COMPLEX AND N-MYC IN NEURONAL DEVELOPMENT AND CARCINOGENESIS

GIUSEPPE GIANNINI
RESEARCH AREA: GENETICS, BIOLOGY AND PATHOPHYSIOLOGY OF EUKARYOTES

Department of Molecular Medicine
@: giuseppe.giannini@uniroma1.it

Failure in maintaining genomic integrity is invariably associated with pathologic events, including inherited diseases known as DNA damage response (DDR)-defective syndromes, and cancer. Many DDR-defective syndromes are characterized by CNS alterations, ranging from developmental problems (i.e. microcephaly) to ataxia or neurodegeneration. Mechanistically, replication stress (RS) and defects in centrosome duplication are the most likely causes of both CNS defects and cancer.
We recently demonstrated that the MRE11/RAD50/NBS1 (MRN) complex, an important component of the DDR, is a transcriptional target of the MYCN proto-oncogene and is essential to control RS associated with MYCN-driven proliferation. Dysfunctions in this axis impair the fast expansion of neural progenitors occurring during CNS development, as we have shown for cerebellar granule cell progenitors (GCPs) (Petroni et al. 2015, Petroni et al. 2016).
Beside its role in neural development, MYCN is frequently deregulated and confers worse prognosis in tumors like neuroblastoma and medulloblastoma. Direct MYCN targeting for therapeutic purposes has not been achieved, yet. Since MYCN regulates a complex network of proteins involved in DNA repair and cell cycle checkpoints, potentially required to restrain the catastrophic effects of RS, targeting one or more of these proteins could raise RS to non-tolerable levels, thus disclosing new therapeutic opportunities for MYCN-dependent tumors. By *in silico* analysis of primary human neuroblastoma gene expression profiles, we have shown that MRE11 and PARP1/2 are negative prognostic factors in neuroblastoma. MRE11 KO or the pharmacological inactivation of its exonuclease activity specifically inhibit growth of MYCN amplified neuroblastoma, raising the level of RS and DNA damage, and causing p53-dependent cell death (Petroni et al. submitted).
PARP inhibitors, which are already used in cancer either in combination with chemical or physical agents or in monotherapy for BRCA-defective ovarian cancer, also increase MYCN induced RS, evoking S-phase and G2/M checkpoints. Nonetheless, MYCN overexpressing cells fail to consolidate these checkpoints and progress through mitosis in the presence of damaged DNA, leading to mitotic catastrophe (Colicchia et al. 2017). We are now exploiting these approaches in mouse models.

Inherited mutations in the NBS1 (or NBN) gene cause the Nijmegen breakage syndrome, an autosomal recessive DDR-syndrome. In mice, CNS-restricted inactivation of the *Nbn* gene results in several abnormalities including microcephaly, growth retardation, cerebellar defects and ataxia. Loss of *Nbn* causes proliferation arrest of cerebellar GCPs, whose postnatal expansion is typically due to Sonic Hedgehog (Shh)-Nmyc pathway. To test whether a constitutive activation of Shh-Nmyc signalling may compensate for the defective GCP proliferation observed in NBN-KO mice, we crossed a Shh-constitutive and neural specific mouse (ND2-SmoA1 mouse) with the NBN KO model. Surprisingly, NBN KO completely suppresses SmoA1 phenotype. While this might be partially due to loss of NBN function in controlling MYCN-dependent RS, our preliminary evidences also suggest that NBN defect is epistatic on SHH pathway. Indeed, SmoA1-NBN-KO GCPs show impaired Shh-pathway activation *in vivo* and *in vitro*. Taking advantage of mice and primary cellular models developed in our unit, we are investigating on the molecular and cellular origins of this phenotype.

Publications

Petroni M, Sardina F, Heil C, Sahún-Roncero M, Colicchia V, Veschi V, Albini S, Fruci D, Ricci B, Soriani A, Di Marcotullio L, Screpanti I, Gulino A, Giannini G. The MRN complex is transcriptionally regulated by MYCN during neural cell proliferation to control replication stress. ***Cell Death and Differentiation***. 2016 Feb;23(2):197-206. doi: 10.1038/cdd.2015.81.

Miele E, Mastronuzzi A, Po A, Carai A, Alfano V, Serra A, Colafati GS, Strocchio L, Antonelli M, Buttarelli FR, Zani M, Ferraro S, Buffone A, Vacca A, Screpanti I, Giangaspero F, Giannini G, Locatelli F, Ferretti E. Characterization of medulloblastoma in Fanconi Anemia: a novel mutation in the BRCA2 gene and SHH molecular subgroup. ***Biomarker Research***. 2015 Jun 6;3:13. doi: 10.1186/s40364-015-0038-z. eCollection 2015.

Petroni M, Giannini G. A MYCN-MRN complex axis controls replication stress for a safe expansion of neuroprogenitor cells. ***Molecular and Cellular Oncology***. 2015 Sep 11;3(2):e1079673. doi: 10.1080/23723556.2015.1079673. eCollection 2016 Mar.

Prodosmo A, Buffone A, Mattioni M, Barnabei A, Persichetti A, De Leo A, Appetecchia M, Nicolussi A, Coppa A, Sciacchitano S, Giordano C, Pinnarò P, Sanguineti G, Strigari L, Alessandrini G, Facciolo F, Cosimelli M, Grazi GL, Corrado G, Vizza E, Giannini G, Soddu S. Detection of ATM germline variants by the p53 mitotic centrosomal localization test in BRCA1/2-negative patients with early-onset breast cancer. ***Journal of Experimental & Clinical Cancer Research***. 2016 Sep 6;35(1):135. doi: 10.1186/s13046-016-0410-3.

Veschi V, Liu Z, Voss TC, Ozbun L, Gryder B, Yan C, Hu Y, Ma A, Jin J, Mazur SJ, Lam N, Souza BK, Giannini G, Hager GL, Arrowsmith CH, Khan J, Appella E, Thiele CJ. Epigenetic siRNA and Chemical Screens Identify SETD8 Inhibition as a Therapeutic Strategy for p53 Activation in High-Risk Neuroblastoma. ***Cancer Cell.*** 2017 Jan 9;31(1):50-63. doi: 10.1016/j.ccell.2016.12.002.

Colicchia V, Petroni M, Guarguaglini G, Ricci B, Sardina F, Sahun Roncero M, Heil C, Capalbo C, Belardinilli F, Coppa A, Screpanti I, Lavia P, Gulino A, Giannini G. The Poly (ADP-ribose) polymerase inhibitor olaparib enhances replication stress and causes mitotic catastrophe in MYCN amplified neuroblastoma. ***Oncogene***. 2017 Aug 17;36(33):4682-4691. doi: 10.1038/onc.2017.40

Research Group

Anna Coppa, researcher; **Valeria Colicchia**, **Marialaura Petroni**, **Francesca Sardina**, post-doc fellows; **Maria Sahún Ronchero**, PhD student.

Collaborations

Zhao-Qi Wang, Leibniz Institute for Age Research, FLI, Jena (D); **Elena Petricci**, Dipartimento. di Chimica, Biochimica e Farmacia, Università di Siena; **Mauro Comes Franchini**, Dip. Chimica Industriele "Toso Montanari", Università di Bologna; **Giulia Guarguaglini**, **Patrizia Lavia**, IBPM, CNR, Roma; **Andrea Prodosmo e Silvia Soddu**, UOSD Network Cellulari e Bersagli Terapeutici Molecolari, Istituto Nazionale Tumori Regina Elena.

