Workshop
BIOMETRA
September 26th, 2017 ~ Aula Magna – LITA, Segrate

Book of Abstracts
Synapses like a starry sky

Immunocytochemical experiment performed in hippocampal cultured neuron obtained from rat’s embryos showing betaIII tubulin (blue) and the excitatory and inhibitory vesicular presynaptic protein, respectively vGlut (green) and vGAT (red).
Organizing and Scientific Committee

Antonucci Flavia
Aureli Massimo
Bifari Francesco
Chiricozzi Elena
Ferrari Luca
Giussani Paola
Marchesi Federica
Pistocchi Anna
Rondelli Valeria
Rusconi Francesco
Samarani Maura
Venturin Marco
Zanchetta Giuliano
Program

9.15 Welcome and introduction

9.30 Session I: Oncology and immunology
Bevilacqua A.
“Antiproliferative and pro-apoptotic activity of melatonin analogues on melanoma and breast cancer cells”
Bruni E.
“The role of tissue-resident γδ-T cells in the pathogenesis of human liver cancer”
Cortese N.
“Exploring neuro-immune networks in colo-rectal cancer”
D’Oria C.
“Dissecting the dynamic interplay between colorectal cancer and T regulatory cells by exploiting the organoid models”
Setten E.
“Macrophages characterization in hypoxic condition related to interstitial fibrosis and tubular atrophy in renal allograft rejection”

10.45 Promega presentation

11.00 Coffee break

11.30 Session II: Molecular mechanisms of diseases
Fazzari M.
“PTC suppression strategy in CDKL5 related disorders: analyzing the feasibility of a “personalized” medicine approach”
Longaretti A.
“Synapse to nucleus cross-talk and its effect on IEG transactivation in response to stress”
Mancini G.
“Role of ganglioside GM1 on CFTR stabilization at plasma membrane: a new challenge for the cystic fibrosis therapy”
Rossetti A. C.
“Role of neuroinflammation in stress-vulnerability and resilience: implication for psychiatric disorders”
Ruocco C.
“Amino acid replacement of dietary protein promotes thermogenesis and energy expenditure in different models of obesity”
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<td>“<em>Brachial artery diameter as a marker of subclinical atherosclerosis: gender differences in the association with risk factors and cardiovascular disease</em>”</td>
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Oral presentations
I discuss here different aspects of human evolution that can help students to understand how the human body and mind work and why they are vulnerable to certain diseases. I examine three main issues: 1) the necessity to consider not only the mechanisms (i.e. the proximate causations) implicated in biological processes but also why these mechanisms have evolved (i.e. the ultimate causations or adaptive significance) in order to understand the functioning and malfunctioning of human body and mind; 2) examples of how human vulnerabilities to disease are caused by phylogenetic constraints, evolutionary tradeoffs reflecting the combined actions of natural and sexual selection, and/or mismatch between past and present environment (i.e., evolution of the eye, teeth and diets, erect posture and their consequences); 3) human pair-bonding and parent-offspring relationships as the result of socio-sexual selection and evolutionary compromises between cooperation and conflict. These psychobiological mechanisms are interwoven with our brain developmental plasticity and the effects of culture in shaping our behavior and mind, and allow a better understanding of functional (normal) and dysfunctional (pathological) behaviors. As the study of human evolution offers a powerful framework for clinical practice and research, the curriculum studiorum of medical (and psychology students) should include in the biology program evolutionary biology and human phylogeny.
ANTIPROLIFERATIVE AND PRO-APOPTOTIC ACTIVITY OF MELATONIN ANALOGUES ON MELANOMA AND BREAST CANCER CELLS

Giuliana Gatti¹, Valeria Lucini², Silvana Dugnani², Angela Calastretti¹, Gilberto Spadoni³, Francesco Scaglione², Gianfranco Canti¹, Annamaria Bevilacqua¹*

¹Department of Medical Biotechnology and Translational Medicine,
²Department of Oncology and Hemato-oncology, Università degli Studi di Milano, Italy
³Department of Biomolecular Sciences, Università degli Studi di Urbino “Carlo Bo”, Urbino, Italy
*e-mail: annamaria.bevilacqua@unimi.it

Melatonin plays fundamental roles in diverse physiological functions ranging from the regulation of circadian rhythms to tumor inhibition, owing to its antioxidant, immunomodulatory and anti-aging properties. The therapeutic potential of melatonin and its analogues prompted us to investigate the in vitro and in vivo antitumor activity of new melatonin derivatives on melanoma and breast cancer cells, and explore the underlying molecular mechanisms.

New indole melatonin analogues were synthetized and tested for their ability to inhibit proliferation and induce apoptosis in DX3 melanoma cells and in MCF-7 and MDA-MB231 breast cancer cells by viability and apoptosis assays. The oncostatic effect of melatonin analogues was also measured on a human melanoma xenograft mouse model. The changes in the expression levels of different proteins in cancer cell lines during treatment with melatonin analogues were investigated by Western blot analysis.

The experiments revealed that the new melatonin analogues inhibited the growth of DX3 melanoma cells in a dose- and time-dependent manner. In addition, the study demonstrated that low concentrations (0.1 mM) of the melatonin analogue UCM 1037 exhibited antiproliferative and cytotoxic effects also in MCF-7 and MDA-MB231 breast cancer cells. The suppression of DX3 tumor growth by the melatonin analogues was further demonstrated in vivo in a xenograft mice model. Caspase 3 resulted to be involved in the pro-apoptotic mechanism induced by UCM 1037 in DX3 and MDA-MB231 cells. A decrease in the activation of both Akt and MAPK pathways was observed in breast cancer cells following UCM 1037 treatment.

In conclusion, this study describes melatonin derivatives showing promising antiproliferative and cytotoxic activity in melanoma and breast cancer cells.
**Keywords:** Melatonin, melatonin receptors, melatonin analogues, melanoma, breast cancer
THE ROLE OF TISSUE-RESIDENT $\gamma\delta$-T CELLS IN THE PATHOGENESIS OF HUMAN LIVER CANCER

Elena Bruni$^{1,2,*}$, Joanna Mikulak$^{2,3}$, Ferdinando Oriolo$^{1,2}$, Paolo Tentorio$^2$, Bruno Silva-Santos$^4$, Matteo Donadon$^5$, Matteo Cimino$^5$, Guido Torzilli$^5$ and Domenico Mavilio$^{1,2}$

$^1$BIOMETRA, Università degli Studi di Milano, Milan, Italy
$^2$Humanitas Clinical and Research Center, Rozzano, Italy
$^3$Institute of Genetic and Biomedical Research (IRGB), CNR, Milan, Italy
$^4$Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon, Lisbon, Portugal
$^5$Department of Hepatobiliary and General Surgery, Humanitas Clinical and Research Center, Rozzano, Italy

*e-mail: elena.bruni@unimi.it

Gamma-delta ($\gamma\delta$) T cells are highly enriched in tissues and represent our first line of defense at epithelial surfaces. Indeed, under homeostatic conditions, $\gamma\delta$-T cells are poorly represented in the peripheral blood while they constitute a predominant population among intrahepatic lymphocytes or intraepithelial intestinal immune cells. Within the tumor microenvironment, $\gamma\delta$-T cells are known to display both cytotoxic and regulatory functions. We previously reported that activated human peripheral blood $V\delta1$-T cells exert cytotoxic activity against different tumor cell lines. Here, we quantified and characterized tissue-resident $\gamma\delta$-T cells from specimens of healthy livers and liver metastasis of colon cancer. We found that $V\delta1$-T cells are enriched in healthy liver compared to $V\delta2$ subset. Inside the tumor tissue, the amount of $V\delta1$-T lymphocytes strongly decrease while the proportion of $V\delta2$ subset remains similar to that of healthy tissue. The majority of both $V\delta1$ and $V\delta2$ T cells are CD4$^{\text{neg}}$/CD8$^{\text{neg}}$ and express high levels of activating receptors CD56, NKG2D and the tissue-resident marker CD69 in both healthy and tumor tissue. The main difference between hepatic $V\delta1$ and $V\delta2$ subset reside in the expression of the inhibitory receptor NKG2A. Indeed in healthy liver $V\delta2$-T cells express high level of NKG2A compared to $V\delta1$ subset and the same pattern of NKG2A expression is kept on these two $\gamma\delta$-T cells subset infiltrated within the tumor tissue. Upon in vitro stimulation, both hepatic $V\delta1$ and $V\delta2$ T cells can release high level of granzyme-B, interferon-gamma (IFN$\gamma$) and tumor necrosis factor-alpha (TNF$\alpha$), while the production of IL-17 seems to be restricted to the $V\delta1$ population. These preliminary data identifying
distinct populations of liver $\gamma$$\delta$-T cells in liver cancer open new perspectives to better understand anti-tumor immune response in liver and to better predict the prognosis of these malignant diseases.

**Keywords:** liver, $\gamma$$\delta$-T cells lymphocytes, tumor
EXPLORING NEURO-IMMUNE NETWORKS IN COLO-RECTAL CANCER

Nina Cortese¹, Giovanni Francesco Castino¹, Giulia Maggi¹,², Diego Morone¹, Marco Erreni¹, Federico Colombo¹, Paola Allavena¹, Federica Marchesi¹,²*

¹ Dipartimento di Immunologia, Istituto Clinico Humanitas, Rozzano, Italy
² BIOMETRA, Università degli Studi di Milano, Milano, Italy
*e-mail: federica.marchesi@unimi.it

Visualization of macrophage (green)-nerve (red) interaction in the colon mucosa.

The central nervous system reflexively regulates the inflammatory response, via a direct modulation of immune cells by peripheral nerves. However, in the context of cancer, where it is already known that inflammation plays a key role, the regulation of immune cells by the nervous system is still largely unexplored. Macrophages, professional phagocytes involved in the inflammatory response, hold a key position also in homeostatic and tissue responses, including neural-mediated circuits. To define whether a neural control of macrophage functions in tumors exists, we have investigated the macrophage-neural interaction in a preclinical model of colo-rectal cancer. In a model of AOM/DSS-induced colorectal cancer, we have visualized the spatial interaction of the complex intestinal neural networks with macrophages through a 3D spatial distribution analysis. F4/80⁺ macrophages were found in closed proximity to S100⁺ neural fibers throughout the
intestinal wall, with distinct distributions according to different layers, suggesting intra-tissue specialization of these phagocytes. Compared to control mice, we found a closer association of macrophages to nerves in tumor bearing mice. Interestingly, macrophages of treated mice also showed an increased expression of the α7 subtype nicotinic acetylcholine receptor (α7nAChR), a neural receptor that plays a key role in the anti-inflammatory reflex in the gut. These results suggest a modification of neural-macrophage networks in colo-rectal cancer that could be important in the regulation of macrophage function in tumors.

**Keywords:** Macrophages, neural networks, colo-rectal cancer
DISSECTING THE DYNAMIC INTERPLAY BETWEEN COLORECTAL CANCER AND T REGULATORY CELLS BY EXPLOITING THE ORGANOID MODELS

G. Della Chiara¹, C. D’Oria¹, ⁵*, C. Godano¹, M. De Simone¹, C. Cordiglieri¹, L. Gianotti², ³, E. Opocher⁴, G. Rossetti, M. Pagani¹, ⁵

¹Istituto Nazionale Genetica Molecolare "Romeo ed Enrica Invernizzi” (INGM), Via F. Sforza 35, Milan 20122 Italy;
²Department of Surgery, San Gerardo Hospital, Monza 20900, Italy;
³School of Medicine and Surgery, Milano-Bicocca University, Monza 20900 Italy;
⁴UO Chirurgia Epatobiliopancreatica e Digestiva Ospedale San Paolo, Milan 20142, Italy;
⁵Department of Medical Biotechnology and Translational Medicine BIOMETRA, Università degli Studi di Milano, Milano 20129, Italy
*e-mail: claudia.doria@unimi.it

The immune response towards tumor specific antigens is rapidly disabled upon cancer progression by the induction of suppressive mechanisms, due to the interplay between malignant cells, tumor milieu and infiltrating T regulatory cells (Tregs). Tregs can indeed upregulate modulatory molecules (immune checkpoints) that have become promising targets of cancer immunotherapy, unleashing the antitumor immune response. Recent experimental evidence showed that their efficacy is related to specific depletion of tumoral Tregs, even if a high fraction of patients still doesn’t respond. Moreover, tumor infiltrating Tregs correlate with a poor prognosis and can adapt their transcriptional program to the surrounding milieu. In line with these findings, a novel transcriptional phenotype of non-lymphoid tissues infiltrating Tregs, implied in metabolic modulation, tissue homeostasis and repair has been recently described. In order to better elucidate the dichotomous role of tumor infiltrating Tregs, we developed a model of 3D culture of normal and colorectal cancer (CRC) derived organoids to study the interplay between these two populations. Preliminary results demonstrate that our culture conditions are suitable to start, amplify and create a biobank of normal and CRC-derived organoids. These libraries can be propagated, frozen and defrosted like any tumor cell line and represent, at the state of the art, a physiological model to approach a co-culture system with Tregs. According to molecular and imaging preliminary analysis, our organoids reproduce the architectural features of the normal and colon carcinoma of origin. In addition, a subsequent detailed single-cell and bulk RNAseq analysis
and epigenome profiling of both normal and CRC-derived organoids and Tregs co-culture will be performed, shedding new light on Tregs plasticity and heterogeneity, and to conceive new specific immunotherapeutic drugs.

