Volume 98/Supplement 1 - 2025

Journal of Biological Research

Bollettino della Società Italiana di Biologia Sperimentale

97th National Congress of the Italian Society for Experimental Biology

Palermo, Italy, 10-13 April 2025

ABSTRACT BOOK

www.jbiolres.org

jbr

pagepress

pagepress

Journal of Biological Research

Bollettino della Società Italiana di Biologia Sperimentale

eISSN 2284-0230

Publisher

PAGEPress Publications
via A. Cavagna Sangiuliani, 5
27100 Pavia, Italy
Tel. +39.0382.1549020
info@pagepress.org – www.pagepress.org

97th National Congress of the Italian Society for Experimental Biology

Palermo, Italy, 10-13 April 2025

COMITATO D'ONORE

Maria Chiara Carrozza, Roberto Lagalla, Francesco Micciché, Massimo Midiri, Roberto Agnello, Angela Bonura, Gioacchino Chimenti, Giovanni Civiltà, Vincenzo D'Anna, Roberta Di Pietro, Giovanni Ferrara, Aldo Gerbino, Cristina Giacoma, Pietro Gizzi, Dario Matranga, Fabrizio Michetti, Domenico Nuzzo, Fiorella Palumbo, Giovanni Perino, Patrizia Perino, Margherita Rizza, Bartolo Sammartino, Don Domenico Saraniti, Roberto Sciarratta, Filippo Sorbello, Augusta Troccoli, Fabio Tulone, Mauro Visconti, Giovanni Zummo

PRESIDENTI DEL CONGRESSO

Francesco Cappello, Stefania Grimaudo, Concetta Maria Messina, Alessandro Pitruzzella

COMITATO SCIENTIFICO

Sara Baldassano, Rosario Barone, Stefano Burgio, Gaetano Felice Caldara, Daniela Carlisi, Celeste Caruso Bavisotto, Danila Di Majo, Sonia Emanuele, Giuditta Gambino, Carla Gentile, Marco Giammanco, Giuseppe Giglia, Antonella Marino Gammazza, Rosaria Maria Pipitone, Patrizia Proia, Francesca Rappa, Federica Scalia, Marta Anna Szychlinska, Sonya Vasto

COMITATO ORGANIZZATORE

Giusi Alberti, Maria Denise Amico, Maria Antonella Augello, Maria Magdalena Barreca, Mattia Biondo, Adelaide Carista, Daniela Coniglio, Giuseppa D'Amico, Anna Barbara Di Stefano, Massimo Fresta, Melania I. Gratie, Alessandra Lo Cicero, Giulia M. G. Lo Piano, Olga M. Manna, Manuela Mauro, Letizia Paladino, Domiziana Picone, Nicolò Ricciardi, Silvia Sarullo, Graziella Serio, Laura Tomasello, Giuseppe Vergilio, Alessandra M. Vitale, Giusy Vultaggio

CON IL PATROCINIO E IL CONTRIBUTO DI

TABLE OF CONTENTS

LECTURES

HOLISTIC BIOLOGY IN THE AGE OF ARTIFICIAL INTELLIGENCE

Franco ANDALORO
Cluster Tecnologico Nazionale Blue Italian Growth and Fondazione Italiana Biologi, Napoli, Italy

The AUV (Autonomous Underwater Vehicle) will be able to operate in the sea without time or depth limits, classifying, measuring and monitoring marine flora and fauna, mapping the seabed and analyzing the sea-water in real time, and with artificial intelligence, which will also have data from ophthalmic sensors capable of perceiving the slightest odors, acoustic detectors sensitive to all frequencies and increasingly high-performance cameras that can operate in absolute darkness, the great mysteries of the sea, that today is yet less known than Mars, will be revealed. All this will represent an enormous opportunity for humanity, not only in terms of the thirst for knowledge but above all for the greed for edible resources, precious metals and new biomolecules that, if we do not know how to manage, we will quickly lead to overexploitation. The new experimental biology, as has already happened to oceanography, will therefore have to be more careful to understand and manage machines, software, instruments and algorithms. Learning to ask questions rather than deciphering answers that will arrive punctually and precisely. In fact, we will all have the same answers if the questions were to be the same. What the collective advantage will be is difficult to predict although we can imagine enormous progress, but the two things do not go together, we have learned this in the last century in which the quality of life, I mean that of everyone not that of the few chosen by the social and geographical scale, has not improved proportionally to scientific results, life expectancy is stagnant if not decreasing in fact, in the face of progress in the medical field, health risks for global contamination, pollution, plastics debris and pandemics increase. But biology has answers to questions that neither artificial intelligence nor robotics will be able to satisfy, and for this reason it will be necessary to place the scientist at the center of the system as a human being, as a fallible actor of the holistic knowledge of a universe made of history and culture as well as memories and errors. Memories and errors that will escape any software that does not know how to have a coffee in a bar by the sea with archaeologists, humanists and dreamers linked together by empathy and emotion. We know that great discoveries do not come from precise questions but from chance and error that generate signals that must be perceived, deciphered, read and rewritten. We do not want to deny the great role that robotics and artificial intelligence will be able to offer but we want to clarify that these are only tools to be used and not objectives to be achieved, they are students not teachers and if we do not understand this, we will slowly let ourselves be dragged into a scientific laziness, into an intellectual poverty that will submit us to the machines as they will never dominate us but we will bend to them. Just think how the debate between thesis and antithesis is already often unraveled downwards by Wikipedia culture that by giving quick certainties eliminates that speculative process that is the antechamber of truth. Field investigations and laboratory observations and above all a holistic vision may be supported by machines and artificial intelligence but not substitutes. Behind every researcher there are his emotions and his memories belonging to a unique, unrepeatable and irreplaceable experience, a vision that is rooted in a memory and a culture that is not digitalized and therefore unavailable to artificial and remote systems. The digital twin of the researcher is not ready yet. We can still "dream and disobey". The reflections that we will make in salt water are vicarial to all experimental biology also because *biös,* the life, (*quam vivimus*), predicts *Thanatos,* the death, which is unknown to the machines.

SUCCESSFUL AGING: THE LESSON FROM SICILIAN CENTENARIANS

Giuseppina CANDORE, Giulia ACCARDI, Anna AIELLO, Anna CALABRÓ, Calogero CARUSO
Laboratorio di Immunopatologia e Immunosenescenza, Dipartimento di Biomedicina, Neuroscienze e Diagnostica avanzata, Università degli Studi di Palermo, Italy

The study of Sicilian centenarians offers valuable insights into successful aging, particularly concerning the roles of immune-inflammatory responses, including oxidative stress. Sicilian centenarians generally exhibit a balanced oxi-inflammatory status, contributing to their health span and longevity (Caruso *et al.*, 2022, doi: 10.37825/2239-9754.1036). Lifestyle choices, genetic factors, and environmental exposures influence this balance. Among the lifestyle choices, the Mediterranean diet, rich in fruits, vegetables, and healthy fats, along with regular physical activity and strong social connections, contribute to the successful aging of Sicilian centenarians. The analysis of the oxi-inflammatory status of Sicilian centenarians showed that this population manifests a grade of inflammation that is comparable with that of the younger population, suggesting a better functionality of the regulatory systems that leads to the control of inflammatory status (Accardi *et al.*, 2024, doi: 10.3390/biology13121010). Regarding oxidative status, studies on Sicilian centenarians reveal that levels of antioxidant markers are similar between centenarians and younger controls (Caruso *et al.*, 2022, doi: 10.37825/2239-9747.1036). The same could be said for the immune system. Our studies highlighted the complex and adaptive nature of immunosenescence in the Sicilian centenarians, emphasizing significant variability influenced by age, Cytomegalovirus serological status, and individual immunological history (Aiello *et al.*, 2019, doi: 10.3389/fimmu.2019.02247). They support the hypothesis that immune aging in centenarians represents a differential adaptation rather than a uniform decline. In fact, the increase in terminally differentiated T cells, natural killer, and the regulation of oxi-inflammatory status appear to reflect adaptive mechanisms by which centenarians effectively respond to a lifetime of immunological challenges (Ligotti *et al.*, 2023, doi: 10.37825/2239-9747.1041). This variability, shaped by genetics, environmental exposures and life history, under-

scores the personalized nature of aging. The oxi-inflammatory and immunological profiles of centenarians, especially the oldest ones, suggest that these changes may not be detrimental but rather represent strategies that promote resilience and exceptional longevity. Furthermore, gender differences also play a role in aging and longevity. Studies have shown that certain markers, including the inflammatory ones, vary between men and women across different age groups, suggesting that gender-specific factors may influence the aging process and the development of age-related diseases (Aiello *et al.*, 2021, doi: 10.14336/AD.2021.0226; Calabrò *et al.*, 2024, doi: 10.37825/2239-9747.1049; Accardi *et al.*, 2024 quoted). In conclusion, understanding the factors that contribute to the longevity of Sicilian centenarians can inform strategies to promote healthy aging in the broader population. By adopting similar lifestyle choices and interventions that modulate inflammation, oxidative stress and immune response, it may be possible to enhance health span and delay the onset of age-related diseases.