MODELING THE HUMAN BONE MARROW NICHE *IN VIVO* AND ITS ROLE IN MALARIA

MARA RIMINUCCI
RESEARCH AREA: BIOLOGY OF MALARIA AND OTHER VECTOR BORNE DISEASES

Department of Molecular Medicine
@: mara.riminucci@uniroma1.it

The project aims to understand the mechanisms of sequestration of the transmission stages of the malaria parasite *Plasmodium falciparum* in the human bone marrow niche by using human bone marrow organoids generated in SCID mice. A scaffold-free version of the human heterotopic ossicle model was developed to improve the analysis of the different marrow tissue and cellular components. In the first year of the project, we fully characterized the novel transplantation system to confirm the functional competence of the human heterotopic bone marrow microenvironment. In the second year, we developed a protocol for the intravenous infusion of labeled human erythrocytes infected with immature (stage II) and mature (stage V) gametocytes in immunodeficient mice. Time course experiments were conducted to investigate gametocyte clearence from the mouse circulation and cell suspensions of mouse bone marrow, spleen and liver were analyzed by FACS to measure parasite clearance from circulation and investigate the distribuition of immature and mature gametocytes in the different organs (Fig. 1A). These experiments detected a fast disappearence from circulation of both immature and mature gametocytes and the localization of immature gametocytes in mouse bone marrow (Fig. 1B). Preliminary immunolocalization studies on paraffin embedded tissues from these experiments, reacted with antibodies specific for the endothelial marker CD31 and the gametocyte-specific protein Pfg27, showed that gametocytes were detectable both in vascular and extravascular sites in the mouse bone marrow. In the third year we worked at improving gametocyte detection in mouse organs and in blood circulation using transgenic *P. falciparum* lines, developed in the laboratory of our collaborator Dr. Pietro Alano (Istituto Superiore di Sanità), whose gametocytes express fluorescent or bioluminescent reporter genes at specific maturation stages. This approach will enable the detection of live parasites and avoid the staining of infected erythrocyte surface with fluorescent labels prior to infusion. We also set up an improved protocol for gametocyte detection and localization in human heterotopic ossicles by preliminary immunostaining of sections of paraffin embedded uninfected ossicles with antibodies against human bone marrow components and mouse endothelial cells. To this aim a collaboration was established with dr. Valeria de Turris (Center fo Life Nano Science, Istituto Italiano di Tecnologia), to analyze thicker ossicle sections (up to 400-

500□m) by two-photon confocal microscopy. A representative image of a 50□m thick section of an uninfected ossicle from preliminary experiments is shown in Fig.1C.
Results and expertise obtained during the project provided the ground for a recently funded two years Seed International Project of the Fondazione Cenci Bolognetti in collaboration with dr Catherine Lavazec (Institute Pasteur, Paris) to investigate erythrocytes infected by mutant gametocytes with altered cell mechanical properties in the ossicle system.

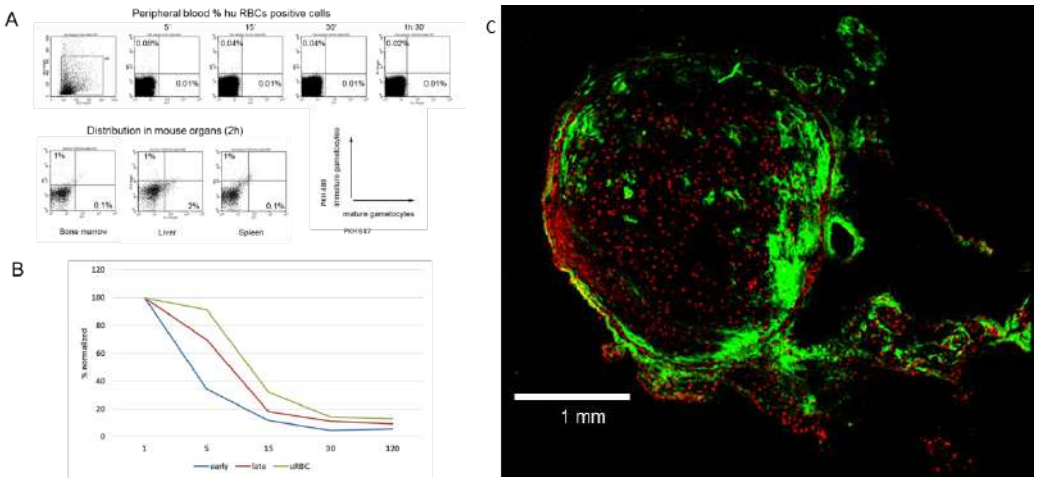


Fig.1: A) A representative FACS analysis of a clearance time course analysis of *P. falciparum* gametocytes infused in SCID mice. B) Kinetics of the clearance of uninfected erythrocytes and of red blood cells infected with immature and mature gametocytes (data normalized on the cell input at inoculation). C) Reconstruction of a TOPRO-labeled 50□m ossicle section by tiling of 100 confocal two-photon microscopy images.

Publications

Remoli C, Michienzi S, Sacchetti B, Di Consiglio A, Cersosimo S, Spica E, Robey PG, Holmbeck K, Cumano A, Boyde A, Davis G, Saggio I, Riminucci M, Bianco P. OSteoblast-specific expression of the Fibrous Dysplasia (FD) causing mutation, Gs□□□□□ produces a high bone mass phenotype but does not reproduce FD in the mouse.
J Bone Miner Res 2014, 30(6):1030-1043.

Riminucci M, Remoli C, Robey PG and Bianco P. Stem cells and bone diseases: new tools, new perspective. *Bone* 2015, 70:55-61.

Pievani A, Sacchetti B, Corsi A, Rambaldi B, Donsante S, Scagliotti V, Vergani P, Remoli C, Biondi A, Robey PG, Riminucci M, Serafini M. Human umbilical cord blood-borne

fibroblasts contain marrow niche precursors that form a bone/ marrow organoid in vivo. *Development* 2017, 144:1035-1044.
Sacchetti B, Remoli C, Funari A, Giannnicola G, G. Robey, P, Kogler G, Liedtke S, Cossu G, Serafini M, Sampaolesi M, Tagliafico E, Tenedini E, Saggio I, Riminucci M, Bianco P. No identical "mesenchymal stem cells" at different times and sites: Human committed progenitors of distinct origin and differentiation potential are incorporated as adventitial cells in microvessels. *Stem Cell Reports* 2016, 6:897-913.

Research Group

Giulia Siciliano, Cristina Remoli, post-doc fellows; **Rossella Labella**, **Biagio Palmisano**, **Samantha Donsanthe**, PhD students; **Alessandro Corsi**, researchers.

Collaborations

Pietro Alano and collaborators, Istituto Superiore di Sanità, Roma.
Valeria de Turris, Center for Life Nano Science, Istituto Italiano di Tecnologia, Roma.

TOWARDS A THERAPY FOR MITOCHONDRIAL TRNA DISORDERS

ARIANNA MONTANARI
UNDER 40 YEARS OLD RESEARCHER
RESEARCH AREA: MOLECULAR INTERACTIONS

Department of Biology and Biotechnologies "Charles Darwin"
@: ari.montanari@uniroma1.it

Defects in mitochondrial (mt) genome can cause a wide range of clinical disorders, mainly neuromuscular diseases. Various strategies have been proposed to address these pathologies but unfortunately, no efficient treatment is currently available. Over 50% of mitochondrial diseases are due to nucleotide changes in the mt tRNA genes (MITOMAP: A Human Mitochondrial Genome Database. http://www.mitomap.org); these mutations are an important cause of human morbidity even if the tRNA genes make up about 10% of the mitochondrial genome.

