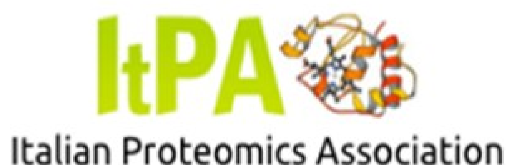

XVII International Italian Proteomics Association Annual Meeting
in partnership with the Hellenic Proteomics Society and Serbian Proteomics Association

Proteomics and Metabolomics towards Global Health

Ospedale Isola Tiberina – Gemelli Isola,
ROMA, ITALY
November 29th -December 1st, 2023

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GENERAL INFORMATION

CONFERENCE REGISTRATION FEES

Before October 13th, 2023: ItPA/EuPA member: 170 euroYPI (< 7years /PhD) ItPA/EuPA member: 150euro- After October 13th , 2023: 200 euro

CONGRESS VENUE
Ospedale Isola Tiberina – Gemelli Isola, Via di Ponte Quattro Capi, 39, Roma, Italy

OFFICIAL LANGUAGE
The Congress official language will be English

POSTER PRESENTATIONS AND AWARDS
Posters will be displayed from 30th November to 1st December 2023. During poster sessions the presence of one of the authors is required. Presentations from young corresponding authors will be candidate for poster prize competition. Awards will be supported by European Proteomics associations (EuPA), Waters S.p.A Global Services and Fondazione ItPA Onlus.

CERTIFICATE OF ATTENDANCE
Certificates of attendance and payment fee receipts will be available at the registration desk.

COFFEE BREAKS AND LUNCHES
Welcome cocktail, coffee breaks and lunches will beserved at the venue.

CONFIRMED INVITED SPEAKERS

JEAN ARMENGAUD, Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS), (Bagnols-sur-Cèze, France)

TANJA CIRKOVIC VELICKOVIC, University of Belgrade, Belgrade, Serbia

HEEYOUN HWANG, Korean Basic Science Institute, (Cheongju-si, South Corea)

***JANNE LETHIO*,** Science for Life Laboratory and Department of Oncology and Pathology, Karolinska Institutet, Stockholm, Sweden.

STEFAN TENZER, Institute of Immunology, University Medical Center University Mainz, Germany

PROGRAM AT A GLANCE

WEDNESDAY 29TH NOVEMBER, 2023	
13:00-18:30	**CONGRESS REGISTRATION**
15:00-15:30	**WELCOME AND OPENING SESSION** **Viviana Greco (Roma, IT), Paola Roncada (Catanzaro, IT), George Tsangaris (Athens, EL), Tatjana Simic (Belgrade, SRB)**
SESSION 1 **Chair: Maria Monti (Napoli, IT), Janne Lethio (Stockholm, SWE)**	
15:30-16:30	**PLENARY LECTURE** ***Janne Lethio,*** *Science for Life Laboratory and Department of Oncology and Pathology, Karolinska Institutet, (Stockholm, Sweden).* ***"Can proteogenomics solve oncologist urgent problems to select combination therapies?"***
16:30-16:50	*Tiziana Bachetti (Genova, IT)* "***Multi-omics analyses reveal altered lipid metabolism and oxidative stress as pathogenic mechanisms in a zebrafish model of Alexander disease"***
16:50-17:10	*Alessandra Morelli (Milano, IT)* "***A Multi-Proteomic Approach to Unravel New Players in Metastatic Melanoma Progression"***
17:10-17:30	*Giulia Robusti (Milano, IT)* "***A Multi-Omics Approach To Dissect Aberrant Epigenetic Mechanisms In Triple Negative Breast Cancer"***
17:30-19:00	**WELCOME COCKTAIL**

THURSDAY NOVEMBER 30TH, 2023- MORNING	
SESSION 2 **Chair: Lorenza Putignani (Roma, IT), Damiana Pieragostino (Chieti, IT)**	
09:00– 09:45	**PLENARY LECTURE**

	Jean Armengaud, *Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS), (Bagnols-sur-Cèze, France)*"***Metaproteomics for the functional analysis of microbiomes: recent achievements & prospects***"
09:45–10:05	*Stefano Levi Mortera (Roma, IT)* "***Metaproteomic investigation on the induced changes of a high fiber, low fat diet intervention in the gut microbiota of patients with Crohn's disease***"
10:05-10:25	*Bruno Tilocca (Catanzaro, IT)* "***Exploring bee metaproteome gather insights on infectious diseases in a One-Health fashion.***"
10:30– 11:00	**COFFEE BREAK AND POSTER EXHIBITION**
11:00-11:45	**INVITED SPEAKER** ***Tanja Cirkovic Velickovic,*** *University of Belgrade, (Belgrade, Serbia)*"***Proteomic insight into allergenic food corona on polyethylene terephthalate microplastics***"
11:45– 12:05	*Aldo Lanzoni (Catania, IT)* "***Proteomic analysis of an Italian Chickpea Genotype by MS-methods***"
12:05 –12:25	*Lavinia Santucci (Roma, IT)* "***Metabolomics evaluation of the effects of Beetroot Juice supplementation in Long Covid patients***"
12:30– 14:00	**LIGHT LUNCH AND POSTER EXHIBITION**
THURSDAY NOVEMBER 30TH , 2023- AFTERNOON	
SESSION 3 **Chair: Alessio Soggiu (Milano, IT), Stefan Tenzer (Mainz, DE)**	
14:00– 14:45	**PLENARY LECTURE** ***Stefan Tenzer****, Institute of Immunology, University Medical Center (Mainz, Germany)* "***Maximizing information content in data-independent acquisition using midia-PASEF***"
14:45-15:05	*Francesca Sacco (Roma, IT)* "***Integrating mass spectrometry-based (phospho)proteomics with prior knowledge networks reveals disease-specific mechanisms.***"
15:05-15:25	*Andrea Graziadei (Milano, IT)* "***Structural systems biology by crosslinking mass spectrometry and deep earning***"

15:25-15:45	*Francesca Pirozzi (Napoli, IT) "**Targeted lipidomics on Glycogen Storage Disease Type Ia (GSDIa) patients' blood samples as new tool for dyslipidemia investigation in GSDIa**"*
15:45-16:30	**COFFEE BREAK AND POSTER EXHIBITION**
16:30-16:50	*Laura Bianchi (Siena, IT) "**New insights into metastatic melanoma exomeres**"*
16:50-17:10	*Maria Concetta Cufaro (Chieti, IT) "**New Proteomics insights in the characterization of Extracellular Vesicle single-phenotype: the role of Leukocyte-derived EVs in Multiple Sclerosis**"*
17:10-17:30	*Annalisa Castagna (Verona, IT) "**Characterization of asthma phenotypes by serum cytokines and extracellular vesicles surface markers analysis**"*
17:30-18:30	**ItPA GENERAL ASSEMBLY**

FRIDAY DECEMBER 1ST, 2023	
SESSION 4 **Chair: Tiziana Alberio (Varese, IT), Heeyoun Hwang (Cheongju-si, South Korea)**	
09:00- 09:45	**PLENARY LECTURE** ***Heeyoun Hwang,*** *Korean Basic Science Institute, (Cheongju-si, South Korea)* ***"Chromosome-centric proteomics study of Cholagiocarcinoma"***
9:45- 10:05	*Marta Lualdi (Varese, IT) "**Dysfunctional microglia in the pathogenesis of Alzheimer Disease: proteome features of the pro-AD phenotype**"*
10:05- 10:25	*Ilaria Iacobucci (Napoli, IT) "**Proteomics profiling of cortical and hippocampal brain regions in dairy cattle**"*
10:25- 10:45	*Giuseppina Catanzaro (Roma, IT) "**CD133 proteolytic processing following brefeldin treatment in medulloblastoma cancer stem cells**"*
10:45- 11:05	*Paolo Grumati (Napoli, IT) "**FAM134A,B,C: The Three Musketeers of ER-phagy**"*
11:05– 11:45	**COFFEE BREAK AND POSTER EXHIBITION**
11:45– 13:00	**ITPA, EUPA AND WATERS CEREMONY AWARDS - CONCLUDING REMARKS** **Paola Roncada (Catanzaro, IT), Viviana Greco (Roma, IT)**

13:00 – 14:00	LIGHT LUNCH- CONGRESS CLOSING

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ABSTRACT VOLUME

PLENARY LECTURES

PL-01

Metaproteomics for the functional analysis of microbiomes: recent achievements & prospects

Jean Armengaud[a]

[a] Laboratory «Innovative technologies for Detection and Diagnostics»,
ProGénoMix IBISA platform
CEA, Bagnols-sur-Cèze, France

Microorganisms play a major role in human, animal and vegetal health, as well as in key terrestrial geochemical processes. To understand how microbial communities function, how they interact with the host and the risks of dysbiosis, we need to identify the diversity of microorganisms present and quantify the numerous molecular players at work, especially proteins which are the workhorses of biological systems. Metaproteomics is a key technology for such functional studies, as it can provide important answers for these highly complex samples: 1) identification of the taxa present, 2) quantification of the biomass of these organisms, 3) identification of the proteins of these taxa and their function, 4) identification of host biomarkers explaining their global response, and 5) overall functional representation of the biological system. The first important key step in metaproteomics is the selection of the database best suited to the specificities of the sample. Proteotyping of organisms enables us to establish the identity of micro-organisms and their corresponding biomass. Several examples will be presented to demonstrate the power of proteotyping. The challenges of metaproteomics in terms of mass spectrometry, interpretation and exploitation of results will be discussed addressed. Moreover, results from recent technologies such as the Astral tandem mass spectrometer will be presented to demonstrate the new power of next generation metaproteomics for deep functional analysis of microbiomes.

PL-02

Proteomic insight into allergenic food corona on polyethylene terephthalate microplastics

Tamara Lujic[a], Nikola Gligorijevic[a,b], Vesna Jovanovic[a], Jelena Acimovic[a], Dragana Mitic[c], Tamara Vasovic[a], Marija Stojadinovic[a], Dragana Stanic-Vucinic[a] and Tanja Cirkovic Velickovic[a,d,*]

[a] Center of Excellence for Molecular Food Sciences, Department of Biochemistry, University of Belgrade – Faculty of Chemistry, Belgrade, Serbia
[b] Department of Chemistry, University of Belgrade – Institute of Chemistry, Technology and Metallurgy, National Institute of Republic of Serbia, Belgrade, Serbia
[c] Microprot Lab, Innovation Center of the Faculty of Chemistry, Ltd., Belgrade, Serbia
[d] Serbian Academy of Sciences and Arts, Belgrade, Serbia

Microplastics is abundant in the environment, food and beverages and get ingested by humans. Its complex interplay with proteins lead to formation of corona. Tightly bound proteins represent hard corona, while weaker binding partners are found in soft corona. Separation of hard and soft corona of allergenic proteins of shrimps, eggs and cow's milk, tropomyosin (TPM), ovalbumin (OVA) and beta-lactoglobulin (BLG) and identification of binding partners by proteomics was aim of our study.
Allergenic proteins were purified from egg white, shrimps and cow's milk. Binding to polyethylene terephthalate microplastics (PET) (70-100 μm) was probed at pH 7 for purified allergens and egg white proteins. After establishment of binding equilibrium, soft and hard corona were separated and analyzed by SDS PAGE, followed by identification of bound proteins by nanoLC-HRMS. Binding of all allergenic proteins was observed in both soft and hard corona. Soft corona contains exclusively intact, full length OVA, TPM and BLG. Hard corona is enriched for truncated OVA and oligomers of TPM. OVA fragments are partially or fully enfolded and have higher level of exposed hydrophobic patches resulting in higher affinity for PET microplastics. In comparison to OVA and TPM, hard corona of BLG is less abundant under similar conditions. BLG is compact globular protein with lower level of exposed hydrophobic patches in comparison to ovalbumin and tropomyosin. In hard corona, trace amounts of contaminating alfa-lactalbumin become enriched. In the presence of egg white protein extract OVA forms both SC and HC on microplastics, being the dominant protein of hard corona (with ovotransferrin). Lysozyme and ovomucin are present only in hard corona. Both proteins are known for their strong bioactivity and represent a small fraction of total egg white proteins.
Our results show that allergenic proteins form hard corona on PET microplastics. Among egg white proteins, minor proteins such as lysozyme and ovomucin become enriched. Denaturing effect of strong binding to microplastics may change functional characteristics of allergens and bioactive proteins of foods and should be further investigated in functional assays.
Acknowledgment: This study was supported by IMPTOX European Union's Horizon 2020 research and innovation program (grant number 965173).

Corresponding author:
Tanja Cirkovic Velickovic.
University of Belgrade – Faculty of Chemistry, Belgrade, Serbia.
e-mail address: tcirkov@chem.bg.ac.rs (T. Cirkovic Velickovic.)

PL-03

Chromosome-Centric Proteomics Study of Cholangiocarcinoma

Heeyoun Hwang[a]

[a] Research Center for Bioconvergence Analysis, Korea Basic Science Institute, Rep. of Korea

In order to explore the biological complexity of humans and promote research for a deeper understanding of human health and diseases, Chromosome-centeric human proteome project (C-HPP) establish a fundamental basis for understanding human biological phenomena through the experimental data of proteome on human chromosomal information. To achieve a deep understanding of human biology of Intrahepatic cholangiocarcinomas (iCCs) which is characterized by their rarity, difficult diagnosis, and overall poor prognosis, we performed comprehensive genomic, transcriptomic, and proteomic analyses on treatment-naïve tumor samples from 102 patients with iCC who underwent surgical resection with curative intent. Three clinically supported subtypes (stem-like, poorly immunogenic, and metabolism) were identified. NCT-501 (aldehyde dehydrogenase 1 family member A1 [ALDH1A1] inhibitor) exhibited synergism with nanoparticle albumin-bound–paclitaxel in the organoid model for the stem-like subtype. The oncometabolite dysregulations were associated with different clinical outcomes in the stem-like and metabolism subtypes.

The poorly immunogenic subtype harbors the non–T-cell tumor infiltration. Integrated multiomics analysis not only reproduced the 3 subtypes but also showed heterogeneity in iCC. In addition, we annotate uncharacterized function of C11orf52 and PROSER2 using the bioinformatics prediction, affinity purification and mass spectrometry (AP-MS) Based on this study, we expect that C11orf52 is related with Wnt signaling pathway via DSG1 from the protein-protein interactions (PPIs) and PROSER2 is an antagonizer tumor progression via the STK25-AMPK pathway in PDAC. The large-scale proteogenomic analysis provides information beyond that obtained with genomic analysis, allowing the functional impact of genomic alterations to be discerned. These findings may assist in the stratification of patients with iCC and in developing rational therapeutic strategies.

PL-04
Connecting cancer genotype with molecular phenotype by proteogenomics

Janne Lehtiö[a]

[a] Karolinska Institutet and Science for Life Laboratory, Stockholm, Sweden

The explosion of genomics data has improved our understanding of cancer greatly in recent years. However, the systems level knowledge on how genomic aberrations affect the functional proteome is still very limited, hindering effective selection of anti-cancer drug combinations in precision medicine. Proteome data represents the combined effect of epigenetic, transcriptional and translational regulation and will therefore provide an important molecular phenotype data layer for multi-omics analysis. To allow effective systems biology analysis including proteomics, we have generated tools that take advantage of massive genomics and transcriptomics data by incorporating sequence information to the proteomics data-analysis pipeline. This will allow protein level analysis of gene variants as well as detection of novel protein coding regions. In cancer, genomics instability creates so called neoantigen when non-canonical proteins and peptides are translated. Our proteogenomics methods offers a view into tumor neoantigen space by controlling error rate in variant peptide detection by combining experimental isoelectric point data from peptide fractions (HiRIEF LC-MS/MS) and bioinformatics approaches into the proteogenomics workflow (IPAW). In same analysis, in-depth quantitative proteome data on tumor tissue provides information both on cancer driving pathways and molecular insights in immune evasion mechanisms.
Here we demonstrate the value of proteogenomics driven multi-omics view of cancer by analysis of several lung cancer cohorts. We could define proteome-based lung cancer subtypes using HiRIEF LC-MS based in-depth proteomics of 141 cases. Interestingly, these subtypes differ on tumor neoantigen burden and types of neoantigens and this was related to immune evasion mechanisms. A lung adenocarsinoma subtype associated with poor prognosis, immune-cold phenotype, despite high neoantigen burden, was identified. This subtype was enriched in STK11 mutations and we could demonstrate that this activated immune evasion mechanism via expression of FLG1 immune inhibitory ligand.
To allow clinical application, we developed a DIA based classifier for lung cancer subtyping. The classifier was tested in two additional tumor cohort, namely in a similar early-stage lung cancer cohort of 208 cases and using a late-stage biopsy cohort of 80 samples.

Janne Lehtiö, Professor,
Dep. Of Oncology and Pathology, Karolinska Institutet and Science for Life Laboratory, Stockholm, Sweden. Email: janne.lehtio@ki.se

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1. **Lehtiö J***, Arslan T, Siavelis I, Pan Y, Socciarelli F , Berkovska O, Umer HM, Mermelekas G, Pirmoradian M, Jönsson M, Brunnström H, Brustugun OT, Pinganksha Purohit K , Cunningham R, Foroughi H, Isaksson S, Arbajian E, Aine M, Karlsson A, Kotevska M, Hansen CG, Drageset Haakensen V, Helland Å, Tamborero D, Johansson HJ, Branca RMM, Planck M, Staaf J, Orre LM. Proteogenomics of non-small cell lung cancer reveals molecular subtypes associated with specific therapeutic targets and immune evasion mechanisms. *Nature Cancer*, **2021** Nov;2(11):1224-1242.
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PL-05

Maximizing information content in data-independent acquistion using midia-PASEF

Ute Distler[1]; Matteusz Krystof Lacki[1]; Michał Piotr Startek[1]; David Teschner[2]; Sven Brehmer[3]; Jens Decker[3]; Thilo Schild[1]; Jonathan Krieger[4]; Florian Krohs[3]; Oliver Raether[3]; Andreas Hildebrandt[2]; Stefan Tenzer[1, 5*]

[1]Institute for Immunology, University Medical Center of the Johannes-Gutenberg University Mainz, Mainz, Germany; [2]Institute for Informatics, Johannes-Gutenberg University Mainz, Mainz, Germany; [3]Bruker Daltonics GmbH & Co.KG, Bremen, Germany; [4]Bruker Ltd, Milton, ON; [5]Helmholtz Institute for Translational Oncology (HI-TRON), Mainz, Germany

midia-PASEF, a novel DIA scan mode uses mobility-specific scanning of overlapping quadrupole windows to optimally cover the ion population in the ion mobility-mass to charge plane. midia-PASEF provides a 2.5-fold increase in fragment ion sensitivity compared to dia-PASEF reference runs, while covering the entire precursor ion mass range without sacrificing cycle time. midia-PASEF thereby maximizes information content in DIA acquisitions which enables the determination of the precursor m/z of each fragment ion with a precision of less than 2 Th. To process the resulting high-complexity datasets, we developed the Snakemake-based midiaID pipeline. midiaID integrates algorithms for multidimensional peak detection and for machine-learning-based classification of precursor-fragment relationships, which are stored in the format of a bipartite graph. Using machine learning approaches to refine the MIDIA graph based on initial database results, we significantly improved the specificity of precursor-fragment relationships, thereby surpassing the spectral purity of DDA in our deconvoluted MIDIA-MSMS spectra as indicated by database search score distributions. Our midiaID pipeline enables fully automated processing and multidimensional deconvolution of midia-PASEF files and exports highly specific DDA-like MSMS spectra with a fragment ion mass accuracy below 10 ppm. In contrast to DDA, midiaPASEF is non-stochastic and thus generates detailed detection profiles of each fragment ion in all dimensions, which facilitates the highly specific deconvolution and scoring of precursor-fragment relationships.
Resulting deconvoluted MIDIA-MSMS spectra are exported as .mgf files which are suitable for *de novo* sequencing and can be searched directly with established tools including PEAKS, FragPipe and Mascot. In short gradients, midiaPASEF acquisition identifies over 100 unique peptides per second and provides powerful library-free DIA analyses including phosphopeptidome and immunopeptidome samples. In addition, midia-PASEF provides all benefits of DIA acquisitions, including efficient ion sampling, high duty cycle and excellent reproducibility[1].

* *Corresponding author*:
Stefan Tenzer.
University Medical Center Mainz, Helmholtz-Institute for Translational Oncology (HI-TRON)
Mainz, Germany
Tel.: +49 6131 17-6199;
fax: +49 6131 17-6202.
E-mail address: tenzer@uni-mainz.de (S. Tenzer)

References:

[1] Distler et al. BioRxiv, https://doi.org/10.1101/2023.01.30.526204

SELECTED ORAL COMMUNICATIONS

O-01 Multi-omics analyses reveal altered lipid metabolism and oxidative stress as pathogenic mechanisms in a zebrafish model of Alexander disease

Deianira Bellitto[a], Simona Candiani[a], Matteo Bozzo[a], Silvia Ravera[b], Nadia Bertola[b], Francesca Rosamilia[c], Paola Barboro[d], Jessica Milla[d], Gabriela Coronel Vargas[e], Erika Iervasi[e], Kateryna Tkachenko[e], Marco Ponassi[e], Aldo Profumo[e], Paolo Romano[e], Camillo Rosano[e], Tiziana Bachetti[e*]

[a] University of Genoa, DISTAV-Department of Earth, Environment and Life Sciences, Genoa, Italy
[b] University of Genoa, Experimental Medicine Department, Genoa, Italy
[c] University of Genoa, DISSAL-Health Science Department- Biostatistic Unit, Genoa, Italy
[d] Centro di Ricerca, Sviluppo e Studi Superiori in Sardegna (CRS4), Pula, Italy
[e] IRCCS Ospedale Policlinico San Martino, Genoa, Italy

Alexander Disease (AxD) is a very rare autosomic dominant leukodystrophy affecting astrocytes, characterized by white matter degeneration and thus neurodegeneration. The genetic cause of AxD relies on heterozygous mutations in the *GFAP* gene, encoding for the Type III Intermediate Filaments of astrocytes Glial Fibrillary Acidic Protein. *GFAP* mutations induce formation of inclusions (Rosenthal fibers, RFs) which accumulate into astrocytes' cytoplasm, processes and around blood vessels. The cellular consequences are the impairment of the proteasome function and the activation of autophagy as cellular response to counteract the aggregation. However, due to the complexity of interaction between cells in the central nervous system, no exhaustive investigations have been able to fully disclose the pathogenetic mechanisms of the disease so far. In this work, we describe the first "omics" studies performed on an entire animal model of AxD, represented by zebrafish (*Danio Rerio*), a small teleost largely employed in studies on human neurodegenerative diseases. In detail, we have used the Tol2 system to create a reporter transgenic line carrying the human *GFAP* p.R239C mutation (p.hR239C-GFP) and a reporter transgenic line expressing only the GFP (GFAP promoter-GFP), both driven only in glial cells by the zebrafish *gfap* promoter.
First, by fluorescence microscopy analysis, we have observed that the mutant embryos showed many intracellular GFAP aggregates, well resembling RFs. Moreover, *omics* approaches based on differential transcriptomics- and proteomics-based comparison between the two zebrafish lines at 10dpf and 20dpf revealed that mutant line showed enrichment in the immune response and in involvement of metabolic processes, especially fatty acid metabolism. In particular, at 20dpf, the proteomic analysis confirmed most pathways emerging at 10dpf and, in addition, showed enrichment in myelination and angiogenesis, two hallmark processes in AxD. Validation of results showed that that in the mutant line there was a higher free radical production and fatty acids peroxidation with respect to the control, in addition to enhanced lipid synthesis in line with the increased lipid peroxidation. All together, these results confirm the reliability of our AxD zebrafish model and open to further investigations about the role of metabolic processes in AxD.