UNLOCKING NERVE REGENERATION: MY RESEARCH JOURNEY THROUGH THE LENS OF SOCIAL MEDIA

Samuele NEGRO[1], Chiara BAGGIO[1] Giorgia D'ESTE[1], Giulia ZANETTI[1], Federico FABRIS[1], Aram MEGIGHIAN[1,2], Alessandro BERTOLI[1], Marilina MASSIMINO[1,3], Manuela BASSO[4], Valentina BONETTO[5], Roberta SCHELLINO[6,7], Marina BOIDO[6,7], Cesare MONTECUCCO[3], Marco PIRAZZINI[1], Michela RIGONI[1]

[1]Department of Biomedical Sciences, University of Padua; [2]Padua Neuroscience Center, University of Padua; [3]CNR Neuroscience Institute, Padua; [4]Department of Cellular, Computational and Integrative Biology - CIBIO, University of Trento; [5]Department of Molecular Biochemistry and Pharmacology, IRCCS-Istituto di Ricerche Farmacologiche "Mario Negri", Milan; [6]Department. of Neuroscience Rita Levi-Montalcini, University of Turin; [7]Neuroscience Institute Cavalieri Ottolenghi, University of Turin, Orbassano (TO), Italy

The peripheral nervous system (PNS) has retained, through evolution, a remarkable ability to regenerate after certain types of damage. My research focuses on uncovering the mechanisms behind this extraordinary capacity. Understanding these processes is crucial for developing therapeutic strategies for neurodegenerative diseases, where these regenerative mechanisms are either impaired or only partially activated. Using a neurotoxin derived from the venom of the black widow spider, we have identified a molecular axis involving the chemokine CXCL12 and its receptor CXCR4 as a pivotal driver of axonal regeneration in response to various types of peripheral nervous system injuries. Currently, we are extending this research to explore how this axis functions in chronic conditions like Amyotrophic Lateral Sclerosis (ALS), which affect the PNS. But, forget the boring charts, figures and graphs,—I'm bringing science to life in a way that's actually fun! I will share my work through funny videos I post on social media (find me at @Samuscientist). These clips capture not just the science but also the everyday realities of life as a researcher, offering a fresh perspective on what drives us and the challenges we face. Because let's be honest—in science sometimes the biggest discovery is realizing your experiment failed *again*.

WHICH CAME FIRST: THE CHICKEN OR THE EGG? LIFE 2.0: THE EVOLUTION OF THE PROTEINS

Patrizia PROIA

Sport and Exercise Sciences Research Unit, Department of Psychology, Educational Science and Human Movement, University of Palermo, Italy

The age-old question 'Did the chicken or the egg come first?' is not only a philosophical table dilemma, but also an intriguing scientific enigma that takes us right to the heart of biological evolution. In this presentation, we will go beyond mere curiosity, exploring the question from a molecular and biochemical perspective: did proteins or DNA come first? We will discover how proteins might have been the true pioneers of life, long before RNA took all the glory, rewriting the classic RNA World Hypothesis narrative in a new era we might call Life 2.0. But we won't stop at theory! Protein evolution is not only about the past, but also about our future... especially on the dish! We will discuss how protein from insects is making its triumphant entry into our diets and how lab-grown meat is revolutionising the very concept of 'steak' (without the need for cows, but with plenty of flavour). Between molecular biology and futuristic gastronomy, we wonder if the next big evolution is not in our eating habits. So let's get ready for a journey from the origin of life to our next meal - and, yes, you may never look at an omelette the same way again!

GIGANTISM AND DWARFISM BETWEEN MYTH, HISTORY AND ANATOMY: A BIOLOGICAL TALE

Francesco Maria GALASSI[1], Elena VAROTTO[2,3]

[1]Department of Anthropology, Faculty of Biology and Environmental Protection, University of Lodz, Poland; [2]Department of Cultures and Societies, University of Palermo, Italy; [3]Archaeology, College of Humanities, Arts and Social Sciences, Flinders University, Adelaide, SA, Australia

This communication examines the topic of gigantism and dwarfism throughout history, using various methodologies and embracing various disciplines, from art to endocrinology, from human anatomy to palaeopathology, with evolutionary and anthropological references to explain the genesis of these conditions. The most famous cases of these pathologies from antiquity to the Modern Age, including mythological references, reported instances from ancient Egypt and the Classical World, will be illustrated, together with a rich

excursus on the evolution of our scientific understanding of these nosological entities and the methodologies implemented to elucidate the presentation and historical trajectory of these pathologies. The talk will show the importance of a multidisciplinary approach to pathological morphology in past eras and open a window on the evolution of diseases.

EXPLORING BIODIVERSITY. THE CONTRIBUTIONS OF THE SPOKE 7 TEAM OF THE UNIVERSITY OF PALERMO TO A MULTIDISCIPLINARY APPROACH UNDER THE FRAMEWORK OF THE NATIONAL BIODIVERSITY FUTURE CENTER (NBFC)

Sabrina LO BRUTTO[1,2], Antonina BADALUCCO[1], Marco BASSI[1,3], Salvatore BONDÌ[1], Fabio CARADONNA[1,4], Melania I. GRATIE[1,5], Alessandro INCARBONA[1,2], Giuliana MADONIA[1,2], Antonella MARINO GAMMAZZA[1,4], Maria Cristina B. SALMERI[1,4], Giovanni SURDI[1], Francesco CAPPELLO[1,5]

[1]National Biodiversity Future Center (NBFC), Palermo; [2]Department DiSTeM, University of Palermo; [3]Department SPPEFF, University of Palermo; [4]Department STeBiCeF, University of Palermo; [5]Department BIND, University of Palermo, Italy

Biodiversity is essential for human well-being, providing ecosystem services such as climate mitigation and nutrient cycling. Its conservation is critical to prevent global disease and environmental crises, as biodiversity loss is responsible for ecological decline.

The NBFC, established under the Post-COVID Recovery and Resilience Plan, promotes nature-based solutions to balance human needs with biodiversity conservation. It is a network of collaborative institutions and private companies focused on environmental protection, particularly in Italy and the Mediterranean region, where biodiversity plays a key role in the sustainable development of developed and developing countries. By integrating scientific outcomes with public initiatives, the NBFC is in line with the Green Deal, which focuses on the Sustainable Development Goals. An integrated interdisciplinary approach is therefore needed to achieve these goals. The Spoke 7 team of the University of Palermo is a multidisciplinary team involved in different types of actions. The activities range from the study of biodiversity, in terms of fauna and flora, including marine, urban and endemic species, to topics related to museology, palaeontological and anatomical collections, through citizen science and actions in the field of sociology. A key objective is public engagement, taking advantage of citizen science and local initiatives to foster awareness and participation. An example is the *BatNight*, a successful outreach initiative. Additionally, efforts to preserve Italian rural commons for biodiversity conservation include policy briefs targeting specific societal groups. Innovative higher education programs have been designed to train students with advanced skills, including specialised training for PhD candidates and undergraduate students. In this context, active learning methodologies play a crucial role in facilitating participatory biodiversity education. The digitisation of historical collections preserved in Italian natural history museums is a further task to be carried out with automated and innovative tools. Overall, the establishment of an interdisciplinary and collaborative research team has proven to be a successful strategy for enhancing specific skills and promoting the regional natural heritage.