In the past the rescue of respiratory defects due to mt tRNA mutations was obtained by overexpression of relevant nuclear genes involved in mt protein synthesis and in particular those encoding the mt protein synthesis elongation factor (Ef-Tu) and the mt aminoacyl-tRNA synthetases (aaRS). Moreover, it was demonstrated that the respiratory defects were relieved by overexpression of yeast and human, cognate and non cognate mt aminoacyl-tRNA synthetases (aaRS) LeuRS, IleRS and ValRS. Moreover shorter sequences of the carboxy-terminal domain (Cterm) of human mt LeuRS responsible for suppression were identified. The peptides of 15 and 16 amino acids (ß30_31 and ß32_33) were endowed with the same suppressing activity of the full Cterm when overexpressed in a broad range of different mt tRNA mutants.

We demonstrate the suppressing ability of ß30_31 and ß32_33 peptides in transfected human cybrids bearing either the m.3243A>G MELAS mutation in the cognate mt $tRNA^{Leu(UUR)}$ or the m.8344A>G MERRF mutation in the non-cognate mt -$tRNA^{Lys}$. The transfected cells show the increasing of the viability and the oxygen consumption and decreasing of the apoptosis. The ability of peptides ß30_31 and ß32_33 to interact with the WT and mutated human mt tRNAs was evaluated by *in vitro* experiments. By the surface plasmon resonance we show that both peptides interact directly and with high affinity with all synthetic mt tRNAs having the most efficient interactions with the mt $tRNA^{Leu(UUR)}$; peptide ß32_33 has higher affinity than ß30_31 towards mt $tRNA^{Lys}$. By thermal denaturation experiments we confirm the highest stabilization effect of the peptide ß32_33 on the thermal stability alterations occurring in synthetic mutated mt $tRNA^{Leu(UUR)}$ and mt $tRNA^{Lys}$.

To possibly develop the above results for therapeutic purposes would be to target peptides to cells and to mitochondria. We used the multi-walled carbon nanotubes to

deliver the suppressive peptide ß32_33 into the mitochondrial compartment. By the fluorescence microscopy we demonstrate the mitochondrial localization in yeast and human peripheral blood mononuclear cells of these constructs, while the control without the peptide is diffused in the cytoplasm, indicating that the peptide sequence maintains its intrinsic mitochondrial targeting activity when conjugated to carbon nanotubes. These constructs do not affect cellular viability and cytotoxicity both *in vitro* and *in vivo*. The *in vitro* viability of monocyte cells was tested by checking the release of the cytosolic enzyme lactate dehydrogenase (LDH), and the possible effect on the cell mitochondrial function by the MTT test. The *in vivo* toxicity was assessed on the simple pluricellular model *Caenorhabditis elegans*: the analyzed constructs do not affect the life-span, the brood size and the reproductive potential of the nematodes.

In order to identify the minimal sequence of the human mt LeuRS Cterm maintaining the rescuing effect we cloned three different esa-nucleotide sequences within the ß30_31 and ß32_33 strands, chosen to preserve the positive charge of the amino acids: NKACGK, LINNKA and KSFLSP. The nucleotide sequence coding for LINNKA overexpressed in the yeast mt tRNA mutants has the best rescuing capability of mutants respiratory defects. As negative control we cloned the sequences corresponding to the α helices within the Cterm (α29_30) that do not contact the tRNAs and two sequences derived from LINNKA obtained by scrambling the amino acids order or by removing the positive charge; the latter variant completely loses the suppressive capability. Moreover we show that the addition of the mitochondrial targeting sequence to LINNKA extends its suppressing capability of different mt tRNA mutants, highlighting a relationship between the rescuing capability of the overexpressed sequences and the amount of suppressor.

The research has extended to the study of the histone acetyltransferase gcn5, required for respiratory growth and mt DNA maintenance. Understanding the mitochondrial role of gcn5 could open interesting perspectives for novel therapeutic approaches to diseases caused by mt DNA depletion.

Publications

Francisci S, Montanari A; Mitochondrial diseases: Yeast as a model for the study of suppressors, *BBA-Molecular Cell Research* 2017, 1864, doi: 10.1016/j.bbamcr.2017.01.008

Di Nottia M, Montanari A, Verrigni D, Oliva R, Torraco A, Fernandez-Vizarra E, Diodato D, Rizza T, Bianchi M, Catteruccia M, Zeviani M, Dionisi-Vici C, Francisci S, Bertini E, Carrozzo R; Novel homozygous mutation in mitochondrial elongation factor EF-Tu associated to dysplastic leukoencephalopathy and defective mitochondrial DNA translation, *BBA- Molecular Basis of Diseases* 2017, 1863, doi: 10.1016/j.bbadis.2017.01.022

Ficociello G, Salemme A, Uccelletti D, Fiorito S, Togna AR, Vallan L, Gonzàlez Domìnguez JM, Da Ros T, Francisci S, Montanari A; Evaluation of the efficacy of carbon

nanotubes for delivering peptides into mitochondria, *RSC Adv* 2016, 6, doi: 10.1039/c6ra14254k

Canzonetta C, Leo M, Guarino SR, Montanari A, Francisci S, Filetici P, SAGA complex and Gcn5 are necessary for respiration in budding yeast, *BBA-Molecular Cell Research* 2016, 1863, doi: 10.1016/j.bbamcr.2016.10.002

Torraco A, Bianchi M, Verrigni D, Gelmetti V, Riley L, Niceta M, Martinelli D, Montanari A, Guo Y, Rizza T, Diodato D, Di Nottia M, Lucarelli B, Sorrentino F, Piemonte F, Francisci S, Tartaglia M, Valente EM, Dionisi-Vici C, Christodoulou J, Bertini E, Carrozzo R; A novel mutation in NDUFB11 unveils a new clinical phenotype associated with lactic acidosis and sideroblastic anemia, *Clin Genet* 2016, 1863, doi: 10.1111/cge.12790

Perli E, Fiorillo A, Giordano C, Pisano A, Montanari A, Grazioli P, Campese AF, Di Micco P, Tuppen HA, Genovese I, Poser E, Preziuso C, Taylor RW, Morea V, Colotti G, d'Amati G; Short peptides from leucyl-tRNA synthetase rescue disease causing mitochondrial tRNA point mutations. *Hum Mol Genet* 2016, 25, doi:10.1093/hmg/ddv619.pp.ddv619.

Research Group

Graziella Ficociello, Post-doc fellow;
Michela Di Nottia, Research fellow;
Manuela Leo, PhD student;
Giulia Trani, PhD student.

Collaborations

Robert N. Lightowlers, **Zofia M. Chrzanowska-Lightowlers**, Wellcome Trust Center for Mitochondiral Research, Newcastle University, UK.
Rosalba Carrozzo, Unit of Muscular and Neurodegenerative Disorders, Laboratory of Molecular Medicine, Bambino Gesù Children's Hospital, IRCCS, Rome.