**Corresponding author*
Dr Tiziana Bachetti, PhD, Dirigente Biologo
UO Proteomica e Spettrometria di Massa IRCCS Ospedale Policlinico San Martino
Largo R. Benzi, 10, 16132 Genova, Italy
Phone 010-5558306
Email: tiziana.bachetti@hsanmartino.it (T. Bachetti)

References

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O-02

New insights into metastatic melanoma exomeres

Felice Maria Accattatis[a], Alfonso Carleo[b], Rossana De Salvo[b], Monica Rodolfo[d], Elisabetta Vergani[d], Paolo Bergese[e], Miriam Romano[e], Marco Brucale[f], Francesco Valle[f], Alberto Corsini[c], Luca Bini[b], Stefano Bellosta[c], Lorenzo Arnaboldi[c], Laura Bianchi[b*]

[a] University of Calabria, Cosenza, Italy;
[b] University of Siena, Siena, Italy;
[c] University of Milano, Milano, Italy;
[d] IRCCS Istituto Nazionale Tumori, Milan, Italy;
[e] DMMT, University of Brescia, Brescia, Italy;
[f] CNR Bologna, Italy.

Extracellular vesicles (EVs) are heterogeneous particles with a lipid bilayer structure that are secreted by various types of cells. Their cargo mediates biological interactions between the releasing cells and the EV targets. Among their several functions, EVs are known to influence disease development and progression, including cancer. Beside promoting proliferation, migration, dissemination, and immune elusion, they have been also suggested to pave the way to metastatic lesions by affecting premetastatic environments and by destroying mechanical defenses, such as the blood-brain barrier.
By applying a specifically developed centrifugation-based separation method, we separated EVs secreted by a metastatic melanoma cell line into five different subpopulations, according to their theoretical size. EVs from different fractions were proved presenting different size, protein cargo, and fatty acid composition, which suggested different possible tropism, rigidity and cargo exchange properties. Their protein cargo MS-based profiling also indicated the population with the smallest diameter values (<50 nm), exomeres, having unique properties and functions. Intriguingly, many of the proteins we identified as unique or significantly enriched in the latter (*e.g.* APP, ATM, SORLA, USP9X) are involved in the development of the nervous system, in its physiology as well as in neurodegenerative disorders, in the onset/progression of melanoma, in patients' overall survival, and in immuno(suppression) and inflammatory responses. Of relevance, more than the 30% of the unique proteins identified exclusively in exomeres and those more abundant in these nanoparticles resulted directly correlated in a functional predictive analysis we performed, thus suggesting their involvement in consecutive steps of specific biochemical processes and molecular pathways.
Since more than 50% of melanoma patients develop brain metastasis during the course of the disease, melanoma exomeres may represent a precious source of biomarkers to unveil the brain metastatic process of this cancer. The selective loading of the above mentioned factors into exomeres could be a process used by melanoma in preparing brain tissue to become receptive for metastatic cells, in increasing colonized tissue tolerance for metastatic cells, and their aggressiveness.

* *Corresponding author*:
Laura Bianchi.
University of Siena, Via A. Moro, 2, Siena, Italy.
Tel.: +39 0577232059;
fax: +39 0577234476.
E-mail address: laura.bianchi@unisi.it (L. Bianchi)

O-03

Characterization of asthma phenotypes by serum cytokines and extracellular vesicles surface markers analysis

Annalisa Castagna[a*], Naila Arif Cheema[a], Francesca Ambrosani[a], Giuseppe Argentino[a], Ruggero Beri[a], Bianca Olivieri[a], Matteo Maule[a], Marco Caminati[a], Simonetta Friso[a].

[a] Department of Medicine, University of Verona, Italy;

Asthma is a common, chronic, inflammatory, respiratory pathological condition characterized by reversible obstruction of airflow, inflammation of the airways and bronchial hyperreactivity. It is usually related to allergic reaction or other forms of hypersensitivity and is further characterized by comorbidities and different response to treatments. The severity of asthma varies and becomes more complex in the presence of other respiratory co-occurring morbidities such as: nasal polyps (NP), eosinophilic granulomatosis with polyangiitis (EGPA), Aspirin sensitive (ASA).
Extracellular vesicles (EVs) are membranous nanoparticles, secreted by all cell types, known to play important roles in the mechanisms involved in the pathophysiology of many diseases including respiratory diseases.
The present study was aimed at identifying the differences in terms of molecular characterization between different subpopulations of patients affected by: 1) asthma; 2) asthma with nasal polyps (NP); 3) asthma with NP and ASA; 4) underlying vasculitis (EGPA) 5) NP 6) Hyper-eosinophilia. Serum samples and extracellular vesicles were analysed. EVs were isolated from sera by ultracentrifugation, then characterized and analyzed in terms of concentration and size by Nanotrack (NTA) and Transmission electron microscopy (TEM). Exosomal surface epitopes were analyzed by MACSPlex Exosome Kit, by flow cytometry. Serum cytokines were measured: TGFβ1, IL-4, IL-5, IL-6, IL-10, IL-17A, IL-33, IL-13, IL-25 and Eotaxin 3, using ELISA assays.
Patients were evaluated at enrolment and at follow up (T=6 months and 12 months) to investigate possible alteration due to biologics treatment.
The results showed that EVs were all in the range of exosomes and were homogeneous in terms of size and concentrations among groups. Cytokines concentrations showed specific trends in the different experimental groups investigated. EVs surface markers characterization showed peculiar features among groups and it was possible to infer specific cells type as EVs progenitor cells. The preliminary findings presented in this work are consistent with other studies in the field and may prove our hypothesis that the EVs and cytokines could bear a unique set of information in the different experimental populations, giving useful insights into the interplay between inflammation and immunity in the investigated pathologies.

* *Corresponding author*:
Annalisa Castagna
Department of Medicine, University of Verona, Italy.
Tel.: +39 0458128417
fax: +39 0458027473
E-mail address: annalisa.castagna@univr.it (A. Castagna)

O-04

CD133 proteolytic processing following brefeldin treatment in medulloblastoma cancer stem cells

Maurizio Ronci[a], Laura Giusti[d], Zaira Spinello[b], Luana Abballe[c], Evelina Miele[c], Elisabetta Ferretti[b], Giuseppina Catanzaro[b*]

[a] Department of Medical, Oral and Biotechnological Sciences, University G. D'Annunzio of Chieti
[b] Departments of Experimental Medicine Sapienza University of Rome, Rome
[c] Paediatric Haematology/Oncology Department, IRCCS Bambino Gesù Children's Hospital, Rome
[d] School of Pharmacy, University of Camerino

Medulloblastoma (MB) is a malignant brain tumor occurring in children and adults, characterized by elevated resistance to conventional chemotherapy. The current clinical approach consists of multimodal strategies with debilitating long-term effects and risk of tumor recurrence. Medulloblastoma stem cells (MBSCs) which are a fraction of the tumor cell population have been associated with poor prognosis and refractoriness to conventional therapies in several kinds of tumors.
CD133 (Prominin 1, PROM1) is a transmembrane protein whose mRNA and glycosylated forms are highly expressed in many human cancer cell types. CD133 also serves as a cancer stem cell (CSC) marker associated with cancer progression.
We recently found that brefeldin A (BrefA) induces major CD133 modifications in MBSCs. The aim of this study was to characterize and understand the modifications of CD133 induced by BrefA. Through state-of-the-art mass spectrometry, we provide structural and functional insights into the mechanisms implied in modification of CD133 induced by BrefA.
Human group 3 MB (G3MB) cell lines, D283, were grown in in stem selective medium (B27™) and treated with BrefA for 24h before collection. Cells were immunoprecipitated using a commercially available anti-CD133 antibody and the bands corresponding to CD133 isoforms were excised, digested with trypsin and analyzed by nanoLC-MS/MS. Raw data were processed using PEAKS XPro.
Our results clearly showed that BrefA treated cells induced the overexpression of an isoform of CD133 that was missing the N-terminus region, cleaved around aa 120. The MS results were confirmed by performing a second IP using a different antibody, targeting the CD133 epitope (aa 41-90).
We report here for the first time, a N-terminus proteolytic processing of CD133, potentially associated to the cell trafficking effects induced by Brefeldin A.

* *Corresponding author*:
Giuseppina Catanzaro, PhD
Department of Experimental Medicine,
Sapienza University of Rome
Viale Regina Elena 324
Rome (Italy)
Tel.: +39 3494917793 ;
E-mail address: giuseppina.catanzaro@uniroma1.it (G. Catanzaro)

O-05

New Proteomics insights in the characterization of Extracellular Vesicle single-phenotype: the role of Leukocyte-derived EVs in Multiple Sclerosis

Maria Concetta Cufaro[a] *, Ilaria Cicalini[a], Marianna Gabriella Rispoli[b], Giulia Catitti[a], Paola Lanuti[a], Valentina Tomassini[b], Damiana Pieragostino[a] and Piero Del Boccio[a]

[a]Analytical Biochemistry and Proteomics and Cytomorphology Laboratory, Center for Advanced Studies and Technology (CAST), University "G. d'Annunzio" of Chieti-Pescara, Chieti, Italy
[b]Department of Neurology, "SS. Annunziata" University Hospital, Chieti, Italy

Extracellular Vesicles (EVs) are small membrane-enclosed particles released by cells which display potent regulatory functions in the whole biological system. EVs can enable extreme reduction of the high dynamic range of detectable protein concentration in whole biofluids, paving the way for the biomarker discovery in the so-called "dark proteome". The best strategy of EVs isolation from biofluids remains an open question for scientific community especially looking at their suitability for proteomics application [1]. Within such a complex and dynamic scenario, we have recently optimized an innovative "FACS-Proteomics" workflow for the isolation and subsequent proteomics characterization of EVs directly from untouched biological fluids taking advantage of a lipophilic cationic dye (LCD) able to probe intact EVs [2,3]. This method offers simultaneously the great possibility of separating cellular specific EVs by sub-typing them with an appropriate panel of antibodies [3]. Here, we provided a successful update of "FACS-Proteomics" application by studing the protein cargo of 140,000 Leukocyte-derived EVs (Leuko EVs, LCD+/CD45+/Phalloidin- events) isolated from tears of Multiple Sclerosis (MuS) patients and Healthy Controls (HC). Our data highlighted a matching between Leuko EV proteins and Vesiclepedia database more than 94%. Leukocyte diapedesis trans-blood brain barrier (BBB) is a crucial immune phenomenon that triggers a subsequent cascade of inflammatory events in MuS pathogenesis and our functional proteomics analysis revealed that this role of leukocyte could be EVs-mediated. In particular, *"migration of endothelial cells"* function is significantly up-regulated via Leuko EVs proteins by *Transforming growth factor beta 1 (TGFB1)* as activated upstream regulator in MuS Leuko EVs. Furthermore, activation of *"angiogenesis"* was predicted as one of the main significant downstream functions in MuS Leuko EVs, according to the complex inflammatory process involving EVs as shuttles in the immune system machinery during neuroinflammation. In conclusion, our "single-vesicles" proteomics approach confirm that Leuko EVs could be considered a useful "liquid biopsy" platform for the assessment of EVs clinical significance even in the lacrimal fluid. In fact, as demonstrated in MuS, tear Leuko EVs proteome reflects pathological events such as neuroinflammation, oxidative stress and pro-angiogenic processes.

* *Corresponding author*:
Maria Concetta Cufaro, PhD
University "G. d'Annunzio" of Chieti-Pescara, Chieti, Italy
Tel.: +39 0871 541596
E-mail address: maria.cufaro@unich.it (M. Cufaro)

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O-06

Structural systems biology by crosslinking mass spectrometry and deep learning

Andrea Graziadei[a*], Francis O'Reilly[b], Christian Forbrig[c], Kolja Stahl[c], Oliver Brock[c], Juri Rappsilber[c]

[a] Fondazione Human Technopole, Milan, Italy
[b] Center for Cancer Research, National Institues of Health, Frederick, MD, USA
[c]Technische Universitaet Berlin, Berlin, Germany

Accurately modeling the structures of proteins and their complexes using machine learning is revolutionizing molecular biology. Experimental data enable a candidate-based approach to systematically model novel protein assemblies. We use a combination of in-cell crosslinking mass spectrometry and co-fractionation mass spectrometry (CoFrac-MS) and AlphaFold2 to identify and model protein–protein interactions in the model Gram-positive bacterium *Bacillus subtilis*. Crosslinking MS data independently validates AlphaFold predictions and scoring. We report and validate novel interactors of central cellular machineries that include the ribosome, RNA polymerase, and pyruvate dehydrogenase, assigning function to several uncharacterized protein[1]. We also introduce AlphaLink, a modified version of the AlphaFold2 algorithm that incorporates experimental distance restraint information into its network architecture. By employing sparse experimental contacts as anchor points, AlphaLink improves on the performance of AlphaFold2 in predicting challenging targets. Our approach uncovers protein–protein interactions inside intact cells, provides structural insight into their interaction interfaces, and is applicable to genetically intractable organisms, including pathogenic bacteria.

* *Corresponding author*:
Andrea Graziadei.
Fondazione Human Technopole, Milano, Italy
E-mail address: andrea.graziadei@fht.org (A. Graziadei)

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O-07

FAM134A,B,C: The Three Musketeers of ER-phagy

Viviana Buonomo[a], Lucia Santorelli[a] and Paolo Grumati[a,b*]

[a]Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy.

[b]University Federico II, Naples, Italy.

Selective autophagy represents a quality control mechanism that ensures a proper turn-over of cellular organelles. The endoplasmic reticulum (ER) is fragmented and delivered to the lysosome via a specific autophagy: ER-phagy. Disassembly of the ER network is a requisite to engulf the ER into autophagosomes; while binding to the mATG8s and a functional autophagy are fundamental for the ER-phagy receptors to fulfil their function. FAM134B was the first characterized mammalian ER-phagy receptor and it belongs to a protein family composed of other two members: FAM134A & FAM134C. The three FAM134s share a poor homology; however, the most conserved region is the same LIR domain. FAM134A and FAM134C are both ER resident proteins, bind mATG8s in a LIR dependent manner and are degraded via lysosomes. Therefore, FAM134A and FAM134C can be annotated as ER-phagy receptors. In basal state, they are in a relatively inactive state and require an activation signal to fragment the ER. All three FAM134s are necessary for ER shape maintenance and a proper collagen homeostasis. Overall, FAM134A,B,C share a parallel function as ER-phagy receptors; however, the loss of one of them is not fully counterweighed by the other two. Fam134a but not Fam134c over-expression is able to recover collagen I levels, in *Fam134b* knockout MEFs, indicating a distinct mode of action in a co-receptor complex with Fam134c. Moreover, we provide evidence that Fam134a has a LIR-independent function in maintaining pro-collagen I homeostasis. This pathway works in parallel to Fam134b, which is self-sufficient to drive mis-folded collagen to lysosomes.

**Corresponding Author:*

Paolo Grumati, PhD
Telethon Institute of Genetics and Medicine
Via Campi Flegrei 34, 80078 Pozzuoli (NA)
Phone: +39 081 1923 0688
E-mail address: p.grumati@tigem.it (P. Grumati)

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O-08 Proteomics profiling of cortical and hippocampal brain regions in dairy cattle

Ilaria Iacobucci[a,b*], Flora Cozzolino[a,b], Luisa Canè[a,c], Claudia Gatta[d], Maria Claudia Gatto[a], Luigi Sacchettino[d], Davide De Biase[e], Evaristo Di Napoli[d], Orlando Paciello[d], Luigi Avallone[d], Danila d'Angelo[d], Francesco Napolitano[a,d], Maria Monti[a,b]

[a]CEINGE-Biotecnologie Avanzate "Franco Salvatore", Naples, Italy
[b]Department of Chemical Sciences, University of Naples, Federico II, 80126, Naples, Italy
[c]Department of Translational Medical Sciences, University of Naples Federico II, Naples, Italy
[d]Department of Veterinary Medicine and Animal Production, University of Naples Federico II 80137-Naples, Italy
[e]Department of Pharmacy, University of Salerno, 84084 Fisciano, Italy

Aging is a physiological process occurring in all living organisms. It is characterized by a progressive deterioration of the physiological and cognitive functions of the organism, accompanied by a gradual impairment of mechanisms involved in the regulation of tissue and organ homeostasis, thus exacerbating the risk of developing pathologies, including cancer and neurodegenerative disorders. From a functional perspective, the processes most involved in brain aging would seem to be mitochondrial dysfunction, hormonal changes, excessive production of ROS, reduced plasticity and synaptic activity, and dysregulation of autophagic activity, resulting in the accumulation of misfolded proteins and potentially toxic species for the organism.
In the present work, for the first time, the influence of aging has been investigated in the brain cortex and hippocampus of grazing the Podolica breed cattle with freedom to wander outdoors, and the autonomy over the access to shelter, water consumption and diet selection mostly by grazing on large areas of pastures and natural resources. We used an LC-MS/MS-based differential proteomics and bioinformatic analysis approach with the aim to identify potential aging or longevity markers, also associated with a specific lifestyle. We compared protein expression profiles in bovine brains in three age groups: young (1-2 years), adult (7-12 years), and aged (16-24 years) for the hippocampus and cerebral cortex regions. We identified 130 and 112 differentially expressed proteins (DEPs) in cortex and hippocampus region, respectively. In both cases, more than 50% of the DEPs were downregulated in aged samples. After the enrichment analysis, we found these proteins involved in synaptic plasticity, myelination, dendritic process, and oxidative stress in the cortex of aged bovine; while in myelination, synaptic vesicle, metabolism, and calcium-related biological pathways in the hippocampal dataset. We confirmed the differential expression of a pool of representative markers by targeted proteomics approach (MRM). Overall, our preliminary data pave the way for future studies for a better understanding of molecular mechanisms, underlying brain aging in grazing cattle, that might allow to develop strategies aimed at improving animal welfare and husbandry practices of dairy cattle from intensive livestock.

* *Corresponding author*:
Ilaria Iacobucci
University of Naples, Federico II, 80126, Naples, Italy
Tel: +39 0813737895
E-mail address: ilaria_iacobucci@unina.it (I. Iacobucci)

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O-09

Proteomic analysis of an Italian Chickpea Genotype by MS-methods

A. Lanzoni[a*], A. Di Francesco[a], M. G. G. Pittalà[a], R. Saletti[a], M. De Santis[b], Z. Flagella[b], V. Cunsolo[a]

[a] Laboratory of Organic Mass Spectrometry, Department of Chemical Sciences, University of Catania, Catania, Italy

[b] Department of Agriculture, Food, Natural Resources and Engineering (DAFNE), University of Foggia, Foggia, Italy

Legumes represent one of the most important sources of human food after cereals[1]. Chickpea (*Cicer arietinum L.*) is one of the most popular legumes crops worldwide. To date, comprehensive studies on agronomic and genetic influences on chickpea protein composition are lacking. Consequently, the aim of this project is the development of a "*shotgun*" proteomics approach[2] to investigate the qualitative and quantitative changes of the protein composition of the Italian chickpea genotype "Pascià" grown under two different water regimes, namely rainfed and irrigated.[3] Proteomic analysis allowed the identification of about 1000 proteins for each sample, revealing a remarkable similarity in the protein composition between the two samples, although some interesting differences were also observed. In this respect, an internal relative quantification revealed that in both samples the most abundant components, as expected, belong to the storage protein family (i.e., legumins, vicilins, convicilins, and albumins), although the rainfed sample, respect to the irrigated one, globally shows a higher content of this class of proteins, and a different proportion of globulins and vicilins. Taking into account that these proteins, may have both health-promoting effects and allergenic properties, these preliminary results will be extended at quantitative level, by a label-free approach, to detect changes in the expression of single proteins between the two samples under investigation. Then, the investigation will be extended to another chickpea genotype, grown under two different water supply, to assess the response (in terms of global protein composition, and expression of single proteins) of different genotypes in relation with different environmental and management conditions.

* *Corresponding author*:
Aldo Lanzoni.
Università di Catania, Viale A. Doria 6, 95125, Catania, Italy.
Tel.: +39 3387566107
E-mail address: aldo.lanzoni@phd.unict.it (A.Lanzoni)

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O-10

Metaproteomic investigation on the induced changes of a high fiber, low fat diet intervention in the gut microbiota of patients with Crohn's disease

Stefano Levi Mortera[a], Valeria Marzano[a], Federica Rapisarda[a], Chiara Marangelo[a], Ilaria Pirona[b], Pamela Vernocchi[a], Marta Di Michele[a], Maria A Quintero [c], O. Damas [c], C. Mangarelli [c], Irina Fernandez [c], Hajar. Hazime [c], Norma. Solis [c], Rose Killian [c], Mailenys Ortega [c], David Kerman [c], Amar Deshpande [c] Siobhan Proksell [c], Ingrid Barrera [c], Maria T. Abreu [c], Lorenza Putignani[d]

[a] Immunology, Rheumatology and Infective Diseases Area, Unit of Human Microbiome, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; [b] GenomeUp S.r.l.
[c] Medicine/Gastro, University of Miami, Miami, FL, United States
[d] Department of Diagnostics and Laboratory Medicine, Unit of Microbiology and Diagnostic Immunology, Unit of Microbiomics - Immunology, Rheumatology and Infective Diseases Area, Unit of Human Microbiome, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

Crohn's's disease (CD) is characterized by chronic intestinal inflammation due to genetic and environmental factors. Microbiome studies have consistently shown dysbiosis in patients with CD, and diet is a key modifiable factor influencing the gut microbiome (GM) and an important risk factor in the development of CD . However, the functional impact of diet modulation on the microbiome in CD is not well elucidated. To that end, we conducted a diet intervention study for Crohn's disease (CD) to test the effect of a high fiber (17g/1000kcal), low fat 35% diet on microbiome function by means of metaproteomic analysis. CD patients chose to receive one-time diet counseling alone (Gr1, n=9), catered food for 8 weeks (Gr2, n=13), or catered food for 8 weeks for a patient (Gr3p, n=19), and a healthy household control (Gr3c, n=20), as well as participated in Dyadic Psychosocial Support (DPS) sessions (Gr3p, Gr3c). Stool samples were collected at week 1 (T0), week 8 (T1) and week 36 (T2). A comprehensive metaproteomic workflow was applied to raw data coming from high throughput nano UHPLC-MS/MS experiments, to get an outcome where differentially regulated metaproteins were assigned to specific taxa and classified by functions and related metabolic pathways [1].Results showed a high heterogeneity between patients, groups of subjects and across the time points, making quite challenging the identification of a modulation of the GM functionality prompted by the diet. Diet-induced changes involving the activation of bacterial metabolic pathways were observed, including those related to carbohydrate metabolism due to an increased fiber uptake. Interestingly, the activity of taxa accomplishing such functions was observed to be affected by the presence or absence of an inflamed environment. Moreover, stratification by disease location provided interesting insight into regionality of the functional microbiome [2]. The ability to compare the effect of a diet intervention in CD patients versus matched household controls will give additional power to understanding how diet can be used to manipulate the microbiome in the context of dysbiosis.