**Keywords:** Colon-derived organoids, Tregs
MACROPHAGES CHARACTERIZATION IN HYPOXIC CONDITION RELATED TO INTERSTITIAL FIBROSIS AND TUBULAR ATROPHY IN RENAL ALLOGRAFT REJECTION

Elisa Setten$^{1,2,*}$ and Massimo Locati$^{1,2}$

$^1$BIOMETRA, Università degli Studi di Milano, Milan, Italy
$^2$Humanitas Clinical and Research Center, Rozzano, Italy
*e-mail: elisa.setten@unimi.it

Interstitial fibrosis and tubular atrophy (IF/TA) is one of the most common chronic alteration of renal tissue after kidney transplant. Tissue fibroblasts play a pivotal role in this process, but immunological cell types, including macrophages and T cells, are also implicated in this process. The objective of this study is to understand the relevance of hypoxia in the cross-talk between macrophages (Mφ) and fibroblasts and the impact of Mφ on extracellular matrix metabolism and tissue remodeling. Model parameters will be extracted from molecular profiling approaches investigating Mφ polarized to M1 and M2 settings under normal and hypoxic conditions in single cell cultures and direct-contact co-cultures with renal fibroblasts.

Hypoxia-responsive genes (VEGF-A, GLUT1, MMP7, CXCR4) have been investigated by qRT-PCR in resting (M0) and activated Mφ (M1/M2) at short (4 h) and extended (24 h) time-points under normoxic or hypoxic conditions (20% and 1% O$_2$ tension, respectively). We observed that Mφ respond to hypoxia inducing specific transcripts and that Mφ polarization (M1/M2) has no impact on the induction of these genes strictly related to hypoxia. On the contrary hypoxia interferes with the expression of M1 transcripts, while having no impact on the regulation of M2 genes. These observations will be implemented with RNA sequencing data obtained for single-culture and co-culture systems in order to better clarify Mφ role in IF/TA and to translate candidate genes into predictive biomarkers and innovative tools to support clinical decisions in transplantation medicine.

**Keywords**: macrophages, hypoxia, fibrosis
PTC SUPPRESSION STRATEGY IN CDKL5 RELATED DISORDERS: ANALYZING THE FEASIBILITY OF A “PERSONALIZED” MEDICINE APPROACH

Maria Fazzari1*, Charlotte Kilstrup-Nielsen2, Nicoletta Landsberger1

1BIOMETRA, Università degli Studi di Milano, Milan, Italy
2Department of Biotechnology and Life Sciences (DBSV), Università dell’Insubria
*e-mail: maria.fazzari@unimi.it

Alterations of CDKL5 give rise to severe disorders identified as CDKL5-related pathologies; to date no cure exists. About 18% of CDKL5-patients carry a non sense mutation and might benefit of a read-through strategy as “personalized” medicine approach.

The read-through process occurs when a near-cognate aminoacyl-tRNA binds a premature stop codon (PTC), allowing its suppression and the subsequent protein elongation. This mispairing event can rarely occur, but can be facilitated using a wide range of drugs.

In order to test PTC suppression, we have chosen some human pathogenic CDKL5 non sense mutations located in the two main domains of the protein: the catalytic N-terminus (R59X, R134X) or in the C-terminus (Q347X, E364X, R550X, S855X) tail. We then evaluated the read-through process using
aminoglycoside and non aminoglycoside drugs in cells transfected with the mutagenized pEGFP-CDKL5 constructs.

Generally, we have found that tested CDKL5 PTCs respond to gentamicin and geneticin but not to PTC124 or GJ072. Then, considering the known aminoglycosides toxicity, we tried to optimize their activity testing the novel molecule CDX5-1 that may strongly enhance their activity. Finally, in order to understand whether the full-length derivatives may maintain the proper function of CDKL5, we analysed some features of full-length read-through products compared to wild-type protein. In particular, while truncated proteins showed an altered subcellular localisation/organisation, after read-through the cellular distribution/phenotype was more similar to wild type meaning that a rescue could be possible.

**Keywords:** Non sense mutation, Read-through, CDKL5-related pathologies
SYNAPSE TO NUCLEUS CROSS-TALK AND ITS EFFECT ON IEG TRANSACTIVATION IN RESPONSE TO STRESS

Alessandra Longaretti1*, Barbara Grillo1, Laura Gerosa2, Emanuela Toffolo1, Chiara Forastieri1, Maria Passafaro2, Francesco Rusconi1, Elena Battaglioli1

1Dept. BIOMETRA, Università degli Studi di Milano, Milan, Italy
2IN-CNR, Milan, Italy
*e-mail: alessandra.longaretti@studenti.unimi.it

Lately the scientific community put a great effort in the study of environmental stress and its effects on brain and behavior. This interest comes from the fact that acute and chronic stress are able to reshape circuits in specific brain areas such as hippocampus, amygdala and PFC, leading to the development of neuropsychiatric disorders like anxiety and depression. Here we present the involvement of the balance between two different isoforms of the epigenetic enzyme LSD1, ubiquitarian LSD1 and its dominant negative isoform neuroLSD1, in the transcriptional homeostasis of the immediate early genes (IEGs) upon neuronal activation. Furthermore, we show the effects of the modulation of neuroLSD1 isoform on neuronal structural plasticity. In mouse hippocampus, the ratio between the transcriptional corepressor LSD1 and its dominant negative isoform neuroLSD1 is dynamic and responds to different paradigms of neuronal activation such as psychosocial stress. Using an in vitro model, we show that neuroLSD1 is downregulated upon activation of the NMDA receptor. To occur, neuroLSD1 reduction requires the ionotropic function of NMDAR. A low dose of neuroLSD1 generates a defective activity-dependent transcription of the IEGs egr1, c-fos and npas4. We suggest that this downregulation is propaedeutic to a refractory window in the induction of IEG transcription. Thus, we introduce a novel role for LSD1 and neuroLSD1 in transcriptional homeostasis of IEGs that occurs upon neuronal activation and its possible role in the prevention of the onset of neuropsychiatric disorders. Supporting this evidence, the genetic ablation of neuroLSD1 in a mouse model leads to the decrease in the mean number of dendritic spines in CA1 area of the hippocampus while its overexpression in primary hippocampal neurons increases their density.

Keywords: IEG, NMDAR, homeostasis, neuroplasticity
Cystic fibrosis transmembrane conductance regulator (CFTR) is a chloride channel expressed at the apical surface of epithelial cells. Mutations in CFTR gene cause Cystic Fibrosis (CF), an autosomal recessive disease characterized by severe lung disease due to the loss of CFTR at plasma membrane (PM) level. Many pharmacological agents have been designed to increase the surface level of mutated CFTR (correctors), as well as its PM stability and activity (potentiators); unfortunately, for the most common CF-causing mutation F508del, their efficacy seems to be time-limited. Many factors contribute to PM CFTR stability, including its compartmentalization in
sphingolipid (SL)-enriched lipid rafts, the interaction with specific lipids such as ganglioside GM1 and multiprotein complex involving ezrin and NHERF1. Based on these findings, we investigated the effects of potentiators, correctors and the ganglioside GM1 on CFTR PM stability. We analysed CFTR expression in CF bronchial epithelial cell lines under the treatment with VX-809 (corrector) and VX-770 (potentiator), individually, in combination and in presence or not of ganglioside GM1. Interestingly the mature form of CFTR is increased in presence of VX-809 individually, but the positive effect of corrector disappears in presence of VX-770. Instead, in cells loaded with GM1, the mature form of CFTR is increased upon the treatment with corrector VX-809 and surprisingly, in the presence of GM1 the negative effect of VX-770 is abolished. This result support the role of sphingolipids in the stabilization of CFTR at plasma membrane level suggesting new therapeutic strategy for the treatment of CF. Future studies will be point to clarify the role of SL and in particular of GM1 on CFTR working at plasma membrane.

Keywords: biochemistry, sphingolipids, cystic fibrosis, therapy
ROLE OF NEUROINFLAMMATION IN STRESS-VULNERABILITY AND RESILIENCE: IMPLICATION FOR PSYCHIATRIC DISORDERS

Andrea Carlo Rossetti\textsuperscript{1*}, Maria Serena Paladini\textsuperscript{1}, Laura Rubini\textsuperscript{2}, Martina Colombo\textsuperscript{1}, Giorgio Racagni\textsuperscript{2}, Mariusz Papp\textsuperscript{3}, Marco Andrea Riva\textsuperscript{2}, Raffaella Molteni\textsuperscript{1}

\textsuperscript{1}BIOMETRA, University of Milan, Milano, Italy
\textsuperscript{2}Department of Pharmacological and Biomolecular Sciences, University of Milan, Milano, Italy
\textsuperscript{3}Institute of Pharmacology, Polish Academy of Sciences, Kracow, Poland
* e-mail: andreacarlo.rossetti@unimi.it

Although it is well-established that stressful life events represent the main environmental risk factor for the development of several psychiatric disorders, most individuals mount adaptive coping strategies that promote resilience in the face of stress. On this base, understanding what distinguishes a vulnerable from a resilient stress-response is fundamental to clarify the pathophysiology of stress-dependent disorders such as major depression (MD).

Since several evidence have linked stress and MD with alterations of the inflammatory system, the goal of our study was to evaluate the role of neuroinflammation on stress-resilience and susceptibility to develop anhedonia, one of the core symptoms of MD.
To this aim, adult male rats were exposed to chronic mild stress (CMS) before being challenged with lipopolysaccharide (LPS, i.p. 250 μg/kg) to trigger an inflammatory response. Sucrose consumption test was used to monitor the insurgence of anhedonic-like phenotype whereas the molecular effects of our paradigm were evaluated using real-time RT-PCR and Western Blot.

In line with our previous studies, 70% of the animals exposed to the CMS paradigm showed a reduction in sucrose intake (“susceptible” rats) whereas the remaining 30% did not show any change (“resilient” rats). LPS reduced the sucrose intake in control unstressed rats with no effect in resilient animals and without worsen the behaviour of susceptible rats, which maintained the anhedonic phenotype. The results of the molecular analyses indicated that, although stress and LPS alter several inflammatory mediators, the differential behavioural response observed after LPS appears to be mainly mediated by microglia. Indeed, a significant increase of markers of microglia activation was detected only in LPS-treated anhedonic animals and not in the resilient rats, suggesting a crucial role for these cells in the mechanisms underlying the different pathological impact of stress exposure.

**Keywords:** depression, stress resilience, lipopolysaccharide, rat, microglia
AMINO ACID REPLACEMENT OF DIETARY PROTEIN PROMOTES THERMOGENESIS AND ENERGY EXPENDITURE IN DIFFERENT MODELS OF OBESITY

Chiara Ruocco1*, Maurizio Ragni1#, Laura Tedesco1, Fabio Rossi1, Francesco Bifari1,2, Luisa Ponzoni3, Ilaria Decimo4, Alessandra Valerio5, Michele O. Carruba1, Enzo Nisoli1

1Center for Study and Research on Obesity, Department of Biomedical Technology and Translational Medicine, University of Milan, Milan, Italy; 2Laboratory of Cell Metabolism and Regenerative Medicine, Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy; 3Fondazione Umberto Veronesi and Department of Biomedical Technology and Translational Medicine, University of Milan, Milan, Italy; 4Department of Diagnostics and Public Health, University of Verona, Verona, Italy; 5Department of Molecular and Translational Medicine, Brescia University, Brescia, Italy.

*e-mail: chiara.ruocco@unimi.it; # contributed equally to the work;

Epidemiological evidence suggests that ingestion of specific dietary macronutrients modifies both metabolic health and health span. In this context, manipulating diet composition has gained increasing attention, despite opposing results concerning food patterns and health have been reported. Indeed, both high-protein and low-protein diets, as well as branched-chain amino acid (BCAA)-enriched and BCAA-restricted diets, have effectively been reported to ameliorate metabolic dysfunction in mice and humans with or without changes in energy balance. Here we show that the long-term exposure to diets containing high ratios of saturated to unsaturated fatty acids (SFA diets) leads to obesity, glucose intolerance, and early mortality, and when the protein portion of the same SFA diet was almost completely substituted with a specific mixture of soluble essential amino acids (SFA-AA diet), this could stimulate thermogenic energy expenditure, preventing and reversing obesity and dysregulated glucose metabolism. Moreover, mice fed with the SFA-AA diet showed an extended survival and improvement of the global health. These effects were due to brown fat activation, through a direct action on the mechanistic target of rapamycin complex 1 in brown adipocytes, independent of the sympathetic nervous system. Furthermore, the SFA-AA diet augmented the circulating
levels of N-acyl amino acids, in association with an increased in the levels of the same molecules in subcutaneous (i.e. inguinal) white adipose tissue (iWAT). These N-acyl amino acids bind mitochondria to function as endogenous uncouplers of respiration in a UCP1-independent manner, and their administration augments energy expenditure. Thus, our data demonstrate, for the first time, that the manipulation of diet, i.e. the amino-acid replacement of dietary protein, could represent a novel approach to prevent obesity and its related disorders, in addition to promote the metabolic health span in humans.

Keywords: thermogenesis, obesity, type 2 diabetes, amino acids, N-acyl amino acids
UNDERSTANDING BARRIER FUNCTION IN BIOLOGICAL NETWORKS

E. Di Cola*, P. Brocca, E. Del Favero, V.M. Rondelli, L.F. Cantù

Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Università degli Studi di Milano, via fratelli Cervi 93, 20090 Segrate (MI), Italia
*e-mail: emanuela.dicola@unimi.it

Schematic: 3 possible routes to cross biological barriers; (1) disruption, (2) penetration without interaction; (3) complexation of nanocarrier with the biopolymer network.