TAMING HIPK2 KINASE ACTIVITY TO TACKLE CELLULAR DISEASES AND CANCER

LINDA CELESTE MONTEMIGLIO
UNDER 40 YEARS OLD RESEARCHER
RESEARCH AREA: MOLECULAR INTERACTIONS

Department of Biochemical Sciences "A. Rossi Fanelli"
@: lindac.montemiglio@uniroma1.it

The homeodomain interacting protein kinase 2 (HIPK2) is a multidomain, tyrosine(Y)-regulated serine/threonine (S/T) kinase mainly found in the nucleus that is involved in the regulation of cell growth, development, morphogenesis, transcription and death. Dysfunctions of HIPK2 result in the development of diseases and abnormalities linked to increased cell proliferation, as in cancer or fibrosis. Regulation of HIPK2 functions is achieved by the complex architecture of its functional domains and by post-translational modifications that include ubiquitylation, sumoylation, acetylation and phosphorylation.
We investigated the effects on the HIPK2 kinase domain of the *cis*-auto-phosphorylation of tyrosine 361 at the activation loop, a specific post-translational modification that controls its dual-specific activity. Previous *in vivo* observations showed that lack of phosphorylation at Y361 results in an aberrant form of HIPK2 with altered activities and specificity on substrates, tumour-like cellular relocalization and accumulation in cytoplasmic aggresomes.
We compared biochemical properties of the kinase domain of the wild type enzyme with the ones of two mutants at key residues, *(i)* Y361F, that mimics the aberrant form of HIPK2 not phosphorylated at this position and *(ii)* K228A, at the catalytic lysine 228, that inactivates the kinase. We used ATP-competitors different in structure to probe structural differences of the activation loop and of the catalytic core due to the Y361 phosphorylation state.
Our results shed some light on the role of Y361 phosphorylation in stabilizing HIPK2, triggering structural changes in the activation loop that we show to be connected to the catalytic pocket. We also showed that Y361-phosphorylation affects the HIPK2 oligomerization state, providing clues on the biochemical basis of the HIPK2 aggregation and aberrant properties observed *in vivo* on cellular systems. Moreover, we observed that ATP-competitors can exert inhibition in a substrate-specific manner, thus opening the possibility to explore this property for HIPK2 activity modulation, as pursued in cancer and fibrosis research that target HIPK2.

Following this evidence, we are exploring the possibility to modulate the phosphorylation activity of HIPK2 on the oncosuppressor p53, one of the crucial HIPK2 partner involved in DNA damage signalling: in the case of severe DNA damage, HIPK2 phosphorylates p53 triggering apoptosis to damaged cells, thus preventing neoplastic transformation and cancer development. We are sieving small libraries of kinase inhibitors and actinomycete natural products to analyse their effect on activity and stability of the complex that will be used also for *in vivo* tests on cellular models of cancer. Additionally, we are studying the effect of such compounds on alternative functional complexes of HIPK2 that affect p53 interaction, such as Axin, which promotes complex formation enhancing the HIPK2-p53 apoptotic functions, and the Human Papilloma Virus (HPV) E6 protein that inhibits it, as in the case of skin carcinogenesis.
Together with functional studies, we are pursuing the structural characterization by cryo-electron microscopy (cryo-EM) integrated by X-ray crystallography of the HIPK2-p53, HIPK2-Axin-p53, and HIPK2-HPV E6 protein complexes. Since HIPK2 and p53 are challenging targets for structural studies due to their intrinsic instability and flexibility, we are devoting much experimental effort in obtaining samples of HIPK2-p53 and HIPK2- HPV E6 protein complexes suitable for cryo-EM characterization, and several negative staining measurements were performed to test sample quality. The biochemical characterization of both complex formation, that we are running in parallel, will guide us in enhancing homogeneity and monodispersion of samples.

Publications

Scaglione A, Monteonofrio L, Parisi G, Cecchetti C, Siepi F, Rinaldo C, Giorgi A, Verzili D, Zamparelli C, Savino C, Soddu S, Vallone B, Montemiglio LC. Effects of Y361-auto-phosphorylation on structural plasticity of the HIPK2 kinase domain. *Protein Sci.* 2018, 27(3):725-737. doi: 10.1002/pro.3367.

Research Group

Antonella Scaglione, **Giacomo Parisi**, **Francesca Malagrinò**, post-doc fellows; **Gabriele Cerutti**, PhD student; **Ida Freda**, post-graduate student.

Collaborations

A. des Georges, The City University of New York (CUNY), New York, USA; **R. Owens**, Oxford Protein Production Factory (OPPF), UK; **M. Ruzzene**, University of Padova, Italy; **J.A. Salas**, Oviedo University, Spain; **C. Savino**, CNR, Rome, Italy; **S. Soddu**, Istituto Nazionale dei Tumori Regina Elena, Rome, Italy; **B. Vallone**, University of Rome Sapienza, Italy.

IMMUNOMETABOLIC CHECKPOINTS OF TREG PERFORMANCE IN HCV-RELATED METABOLIC INFLAMMATION AND CANCER

SILVIA PICONESE
UNDER 40 YEARS OLD RESEARCHER
RESEARCH AREA: CELLULAR AND MOLECULAR IMMUNOLOGY

Dipartimento di Medicina Interna e Specialità Mediche
@: silvia.piconese@uniroma1.it

Regulatory T cells (Tregs), which are physiologically devoted to the suppression of unwanted immunity and inflammation, inhibit protective anti-tumor responses. Our data in human cancer demonstrate that activated Tregs express high levels of the OX40 receptor and accumulate in hepatic microenvironments affected by premalignant condition (cirrhosis) or hepatocellular carcinoma (HCC). T cell promply adapt ther metabolism according to external stimuli, shifting from oxidative to glycolytic metabolism in resting/memory or proliferating conditions, respectively. However, in the tumor microenvironment, the high levels of glucose consumption by tumor cells leads to glucose deprivation and to functional paralysis of anti-tumor effector cells. The goal of this project was to define whether Tregs could resist the hostile tumor microenvironment through metabolic adaptation.

First, we analyzed tumor-infiltrating Tregs in a short-term experimental model of tumor growth. We observed that tumor-infiltrating Tregs accumulated higher levels of intracellular neutral lipids, compared to tumor-infiltrating conventional T cells (Tconvs) and to peripheral counterparts, as revealed by incorporation of the lipophilic dye Bodipy. Multicolor flow cytometry allowed visualizing, in tumor-Tregs, a higher mRNA content of genes involved in fatty acid synthesis, uptake or usage. A higher lipid synthesis may be fueled, in tumor-Tregs, by a more active glucose metabolism: supporting this hypothesis, we found that i) Tregs did not show defective glucose uptake in the tumor microenvironment, contrary to Tconvs; ii) tumor-Tregs expressed higher levels of genes related to glycolysis, compared to Tconvs; iii) tumor-Tregs engaged both glycolysis and oxidative phosphorylation, at higher levels compared to Tconvs. The reliance of Tregs on both pathways is testified by the observation that, in vitro, the blockade of either pathway inhibited Treg proliferation.

To ascertain whether the preferential lipid accumulation in Tregs was an exclusive feature of tumor, we analyzed Treg frequency and intracellular lipid content in two distinct models of liver disease, namely the *Mdr2*$^{-/-}$ mouse (developing from an early age inflammatory cholangitis that progresses into cirrhosis and cancer), and the HCV-transgenic (HCVTg) mouse (spontaneously developing steatosis at advanced ages with no evidence of inflammation): we found hepatic Treg expansion and intracellular lipid

accumulation, in *Mdr2*$^{-/-}$ but not in HCVTg mice, demonstrating that Tregs enlarge their intracellular lipid pool when prompted to expansion, irrespective of whether triggered by inflammation or tumor.
Finally, we could demonstrate that a circuit of glycolysis/lipid biosynthesis may support the tumor-specific expansion also of human Tregs: a gene expression analysis indicated that OX40+ Tregs, estracted from human HCC samples, display molecular signs of metabolic reprogramming, mostly involving glycolysis and lipid biosynthesis/modification.
In summary, our results indicate that activation of specific metabolic pathways may support Treg advantage in the tumor microenvironment in both mouse and human cancer.