* Corresponding author:
Stefano Levi Mortera.
Bambino Gesù Children's Hospital, Viale di San Paolo 15, Roma, Italy.
Tel.: +39 68594285
E-mail address: stefano.levimortera@opbg.net (S. Levi Mortera)

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O-11

Dysfunctional microglia in the pathogenesis of Alzheimer Disease: proteome features of the pro-AD phenotype

Marta Lualdi [a*], Luisa Pieroni [b,c], Lucia Scipioni [c,d], Daniel Tortolani [c,e] Francesca Ciaramellano [c,e], Roberto Coccurello [c], Debora Cutuli [f], Fabio Ferlazzo [f], Laura Petrosini [f], Sergio Oddi [c,e], Mauro Maccarrone [c,d], Tiziana Alberio [a]

[a] University of Insubria, Department of Science and High Technology, Busto Arsizio (VA), Italy
[b] UniCamillus —International Medical University, Rome, Italy
[c] European Center for Brain Research (CERC)/Santa Lucia Foundation IRCCS, Rome, Italy
[d] University of L'Aquila, Department of Biotechnological and Applied Clinical Sciences, L'Aquila, Italy
[e] University of Teramo, Department of Veterinary Medicine, Teramo, Italy
[f] La Sapienza University, Department of Psychology, Rome, Italy

Accumulating evidence reveals a pivotal role of dysfunctional microglia in the pathogenesis of Alzheimer Disease (AD). The acquisition of a peculiar "pro-AD" phenotype by microglial cells exacerbates the β amyloid (Aβ) cascade, which in turn contributes to microglial activation and shift towards pathogenetic hallmarks. From a molecular point of view, there is an urgent need to characterize these CNS primary immune cells, when contributing to AD onset.

The aim of the present study was the proteomics characterization of primary microglial cells, challenged or not with lipopolysaccharide (LPS), isolated from neonatal Tg2576 (Tg) and wild-type (WT) mice. This allowed us to evaluate the proteome alterations due to the exposure of microglia to soluble forms of Aβ peptides within the brain of Tg mice during intrauterine life. The four groups of samples (WT Ctrl, WT LPS, Tg Ctrl, Tg LPS) have been analyzed in a shotgun LC-MS/MS experiment on a Synapt G2-Si Mass spectrometer (Waters). PCA evidenced a full segregation of Tg cells from WT cells. Moreover, LPS treatment in vitro induced a marked change in Tg cells, while a milder effect was observed on the proteome of WT cells. By comparing WT LPS and Tg LPS samples, over-representation analysis and Gene Set Enrichment Analysis evidenced lipid metabolism and trafficking, pattern recognition receptors biosignalling, and cellular senescence as enriched categories.

In conclusion, these mechanisms (and related proteins) may be those that are mainly altered in the "pro-AD" microglial phenotype and contribute to AD pathogenesis.

* *Corresponding author*:
Marta Lualdi.
Department of Science and High Technology, University of Insubria, Busto Arsizio (VA), Italy.
Tel.: +39 0331 339423;
E-mail address: marta.lualdi@uninsubria.it (L. Lualdi)

O-12

A Multi-Proteomic Approach to Unravel New Players in Metastatic Melanoma Progression

Alessandra Morelli[a*], Vittoria Matafora[a], Angela Bachi[a]

[a] IFOM ETS - The AIRC Institute of Molecular Oncology, Via Adamello 16, 20139 Milano, Italy.

Amyloid-like fibrils have been recently discovered as characteristic of metastatic melanoma, both *in vitro* and in patient samples [1]. Although amyloids are typically harmful, physiological fibrils of melanocyte-specific protein (PMEL) in melanocytes serve as a scaffold for melanin deposition. Some proteases, including the beta-secretase BACE2, generate the amyloidogenic peptides, and in normal conditions this process is finely regulated [2]. Elevated expression and activity of BACE2, correlating with a poorer prognosis, have been reported in numerous tumors, including melanoma [3]. Our group demonstrated that BACE2-dependent PMEL fibrils in metastatic melanoma promote cancer cell growth and invasion through mechanotransduction activation [1]. Preliminary results had also detected increased secretion of ECM components in high BACE2 cells. To explore the structural rearrangements occurring in the secretome of high-BACE2 metastatic melanoma cells, we employed Limited Proteolysis coupled to Mass Spectrometry (LiP-MS) [4]. Moreover, to reveal BACE2-dependent cleavage targets, and clarify whether their processing might influence the metastatic phenotype, we applied the N-Tails degradomics approach [5]. Our N-Tails experiments unveiled numerous N-Termini regulated by BACE2 activity, many of which are found in proteins involved in cell adhesion. Moreover, LiP-MS analysis confirmed structural rearrangements in some BACE2-dependent cleaved proteins. Among those, amyloidogenic PMEL peptides resulted conformationally perturbed in metastatic melanoma models. Other known amyloidogenic proteins were also detected by LiP-MS. This suggests a potential role of PMEL as seed, influencing the stability of other proteins and their fibrillation. Further studies are required to elucidate whether processing of BACE2 targets may sustain a pro-metastatic phenotype and to clarify whether fibrils contribute to the creation of a solid fibril network, which could impede drug diffusion or create a hypoxic and stressful microenvironment.

* *Corresponding author*:
Alessandra Morelli
PhD student
IFOM ETS - The AIRC Institute of Molecular Oncology
Via Adamello 16, 20139 Milano, Italy.
E-mail address: alessandra.morelli@ifom.eu (A. Morelli)

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O-13

Targeted lipidomics on Glycogen Storage Disease Type Ia (GSDIa) patients' blood samples as new tool for dyslipidemia investigation in GSDIa

Francesca Pirozzi[a,b*], Roberta Fedele[b], Carmen Rosano[c], Renata Auricchio[c], Daniela Melis[d], Pietro Strisciuglio[c], Maaike H. Oosterveer[e], Terry G. J. Derks[f], Giancarlo Parenti[c,g], Margherita Ruoppolo[a,b], Alessandro Rossi[c], Marianna Caterino[a,b]

[a] Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Naples, Italy;
[b] CEINGE - Biotecnologie Avanzate Franco Salvatore s.c.a r.l., Naples, Italy;
[c] Department of Translational Medicine, Section of Pediatrics, University of Naples Federico II, Naples, Italy;
[d] Department of Medicine, Surgery and Dentistry 'Scuola Medica Salernitana', University of Salerno, Salerno, Italy;
[e] Department of Pediatrics, University of Groningen, Groningen, The Netherlands;
[f] Division of Metabolic Diseases, Beatrix Children's Hospital, University of Groningen, Groningen, The Netherlands;
[g] Telethon Institute of Genetics and Medicine, Pozzuoli, Italy

Glycogen Storage Disease Type Ia (GSDIa) is an autosomal recessive inborn error of carbohydrate metabolism caused by a deficiency in the catalytic subunit of the Glucose-6-phosphatase complex, selectively expressed in liver, kidney, and intestine and involved in the glucose homeostasis by catalyzing the final step of gluconeogenesis and glycogenolysis. Common GSDIa clinical features include hypoglycemia, hypercholesterolemia, hypertriglyceridemia, hyperuricemia, lactic acidemia and hepatomegaly. Strict dietary management based on frequent daytime feedings combined with uncooked cornstarch, extended-release cornstarch (Glycosade®) and/or continuous gastric drip-feeding is the cornerstone of the treatment[1]. Despite dietary regimen, long term complications may occur. Therefore, detailed understanding of the mechanistic link between glycemic control and hyperlipidemia is of great importance to further improve and personalize GSDIa patient care[2]. Since the extent of lipid metabolism disruption and its potential consequences remain incompletely understood, a comparative lipidomic study was performed in order to better characterize hyperlipidaemia in GSDIa. LC-MS/MS targeted lipidomics analysis was carried on plasma samples from 12 GSDIa patients (8 males, 4 females, age 5.2-34.9 years), 12 age- and gender-matched healthy controls (HC) and 6 age-matched subjects with hyperlipidaemia. 550 lipids were identified, quantified and correlated with biochemical markers of metabolic control. The extensive plasma lipidomic profiling of GSDIa patients showed broader lipid metabolism involvement in GSDIa, putting the light on new lipid species involved in GSDIa.

* *Corresponding author*:
Francesca Pirozzi.
Department of Molecular Medicine and Medical Biotechnology
University of Naples Federico II, Naples, Italy.
Tel.: +39 0813737807.
E-mail address: francesca.pirozzi2@unina.it (F. Pirozzi)

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O-14

A Multi-Omics Approach To Dissect Aberrant Epigenetic Mechanisms In Triple Negative Breast Cancer

Giulia Robusti[a], Roberta Noberini[a], Alessandro Vai[a], Giulio Pavesi[b], and Tiziana Bonaldi[a,c]

[a] Department of Experimental Oncology, European Institute of Oncology IRCCS, Milan, Italy
[b] Department of Biosciences, University of Milan, Milan, Italy
[c] Department of Oncology and Haematology-Oncology, University of Milano, Milan, Italy

Although cancer was long considered a genetic disease, the contribution of epigenetics to various aspects of cancer biology is now being increasingly recognized. In particular, aberrations in the deposition and maintenance of histone post-translational modifications (PTMs) can result in the inappropriate expression or silencing of genes, potentially leading to cancer. We applied a quantitative mass-spectrometry-based method to study histone PTMs in different breast cancer subtypes, with a focus on triple-negative breast cancers (TNBCs), which comprise a heterogeneous group of tumors lacking well-defined molecular targets and targeted therapies. By mass spectrometry, we profiled > 100 breast cancer samples and identified changes in the methylation levels of specific residues (H3K4me2, H3K9me3, and H4K20me3) in TNBC compared to the other breast cancer subtypes and in particular Luminal A tumors. With the aim of dissecting the molecular mechanisms underlying these epigenetic changes, we undertook two different experimental strategies. First, we carried out a multi-OMICs analysis involving the integration of epi-genomics, transcriptomics, and epi-proteomics data from matched breast cancer samples and public repositories, and found that the presence of H3K4me2 at promoters and super-enhancers in TNBC samples drives the expression of genes associated with the TNBC aggressive phenotype. Second, in order to characterize possible upstream mechanisms, we focused on the histone modifying enzymes involved in the deposition and removal of methylation. In particular, we are investigating H3K9-selective methyltransferases whose knockdown seems to have a potential effect not only on levels of H3K9me3 methylation but also on modulation of H4K20me3 in model breast cancer cell lines, suggesting a potential mechanism of crosstalk.

* *Corresponding author*:
Giulia Robusti.
European Institute of Oncology, Via Adamello 16, Milano, Italy.
E-mail address: giulia.robusti@ieo.it (G. Robusti)

O-15

Integrating mass spectrometry-based (phospho)proteomics with prior knowledge networks reveals disease-specific mechanisms.

Giorgia Massacci[a], Veronica Venafra[a], Valeria Bica[a], Simone Graziosi[a], Livia Perfetto[b], Francesca Sacco[a,c*]

[a] University of Tor Vergata, Via della Ricerca Scientifica, Rome, Italy;
[b] University of La Sapienza, Piazzale Aldo Moro, Rome, Italy;
[c] TIGEM, Via Campi Flegrei 34, Pozzuoli, Italy;

A complex and highly interconnected network of molecules govern cellular physiology and its ability to properly respond to external and internal stimuli. In this scenario, protein phosphorylation plays a crucial role, by propagating signals through the finely tuned regulation of protein function. Indeed, protein phosphorylation networks are implicated in the control of almost every biological function, being often deregulated in complex diseases and consequently targeted by novel therapeutic drugs.

The systematic characterization of disease-specific pathways is crucial to identify novel drug targets and uncover the biological significance of disease-associated mutations. Here, I will discuss how it is possibile to combine mass spectrometry (MS)-based (phospho)proteomics with literature-derived signaling networks to build context-specific models, describing the molecular mechanisms driving complex phenotypes. Specifically, I will focus on a subset of leukemia patients with an impaired sensitivity to first-line therapeutic drugs. State-of-the-art MS-based phosphoproteomics enables us to stratify leukemia cells according to their responsiveness. Combining our deep dataset with prior-knowledge network reveals crucial pathways altered in drug resistant leukemia cells. Pharmacological targeting of “resistant”-specific pathways restores the ability of leukemia cells to properly respond to chemotherapeutic drugs.

Finally, our work demonstrates how unbiased, MS-based based, system-level studies have the potential to accelerate the discovery of more granular, patient-specific mechanisms of disease toward the identification of more effective therapeutic approaches.

* *Corresponding author*:
Prof. Francesca Sacco
University of Rome Tor Vergata, Rome, Italy.
Tel.: +39 0672594305;
E-mail address: francesca.sacco@uniroma2.it (F. Sacco)

O-16 Metabolomics evaluation of the effects of Beetroot Juice supplementation in Long Covid patients

Lavinia Santucci[a,b*], Francesca Canu[a,b], Giulia Cipriani[b], Riccardo Calvani[a], Aniello Primiano[a], Silvia Persichilli[a,b], Matteo Tosato[a], Federico Marini[c], Alfredo Miccheli[c], Fabio Sciubbia[c], Alberta Tomassini[d], Walter Aureli[d], Francesco Landi[a,b], Andrea Urbani[a,b], Jacopo Gervasoni[a]

[a] Fondazione Policlinico Universitario A. Gemelli, IRCCS, Rome, Italy
[b] Catholic University of Sacred Heart, Rome, Italy
[c] Sapienza University of Rome, Rome, Italy
[d] R&D Aureli Mario S. S. Agricola, Ortucchio, Italy

Long Covid is a multi-system syndrome reported by many Covid 19 survivors with persistent effects and complex symptoms that could lead to significant chronic morbidity. The pathogenesis of late sequelae of COVID-19 is undefined and can be caused by different mechanism as chronic inflammation, autoimmune processes, alterations in immunometabolic pathways and endothelial damage [1]. Beetroot juice obtained from red beetroot (Beta Vulgaris), has potential beneficial effects on cardiovascular health, on metabolic homeostasis, inflammation and lung function, due to its high nitrates (NO_3^-) content. Our aim was to evaluate the effects of beetroot juice supplementation in Long Covid patients, reporting faitgue, muscle weakness and dyspnea. To evaluate the effects of beetroot juice supplementation, 28 patients with Long Covid (16 receiving treatment and 12 receiving placebo) were enrolled. Serum samples were collected at baseline and at the end of treatment after 14 days. A panel of 26 inflammatory mediators were determined using a Bio-Plex test. Kairos kit (Waters Corporation) was used for the analysis of 44 amino acids through UPLC/MS. Fatty acids concentration was determined through a GC-MS method, using Eureka Lab division diagnostic kit. Homemade analytical methods were used for thiols and asymmetric-dimethylarginine (ADMA), through HPLC-FL and LC-MS/MS respectively. Chemometric classification models were built using Partial least squares discriminant analysis (PLS–DA) to characterize the variation of metabolism in patients ($\Delta_{t0,t14}$). Each metabolic pathway was studied independently. Using CovSel algorithm for variables selection, we obtained 4 different classification models with accuracy ranges from 53.1% and 75.3%. At least a multi-block classification models was built selecting 4 amino acids (aspartic acids, citrulline and kynurenine) and a fatty acid (palmitoleic acid). This model allowed discrimination between treated and placebo subjects with an accuracy of 81.8%. Due to our results, especially relevant was the alteration between treated and placebo in dyad kynurenine-triptophan, notoriously involved in many disease characterized by high inflammation state. Increased kynurenine levels in patients who takes beetroot juice showed how the supplementation was effective. In conclusion we assume that beetroot juice supplementation may be brought as a successful approach to contrast inflammatory states in people with Long Covid.

Corresponding author:
Dr. Lavinia Santucci
Fondazione Policlinico Universitario A. Gemelli, IRCCS, Rome, Italy
Tel.: +39-06 30154222
E-mail address: lavinia.santucci@guest.policlinicogemelli.it (L. Santucci)

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O-17

Exploring bee metaproteome gather insights on infectious diseases in a One-Health fashion.

Bruno Tilocca[a*], Viviana Greco[b,c], Mariachiara Paonessa[a], Domenico Britti[a], Andrea Urbani[b,c], Paola Roncada[a*]

[a] University "Magna Graecia" of Catanzaro, Viale Europa, Catanzaro, Italy;
[b] Catholic University of the Sacred Hearth, Largo F. Vito n 1, Roma, Italy;
[c] Policlinico Universitario "A. Gemelli", Largo A. Gemelli n1, Roma, Italy;

By flying over short-to-mid distances, bee helds the potential to "move" microorganisms across the three spheres of life (i.e. human, animals and environment) with strong implication over the whole ecosystem. As of today, only a handful of studies deal with bee gut metaproteomics to highlight the microbiological potential of bees under the human animal and environmental perspective, and no standardized or reference model exists for the study of the gut microbiota of this important ecological player.
Here, the functional gut microbiota of four adult bee workers representatives is investigated by mass spectrometry-based metaproteomics and bioinformatics data analysis to explore the structure and activities of bacteria, fungi and protists, meant as the major gut colonizers. The outcomes of our explorative research underline the massive presence of bacteria in relation to the other investigated taxa. Interestingly, pathogenic or potentially pathogenic specimens have been identified in all the surveyed taxa, supporting the hypothesized role of bees while bridging the microbial routes across the pillars of life. Functional featuring of the bee gut microbiota is also detailed for the bacteria, fungi, and protists members providing evidence of the metabolic strength of the bee microbiota and its involvement in conditioning microbial phenotypic behaviours, which unavoidably condition the onset and progression of infective outbreaks in the animal, human and environmental field. Altogether, our study contributes to the pioneering knowledgebase on the functional bee gut microbiota and provides a first glimpse into the future modulation of the structure and function of such microbial communities towards a reduced diffusion of pathogens and antimicrobial resistance traits via this yet underestimated route.

* *Corresponding author*:
Bruno Tilocca and Paola Roncada.
University "Magna Graecia" of Catanzaro, Viale Europa snc, Catanzaro, Italy
Tel.: +39 09613694284;
E-mail address: tilocca@unicz.it (B. Tilocca); roncada@unicz.it (P. Roncada)

POSTERS

P-01

Iron homeostasis related parameters in a large cohort of patients hospitalized for covid-19 pneumonia: focus on protein markers

Elisa Antinori[a*], Lorenzo Bertolone[a], Alice Vianello[a], Giacomo Marchi[a], Fabiana Busti[a], Camila Dos Santos Lima[a], Claudia Bozzini[a], Annalisa Castagna[a], Acaynne Zidanes Lira[a], Patrizia Pattini[a], Chiara Stranieri[a], Giulia Sartori[a], Ernesto Crisafulli[a], Anna Maria Fratta Pasini[a], Simonetta Friso[a], Giuseppe Lippi[a], Domenico Girelli[a].

[a]Department of Medicine, Università degli Studi di Verona, Verona, Italy.

The COVID-19 pandemic, caused by SARS-CoV-2, has led to millions of deaths worldwide. The disease's severity ranges from asymptomatic to critical, with around 5% of cases developing acute respiratory distress syndrome (ARDS) and multiple organ failure (MOF) due to an excessive response of their immune system. This excessive response is hallmarked by an abnormally high release of circulating cytokines, termed cytokine release syndrome (CRS) which is one of the main causes of disease severity and death in patients (Hu et al., 2021).
As the control of iron metabolism is crucial in infections and because several cytokines involved in the cytokine storm are also regulators of iron homeostasis, we aim to investigate the relationships between inflammatory indexes and iron metabolism in patients hospitalized with severe COVID-19.
Blood samples of 392 patients hospitalized for COVID-19 pneumonia, were taken at three time points, and various assays, including cytokine analysis, hepcidin, erythroferrone, and soluble transferrin receptor (sTfR) quantifications, were performed. Statistical analysis, including t-tests, non-parametric tests, and correlation assessments, was conducted to evaluate associations with disease severity.
The population primarily comprised older male patients. Cytokine analysis showed higher IL-6, IL-8, and IL-10 levels in severe cases. Iron-related markers confirmed low iron and high ferritin in severe cases. Iron levels negatively correlated with markers of inflammation and cytokines, especially in milder cases.
Logistic regression identified N/L ratio, IL-6, and hemoglobin as severity predictors. Multivariate analysis linked ferritin, IL-1RA, and P/F to serum iron and N/L, ferritin, IL-1R-A, and IL-6 to hepcidin. Surprisingly, no significant correlations were found between hepcidin and iron or between hepcidin and erythroferrone, suggesting ERFE-independent iron regulation mechanisms in response to COVID-19-related hypoxia.

* *Corresponding author*:
Elisa Antinori.
Università degli Studi di Verona, Verona, Italy
Tel.: +045 812 8422.
E-mail address: elisa.antinori@univr.it (E. Antinori)

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P-02

BACE2 regulates tumor-microenvironment metabolic interactions

Vittoria Matafora[a], Francesco Farris[a], Alice Elhagh[a], Alessandra Morelli[a] and Angela Bachi[a*]

[a] IFOM ETS - The AIRC Institute of Molecular Oncology, Via Adamello 16, 20139 Milan, Italy

Lipid metabolism is often deregulated in cancer. Indeed, enhanced lipid synthesis, storage, and uptake contribute to cancer proliferation, survival, invasion and metastasis[1]. Lipids are used to obtain energy, constitute biological membranes, and act as signaling molecules. Rewiring of lipid metabolism in cancer has been linked to the activation of oncogenic signaling pathways as well as to the cross talk with tumor microenvironment[2]; however, the mechanism adopted by cancer cell to became addicted to lipids is still largely unknown. By comparative analysis of proteins secreted by metastatic and primitive melanoma cell lines, our group recently found that proteins involved in lipid metabolism such as ApoE, together with amyloidogenic proteins, accumulate markedly in the metastatic extracellular environment[3]. We also observed that BACE2 sheddase activity is responsible for amyloid maturation, and that its inhibition increases the secretion of proteins related to lipid metabolism in metastatic cells. From global lipidomic profiles of melanoma cell lines using an in-house optimized LC-MS method, Opti-nQL [4], we found that metastatic melanoma cells, which express higher levels of BACE2 compared to the primitive ones, are enriched in free cholesterol and triglycerides and accumulate lipid droplets. By proteomic analysis, we also found an increased expression of enzymes related to cholesterol biosynthesis and fatty acids regulation. Of note, upon BACE2 inhibition, lipidomic profiles of melanoma metastatic cell lines showed an increase of free cholesterol and phospholipids and a decrease in triglycerides. Coherently, a decrease in lipid droplets content was observed, suggesting the involvement of BACE2 activity in fatty acid homeostasis. As BACE2 is a sheddase, we focus on its specific role and, by spatial proteomic, we discovered that BACE2 regulates the level of lipids transporters on the membrane, tuning lipid uptake from the extracellular space. We found the same mechanism also present in pancreatic cancer cells displaying high BACE2 level. Our study reveals the role of BACE2 as modulator of lipid metabolism in cancer.