Biological networks are formed by biopolymers/proteins matrices with a high content of water. They play the fundamental role of barriers in cells, tissues and organs regulating the diffusion/exchange of molecules. Though biological networks perform such protective tasks, there is still a limited understanding of their internal organisation and filtering mechanisms. Indeed, the dysfunction of such mechanisms can lead to an abnormal increase of the barrier function. For instance, the alterations in mucus permeability can result in severe pathologies ranging from viral and parasitic infections, cystic fibrosis to some form of woman infertility. The design of nanoparticles (NPs) capable of interacting with the biopolymers of the networks represents currently a promising strategy to achieve the penetration of active ingredients through such barriers and access the interior of the cell. In this context, we present a study on the development of mucus-penetrating NPs for transmucosal nasal administration, in interaction with a model mucus
of that epithelium (mucin type II hydrogel) at physiological conditions. Structural issues are of primary importance to control the mechanism of action and thus to rationally engineer particles able to overcome mucosal clearance mechanisms. Whereas overall properties, such as mean size and surface charge can be obtained by bench instruments (DLS, SLS and Zeta potential), the complementarity of techniques with higher space resolution (SAXS and SANS) is employed here to gain a deeper knowledge of the behaviour of the NPs in the mucus matrix. In particular, we focus on their physical interactions with Mucin type II (muco-adhesion, stability, and penetration) and discuss them as a function of the properties of their engineered surface.

**Keywords**: biological fluids, mucin, nanoparticles, drug-delivery
SYNTHESIS OF *STREPTOCOCCUS PNEUMONIAE* 19A CAPSULAR POLYSACCHARIDE FRAGMENTS AND THEIR BIOLOGICAL EVALUATION

Laura Morelli1*, Silvia Fallarini2, Luigi Lay3, Federica Compostella1

1Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy.
2Department of Pharmaceutical Sciences, Università del Piemonte Orientale, Università degli Studi del Piemonte Orientale, Novara, Italy.
3Department of Chemistry, University of Milan, Milan, Italy
*e-mail: laura.morelli@unimi.it

*Streptococcus pneumoniae* (SP) is a common human pathogen associated with a broad spectrum of disease and it is still a leading cause of mortality and morbidity worldwide [1]. Nowadays the most effective strategy to reduce the burden of disease caused by SP is vaccination. Administered vaccines contain fragments of bacterial capsular polysaccharide (CP), which are important surface antigens of these invading bacteria. New research strategies in this field aim at developing nanomaterials loaded with carbohydrate antigens, which are emerging as synthetic vaccine candidates [2,3]. The prerequisite of this approach is the identification of the shortest polysaccharide fragment able to be recognized by the natural antibody and to be immunogenic. In this framework has emerged our interest in SP 19A serotype, which is one of the serotype responsible for the bulk of pneumococcal disease [4]. In particular, we aim at developing a derivative of the SP19A repeating unit, β-D-ManpNAc-(1→4)-α-D-Glcp-(1→3)-Rhap-(1-O-
phosphate), still endowed with biological activity and suitable for conjugation to different nanomaterials.

![Chemical structure of SP 19A synthetic trisaccharide 1.](image)

**Figure 1. Chemical structure of SP 19A synthetic trisaccharide 1.**

Herein we will describe the synthesis of compound 1, the saccharide portion of SP 19A repeating unit conjugated at the reducing end to an aminopropyl linker. This compound has been obtained through a glycosylation reaction between a properly protected thiophenyl β-D-ManpNAc-(1→4)-α-D-Glcp disaccharide and a new suitable α-amino propyl rhamnoside acceptor. Classical competitive Elisa assay demonstrated that compound 1 has inhibitory properties and is recognized by the anti-19A antibody.

**Keywords:** *Streptococcus pneumoniae*, Pneumococcal vaccine, glycosylations, synthetic polysaccharides

**References**


MICROMECHANICAL PROPERTIES OF DNA NETWORKS MEASURED BY A NOVEL MICROFLUIDIC TOOL

Giovanni Nava\textsuperscript{1*}, Valerio Vitali\textsuperscript{2}, Paolo Minzioni\textsuperscript{2}, Francesca Bragheri\textsuperscript{3}, Roberto Osellame\textsuperscript{3} and Tommaso Bellini\textsuperscript{1}

\textsuperscript{1}BIOMETRA, Università degli Studi di Milano, Milan, Italy
\textsuperscript{2}Department of Information and Industrial Engineering, Università di Pavia, Pavia, Italy
\textsuperscript{3}Istituto di Fotonica e Nanotecnologie (IFN-CNR), Politecnico di Milano, Milano, Italy
*e-mail: giovanni.nava@unimi.it

(\textit{left}) Microfluidic chip for micro-rheological measurements in which a micrometric bead (\textit{right}) can be manipulated inside the channel using two laser beams. The observation of the bead motion (numbers indicate progressive positions) enables measuring mechanical stability and viscoelastic forces (\textit{bottom}).

Fluids hosting reversible three-dimensional molecular networks are systems of crucial importance both for understanding the structure of cells and tissues and as innovative biomimetic materials. In these systems, the connectivity of the elements (e.g. proteins, nucleic acids, polymers) give rise to a set of interesting properties including scaffolding for biochemical reactions, self-healing, and plastic/elastic mechanical stability. To study the mechanical properties of these materials at a microscopic level and in small volumes, we developed a novel microfluidic device in which we operate with optical forces. The device consists in a straight microchannel in which light is transported by optical waveguides written in the chip via laser inscription. Thanks to such geometry, a bead ($r = 5 \ \mu m$) can be optically trapped and
manipulated in the middle of the channel [1]. By modulating the optical force in the available range of 0.1-100 pN, we can push the bead in a tiny amount of fluid (<1 μL), and measure viscosity and elasticity of the material.

We applied this method to study a DNA hydrogel. These are materials obtained by sequence directed bonding of multiple DNA strands, which are currently investigated both as highly controlled model systems of 3D molecular networks, and as smart materials able to combine structural scaffold stability and genetic coding [2]. Specifically, we studied the evolution of the mechanical properties of a model model system consisting in DNA star-shaped nano-construct that, upon lowering the temperature, aggregate via hybridization of overhangs, into a highly-controlled space-filling hydrogel [3,4]. We observe that the development of the 3D molecular network deeply affects the mechanical properties of this material: its viscosity enormously increases and the friction becomes independent from bead velocity. We demonstrate that all the mechanical behaviour of this system can be quantitatively explained on the sole bases of the DNA network geometry and of the thermodynamics of DNA hybridization.

**Keywords:** DNA Nanotechnology, Hydrogels, Microrheology, Microfluidics

**References**
TUBEROUS/STENOTIC BREAST MALFORMATION AND ITS RECONSTRUCTIVE SURGICAL CORRECTION: EPIDEMIOLOGY AND NEW CLASSIFICATION SYSTEM

Marco Klinger¹ *, Valeria Bandi¹, Valeriano Vinci¹, Andrea Lisa¹, Micol Giaccone¹, Federico Barbera¹, Luca Maione¹

¹BIOMETRA, Università degli Studi di Milano, Milan, Italy - Department of Medical Biotechnology and Translational Medicine BIOMETRA – Plastic Surgery Unit, Humanitas Research Hospital, Rozzano (Milan), Italy
*e-mail: marco.klinger@unimi.it

The tuberous breast represents a mammary deformity characterized by short lower pole, nipple areola herniation and reduced mammary horizontal diameter and its prevalence still remains undetermined. Moreover, several classification systems have been reported to define the spectrum of tuberous breast deformity and a range of surgical techniques has been described. In the present study we report tuberous breast deformities prevalence as observed in our clinical practice and we propose a new classification based on the type of stenosis, glandular trophism and degree of ptosis in order to help surgical planning.

A retrospective analysis was performed on 1600 female patients presented to our clinic from 2009 to 2014 for augmentation or reduction mammoplasty and other breast conditions. Data were collected according to three groups: breast augmentation group (AUG), breast reduction group (RED) and general population group (POP). We also evaluated 246 patients meeting our definition of stenotic breast classifying them in 8 types according to the clinical presentation and we reported the key surgical maneuvers, aimed at correcting the different breast deformities, according to the Stenotic Breast Type. 400 patients were analyzed in AUG group and in RED group; 194 patients (48,5 percent) and 189 cases (47,3 percent) respectively demonstrated at least one tuberous breast deformities; in 800 patients of POP group we found 221 patients (27,6 percent) with at least one tuberous breast deformity. We distinguished eight different groups of stenotic breast and we reported the surgical maneuvers adopted in order to correct each type. We observed a satisfying long term aesthetic outcome with 4,9% reintervention rate.
In conclusions the retrospective analysis reveals a high prevalence of tuberous breast deformity. We believe this new classification to be extremely useful to select the most suitable surgical maneuvers for each breast deformity, thus improving the global surgical outcome.

**Keywords:** Stenotic breast; Tuberous breast; Breast Malformation; Epidemiology; Classification
EPIGENETICS AND PROTEOMICS OF PSORIASIS AND PSORIATIC ARTHRITIS IN MONOZYGOTIC TWINS

Angela Ceribelli¹, Elvezia M. Paraboschi², Natasa Isailovic¹, Elena Generali¹, Michela Robusto², Maria De Santis¹, Giulia Cardamone², Francesco Sacrini³, Antonio Costanzo³, Stefano Duga², Carlo Selmi¹,4*

¹Rheumatology and Clinical Immunology, Humanitas Research Hospital, Rozzano (MI); ²Laboratory of Medical genetics and RNA biology, Humanitas Research Hospital, Rozzano (MI);
³Dermatology Unit, Humanitas Research Hospital, Rozzano (MI)
⁴BIOMETRA Department, University of Milan
*e-mail: carlo.selmi@unimi.it

Background. Psoriatic disease is a chronic inflammatory disorder spanning from skin disease (PsO) to psoriatic arthritis (PsA). The genetic background is insufficient to explain disease onset, and epigenetics, partially resulting from the interaction with the environment, represents a potential process modulating disease susceptibility. Moreover, proteomic analyses are crucial for the comprehension of the molecular mechanisms involved in the progression of the disease.

In this frame, our aim is to analyze the epigenetics signatures and proteomics profiles of PsO/PsA in a cohort of monozygotic (MZ) twins discordant for the disease.

Methods. We performed DNA methylation analysis (Infinium MethylationEPIC BeadChip), and transcriptome profile (Illumina TruSeq Stranded mRNA kit) in whole blood of MZ twins, whereas proteomic analyses (www.somalogic.com) were conducted on twins’ serum.

Results. The epigenetics analysis identified 19 genes consistently differentially methylated and mostly involved in the pathway of TGF-β and IFN response. Pathway analysis of integrated methylome and transcriptome data evidenced an enrichment in “transcription regulation”, “innate immunity”, “ATP-binding” and, “Srp-dependent co-translational proteins”, that may be involved in the psoriatic condition. Moreover, serum proteomics of PsO/PsA versus healthy twins showed a significant up/downregulation of 10 and 3 proteins, respectively, involved in the innate and adaptive immune response, DNA repair and DNA damage sensors pathways.
Conclusions. This omics approach allowed the identification of biological pathways and target proteins that could have a potential pathogenic role and may prove useful as disease biomarkers.

Keywords. Twin studies, psoriatic disease, proteomics, epigenetics
BRACHIAL ARTERY DIAMETER AS A MARKER OF SUBCLINICAL Atherosclerosis: GENDER DIFFERENCES IN THE ASSOCIATION WITH RISK FACTORS AND CARDIOVASCULAR DISEASE

Daniela Coggi¹, Mauro Amato¹, Daniela Sansaro¹, Alessio Ravani¹, Beatrice Frigerio¹ and Damiano Baldassarre¹,²*

¹Centro Cardiologico Monzino, Milan, Italy
²BIOMETRA, Università degli Studi di Milano, Milan, Italy
*e-mail: damiano.baldassarre@unimi.it

During the atherogenic process, arterial diameter measured in plaque free areas tends to enlarge. Several studies, focused on the brachial artery diameter (BAD), indicate that arterial enlargement is a generalized phenomenon, and may be useful to improve the prediction of vascular events. BAD is associated with vascular risk factors (VRFs), cardiovascular risk-estimation, subclinical atherosclerosis (as indexed by carotid intima media thickness, IMT), as well as with the prevalence/incidence of vascular
events. Few studies have evaluated the association between BAD and VRFs according to gender differences. Aim of the present study was to assess gender differences in the association between BAD, VRFs and subclinical atherosclerosis, and to investigate the association between BAD and incidence of cardiovascular events. 4644 subjects (46.1% women; age (mean±SD) 57±13 years) had their BAD and carotid IMT measured by B-Mode ultrasound. BAD was measured in plaque free areas. A total of 37 subjects experienced a vascular event during a median follow up period of 5.23 years (IQR; 3.03 – 8.39). The associations between BAD and some VRFs (i.e. BMI and hypertriglyceridemia), Framingham risk score and number of VRFs were significantly different in men and women. With the exception of BMI, which seems to influence BAD more in men, all other VRFs tested seemed to influence BAD more in women. The correlation between BAD and carotid IMT, reported in literature, seems to be a spurious correlation, indeed it disappeared after adjustment for confounders. Finally, BAD was a predictor of vascular events independently from gender, VRFs, patient’s atherosclerotic profile and arterial enlargement in other vascular districts. In conclusion, BAD is a promising biomarker of atherosclerosis. Since gender influences several associations with VRFs, gender differences must not be ignored when BAD enlargement is used in preventive strategies as an early biomarker of atherosclerosis.