Publications

Donninelli G, Del Cornò M, Pierdominici M, Scazzocchio B, Varì R, Varano B, Pacella I, Piconese S, Barnaba V, D'Archivio M, Masella R, Conti L, Gessani S. Distinct Blood and Visceral Adipose Tissue Regulatory T Cell and Innate Lymphocyte Profiles Characterize Obesity and Colorectal Cancer. *Front Immunol.* 2017 8:643. doi: 10.3389/fimmu.2017.00643

Timperi E, Barnaba V, Piconese S. Phenotypic and Functional Analysis of the Suppressive Function of Human Regulatory T Cells. *Methods Mol Biol.* 2017;1514:139-151. doi: 10.1007/978-1-4939-6548-9_12

Timperi E, Folgori L, Amodio D, De Luca M, Chiurchiù S, Piconese S, Di Cesare S, Pacella I, Martire C, Bonatti G, Perrone S, Boni T, Marcovecchio GE, Reale A, Parisi F, Dotta A, Barnaba V, Rossi P. Expansion of activated regulatory T cells inversely correlates with clinical severity in septic neonates. *J Allergy Clin Immunol.* 2016 May;137(5):1617-1620.e6. doi: 10.1016/j.jaci.2015.10.048.

Timperi E, Pacella I, Schinzari V, Focaccetti C, Sacco L, Farelli F, Caronna R, Del Bene G, Longo F, Ciardi A, Morelli S, Vestri AR, Chirletti P, Barnaba V, Piconese S. Regulatory T cells with multiple suppressive and potentially pro-tumor activities accumulate in human colorectal cancer. *Oncoimmunology.* 2016 Apr 25;5(7):e1175800. doi: 10.1080/2162402X.2016.1175800.

Pacella I, Timperi E, Accapezzato D, Martire C, Labbadia G, Cavallari EN, D'Ettorre G, Calvo L, Rizzo F, Severa M, Coccia EM, Vullo V, Barnaba V, Piconese S. IFN-α promotes rapid human Treg contraction and late Th1-like Treg decrease. *J Leukoc Biol.* 2016 Sep;100(3):613-23. doi: 10.1189/jlb.5A0415-140R.

Schinzari V, Barnaba V. Piconese S. Chronic HBV and HCV infections and cancer: synergy between viral and host factors. *Clin Microbiol Infect.* 2015 Nov;21(11):969-74. doi: 10.1016/j.cmi.2015.06.026. Review.

Piconese S, Barnaba V
Regulation of immunopathology in hepatitis B virus infection.
Nat Med. 2015 Jun 4;21(6):548-9. doi: 10.1038/nm.3873. Commentary.

Research Group

Ilenia Pacella Post-Doc fellow
Chiara Focaccetti Post-Doc fellow.

Collaborations

Giuseppe Matarese II Università di Napoli
Maryline Bourgine Institut Pasteur Paris.

A DROSOPHILA MODEL FOR SPINAL MUSCULAR ATROPHY (SMA): IDENTIFICATION AND CHARACTERIZATION OF SMN INTERACTORS AND PHENOTYPIC MODIFIERS

GRAZIA DANIELA RAFFA
UNDER 40 YEARS OLD RESEARCHER
RESEARCH AREA: GENETICS, BIOLOGY AND PATHOPHYSIOLOGY OF EUKARYOTES

Department of Biology and Biotechnology "C. Darwin"
@: graziadaniela.raffa@uniroma1.it

SMN is the gene responsible for spinal muscular atrophy, a devastating neuromuscular disease, characterized by defects in RNA metabolism and loss of motor neurons. We developed an RNAi-based model to study SMN function in *Drosophila* and found two novel SMA modifiers: WDR79 and TGS1.

1) *WDR79/TCAB1 is a conserved SMN modifier.*

We found that *WDR79/TCAB1 Drosophila* mutants exhibit downregulation of the SMN transcript and protein, causing defects in locomotion behavior in both mutant larvae and adults. Importantly, WDR79 overexpression significantly ameliorates the locomotion defects of *SmnRNAi* mutant larvae. We also found that *SmnRNAi* flies exhibit defects in wing expansion, that are significantly improved by increasing the WDR79 dosage. We have also shown that the genetic interaction between SMN and WDR79 is conserved in the nematode *C. elegans*. Our results collectively suggest that WDR79 and SMN play evolutionarily conserved cooperative functions in the nervous system

2) *Investigating the role of TGS1 in the SMN pathway*

TGS1, Trimethyl Guanosine Synthetase catalyzes the formation of the TMG cap at the 5' end of several types of RNAs, including the snRNAs, snoRNAs and telomerase RNA. SMN is thought to be upstream of TGS1 in the pathway required for cap hypermethylation, thus we expect the SMN- and TGS1- depleted cells to be defective for at least a subset of RNA targets/Ribonucleoproteins that require this modification. We generated novel null alleles of *Drosophila Tgs1 (dTgs1)* using CRISPR-Cas9 mutagenesis. Homozygosity for these mutations causes early lethality. Notably, the expression of a human *TGS1* transgene in these mutants completely rescued lethality, demonstrating that TGS1 function is conserved. Hypomorphic *dTgs1* mutants exhibit phenotypes similar to those observed in *SmnRNAi* flies, including defects in locomotion and wing expansion. In addition, the SMN-dependent wing expansion phenotype is significantly corrected by expression of either dTgs1 or human TGS1, suggesting that Tgs1 is in involved in the same neural circuits controlled by Smn. We also found that human HeLa cell lines carrying mutations in *TGS1* have reduced Cajal bodies, defects

in SMN localization and in the splicing of transcripts containing U12 type introns. Our studies show that loss of TGS1 function elicits defects similar to those observed upon loss of SMN both in *Drosophila* and in humans.

Publications

Di Giorgio ML, Esposito A, Maccallini P, Micheli E, Bavasso F,…, **Raffa GD**. (2017) WDR79/TCAB1 plays a conserved role in the control of locomotion and ameliorates phenotypic defects in SMA models. Neurobiology of disease, 105, 42-50. DOI: 10.1016/j.nbd.2017.05.005

Cicconi A, Micheli E, Verni F, Jackson A, Gradilla AC,…, **Raffa GD**. (2017) The Drosophila telomere-capping protein Verrocchio binds single-stranded DNA and protects telomeres from DNA damage response. Nucleic acids research, 45, 3068-3085. DOI: 10.1093/nar/gkw1244

Cipressa F, Morciano P, Bosso G, Mannini L, Galati A, **Raffa GD**, . . . Cenci G. (2016) A role for Separase in telomere protection. Nature communications, 7, 10405. DOI: 10.1038/ncomms10405

Burla R, Carcuro M, Raffa GD, Galati A, Raimondo D , Rizzo A, La Torre M, Micheli M, Ciapponi L, Cenci G, Cundari E, Musio A, Biroccio A, Cacchione S, Gatti M, Saggio I. AKTIP/Ft1, a new shelterin-interacting factor required for telomere maintenance. PLOS Genetics 2015, 11:e1005167. doi: 10.1371/journal.pgen.1005167.

Cenci G, Ciapponi L, Marzullo M, Raffa GD, Morciano P, Raimondo D, Burla R, Saggio I, Gatti G. The analysis of pendolino (peo) mutants reveals differences in the fusigenic potential among Drosophila telomeres. PLOS Genetics 2015, 11:e1005260. doi: 10.1371/journal.pgen.1005260.