* *Corresponding author*:
Angela Bachi
IFOM, Istituto Fondazione di Oncologia Molecolare ETS
Via Adamello 16, 20139, Milan, Italy
E-mail: angela.bachi@ifom.eu (A. Bachi)

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P-03 In-depth phosphoproteomics reveals novel mechanisms of tyrosine kinase inhibitor resistance in chronic myeloid leukemia

Valeria Bica[a], Giorgia Massacci[b], Sara Gualdi[c], Veronica Venafra[a], Simone Graziosi[a], Sara Latini[a], Patrizia Chiusolo[d], Gessica Minnella[d], Livia Perfetto[b,e], Francesca Sacco[b,f*]

[a]PhD Program in Cellular and Molecular Biology, University of Rome "Tor Vergata", Rome, Italy.
[b]Department of Biology, University of Rome Tor Vergata, Rome, Italy
[c]Dep. of Biology and Biotechnology "Charles Darwin", University of Rome La Sapienza, Rome, Italy.
[d]Dip. di Scienze Radiologiche ed Ematologiche, Università Cattolica del Sacro Cuore, Rome, Italy
[e]Fondazione Human Technopole, Department of Biology, Milan, Italy
[f]Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy

Chronic Myeloid Leukemia (CML) is a myeloproliferative neoplasm that affects the stem cell precursors of the myeloid lineage. The cytogenetic hallmark of CML is BCR-ABL, a constitutively active fusion oncogene that promotes proliferation and survival of leukemic blasts [1,2]. Treatment of CML has been revolutionized by the introduction of Imatinib, a BCR-ABL tyrosine kinase inhibitor (TKI), which has significantly improved patient clinical outcome. However, approximately 15% of patients fails treatment because of disease relapse caused by the occurrence of BCR-ABL-dependent or -independent mechanisms of resistance [3,4]. My project aims to obtain a systemic and comprehensive characterization of the BCR-ABL-independent mechanisms of resistance, with the final goal to identify novel therapeutic strategies to improve clinical outcomes of unresponsive CML patients. I generated an imatinib-resistant cell model by exposing K562 cells to gradually increased concentrations of imatinib. Biological characterization revealed a BCR-ABL independent type of resistance. To obtain a comprehensive picture of how signaling pathways rewiring leads to drug resistance, we have combined mass spectrometry-based (phospho)proteomics with network-based approaches. Specifically, by applying our in-house developed *Signaling Profiler* pipeline, which integrates omics datasets with prior knowledge signaling network, we obtained CML resistant cells specific models [5]. Exploiting the Signaling Profiler output, we selected hyper-activated proteins based on the availability of a specific FDA-approved inhibitor. From this analysis we identified as potential targets BCL2, BTK, DNMTs and JAK2. Preliminary data show promising results. In fact, exposure to venetoclax (BCL2i), ibrutinib (BTKi) or azacytidine (DNMTi) can significantly induce apoptosis in resistant cells. In the future, we plan to perform combinatorial treatment to individuate optimal therapeutical strategy to restore sensitive phenotype. Moreover, we plan to assess the clinical relevance of this study by validating our finding in responder and non-responder patients' derived primary blasts.

** Corresponding author:*
Francesca Sacco
Department of Biology, University of Rome Tor Vergata, Rome, Italy
E-mail address: francesca.sacco@uniroma2.it (F.Sacco)

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P-04 Unique Peptides of Cathelicidin-1 in the Early Detection of Mastitis in Sheep

Maria V. Bourganou[a,b1*], Evangelos Kontopodis[b1], George Th. Tsangaris[b1], Vasileios Pierros[b], Natalia G.C. Vasileiou[c], Vasia S. Mavrogianni[d], George C. Fthenakis[d], and Angeliki I. Katsafadou[a]

[a]Faculty of Public and One Health, University of Thessaly, Karditsa, Greece
[b]Proteomics Research Unit, Biomedical Research Foundation of the Academy of Athens, Athens, Greece
[c]Faculty of Animal Science, University of Thessaly, Larissa, Greece
[d]Veterinary Faculty, University of Thessaly, Karditsa, Greece
[1] Equal contribution

Objective. The study refers to the investigation of the sequence of cathelicidin-1 present in ewes' milk, with the aim to identify its unique peptides and core unique peptides, which would reveal potential targets for accurate detection of the protein for early diagnosis of mastitis in ewes.

Methods. Potential uniqueness of each peptide of cathelicidin-1 was investigated against those in all proteins reported to have been detected in sheep, goats or cattle, thus creating a set of Core Unique Peptides (CUP). The analysis was performed by using a bioinformatics tool, built on a big-data algorithm. Thereafter, Composite Core Unique Peptides (CCUP) that were constructed based on a sequence of two or more consecutive or overlapping core unique peptides, were also searched. Finally, the three-dimensional (3D) structure of the protein was analyzed, using the AlphaFold predicted 3D model. In addition, the detection of unique sequences among the tryptic digest peptides of cathelicidin-1 was achieved, which would improve accuracy of identification of the protein during targeted MS-based proteomics.

Results. In total, 59 CUPs and four CCUPs were detected in cathelicidin-1 of sheep origin. During the 3D structure analysis of the protein, 35 CUPs were found on the core of the protein and, of these, 29 CUPs were located on amino acids in regions of the protein with 'Very high' or 'Confident' estimates of confidence of the structure. Finally, it was found that six CUPs were located entirely on loop or α-helix motifs of the secondary structure of the protein and were considered to be suitable as potential antigenic targets for accurate detection of the protein. Among tryptic digest peptides, there were six unique peptides in that protein.

Conclusion. The detection of unique peptides in cathelicidin-1 provided potential targets for accurate detection of the protein as biomarker. The selection of six CUPs as potential antigenic targets may be used to improve early diagnosis of mastitis in sheep. Moreover, another six unique peptides were detected in tryptic digests and may offer novel mass tags, which would facilitate the detection of cathelicidin-1 during MS-based diagnostics.

* *Corresponding author*:
Maria V. Bourganou.
Faculty of Public and One Health, University of Thessaly, Karditsa, Greece.
Tel.: +30 2441064761;
E-mail address: mbourganou@uth.gr (M. Bourganou)

P-05 Targeted proteomics approach for qualitative and quantitative monitoring of human TSLP proteoforms in patients with asthma

Luisa Canè[a,b], Remo Poto[a,c], Stefania Loffredo[a,c,e], Ilaria Iacobucci[b,d], Piero Pucci[b], Gianni Marone[a,c,e], Gilda Varricchi[a,c,e], Maria Monti[b,d]

[a]Department of Translational Medical Sciences, University of Naples Federico II, Naples, Italy
[b]CEINGE-Advanced Biotechnologies "Franco Salvatore", Naples, Italy
[c]World Allergy Organization (WAO), Center of Excellence (CoE), Naples, Italy
[d]Department of Chemical Sciences, University of Naples Federico II, Naples, Italy
[e]Center for Basic and Clinical Immunology Research (CISI), University of Naples Federico II, Naples, Italy.

Thymic stromal lymphopoietin (TSLP) is a pleiotropic cytokine, also called alarmin, highly expressed by bronchial epithelial cells (BECs) and certain cells of the immune system (**1**). TSLP exerts its biological roles by binding to the heterodimeric receptor (TSLPR-IL-7Rα) present on a plethora of immune cells. The TSLP/TSLPR axis plays a central role in inflammatory diseases of the airways and in certain cancers[1]. Overexpression of TSLP has been involved in orchestrating allergic inflammation in asthma and other allergic disorders. The key role of TSLP in the pathobiology of asthma was demonstrated by the efficacy and safety of an anti-TSLP monoclonal antibody (mAb) (tezepelumab) in the treatment of severe asthma with T helper 2-like (T2-high) and T2-low phenotypes[2]. Although tezepelumab is effective across the spectrum of asthma phenotypes, at present, there are no predictive biomarkers to identify candidates for anti-TSLP therapy. An additional layer of complexity is the identification of two proteoforms of TSLP expressed in human BECs: the long form (lfTSLP) and a shorter TSLP isoform (sfTSLP), which overlaps the C-terminus of lfTSLP[3]. Inflammatory stimuli specifically upregulate lfTSLP, but not sfTSLP in human BECs and lung macrophages[4]. At present, antibodies specifically recognizing epitopes of the two TSLP proteoforms are not available. In addition, at site of inflammation and in biological fluids, TSLP can be rapidly cleaved by mast cell-derived proteases (tryptase and chymase)[5]. For the above reasons, the qualitative and quantitative monitoring of TSLP proteoforms in biological fluids and immune cells is extremely difficult. We developed a qualitative and quantitative targeted proteomics technique based on multiple reaction monitoring (MRM) strategy[6] to unequivocally detect the two TSLP proteoforms. MRM analysis of human recombinant TSLP allowed to identify peptide sequences specific for the two proteoforms and shared peptides in both human lung macrophages and peripheral blood monocytes. The same technique was applied to the identification of the two proteoforms of TSLP in plasma of normal subjects and patients with different severity of asthma. An additional objective of this study will be the correlation between the plasma concentrations of TSLP proteoforms and the severity of different asthma phenotypes.

* *Corresponding author*:
Luisa Canè
Department of Translational Medical Sciences, University Federico II, Naples, Italy
Tel.: +39 3392685890;
E-mail address: canel@ceinge.unina.it (L. Canè)

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P-06

Improving clustering methods with spatial multi-omics information to detect hidden patterns in mass spectrometry imaging data

Giulia Capitoli[a*], Vanna Denti[b], Andrew Smith[b], Francesco Denti[c]

[a] Department of Medicine and Surgery, Bicocca Bioinformatics Biostatistics and Bioimaging B4 Center, University of Milan-Bicocca (UNIMIB), Monza, Italy

[b] Department of Medicine and Surgery, Clinical Proteomics and Metabolomics Unit, University of Milan-Bicocca, Monza, Italy

[c] Department of Statistics, Università Cattolica del Sacro Cuore, Milano, Italy

Mass spectrometry records molecular mass abundance for a broad set of different molecules, i.e., lipids, N-glycans, and tryptic peptides, given a sample of a specific biological tissue [1]. In particular, the MALDI-MSI technique produces imaging data where, for each pixel, a mass spectrum is recorded for each molecule.
This methodology could be used to identify groups of pixels that present similar patterns to extract interesting insights, such as anomalies that pathologists cannot capture from the original morphological image. This task is particularly challenging given the high dimensionality of the data and the spatial correlation among pixels.
The standard statistical methods do not fully address the pixel spatial dependencies and the networks of different molecules. We developed a method of spatial penalization within Bayesian clustering algorithms to consider the pixels' geographical location, thus improving the consistency and interpretation of the results obtained. We compared the new methodology with the most standard clustering approach, the K-means algorithm.
Based on the proposed approach, we are able to integrate data of various types of molecules extracted from the same tissue, highlighting and uncovering hidden patterns resulting from the spatial correlation among pixels and the relationship between multiple molecular layers.
We demonstrated the validity of our proposed approach on a murine brain and clear cell renal cell carcinoma (ccRCC) tissue. The three molecular levels sequentially extracted through MALDI-MSI from the single FFPE tissue section provided complementary information and characterized different histological regions. Compared to the standard K-means approach, we can appreciate how the Bayesian models eliminate sampling impurities better than the K-means, improving the quality and stability of the clustering segmentation.
Moreover, when the spatial omics data was integrated, the different histopathological regions of the ccRCC tissue could be better discriminated with respect to the imaging data set of any single omics class. Taken together, these promising findings demonstrate the capability to more comprehensively map the molecular complexity within pathological tissue.

* *Corresponding author*:
Giulia Capitoli
University of Milan-Bicocca, Via Raoul Follereau 3, Vedano al Lambro (MB), Italy.
Tel.: +39 3381801181
E-mail address: giulia.capitoli@unimib.it (G. Capitoli)

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P-07

Biomarkers discovery through multivariate statistical methods to face clinical issues concerning thyroid tumour variants classification

Giulia Capitoli[a*], Isabella Piga[b], Vincenzo L'Imperio[c], Davide Bernasconi[a], Fabio Pagni[c]

[a] Department of Medicine and Surgery, Bicocca Bioinformatics Biostatistics and Bioimaging B4 Center, University of Milan-Bicocca (UNIMIB), Monza, Italy

[b] Department of Medicine and Surgery, Clinical Proteomics and Metabolomics Unit, University of Milan-Bicocca, Monza, Italy

[c] Department of Medicine and Surgery, Pathology, Fondazione IRCCS San Gerardo dei Tintori, University of Milan-Bicocca, Monza, Italy

Thyroid nodules are common among Western populations, with an estimated prevalence of 50% among individuals aged above 60. However, only 5-10% of the nodules are cancerous, making identifying malignant lesions a substantial health concern for pathologists searching for novel and more accurate diagnostic tools and techniques. Machine Learning (ML) algorithms have emerged as a transformative force in healthcare, improving medical practice in several aspects, including diagnosing tumours. Among the possible biomarkers of thyroid cancer, molecular features obtained through Matrix Assisted Laser Desorption Ionization Mass Spectrometry Imaging (MALDI-MSI) are the most promising. This work presents an application of several ML algorithms using molecular features to build an accurate diagnostic tool for the classification of thyroid nodules [1]. The primary goal of this research is to discover discriminatory molecular signals that can serve as valuable biomarkers. These tumour markers play a crucial role in accurately classifying undefined thyroid cancer variants, such as the Non-Invasive Follicular Thyroid Neoplasm with Papillary-like nuclear features type (NIFTP), shedding light on their behaviour and establishing connections to malignancy or benignity. Regarding the ML methods considered for the task, the implementation and comparison of Linear Discriminant Analysis (LDA), Diagonal Discriminant Analysis (DDA), and sparse Partial Least Squares Discriminant Analysis (sPLS-DA) in this work have provided valuable insights into understanding the behaviour of NIFTP. The noteworthy aspect is that all three techniques discover common and relevant features as biomarkers for the NIFTP class, thus improving the reliability of the results from a statistical point of view. These supervised approaches have enabled the identification of specific molecular signals that effectively distinguish thyroid tumour classes, shedding light on NIFTP-type characteristics within this context, achieving accuracy greater than 0.9. This synergy between the medical and machine learning domains can also catalyze further exploration in biomarker discovery. Expanding the applications of supervised learning approaches to address clinical issues in the omics field is a pivotal aspect that can foster cutting-edge research and provide a reliable starting point for researchers to implement and enhance machine learning techniques.

* *Corresponding author*:
Giulia Capitoli
University of Milan-Bicocca, Via Raoul Follereau 3, Vedano al Lambro (MB), Italy.
Tel.: +39 3381801181
E-mail address: giulia.capitoli@unimib.it (G. Capitoli)

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P-08

Analysis of the oral fluid of human preterm newborns after regurgitation evidences the cleavage pathway of the proteins of breast milk during the fetal digestion.

Chiara Tirone[a]*, Giovanni Vento[a], Federica Iavarone[b], Nicoletta Menzella[a], Davide De Tomaso[a], Simona Fattore[a]. Claudia Desiderio[c], Tiziana Cabras[d], Barbara Manconi[d], Alessandra Olianas[d], Mozhgan Boroumand[e], Irene Messana[d], Gavino Faa[d], Massimo Castagnola[g].

[a]Neonatology Unit, Department of Woman and Child Health and Public Health, Fondazione Policlinico Univ. "Agostino Gemelli", Dipartimento di Scienze della Vita e Sanità Pubblica
[b] Dipartimento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, Rome, Italy.
[c]Istituto di Scienze e Tecnologie Chimiche "Giulio Natta", CNR, Rome, Italy;
[d]Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy
[e] National Institute on Aging, NIH, Baltimore, MD 21224, USA;
[g]Centro Europeo di Ricerca sul Cervello-IRCCS Fondazione Santa Lucia, Rome, Italy;

The oral fluid of preterm newborn is a very precious specimen to investigate various molecular mechanisms happening during the late fetal development [1]. It can provoke respiratory problems to the preterm and it is commonly aspired by the NICU (Neonatal Intensive Care Unit) personnel and discharged as a waste. For this reason, its use for analytical purposes has been allowed by the Ethical committees of our universities. We had the occasion to take this fluid few minutes after the breastmilk feeding because the newborn had a dangerous regurgitation. The analysis of this sample using a high-resolution nano-chromatography coupled to the Orbitrap-Lumos MS apparatus with a top-down pipeline (without any proteolytic treatment of the sample [2]) allowed to detect a multitude of fragments deriving from β-casein, α-S_1-casein, κ-casein and lactotransferrin. They indicated that in the gastric tract of preterm several trypsin- and chimotrypsin-like proteinases as well as many carboxy- and amino-exopeptidases operate with a complex and coordinate fragmentation pathway. The preliminary list of fragments detected are visible in the poster and many of them have probably an autonomous function. They are indeed potential cryptides, i.e. small peptides encrypted inside the sequence of bigger proteins, with a function different from that one of the parent protein [3]. Further experiments are necessary to clarify the entire pathway of proteolytic events happening during fetal digestion and to establish the function of several fragments. These studies can also give suggestions on new artificial milk-based food to use in substitution to human breast milk, when not-available.

* *Corresponding author*:
Chiara Tirone, Università Cattolica e Policlinico Gemelli, Largo Francesco Vito 1, 00168, Rome, Italy
Tel. e fax: +39 06-3015-4357.
E-mail address: chiara.tirone@policlinicogemelli.it (C. Tirone)

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P-09

Resolving phenotypic variability in two mitochondrial patients with *TWNK* variant: a proteomics approach.

Michela Cicchinelli[a*], Guido Alessandro Primiano[b], Serenella Servidei[b], Andrea Urbani[a,c], Federica Iavarone[a,c]

[a]Dipartimento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, Rome, Italy, [b]Dipartimento di Neuroscienze, Organi di Senso e Torace, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome,Italy,[c]Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Italy

Chronic progressive External Ophtalmoplegia (CPEO) is the most frequent primary mitochondrial myopathies (PMM), genetically defined disorders leading to defects of oxidative phosphorylation affecting predominantly, but not exclusively, skeletal muscle [1]. Mitochondrial diseases associated with mtDNA instability are due to autosomal recessive or dominant variants in nDNA genes (e.g., *TWNK*) encoding proteins playing role in the maintenance pathway. While enormous progress has been made in genetic characterization of these neurogenetic disorders, similarly significant results have not been obtained in the deep understanding of the pathogenetic mechanisms. Based on these premises, we collected urinary samples of two sisters affected by CPEO and c.1075G>A [p.Ala134Thr] variant in *TWNK* gene, characterized by a different clinical severity, and a control group, in order to evaluate the metabolic mechanisms and explore the causes of the phenotypic variability. Patients were recruited in collaboration with the Neurophysiopathology Unit at the Fondazione Policlinico Universitario Agostino Gemelli IRCCS. The disease burden was assessed by the Newcastle Mitochondrial Disease Adult Scale (NMDAS), assigning a different severity score between the two patients: 12 (less severe) and 31 (more severe) [2]. The analysis was based on a bottom-up proteomic approach, performed using an Orbitrap Fusion LumosTribrid mass spectrometer (Thermo Scientific), and label-free quantification. Raw data were analyzed using the Proteome Discoverer 2.4 software, based on SEQUEST HT algorithm and UniprotKB/Swiss-prot *Homo Sapiens* database. The bioinformatics analysis was developed using MATLAB tools, Reactome and FunRich open access databases. We carried out differential expression analysis, supported by a pathways enrichment, to identify the disease-specific metabolic response. Preliminary results show an increase of the antioxidant response in both patients compared with healthy controls. Significant differences, especially in the activation of both anaerobic and aerobic metabolisms, were also found between the two sisters. These data document how the proteomic approach can significantly contribute to reveal the mechanisms underlying phenotypic variability in mitochondrial diseases, also with the aim of identifying potential therapeutic targets.

**Corresponding author:*
Michela Cicchinelli,
Università Cattolica del Sacro Cuore, Largo Francesco Vito 1, Roma, Italia,
E-mail address: michela.cicchinelli@unicatt.it (M. Cicchinelli)

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P-10

Telomeric G-quadruplex for the fishing and the identification of potential protein targets in breast cancer

Irene Cipollone[a,b*], Ilaria Iacobucci[a,b], Flora Cozzolino[a,b], Chiara Platella[a], Domenica Musumeci[a], Daniela Montesarchio[a], Maria Monti[a,b]

[a]Department of Chemical Sciences, University of Naples "Federico II", Naples, Italy;
[b]CEINGE Advanced Biotechnologies "Franco Salvatore", Naples, Italy;

Precision medicine seeks to treat and prevent disease by considering the genetic variability, environment, and lifestyle of each individual. Among other things, point-of-care testing (POCT) devices are required to determine the most suitable medicines since they seek to obtain quick diagnostic data for every patient. The available POCT instruments and regular diagnostic kits are often based on antibodies, but, in the last years, aptamers have become a useful tool in the diagnostic field. Aptamers are single-stranded DNA or RNA oligonucleotides that bind proteins and small molecules with high affinity and specificity by identifying tertiary or quaternary structures as antibodies. By enabling a highly specific molecular recognition, oligonucleotide aptamers provide a valuable alternative to antibodies to obtain quick diagnostic results for patients. Numerous guanine-rich oligomers are among the known aptamers. In fact, guanine-rich oligonucleotide aptamers have the unique capacity to fold stably in G-quadruplexes, non-canonical nucleic acid conformations that are stabilized by guanine tetrads. These structures are highly recurrent in human telomers, and it has been proved they have a role in the pathologic development of cancer cells. In this context, by using an AP-MS approach, we isolated and identified G-quadruplex interacting proteins with the aim of identify potentially early cancer markers. More specifically, a DNA pulldown approach has been carried out by immobilizing a specific G4 human telomeric structure onto beads and by incubating them with the protein nuclear extracts from two different cell lines: MCF-7, a breast cancer cell line, and MCF-10a, an epithelial breast cell line (control). 112 interactors were identified in MCF-7 and 65 in MCF-10a cells; of which, 30 were in common between the two groups. The functional clusterisation analysis allowed us to connect the largest number of identified proteins to transcription and translation processes. For the validation of the expression levels of some proteins of interest, a Multiple Reaction Monitoring (MRM) analysis has been carried out. The ongoing experiments are concerning the validation of direct interactions between selected potential protein biomarkers and G4 oligonucleotide by using SPR based technology and limited proteolysis-MS for *in vitro* experiments and immunofluorescence assays for in cell analysis.

**Corresponding author*:
Irene Cipollone,
Department of Chemical Sciences,
University of Naples "Federico II", Naples, Italy.
Email: irene.cipollone@unina.it (I. Cipollone)

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Mapping the interactomic network of Neuroglobin

Michele Costanzo[a,b,*], Valeria Manganelli[c], Tina Garofalo[c], Roberta Misasi[c], Margherita Ruoppolo[a,b]

[a] Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II,
Naples, Italy;
[b] CEINGE–Biotecnologie Avanzate Franco Salvatore S.C.Ar.L, Naples, Italy;
[c] Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy.