ANTHROPOLOGY

HOW NEW METHODOLOGICAL APPROACHES IN THE STUDY OF ACTIVITY MARKERS AND NONMETRIC SKELETAL TRAITS CAN HELP IN THE ANTHROPOLOGICAL DEFINITION OF COMMINGLED INHUMATIONS

Claudia FIORENTINO[1], Luca SINEO[2], Margherita MICHELETTI CREMASCO[3]
[1]Dipartimento Culture e Società, Università degli Studi di Palermo; [2]Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche, Università degli Studi di Palermo; [3]Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università degli Studi di Torino, Italy

Classical bioanthropology concerns the interpretation of the biological profile of ancient populations, their state of health, and quality of life through the analysis of specific markers present on skeletal remains. The interpretation of these markers can be complex and/or compromised sometimes, especially when the research is focused on poorly preserved commingled remains. The study of commingled human remains poses many difficulties in reconstructing the population dynamics of ancient communities, given the frequent impossibility of determining the skeletal conformation individual per individual. In the study of Sicilian protohistoric populations, similar conditions are typically encountered due to the multiple burial traditions of the time. Therefore, resourcefulness is indispensable from a methodological point of view, in order to record as much information as possible. Because of these circumstances, we propose the use of non-metric traits of the postcranial skeleton, in particular the lower limbs – including markers of biomechanical overload -, as an advantageous tool for interpreting the habitual body posture and gesture during human-environment interaction, presumably in relation to their occupational activities. The aim is to change the approach to data presentation of these traits to reinforce their functional and postural interpretation. The osteological analysis was conducted on two Sicanian indigenous populations diachronically close to each other and whose settlements were located in two different areas of Sicily: the necropolis of Ponte della Paolina (MNI: 77) in the South-Eastern (Ragusa, RG) during the Late Bronze Age, and the necropolis of Baucina (MNI: 59) in the Western (Palermo, PA) between 7th-5th centuries BCE. Both the communities were found inside an artificial cave tomb of Sicanian tradition, namely an intermixing of remains. We documented the presence of morphological non-metric traits on adult lower limb bones -femur, tibia, patella, calcaneus, talus, in literature referable as a consequence of postures and activities prolonged over time. We recorded the presence of Poirier's facet, vastus notch, tibial, talar, and calcaneal accessory articular facets, and calculated their relative frequencies separately for each bone and side, to maintain the reference to the number of individuals. Then we have coupled their frequencies by joint relationship and we have interpreted by the combination of presence and function of each trait. What we have obtained is a series of hypotheses of habitual static and dynamic postures, that seems to be coherent with a static position of kneeling and dorsiflexion of the foot for Ponte della Paolina, present as well in the Baucina sample, although in this case in combination with markers that suggest an alternation of dynamic activities such as walking on rough terrain, as demonstrated by the presence of biomechanical stress markers of the plantar heels. In conclusion, we propose a new role and perspective for morphological non-metric traits in bioanthropological studies, by not limiting them to the classical recording method "presence/absence", but making them useful to functional interpretations.

BRCA2 ALTERATION IN AN OVARIAN NEOPLASM FROM A 19TH CENTURY ITALIAN MUMMY DETECTED BY NEXT GENERATION SEQUENCING

Veronica FORTE[1], Luca VENTURA[1], Cinzia MERCURIO[1], Elisabetta CILLI[2], Mirko TRAVERSARI[3], Francesco PEPE[4], Giancarlo TRONCONE[4], Claudio BELLEVICINE[4]
[1]UOC Anatomia Patologica, Ospedale San Salvatore, L'Aquila; [2]Dipartimento Beni Culturali, Università di Bologna; [3]Dipartimento Scienze Mediche e Chirurgiche, Università di Bologna; [4]Dipartimento Sanità Pubblica, Università di Napoli Federico II, Napoli, Italy

Recent advances in molecular biology have revolutionized the understanding and treatment of cancer, especially with the advent of targeted therapies. However, cancer is not a modern phenomenon, and its presence in ancient populations is increasingly recognized through paleopathological studies. This research explores the potential of next-generation sequencing (NGS) in analyzing ancient tumor tissues from an ovarian neoplasm in a 19th-century mummified female from Goriano Valli, Italy. We studied a naturally mummified woman (GVSG01) dating back to 19th century, through computed tomography (CT) scanning and autopsy examination. Tissue samples obtained at autopsy were submitted to histological examination. Ancient DNA (aDNA) was extracted from the tumor tissue, followed by NGS analysis using the Oncomine™ BRCA Research Assay to identify potential genetic alterations. CT scans revealed a large solid-cystic mass in the pelvis suggestive of ovarian cancer. Histologic analysis identified fibrous walls and necrotic debris, supporting the diagnosis of papillary cystic neoplasm. NGS analysis detected a variant of uncertain significance (VUS) in the BRCA2 gene (c.7093C>T; p.His2365Tyr), suggesting a potential association with genomic instability and ovarian cancer, though the variant's significance remains unclear. Molecular techniques in oncology have transformed cancer management in the last decade, enabling targeted therapies that improve survival and quality of life. Despite its ancient origins, cancer remains a critical health issue, as evidenced by its presence in archaeological findings. Paleopathology and paleoradiology have significantly advanced the study of ancient tumors, with a particular focus on the challenges of aDNA extraction and analysis. This case provides one of the rare instances of soft tissue ovarian neoplasm identified in the paleopathological setting, contributing to the limited under-

standing of ancient ovarian tumors. The BRCA2 variant identified is consistent with mutations implicated in high-grade serous ovarian carcinoma, underscoring the relevance of molecular paleo-oncology in the study of ancient neoplastic diseases. Moreover, the study highlights the potential of molecular techniques to advance the understanding of ancient cancer biology, providing insights into the genetic bases of neoplastic disease through the analysis of aDNA. Further research is needed to refine the interpretation of variants of uncertain significance in ancient cancer studies.

ATLANTO-AXIAL JOINT IN SICILIAN ARCHAEOLOGICAL POPULATIONS: A MULTIDISCIPLINARY ANALYSIS OF ANATOMICAL VARIATIONS USING 3D IMAGING, MORPHOMETRY, BIOARCHAEOLOGICAL AND FORENSIC-ODONTOLOGICAL TECHNIQUES

Francesca MELI[1,5], Salvatore FICARRA[2], Margherita MICHELETTI CREMASCO[3], Emilio NUZZOLESE[4], Giuseppe CAROTENUTO[5], Luca SINEO[5]

[1]Department of Culture and Society, University of Palermo, Italy; Analytical and Isotopic Mass Spectrometry Lab, Department of Cultural Heritage, University of Salento, Lecce, Italy; Aix Marseille University, CNRS, Minist Culture, LAMPEA, Aix-en-Provence, France; [2]Alma Mater Studiorum - CeSIA, University of Bologna, Italy; [3]Department of Life Sciences and Systems Biology, University of Turin, Italy; [4]Human Identification and Forensic Odontology Laboratory, Section of Legal Medicine, Department of Public Health and Pediatric Sciences, University of Turin, Italy; [5]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy

The axis, the second cervical vertebra (C2), forms a pivot upon which the atlas (C1) rotates, supporting the head. Although this region is anatomically compact, it can lead to serious complications due to the complex anatomy of the cranio-cervical junction. Instability at the cranio-cervical junction can arise from age-related (cumulative) degenerative processes or underlying pathologies such as rheumatoid arthritis. While the influence of these conditions on the apical and alar ligaments has been investigated, the relationship between these ligaments and anatomical variations of the dens remains largely unexplored. Furthermore, levels of cumulative stress and other pathological manifestations are also observed in the odontoid process of the axis, a key anatomical district involved in the so-called Crowned Dens Syndrome (CDS). This study aimed to investigate the anatomical variations of the anterior atlanto-axial joint and their association with the alar and apical ligaments, discussing potential etiologies in an osteological collection from three different ancient indigenous populations of Sicily: Contrada Colombardo (Copper Age), Ponte della Paolina (Early Bronze Age), and Baucina (Iron Age/Antiquity). The objective was to measure and present detailed morphometric parameters of the body of the axis and its odontoid process. Thirty dry axis and atlas vertebrae were obtained for anatomical evaluation, focusing on the body and odontoid process. Morphometric measurements included linear dimensions of length, breadth, and height. The vertebrae were examined using archaeological photogrammetry and forensic-odontological techniques, involving an intraoral scanner (Shining 3D) and a portable X-ray device coupled with a digital sensor (Vatech) for dental radiology. The intraoral scanner, typically used in dentistry but also in dental autopsies, captures optical impressions of dental arches through photogrammetry, while the periapical digital sensor facilitated digital radiographs of the odontoid process, compatible in dimension with the sensor. This enabled the easy acquisition onsite of 3D scans of the vertebrae and X-ray images of the of the odontoid process, for future and remote observation and examination.