Research Group

Bavasso Francesca; **Maccallini Paolo**, PhD Students;
Cicconi Alessandro, **Valeria Palumbo**, Post-doc fellows

Collaborations

Stefano Cacchione, **Alessandra Galati**-Sapienza Università di Roma, Department of Biology and Biotechnology
Elia Di Schiavi-IBBR, CNR, Naples
Steven Artandi-Stanford University, USA

STUDY OF THYROID HORMONE T3 IN SKELETAL MUSCLE HOMEOSTASIS: POSSIBLE ROLE IN MUSCLE WASTING

CECILIA VERGA FALZACAPPA
UNDER 40 YEARS OLD RESEARCHER
RESEARCH AREA: MOLECULAR INTERACTIONS

Department of Medical-Surgical Sciences and Biotechnologies
@: cecilia.vergafalzacappa@uniroma1.it

Skeletal muscle has been recognized as a thyroid hormones (THs) target for contractile function, regeneration, metabolism and glucose disposal. However, a possible role for THs in muscle homeostasis and cellular metabolism in a pathological state such as food deprivation has never been investigated. Food deprivation induces skeletal muscle atrophy, causing metabolic changes, and forcing the tissue to utilize fatty acid as the main oxidation substrate. The purpose of this study is to evaluate whether thyroid hormones may hamper the fasting induced skeletal muscle atrophy and to investigate the mechanisms involved.

On the first year of the project, we demonstrated that the daily injection of high dose thyroid hormone was able to protect Balb-c male mice (8 weeks) from fasting induced skeletal muscle atrophy.
In the second year of the project we characterized the molecular mechanisms underlying the morphological effects, we excluded hyperthyroidism in our model and evidenced that, interesting, the atrophic pathway was not hampered by the hormone treatment. On the other hand, we obtained some interesting indications on the possible induction of metabolic adaptation in skeletal muscle by hormone treatment.
Based on the results of transcriptome analyses, which evidenced that T3 mostly regulates metabolic process related genes, in the third year we focused on the effects of thyroid hormone treatment on the metabolic pathway. We evidenced that the hormone treatment was able to significantly reduce the shift in the metabolism towards a more oxidative state ($p<0,05$) and a less glycolytic one ($p<0,01$) induced by fasting. The decrease of the amount in oxidative muscle fibers during T3 treatment in STV agrees with a regulation of PGC-1 family members genes, molecular markers of mitochondriogenesis and fatty acid oxidation.
To definitely exclude any other action, we confirmed that the hormone treatment did not affect the proteasome pathway, nor influenced the anabolic pathway. These finding confirmed that the observed effects did not depend on a direct regulation of the atrophic pathway. Interestingly we also made evidence that myofibers neogenesis was not

induced by T3, excluding that the hormone action could involve the skeletal muscle regeneration.
In conclusion, our work demonstrate that thyroid hormone T3 can protect myofibers from a starvation induced atrophy in a mouse model and that this effect is mainly due yo the induction of a metabolic adaptation in myofibers. Therefore, T3 treatment during fasting seems to exert a protective role on the metabolism of muscle fibers

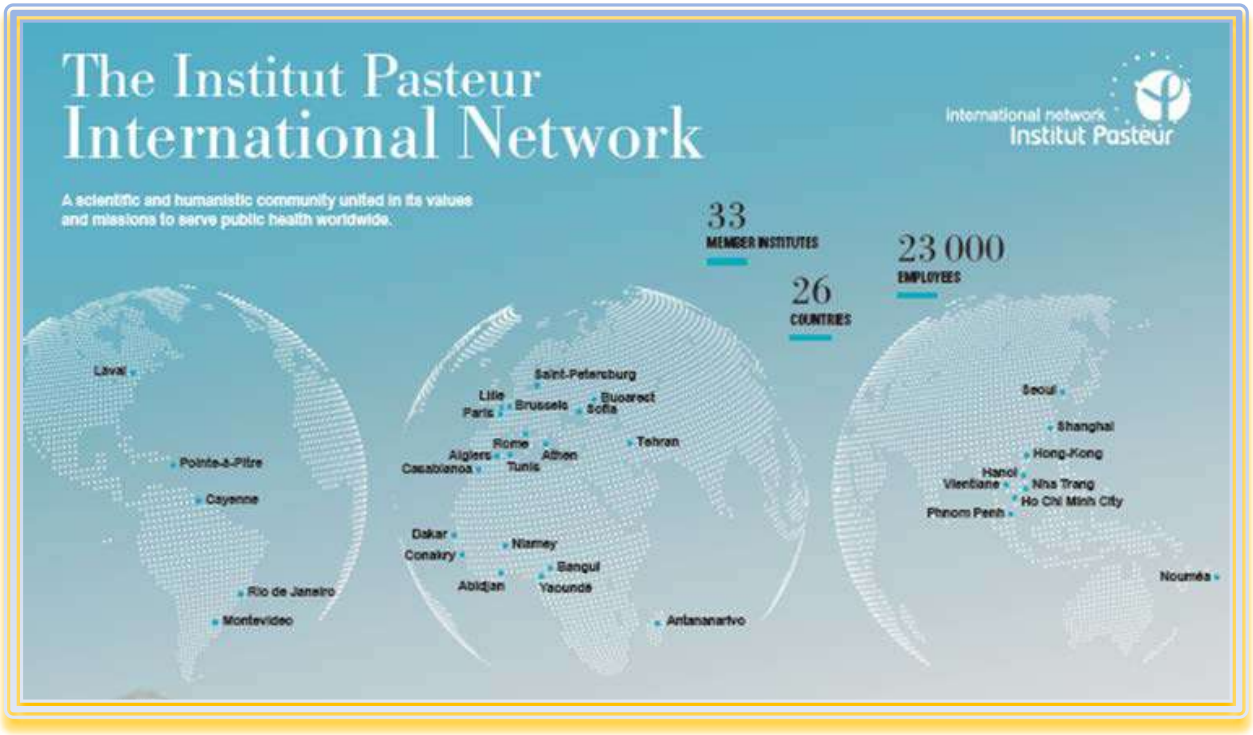
Research Group

Sara Ucci, post-doc fellow; **Valentina Russi,** post graduate fellow

Collaborations

Viviana Moresi, Sapienza Università di Roma.

COLLABORATIONS WITHIN THE INTERNATIONAL NETWORK OF INSTITUTES PASTEUR



PTR – PROJETS TRASVERSAUX DE RECHERCE

Funded by Institut Pasteur Paris.

PTR 2017 - 2018
COMPLEXITY OF IMMUNE INTERACTIONS IN CHRONIC HEPATITIS B VIRUS INFECTION: HOW THE EXACERBATED INFLAMMATORY RESPONSE BY SELF ANTIGEN-SPECIFIC CD8 T CELLS AND REGULATORY T CELLS DICTATE THE FATE OF HBV-SPECIFIC RESPONSES.

Maryline BOURGINE[1], Yu WEI[1,2], Silvia PICONESE[3], Vincenzo BARNABA[3]
1. IP Paris, 2. IP Shanghai, 3. IP Rome.

Hepatocellular carcinoma (HCC) is the second cause of death by cancer in the world. Chronic infection with hepatitis B virus (HBV) is a major etiology factor for the development of HCC, being associated with more than half death by HCC each year. The onset of HCC is principally linked to chronic inflammation due to the host immune response to infected liver cells. It is well known that in chronically infected HBV patients, virus-specific T-cell responses are weak and impaired, with T cell exhaustion that is characterized by a high susceptibility to apoptosis. Apoptotic cells are engulfed by dendritic cells that process and cross-present apoptosis-derived epitopes (AE) to autoreactive CD8 T cells. In the long term, these CD8 self-reactive cells lead to the dysfunction/generalized depletion of T cells by a mechanism of apoptosis. The autoimmune response against apoptotic T cells may play a role in the state of chronic inflammation in chronic HBV infection.