Neuroglobin (Ngb) is a 17 kDa monomeric hexa-coordinated heme protein belonging to the family of globins. As a globin, the main physiological functions of Ngb involve the binding of O_2, and the scavenging and detoxification of oxygen reactive species. In our previous investigations, we have proposed that autophagy represents a novel mechanism by which Ngb may exert its protective roles in Ngb-overexpressing cells [1]. In addition, also the effects of Ngb upregulation were investigated at the proteome level [2]. Nonetheless, the molecular interactions that drive Ngb-related processes still remain unknown.

To provide insights into the molecular network involving Ngb, we adopted a quantitative interactomics approach. Thus, the interactome of Ngb protein was investigated combining proteomics and bioinformatics analyses with co-immunoprecipitation experiments.

Cells overexpressing a Ngb-FLAG protein were grown in parallel with cells containing a GFP-FLAG construct, used as controls for the experiments. An affinity purification step was performed to capture FLAG-tagged proteins-binding complexes using a specific antibody. Thus, immunoprecipitated NGB-complexes were analyzed by LC-MS/MS and putative interactors were selected according to their abundance in Ngb-FLAG cells in comparison with GFP-FLAG samples. After exclusion of the contaminants commonly identified in affinity purification experiments within the CRAPome database, a shortened list of high-confidence Ngb-interacting partners was obtained. The bioinformatic analysis of such candidates enriched many significant gene ontology terms related to cellular signaling pathways, including autophagy.

The main results of this study include the identification of RPTOR as one Ngb-binding protein and the demonstration of Ngb-RPTOR association following Ngb upregulation. Other putative partners of Ngb will be validated using co-immunoprecipitation procedures.

* *Corresponding author*:
Michele Costanzo, PhD.
Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II,
Naples, Italy.
Tel.: +39 0813737807
E-mail address: michele.costanzo@unina.it (M. Costanzo)

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P-12

QProMS: Quantitative Proteomics Made Simple
A Web-Based Application for Quantitative Proteomics Analysis

Giorgia Cucina[a], F. Bedin[b], A. Graziadei[c], A. Cuomo[b*]

[a] University of Milan, Via Festa del Perdono 7, Milan, Italy;
[b] Department of Molecular Oncology, European Institute of Oncology IRCCS, Milan, Italy;
[c] Fondazione Human Technopole, Milan, Italy

The significance of mass spectrometry-based proteomics in biological and clinical research has steadily increased, becoming one of the most powerful tools[1].
Therefore, modern proteomics experienced a rapid evolution of algorithms and data analysis approaches aimed at extracting robust statistical insights and identifying potentially intriguing biological outliers. However, each of these workflows comes with its dedicated data analysis package, often customized to suit specific applications and not having a user-friendly interface, making them less accessible to researchers.
We present here Quantitative Proteomics Made Simple (QProMS), a user-friendly, data analysis and visualisation pipeline aimed to guide the scientist through all steps from preprocessing and statistical analysis to visualization in a graphical interface.
QproMS is capable of importing and processing proteomics data generated from various software platforms including MaxQuant, FragPipe and Spectronaut.
Statistical tests rely on established R functions and are compatible with all types of label-free quantification experiments. The pipeline recapitulates features from different available software packages and introduces mixed imputation, an improved framework for handling missing values. QProMS can also perform interaction analyses based on gene ontology, or by querying protein-protein interaction databases. All figures in QProMS are interactive, allowing for investigation of individual proteins of interest before exporting. The analysis steps and parameters can be saved within a standalone report, ensuring reproducibility for future analyses.
In summary, QProMS serves as a user-centric, all-in-one solution for proteomics data analysis and interpretation. It is built to offer a comprehensive and robust platform for both beginner and experienced researchers, enabling state-of-the art data analysis of a wide variety of label-free proteomic workflows ranging from global proteome profiling to targeted methods such as proximity labelling.

* *Corresponding author*:
Alessandro Cuomo.
Department of Molecular Oncology, IEO, European Institute of Oncology IRCCS, Milan, Italy.
E-mail address: alessandro.cuomo@ieo.it

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P-13

Proteomics functional characterization of growth hormone–secreting and non-functional pituitary adenomas

Rossana De Salvo[a*], Cettina Saoca[b], Alfonso Carleo[1], Laura Licitri[a] , Lorenza Vantaggiato[a], Loredano Grasso[b], Francesco Ferrau[b], Enxhi Shaba[a], Vincenzo Maccaione[b], Luca Bini[a], Salvatore Cannavò[b], M'hammed Aguennouz[b], Laura Bianchi[a]

[a] University of Siena, Department of Life Science, Siena, Italy;

[b] University of Messina, Messina, Italy.

Pituitary adenomas (PAs), generally benign lesions, can sometimes present aggressive characteristics: rapid growth, local invasiveness, and poor response to conventional treatments. Only a few genetic alterations have been reported to be associated with this clinical behaviour, however, recently, the epigenetics impact on the PAs proteome was demonstrated. We the aim to identify affections in the proteomic profile of growth hormone (GH)-secreting and non-functional pituitary adenomas (GH-SPA and NFPA, respectively) to delineate altered pathways that may imbalance pituitary gland homeostasis. Seeking for molecular biomarkers with a possible prognostic and/or predictive role, we investigated GH-SPA, NFPA, and control samples by applying a two-dimensional-electrophoresis (2DE)/mass spectrometry (MS) approach. Despite patient-dependent variability, we delineated protein-patterns significantly distinguishing the two types of adenomas. Interestingly, a number of identified differences, occurring among these tumors and among them and control subjects, resulted in proteoforms of the same unique proteins, hence suggesting an involvement of co- and post-translational modifications in differentiating the pathological properties of the investigated adenomas. In particular, the functional processing of the identified protein differences evidenced their involvement in cell adhesion pathways, protein folding, and glycolytic and beta-oxidation pathways. Noteworthy, many of identified proteins are mitochondrial factors, hence suggesting an impairment in energy supply and a failure in oxidative stress protection. Some of these factors, by coordinating aerobic glycolysis and tricarboxylic acid cycle, may promote tumor growth and chemoresistance.

** Corresponding author:*

Rossana De Salvo.
Siena University, Via Aldo Moro 2, Siena (Italy).
Tel.: +39 3312290422.
Email address: rossana.desalvo@student.unisi.it (R. De Salvo).

P-14 Proteomics to profile the molecular phenotype of amniotic membrane mesenchymal stromal cells (hAMSCs) secretome

Alexandra Muntiu[a], Andrea Papait[b,c], Federica Vincenzoni[b,d], Diana Valeria Rossetti[a], Alberto Vitali[a], Valentina Marassi[e], Giampiero La Rocca[f], Barbara Roda[e], Tullia Maraldi[g], Pietro Romele[h], Anna Cargnoni[h], Antonietta Silini[h], Ornella Parolini[b,c], Claudia Desiderio[a]*

[a]Istituto di Scienze e Tecnologie Chimiche "Giulio Natta" (SCITEC), Consiglio Nazionale delle Ricerche, Rome, Italy
[b]Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy
[c]Department of Life Science and Public Health, Università Cattolica del Sacro Cuore, Rome, Italy
[d]Dipartimento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, Rome, Italy
[e]Department of Chemistry, University of Bologna, Bologna, Italy
[f] Department of Biomedicine, Neuroscience, and Advanced Diagnostics, University of Palermo, Palermo, Italy
[g]Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy
[h]Centro di Ricerca E. Menni, Fondazione Poliambulanza, Brescia, Italy

The hAMSCs secretome exhibits potent immunomodulatory and pro-regenerative actions, as demonstrated in *in vivo* disease models where altered immune responses played a pivotal role. In this regard, identifying the secreted factors responsible for these therapeutic actions is of paramount importance [1-3]. To this end, we have employed combined proteomic platforms to elucidate the protein and peptide content of the secretome. 10 kDa FASP modified protocol was applied for analysis of the whole secretome and its EVs fractions by LC-MS proteomics in bottom-up approach. Differently, the FASP filtrate (<10 kDa fraction) was submitted to direct LC-MS analysis by top-down approach. 137 proteins repeatedly characterized the hAMSCs whole secretome and showed the enrichment of Immune system, Hemostasis and Extracellular Matrix Organization pathways and protein domains with a role in extracellular matrix and in cytoskeletal signaling and dynamics. Top-down proteomic analysis identified peptides and protein fragments repeatedly characterizing the whole secretome which evidenced pre-/post-secretion proteolytic events. The analysis of EVs fractions, native FFF obtained, identified 200 shared proteins and exclusive elements, which were submitted to GO analysis and Extracellular Exosome classification. Top-down analysis found albumin and pancreatic alpha-amylase peptide fragments marking EVs fractions. These data provided a comprehensive characterization of the hAMSCs secretome combining different approaches and providing unexplored insights into the molecular features of this intriguing bio-product.

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**Corresponding author:*
Claudia Desiderio
Istituto di Scienze e Tecnologie Chimiche (SCITEC) "Giulio Natta"
Consiglio Nazionale delle Ricerche,
Largo F.Vito 1, 00168, Rome, Italy
E-mail address: claudia.desiderio@scitec.cnr.it (C. Desiderio)

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P-15

A Meta-omics approach to assess the *Lactobacillus casei* effects on gut microbiota modulation in healthy C-section newborns

Marta Di Michele[a*], Pamela Vernocchi[a], Federica Del Chierico[a], Giuseppe Pontrelli[b], Francesca Rocchi[b], Ravà Lucilla[c], Silvia Porta[d], Walter Fiore[d], Paolo Rossi[b], Lorenza Putignani[e]

[a]Immunology, Rheumatology and Infectious Diseases Research Area, Unit of Human Microbiome, Bambino Gesù Children’s Hospital, IRCCS, Rome, Italy
[b]Academic Department of Pediatrics, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; University of Tor Vergata, Rome, Italy
[c]Unit of Epidemiology and Biostatistics, Bambino Gesù Children’s Hospital, IRCCS, Rome, Italy
[d]Sofar S.p.A., Trezzano Rosa, Milan, Italy
[e]Unit of Microbiology and Diagnostic Immunology, and Immunology, Rheumatology and Infectious Diseases Research Area, Unit of Human Microbiome, Bambino Gesù Children’s Hospital, IRCCS, Rome, Italy

The different types of delivery modes play a crucial role on the establishment of early gut microbiota (GM) colonization in newborns. Infants born by caesarean section (CS) have an altered microbial colonization and a reduced growth and biodiversity of GM beneficial bacteria, leading to subsequent health outcomes. Our aim was to determine the *Lactobacillus casei* DG® probiotic activity on GM modulation in healthy CS newborns within 48 hours after birth, to assess the probiotic persistence in the gut and to evaluate the effect on GM ecological and functional profile. A randomized, double-blind, placebo-controlled clinical trial was performed. Sixty healthy CS newborns were enrolled and stratified into three birth weight-based groups, each of them was randomized 1:1 with *L. casei* DG® (Tx group) or placebo (Placebo group). Faecal samples were collected during clinical visits at five different time points (V_1 to V_5) over 84 days, and analysed by Real-Time PCR, 16S rRNA targeted-metagenomics and metabolomics by gas chromatography mass spectrometry (GC-MS). A multivariate, univariate and correlation statistical analysis were applied to compare Tx with Placebo over time.

The probiotic *L. casei* DG® was detected in Tx since administration starting point (V_2) until the latest time point (V_5), confirming the ability of the strain to overcome the gastrointestinal barrier and to persist in the gut. The GM ecological and metabolic profiles evidenced an increase of *Lactobacillus* spp., butanoic and propanoic acids in Tx during the first days of intake. A positive correlation ($p \leq 0.05$) was observed in Tx between *L. casei* DG® with *Lactobacillus* and *Granulicatella*; butanoic acid with *Ruminococcus, Prevotella, Collinsella, Faecalibacterium*; propanoic acid with *Oscillospira, Eggerthella*, *Bacteroides* and *Bifidobacterium*. Our meta-omics approach revealed that *L. casei* DG® administration has an effect on the modulation of GM ecology and functional activity, highlighting association between beneficial bacteria and SCFAs.

* *Corresponding author*:
Marta Di Michele.
Bambino Gesù Children’s Hospital, Viale di San Paolo 15, Rome, Italy.
Tel.: +39 68594285;
E-mail address: marta.dimichele@opbg.net (M.Di Michele)

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P-16

Study of *Listeria monocytogenes*: a proteomic and transcriptomic approach

Ivanka Krasteva[a], Federica D'Onofrio[ab*], Maria Schirone[b], Luigi Iannetti[a], Francesco Pomilio[a], Manuela Tittarelli[a], Francis Butler[c], Mirella Luciani[a]

[a]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy;
[b]University of Teramo, Department of Bioscience and Technology, Teramo, Italy;
[c]University College Dublin, School of Biosystems and Food Engineering, Agriculture and Food Science Belfield, Dublin, Ireland

Listeriosis is a zoonosis caused by *Listeria monocytogenes*, which is a foodborne pathogen posing a significant health risk, especially to individuals with weakened immune systems, including pregnant women, newborns, young children, and the elderly. The consumption of several food products has been linked to numerous outbreaks, including raw milk, soft cheeses, processed meats, seafood, and ready-to-eat foods like salads and raw vegetables. One of the concerning aspects of *L. monocytogenes* is its ability to thrive and reproduce in a wide range of environmental conditions, which poses challenges for food safety and contamination control. This adaptability allows the pathogen to persist in conditions with low pH, refrigeration temperatures, and high salt concentrations [1].

The following study aims to identify differentially expressed genes and pathogenic proteins encoded by *L. monocytogenes* during its exposure to stress growth conditions (37°C, 0.5%, pH 7 vs A2 - 12°C, 7.0%, pH 5.5). Bacterial cells were collected during late exponential growth phase to extract, purify and quantify RNA and protein samples as well. Transcripts were analyzed by RNAseq technique while proteins by nLC-MS/MS approach. Proteins identification required a minimum of 2 peptides per protein against the *L. monocytogenes* Uniprot database. Differential expression results were obtained by TopHat and Cufflinks software and were correlated with proteomic data, which were analyzed and clustered using an immunoinformatic pipeline and STRING v11.05, respectively.

By the analysis performed it was identified a specific panel of genes which encoded for several specific proteins associated with cold tolerance (lmo0823), acidic (lmo2005) and oxidative stress. Furthermore all the proteins identified are involved in pathogenic and virulent pathways and most of them are well known to be regulated by *Prfa* gene (lmo2157).
These results are supported by prior literature reports and could be useful for future research focused on understanding the pathogenesis of listeriosis.

* *Corresponding author*:
Federica D'Onofrio.
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy.
Tel.: +39 0861 332487;
fax: +39 0861 332451.
E-mail address: f.donofrio@izs.it (F. D'Onofrio)

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P-17

Untargeted Metabolomics Reveals a Multi-Faceted Resistance Response to Fusarium Head Blight Mediated by the Thinopyrum elongatum Fhb7E Locus Transferred via Chromosome Engineering into Wheat

Giuseppina Fanelli [ab], Ljiljana Kuzmanović [b], Gloria Giovenali [b], Silvio Tundo [c], Giulia Mandalà [b], Sara Rinalducci [a*], Carla Ceoloni [b]

[a]Department of Ecological and Biological Sciences (DEB), University of Tuscia, Viterbo, Italy.
[b]Department of Agriculture and Forest Sciences (DAFNE), University of Tuscia, Viterbo, Italy.
[c]Department of Land, Environment, Agriculture and Forestry (TESAF), University of Padova, Legnaro, Italy.

The Thinopyrum elongatum Fhb7E locus has been proven to confer outstanding resistance to Fusarium Head Blight (FHB) when transferred into wheat, minimizing yield loss and mycotoxin accumulation in grains. Despite their biological relevance and breeding implications, the molecular mechanisms underlying the resistant phenotype associated with Fhb7E have not been fully uncovered. To gain a broader understanding of processes involved in this complex plant-pathogen interaction, we analysed via untargeted metabolomics durum wheat (DW) rachises and grains upon spike inoculation with Fusarium graminearum (Fg) and water. The employment of DW near-isogenic recombinant lines carrying or lacking the Th. elongatum chromosome 7E region including Fhb7E on their 7AL arm, allowed clear-cut distinction between differentially accumulated disease-related metabolites. Besides confirming the rachis as key site of the main metabolic shift in plant response to FHB, and the upregulation of defence pathways (aromatic amino acid, phenylpropanoid, terpenoid) leading to antioxidants and lignin accumulation, novel insights were revealed. Fhb7E conferred constitutive and early-induced defence response, in which specific importance of polyamine biosynthesis, glutathione and vitamin B6 metabolisms, along with presence of multiple routes for deoxynivalenol detoxification, was highlighted. The results suggested Fhb7E to correspond to a compound locus, triggering a multi-faceted plant response to Fg, effectively limiting Fg growth and mycotoxin production.

**Corresponding Author*:
Prof.ssa Sara Rinalducci, Associato di Biologia Molecolare
Università degli Studi della Tuscia, Dipartimento di Scienze Ecologiche e Biologiche (DEB)
Largo dell'Università snc – Viterbo, Italy
Phone: 0761 357101
Email: sara.r@unitus.it (S. Rinalducci)

P-18

Proteomics Cell Surface Profiling of Differentiating Leukemia Cells

Maurine Fucito[a*]

[a] Analytical Chemistry, Department of Chemical, Pharmaceutical and Agricultural Science, University of Ferrara, Italy

Around 70% of modern pharmaceuticals have been developed to target cell surface proteins. Therefore, proteomic profiling of cell surface proteins is highly popular as it leads to the discovery of new biomarkers and drug targets. There are two major barriers from detecting cell surface proteins namely their poor solubility and their low relative abundance compared to intracellular proteins. To overcome these, the Cell Surface Capturing (CSC) technology has been developed to enrich for the cell surface proteins [1]. It consists of biotinylation, i.e. adding biotin, to increase the cell surface proteins polarity, followed by the addition of streptavidin which has affinity for biotinylated proteins. We used this technology on the K562 leukemia cell line. The cells were treated with dimethyl sulfoxide (DMSO), for three days which induced the erythrocytic differentiation and lead to functional erythrocytes. A triplicate of cell pellets with and without the three-day DMSO exposure respectively, was collected and processed with the CSC technology. After the cell surface enrichment, proteins were filter-digested with trypsin and analysed through LC-MS/MS. In this study, we aim to shed light on the regulations of cell surface proteins during the erythrocytic differentiation induced by DMSO and identify new putative biomarkers.

* *Corresponding author*:
Maurine Fucito.
Analytical Chemistry, Department of Chemical, Pharmaceutical and Agricultural Science, University of Ferrara, Italy.
Tel.: +39 3924547653
E-mail address: maurine.fucito@unife.it (M. Fucito)

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P-19

The Neprilysin protease: mitochondrial localization, novel targets, and a possible role in Parkinson's disease

Giulia Gaiferri[a], Vittoria Monaco[b,c], Maria Monti[b,c], Heather Bondi[a,d], Mauro Fasano[a,d], Tiziana Alberio[a,d], and Marta Lualdi[a,d]*

[a] Department of Science and High Technology, University of Insubria, Busto Arsizio (VA), Italy;
[b] Department of Chemical Sciences, University Federico II of Naples, Naples, Italy;
[c] CEINGE Advanced Biotechnologies "Franco Salvatore", Naples, Italy;
[d] Center of Research in Neuroscience, University of Insubria, Busto Arsizio (VA), Italy

Neprilysin is a zinc-dependent endopeptidase ubiquitously distributed. It exists in both transmembrane and soluble catalytically active forms, and it is responsible for the cleavage of angiotensin, bradykinin, substance P, glucagon, insulin β-chain, amyloid-beta, enkephalins and oxytocin. Its role is central both at the periphery and in the central nervous system [1]. In 2019, we developed and applied the mitochondrial dimethylation-TAILS (Terminal Amine Isotopic Labeling of Substrates) degradomics approach in a cellular model of Parkinson's disease (PD) mimicking dopamine dyshomeostasis in SH-SY5Y cells [2]. This led us to the identification of neprilysin as a candidate protease involved in mitochondrial dysfunction related to PD. Thus, we decided to further investigate its role within mitochondria.

First, by using a mitochondrial sub-fractionation protocol and immunofluorescence we demonstrated for the first time that neprilysin displays mitochondrial localization. More specifically, it is present as a soluble protein in the mitochondrial matrix. In addition, when dopamine dyshomeostasis was induced, the fraction of protein co-localized with mitochondria significantly increased. Second, we applied the dimethylation-TAILS approach on the total proteome of SH-SY5Y cells, to identify the complete set of substrates of neprilysin in this cellular model. We obtained a list of 209 substrate proteins, that we used as input to perform a functional enrichment analysis, using Reactome as reference pathway database. The significantly enriched pathways resulted to be related to protein translation, ribosomal function, processing of RNAs and proteins, and neurodegeneration. To identify the mitochondrial substrates of neprilysin, we cross-checked the list of neprilysin targets with the MitoCarta2.0 database and we identified twenty mitochondrial targets, including some ribosomal proteins, heat shock proteins, membrane transporters, and metabolic enzymes.

In conclusion, we demonstrated that neprilysin exists as a mitochondrial protease and may play a role in mitochondrial dysfunction linked to dopamine imbalance in PD.

* *Corresponding author*:
Marta Lualdi.
Department of Science and High Technology, University of Insubria, Busto Arsizio (VA), Italy.
Tel.: +39 0331 339423;
E-mail address: marta.lualdi@uninsubria.it (M. Lualdi)

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P-20

Triple negative breast cancer: novel therapeutic strategies investigated by proteomics

Sveva Germini[a,b], Serena Camerini[a] , Irene Ruspantini[a], Maria E. Pisanu[a], Mattea Chirico[a], Sara Baccarini[a], Rosa Vona[a], Donatella Pietraforte[a], Egidio Iorio[a], , Marialuisa Casella[a]*

[a] Istituto Superiore di Sanità, Rome, Italy;
[b] Università di Tor Vergata, Dipartimento di Medicina Sperimentale e dei Sistemi, Rome, Italy;

Triple negative breast cancer (TNBC) is the most aggressive, with the poorest prognosis, of all BC subtypes. TNBC lacks estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2; hormonal therapies have no effect and chemotherapy resistance develops [1]. Metformin (MF), an anti-diabetic drug, is known to lower cancer incidence. This anti-cancer activity is not completely understood. We are investigating a novel drug repurposing strategy coupling MF with D609 (inhibitor of phosphatidylcholine-specific phospholipase C, PC-PLC [2]). Preliminary results on MDA-MB-231 cells reveal that the combination treatment (MF and D609) inhibits cells migration more powerfully compared to the effect due to MF alone and that PC-PLC inhibition by D609 enhances the anti-proliferative effects exerted by MF singularly. We investigated the proteome changes following MF treatment alone and/or in association with D609, using qualitative and quantitative (label-free) LC-MS/MS bottom-up proteomic approaches carried on applying both data-dependent (DDA) and data-independent (DIA) acquisition methods. We identified and quantified more proteins in samples acquired in DIA mode with a reduction in the number of missing values, compared to DDA mode. Principal Component Analysis of the acquired data groups together MF and combination treatment, separating them from control and D609; this result suggests that MF is the key modulator of the proteome in the combined treatment. MF and combination treatment up- and down-regulate the concentration of several proteins with a greater number recognized from DIA data. Enrichment analysis of the regulated proteins derived from DIA shows more significantly enriched pathways than DDA. Oxidative phosphorylation and cell cycle are two of the most significant up- and down-regulated pathways respectively, for both MF and combination treatment. Moreover, these two conditions share several pathways in agreement with the observed predominant MF effect. Additional proteomic and phosphoproteomic experiments are in progress together with metabolomic analyses: the integration of these –omics data will show a multifaceted mechanism of action of the drug repurposing strategy on TNBC cells.