**Keywords:** brachial artery diameter, gender differences, vascular risk factors, cardiovascular events, atherosclerosis
Poster presentations
IDENTIFICATION OF THE ANTIGEN RECOGNIZED BY RHIGM22, A REMYELINATION-PROMOTING HUMAN MONOCLONAL ANTIBODY

Livia Cabitta¹*, Sara Grassi¹, Simona Prioni¹, Laura Mauri¹, Maria Grazia Ciampa¹, Yana Zorina², Sandro Sonnino¹, Alessandro Prinetti¹

¹BIOMETRA, Università degli Studi di Milano, Milan, Italy
²Acorda Therapeutics, Inc., Ardsley, NY, USA
*e-mail: livia.cabitta@unimi.it

Recombinant human IgM22 (rHIgM22) binds to myelin and oligodendrocytes (OLs), and promotes remyelination in mouse models of multiple sclerosis. rHIgM22 preferentially reacts with sulfatide-positive (O4⁺) OLs, and binding of rHIgM22 is abolished in CNS tissue slices from Cst (-/-) mice, suggesting that its binding requires the presence of a product of cerebroside sulfotransferase, possibly sulfatide, highly expressed in OLs and myelin. However the identity of the antigen recognized by this antibody remains to be elucidated.

We tested the binding of rHIgM22 to purified lipids and lipid extracts from mouse brain, CNS myelin, mixed glial cells, and O4⁺ OLs using TLC immunostaining and SPR with lipid monolayers.

Our preliminary results show that IgM22 binds to sulfatide in vitro, while it does not bind to other myelin sphingolipids suggesting that sulfatide at the OLs surface might be important for the binding of rHIgM22 to these cells and to myelin. However, IgM22 does not bind structures expressing sulfatide outside the nervous system, so additional factors are likely relevant for the immunoreactivity of IgM22 in CNS. Indeed, in lipid extracts from different sources we found another lipid antigen selectively recognized by rHIgM22, whose identity is under investigation. This lipid is also present in the extracts from mixed glial cultures, which do not contain mature O4⁺ OLs, suggesting that other glial cells in addition to OLs might be important in the response to rHIgM22.

Keywords: sphingolipids, remyelination
ENDOTHELIAL DYSFUNCTION IN IDIOPATHIC THROMBOEMBOLISM INVESTIGATED THROUGH GENE EXPRESSION PROFILING OF ENDOTHELIAL COLONY FORMING CELLS

Francesca Calcaterra¹,²*, Corrado Lodigiani³, Claudia Carenza¹,², Chiara Pandolfo¹, Luca Librè³, Paola Ferrari³, Silvia Della Bella¹,², Domenico Mavilio¹,²

¹BIOMETRA, Università degli Studi di Milano, Milan, Italy; ²Laboratory of Clinical and Experimental Immunology, Humanitas Clinical and Research Center, Rozzano (MI), Italy; ³Thrombosis Center, Humanitas Clinical Institute, Rozzano (MI), Italy
*e-mail: francesca.calcaterra@unimi.it

Venous thromboembolic events are defined idiopathic (ITE) when occurring without triggering and/or favoring circumstances. The integrity and the proper function of vessel endothelial lining are critical to prevent thrombi formation. Endothelial colony-forming cells (ECFCs) are bone-marrow-derived progenitors essential in endothelial homeostasis and repair. By using ECFC characterization as non-invasive strategy, this study investigated whether endothelial dysfunction may play a pathogenic role in ITE.

ECFCs were isolated and cultured from ITE patients and healthy controls using a protocol optimized in our lab. Efficiency of ECFC isolation and cell behavior of ECFC cultures, assessed as cell senescence and proliferation, were compared between ITE and controls. ECFC gene expression profile was analyzed by Illumina HumanHT-12 v4 Expression BeadChip arrays, and Gene Set Enrichment Analysis (GSEA). Candidate genes were validated by Q-PCR and ELISA.

ECFCs isolated from ITE patients were characterized by earlier senescence and reduced proliferation than control ECFCs. Genearray analysis showed that ECFCs isolated from ITE patients and controls were characterized by a different gene expression profile, with 2905 genes differentially expressed. GSEA analysis indicated 208 gene sets differentially expressed, with 8 gene sets up- and 200 down-regulated in ITE patients. 5 candidate genes, up-regulated in ITE patients and encoding for molecules that inhibit cell proliferation and survival (TNFSF15, TNFRSF25, CREB1, DUSP6 and CCNG1), were validated by Q-PCR. Higher expression of TNFSF15 in ITE patients was also confirmed by ELISA.
This study showed that ECFCs obtained from ITE patients differ from control ECFCs in their in vitro behavior and gene expression profile. Blocking experiments aimed at elucidating the role of TNFSF15 and its receptor (TNFRSF25) in ECFC proliferation and apoptosis are in progress, in order to investigate the mechanisms possibly involved in ITE pathogenesis.

**Keywords:** Endothelial Colony-Forming Cells, Idiopathic thromboembolism, cell proliferation, apoptosis, gene expression profiling
THE PROGESTIN DESOGEZTREL AFFECTS PHOX2B AND ITS TARGET GENES EXPRESSION: IMPLICATIONS IN THE THERAPEUTICAL APPROACHES IN CONGENITAL CENTRAL HYPOVENTILATION SYNDROME (CCHS)

S. Cardani¹*, S. Di Lascio¹, D. Belperio¹, E. Di Biase¹, R. Benfante¹,², D. Fornasari¹,²

¹BIOMETRA, University of Milan, Milan (Italy)
²CNR - Institute of Neuroscience, Milan (Italy)
*e-mail: silvia.cardani@unimi.it

Congenital Central Hypoventilation Syndrome (CCHS, MIM 209880) is a rare neonatal disease characterized by abnormal ventilatory response to hypoxia and hypercapnia, owing to failure of autonomic respiratory control. Frameshift mutations (5%) and polyalanine triplet expansions (95%) have been detected in the coding region of the transcription factor PHOX2B, responsible for the proper development and function of the ANS. Consistent with its role as transcriptional regulator, transcriptional dysregulation might be an important mechanism of CCHS pathogenesis. CCHS is a neurodevelopmental disorder, and current CCHS treatment research is aimed at counteracting the toxic effects of the mutated PHOX2B protein. Very recently it has been fortuitously observed that two females patients, using
the progestin Desogestrel, for contraceptive purposes, dramatically ameliorated the clinical symptoms of CCHS, showing chemosensitivity recovery. The molecular mechanism of this unexpected pharmacological effect is completely unknown, but this observation has strong proof of concept value, in the perspective of a pharmacological intervention in CCHS, at least for ameliorating respiratory symptoms. Recent data in the literature reported that Desogestrel, by improving resting ventilation, increases baseline respiratory frequency of CCHS patients leading to a decrease in their $\text{PCO}_2$. The same authors reported that in mice this increase necessitates the functioning of the serotoninergic system, thus suggesting that other neuronal systems, not depending on PHOX2B, play a key role in the amelioration of breathing defects. In contrast to this studies, here we show that Desogestrel affects directly PHOX2B expression and consequently the expression of some of its target genes, in a cellular context dependent manner, thus reinforcing the role that PHOX2B has in the pathogenesis of CCHS and in therapy response.

**Keywords**: molecular biology, transcriptional regulation, pharmacology
DESIGN OF FUNCTIONAL DNA ASSEMBLIES FOR NEXT GENERATION BIOSENSORS

Thomas Carzaniga\textsuperscript{1*}, Giuliano Zanchetta\textsuperscript{1}, Tommaso Bellini\textsuperscript{1}, Marco Buscaglia\textsuperscript{1}

\textsuperscript{1}Department of Medical Biotechnologies and Translational Medicine, Università degli Studi di Milano, LITA Segrate.

*e-mail: thomas.carzaniga@unimi.it

Detection and quantification of specific biomolecules in fluid samples are constantly needed in many fields, including human diagnostics, environmental monitoring and food safety. In the last decades, the development of novel detection methods and the increased availability of custom biomolecular probes has stimulated the research on innovative biosensor solutions. Most recently, the design of DNA-based molecular machineries is emerging as a further breakthrough to add controlled functionality at the nanoscale and achieve \textit{in-situ} amplification of molecular recognition events. Here we describe our research on the development of a new class of biosensors exploiting the tools of DNA nanotechnology in combination with a previously proposed optical label-free detection method. This approach, called Reflective Phantom Interface, is based on the imaging
of an interface with very weak reflectivity, where different molecular probes, typically antibodies, are immobilized. The local change of reflectivity provides a direct and real-time quantification of different molecular targets in solution without the need of fluorescent or colorimetric markers. We extended this method to DNA-DNA and DNA-protein interactions, with a two-fold interest: (i) we propose it as a powerful tool to study and model in detail the strengths and the kinetics of the interactions between DNA or RNA fragments with different mismatches or between nucleic acids and transcriptional regulator proteins; (ii) we realized DNA nanostructures on the biosensor surface in order to provide an effective amplification of the recognition process. In particular, we studied the growth of repeated filaments by the so-called Rolling Circle Amplification, forming a network of aptamers specific for capturing bacteria, and we studied the self-assembly of a responsive hydrogel undergoing conformational changes upon binding of specific targets.

**Keywords:** nucleic acids; transcriptional regulators; Rolling Circle Amplification; DNA nanotechnology; biosensors
PD1+ CELLS ACCUMULATE IN HIGHLY METABOLIC TUMORS IN PANCREATIC ADENOCARCINOMA

Giovanni Francesco Castino¹, Nina Cortese¹, Giulia Maggi¹,², Marco Erreni¹, Giovanni Capretti³, Cristina Ridolfi³, Francesca Gavazzi³, Paola Spaggiari⁴, Massimo Roncalli⁴, Alessandro Zerbi³, Paola Allavena¹, Federica Marchesi¹,²,*

¹ Dipartimento di Immunologia, Istituto Clinico Humanitas, Rozzano, Italy
² BIOMETRA, Università degli Studi di Milano, Milano, Italy
³ Sezione di Chirurgia Pancreatica, Istituto Clinico Humanitas, Rozzano, Italy
⁴ Sezione di Anatomia Patologica, Istituto Clinico Humanitas, Rozzano, Italy
* e-mail: federica.marchesi@unimi.it

The connection between tumor metabolism and immune infiltration.

The pathways that regulate immune cell function and metabolism are tightly linked and in many pathologic states, including cancer, metabolic dysfunction can severely impact on the efficacy of the immune response. Here we investigated the association of tumor metabolic activity and immune infiltration in Pancreatic Ductal Adenocarcinoma (PDAC), a microenvironment characterized by a strong immunosuppression. Tumor glycolysis was targeted in a murine PDAC cell line by knocking down the gene encoding for Phosphofructokinase (PFK⁶⁴), a key glycolytic enzyme. Glucose metabolism and tumor infiltrating leukocytes (TILs) were analyzed by multicolor flow
cytometry comparing PFK\textsuperscript{KD} tumors and control tumors. Immunohistochemical evaluation of metabolic and immune infiltrate markers was performed in a cohort of 40 PDAC patients, surgically operated at Humanitas Clinical and Research Center. PDAC tumors obtained from cell lines with different metabolic consumptions were differently infiltrated by T cells, with PD1+ CD8-TILs accumulating in highly metabolic tumors. Our glycolysis-targeting strategy revealed that cytotoxic T cells were recruited at almost the same extent in both PFK\textsuperscript{KD} and control tumors, however the percentage of PD1 expressing CD8+ TILs (PD1-TILs) was significantly decreased in PFK\textsuperscript{KD} tumors. Moreover, PD1-TILs from both PFK\textsuperscript{KD} and control tumors exhibited characteristics of effector cells including production of IFN\textgamma. In human PDAC sections, a higher density of PD1-TILs correlates with tumors expressing higher levels of GLUT-1, suggesting that PD1-TILs accumulate in highly glycolytic tumors. Both in human and preclinical models of PDAC, tumor cell glycolysis impacts on the type of immune cell infiltration, underlining a key interplay between glucose metabolism and the antitumor immune response.

**Keywords:** Pancreatic cancer, tumor-infiltrating leukocytes, metabolism, biomarkers
THE ENLARGEMENT OF BRACHIAL ARTERY DIAMETER AS A NOVEL MARKER OF ATHEROSCLEROTIC RISK

Daniela Coggi¹, Mauro Amato¹, Daniela Sansaro¹, Alessio Ravani¹, Beatrice Frigerio¹ and Damiano Baldassarre¹,²*

¹Centro Cardiologico Monzino, Milan, Italy
²BIOMETRA, Università degli Studi di Milano, Milan, Italy
*e-mail: damiano.baldassarre@unimi.it

BAD enlargement is a novel marker of atherosclerotic risk.

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<th>Study group:</th>
<th>4641 patients</th>
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<td>(44.6% women and 55.4% men; age (mean±SD) 58±13 and 55±13, respectively).</td>
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BAD associated with the prevalence of vascular events just in women.

The significant association was mainly due to association with myocardial infarction

BAD was closely associated with ICCAD, but determinants of the enlargement of the two vascular districts were very different.

BAD is an independent marker of myocardial infarction, which, at least in women, may provide information complementary to that coming from VRFs and ICCAD.

The enlargement of inter-adventitia common carotid artery diameter (ICCAD) measured in plaque free areas improves patient's risk stratification when added to algorithms for the assessment of global cardiovascular risk. The enlargement of brachial artery diameter (BAD) has also been successfully associated to vascular events. Whether the simultaneous measurement of arterial enlargement in two distinct vascular districts improves risk stratification or not is currently unknown. To address this issue, we measured BAD and ICCAD and carotid intima media thickness (C-IMT) by B-Mode
ultrasound in 4641 patients (44.6% women and 55.4% men; age (mean±SD) 58±13 and 55±13, respectively). BAD and ICCAD were measured in plaque free areas. Among the participants, 4271 were asymptomatic, 335 (64 women) experienced a myocardial infarction and 35 (11 women) a stroke. BAD associated with the prevalence of vascular events in both women and men. After adjustment for age, traditional risk factors, C-IMT and ICCAD, this association persisted in women (O.R and CI: 2.2 [1.1-4.4]; p<0.05) but not in men (O.R and CI: 1.1 [0.8-1.7]; p=NS). When the analysis was performed considering myocardial infarction and stroke separately, it becomes clear that the previously observed significant association was mainly due to association with myocardial infarction (O.R and CI: 2.6 [1.2-5.6]; p<0.05). BAD was closely associated with ICCAD (Beta of about 0.30±0.03; P<0.0001, in both sexes). Despite this, determinants of the enlargement of the two vascular districts were very different. For example, the relationship between BAD and the Framingham risk score was two times lower than that observed with ICCAD. BAD is an independent marker of myocardial infarction, which, at least in women, may provide information complementary to that coming from vascular risk factors and ICCAD.