The project proposes to identify and characterize the CD8 cells specific for self epitopes derived from apoptotic T cells, in maintaining the state of chronic inflammation associated to HBV infection and the effects of the regulatory T cells to balance the activity of autoreactive CD8 T cells. To reach this goal, we propose to study the AE-specific CD8 response in:
1. An innovative mouse model for chronic hepatitis B, i.e. wild-type mice transduced with an adeno-associated vector carrying a replication-competent HBV DNA genome (AAV-HBV) vector.
2. Patients (HLA A*0201-positive) with HBV infection at different stages of the chronic disease, treated or not with nucleoside analogues.
Understanding the mechanisms that regulate the frequency and phenotype of apoptotic epitope-specific T cells may cast novel insight into the pathogenesis, progression and clinical evolution towards HCC associated with HBV infection.

PTR 2017 - 2018
UNDERSTANDING THE SELECTIVE BENEFIT OF THE SHIGELLA GENOME SPECIFIC ARCHITECTURE.

Bianca COLONNA[1], Didier MAZEL[2] ,
1. IP Rome 2. IP Paris

Current knowledge incorporates the notion that the bacterial genome can be made up of more than one chromosome, that mobile genetic elements play a key role in the adaptation of bacteria to new environments, including the human host, and that the distinction between chromosome and very large plasmid has become a subtle one. All this is particularly relevant when addressing the genome arrangement and evolution of bacterial pathogens which often harbour virulence genes on a large plasmid. A paradigmatic case in this respect is constituted by *Shigella* and enteroinvasive *E. coli* (EIEC), the etiological agents of bacillary dysentery, a life-threatening syndrome for the child population of developing countries. In these bacteria the pathogenicity process depends on the presence of a large plasmid (pINV) which carries a 32 kb pathogenicity island (PAI) encoding most genes required for the invasive process. The pINV is able to integrate into the chromosome and in this condition virulence traits are no longer expressed.
The project tackles several questions concerning the genetic and evolutionary relationships between the virulent phenotype and the genome arrangement of *Shigella* and EIEC: how stringent is the need for virulence genes to reside on a plasmid? Are these genes always silenced when they become part of a chromosome? Which mechanism controls their switch-off? Which are the consequences of domestication of the pINV in terms of stability and gene expression? Can an avirulent phenotype be established by affecting plasmid stability with specific drugs?
The project develops as a collaboration between two groups (B.Colonna, Rome and D.Mazel, Paris), and relies on their longstanding expertise in molecular genetics and genomics in the addressed fields.
In terms of expected impact, gaining new insight into bacterial genome organization and defining how genomes are arranged, besides opening new developments in the field of synthetic biology, will contribute to the design of new therapeutic strategies targeting the stability of genome elements.

Participants

Bianca Colonna, Gianni Prosseda, Maria Rita Stirpe, Martina Pasqua
Didier Mazel, Marie Eve Val -Kennedy

PTR 2017 - 2018
A MULTIDISCIPLINARY INVESTIGATION OF THE NEGATIVICUTES: ATYPICAL FIRMICUTES WITH LPS-OUTER MEMBRANES THAT INHABIT THE HUMAN GUT.

Simonetta GRIBALDO[1], Christophe BELOIN1 Maria L. BERNARDINI[2]
1. IP Rome 2. IP Paris

The *Negativicutes* are a poorly studied lineage of bacteria that include common inhabitants of the human oral and gut microbiome such as the anaerobe *Veillonella*, and which can also develop into opportunistic pathogens. Despite belonging phylogenetically to the Firmicutes (low G+C Gram positives), they surprisingly harbour outer membranes (OM) with lipopolysaccharide (LPS), which are characteristics typical of Gram-negative bacteria, making them an evolutionary conundrum and also promising new models to study the diversity and function of bacterial cell envelopes. However, very few and scattered information is available on the nature and origin of the OM in these atypical diderm Firmicutes and on their role in community behaviour in the gut environment, as well as their interaction with the immune system. This project is aimed at fulfilling this gap by carrying out a highly interdisciplinary study merging bioinformatics and evolutionary analyses with experimental work.
To achieve this goal the available complete genomes of *Negativicutes* will be screened through a bioinformatics pipeline to detect all potential OM components. This analysis will be complemented by a comparative proteomic analysis of the major components of the cell surface of strains representing the diversity of *Negativicutes*. The resulting list of candidates will be subjected to functional annotation and evolutionary analysis to establish a picture of the nature of the OM of *Negativicutes* and to understand how it originated. In parallel, the cell surface components, which are involved in the sessile life of *Veillonella* and its interaction with other relevant members of the gut microbiota community will be analysed.
The impact of life in biofilm will be studied focusing the attention on its influence on OM composition and structure, including LPS, which is essential for the interaction of *Veillonella* with host components and notably the immune system. The inflammatory potential of *Veillonella* will be assessed by analysing the immune impact of its LPS, which will be purified and structurally and immunologically characterized.
This exploratory project will pioneer a novel research line on an understudied branch of the bacterial tree harbouring unique characteristics and with obvious relevance for both evolutionary and biomedical research.

PTR 2016 - 2017
BEYOND ACID RESISTENCE: ROLE OF BACTERIAL GAMMA-AMINOBUTYRATE (GABA) IN COMMUNITY AND HOST INTERACTIONS (GABACTERIA)

Jean Marc GHIGO[1], Daniela DE BIASE[2]
1. IP Rome 2. IP Paris

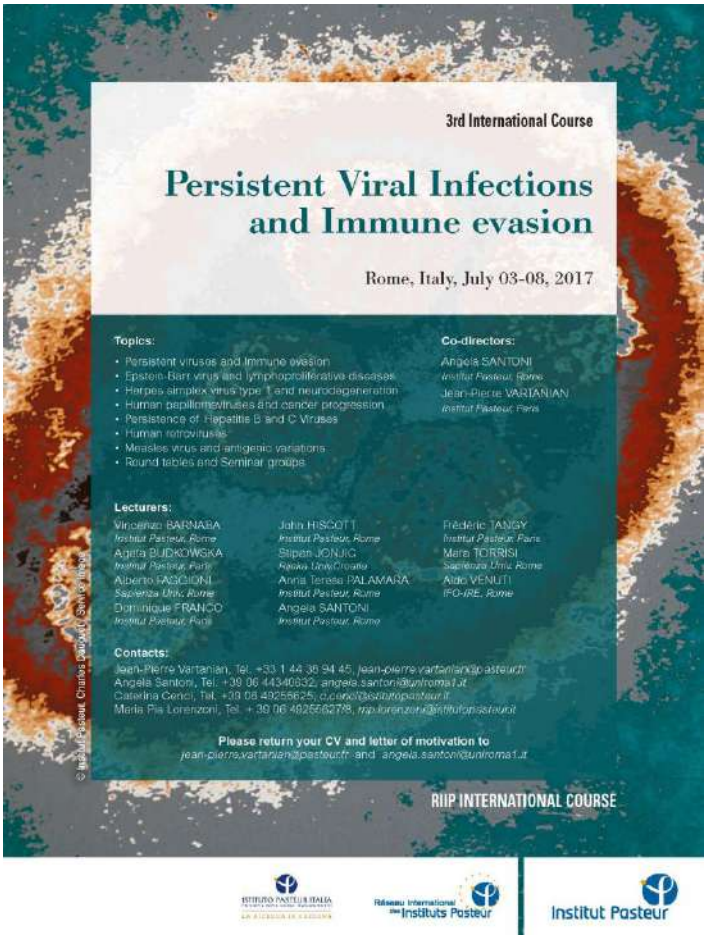
The role of bacterial GABA, a non-proteinaceous amino acid, on bacterial and host physiology was investigated in the frame of the PTR 540. The role of the acid resistance genes coding for the regulators and enzymes involved in GABA synthesis in *Escherichia coli* in phenotypes (motility, aggregation and biofilm formation) that are not directly related to acid resistance, for which these genes are most well known, were investigated. IP-Paris and IP Roma team analyzed these community-associated phenotypes in wildtype and mutant *E. coli* generated by IP-Roma. Whereas some mutants in the genes involved in GABA synthesis and regulation display an increased frequency of aggregation, other analyses did not reveal any strong phenotypic differences compared to wild type. IP-Paris and IP Roma also studied how these phenotypes are affected in the presence of known concentration of extracellular GABA in commensal and pathogenic *E. coli*. These analyses did not evidence drastic changes in biofilm formation ability. However, while carrying out biofilm studies in different media and temperatures, IP Roma noticed strong increase in biofilm ability in some specific media and temperatures. This is currently under further investigation. In parallel of these in vitro approaches, IP-Roma performed calibration tests and developed an HPLC assay sensitive enough to detect GABA from biological samples prepared by IP-Paris, corresponding to conventional and germ-free mouse sera. The preliminary results are promising and going to be further developed. IP-Paris also developed a set-up to study simple behaviors (flight or attraction towards light) in conventional and germ-free zebrafish larvae colonized. At least under these assay conditions, IP-Paris did not observe any GABA-associated change in behavior. We are now assembling common results and planning to deepen our understanding on preliminary but promising results. Overall this project has set the basis to address the evolution and consequence of GABA produced by the gut microbiota.