ISOTOPIC INSIGHTS INTO EPIGRAVETTIAN DIET AT SAN TEODORO CAVE (SICILY): A MULTIPROXY APPROACH

Francesca MELI[1-3,5], Margherita MICHELETTI CREMASCO[6], Cecilia TRAMATI[4], Arianna ROMANO[1,5], Salvatrice VIZZINI[4], Luca SINEO[5]

[1]Department of Culture and Society, University of Palermo, Italy; [2]Department of Cultural Heritage, Salento University; Analytical and Isotopic Mass Spectrometry Lab, Lecce, Italy; [3]Aix Marseille University, CNRS, Minist Culture, LAMPEA, Aix-en-Provence, France; [4]Department Earth and Sea Sciences (DISTEM) - Laboratory of Isotopic Ecology, University of Palermo, Italy; [5]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy; [6]Department of Life Sciences and Systems Biology, University of Turin, Italy

The Cave of San Teodoro (Acquedolci, Messina), located on Pizzo Castellaro, represents the oldest *Homo sapiens* settlement in Sicily. Excavations, conducted throughout the 20th century and recently (2021-2024), have revealed Epigravettian hunter-gatherer (HG) burials dating back approximately 15,000 years. These burials are stratified within layers containing abundant evidence of human activity, including charcoal, animal remains (primarily *Cervus elaphus*, *Bos primigenius*, and *Sus scrofa*), gastropod shells, and lithic debris. The rich faunal assemblage, coupled with the site's inland location (over 6 km from the Epigravettian coastline), raises questions about the dietary reliance on animal protein. To address this, we analysed stable carbon and nitrogen isotopes ($\delta^{13}C$ and $\delta^{15}N$) in collagen extracted from human and animal remains recovered during the recent (2021-2023) excavations. These results were compared with previously published data from San Teodoro and two others Sicilian Epigravettian sites: Addaura and Grotta d' Oriente (Burial C). The San Teodoro samples yielded isotopic compositions ranging from -20.5° to -20.0° for $\delta^{13}C$ and 12.5° to 11.5° for $\delta^{15}N$. These values indicate a diet incorporating proteins from large herbivores foraging in a C3-dominated environment, alongside a significant and diverse plant component. The nitrogen isotope values do not suggest a predominantly meat-based diet. This is corroborated by dental

microwear analysis, which also points to a substantial plant intake. Our findings align with current understanding of Epigravettian HG paleoecology, highlighting a balanced dietary strategy adapted to the available resources within the postglacial landscape.

THE ANCIENT PEOPLING OF SICILY FROM A MITOGENOMIC PERSPECTIVE

Giulio CATALANO[1], Anna TOMMASI[2], Rajiv BOSCOLO AGOSTINI[3], Giacomo VILLANI[2], Nicola RAMBALDI MIGLIORE[2], Maria Teresa VIZZARI[3], Irene CARDINALI[4], Rosalinda DI GERLANDO[2], Valeria NICOLINI[2], Francesca MELI[1], Patricia SANTOS[3], Anna OLIVIERI[2], Ugo A. PEREGO[2], Nicoletta VOLANTE[5], Lucia SARTI[5], David CARAMELLI[6], Hovirag LANCIONI[4], Alessandra MODI[6], Silvia GHIROTTO[3], Alessandro ACHILLI[2], Luca SINEO[1]

[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo; [2]Department of Biology and Biotechnology "L. Spallanzani", University of Pavia; [3]Department of Life Sciences and Biotechnology, University of Ferrara; [4]Department of Chemistry, Biology and Biotechnology, University of Perugia; [5]Department of History and Cultural Heritage, University of Siena; [6]Department of Biology, University of Florence, Italy

The application of massive ancient DNA sequencing in evolutionary-anthropological, paleopathological and archaeological questions is a consolidated reality supported by hundreds of highly visible editorial products. The approaches are currently truly sophisticated and aim both at the reconstruction of large demographic and genetic scenarios of past populations and their biogeography, and at the individual characterization and geno-phenotypic reconstruction of individuals, especially, in this case, if they are anthropological samples of some phylogenetic interest. In this communication we want to trace the most recent results regarding the reconstruction of the mitochondrial (matrilineal) genomic history of the human population of Sicily in the period from the evolved Epigravettian, in which the first entries of *Homo sapiens* into the region were recorded, through the Strait of Messina, to the demographic-cultural transition of the Mesolithic-Neolithic and to the great demic migrations of the Metal Ages. The overlap of chronology and haplotypes geography results in a very convincing scenario that reconstructs the human mitochondrial genomic history in Sicily since the early colonization of the island to the first millennium BC.

PALEOPATHOLOGICAL EVIDENCE OF A TERMINAL ULCER (TU) ON THE MUMMIFIED BODY OF THE VENERABLE GIACOMO TORNO

Mirko TRAVERSARI[1*], Luca VENTURA[2*], Aleksander Sebastian IWASZCZONEK[3], Elisabetta CILLI[4], Marco LONGONI[5], Gianandrea PASQUINELLI[1,6], Giancarlo TRONCONE[7], Arturo BRUNETTI[8], Davide MELANDRI[1,9], Claudio BELLEVICINE[7]

[1]Department of Medical and Surgical Sciences, University of Bologna; [2]Division of Pathology, San Salvatore Hospital, L'Aquila; [3]Regular Theatine Clerics, Basilica of San Paolo Maggiore, Napoli; [4]Department of Cultural Heritage, University of Bologna, Ravenna; [5]Neurology Operative Unit, Bufalini Cesena City Hospital and Morgagni Pierantoni Forlì city Hospital, AUSL della Romagna; [6]IRCCS AOUBO, Bologna; [7]Department of Public Health, University "Federico II", Napoli; [8]Department of Advanced Biomedical Sciences, University "Federico II", Napoli; [9]Department of Emergency, Burn Center and Dermatology, AUSL della Romagna, Cesena, Italy

[*]Denote co-first author.

Giacomo Torno was born in the vibrant city of Naples in the year 1539, though some historical accounts suggest his birth date may have been in 1541. At the young age of 18, he embraced a life of religious service by joining the Clerics Regular of the Theatine Order, a Catholic religious organization dedicated to reform and the spiritual development of the clergy. He was officially welcomed into the order on 30 October 1558 in the Church of San Paolo Maggiore, a significant event that marked the beginning of his commitment to the Theatine community. Torno lived a life largely shrouded in devotion and religious practice, but his later years were marked by substantial physical suffering. On 4 December 1608, he suffered a debilitating stroke that severely impacted his health. Over the course of the next 45 days, as he struggled with his illness, contemporary accounts depicted him as being tormented, purportedly by the devil. Witnesses noted that he was afflicted by constant spasms in his right arm, which led to significant discomfort and contributed to an overall sense of distress during his final days. Upon his death, investigations conducted on his mummified remains revealed intriguing and concerning findings. A careful analysis uncovered a distinct irregularity in the skin surface at the sacral region, which indicated a deeper issue. Medical experts determined that the morphological features of this abnormality pointed toward a wound that had developed during his lifetime. Historical records reveal that he had, in fact, suffered a fracture of the first coccygeal vertebra. Further examination suggests that the appearance of the lesion correlating with the time of his demise, alongside its particular shape, can be categorized as an instance of the Kennedy terminal ulcer. This condition, recognized in medical discussions, is characterized by skin deterioration resulting from persistent pressure, often in patients who are bedridden or gravely ill. Thus, Giacomo Torno's case represents one of the earliest recorded instances of this phenomenon, drawing connections from both direct clinical observations and indirect historical accounts that highlight the complexities of his medical condition and its remarkable implications for the study of terminal illnesses in the early modern era.