**Keywords:** brachial artery diameter, carotid artery diameter, prevalence of vascular events, atherosclerosis
LncFOXP3 EPIGENETICALLY REGULATES CELL IDENTITY AND FUNCTION OF CD4⁺ T REGULATORY LYMPHOCYTES

Alessia Dardanelli¹,²*, Mariangela Lorenzo², Valeria Bevilacqua², Silvia Vangelisti², Paola Gruarin², Alessandra Albertelli², Valeria Ranzani², Federica Gervasoni², Marialucia Sarnicola², Serena Curti², Grazisa Rossetti², Massimiliano Pagani¹,²

¹BIOMETRA, Università degli Studi di Milano, Milan, Italy
²Istituto Nazionale di Genetica Molecolare INGM-Romeo ed Enrica Invernizzi, Milan, Italy
*e-mail: alessia.dardanelli@unimi.it

The human immune system is a suitable context for the study of cell plasticity in response to environmental stimuli. Differentiation of naïve cells into specialized subsets guarantees the proper immune system function. These subsets were once considered as terminally differentiated cells but recently demonstrated a high degree of plasticity, whose underlying molecular mechanisms are still poorly understood. In this context, CD4⁺ regulatory T lymphocytes (Treg) play a crucial role in the maintenance of immunological self-tolerance thanks to their peculiar suppressive function. Treg cells dysfunction is associated to autoimmune pathologies, inflammatory diseases and cancer. Their striking plasticity could be exploited as a promising therapeutic opportunity to modulate their differentiation and function in the context of several immune-mediated diseases. A better characterization of the underlying molecular mechanism is thus compelling. We published findings showing a key involvement of long non coding RNAs (lncRNAs) in the modulation of cell plasticity within the human immune system. These molecules proved to be highly specific and fundamental for lymphocytes cell-identity and in literature are also reported to be aberrantly expressed in a plethora of human diseases. These features brought lncRNAs to the fore as novel and promising therapeutic targets. On these premises, we are now collecting evidences regarding lncRNAs specifically expressed in human Treg lymphocytes. We are particularly investigating the role and function of Lnc-FOXP3 that is transcribed upstream FOXP3 gene: the key transcription factor, stably expressed in Treg cells and fundamental for their proper function. Due to the close proximity of Lnc-FOXP3 to FOXP3 gene and the preliminary
evidence we collected we want to investigate more in detail the role of Inc-FOXP3 in Treg cells and its mechanism of action to better understand if it could be considered a promising therapeutic target of Treg-mediated diseases.

Keywords: Immune system, T regulatory lymphocytes, long non coding RNAs
SERUM PROTEOMICS PROFILING IDENTIFIES NEW BIOMARKERS ASSOCIATED WITH SYSTEMIC SCLEROSIS

Maria De Santis¹, Elena Generali¹, Angela Ceribelli¹, Natasa Isailovic¹, Marta Caprioli¹, Giacomo Maria Guidelli¹, Carlo Selmi¹,²*

¹Rheumatology and Clinical Immunology, Humanitas Research Hospital; ²BIOMETRA Department, University of Milan, Italy.
*e-mail: carlo.selmi@unimi.it

Background and aim. Systemic sclerosis (SSc) is an autoimmune disease associated with serum anti-nuclear antibodies (ANA) and anti-centromere (ACA), anti-topoisomerase I (ant-Scl70), and anti-RNA polymerase III antibodies, identifying patient subgroups. However, no reliable biomarkers can predict SSc susceptibility and internal organ involvement. Therefore, we aimed to identify serum protein biomarkers associated with SSc and interstitial lung disease (ILD).

Methods. We analyzed serum samples of 3 patients with SSc and ILD and 3 patients with SSc and no ILD, and 4 healthy controls (HC). All subjects were women and age matched. Serum proteomics profiling was performed using the SOMAscan platform (SomaLogic, Inc., Boulder, CO, USA).

Results. Proteomic analysis identified 33 proteins which differentiated SSc from HC and 9 proteins which differentiate SSc patients with and without ILD. Compared to healthy controls, SSc cases showed an altered expression of proteins involved in extracellular matrix formation and cell-cell adhesion, angiogenesis, and lymphocyte recruitment, activation, and signaling, including interferon and IL-1 signatures, while an overall inhibition of markers of neutrophil function was noted. Patients with SSc and ILD manifested increased protein levels related to intracellular signaling and cell cycle, along with an increase of monocyte chemoattractants and ligands for the leukocyte adhesion compared to SSc without ILD. We further observed a decrease in B cell stimulating factor and IL-22 signaling in SSc and ILD.

Conclusions. Serum proteomic profiles can differentiate SSc from healthy controls and SSc patients with and without interstitial lung disease; moreover, our results identify biomarkers with a putative pathogenic significance.

Keywords: biomarkers, proteomics, fibrosis
DISSECTING MOLECULAR BASIS OF GANGLIOSIDE GM1 NEURO-PROPERTIES: EFFECTS OF GM1 OLIGOSACCHARIDE ON DIFFERENTIATION OF MOUSE PRIMARY NEURONS

Erika Di Biase*, Margherita Maggioni, Maura Samarani, Simona Prioni, Elena Chiricozzi and Sandro Sonnino

Department of Medical Biotechnology and Translational Medicine, University of Milano, Segrate (MI), Italy
*e-mail: erika.dibiase@unimi.it

Neuroprotective and neurotrophic properties of ganglioside GM1 have been reported both *in vitro* and *in vivo*. In cultured neurons the overexpression of sialidase Neu3 induces the local enrichment of GM1 in plasma membrane lipid rafts allowing the dimerisation and activation of Trk family receptors. This event in turn triggers a specific signalling cascade resulting in actin depolymerisation that allows axon protrusion and elongation. Despite this evidence, the mechanism of action of GM1 is still unknown. Recently we demonstrated that GM1 oligosaccharide directly binds TrkA, triggering the TrkA-Map kinases pathway activation which leads to neurodifferentiation of neuroblastoma cells. Here, we characterize the effect of GM1 oligosaccharidic chain on the differentiation of mouse primary neurons. Exogenously administered GM1 oligosaccharide accelerates differentiation...
processes by enhancing cell clusterization and networking. Accordingly, biochemical analysis pointed out increased expression level of neurodifferentiation markers in oligosaccharide treated cells. GM1 oligosaccharide interacts with cell surface without entering cells, suggesting a presence of biological target on neuronal plasma membrane. Interestingly, we show the direct involvement of Trk pathway activation as the early event underling GM1 oligosaccharide effects in neurons. This means that the specific role of ganglioside GM1 in neuronal differentiation, described in the past, is determined by a direct interaction between the oligosaccharide portion and specific proteins.

**Keywords:** glycosphingolipids, neurodifferentiation, Trk receptors
NEWLY IDENTIFIED PHOX2B TARGET GENES AS DRUG TARGETS IN CONGENITAL CENTRAL HYPOVENTILATION SYNDROME (CCHS)

Simona Di Lascio¹*, Silvia Cardani¹, Debora Belperio¹, Laura Gritti¹,², Chiara Verpelli¹,², Roberta Benfante¹,², Diego Fornasari¹,²

¹BIOMETRA, Università degli Studi di Milano, Milan, Italy
²CNR – Neuroscience Institute, Milan, Italy
*e-mail: simona.dilascio@unimi.it

Heterozygous mutations in the coding region of PHOX2B gene are associated with Congenital Central Hypoventilation Syndrome (CCHS), a rare disorder characterized by a broad variety of symptoms of autonomic nervous system dysfunction including inadequate control of breathing. PHOX2B is a transcription factor that plays a crucial role in autonomic nervous system development. In vivo and in vitro studies suggest that a loss of function mechanism, combined with a dominant-negative effect and/or toxic gain of function of the mutated proteins, is responsible for the entire disease spectrum. We have recently reported that several mutant proteins interfere with the transcriptional activity of the wild-type protein in a promoter-specific manner. Of note, we showed that PHOX2B is capable of controlling its own transcription by binding its own promoter, and PHOX2B mutants can negatively interfere with the expression of the normal allele, thus further reducing the amount of normal PHOX2B protein. Absolute limitations to the
comprehension of the pathogenesis of CCHS, and the development of new and effective treatments for this disease, is the missing knowledge of target genes regulated by PHOX2B, whose expression may be eventually dysregulated in the disease. Our general hypothesis is that impaired PHOX2B gene expression combined with more general transcriptional dysregulation plays a major pathogenetic role in CCHS. ChIP-seq analysis in IMR32 neuroblastoma cell line allowed us to identify many PHOX2B target gene candidates that are under validation by comparing wild-type and CRISPR-CAS9 Knocked-down PHOX2B expressing IMR32 cells, and in iPS-derived autonomic neurons. Gene Ontology analysis of the set of peak-associated genes identified several enriched terms, such as synaptic transmission, regulation of embryonic development, cell-cell signaling, axonogenesis, and neuron development, consistent with PHOX2B role during Autonomic Nervous System development and maintenance.

**Keywords:** Molecular biology, transcriptional regulation, ChIP-seq, CRISPR-CAS9, sympathetic neuron
DDM MICRORHEOLOGY OF COMPLEX FLUIDS

P. Edera\textsuperscript{1*}, D. Bergamini, F. Giavazzi\textsuperscript{1}, R. Cerbino\textsuperscript{1}

\textsuperscript{1}BIOMETRA, Università degli Studi di Milano, Milan, Italy
*e-mail: paolo.edera@unimi.it

Mechanical deformability of cytoplasm has a critical importance in many cellular and subcellular processes, form cell amoeboid movement to chromosome segregation.
On the other hand, it has been shown that an alteration of cellular mechanical properties can lead to a dysfunction of the whole organism (e.g. the lack of lamine that modifies nuclear rigidity in Progeria).
For these reasons, several efforts have been made to develop techniques allowing to access \textit{local viscoelastic properties} of cells.
An elegant and minimally invasive approach to measure viscosity and elasticity of a material consists in embedding micrometric particles (tracers) and in observing, with an optical microscope, their erratic motion, driven by spontaneous thermal fluctuations.
If the embedded tracers have a good optical contrast and there are no other sources of optical signal, their trajectories can be individually identified by Single Particle Tracking. However, these conditions can be hardly met in several systems of biological interest, where tracers are too small or too crowded to be resolved and tracked individually.

We present a new method, based on Differential Dynamic Microscopy, that operates on a sequence of microscopy images and, through the direct quantification of key statistical properties of motion of the tracers, enables the measurement of viscosity and elasticity of complex matrices. Our method, as it does not require to reconstruct single particle trajectories, enables the investigation of complex biological systems, that are intractable with Particle Tracking.

**Keywords**: Optical Microscopy, Micro-rheology
Polymicrogyria (PMG) is a condition characterized by abnormal prenatal brain development and excessive number of ectopic small gyri in the cerebral cortex. Symptoms range from isolated impairment of cognitive function to severe encephalopathy and intractable epilepsy. The pathogenesis is still poorly understood. Experimentally, PMG can be reproduced by the focal freeze-lesion model in neonatal rodents. Previous studies in the model showed unbalanced synaptic transmission with higher susceptibility to epilepsy. Biochemical, morphological and behavioral analysis of the PMG
model revealed a significant astrogliosis, microglial activation and increased levels of inflammatory cytokines. Furthermore, a diffuse cortical hypomyelination is evident. Adult PMG mice display altered EEG profile and defective motor skills. Our first approach showed that transplantation of human CNS neural stem cells has beneficial effect in PMG mice ameliorating the myelin defects, restoring normal EEG brain activity and improving motor functions. As second strategy we performed the pharmacological inhibition of IL-1R pathway by the IL-1R antagonist anakinra. We obtained a significant improvement of EEG and motor skills in adult PMG mice thus indicating a role of inflammation at the root of the pathology and identifying a therapeutic time window for the treatment. Recently, a homozygous missense mutation, causing a partial loss of FIG4, was found to co-segregate with PMG. FIG4 is a phosphatase interacting with phosphatidylinositol-5-phosphate (PI5P) kinase PIKfyve and Vac14 to control the production of phosphatidylinositol-3,5-diphosphate (PI3,5P2) a regulator of membrane and protein trafficking in the endosome–lysosome axis. Recently the PIKfyve/FIG4/VAC14 complex has been involved in AMPARs trafficking. We are currently carrying out experiments (patch clamp electrophysiology and single cell calcium imaging) to investigate the impact of FIG4 downregulation in neuronal function.