* *Corresponding author*:
Marialuisa Casella. PhD
Core Facilities, Istituto Superiore di Sanità, Rome, Italy.
Tel.: +390649902294.
E-mail address: marialuisa.casella@iss.it (M. Casella)

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P-21

Plasma-Based Proteomics Profiling in Patients with Hyperplasia and Endometrial Cancer

Hicham Benabdelkamel[a]*, Khalid Akkour[b], Assim A. Alfadda[a,c], Hani Alhalal[b], Afshan Masood[a], Moudi A. Alwehaibi[a] , Maria Arafah[d] , Eman Alshehri[b], and Ibrahim O. Alanazi[e]

[a] Proteomics Resource Unit, Obesity Research Center, College of Medicine, King Saud University, Riyadh 11461, Saudi Arabia.
[b] Obstetrics and Gynecology Department, College of Medicine, King Saud University, Riyadh 11461, Saudi Arabia.
[c] Department of Medicine, College of Medicine and King Saud Medical City, King Saud University, Riyadh 11461, Saudi Arabia.
[d] Department of Pathology, College of Medicine, King Saud University, King Saud University Medical City, Riyadh 11461, Saudi Arabia.
[e] The National Center for Biotechnology (NCB), Life Science and Environment Research Institute, King Abdulaziz City for Science and Technology (KACST), Riyadh 11442, Saudi Arabia.

Uterine cancers are among the most prevalent gynecological malignancies, and endometrial cancer (EC) is the most common in this group. This study used plasma-based proteomic profiling analysis in patients with endometrial cancer and hyperplasia, and control patients. Conventional 2D gel electrophoresis, followed by a mass spectrometry approach with bioinformatics, including a network pathway analysis pipeline, was used to identify differentially expressed proteins and associated metabolic pathways between the study groups. Thirty-six patients (12 with endometrial cancer, 12 with hyperplasia, and 12 controls) were enrolled in this study. The mean age of the participants was 46–75 years. 120 proteins were significantly differentially expressed between the study groups, of which 85 were significantly differentially regulated (37 upregulated and 48 downregulated) in the plasma samples of EC patients compared to the control (Ctrl). Furthermore, 81 proteins were significantly dysregulated (37 upregulated and 44 downregulated) in plasma samples of hyperplasia (HY) patients compared to Ctrl. Sixty-two proteins (8 upregulated and 54 downregulated) in the EC group compared to the HY group. The functional category showed that most of the differentially expressed proteins identified were enzymes with catalytic activity (43%) followed by Binding (39%). The PPI network of differentially expressed proteins between EC and control constructed through the STRING database. The panels of protein markers identified in this study could be used as potential biomarkers for distinguishing between EC and HY and early diagnosis and progression of EC from hyperplasia and normal patients.

* *Corresponding author:*
Dr. Hicham Benabdelkamel, PhD.
Proteomics Resource Unit,
Obesity Research Center, College of Medicine,
King Saud University, Riyadh, Saudi Arabia.
Email: helkamel@ksu.edu.sa (H. Benabdelkamel)

P-22

Detection of the α-hemoglobin stabilizing protein in the oral fluid of preterm newborns by a top-down proteomic pipeline.

Federica Iavarone[a,e,*], Chiara Tirone[b], Simona Fattore[b], Davide De Tomaso[b], Nicoletta Menzella[b], Giovanni Vento[b], Alessandra Olianas[c], Barbara Manconi[c], Tiziana Cabras[c], Irene Messana[c], Gavino Faa[c], Massimo Castagnola[d].

[a]Dipartimento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, Rome, Italy;
[b]Neonatology Unit, Department of Woman and Child Health and Public Health, Fondazione Policlinico Universitario "Agostino Gemelli", Dipartimento di Scienze della Vita e Sanità Pubblica, Facoltà di Medicina e Chirurgia, Università Cattolica del Sacro Cuore, Rome, Italy
[c]Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy
[d]Centro Europeo di Ricerca sul Cervello-IRCCS Fondazione Santa Lucia, Rome, Italy[e] Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Italy

The oral fluid of preterm new-borns is commonly a waste specimen, aspired and eliminated during the management of the baby in the NICU (Neonatal Intensive Care Unit) to avoid its dangerous pouring inside the bronchus and the lung. For this reason, it is a sample ethically utilizable for analytical aims and the Ethical Committee of our Universities authorized this research. Sometimes the newborns that are assisted upon the NICU can have less of 180 days of post conceptional age (PCA) [1]. Therefore, these specimens, especially when serially collected, can give precious information to investigate the molecular events occurring in last period of fetal development in the period spanning from 6 months of PCA until the normal time of delivery. During the analyses, carried out on the preterm oral fluid investigated by a high-resolution HPLC-MS approach (top-down pipeline) we were able to detect the **α-hemoglobin stabilizing protein** (AHSP), an erythroid-specific protein. AHSP forms a stable complex with free α-hemoglobin but not with β-hemoglobin or hemoglobin A ($\alpha_2\beta_2$); it was found few years ago by Kihm and colleagues using a screening for genes induced by the essential erythroid transcription factor GATA-1 [2]. However, our MS data were incongruent with the protein sequence reported in literature. Indeed, the protein has a monoisotopic mass $[M+H^+]^{1+}$ = 11744.958 *m/z* and this mass as well the CID and HCD MS/MS fragmentation patterns of the intact protein agree with N-terminal methionine residue loss and N-terminal acetylation, a post-translational modification very common in secretory proteins. Samples having sensible amounts of AHSP resulted from preterm newborns with several transient hematological problems. Since this data have not statistical significance, further studies are necessary to verify if AHSP can be utilized as an early marker of several neonatal hematological diseases.

* *Corresponding author*:
Federica Iavarone
Dipartimento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, Roma, Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Facoltà di Medicina, Università Cattolica, Largo F. Vito 1, 00168, Roma, Italy.
Tel. and/or Fax: ++39-06-3053598;
e-mail: federica.iavarone@unicatt.it (F. Iavarone)

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P-23

UnravelinG the proteomic signature of prostate cancer evolution from normaL to ADvanced castration-resIstAnT phenOtypes: a diffeRential proteomic approach (GLADIATOR)

Erika Iervasi[a]*, Camillo Rosano[a], Marco Ponassi[a], Aldo Profumo[a], Gabriela Coronel Vargas[a], Tiziana Bachetti[a], Anna Aprile[a], Paolo Romano[a], Paola Barboro[a]

[a] U.O. Proteomica e Spettrometria di massa - IRCCS Ospedale Policlinico San Martino, Genova, Italy

Prostate cancer (PCa) is a heterogeneous and multifactorial disease. Today in Italy it remains the fourth cause of death (5.9% mortality, 2020). The development of the primary tumor starts from some forms of benign prostatic hypertrophy (BPH) which can progress to an advanced stage and then into metastasis. The mainstay treatment for androgen-sensitive primary tumors and metastatic PCa is androgen deprivation therapy (ADT). However, tumor cells can become resistant to treatment and the disease progresses to castration-resistant PCa (CRPC) [1]. Currently, the only circulating marker used in clinical practice to monitor resistance to mPCa therapy is prostate specific antigen (PSA). For this reason, the aim of our research is the identification of new markers that can follow the evolution of phenotypic changes in different conditions: from healthy control to the advanced stage of the tumor. This result will allow us to have a hypothetical time map for diagnostic/prognostic purposes and to act with a more adequate therapeutic strategy. The characterization of a group of proteins that can be down- or over-expressed will constitute the key points of the qualitative and quantitative screening panel. Our future objectives will be proteomic and bioinformatics analyzes on tumor phenotypes compared to controls and the hyperplastic condition, with the aim of identifying new potential biomarkers that allow us to understand in detail the progress of tumor progression. The same approach will be applied to try to understand the metastatic condition.
We have selected specific cell lines that mimic prostate cancer at different stages. A differential proteomic analysis was performed using a bottom up approach. Tandem mass spectrometry analysis was performed on an Ultimate 3000 nanochromatography system, equipped with a PepMap RSLC18 column (75µm x 50cm) (Thermo). After separation, the flow was sent directly to an Easyspray source connected to an Exactive Plus Orbitrap Q mass spectrometer (both Thermo). Xcalibur software (version 4.1) (Thermo) was used to operate the UHPLC/HR-MS. Raw data were analyzed with Proteome Discoverer 2.4.1.15 software (Thermo). DEP identification analysis was performed using protein linear models combined with empirical Bayes statistics and the Limma.9 package and their expression was compared with the LNCaP reference line.

* *Corresponding author*:
Erika Iervasi.
Genova IRCCS Ospedale Policlinico San Martino, UO Proteomica e Spettrometria di massa, Largo Rosanna Benzi, Genova Italy.
Tel.: +39 3467859300;
E-mail address: erika.iervasi@hsanmartino.it (E. Iervasi)

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P-24

Proteomic and acetylomic profiles explaining the role of RICTOR/mTORC2 downregulation in BRAF^{V600E} melanoma cells

Lorenza Vantaggiato[a], Enxhi Shaba[a], Luca Ponzone[b,c], Luca Bini[a], Enzo Calautti[b,c] , Claudia Landi[a*]

[a] Functional Proteomic Section, Department of Life Sciences, University of Siena, Siena, Italy
[b] Molecular Biotechnology Center "Guido Tarone", University of Turin, Turin, Italy
[c] Department of Molecular Biotechnology and Health Sciences, University of Turin, Turin, Italy

Malignant cutaneous melanoma (MM) is the deadliest form of skin cancer characterized by an activating mutation of BRAF (BRAFV600E) in approximately 50% of MM. The combination of BRAF- and MEK-inhibitors (BRAF/MEKi) is frequently used for their clinical management but one major drawback is the rapid development of therapeutic resistance which often converge on the upregulation of mitochondrial bioenergetics and NAD+ biosynthetic pathways. During tumor progression, the mechanistic target of rapamycin complex 2 (mTORC2) is frequently activated, with important impacts on cancer cell growth and metabolism, therefore, it is generally regarded as an oncogenic driver and a prospective therapeutic target in many tumors, including MM. Contrary to this mainstream view, bioinformatics analysis of TCGA melanoma patients' database revealed that low Rictor levels in tumors, an essential subunit of mTORC2, correlate with an overall worse clinical outcome. GSEA analysis of low-RICTOR tumors also evidenced a gene expression signature suggestive of activation of mitochondrial energy producing pathways playing a key role in the metabolic reprogramming miming mechanisms of resistance to BRAFi/MEKi of MM cells. In light of these, we propose a differential proteomic study on MM BRAFV600E cells with decreased Rictor expression *vs* control cells, in order to defining the mechanistic bases of the functions of Rictor/mTORC2 in melanoma M14 cells. Proteomic analysis reveals proteins related to oxidative stress protection, mitochondrial functions and NAD+ biosynthesis. Because enhanced mitochondrial respiration is a hallmark of the BRAF/MEKi-resistance, we found the changes in proteins involved in TCA cycle and electron transfer chain (ETC) of particular interest. Since we also highlighted molecular pathways leading to the production of acetil Co-A, we suppose a modulation of intracellular acetate confirmed by cytosolic and mitochondrial analyses. Diverse levels of intracellular acetate in Rictor deficient cells lead us to suppose a differential proteome modulation by acetylation confirmed by the acetylomic analysis.
Taken together these results shed light on potential mechanisms of resistance to the target therapy mimed by mTORC2 silencing, also highlighting new molecular targets for MM therapy.

* *Corresponding author*:
Claudia Landi PhD,
University of Siena, Via Aldo Moro 2 53100 Siena, Italy.
Tel: +39 0577232449
E-mail address: claudia.landi@unisi.it (C. Landi)

P-25

Supplementation With Uric And Ascorbic Acid Protects Stored Red Blood Cells Through Enhancement Of Non-Enzymatic Antioxidant Activity And Metabolic Rewiring

Vassilis L Tzounakas [a], AlkminiT Anastasiadi [a], Vasiliki-Zoi Arvaniti [a], Veronica Lelli [b], Giuseppina Fanelli [b], Efthymios C Paronis [c], Anastasia C Apostolidou [c], Evangelos G Balafas [c], Nikolaos G Kostomitsopoulos [c], Effie G Papageorgiou [d], Issidora S Papassideri [a], Konstantinos Stamoulis [e], Anastasios G Kriebardis [d], Sara Rinalducci [b*], Marianna H Antonelou [a]

[a] Department of Biology, School of Science, National and Kapodistrian University of Athens (NKUA), Athens, Greece.
[b] Department of Ecological and Biological Sciences, University of Tuscia, Viterbo, Italy.
[c] Center of Clinical, Experimental Surgery & Translational Research, Biomedical Research Foundation, Academy of Athens (BRFAA), Athens, Greece.
[d] Laboratory of Reliability and Quality Control in Laboratory Hematology (HemQcR), Department of Biomedical Sciences, School of Health & Welfare Sciences, University of West Attica (UniWA), Egaleo, Greece.
[e] Hellenic National Blood Transfusion Centre, Acharnes, Greece.

Redox imbalance and oxidative stress have emerged as generative causes of the structural and functional degradation of red blood cells (RBC) that happens during their hypothermic storage at blood banks. The aim of the present study was to examine whether the antioxidant enhancement of stored RBC units following uric (UA) and/or ascorbic acid (AA) supplementation can improve their storability as well as post-transfusion phenotypes and recovery by using in vitro and animal models, respectively. For this purpose, 34 leukoreduced CPD/SAGM RBC units were aseptically split in 4 satellite units each. UA, AA or their mixture were added in the three of them, while the fourth was used as control. Hemolysis as well as redox and metabolic parameters were studied in RBC units throughout storage. The addition of antioxidants maintained the quality parameters of stored RBCs, (e.g., hemolysis, calcium homeostasis) and furthermore, shielded them against oxidative defects by boosting extracellular and intracellular (e.g., reduced glutathione; GSH) antioxidant powers. Higher levels of GSH seemed to be obtained through distinct metabolic rewiring in the modified units: methionine-cysteine metabolism in UA samples and glutamine production in the other two groups. Oxidatively-induced hemolysis, reactive oxygen species accumulation and membrane lipid peroxidation were lower in all modifications compared to controls. Moreover, denatured/oxidized Hb binding to the membrane was minor, especially in the AA and mix treatments during middle storage. The treated RBC were able to cope against pro-oxidant triggers when found in a recipient mimicking environment in vitro, and retain control levels of 24h recovery in mice circulation. The currently presented study provides (a) a detailed picture of the effect of UA/AA administration upon stored RBCs and (b) insight into the differential metabolic rewiring when distinct antioxidant "enhancers" are used.

**Corresponding Author*
Sara Rinalducci
Università degli Studi della Tuscia, Dipartimento di Scienze Ecologiche e Biologiche (DEB)
Largo dell'Università snc – Viterbo, Italy
Email: sara.r@unitus.it (S.Rinalducci)

P-26 Gut microbiota functions in autism spectrum disorders: potential metabolic biomarkers

Pamela Vernocchi[a], Chiara Marangelo[b*], Silvia Guerrera[b], Federica Del Chierico[a], Valerio Guarrasi[c], Simone Gardini[c], Federica Conte[d], Paola Paci[e], Gianluca Ianiro[f], Antonio Gasbarrini[f], Stefano Vicari[b], Lorenza Putignani[g]

[a]Research Unit of Human Microbiome, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; [b]Child and Adolescent Neuropsychiatry Unit, Department of Neuroscience, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; [c]GenomeUp, Rome, Italy; [d]Institute for Systems Analysis and Computer Science "Antonio Ruberti", National Research Council, Rome, Italy; [e]Department of Computer, Control and Management Engineering, Sapienza University of Rome, Rome, Italy; [f]CEMAD Digestive Disease Center, Fondazione Policlinico Universitario "A. Gemelli" IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy; [g]Unit of Microbiomics and Research Unit of Human Microbiome, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

Autism Spectrum Disorder (ASD) is a multifactorial disorder of neurological development characterized by intellectual and language impairment. ASD patients frequently show gastrointestinal (GI) symptoms. The bidirectional communication between the gut and the brain is driven by the gut microbiota (GM). Herein, we describe the alteration in GM metabolomic profile and its functions of ASD patients, considering clinical, anamnestic and nutritional variables. Forty-one and 35 faecal samples collected from ASDs and neurotypical children (CTRLs), respectively, (age range, 3–15 years) were analysed by gas-chromatography mass spectrometry coupled with solid phase micro extraction (GC-MS/SPME) [1]. Untargeted metabolomics data were correlated with operational taxonomic units (OTUs) from targeted-metagenomics [2] and with subjects' metadata by computational models to discover new potential biomarkers for ASD patients on the basis of GM patterns. Statistical analysis were applied for omics and clinical data to compare ASD cohort versus CTRLs also considering the patient's potential cofounding factors as: GI symptoms, autism severity symptoms and Child Behavior Checklist (CBCL) internalizing (INT) and externalizing (EXT) scales. The ASD gut metabolic profile, compared with CTRLs, was characterized by over-expressed levels of SCFAs, ketones, indoles, alcohols and aldehydes. Interestingly, ASD without GI symptoms and with low autism severity symptoms showed higher value of indole thus increasing its protection activity against gut inflammation. Moreover, 2-decanol, butanoic acid-ethyl ester, hexanoic acid, propanoic acid, 2-tridecanol, butanoic acid- propyl ester, butanoic acid- butyl ester and 2-pentadecanone were associated to ASD at risk for CBCL-EXT compared to ASD without clinical CBCL-EXT symptoms. In addition, 2-pentananone and 2-butanone were higher in patients at risk CBCL-INT than ASD with no clinical CBCL-INT symptoms. In conclusion, our findings highlighted the presence of altered microbial metabolites, with potential neuroactive effects that could lead to new insights into ASD progression, as well as the discovery of new risk predictors.

** Corresponding author:*
Chiara Marangelo
Research Unit of Human Microbiome, Bambino Gesù Children's Hospital, IRCCS
Viale di San Paolo 15 - 00146 – Rome
Tel.: +39 3403061748
E-mail address: chiara.marangelo@opbg.net (C. Marangelo)

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P-27

The assessment of gut microbiota modulation in patients after ischemic stroke

Riccardo Marsiglia [a*], Chiara Marangelo[a], Pamela Vernocchi[a], Stefania Pane[a], Alessandra Russo[a], Eleonora Guanziroli[c], Franco Molteni[c], Lorenza Putignani[b]

[a] Immunology, Rheumatology and Infectious Diseases Research Area, Unit of Human Microbiome, Bambino Gesù Children's Hospital, IRCCS, 00146 Rome, Italy, [b]Unit of Microbiology and Diagnostic Immunology, Rheumatology and Infectious Diseases Research Area, Unit of Human Microbiome, Bambino Gesù Children's Hospital, IRCCS, 00146 Rome, Italy, [c]Villa Beretta Rehabilitation Center, Costa Masnaga, Italy

Neurological damage as ischemic stroke can be caused by perturbation of the gut-brain axis, a bidirectional communication system between the gut and the brain. Dysbiosis is caused by an imbalance in the gut microbiota (GM), which also may be linked to several risk factors for ischemic stroke, such as diabetes, obesity, and hypertension. In particular, the GM can influence the brain through the production of different metabolites such as short chain fatty acids (SCFAs), indole and 3-methyl indole (skatole) that could be involved in stroke outcomes. The aim of this study was to examine changes in metabolic activities related to GM after stroke event and their possible associations with clinical values. Faecal samples of 10 patients after ischemic stroke and 6 healthy subjects controls (CTRLs) were analysed. The samples have been evaluated in term of ecological profile by 16S rRNA and for functional profile by metabolomics analysis performed with gas chromatograph coupled to a mass spectrometer (GC-MS), while the zonulin, marker of gut permeability, was detected by ELISA. Furthermore, each metaomic dataset underwent univariate and multivariate statistical analysis, and their correlations with clinical features and biochemical variables were evaluated using correlation analysis and nonparametric tests. GM ecology composition, evaluated by the study of the Amplicon Sequence Variant (ASV), showed that Ruminococcaceae, Clostridiaceae and *Oscillospira* were statistically associated with CTRLs, while *Blautia*, *Akkermansia* and *Sutterella,* recognized as SCFA-producing bacteria, were related to patients after stroke event. The metabolomic analysis revealed that the profile of GM in stroke patients was characterized by high concentrations of SCFAs, that seems to be implicated in the amplification of the inflammatory process. Moreover, all patients revealed a complete reduction of indole compared to CTRLs. The results of this study highlighted the presence of altered microbial metabolites, especially of SCFAs concentrations. This signature may be a potential biomarker profile for ischemic stroke and could explain a possible relation with patients' prognosis and in clinical outcome.

** Corresponding author:*
Riccardo Marsiglia.
Bambino Gesù Children's Hospital, IRCCS, Unit of Human Microbiome, Rome, Italy
Tel: 3661023867
E-mail address: riccardo.marsiglia@opbg.net (R. Marsiglia)

P-28

The metaproteome of the gut microbiota in pediatric patients affected by COVID-19

Valeria Marzano[a*], Federica Rapisarda[a], Chiara Marangelo[a], Stefano Levi Mortera[a], Antonia Piazzesi[a], Pamela Vernocchi[a], Stefania Pane[b], Lorenza Romani[c], Paolo Palma[d,e], Lorenza Putignani[f] and the Cactus Study Team

[a]Research Unit of Human Microbiome, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; [b]Unit of Microbiomics, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; [c]Unit of Infectious Disease, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; [d]Research Unit of Congenital and Perinatal Infections, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy; [e]Chair of Pediatrics, Department of Systems Medicine, University of Rome "Tor Vergata", Rome, Italy; [f]Unit of Microbiomics and Research Unit of Human Microbiome, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

The gut microbiota (GM) play a significant role in the infectivity and severity of COVID-19 infection [1]. Children with COVID-19 present milder symptoms and are at lower risk of hospitalization and life-threatening complications [2]. Thus, the aim of this study was to highlight the relationship among the composition of GM and COVID-19 to uncover the biological mechanisms that may underlie the protective effect of the GM.

LC-MS/MS analyses were performed on stool samples collected from 21 paediatric patients (COVID-19s) and 21 age-and sex-matched healthy subjects, acting as controls (CTRLs), to evaluate GM metaproteome signatures by label free quantification of bacterial protein groups (PGs), along with functional annotations via COG and KEGG databases and taxonomic assignment through the use of the lowest common ancestor algorithm.