REFERENCES

Del Tufo GB. Supplimento alla Historia della religione de' padri

cherici regolari raccolta e posta in luce da monsignor Gio. Battista del Tufo. Mascardi appresso I, editor. Roma; 1616.
Traversari, M., Ventura, L., Iwaszczonek, A. S., Cilli, E., Longoni, M., Pasquinelli, G., Troncone, G., Brunetti, A., Melandri, D., & Bellevicine, C. (2024). A case of Kennedy terminal ulcer in a 17th-century Italian mummy. Dermatology Reports, 16(4). https://doi.org/10.4081/dr.2024.9956

FORENSIC ANTHROPOLOGY IN SICILY: THE FAPAB RESEARCH CENTER EXPERIENCE

Elena VAROTTO[1], Carlo ROMANO[2], Giovanni MARCÌ[3], Francesco Maria GALASSI[4]
[1]Forensic Anthropology, Paleopathology and Bioarchaeology (FAPAB) Research Center, Avola, Italy; [2]Comandante della Sezione Biologia del RIS Carabinieri di Messina, Italy; [3]Sezione Investigazioni Scientifiche Carabinieri, Catania, Italy; [4]Department of Anthropology, Faculty of Biology and Environmental Protection, University of Lodz, Poland

Since its foundation in 2019, the FAPAB Research Center (Avola, Sicily) has been very active in the forensic anthropological scenario in Sicily, Calabria and Apulia. During these years, thanks to the fruitful collaboration with law enforcement agencies, public prosecutors' offices and courts, many anthropological cases have been dealt with as technical consultants. This presentation shows how some interesting forensic cases of different types have been approached on a methodological level, illustrating a set of cases examined by us in Sicily as consultants to the prosecutor's office, combining theoretical and practical aspects by utilizing anthropological, taphonomical, radiological (X-ray and CT scan imaging) techniques as well as radiocarbon-dating approaches. We endeavour to underline how a traditional view that identifies forensic anthropology with the drafting of a biological profile is not enough to answer the questions that, for instance a prosecutor, may ask you. This talk will show a set of four peculiar cases from terrestrial and aquatic environments: one consists of out-of-context human remains found in a Sicilian city centre; one from a cemetery; another one from a marine scenario and the last one from a transitional fluvial-marine location. As a simple example, human remains retrieved in aquatic environments differ from that found in terrestrial ones and, besides taphonomic alterations which are contextual with the local conditions in which the remains lie, also the presence of adhering marine taxa should be considered. This presentation demonstrates how important the interaction between the judicial system and a forensic anthropological team can greatly increase the capacity to perform correct assessments, hence avoiding unnecessary costs and longer timescales for the judicial system and providing the correct scientific answers to complex cases.

AQUATIC ENVIRONMENT AND ECOSYSTEM

IMPROVING THE SUSTAINABILITY OF FISHERIES THROUGH THE VALORIZATION OF UNDERUTILIZED SPECIES WITH INNOVATIVE PROCESSING TECHNOLOGIES

Alessandra AIELLO[1], Rosaria ARENA[1], Davide ANGILERI[1], Laura LA BARBERA[2], Simona VALENZA[2], Andrea SANTULLI[1], Concetta Maria MESSINA[1,2]

[1]Università degli Studi di Palermo, Dipartimento di Scienze della Terra e del Mare DiSTeM, Laboratorio di Biochimica Marina ed Ecotossicologia, Trapani; [2]Istituto di Biologia Marina, Consorzio Universitario della Provincia di Trapani, Italy

In recent years, consumption of seafood products has increased significantly, driven by growing consumer demand and the expansion of international markets. However, this trend has generated concerns about the sustainability of marine resources, highlighting the need for solutions that can balance global food needs with the conservation of marine ecosystems. In line with global, European and national policies and the initiatives of organisations such as the FAO, this research would contribute to improve sustainable fisheries and food production by reducing pressure on overfished target species and promoting the consumption of underutilized species. *S. smaris* is a common Mediterranean demersal species characterized by a high and significant content of polyunsaturated fatty acids of the omega-3 series, associated with beneficial effects on human health, such as DHA and EPA, and a good protein content. This study investigated the use of advanced processing techniques to produce *S. smaris* salty powder through a salting-drying method. This product is recognized as a Traditional Agro-food Product, holding significant commercial value. Salting-drying method induces product dehydration, which represents a key advantage as it is associated with a reduction in bacterial and enzymatic activity, thereby contributing to extended shelf life. A multidisciplinary approach was used to evaluate the sensory, physicochemical, biochemical and nutritional properties of the final product. The results showed that the processed product had good sensory characteristics and a high protein content. In terms of fatty acid profile, n-3 PUFAs were the most abundant class, with the main fatty acids being docosahexaenoic The innovative processing allowed for a good shelf life of the salted *S. smaris* powder, improving its potential as a processed fish product of high nutritional and commercial value. This study contributes to reduce the pressure on target species and can also provide economic advantages for the seafood industry through the creation of new production increasing the competitiveness of the fishery value-chain.

EFFECTS OF DIFFERENT SIZES OF POLYLACTIC ACID NANOPLASTICS (PLA-NPS) ON ZEBRAFISH

Maria Denise AMICO[1], Federica SCALIA[1], Fabiano CAPPARUCCI[2], Carmelo IARIA[2], Marianna MARINO[3], Antonietta SANTORO[3], Francesca RAPPA[1], Francesco CAPPELLO[1,4], Fabio BUCCHIERI[1], Antonella MARINO GAMMAZZA[1], Marta Anna SZYCHLINSKA[5]

[1]Department of Biomedicine, Neuroscience and Advanced Diagnostics (BIND), University of Palermo; [2]Department of Chemical, Biological, Pharmacological and Environmental Sciences, University of Messina; [3]Department of Medicine, Surgery and Dentistry, "Scuola Medica Salernitana", University of Salerno; [4]Euro-Mediterranean Institute of Science and Technology (IEMEST), Palermo; [5]Department of Precision Medicine in Medical, Surgical and Critical Care (Me.Pre.C.C.), University of Palermo, Italy

Since its discovery, the plastic polymer has found great application in human daily life. Unfortunately, improper disposal of plastic has led to its increasing accumulation in the environment. Various environmental agents cause the fragmentation of plastic, leading to the formation of nanoplastics (NPs) having a size of less than 1 µm. Recently, numerous studies have highlighted that NPs represents a huge threat not only to the environment, but also to living organisms included humans. Indeed, NPs can reach humans through biomagnification phenomena, inducing toxicity at different levels. For about a decade, to counteract and contain the deleterious effects of plastic, biodegradable plastics such as polylactic acid (PLA) have been introduced. However, recently some studies have questioned the real biodegradability of PLA and compared its effects to those of conventional plastics. In recent years, the zebrafish (*Danio rerio*) has been established as a model of choice in biomedical research and toxicological analyzes thanks to numerous advantages over mammalian models, such as high fecundity, short life cycle, high genetic homology with humans and the transparency of the embryos and larvae, which allows the *in vivo* visualization of organ development, and the distribution of contaminants marked with fluorochromes. Several studies have established that exposure of zebrafish to conventional plastics leads to various behavioral and morphological alterations, as well as tissue toxicity at different levels. More recent studies aim to investigate the effects of biodegradable plastics such as PLA on the zebrafish model. For these reasons zebrafish embryos and larvae have been exposed to polylactic acid nanoplastics (PLA-NPs) labeled with rhodomine at two different concentrations (0.1 mg/L and 1 mg/L) from 0 to 120 hours after fertilization (hpf). To investigate and compare the effects of the PLA-NPs in relation to the NPs size, two different batches of PLA-NPs, sized150 nm and 250 nm, were used. The toxicity of PLA-NPs was evaluated according to OECD test no. 236: Fish embryo acute toxicity test (FET). The distribution and bioaccumulation of PLA-NPs were evaluated *in vivo* by using a fluorescence microscope at different time points (24, 48, 72, 96 and 120 hpf) and the morphology was evaluated through histological analysis. Furthermore, to give translational value to the study, the internalization potential of PLA-NPs was evaluated in human fibroblasts (HDF) by flow cytometry and immunofluorescence. The results demonstrated that PLA-NPs accumulated within the larvae in a size-dependent manner, determining alterations in heartbeat and changes in intes-

tinal morphology. Moreover, HDF showed size-dependent up-take ability of PLA-NPs. Here, we report data suggesting size-dependent harmful effects of PLA-NPs on zebrafish development. However, further investigations are necessary to confirm the evidence on the zebrafish model, and it is also necessary to extend the investigation to other aquatic models to understand the real effects of PLA-NPs.