Keywords: Polymicrogyria, inflammation, myelin, synapses, neurodevelopmental disease
MORPHOLOGICAL AND FUNCTIONAL CILIARY DEFECTS IN MECP2 NULL SYSTEMS: A NOVEL DRUGGABLE MACHANISM IN RETT SYNDROME

Angelisa Frasca¹*, Barbara Leva², Anna Bergo², Eleonora Spiombi¹, Michela Palmieri³, Charlotte Kilstrup-Nielsen², Nicoletta Landsberger¹

¹BIOMETRA, Università degli Studi di Milano, Milan, Italy
²Department of Biotechnology and Life Science, University of Insubria, Busto Arsizio, Italy
³San Raffaele Rett Research Unit, Division of Neuroscience, San Raffaele Scientific Institute
*e-mail: angelisa.frasca@unimi.it

Rett syndrome (RTT) is a devastating neurological disorder that to date is still lacking any cure. However, it has been established that phenotypic rescue is possible in MeCP2 null animal model upon reactivation of the endogenous gene. MeCP2 is a multi-functional protein that, in addition to a nuclear activity, is functionally and molecularly associated to the centrosome. Considering the important function of the centrosome to the primary cilium, the fact that the primary cilium is a signalling device exploiting microtubule (MT) track ways and Mecp2-null cells have impaired microtubule stability, we investigated if MeCP2 is required for primary cilium formation and if impaired ciliary structures/functions are implicated in RTT. The primary cilium acts as an antenna to probe extracellular signals, controlling cell proliferation, differentiation and migration. Its relevance for normal development is highlighted by a growing list of diseases linked to primary cilia dysfunctions. Our data demonstrate that depletion of Mecp2 in cultured cells causes a
reduction in the % of ciliated cells and in the length of cilium, which are normalized by the exogenous expression of MeCP2. In support of a role for MeCP2 in cillum formation, immunostaining of cortical brain slices from Mecp2 null mice indicates a reduction in ciliary volume. Further, we found that Mecp2 null cells are defective in the SHH pathway, that is exclusively associated with primary cilium. Since primary cilia are extended and maintained by the transport of particles along the MT, we tested and proved that pharmacological stabilization of MT rescues the observed ciliary defect. In conclusion, our data demonstrate that cilium defects are linked to Mecp2 deletion and that cilium formation can be pharmacologically rescued, highlighting this organelle as an interesting candidate for novel therapeutic approaches in RTT.

**Keywords:** cilium; SHH pathway; Rett neurobiology
SERUM ANTI-PHOSPHOLIPID ANTIBODY PREVALENCE AND CARDIOVASCULAR SIGNIFICANCE IN THE GENERAL POPULATION OF THE CAMELIA STUDY

Elena Generali¹, M. De Santis¹, Pier Maria Battezzati²,³, A. Ceribelli¹, Pierluigi Meroni², Massimo Zuin²,³, Carlo Selmi¹,²*

¹Rheumatology and Clinical Immunology, Humanitas Research Hospital, Rozzano (MI), 
²University of Milan 
³Gastroenterology and Liver Unit, San Paolo Research Hospital, University of Milan 
*e-mail: carlo.selmi@unimi.it

Background and aims. The serum anti-phospholipid antibody (aPLs) prevalence in the general population and the association with cardiovascular (CV) disease is unclear. We aimed to determine the prevalence of aPLs and CV and metabolic comorbidities in a Northern Italian city.

Methods. We performed a cross-sectional study on 1,712 adult subjects randomly enrolled in 2010 from the voting lists of Abbiategrasso. All subjects completed a questionnaire for medical history and ongoing/past medications and underwent physical examination and abdomen and carotid ultrasound. Anti-cardiolipin (aCL), anti-beta2 glycoprotein I (aGPI), antiphosphatidylserine-prothrombin (aSP) IgG, IgM, and IgA antibodies were tested in all subjects by ELISA.

Results. APLs were positive in 15.1% of subjects, with no differences between sexes and with highest prevalence rates in older groups. A history of CV events was more frequent in aPLs positive subjects (odds ratio (OR) 1.67, 95% confidence interval (CI) 1.08-2.54, p=0.012), particularly peripheral vasculopathy (crude OR in aPLs positive subjects 2.02; 95CI 1.14-3.57, p=0.015). In subjects with the highest CV risk profile (i.e. with a Framingham risk score >20 and/or diabetes and/or BMI >35), aPLs positivity was associated with the highest risk of CV events (OR 2.52, 95% CI 1.24-5.11, p=0.011). Of interest, aGPI IgA were associated with increased carotid intima-media thickness (adjusted beta 0.51, p= 0.003).
**Conclusions.** APLs prevalence in our cohort is higher than previously reported, especially in older subjects, but with an equal distribution between sexes. CV events are more frequent in aPLs positive subjects, especially when combined with a high CV risk profile.

**Keywords.** Epidemiology, autoantibodies, cardiovascular risk
MUTATION ADAPTED U1/U6 snRNAs ACTING ON CDKL5 pre-mRNA: “A PROOF OF CONCEPT” FOR TREATMENT OF SPLICING-ASSOCIATED MUTATIONS IN CDKL5 DISORDERS

Domenico Giorgio1*, Mirko Pinotti 2, Dario Balestra 2, Nicoletta Landsberger1

1BIOMETRA, Università degli Studi di Milano, Milan, Italy
2Department of Biochemistry and Molecular Biology, University of Ferrara, Italy
*e-mail: domenico.giorgio@unimi.it

Genetic lesions of various kinds in the X-linked CDKL5 gene are responsible for a suite of disorders affecting both genders, which generally share the common feature of early drug-resistant epilepsy, emerging in the first months of life. No cure is currently available for CDKL5 conditions and treatment is usually based on support therapy for the comorbidities, and on rehabilitation. Our knowledge on CDKL5 functions and the consequences of its deficiency, are still very limited; therefore, the identification of “rationally-designed” therapeutic approaches will probably not occur in the immediate future. We found that almost 15% of pathogenic mutations might benefit of therapeutic approaches normalizing splicing. By acting on pre-mRNA, these approaches circumvent the overexpression problem, the packaging limitations imposed by gene therapy as well as the necessity of transducing, in heterozygous female patients, mainly those cells that express the mutated allele.

To identify therapies correcting splicing, we have initially focused on mutations at donor sites, which reduce their complementarity to the 5′ tail of
the U1 snRNA. Several mutant minigenes carrying different CDKL5 splicing mutations were generated and expressed either in non-neuronal and neuronal human cell lines. Splicing defects have been characterized at the nucleotide level. Then we designed and tested the capacity of modified U1 snRNAs to positively affect in vitro aberrant CDKL5 RNA splicing. The splicing pattern analysis of U1 snRNAs, cotransfected with the corresponding minigene variants, showed that all mutations were rescued by the corresponding complementarity U1. Since this correction approach appears to be limited to single mutations, we screened also U1 snRNAs shifted, binding at non-conserved intronic sequences downstream the exon identified, searching for a unique molecule able to restore different mutations affecting the same exon.

**Keywords:** CDKL5, splicing, U1 snRNA
rHlgM22, A REMYELINATION-PROMOTING ANTIBODY, INHIBITS ACID SPHINGOMYELINASE ACTIVITY IN MIXED GLIAL CELL CULTURES

Sara Grassi1*, Simona Prioni1, Yana Zorina2, Livia Cabitta1, Sandro Sonnino1, Alessandro Prinetti1

1BIOMETRA, Università degli Studi di Milano, Segrate (MI), Italy
2Acorda Therapeutics, Inc., Ardsley, NY, USA
*e-mail: sara.grassi@unimi.it

Recombinant human IgM22 (rHlgM22) binds to myelin and oligodendrocytes (OLs), and promotes remyelination in mouse models of multiple sclerosis. The identity of its antigen is still under investigation but we have shown that different sphingolipids are potentially involved in its ability to bind at the cell surface. Literature strongly suggests that rHlgM22 biological activity is mediated by the reorganization of Lyn, integrin αvβ3 and PDGFαR at the cell surface to form a signaling complex triggering Lyn activation which, in turn, promotes oligodendrocyte precursor cells (OPCs) survival and proliferation [1]. However, rHlgM22-mediated OPC proliferation is only detectable in mixed glial cultures (MGC), but not in purified OPCs [2]. Previous studies in OLs showed that the anti-apoptotic effect of Lyn activation might be due to reduced activity of acid sphingomyelinase (ASMase) and consequent reduced ceramide generation [3]. Ceramide generated by the action of ASMase represents an important pro-apoptotic signal, but also a signal for the re-arrangement of sphingolipid-rich signaling platforms. We assessed ASMase activity in MGC following a dose treatments of different duration with rHlgM22. Two different non-immunogenic human IgMs were used as a negative control. The data we obtained show a significant decrease of total ASMase activity in MGC treated with rHlgM22, with respect to control. Moreover, we observed that, in MGC, Lyn is enriched in sphingolipid-enriched membrane fractions, which are also enriched in ASMase. rHlgM22-mediated increased Lyn expression and activation could result in a decrease in ASMase activity and in ceramide generation, thus inhibiting pro-apoptotic signaling and/or the organization of sphingolipid-dependent signaling platforms.

Keywords: Multiple Sclerosis, Demyelination, Glycosphingolipids, ASMase, Lyn
References
NUCLEIC ACID SENSORS IN THE PATHOGENESIS OF PSORIATIC DISEASE: LL37 AND IFI16

Natasa Isailovic1, Maria De Santis1, Angela Ceribelli1, Elena Generali1, Lorenzo Altamore2, Giuseppina Sabatino2, Paolo Rovero2, Anna Maria Papini2, Valeria Caneparo3, Santo Landolfo3, Marisa Gariglio4, Marco De Andrea3, Carlo Selmi1,5*

1Rheumatology and Clinical Immunology, Humanitas Research Hospital, Rozzano (MI)
2Laboratory of Peptide and Protein Chemistry and Biology, Departments of Chemistry “Ugo Schiff” and NeuroFarBa, University of Florence, Italy
3University of Torino, Italy
4University of Piemonte Orientale, Italy
5BIOMETRA Department, University of Milan, Italy
*e-mail: carlo.selmi@unimi.it

Background and aims. The etiopathogenesis of psoriatic disease, including psoriasis and psoriatic arthritis (PsA), is still unclear, but recently it has been clarified why self-DNA become immunogenic in psoriatic disease, suggesting an important role of nucleic acid sensors such as LL37 and IFI16, in psoriatic disease. We aimed at investigating humoral and cellular response to LL37 and IFI16 in PsA.

Methods. Serum samples from PsA patients and age- and sex-matched healthy controls (HC) have been tested for anti-LL37 (human synthetic LL37-based ELISA), IFI16 (capture ELISA) and anti-IFI16 (human recombinant IFI16-based ELISA). Confirmation of anti-IFI16 with protein radio-immunoprecipitation using marked 35S- K562 cell extract and Western blot.

Results. Anti-LL37 IgM antibodies were detected in 22/35 (63%) PsA sera, compared to 2/34 (6%) of HC (p<0.001). Two/22 (9%) anti-hLL37-positive subjects were in remission according to DAS28-CRP, compared to 5/13 (39%) anti-hLL37-negative patients (p=0.036). IFI16 was detected in 73/158 (46.2%) of PsA sera and correlated significantly with high levels of C reactive protein (50.7% vs 30.6%, p=0.01). Anti-IFI16 IgG was positive in 12% of PsA cases and correlated significantly with high levels of C reactive protein (63.2% vs 36.7%, p=0.027). Anti-IFI16 IgA was positive in 14.6% and were significantly increased in subjects with skin psoriasis (95.7% vs 77%, p=0.045). IFI16 was detected in 1/7 synovial fluid, while anti-IFI16 IgG antibodies in 3/7. IFI16 declined during anti-TNFalpha treatment.
Conclusions. Nucleic acid sensors LL37 and IFI16 elicit an adaptive immune response in PsA, and autoantibodies against LL37 and IFI16 correlate with disease activity.

Keywords: innate immunity, adaptive immunity, DNA sensors
MOUSE NEUROBLASTOMA CELLS NEURODIFFERENTIATION PROMOTED BY GM1 GANGLIOSIDE IS MEDIATED BY THE INTERACTION BETWEEN ITS OLIGOSACCHARIDE AND TrkA RECEPTOR

Margherita Maggioni\textsuperscript{1*}, Erika Di Biase\textsuperscript{1}, Chiara Parravicini\textsuperscript{2}, Ivano Eberini\textsuperscript{2}, Elena Chiricozzi\textsuperscript{1} and Sandro Sonnino\textsuperscript{1}

\textsuperscript{1}BIOMETRA, Università degli Studi di Milano, Milan, Italy
\textsuperscript{2}Department of Pharmacological and Biomolecular Science, University of Milan, Italy
\*e-mail: margherita.maggioni@unimi.it

GM1 ganglioside is known for promoting neurodifferentiation and neuroprotection in neuronal cells. GM1-stimulated Trk receptor activity has been proved for explain its neurotrophic functions. Recently we found that the oligosaccharide portion of GM1, such as the entire molecule, but not the ceramide, directly activates TrkA-MAPK pathway leading to neurodifferentiation processes in neuroblastoma cells.

Here, we reported that the GM1 oligosaccharide directly interact with TrkA receptor.

The GM1-TrkA interaction was investigated by photo-labeling experiments performed with analogues of GM1 and its oligosaccharide. These photoactivable compounds have been administered to N2a cells for 3 hours.
Subsequently, cells were illuminated and the nitrophenyl azide photoactivable group, linked to the terminal portion of ceramide or to the sugar moiety, get to a very reactive intermediate able to bind covalently the molecules in the close environment.

For the first time, we showed a direct connection between the oligosaccharide portion of GM1 and the extracellular domains of TrkA using both GM1 and OligoGM1 photoactivable on the sialic acid, but no interaction are detectable between TrkA and GM1 photoactivable on ceramide moiety. Moreover, molecular docking analysis confirmed that GM1 oligosaccharide binds the TrkA-NGF complex leading to a binding free energy of approx. -11.5 kcal/mol, acting as a bridge able to increase and stabilize the TrkA-NGF molecular interaction.