By t-test univariate analysis, COVID-19s exhibited differential expression of 698 PGs in comparison to CTRLs, whose clustering distinctly separates the two groups. Our data demonstrated how SARS-CoV-2 infection affects the functionality of the GM at multiple levels. Specifically, it modifies metabolic pathways, including those associated with tryptophan, and impacts the mechanisms of antibiotic resistance and bacterial virulence. Many of these pathways are associated with protective bacterial GM species, and were also negatively correlated with clinical parameters known to increase with COVID-19 severity, such as white blood cell count.

These findings highlight the mechanisms by which the pediatric GM could contribute to protection against the more severe manifestations of the disease in children. To our knowledge, this is the first study to exclusively investigate the pediatric metaproteome in patients infected with SARS-CoV-2, uncovering new metabolic profiles relevant to the development of adjuvant therapies for COVID-19.

* *Corresponding author*:
Valeria Marzano, PhD
Bambino Gesù Children's Hospital, IRCCS,
Viale Ferdinando Baldelli 38, 00146 Rome, Italy
Tel.: +39.066859/4061/4285/4372
E-mail address: valeria.marzano@opbg.net (V.Marzano)

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P-29 Mass spectrometry-based phosphoproteomics reveals a key role of the WEE1-CDK1 axis in mediating tyrosine-kinase inhibitors resistance in AML patients.

Giorgia Massacci[a*], Veronica Venafra[b], Sara Latini[b], Valeria Bica[b], Giusj Monia Pugliese[a], Simone Graziosi[b], Felix Klingelhuber[c], Natalie Krahmer[c], Thomas Fischer[d,e], Dimitrios Mougiakakos[f], Martin Boettcher[d,e], Livia Perfetto[g] and Francesca Sacco[a,h*]

[a] Department of Biology, University of Rome Tor Vergata, Rome, Italy.
[b] PhD Program in Cellular and Molecular Biology, Department of Biology, University of Rome "Tor Vergata", Rome, Italy.
[c] Institute for Diabetes and Obesity, Helmholtz Zentrum München, German Center for Diabetes Research, Neuherberg, Germany.
[d] Institute of Molecular and Clinical Immunology, University of Magdeburg, Magdeburg, Germany.
[e] Healthcampus for Inflammation, Immunity and Infection (GCI3), University of Magdeburg, Magdeburg, Germany.
[f] Department of Hematology and Oncology, University of Magdeburg, Magdeburg, Germany.
[g] Department of Biology, Fondazione Human Technopole, Milan, Italy.
[h] Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy

Despite the development of new therapeutic strategies combining standard chemotherapy and targeted therapies, Acute myeloid Leukaemia (AML) continues to be a devastating disease with a 5-year survival rate lower than 25%. Internal tandem duplications (ITDs) in the FLT3 gene occur in about 30% of AML patients and are generally associated with a very poor prognosis characterized by high relapse rate and short remission time (1). Even if the molecular landscape of the ITD mutations is complex and characterized by high heterogeneity, all FLT3-ITD positive patients receive the same treatment consisting of the combination of chemotherapy and the multikinase inhibitor midostaurin. Remarkably, a recent retrospective study revealed that the favorable impact of midostaurin treatment is specifically limited to patients harboring the insertion in the juxtamembrane domain ($FLT3^{ITD\text{-}JMD}$), whereas those with the mutation in the tyrosine kinase domain ($FLT3^{ITD\text{-}TKD}$) exhibited chemoresistance and decreased overall survival (2). Here, we employed deep sequencing and mass spectrometry-based (phospho)proteomics to perform the first unbiased multi-layered analysis aimed at deciphering the molecular basis underlying these distinct clinical phenotypes. Integrating transcriptomic, proteomic and phosphoproteomic data with prior knowledge network, by using the newly developed *Signaling Profiler* computational pipeline (3), we provided a global and accurate map of the signaling rewiring occurring in FLT3-ITD cells upon tyrosine kinase inhibitors treatment. Our approach revealed a novel mechanism of resistance mediating by the WEE1-CDK1 axis in FLT3-ITD positive cells and we demonstrated that the pharmacological inhibition of the WEE1 kinase significantly increased midostaurin sensitivity of $FLT3^{ITD\text{-}TKD}$ cells and patient-derived blasts.

* *Corresponding author*:
Prof. Francesca Sacco
University of Rome Tor Vergata, Rome, Italy.
Tel.: +39 0672594305;
E-mail address: francesca.sacco@uniroma2.it (F. Sacco)

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P-30 Redox status of critical disulfides of SARS-CoV-2 receptor-binding-domain exposed to bioactive chomophore phycocyanobilin

Ana Simovic[a], Masa Bicanin[a], Mirjana Radomirovic[a], Jelena Acimovic[a], Dragana Mitic[b*], Dragana Stanic-Vucinic[a], Tanja Cirkovic Velickovic[a,c,d,e]

[a] Center of Excellence for Molecular Food Sciences, Department of Biochemistry, University of Belgrade – Faculty of Chemistry, Belgrade, Serbia
[b] Microprot Lab, Innovation Center of the Faculty of Chemistry, Ltd., Belgrade, Serbia
[c] Serbian Academy of Sciences and Arts, Belgrade, Serbia
[d] Department of Food Technology, Safety, and Health, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium
[e] Center for Food Chemistry and Technology, Ghent University Global Campus, Incheon, Republic of Korea

The emergence of the novel coronavirus SARS-CoV-2 has attracted the attention of the whole scientific community. However, as there are significant concerns regarding the effectiveness of vaccines and drugs against novel SARS-CoV-2 variants, naturally derived broad-spectrum of antivirals seems to be precious adjuvant agents to assist in combat against this disease. Phycocyanobilin (PCB) is an open-chain tetrapyrrole chromophore of phycocyanin (PC), chromoprotein derived from Spirullina, with strong anti-oxidative action. The role of disulfide bonds and thiol-disulfide balance in RBD is considered to play a significant role in the binding of S protein to ACE2 receptor. In RBD, in contrast to C480–C488 disulfide, which is thermodynamically stable, C379–C432 and C391–C525 disulfides are in dynamic equilibrium with their thiol states and, thus these two pairs of disulfides are more sensitive to changes in redox poise. Our study aimed to investigate impact of PCB on disulfide balance of RBD by redox proteomics and to investigate structural changes in the protein exposed to PCB. The effect of PCB on RBD secondary structures was examined by far-UV CD spectroscopy after titration of RBD with increasing concentrations of PCB. The presence of PCB had a pronounced effect on the spectral shape. RBD is dominantly composed of random coils and β-sheets. In the presence of PCB a slight increase of α-helical and random coils content, while the content of β-sheets and β-turns is decreased. Mapping redox-active disulfides and reactive cysteines in recombinant SARS-CoV-2 RBD was done using redox proteomics on both recombinant RBD and PCB-exposed RBD. A mass shift caused by alkylation of free Cys residues was detected on three Cys residues demonstrating disulfides C379–C432 and 432-391 to be semi-stable in both RBD and PCB-exposed RBD. Our results demonstrate that RBD exposed to PCB undergo structural changes but does not change the redox state of its critical semi-stable disulfides.
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**Corresponding author*:
Dragana Mitic.
Innovation Center of the Faculty of Chemistry, Ltd., Belgrade, Serbia.
E-mail address: dmitic@chem.bg.ac.rs (D. Mitic)

P-31 Preliminary comparison of the salivary peptidome of patients with newly diagnosed and recurrent glioblastoma multiforme by a top-down proteomic pipeline

Alexandra Muntiu[a], Diana Valeria Rossetti[b], Federica Vincenzoni[a,c], Irene Messana[b], Massimo Castagnola[d], Giuseppe La Rocca[c,e], Alessandro Olivi[c,e], Andrea Urbani[a,c], Giovanni Sabatino[c,e] and Claudia Desiderio[b]

[a] Dipartimento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, Rome, Italy;
[b] Istituto di Scienze e Tecnologie Chimiche "Giulio Natta", Consiglio Nazionale delle Ricerche, Rome, Italy;
[c] Fondazione Policlinico Universitario A. Gemelli IRCCS, Università Cattolica del Sacro Cuore Rome, Italy;
[d] Centro Europeo di Ricerca sul Cervello-IRCCS Fondazione Santa Lucia, Rome, Italy;
[e] Institute of Neurosurgery, Fondazione Policlinico Universitario A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy.

Glioblastoma multiforme (GBM) is the most common and invasive primary malignant brain tumour of the central nervous system (CNS). Surgery is the first-line therapy followed by co-adjuvant chemo-radiotherapy combined treatment, and the prognosis for patients with this disease is still extremely poor with a 5-year survival of 7.2% [1]. The present study provides the first attempt to investigate and compare the peptidome of pool samples of saliva collected before surgery from patients affected by newly diagnosed (ND) and recurrent (R) GBM. Top-down proteomics is the strategy of choice for characterizing small proteins and peptides in their intact form with related proteoforms differing by post-translational modifications and to investigate the naturally occurring protein fragmentome that includes cryptides with proper biological activity [2]. Salivary samples were analyzed by nano-LC-ESI-Orbitrap MS platform after filtering on FASP device equipped with 10kDa membrane filter. The resulting data, obtained by replicate analysis, were filtered to ensure high confident identification and analytically reliable results for discovery of potential biomarkers. The analyses identified peptides of 48 and 42 protein elements in ND and R GBM saliva, respectively. Grouping analysis revealed 24 proteins common to all pools, while 24 and 18 resulted exclusively classified in ND and R GBM, respectively. Noteworthy correlations have been found between the present results and previous data obtained from GBM cavitational ultrasonic surgical aspirate fluid proteomic analysis [3]. Some of the common peptides identified in ND and R tumor saliva belong to proteins whose genes have non-elevated expression in the salivary gland and have been previously associated with cancer or glioblastoma tumor. Among the peptides identified as exclusive, the PEBP1, the TTR and PFN1 peptides, identified in R GBM, and CFL1 and PTMA peptides, identified in the ND GBM saliva, could have an interesting role will be further investigated. This pilot investigation revealed tumor histotype-specific salivary peptidome alterations, demonstrating the potential of saliva biofluid to discover GBM disease biomarkers for future perspectives of clinical application.

* *Corresponding author*: Alexandra Muntiu
Università Cattolica del Sacro Cuore, L.go F. Vito 1, 00168, Roma, Italia.
Tel.: +39 392594391; E-mail address: alexandra.muntiu@gmail.com (A.Muntiu)

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P-32
Effects of allosteric or genetic inhibition of ERAP1 on the proteome of A375 melanoma & THP-1 acute leukaemia cells

Martha Nikopaschou [a,b], Martina Samiotaki [c], Kamila Król [d], Doriana Fruci [d], George Panayotou [c], Efstratios Stratikos [a,b,*]

[a] National Center for Scientific Research Demokritos, Athens, Greece
[b] National & Kapodistrian University of Athens, Athens, Greece
[c] Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece
[d] Ospedale Pediatrico Bambino Gesù, Rome, Italy

Endoplasmic reticulum aminopeptidase 1 (ERAP1) is an ER-resident enzyme with important roles in adaptive immune responses. ERAP1 removes N-terminal amino acids from antigenic peptide precursors, to optimize presentation by Major Histocompatibility Complex I molecules. While ERAP1 activity is essential for the presentation of many antigenic peptides, over-trimming and destruction of cancer-specific epitopes can reduce cytotoxic T-cell responses and facilitate cancer evasion[1]. Its role in cancer evasion, together with prior observations that ERAP1 inhibition alters the immunopeptidome of cells, have brought ERAP1 to the spotlight as a promising target for cancer immunotherapy[2,3]. Although these immunopeptidome shifts have been interpreted to be a direct outcome of inhibition of ERAP1 function, the immunopeptidome is also influenced by the proteomic state of the cell and changes in the proteome may also contribute but have not been explored till now.

To address this question, we used a library-free data-independent acquisition (DIA) strategy to explore changes in expressed proteins after genetic (ERAP1 KO) or allosteric inhibition of ERAP1 in two different human cancer cell lines: A375 melanoma and THP-1 leukaemia cells.

Proteomic analysis identified 5575 proteins for A375 and 5758 proteins for THP-1 cells. ERAP1 disruption resulted in significant proteome shifts, with 1375 proteins in A375 cells and 1659 in THP-1 being differentially expressed in the inhibitor treated and KO cells, compared to the wild-type cells (FDR= 0.05, S0=0.5). In both cell lines, inhibitor treatment resulted to less prominent proteome changes and the majority of these changes was also observed in the KO cells. Pathway analysis of the differentially expressed proteins revealed key pathways affected in multiple experimental conditions, including pathways related to cellular responses to stress, vesicle-mediated transport, intracellular protein transport, as well as immune response pathways. Our findings suggest that ERAP1 inhibition can indeed affect the cellular proteome, primarily when the enzyme is genetically removed. Further research is necessary to explore the biological significance of the observed changes as well as their potential impact on the use of ERAP1 as a pharmacological target in cancer immunotherapy.

* *Corresponding author*:
Efstratios Stratikos
National & Kapodistrian University of Athens, Athens, Greece
Tel.: +30 2107274471
E-mail address: estratikos@chem.uoa.gr (E. Stratikos)

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P-33 Exploring proteomics hallmarks of *SOD1*-mutated and sporadic Amyotrophic Lateral Sclerosis

Anna Percio[a,b,*], Michela Cicchinelli[a,b], Mario Sabatelli[c, d] , Andrea Urbani[a,b], Viviana Greco[a,b]

[a] Department of Basic Biotechnological Sciences, Intensivological and Perioperative Clinics, Università Cattolica del Sacro Cuore, Roma, Italia
[b] Unit of Chemistry, Biochemistry and Clinical Molecular Biology, Fondazione Policlinico Universitario A. Gemelli IRCSS, Roma, Italia
[c] Adult NEMO Clinical Center, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy[d] Department of Neuroscience, Università Cattolica del Sacro Cuore, Rome, Italy.

Amyotrophic Lateral sclerosis (ALS) is a multifactorial neurodegenerative disease characterized by the progressive death of motorneurons. Despite considerable research efforts, the exact mechanism triggering the pathological process is not yet fully understood. A complex interaction of genetic and environmental factors contributes to motorneurons damage. Recent evidence points out that strong redox dysregulation, associated to mitochondrial dysfunction, may act as key driver for motorneuron degeneration [1]. In addition, mutations of several genes (e.g., *SOD1* in 20% of the cases, as well as *C9orf72, TARDBP, FUS,*) have been associated with ALS, however familial ALS (fALS) accounts for about 10% of all cases, while the remaining 90% of cases are sporadic ALS (sALS). Both forms, familiar and sporadic, are clinically indistinguishable with the same phenotype. In this perspective, this study aims at an in-depth characterization of ALS proteome in order to highlight common and different molecular features depending on gene variants mainly looking at the *SOD1* variant.
To achieve this purpose, fibroblasts derived from human skin biopsies deposited in the AISLA ALS National Biobank at the Nemo Clinical Center, (Fondazione Policlinico A. Gemelli, Rome, Italy), have been used. In particular, fibroblasts from patients with both sALS and fALS, including patients with *SOD1*, and healthy donors have been selected. Label free proteomic analysis has been performed using HR-DDA approach on Orbitrap Fusion Lumos Tribrid Mass Spectrometer (Thermo Scientific). Peptide identification and label free quantification have been performed by Peaks X Pro software; statistics and bioinformatics analysis have been carried out by MATLAB, Reactome and QIAGEN Ingenuity Pathway Analysis (QIAGEN IPA) softwares. This investigation provides a comprehensive characterization of the redox landscape of ALS, revealing molecular features hitherto unexplored.
Significant protein hallmarks and a metabolomic mitochondrial reshaping have been shown by making comparisons between ALS and healthy patients, as well as within the ALS group. In particular, an impairment of networks related to autophagy and antioxidant mechanisms has been highlighted in the sporadic form and, mainly, in the familial *SOD1*-ALS patients.

**Corresponding Author:*
Anna Percio, Pharmacy Graduate
Dipartimento di Scienze Biotecnologiche di Base Cliniche Intesivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, Largo F. Vito,1, Rome, Italy
E-mail address: anna.percio01@icatt.it (A.Percio)

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Proteogenomic Characterization of High Grade Serous Ovarian Cancer

Diletta Piana [a*], Viviana Greco [a,b], Federica Iavarone [a,b] , Elisa de Paolis[c], Camilla Nero[d], Giovanni Scambia[d], Angelo Minucci[c], Andrea Urbani[a,b,c]

[a]Dipartimento di Scienze Biotecnologiche di Base Cliniche Intesivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, Rome,
[b]Fondazione Policlinico Universitario A. Gemelli IRCSS, Rome,
[c]Molecular and Genomic Diagnostics Unit, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome,
[d]Division of Oncological Gynecology, Department of Women's and Children's Health, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome.

High-Grade Serous Ovarian Carcinoma (HGSOC) is one of the deadliest gynaecological neoplasm. Among the numerous cancer-related genes, the Loss Of Function (LOF) of germline and somatic variants of the *BRCA1* gene is clinically relevant. *BRCA1* is a gene linked to DNA repair by Homologous Recombination (HR) and its deficiency makes these tumour types candidates for platinum-based chemotherapy and poly-ADP inhibitors (PARPi)[1]. Despite an initial benefit from PARPi some HGSOC subclones become resistant to the therapy[2]. This could be due to the high molecular and genetic heterogeneity within these HGSOC.

Therefore, the aim of this study was to identify putative molecular signatures related to *BRCA1* 'functional loss' by proteogenomic approach.

Based on the genomic classification obtained by Next-Generation Sequencing (NGS) analysis, fresh-frozen tissues from patients with HGSOC have been classified into 3 groups according to their different LOFs. Specifically, Frameshift (p.Gln172AsnfsTer62), Missense (p.Leu22Ser) and several Non-sense mutations of *BRCA1* have been considered compared to *BRCA1* Wild-Type (WT).

An in-depth label-free proteomic analysis have been performed on Orbitrap Fusion Lumos Tribrid mass spectrometer; PeaksXPRO software have been used for protein identification and quantification, and REACTOME and STRING software for pathway analysis and functional interaction.

Several proteins related to key pathways such as mechanisms of DNA repair, and energetic metabolism are downregulated in all *BRCA1* mutated groups compared to the *BRCA1* WT, suggesting there might be a correlation with *BRCA1* loss. Conversely, a similar pattern of up-regulation has been shown in tissues with both frameshifts and non-sense mutations compared to WT. On the other hand, significant proteins mainly related to RNA metabolism are downregulated in the sample group with missense mutations.

Although the loss of *BRCA1* is common to all these groups, these preliminary results shed light on the different molecular signatures and pathways that might be related to the different mutations.

** Corresponding author:*
Diletta Piana, PhD student
Università Cattolica del Sacro Cuore, Largo Francesco Vito 1, Roma, Italia.
E-mail address: diletta.piana@unicatt.it (D.Piana)

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P-35

Detection and quantitation of antimicrobial proteins/peptides and resistome proteins in milk of different bovine breeds

Cristian Piras[a*], Rosario De Fazio[a], Carlotta Ceniti[a], Bruno Tilocca[a], Vincenzo Cunsolo[b], Antonella Di Francesco[b], Francesca Oppedisano[c], Rainer Cramer[d], Paola Roncada[a] and Domenico Britti[a]

[a] Department of Health Sciences, "Magna Græcia University" of Catanzaro, Catanzaro, Italy
[b] Laboratory of Organic Mass Spectrometry, Department of Chemical Sciences, University of Catania, Italy.
[c] Institute of Research for Food Safety & Health (IRC-FSH), Department of Health Sciences-University of Catanzaro Magna Græcia, Catanzaro, Italy.
[d] Department of Chemistry, University of Reading, Whiteknights, Reading, UK

Milk, a complex and nutritionally rich biological fluid, is gaining recognition for its role as a source of bioactive compounds with diverse functional properties. This study explores the presence and abundance of antimicrobial proteins/peptides (AMPs) and resistome proteins in bovine milk.
Liquid atmospheric pressure matrix-assisted laser desorption/ionization (LAP-MALDI) mass spectrometry (MS) profiling, bottom-up proteomics, and metaproteomics were used to comprehensively analyze milk samples from various bovine breeds to undertake the identification and characterization of AMPs and the investigation of resistome proteins along with a broader discussion of the implications for food safety, public health, and the dairy industry.
The results of LAP-MALDI MS analysis of milk coupled with Linear Discriminant Analysis (LDA) demonstrate the potential of distinguishing between Podolica and control milk samples based on MS profiles. The classification accuracy achieved in the training set is 87.5%, and in the test set, it reaches 96.7%.
Bottom-up proteomics revealed approximately 220 quantified proteins, with cathelicidins and annexins exhibiting higher abundance levels in control cows. These findings suggest functional differences between milk from different bovine breeds.
Analysis of the bottom-up proteomics data using the Comprehensive Antibiotic Resistance Database (CARD) detects beta-lactamases and tetracycline resistance proteins in both control and Podolica milk samples, with no significant breed-specific differences observed.
This study provides insights into the biomolecular complexity of milk composition, emphasizing the roles of AMPs, resistome proteins, genetics, and the potential risks associated with AMR in dairy products. The findings contribute to our understanding of milk as a source of bioactive compounds with implications for human health and food safety.

* *Corresponding author*:
Cristian Piras
Department of Health Sciences, "Magna Græcia University" of Catanzaro
Campus Universitario "Salvatore Venuta" Viale Europa - 88100, CATANZARO
phone +393281318896
E-mail address: c.piras@unicz.it (C.Piras)

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Proteomic profile of Extracellular Vesicles secreted by Astrocytes using a shotgun proteomic approach and high resolution mass spectrometry

Maria G. G. Pittalà[a], Antonella Di Francesco[a], Vincenzo Cunsolo[a], Aldo Lanzoni[a], Loredana Leggio[b], Greta Paternò[b], Nunzio Iraci[b], Rosaria Saletti[a]

[a] Laboratory of Organic Mass Spectrometry, Department of Chemical Sciences, University of Catania;
[b] Laboratory of Molecular Biology, Department of Biomedical and Biotechnological Sciences, University of Catania.