The experiments were conducted within the PRIN project "Plastic Contamination by Poly(Lactic Acid) (PLASTAMINATION): organ lesions and underlying molecular mechanisms", MUR, PRIN-PNRR2022 CODE NUMBER: P2022AA47Y-CUP MASTER D53D23021910001.

REFERENCES

Lamparelli, E.P *et al.* Pharmaceutics 2023 doi: 10.3390/pharmaceutics15112549.

Alberti G, Amico MD *et al.* IJMS 2024 doi: 10.3390/ijms25105394.

Bellipanni G *et al.* Cell Physiol. 2016 doi: 10.1002/jcp.25319.

Bhagat J *et al.* Sci Total Environ. 2020 doi: 10.1016/j.scitotenv.2020. 138707.

EFFECTS OF VALSARTAN ON THE GOLDFISH (*CARASSIUS AURATUS*) HEART: FROM THE WHOLE ORGAN TO TISSUE LEVEL

Alessia CAFERRO[1], Mariacristina FILICE[1], Maria Assunta IOVINE[1], Ellie ENGLAND[2], Alfonsina GATTUSO[1], Maria Carmela CERRA[1], Holly A. SHIELS[2], Sandra IMBROGNO[1]

[1]Department of Biology, Ecology and Earth Science, University of Calabria, Rende (CS), Italy; [2]Division of Cardiovascular Sciences, Faculty of Biology, Medicine and Health, University of Manchester, United Kingdom

In recent years, the presence of antihypertensive drugs in the aquatic ecosystems has significantly increased, raising concerns about their potential risk to non-target organisms. Among these drugs, the AT_1 receptor inhibitor, Valsartan is a concern due to its high consumption by human populations and its incomplete removal by wastewater treatment plants. It has been detected in surface waters at concentrations ranging from ng/L to ug/L. However, information about its influence on aquatic organisms is limited. By using isolated working hearts and cardiac muscle strips, this study investigated the putative effects of Valsartan on the cardiac function of the goldfish *Carassius auratus*. *Ex-vivo* working heart preparations exposed to increasing concentration of Valsartan (from 10^{-10}M to 10^{-5}M) under basal conditions did not show changes in cardiac output (CO), stroke volume (SV), and stroke work (SW). In contrast, under conditions of increasing preloads (*i.e.* Frank-Starling mechanism), isolated hearts exposed to Valsartan reached values of CO, SV, and SW higher than controls. Isometric cardiac muscle strips showed changes in the developed contraction force in response to Valsartan exposure. Particularly, a reduction in force (Fmax), as well as in the rate of contraction and relaxation was observed in ventricular strips exposed to a drug concentration of 10^{-6}M. A different behaviour was shown by atrial strips, in which a decrease in the rate of contraction and an increase in the rate of relaxation, with no significant change in Fmax, has been observed. Overall, our results showed that the exposure to Valsartan affects the cardiac function of the goldfish *C. auratus*. Although the molecular mechanisms underlying the responses here observed remain to be analysed, our data provide evidence about the capacity of antihypertensive drugs to influence heart physiology of non-target organisms with possible impact on animal performance.

STRUCTURE, ULTRASTRUCTURE AND FUNCTION OF THE MUCOUS GLANDS AND VENTRAL PHOTOPHORES IN CHAULIODUS SLOANI BLOCH & SCHNEIDER, 1801 (PISCES: STOMIIDAE): A COMBINED DEFENSIVE STRATEGY

Mauro CAVALLARO, Maria Cristina GUERRERA, Maria Luisa ARAGONA, Lidia PANSERA, Kamel MHALHEL, Rosaria LAURÀ, Maria LEVANTI, Antonino GERMANÀ

Dipartimento di Scienze Veterinarie Università degli Studi di Messina, Italy

The species *Chauliodus sloani* Bloch & Schneider, 1801 a mesopelagic fish occasionally stranding along the coasts of the Strait of Messina, plays a crucial ecological role, representing a significant portion of the biomass consumed by higher trophic-level predators This study focuses on the mucous glands and ventral photophores of *C. sloani*, two unique anatomical structures that are central to the species' defensive behavior. The mucous glands, whose morphology and function are analyzed in detail, secrete a dense mucus that envelops the animal when threatened. The ventral photophores, arranged in a well-defined pattern, are used for bioluminescence production, which, in combination with the mucus, functions as a defensive strategy against predators. The light emission from the photophores, coupled with the mucous substance, creates a "smoke screen" effect that disorients predators, reducing the prey's visibility. Our research particularly examines how bioluminescence and mucus may interact, amplifying the effectiveness of this defense mechanism. Furthermore, the defensive behavior observed in nature, documented by Widder (2022), suggests that the animal simultaneously emits light and mucus in response to an attack, indicating a synergistic function between the two structures. These findings provide new insights into the understanding of defensive anatomical adaptations in mesopelagic species and the evolution of complex defense mechanisms in pelagic environments.

EMBRYO ARCHITECTURE UNDERGOES REMODELING IN RESPONSE TO VANADIUM AND HIGHER TEMPERATURES: A SHIFT FROM MORPHOMETRIC TO MOLECULAR PERSPECTIVES

Gabriele SANGIORGI[1], Chiara MARTINO[1,2], Fabiana GERACI[1], Rosaria SCUDIERO[3], Roberto CHIARELLI[1]

[1]Department of Biological, Chemical and Pharmaceutical

Sciences and Technologies (STEBICEF), University of Palermo; [2]NBFC, National Biodiversity Future Center, Palermo; [3]Department of Biology, University Federico II, Napoli, Italy

The study of ecotoxicity induced by vanadium (V) represents an area of increasing interest due to the growing use of V in both the industrial and pharmaceutical areas. This leads to its introduction into water environments, marking a developing problem, especially since rising global temperatures appear to intensify its toxic properties. Cytotoxicological approaches carried out on whole marine embryos represent a valid research tool since they grow directly in contact with the pollutants and are equipped with highly responsive cells to stressors. Here, we discuss the detrimental impact on *Paracentrotus lividus* sea urchin embryos resulting from the combination of V and higher temperatures, reflecting the effects of climate variation. The results demonstrate the remodeling of embryonic architecture at the morphometric level, revealing developmental delays and anomalies. These malformations involve variations in the total skeletal mass due to the almost total absence of the skeleton, with the exception of small calcareous aggregates. Furthermore, both a modulation in total tissue remodeling enzymatic activities and a variation in the amount of three MMP-like gelatinases (MMP-2, -9, and -14) were observed. This research demonstrates that climate change significantly increases the harmful effects of V, emphasizing the necessity for comprehensive toxicity assessments in environmental evaluations.

REFERENCES

Martino *et al.* Between Life and Death: Sea Urchin Embryos Undergo Peculiar DNA Fragmentation after Exposure to Vanadium, Cadmium, Gadolinium, and Selenium. Life (Basel). 2024 Oct 12;14(10):1296. doi: 10.3390/life14101296. PMID: 39459596; PMCID: PMC11508963.

Chiarelli *et al.* Vanadium Modulates Proteolytic Activities and MMP-14-Like Levels during *Paracentrotus lividus* Embryogenesis. Int J Mol Sci. 2022 Nov 17;23(22):14238. doi: 10.3390/ijms 232214238. PMID: 36430713; PMCID: PMC9697301.