**Keywords:** GM1 ganglioside, GM1 oligosaccharide, TrkA receptor, neuron differentiation
**NIPBL, A NEW PLAYER WITH NPMC+ IN THE ONSET OF ACUTE MYELOID LEUKEMIA**

Mara Mazzola¹*, Grazia Fazio², Gianluca Deflorian³, Laura Ferrari³, Claudia Saitta², Erica Bresciani⁴, Andrea Biondi², Anna Marozzi¹, Gianni Cazzaniga², Anna Pistocchi¹

¹BIOMETRA, Università degli Studi di Milano, Milan, Italy
²Fondazione Tettamanti, Ospedale San Gerardo, Monza; Università di Milano-Bicocca, Milan Italy
³Istituto FIRC di Oncologia Molecolare (IFOM), Milan, Italy
⁴Oncogenesis and Development Section, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD

*e-mail: mara.mazzola@unimi.it

Cohesins form a multimeric protein complex involved in the cohesion of sister chromatids, post-replicative DNA repair and transcriptional regulation. Recently, recurrent mutations and deletions involving multiple components of the cohesin-complex have been reported in Acute Myeloid Leukemia (AML), and other myeloid neoplasms. These genetic lesions are mostly mutually exclusive and occur in 9% of AML patients, suggesting a role for the cohesin-complex in the pathogenesis of AML. Frequently, mutations in
cohesin genes co-occurred with the known AML-associated gene *nucleophosmin* (*NPM1*). NPM1 is a ubiquitous multifunctional phosphoprotein normally detected primarily in the nucleus. However, when mutated, it aberrantly relocates to the cytoplasm (NPMc+). Forced NPMc+ expression in zebrafish causes an expansion of hematopoietic stem cells (HSCs) and indifferented myeloid cells in line with AML patient features. We analysed the expression of cohesin genes in our AML cohort (available at Fondazione Tettamanti) and, surprisingly, only the expression of *NIPBL* was decreased with NPMC+ mutation. Using our valid zebrafish model of *nipblb* haploinsufficiency previously generated for the study of the Cornelia de Lange Syndrome in which germinal mutations in cohesin genes have been reported, we observed an increase in undifferentiated myeloid cells and a significant decrease in differentiated neutrophils, a phenotype resembling the NPMc+ zebrafish model and AML development. Therefore, we investigated a possible synergy between NPMC+ and *NIPBL* in the onset of the aberrant hematopoietic phenotype in zebrafish and we identified the canonical Wnt pathway as an active player in this process, as already demonstrated in NPMC+ AML patients.

**Keywords**: cohesins, *NIPBL*, NPMC+, AML, zebrafish
CHARACTERIZATION OF A NOVEL NKp46$^{\text{pos}}$/V$\delta$1-RESTRICTED $\gamma\delta$ TCR LYMPHOCYTES RESIDENT WITHIN THE HUMAN INTESTINE

Ferdinando Oriolo$^{1,6,*}$, Joanna Mikulak$^{1,2}$, Alessandra Roberto$^{1}$, Elena Bruni$^{1}$, Paolo Tentorio$^{1}$, Federico Colombo$^{3}$, Anna Villa$^{1,2,4}$, Bruno Silva-Santos$^{5}$, Silvia Della Bella$^{1,6}$ and Domenico Mavilio$^{1,6}$

$^1$Unit of Clinical and Experimental Immunology, Humanitas Clinical and Research Hospital, Rozzano, Milan, Italy
$^2$Institute of Genetic and Biomedical Research (IRGB), CNR, Milan, Italy
$^3$Flow Cytometry and Cell Sorting Unit, Humanitas Clinical and Research Center, Rozzano, Milan, Italy
$^4$Telethon Institute for Gene Therapy, Division of Regenerative Medicine, Stem Cells and Gene Therapy, Istituto di Ricovery e Cura a Carattere Scientifico (IRCCS) San Raffaele Scientific Institute, Milan, Italy
$^5$Institute of Molecular Medicine, Faculty of Medicine, University of Lisbon, Lisbon, Portugal
$^6$Department of Medical Biotechnologies and Translational Medicine (BioMeTra), University of Milan, Milan, Italy
*e-mail: ferdinando.oriolo@unimi.it

NKp46 is a member of Natural Cytotoxic Receptors (NCRs) triggering Natural Killer (NK) cell cytotoxicity and production of inflammatory cytokine against harmful cells. Although NKp46 had been first identified as a specific NK cell receptors, recent studies revealed that it is also expressed on other immune cells such as Innate Lymphoid cells, $\alpha\beta$ T and NKT lymphocytes. We previously reported that NKp46 expression is inducible upon in vitro activation on a subset of human peripheral blood V$\delta$1 T cells. Here, we identify a novel NKp46$^{\text{pos}}$/V$\delta$ T lymphocytes subset resident in human intestine under homeostatic conditions within the intraepithelial (IEL) compartment (average of 50 % ± 8.34 of all V$\delta$ T IELs) and, to a lower degree, in lamina propria (LP) (average of 10 % ± 5.56 of all V$\delta$ T LPLs). This NKp46$^{\text{pos}}$/V$\delta$ T IEL subset is characterized by a cytotoxic phenotype expressing CD56, CD8, CD69, NKG2D and NKG2C. Expression of NKp46 in intestinal V$\delta$ IELs was restricted to V$\delta$1 TCR chain repertoire that distinguishes their tissue-like phenotype from peripheral blood V$\delta$ T cells which mainly exhibited a V$\delta$2 TCR distribution. NKp46$^{\text{pos}}$/V$\delta$T phenotype reflects higher cytotoxic effector activity against human leukemia cell line K562 as well as GRZB and...
IFNγ production compared to the NKp46\textsuperscript{neg} counterpart. In addition, expression of NKp46 was specific for IL15/IL2-dependent differentiation of human infant thymic γδ T cells precursors and not for Tγδ cells of adult healthy donors. Therefore, a novel identified NKp46\textsuperscript{pos} human gut-specific phenotype is a feature of Tγδ differentiation of thymic precursors’ that results with high cytotoxic anti-tumor activity.

**Keywords:** γδT cells, NKp46, IELs, cytotoxicity, Thymocytes
DEPRESSION AND MULTIPLE SCLEROSIS COMORBIDITY: POTENTIAL ROLE OF EARLY LIFE ADVERSTIES

Maria Serena Paladini¹*, Giusy Tindara Coppolino², Andrea Carlo Rossetti¹, Davide Marangon², Alice Guidi¹, Marta Fumagalli², Davide Lecca², Maria Pia Abbracchio², Raffaella Molteni¹

¹BIOMETRA, University of Milan, Milano, Italy.
²Department of Pharmacological and Biomolecular Sciences, University of Milan, Milano, Italy.
*e-mail: maria.paladini@unimi.it

Multiple sclerosis (MS) is an inflammatory demyelinating disease with a high incidence of psychiatric comorbidity, in particular almost one-quarter of MS patients suffer from depression. It has been reported that psychosocial/pharmacological support to relieve depression has a beneficial impact on quality of life and also survival time of MS patients. Nevertheless, the diagnosis and the treatment of the psychopathology is still neglected, leading to a critical unmet need that might be addressed by deepen studies on the connection between MS and depression.

To this aim, we generated a model of depression-associated MS by inducing experimental autoimmune encephalomyelitis (EAE) in control mice or in mice prenatally exposed to stress, the main risk factor for depression. Specifically, since both depression and MS affect women twice as often as men, EAE was induced by immunization with MOG₃₅-₅₅/CFA and treatment with pertussin...
toxin (PTX) in adult female mice born from control or stressed dams, the latter exposed to restraint stress during the last days of gestation. Next, to investigate the reciprocal impact of stress exposure and MS we performed several behavioural evaluations.

The sucrose intake test revealed that prenatal stress (PNS) induced an anhedonic-like phenotype only in the EAE-mice, which show a tendency - although not statistically significant- to drink less sweet solution. Alongside this observation, PNS increased the severity of the EAE clinical scores, particularly in the acute phase and during the recovery phase, confirming the hypothesis of a mutual interaction between the two conditions.

Our results clearly suggest an interplay between EAE-induction and PNS exposure and indicate this combined model as a new promising candidate in the study of depression-associated MS. Further analyses are demanded to better characterize the feature of EAE and PNS combination, especially at molecular level.

**Keywords:** Multiple Sclerosis, Depression, EAE, Prenatal Str
NEW ROLE OF ATM IN HIPPOCAMPAL NEURONS DURING DEVELOPMENT

Lara Pizzamiglio\(^1\)*, Elisa Focchi\(^1,3\), Luca Murru\(^2\), Matteo Tamborini\(^3\), Maria Passafaro\(^2\), Elisabetta Menna\(^2,3\), Michela Matteoli\(^2,3\) and Flavia Antonucci\(^1,2\)

\(^1\)BIOMETRA, University of Milan, Milan, Italy; 
\(^2\)Institute of Neuroscience, C.N.R., Milan, 20129, Italy; 
\(^3\)Humanitas Clinical and Research Center, IRCCS Rozzano, Italy; 
*e-mail: lara.pizzamiglio@unimi.it

ATM (Ataxia Telangiectasia mutated) is a serine/threonine protein kinase linked to DNA damage response. Despite its activity in dividing cell has been largely investigated, a high number of evidences have demonstrated new roles of ATM also in adult neurons, such as its involvement in adult neurogenesis, its crucial function in oxidative stress response and its interaction with two synaptic vesicle proteins, VAMP2 and synapsin-I. Coherently, the neurodegenerative condition associated to genetic mutations in \textit{Atm} gene, the Ataxia Telangiectasia (A-T), exhibits a variable phenotype with impairment in cognition. Thus, ATM may impact brain functions through distinct mechanisms, in different brain regions and cell populations.
Taking advantages of neuronal cultures and by a combination of different experimental approaches, we found in ATM het hippocampal neurons a significant excitatory/inhibitory unbalance toward inhibition as indicated by: the higher frequency of mIPSCs, the increased number of GABAergic synapses and the more precocious development of the inhibitory system (i.e. excitatory to inhibitory GABA switch). *In vivo*, the enhanced inhibition still persists as well as a reduced neuronal excitability. One of the mechanisms underlying these rearrangements consists in the higher levels of ERK1/2 phosphorylation found in het hippocampi in association with a lower expression of its phosphatase, PP1. These data unveil an unexpected role of ATM in the maintenance of an appropriate GABAergic development and transmission in hippocampal formation, laying the basis for a more clear comprehension of cognitive defects occurring in A-T. Our preliminary results obtained treating het neurons with the KCC2 selective antagonist, VU0240551, indicate a recovery of the defective GABA-switch associated to the complete restoration of hippocampal functional properties.

**Keywords:** ATM, hippocampus, KCC2, GABAergic neurotransmission, neurodevelopment
DEVELOPING MRNA-PROFILING SYSTEM FOR THE FORENSIC IDENTIFICATION OF BODY FLUIDS

Giorgio Portera, Luca Ferrari, Paola Riva*

BIOMETRA, Università degli Studi di Milano, Milan, Italy
*e-mail: paola.riva@unimi.it

In the present forensic practice, the information about the biological origin of forensic traces is usually determined by enzymatic tests. The few types of biological traces that is possible to diagnose with protein-based testing are peripheral blood, semen and saliva. Development of specific tools should improve biological traces diagnosis. The Genetisti Forensi Italiani (GEFI) group, with National Forensic Institute of Netherlands (NFI) started a collaborative project on mRNA-based body fluid/skin typing, focused on identification of specific gene expression profiling, specifically associated to body fluids allowing to determine the biological origin of traces. In particular, we have tested methods and primers for many types of tissue proposed by an European Forensic Genetics Group (EUROFORGEN-NoE) to identify blood, saliva, semen, menstrual secretion, vaginal mucosa, skin and nasal mucosa. The isolation of RNA was performed by DNA/RNA co-isolation protocol, which uses silica-based columns. RT-PCR multiplex assay was developed for simultaneous detection and typing of 19 mRNA markers. RNA profiling was carried out by ABI3130XL genetic analyzer on blind, aged and fresh biological traces and samples from crime scene. The assay has good sensitivity for fresh traces and for some RNA markers analysed, precisely from semen, blood, menstrual secretion and skin of blind and aged samples, while no data from vaginal mucosa of aged samples have been provided. Now we are testing the assay sensitivity in function of time and temperature of conservation of the traces with the aim of identifying criteria of eligibility of the biological traces picked up from the crime scene, for the above assay, to provide the detective an efficient test in suitable conditions. Data typing highlights the importance of the method improving the interpretation of forensic stains by providing information not only concerning the donor of the sample, but also indicating the cell types present on the stain of the crime scene.
Keywords: RNA profiling, RT-PCR multiplex, Forensic DNA, Body fluid identification
SEAHORSE XF ANALYZER: AN ADVANCED TECHNOLOGY TO STUDY THE CELLULAR METABOLISM IN REAL-TIME

Fabio Rossi1*, Maurizio Ragni1, Laura Tedesco1, Chiara Ruocco1, Alessandra Valerio2, Michele O. Carruba1, Enzo Nisoli1

1Department of Biomedical Technology & Traslational Medicine, University of Milan, Milan, Italy; 2Department of Molecular and Translational Medicine, Brescia University, Brescia, Italy.
*e-mail: fabio.rossi@unimi.it

Cells generate energy via two mechanisms, mitochondrial respiration and glycolysis. During the mitochondrial respiration, substrates such a glucose, fatty acids, and amino acids are oxidized with oxygen consumption and ATP production, while during glycolysis glucose fermentation with production of protons and extra cellular acidification are observed. The Seahorse XF assays are considered key experimental approaches to investigate cell metabolism in a non-invasive/label free and real-time method. Using this kind of method, we are able to analyze the mitochondrial activity, by measuring the type of fuels used by the cells, and the amount of coupled and uncoupled respiration generated by the respiratory chain. We can compare oxygen consumption, ATP production, oxidative vs. glycolytic metabolism, and oxidative stress in different cell types (for example, control vs. gene-silenced cells, treated or not with drugs or nutrients). We will show results, we have recently obtained in cultured cardiac HL-1 cells and brown adipocytes exposed to different doses and different times of multiple amino acid mixtures. Seahorse XF technology simplifies analysis of cell energy metabolism, telling us what our cells are, and revealing a clear picture of what they do.