TRAINING AND EDUCATION

- **RIIP INTERNATIONAL COURSE**
- **PASTEUR SEMINAR SERIES**

RIIP INTERNATIONAL COURSE

ROME, JULY 03 – 08 2017



Istituto Pasteur Italia has hosted the 3rd International Course on "***PERSISTENT VIRAL INFECTIONS AND IMMUNE EVASION***" organized in collaboration with Institut Pasteur of Paris.

Co - Directors: Angela Santoni (IP Italia) and Jean Pierre Vartanian (IP Paris).

Topics: Persistent viruses and Immune evasion; EBV and lymphoproliferative diseases; HSV type 1 and neurodegeneration; HPV and cancer progression; Pesistence of HBV and HCV; Human retroviruses; Measles virus and antigenic variations; Round tables and Seminar groups.

Lecturers: Vincenzo Barnaba (IP Italia); Agata Budkowska (IP Paris); Alberto Faggioni (Sapienza Univ); Dominique Franco (IP Paris); John Hiscott (IP Italia); Stipan Jonjic (Croatia Univ); Anna Teresa Palamara (IP Italia); Angela Santoni (IP Italia); Carolina Scagnolari (Sapienza Univ); Frédéric Tangy (IP Paris); Mara Torrisi (Sapienza Univ); Aldo venuti (IFO-IRE Rome).

Tutors: Cristina Cerboni (IP Italia); Roberta Gonnella and Roberta Santarelli (Sapienza Univ); Silvia Piconese (IP Italia); Gianluca Russo (Sapienza Univ).

14 selected students attended and successfully terminated the course.

2017 PASTEUR SEMINARS - Scientific Organizer: John Hiscott

February

02 feb **Paola Vittorioso** (Dept. of Biology and Biotechnology – Sapienza)
From seed to seedling: how Arabidopsis makes the decision to start a new life cycle.

16 feb **Francesca Cutruzzolà** (Dept. of Biochemical Science – Sapienza)
Cyclic dinucleotides: from second messengers in bacterial biofilm formation to modulators of host immune response.

23 feb **Giuseppe Sciumé** (Dept. of Moelcualr Medicine – Sapienza)
Transcriptional and Epigenetic regulation of Innate lymphoid cell biology.

March

02 march **Cristina Limatola** (Dept. of Physiology and Pharmacology – Sapienza)
New strategies to reprogram microglia/ macrophages in brain tumors: the effect of the environment.

17 march **Adane Achour** (Karolinska Institute – Stockolm)
Bases for induction of efficient CTL responses against viral escape variants; Implications for future vaccination studies.

20 march **Howard Young** (National cancer Institute - Frederick MD – USA)
Interferon-gamma: a Look at the Dark side.

30 march **Giuseppe Matarese** ("Federico II" University of Naples)
Metabolic control of human Treg cell generation in health and autoimmunity.

April

20 april **Michael L. Goldberg** (Cornell University, Ithaca, NY, USA).
Phosphatase regulation by unfair competition.

May

05 may **Raymond Kaempfer** (Hebrew University – Jerusalem – Israel)
Targeting B7/CD28 costimulatory axis: Novel strategy to block lethal host responses to severe infections.

11 may **Frédéric Barras** (CNRS - University Aix – Marseille – France)
Antibiotics and metal: new facts to an old story.

18 may **Monsef Benkirane** (CNRS- University of Montepellier – France)
Finding, Understanding and Eliminating the HIV Reservoir.

September

21 september **Barbara Seliger** (Martin Luther University – Halle – Germany)
Many ways leading to immune escape of tumors.

October

23 october **Mark Willcox** (University of new South Wales, Sydney – Australia)
Development of antimicrobial contact lenses.

November

23 november **Gennaro Ciliberto** (National Institute of Tumours Regina Elena – Rome)
Non mutational events driving drug resistance in melanoma.

07 december **Antonio Musarò** (Dept. of Anatomical and Hystoloical Sciences – Sapienza)
Stem cells and tissue niche: two faces of the same coin of muscle regeneration.

PUBLIC DISSEMINATION OF SCIENCE

FOR THE SCHOOLS

Istituto Pasteur Italia has also carried on a well-established **educational project** (established in 2009) for **schools students** involving a series of meetings and practical activities in the laboratories, so that students can learn to appreciate the importance Science has for all. Staring from 2016 the Institute has also started a collaboration with IBSA Foundation for scientific Research for the publication of the book series "I Ragazzi di Pasteur" coupling Science with Comics. The books published in 2017 cover different subjects such as Infectious Diseases, Vaccination, Drug addiction, Central Nervous System. This project was also born to teach students to discriminate against the fake news about scientific topics circulating on the internet.

FOR THE WIDER PUBLIC

Several cultural events have also been organized for an audience of non-experts to meet the scientists and discuss about some of the most important and interesting topics for science and society (such as microbial resistence, vaccination, physical and brain activities, pollution, autoimmune diseases, emerging viral diseases…).

These events have been carried out in coffee bars and book shops.

ADMINISTRATIVE BOARD

The Board of Administration is chaired by a President.
President
Luigi Frati
Members
Angela Santoni (Scientific Director), **Vincenzo Barba**, **Corrado Gatti**, **Paolo Sarti**
Administrative Secretary
Nicoletta Silvestri
Auditors
Ugo La Cava, Carla Vassallo, Adriana Vittazzi

SCIENTIFIC BOARD

The Scientific Council is a board of scientists active in the field of the pasteurian sciences.
Scientific Director
Angela Santoni (Immunology)
Members
Vincenzo Barnaba (Molecular Medicine), **Francesca Cutruzzolà** (Molecular Biology), **Cristina Limatola** (Neurosciences), **Anna Teresa Palamara** (Microbiology and Infectious Diseases), **Sergio Pimpinelli** (Genetics), **Romano Silvestri** (Drug Sciences), **Marco Tripodi** (Cell and Developmental Biology)

STAFF

Sara Atzeni, Caterina Cenci, **Maria Pia Lorenzoni**, **Lynda Romani**, **Nicoletta Silvestri**

Istituto Pasteur Italia – Fondazione Cenci Bolognetti
Viale Regina Elena 291 00161 Roma
pasteuritalia@istitutopasteur.it

ISTITUTO PASTEUR ITALIA
FONDAZIONE CENCI BOLOGNETTI