FLOW CYTOMETRY AND ARTIFICIAL INTELLIGENCE: A SUCCESSFUL COMBINATION FOR NANOPLASTIC DETECTION IN WATER AND BIOLOGICAL FLUIDS

Francesca D'ASCANIO[1,2,3], Francesco DOTTA[1,4], Annalisa BRUNO[1], Cristina MILILLO[1], Melania DOVIZIO[1], Domenico DE BELLIS[1,2], Giulia COLASANTE[1,2], Arianna AQUILINI-MUMMOLO[1,2], Sabino PORRO[1,2], Tamer Shibl Ali ESMAIL[1,2], Ayesha YOUNAS[1,2], Pasquale SIMEONE[1,2], Bruna SINJIARI[4], Piero CHIACCHIARETTA[1,4], Patrizia BALLERINI[1], Piero DI CARLO[1,4], Paola LANUTI[1,2]

[1]Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University of Chieti-Pescara, Chieti; [2]Department of Medicine and Aging Sciences, "G. d'Annunzio" University of Chieti-Pescara, Chieti; [3]Department of Humanities, Law and Economics, "Leonardo da Vinci" University, Torrevecchia Teatina (CH); [4]Department of Innovative Technologies in Medicine and Dentistry, University "G. d'Annunzio" of Chieti-Pescara, Chieti, Italy

Nanoplastics (NPs) are small particles, ranging from 1 to 1000 nm, resulting from plastic degradation. NPs have been detected in the air, water, and soil, therefore contaminating food and water. Recently, NPs have been identified in human peripheral blood and their impact on human health has been pointed out, given that NP long-term effects are still unknown. Conventional methods for detecting and quantifying NPs are often time-consuming and labor-intensive. This study aimed to develop a rapid and efficient method for analyzing biological and water samples using flow cytometry combined with artificial intelligence (AI), enabling extensive population screening. We collected pure water samples and water samples spiked with polystyrene NPs of two different sizes: 50 nm and 800 nm. Samples were further analyzed by flow cytometry. Flowkit python package was used to analyze flow cytometry data. Using the seaborn visualization python package, we analyzed the correlation among the dataset features, which were further plotted into 6 different heatmaps, one for each sample. Results were used to further identify NPs in real water and urine samples from mice (N=5) and humans (N=5). The analysis of the heatmaps revealed distinct bright regions in the emission channel of FITC (Ex=488 nm; Em=525/40 BP) as well as in the channels excited by the violet laser (Ex=405 nm; Em=450/45 BP, Em=525/40), allowing to clearly identify both the 50 nm and 800 nm NPs spiked in the samples. These features allowed to develop more advanced algorithms, further applied to identify NPs in water and urine samples. We developed a new AI/Flow Cytometry combined method for the rapid identification of NPs in water and biological samples, therefore providing a robust tool for addressing the global issue of NP contamination and their impact on human health.

ASSESSMENT OF PESTICIDE CONTAMINATION AND ITS ECOLOGICAL IMPACTS ON AQUATIC ENVIRONMENTS

Davide DI PAOLA

Department of Chemical, Biological, Pharmaceutical, and Environmental Science, University of Messina, Italy

Pesticide contamination in aquatic environments poses significant ecological risks, affecting water quality, biodiversity, and ecosystem stability. This study assesses the extent of pesticide pollution in aquatic systems, examining its sources, distribution, and ecological consequences. Through controlled laboratory experiments, testing the toxicity of selected pesticides and their mixtures on various aquatic model organisms, including fish, invertebrates. We evaluated multiple toxicity pathways, such as oxidative stress induction, neurotoxicity, endocrine disruption, and immune system impairment, using biochemical assays, behavioral analyses,

and gene expression profiling. Data collected reveal that both acute and chronic pesticide exposure contribute to biodiversity loss, bioaccumulation in aquatic organisms, and disruptions in trophic interactions. Sensitive species, particularly invertebrates and fish, exhibit physiological and behavioral alterations linked to pesticide toxicity. Moreover, interactions between pesticides and other environmental contaminants, such as pharmaceuticals and industrial chemicals, raise concerns about potential synergistic effects. Even at sub-lethal concentrations, these chemical mixtures can amplify toxicity, leading to unforeseen impacts on aquatic biota, including endocrine disruption, oxidative stress and immune suppression. Our research underscores the urgent need for comprehensive risk assessments that account for the combined effects of multiple pollutants. Strengthening monitoring programs, promoting sustainable agricultural practices, and refining regulatory frameworks are essential steps toward mitigating pesticide-induced ecological disturbances and preserving aquatic ecosystem health.

THE ECOLOGICAL SITUATION AND THE ASSESSMENT OF SOME BIOCENOSIS ON THE BUNA DELTA RIVER AND VILUNI LAGOON

Aurora DIBRA[1], Caterina FAGGIO[2], Valbona ALIKO[3], Xheni QYTYKU[4]
[1]University of Shkodra, Luigj Gurakuqi, Shkoder, Albania; [2]UNIME - Università degli Studi di Messina, Italy; [3]University of Tirana, Faculty of Natural Sciences, Albania; [4]University of Tirana, Faculty of Natural Sciences, Albania

The Viluni lagoon and the Ramsar site Buna River and its estuary are very complex water ecosystems. They are located near the administrative border between Albania and Montenegro. An ecosystem rich in natural resources, a lot of biodiversity, landscape, agriculture potentials, and cultural heritage. The main economic development in the villages Dajci, Pentar, Velipoja and Rrjolli is agriculture and fishery combined with rural tourism. A lot of forests and medical plants, pines and shrubs, dunes, and their specific biota are very important for ecological research studies. The paper presents data and the characteristics of biocenosis, marine biodiversity, the water quality in the Lagoon, and also the effects of pollution on biodiversity. Based on data collection, maps, and graphics present the ecological characteristics of biocenosis, dominant and rare species, and the abundance and frequency of specific species. Pollution is assessed in water and sediments. All data is collected during the expeditions in the field and elaborated in Excel. Also, in the area, there is a big potential for livestock production and handicraft products. Ecosystem services are assessed to enlarge the communities' knowledge about ecosystems and also to practice tools for sustainable use of land and biodiversity. Using the landscape, the biota, and the climate of the area, protecting natural resources, and developing sustainable tourism development could be a good perspective for the social and economic development in the zone. This new perspective of sustainable development should introduce new concepts of environmental education to the community and help them to transform their daily economic activities into coherence with nature protection and ecosystem services. The community should know the benefits of biodiversity use and agrotourism and ecotourism development as they will be in harmony with nature, and if they want to live a sustainable life in the area of Viluni Lagoon and Buna Rives shores.

PRELIMINARY DATA ON BACTERIAL ANALYSIS IN *CARETTA CARETTA* NESTS IN SICILY (ITALY)

Samira CACCIATORE[1], Paola GALLUZZO[1], Giorgia SCHIRÒ[1], Gianluca GIOIA[1], Oleana Olga PRATO[2], Francesco Paolo PALAZZO[2], Salvatrice VIZZINI[3], Anna CARROZZO[4], Vincenzo MONTEVERDE[1], Salvatore DARA[1], Rosaria DISCLAFANI[1,3]
[1]Centro di Referenza Nazionale sul Benessere, Monitoraggio, Diagnostica delle Malattie delle Tartarughe Marine (C.Re.Ta.M.), Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Palermo; [2]WWF Italia, Roma; [3]Centro di Sostenibilità e Transizione Ecologica, University of Palermo; [4]Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Palermo, Italy

The loggerhead sea turtle (*Caretta caretta*) is the only known species nesting along the Sicilian coasts. It is a vulnerable and threatened species, mostly due to anthropic activities (1) and, for this reason, the monitoring of its nesting is crucial for the conservation of this species. The aim of this study was to evaluate the presence of zoonotic bacteria in *C. caretta* nests in order to evaluate eventually sanitary risks for human health. A total of 820 unhatched eggs collected from n. 14 nests were sampled and bacteriological analysis were performed. The nests were located in different provinces of Sicily, (Italy): n. 3 in Trapani, n. 6 in Palermo, n. 3 in Agrigento, n. 1 in Siracusa and n. 1 in Caltanissetta. Samples were plated on selective and differential agar media and incubated at 37°C for 24h. The identification of isolated strains was carried out with the biochemical APIÒ system. All turtle nests analysed tested positive for bacterial culture and six species were identified: *Aeromonas hydrophila* (7/14; 50%), *Pseudomonas aeruginosa* (4/14; 28,57%), *Yersinia enterocolitica* (4/14; 28,57%), *Pseudomonas stutzeri* (3/14; 21,43%), *Serratia marcescens* (1/14; 7,14%), *Vibrio alginolyticus* (1/14; 7,14%). Our results showed a prevalence of *Aeromonas spp*. and *Pseudomonas spp*. in most of the nests analysed, with a higher percentage in the nests found in Palermo (n. 2 Mondello; n. 1 Balestrate; n.1 Isola delle Femmine and n.1 Cinisi): and Trapani (n. 1 Sibiliana and n. 1 Trapani). In 42,86% of the analyzed nests (n. 6/14), multiple bacteria strains were found simultaneously. The presence of the genera *Aeromonas* and *Pseudomonas* could be related to passage of the bacteria from female turtles to the eggs. Moreover, like other bacteria, they could infect the eggs by penetrating the pores of the shell, where can exploit internal substrates, facilitating bacterial proliferation (2, 3). *Aeromonas hydrophila* is frequently isolated from seawater and sand samples (4), and is generally considered a zoonotic bacterium (5). *Pseudomonas* is an opportunistic environ-