Astrocytes (AS) play a crucial role in maintaining the homeostasis of dopaminergic (DAergic) neurons. In Parkinson's disease (PD), which is characterized by the progressive loss of DAergic neuronal cell bodies in the ventral midbrain (VMB), and their terminals in the striatum (STR), extracellular vesicles (EVs) secreted by astrocytes (AS-EVs) can have either destructive or beneficial effects [1]. To identify the mechanisms involved in the neuroprotective effects of AS-EVs, we have investigated their proteomic profile, comparing vesicles secreted by astrocytes from the VMB vs. the STR, both in basal conditions (AS-EVs CTRL) and activated by the pre-treatment with the neuroprotective chemokine CCL3 (AS-EVs CCL3) [2].
Intact EVs were purified from primary cultures of postnatal VMB and STR astrocytes. EVs were lysed using 0.1% RapiGest SF and proteins were reduced with DTT, alkylated with iodoacetamide and digested using trypsin. Analysis was conducted in triplicate by a shotgun approach and nanoUHPLC/High-Resolution nanoESI-MS/MS [3]. Finally, Gene Ontology (GO) term enrichment analysis to find statistically over- and under-represented categories was carried out with BiNGO 3.0.5 as a plugin for Cytoscape 3.9.1.
The results showed a different proteomic profile of AS-EVs CTRL and AS-EVs CCL3 extracted from VMB and STR. In particular, many of the proteins identified exclusively in the AS-EVs CCL3 are involved in regulation and development of nervous system, in secretion of synaptic vesicles and in differentiation and proliferation of neurons and glia cells. GO analysis revealed that EV proteins correlated with neurological disease pathways including neurodegeneration, loss of synapse and neuroinflammation; interestingly, they are also enriched in proteins related to cellular growth, neural and glial proliferation, in line with the neuroprotective potential of the donor astrocytes.
Our results suggest the existence of a molecular machinery capable of organizing the sorting of specific proteins towards EV-mediated secretion in astrocytes. Furthermore, it implies the possibility that secreted proteins may have specific functions when transferred to target cells and the possibility to reprogram AS-EVs for brain repair. Thus, knowledge generated within this study could pave the way for the development of innovative therapeutic approaches to address PD.

Corresponding Author:
Maria Gaetana Giovanna Pittalà,
University of Catania, Via Santa Sofia 64, 95123 Catania, Italy.
E-mail address: mariagaetana.pittala@unict.it (M. G. G. Pittalà)
Tel. +393283415128

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Proteomics signature of autoimmune atrophic gastritis: towards a link with gastric cancer

Ombretta Repetto[a*], Valli De Re[a] , Paolo Giuffrida[b] , Marco Vincenzo Lenti[b] , Agostino Steffan[a] , Antonio Di Sabatino[b] , Renato Cannizzaro[c]

[a] Facility of Bio-Proteomics, Immunopathology and Cancer Biomarkers, Centro di Riferimento Oncologico di Aviano (CRO) IRCCS, Aviano, Italy;
[b] Department of Internal Medicine, San Matteo Hospital Foundation, University of Pavia, Pavia, Italy;
[c] Department of Gastroenterology, Centro di Riferimento Oncologico di Aviano (CRO) IRCCS, Aviano, Italy

Autoimmune atrophic gastritis (AAG) is a chronic inflammatory disease that can progress to gastric cancer (GC). To better understand AAG pathology, this proteomic investigation analyzed gastric proteins whose abundance levels are altered in this disease and also in GC.

By two-dimensional difference gel electrophoresis (2D-DIGE), we compared protein maps of gastric corpus biopsies from AAG patients and controls. Differentially abundant spots (|fold change| ≥1.5, P<0.01) were selected and identified by LC-MS/MS. The spots were further assessed in gastric antrum biopsies from AAG patients (without and with Helicobacter pylori infection) and from GC patients and unaffected first-degree relatives of GC patients.
2D-DIGE identified a total of 67 differentially abundant spots, which were identified by LC-MS/MS as 53 distinct proteins. The most significant (adjusted P<0.01) biological process associated with the less abundant proteins was “tricarboxylic acid cycle”. Of the 67 spots, 57 were similarly differentially abundant in AAG-antrum biopsies irrespective of H. pylori infection status. The differential abundance was also observed in GC biopsies for 63 spots, and in normal gastric biopsies of first-degree relatives of GC patients for 31 spots. The different abundance levels of two more abundant proteins (PDIA3, GSTP gene products) and four less abundant proteins (ATP5F1A, PGA3, SDHB, PGC) were confirmed by immunoblotting.

This study identified a proteomics signature of AAG. Many differential proteins were shared by GC and may be involved in the progression of AAG to GC. These findings should help decipher the molecular scenario underlying these related diseases.

**Corresponding author:*
Ombretta Repetto, PhD
Facility of Bio-Proteomics, Immunopathology and Cancer Biomarkers, Centro di Riferimento Oncologico di Aviano (CRO) IRCCS, Via Franco Gallini 2, Aviano (PN) Italy
Tel.: +39 0434 659325
E-mail address: orepetto@cro.it (O. Repetto)

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XL-MS strategy to reveal novel protein-protein interactions at plasma membrane compartment

Lucia Santorelli[a*], Sara Petrosino[a], Michele Santoro[a], Paolo Grumati[a,b]

[a] Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy
[b] Department of Clinical Medicine and Surgery, Federico II University, Naples, Italy

Functional interactions among proteins are fundamental to cell biology. Constituting ~30% of the mammalian proteome and 60% of all drug targets, plasma membrane proteins (PMPs) playing important roles in the modulation of diverse molecular processes, including transport, signal transduction, endocytosis and secretion [1]. Revealing the interactome of PMPs under different biological conditions is an unbiased approach to depict regulatory pathways controlling cell behaviour. Despite the biochemical impact, knowledge gaps regarding protein-protein interactions (PPIs) at PM persist.
Recently, cross-linking mass spectrometry (XL-MS) emerged as a powerful tool for PPIs discovery and characterisation, driving to the enlightenment of novel binding partners otherwise undetected [2,3].Thus, we decided to apply the XLMS strategy to map, on a system-wide scale, the PMPs networks of He-La cancer cells, used as cellular reference model.
Starting from cells lysate under normal conditions, PM-enriched fractions were cross-linked with disuccinimidyl dibutyric urea (DSBU) in both intact and disrupt state and analysed via label-free nLC-MS/MS. A DSBU titration was carried out to elucidate the best crosslinker-proteins ratio. The acquired data were inspected with MeroX tool [4], exploiting a refined in-house PM database, derived from the MS non-cross-linked fraction dataset.
Out of the 250 unique residue-to-residue cross-links, 108 identifications, originated from 86 proteins assigned to PM compartment, while the remaining cross-links were identified for proteins which are currently not connected to PM, presenting potentially novel interaction partners. At present, we are applying XL-MS workflow for the characterisation of changes occurring to the PM in response to micro-environment stimuli in metabolic disease context.

* *Corresponding author*: Lucia Santorelli.
Grumati's Lab and Mass Spectrometry Facility
Telethon Institute of Genetics and Medicine (TIGEM), Pozzuoli, Italy
E-mail address: l.santorelli@tigem.it (L.Santorelli)

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P-39

Modern superheroes: the tardigrade Damage Suppressor protein improves tolerance in human transfected cells exposed to oxidative stress

Enxhi Shaba[a*], Lorenza Vantaggiato[a], Luca Bini[a], Carlotta Marzocchi[b], Claudia Ricci[b], Silvia Cantara[b] and Claudia Landi[a]

[a]Functional Proteomics Lab, Life Sciences Dept., University of Siena
[b]Medical, surgical and neurological sciences Dept., University of Siena

Of recent discovery, the genome sequencing of the *Ramazzottius varieornatus* revealed a unique nucleosome-binding protein, named the damage suppressor protein (Dsup). As a tardigrade, it has been acquiring great interest as these organisms are small invertebrates capable of surviving extreme environments, such as drought, severe temperatures and damaging stresses. Because of its extraordinary abilities, several studies have been investigating Dsup role in protection against stress like X-ray, oxidative stress, genomutagens or UV-C radiation by transfection of plants or human cells. Interestingly, the findings suggest that Dsup not only indirectly interact with DNA via chromatin binding mediating DNA protection from ROS and other damaging agents, but also triggers an overall response of DNA damage signaling, DNA repair, antioxidant defense and cell cycle regulation by the transcriptional modulation of endogenous genes, resulting in an acquired resistance to stress [1,2,3]. Given these promising outcomes, our functional proteomic study attempts to provide a wider comprehension of the molecular mechanisms modulated by Dsup in human cells exposed to oxidative stress. We performed a differential proteomic analysis of control human HEK293 cells (Dsup–) and human HEK293 cells transfected with Dsup (Dsup+), after overnight H_2O_2 exposure. Proteomic results identified 60 statistically significant differential proteins, which were analysed by enrichment and pathway analyses. Enrichment analysis by GO terms of biological processes show that mRNA splicing processes and redox homeostasis are mainly represented by high abundant proteins in Dsup+, whereas ubiquitin-dependent proteasome-mediated degradation processes and metabolic processes are mainly represented by low abundant proteins in Dsup+. Furtherly, enrichment analysis by GO terms of cellular compartments highlights a different sub-cellular localization of differential proteins, as the high abundant ones in Dsup+ are mainly associated with ribonucleoproteins complexes, spliceosome and nucleoplasm, whereas the low abundant ones in Dsup+ are associated with proteasome complexes and mitochondria. Interestingly, pathway analysis highlights the involvement of the differential proteins in transcriptional regulation mechanisms, oxidative stress response and energetic metabolism. In conclusion, our preliminary data provide new insights in Dsup functional impact and involvement in protection against stress.

* *Corresponding Author:*
Enxhi Shaba
Functional Proteomics Lab., Life Sciences Dept. University of Siena, Siena, Italy
Tel.: +39 338 4177848; +39 0577 232368
E-mail address: enxhi.shaba@unisi.it (E. Shaba)

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The cultivable microflora of raw cow milk and the associated resistome: insights from a multi-omic approach

Giulia Laterza[a], Gabriele Meroni[a], Vincenzo Cunsolo[b], Maria G. G. Pittalà[b], Francesca Zaghen[a], Valerio Sora[a], Piera Anna Martino[a], Alfonso Zecconi[a], Paola Roncada[c], Luigi Bonizzi[a], Alessio Soggiu[a*]

[a] Dipartimento di Scienze Biomediche, Chirurgiche e Odontoiatriche, Sezione One-Health, Università degli Studi di Milano, Milano, Italy
[b] Laboratory of Organic Mass Spectrometry (LOMS)
Department of Chemical Sciences - University of Catania, Catania, Italy
[c]University "Magna Graecia" of Catanzaro, Viale Europa, Catanzaro, Italy;

Raw milk is a reservoir of nutrients and a multitude of beneficial microorganisms but also a possible vehicle of antimicrobial resistant (AMR) pathogens. Multi-omic methods now make it possible to obtain an increasingly complete overview of the microbial diversity associated with milk quality and safety. In raw milk, however, the high number of somatic cells and the presence of highly abundant proteins poses methodological limitations for a multiomic investigation focused on detecting the microbiome capable of multiplying in the host under normal conditions. The purpose of this work was to evaluate the cultivable microflora and the culturable resistome derived from raw milk to obtain a comprehensive functional profile of proteins implicated in the phenomenon of AMR. Through the nanopore approach the microbiome profile of each sample has been recovered. Indeed, a specific bioinformatic pipeline for the MinION Mk1C long-reads sequencing has been applied (MetONTIIME-MicrobiomeAnalyst) to obtain a comprehensive WGS profiling. The metaproteomic approach has been focused on the detection of principal AMR proteins expressed in cultivable raw milk microbiome (resistome)[1]. High resolution Orbitrap mass spectrometry-based analysis with AMR targeted databases (WGS-NCBI AMRFinder-CARD) and the Unipept desktop application allowed the monitoring of antimicrobial resistance genes (ARGs)-related proteins and related bacterial genera. To the best of our knowledge, this multi-omic investigation provided for the first time, a complete description of cultivable raw milk microbial communities and expressed AMR proteins. The new strategy applied could be of practical importance to detect and monitor cultivable resistome at taxonomic and functional level to improve the quality and safety of dairy productions in a one-health perspective.

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**Corresponding author*:
Alessio Soggiu
Dipartimento di Scienze Biomediche, Chirurgiche e Odontoiatriche, Sezione One-Health, Università di Milano, via Pascal 36
Tel.: +39 0250315138
E-mail address: alessio.soggiu@unimi.it (A. Soggiu)

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P-41 Proteomics assessment of early treated adults with Phenylketonuria: a perspective study to assess the disease status

Maria Lucia Tommolini[a*], Maria Concetta Cufaro[a], Silvia Valentinuzzi[a], Ilaria Cicalini[a], Federica Di Marco[a], Filippo Manti[b], Vincenzo Leuzzi[b], Francesca Nardecchia[b], Claudia Carducci[c], Tiziana Pascucci[d], Vincenzo De Laurenzi[a], Luisa Lo Iacono[b], Luca Federici[a], Piero Del Boccio[a], Damiana Pieragostino[a]

[a] Analytical Biochemistry and Proteomics Laboratory, Center for Advanced Studies and Technology (CAST), University "G. d'Annunzio" of Chieti-Pescara, Chieti, Italy
[b] Child Neuropsychiatry Unit, Department of Human Neurosciences, University of Rome "La Sapienza"- AOU Policlinico Umberto I Rome, Italy
[c] Department of Experimental Medicine, University of Rome "La Sapienza", Rome, Italy
[d] Department of Psychology, University of Rome "La Sapienza", Rome, Italy

Phenylketonuria (PKU) is a deficiency of phenylalanine hydroxylase (PAH), it is responsible for the conversion of Phe to Tyr. PKU is classified as an autosomal recessive disorder of phenylalanine metabolism, where elevated phenylalanine levels cause irreversible neurological damage, people with PKU also have generalized skin hypopigmentation, and a musty sweat odour. In this context, in untreated PKU patients, blood Phe concentration increases, resulting in phenyl pyruvic acid excreted in the urine, while Tyr concentration is low. Early diagnosis is based on newborn screening, if treatment is started immediately and continued, some intellectual abilities are preserved. Nowadays, there are 0.45 million people worldwide with the inherited metabolic disorder PKU. Even if pharmaceutical therapies have been developed, a phenylalanine restricted diet is the only effective treatment. Our study aimed to investigate the protein cargo of lymphocytes of PKU early treated adult patients. 3 million of lymphocytes were purified from plasma samples of 10 early treated PKU patients compared to 10 healthy controls (HC). The protein lysates were tryptically digested by Filter-aided sample preparation (FASP) for label free proteomics. Peptides were acquired by nanoLC-Orbitrap-Fusion-Tribrid Mass Spectrometer. Quantitative proteomics data obtained from Proteome Discoverer were used for functional analysis through Ingenuity Pathway Analysis (IPA). Data obtained from lymphocytes protein cargo revealed proteins involved in neurological damage and oxidative stress. Afamin (AFM) was quantified only in PKU lymphocytes, it may be involved in the transport of vitamin E which has antioxidant properties, on the contrary, Dihydropteridine reductase (DHPR), related to tetrahydrobiopterin (BH4) and thus hyperphenylalaninemia, was identified exclusively in HC lymphocytes. The IPA analysis highlighted that "cell viability of nervous tissue cell lines" was one of the most inhibited biofunction in PKU lymphocytes, instead, "apoptosis of neurons" was strongly activated. In support of these results, the downstream prediction reports a strong upstream inhibition of neurogenin-1, a neurogenesis transcriptional regulator. These first findings confirm that PKU lymphocytes would represent a useful platform for "liquid biopsy" to assess the clinical status of the patient. Targeted metabolomics could be used for correlation analysis between protein markers and metabolites.

** Corresponding author:*
Maria Lucia Tommolini
University "G. d'Annunzio" of Chieti-Pescara, Chieti, Italy
Phone: +39 0871 541333
e-mail: maria.tommolini@unich.it (M.Tommolini)

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Proteomic analysis of A375 BRAF^{V600E} melanoma cells to highlight the protein modulation following Rictor/mTORC2 downregulation

Lorenza Vantaggiato[a*], Enxhi Shaba[a], Luca Ponzone[b,c], Luca Bini[a], Enzo Calautti[b,c], Claudia Landi[a]

[a] Department of Life Sciences, Lab of Functional Proteomics, University of Siena.

[b] Molecular Biotechnology Center "Guido Tarone", University of Turin, Turin, Italy

[c] Department of Molecular Biotechnology and Health Sciences, University of Turin, Turin, Italy

Cutaneous malignant melanoma (CMM) is a tumour of melanocytic origin; it is the fifth most common form of cancer in adults with a significantly high morbidity and mortality rates. About 50 % of CMM harbors activating BRAF mutations (over 90 % BRAF^{V600E}), making it possible to apply different target therapies inhibiting BRAF/MEK. However, the efficacy of these therapies is limited by the onset of therapeutic resistance characterized by CMM metabolic rewiring passing from the glycolytic to the Oxidative Phosphorylation (OXPHOS)-. The mechanistic target of rapamycin complex 2 (mTORC2) represents a key element in cellular growth and metabolism. Many studies report the over-activation of mTORC2 in several tumor types, including malignant melanoma.Contrary to this, bioinformatics analysis of TCGA melanoma patients' database highlighted that patients with melanoma and low levels of Rictor, a key component of mTORC2, have worse clinical outcomes; these results are related to a modified genetic expression which leads to the activation of mitochondrial energy production miming the target therapy resistance mechanisms. Moreover, our previous proteomic study on M14 CMM BRAFV600E cell line proficient and deficient for Rictor, corroborate these data reporting an up-regulation of proteins related to oxidative stress protection, mitochondrial functions, NAD+ biosynthesis, TCA cycle and electron transfer chain (ETC) in Rictor deficient cells. On these bases and taking in consideration that M14 cells are characterized by a glycolytic metabolism, we report a new proteomic analysis on A375 CMM BRAF^{V600E} cells proficient and deficient for Rictor. The aim of the study was to perform a differential proteomic analysis on a cell culture characterized by an OXPHOS metabolism such as the A375 cell line in order to identify the operating mechanism of Rictor/mTORC2 in melanoma cells with an alternative metabolic way to shed light on new potential biomarkers useful for future therapeutic perspectives to counteract target therapy resistance.

**Corresponding Author:*
Dr. Lorenza Vantaggiato, PhD
Siena University, Via Aldo Moro, 2, 53100 Siena, Italy
E-mail address: lorenz.vantaggiato@unisi.it (L .Vantaggiato)

P-43 Influence of female sex hormones on salivary protein secretion in test anxiety

Lorenzo Zallocco[a,b*], Maurizio Ronci[c], Antonio Lucacchini[b], Maria Rosa Mazzoni[d], Laura Sebastiani[a] and Laura Giusti[e]

[a] Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa
[b] Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy
[c] Department of Medical, Oral and Biotechnological Sciences, University "G. d'Annunzio" of Chieti-Pescara, Italy
[d] Department of Pharmacy, University of Pisa, Pisa, Italy
[e] School of Pharmacy, University of Camerino, Camerino, Italy

Social anxiety is a psychological disorder derived from the fear of being judged by others that leads to a strong state of stress. The physiological response to a stressful condition occurs mainly through the involvement of the endocrine, nervous and immune systems. In particular, the hypothalamic-pituitary-gonadal and hypothalamus-pituitary- adrenal axis are involved.
This study aims to investigate the modulation of salivary protein secretion by female sex hormones, during an acute psychological stress. Twenty healthy females recruited either in the pre-ovulatory (Pre-Ov) or post-ovulatory (Post-Ov) phase of the menstrual cycle, characterized, respectively, by low and medium-high levels of estradiol and progesterone, participated to a test anxiety task. The task simulated an oral exam and consisted of 3 phases: relaxation, study of a written text, and oral exposition in front of a “professor” of the studied text. Four saliva samples were collected: before starting the test (T1), after the relaxation phase (T2), after the exam simulation (T3), and 20 minutes after the exam end (T4). Anxiety perception during the various phases was evaluated by means of specific questionnaires. Progesterone and Estradiol concentrations were analyzed through kit ELISA. Comparative proteomic analysis of saliva samples was performed by 2-DE/MS. Significant differences of protein expression were observed at different times both in Pre-Ov and Post-Ov groups suggesting a different response to acute stress linked to sex hormone levels. Furthermore, for all the observed spots, correlations were carried out between the ratios T3/T2 and T4/T2 of the normalized optical densities and the levels of estradiol and progesterone. The comparisons between Pre-Ov and Post-Ov highlighted 29 spots in common between groups, 17 spots exclusively dysregulated in Pre-Ov (i.e., YWHAE, YWHAZ, SFN, CST2, and CST4) and 53 spots exclusively dysregulated in Post-Ov (i.e., PIGR, IGJ, IGLC/IGKC, PIP, and PFN1).
The correlation analysis pointed out a link between progesterone, estradiol and protein levels of 8 (i.e., AMY1, and CST1) and 11 spots (i.e., CA), respectively.
In conclusion, this study highlighted that female sex hormone levels modulate the secretion of salivary proteins during an acute psychological stress.

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* *Corresponding author*:
Lorenzo Zallocco
Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa
Tel: 050 2213460
E-mail address: l.zallocco@gmail.com (L. Zallocco)

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Analysis of the oral fluid of human preterm newborns after regurgitation evidences the cleavage pathway of the proteins of breast milk during the fetal digestion.

Chiara Tirone[a]*, Giovanni Vento[a], Federica Iavarone[b], Nicoletta Menzella[a], Davide De Tomaso[a], Simona Fattore[a]. Claudia Desiderio[c], Tiziana Cabras[d], Barbara Manconi[d], Alessandra Olianas[d], Mozhgan Boroumand[e], Irene Messana[d], Gavino Faa[d], Massimo Castagnola[g].

[a]Neonatology Unit, Department of Woman and Child Health and Public Health, Fondazione Policlinico Univ. "Agostino Gemelli", Dipartimento di Scienze della Vita e Sanità Pubblica
[b] Dipartimento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, Rome, Italy.
[c]Istituto di Scienze e Tecnologie Chimiche "Giulio Natta", CNR, Rome, Italy;
[d]Department of Life and Environmental Sciences, University of Cagliari, Cagliari, Italy
[e] National Institute on Aging, NIH, Baltimore, MD 21224, USA;
[g]Centro Europeo di Ricerca sul Cervello-IRCCS Fondazione Santa Lucia, Rome, Italy;

The oral fluid of preterm newborn is a very precious specimen to investigate various molecular mechanisms happening during the late fetal development [1]. It can provoke respiratory problems to the preterm and it is commonly aspired by the NICU (Neonatal Intensive Care Unit) personnel and discharged as a waste. For this reason, its use for analytical purposes has been allowed by the Ethical committees of our universities. We had the occasion to take this fluid few minutes after the breastmilk feeding because the newborn had a dangerous regurgitation. The analysis of this sample using a high-resolution nano-chromatography coupled to the Orbitrap-Lumos MS apparatus with a top-down pipeline (without any proteolytic treatment of the sample [2]) allowed to detect a multitude of fragments deriving from β-casein, α-S_1-casein, κ-casein and lactotransferrin. They indicated that in the gastric tract of preterm several trypsin- and chimotrypsin-like proteinases as well as many carboxy- and amino-exopeptidases operate with a complex and coordinate fragmentation pathway. The preliminary list of fragments detected are visible in the poster and many of them have probably an autonomous function. They are indeed potential cryptides, i.e. small peptides encrypted inside the sequence of bigger proteins, with a function different from that one of the parent protein [3]. Further experiments are necessary to clarify the entire pathway of proteolytic events happening during fetal digestion and to establish the function of several fragments. These studies can also give suggestions on new artificial milk-based food to use in substitution to human breast milk, when not-available.

* *Corresponding author*:
Chiara Tirone, Università Cattolica e Policlinico Gemelli, Largo Francesco Vito 1, 00168, Rome, Italy
Tel. e fax: +39 06-3015-4357.
E-mail address: chiara.tirone@policlinicogemelli.it

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