Keywords: Metabolism, Mitochondria, Respiration, Glycolysis
ATP-GATED P2X7 RECEPTOR AS A NOVEL CHECKPOINT MOLECULE IN T EFFEC TOR/MEMORY CELL FUNCTION

Elsa Rottoli¹*, Fabio Grassi¹

¹BIOMETRA, Università degli Studi di Milano, Milan, Italy
*e-mail: elsa.rottoli@unimi.it

Adenosine triphosphate is a ubiquitous extracellular messenger, which activates purinergic receptors in the plasma membrane of eukaryotic cells termed P2 receptors. We showed that T effector/memory (TEM) cells express high levels of P2rx7 encoding for the ATP-gated ionotropic P2X7 receptor subtype. The deletion of the gene in these cells results in increased survival and proliferation rate both in vitro and in vivo. P2rx7⁻ TEM cells are characterized by a bioenergetic advantage compared to wild-type (WT) cells and increased mitochondrial mass at the electron microscope. Microarray gene expression analysis showed that P2rx7⁻ TEM cells clustered together and separately from WT cells. Among differentially expressed genes we identified cyclin-dependent kinase inhibitor 1A (Cdkn1a), encoding for p21Waf1/Cip1, as a transcript upregulated in WT cells. P21 regulates progression through G₁ to S phase in mammalian cells. To address whether P2X7 signaling directly regulated Cdkn1a expression we stimulated WT TEM cells with BzATP as a selective P2X7 agonist. This resulted in significant increase in Cdkn1a transcripts with respect to unstimulated cells and this increase was abrogated by the selective P2X7 antagonist A-438079. Western blot analysis showed that WT TEM stimulated with BzATP are characterized by increased phosphorylated p53 at serine 15, a fundamental site for the transcription of p21, and in phosphorylated p38 (Thr180/Tyr182). The activation of P2X7 in WT TEM cells induces an increase in mitochondrial ROS production and an increase in histone H2Ax phosphorylation, a marker of DNA damage. We tested if P2X7 receptor has a role in the induction of senescence in TEM cells; the analysis of β-galactosidase activity upon BzATP stimulation showed that this was the case. We hypothesize P2X7 constitutes a checkpoint for CD4 TEM cells to limit their expansion in inflammatory environments.

Keywords: purinergic signaling, T cell activation, cell death, senescence
CAN THE USE OF A SINGLE INTEGRATED UNITARY AUTONOMIC INDEX PROVIDE EARLY CLUES FOR EVENTUAL ELIGIBILITY FOR OLYMPIC GAMES?

Roberto Sala¹, Mara Malacarne¹,², Davide Fusetti¹,³, Gianluigi Oggionni¹,³, Massimo Pagani¹, Daniela Lucini¹,²,³*

¹BIOMETRA, Università degli Studi di Milano, Milan, Italy
²Humanitas Clinical and Research Center, Sezione Medicina dell’Esercizio e Patologie Funzionali Rozzano, Italy
³Scuola di specializzazione in Medicina dello Sport e dell’Esercizio Fisico, Università degli Studi di Milano, Milan, Italy
*e-mail: daniela.lucini@unimi.it

Optimal autonomic nervous system (ANS) control and stress resilience might be considered critical elements of athletic performance. Autoregressive spectral analysis of heart rate variability (HRV) may represent a convenient tool in order to define ANS control. It furnishes a set of different variables and ANS information may be unevenly distributed through derived indices, influenced by age and gender, thus impairing a friendly and consistent use of HRV in clinical settings. In order to overcome these problems, we developed a novel unitary autonomic index for sports (ANISIs). In this study we hypothesize that this Index, together with a somatic stress related symptom score (4SQ), might help characterize athletes who were eventually selected for the Rio 2016 Olympic Games Italian team (Rio+).

We examined 778 athletes who underwent ANS and exercise ECG evaluation, months before the selection. The combination of vagal and sympathetic indices from HRV into ANISIs was performed by radar plot and percent ranking of index variables. We assessed (Rio+) vs (Rio-) athletes also after subdivision into three sport intensity groups (Low, Mid and High intensity).

Overall there were no significant differences when considering single individual spectral derived variables. Conversely, the unitary Index ANISIs was significantly higher in (Rio+) compared to (Rio-) (54.5±29.5 vs 47.9±28.4 p=0.014). This difference was particularly evident (p=0.017) in H intensity groups. 4SQ was significantly more elevated in the (Rio-) group of athletes competing in specialties with L or M intensity (L 22.9±22.8 vs 7±16.6, p=0.023; M 22.4±20.0 vs 15.76±18.8, p=0.016), no difference being observed in the H groups.
ANSIs, a proxy of quality of cardiac autonomic regulation and simple assessment of resilience to stress, may differentiate athletes who were eventually selected for participation in the 2016 Rio Olympic Games from those who were not, suggesting the possibility of a “winning functional phenotype”

Keywords: autonomic nervous system, stress, athletes, performance, Olympic games
**β-GLUCOCEREBROSIDASE GBA2: A NEW PROMISING TARGET TO REDUCE CYSTIC FIBROSIS LUNG INFLAMMATION**

Domitilla Schiumarini\(^1\)*, Nicoletta Loberto\(^1\), Paola Brocca\(^1\), Silvia Munari\(^2\), Giulia Mancini\(^1\), Rosaria Bassi\(^1\), Maria Cristina Dechecchi\(^2\), Sandro Sonnino\(^1\), Massimo Aureli\(^1\)

\(^1\) Dep. Medical Biotechnology and Translational Medicine, University of Milano, Italy
\(^2\) Laboratory of Molecular Pathology, University Hospital of Verona, Italy

*e-mail: domitilla.schiumerini@unimi.it*

Cystic fibrosis (CF) is characterized by progressive chronic infection and inflammation of the airways, which cause the obstructive lung disease in CF patients. Resultant progressive remodeling leads to irreversible damage and fibroses, which is a major cause of mortality in patients. Several evidences highlight the role of sphingolipids (SLs) in CF. Interestingly, an accumulation of ceramide has been demonstrated in lower airway of CF patients. Moreover, the infection of bronchial epithelial cells with Pseudomonas aeruginosa (PAO) activated host acid sphingomyelinase leading to ceramide generation at cell surface. This ceramide regulates the release of inflammatory cytokines, such as IL-8. Ceramide production at the plasma membrane level could be triggered also by the action of the non-lysosomal glycohydrolase β-glucosylceramidase GBA2. Here, we report the importance of GBA2 in regulating the pro-inflammatory state of CF cells as well as the inflammatory response after PAO infection. In particular, we examined the impact of lowering the expression of GBA2 in CF cells exposed to PAO by siRNA and we found that the IL-8 expression is reduced in both uninfected and infected cells.

For these experiments, we developed nanoparticles (NP) for the systemic delivery of siRNA targeting GBA2 as a possible promising new anti-inflammatory therapy for CF lung disease.

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ROLE OF ACIDIFICATION IN THE MODULATION OF NEURONAL GANGLIOSIDE PATTERN

Francesca Testa*, Maura Samarani, Giulia Mancini, Massimo Aureli, Simona Prioni, Alessandro Prinetti, Sandro Sonnino

1Dipartimento di Biotecnologie Mediche e Medicina Traslazionale, Università degli Studi di Milano, Via Fratelli Cervi 93, 20090, Segrate (Mi).
Tel: 02 50330380 Fax: +39 0250330365
*e-mail: francesca.testa1@unimi.it

The glycosphingolipids (GLSs) pattern expressed on the cell surface is the result of intracellular metabolic events; however, some enzymes involved in these processes, particularly glycohydrolases characterized by an acidic optimum pH for their activity, are also present in PM (1). The regulation of intracellular pH implies the extrusion of protons, from the cytosol to the extracellular space, achieved in neurons by the Na+/H+ exchangers (NHEs) isoforms 1 and 5 (2, 3). Since the PM-glycohydrolases and the proton extruders are both enriched at the cell surface within lipid rafts; regulated activation of the proton pumps in specific membrane microdomains could be an effective way to activate specific PM-glycohydrolases, thus affecting the local membrane GLSs composition.

We assessed the expression pattern of NHEs in different cells by western blot and PCR analysis. Moreover, we measured the pH at the cell surface and the enzymatic activities, in cell homogenate and at the cell surface, after treatment with NHEs specific inhibitors. Then, we performed metabolic labeling experiments followed by a HPTLC analysis of the total lipid extracted from the cells, put under hypoxic conditions to induce PM environment acidification. Further, we evaluated the possible formation of GD1b-lactone, using a photoactivable ganglioside.

We focused on the possible role of PM-glycohydrolases as modulators of PM-GLSs composition in human fibroblasts, glioma and neuroblastoma cells. We verified the presence of the glycohydrolases on the cell surface, the association of their activity at the PM and that all of them work best at acidic pH. We also demonstrated that the inhibition of the NHEs induced a reduction of the PM-associated activity of the enzymes involved in GLSs metabolism.
The acidification of the PM could play a role in the formation of GD1b-lactone and this project could help us to understand which may be the mechanism underlying this process.

References
SELF-ASSEMBLY OF SHORT RNA/DNA OLIGOMERS AS AUTOCATALYTIC PATHWAY FOR THE ORIGIN OF LIFE

Todisco, M¹*; Fraccia, T. P¹,3; Smith, G². P.; Zanchetta¹, G.; Clark, N. A², Bellini, T¹

¹Biotechnology and Translational Medicine Dept. Università degli Studi di Milano, IT; ²Soft Material Research Center, Physics Dept. University of Colorado, Boulder (CO), USA; ³Promotion of Human Sciences and Life Quality Dept., Università San Raffaele, Roma, IT
*e-mail: marco.todisco@unimi.it

In a series of recent works, it has been shown that short DNA oligos (down to 4nt long) can spontaneously segregate from molecular pools and organize into liquid crystals domains, in which molecules are still fluid but highly organized in ordered columns. In such domains, DNA has a greatly enhanced yield of non-enzymatic polymerization, meaning that the proximity between DNA terminals, held in contact by the liquid crystalline order enhances the chance of chemical ligation. Liquid crystals thus favor the abiotic elongation of DNA chains.

Given the relevance of this finding in the context of the first appearance of biopolymers on the prebiotic Earth, (i) we tested whether these phenomena of order and catalysis could also be verified in solutions of oligomers of RNA,
a molecule considered more ancient than DNA, and (ii) we investigated what is the minimum oligomer length required for the spontaneous formation of liquid crystalline order. We have thus studied the self-assembly in solutions of 12nt and 6nt long RNA oligomers and found that their self-assemble is easier than for DNA, and confirmed that the liquid crystalline supramolecular order enhances the ligation efficiencies: in the presence of condensing agents, RNA oligomers in liquid crystalline environment bind into polymers much longer than obtained in unstructured solutions. Moreover, liquid crystal order prevents the formation of circular chains. By studying the self-assembly of nucleotides in various conditions of charge and pH, we found that even single nucleobases with a triphosphate group can form liquid crystal structures. Nucleosides triphosphate are indeed able to base-pair according to the Watson-Crick role and stack onto each other forming linear aggregates that in turn organize into liquid crystal solutions when sufficiently concentrated. Altogether, these data suggest the possibility of a prebiotic scenario in which spontaneous self-assembly of single activated nucleotides could have led to the formation of a pool of long RNA polymers. This process is mediated by the liquid crystal ordering that in turn is stabilized in the presence of longer molecules, giving rise to a positive feedback loop the could have eventually led to the RNA-world.

Keywords: origin of life, self-assembly, RNA-world
FATTY ACIDS RESCUE IN VITRO ANGIGENESIS IN HUMAN ENDOTHELIAL CELLS CULTURED IN HORMONE-DEPRIVED MEDIUM

C. Vanetti*, F. Bifari, L. M. Vicentini and M. G. Cattaneo

Dept of Medical Biotechnology and Translational Medicine, University of Milan
*e-mail: claudia.vanetti@unimi.it

Hormone-deprived serum (charcoal-stripped serum, CSS) is a well-accepted method to model effects of sex hormones in cell cultures. We have recently shown that CSS decreases human endothelial cell (EC) growth and in vitro angiogenesis even when estrogen concentration is restored\(^1\). Here, by independently studying male and female ECs (M-ECs and F-ECs, respectively), we found that CSS inhibited growth and angiogenesis in cells from both sexes, with a more pronounced effect on M-ECs. Reconstitution of CSS with 17-β estradiol, dihydrotestosterone, or the lipophilic thyroid hormone - all depleted in CSS-containing medium - did not prevent the CSS-induced M-EC and F-EC inhibition.

Since essential metabolites other than hormones are lost in CSS, we focused our attention on fatty acids (FAs) that are fully depleted in CSS. We found that supplementation with the FA palmitic acid or the acetyl-CoA precursor acetate significantly rescued the CSS-induced inhibition of growth and sprouting in ECs of both sexes.

In conclusion, our results show that the loss of metabolic precursors (e.g., FAs) rather than hormones is involved in the impairment of in vitro proliferative and angiogenic properties of M-ECs and F-ECs cultured with CSS. Consequently, in vitro biological effects of hormones (commonly studied in CSS-containing media) might be affected by the concurrent loss of essential metabolites, thus resulting in some metabolic rather than endocrine responses. For that reason, it is important to have knowledge of the undervalued contribute that CSS may give to experimental outcomes from in vitro experiments and to their interpretation.

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