mental bacterium but it is commonly isolated from animal samples; it is recognized as cause of several diseases both in humans and animals, causing also abortion in mammals (6). *Pseudomonas* is also responsible for nosocomial infection and due to its capacity to develop antibiotic resistance, it is often particularly challenging to treat (7). All bacterial strains isolated from *Caretta caretta* eggs are zoonotic; the presence of zoonotic bacteria in sea turtle nests underscores the critical need for continuous monitoring of sea turtle populations, not only for their conservation but also to mitigate potential risks to human health, particularly in vulnerable individuals.

REFERENCES

1. Simantiris N. Sci Total Environ. 1 maggio 2024;923:171354.
2. Soslau G, *et al.* Journal of Experimental Marine Biology and Ecology 2011; doi: 10.1016/j.jembe.2011.10.018.
3. Orós J, *et al.* Dis Aquat Organ. 2005; doi: 10.3354/dao063013.
4. Alduina R, *et al.* Antibiotics (Basel). 2020; 10.3390/antibiotics9030116.
5. Fiorentini C, *et al.* J Appl Microbiol. 1998; 10.1046/j.1365-2672.1998.853517.x.
6. Yaeger M. J. and Holler L. D. CHAPTER 49 Bacterial Causes of Bovine Infertility and Abortion. Current Therapy in Large Animal. ISBN 13: 978-0-7216-9323-1.
7. Diggle SP, Whiteley M. Microbiology (Reading). 2020; 10.1099/ mic.0.000860.

RAMAN MICROSCOPY UNVEILS POLYSTYRENE NANOPARTICLES IN ZEBRAFISH: A NON-INVASIVE APPROACH TO NANO-BIO INTERACTIONS

Valentina IEZZI[1], Federico PERRELLA[2], Annamaria IANNETTA[3], Ludovica SULCANESE[3] Tommaso SILVESTRINI[3,4], Roberta Maria FERRI[1], Monia PERUGINI[3], Elisabetta BENEDETTI[1], Carino FERRANTE[5]

[1]Department of Life, Health and Environmental Sciences, University of L'Aquila; [2]Department of Physicol and Chemical Sciences University of L'Aquila; [3]Department of Bioscience and Agro-Food and Environmental Technology, University of Teramo; [4]Advanced Study "Theoretical and Applied Neuroscience" Preclinical, clinical and Translational neuroscience, University of Camerino (MC); [5]CNR-SPIN, c/o Department of Physicol and Chemical Sciences University of L'Aquila, Italy

Nanoplastic (NP) pollution represents a growing environmental threat, with potential toxic effects on aquatic organisms. In this study, Raman microscopy was used to quantitatively monitor NP bioaccumulation during the early stages of development. Raman microscopy has proven to be an effective non-invasive and label-free analytical technique for investigating nanoparticle-biosystem interactions, enabling the detection and localization of polystyrene (PS) nanoparticles within zebrafish. The sensitivity of Raman spectroscopy to molecular vibrations, combined with the high spatial resolution provided by an integrated microscopy system, allows for the acquisition of spatially resolved chemical information at the sub-cellular scale. Advanced data processing techniques, such as multivariate analysis and Principal Component Analysis (PCA), were subsequently used to better interpret the Raman spectral features. Specifically, a confocal micro-Raman setup was employed, enabling the isolation of the focal plane of interest with an in-plane spatial resolution of approximately 1 µm. This setup allows for non-invasive analysis without requiring external markers such as fluorophores. A 532 nm continuous-wave (CW) laser excitation source was used, providing an optimal signal-to-noise ratio while minimizing sample damage in biological specimens. Additionally, this setup allows for subsequent analyses on the same sample, such as fluorescence imaging or biochemical assays. Zebrafish larvae at 96 hours post-fertilization (hpf) were exposed to 150 nm PS beads, a size previously used in other studies (Lee *et al.*, 2019; Van Pomeren *et al.*, 2017; Qiang and Cheng, 2019). *In vivo* imaging enabled the detection of PS in key target organs, including the intestine and eye, as previously reported in the literature (Qiang and Cheng, 2019). FET tests were performed according to OECD guideline No. 236 (OECD, 2013). PS nanoparticles were sonicated and dispersed in distilled water (DW) at concentrations of 1 µg/ml, 5 µg/ml, 50 µg/ml, 250 µg/ml, and 500 µg/ml. PS exposure at these concentrations did not induce significant toxicological effects, making it impossible to determine lethal doses (LD10, LD20, and LD50), as high concentrations led to nanoparticle aggregation. Sublethal effects observed included blood stasis, yolk edema, and scoliosis. In zebrafish samples, point measurements revealed that PS specifically accumulated within and around the pupil, even up to 15 µm deeper than the iris focal plane. Raman mapping of extended sample regions is hindered by the presence of melanin deposits, whose fluorescence signal obscures polystyrene detection. The mapping procedure involves raster-scanning a sample region while acquiring Raman spectra at each point to create a spatially resolved chemical image of the sample. To overcome this limitation, future analyses will be conducted on nacre zebrafish to enable more detailed Raman measurements.

REFERENCES

Lee *et al.*, Nanoscale 11, 3173-3185 2019.
Qiang and Cheng, Ecotoxicol. Environ. Saf. 176, 226-233 2019.
Van Pomeren *et al.*, Aquatic Toxicology 190, 40-45 2017.

HIDDEN COSTS OF POLYVINYL ALCOHOL POLLUTION: EFFECTS ON MUSSEL PHYSIOLOGY, OXIDATIVE BALANCE, AND ENERGY ALLOCATION

Federica IMPELLITTERI[1], Alessia CAFERRO[4], Mariacristina FILICE[4], Sandra IMBROGNO[4], Caterina FAGGIO[2,3]

[1]Department of Veterinary Sciences, University of Messina; [2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina; [3]Department of Eco-sustainable Marine Biotechnology,

Stazione Zoologica Anton Dohrn, Naples; [4]Department of Biology, Ecology and Earth Sciences, University of Calabria, Cosenza, Italy

Aquatic environments are increasingly threatened by a diverse range of pollutants, with personal care products (PCPs) emerging as significant contributors. Among the numerous chemicals in PCPs, polyvinyl alcohol (PVA) stands out due to its widespread use in cosmetics and household products. Each year, over 65,000 tons of PVA are released into the environment. To evaluate the toxic effect of PVA, mussels were exposed to two concentrations of PVA (PVA1: 0.1 mg/L and PVA2: 10 mg/L), plus control, over 14 days. The experimental design evaluated haemocytes and digestive cells viability, osmoregulatory capacity through regulation volume decrease (RVD) assay, oxidative stress markers in gills and digestive glands (DG), and byssal plaque production. Cell viability was assessed using the Trypan Blue (TB) exclusion assay and the Neutral Red (NR) retention test. Haemocytes, extracted from the adductor muscle, and digestive cells, isolated from DG tissue, were analyzed using a Bürker chamber. The TB assay indicated a significant reduction in haemocytes viability at PVA2 ($p<0.05$), while digestive cells showed a minor but significant decrease at the same concentration ($p<0.05$). The NR assay confirmed these trends by highlighting lysosomal membrane destabilization in both cell types exposed to PVA2. The RVD assay was conducted on DG cells to assess their osmoregulatory response following exposure to a hypotonic challenge. Cell swelling and subsequent volume recovery were recorded through videometric analysis. While no significant differences were observed between groups in the initial volume decrease, cells exposed to PVA2 exhibited impaired volume recovery, indicating compromised osmoregulatory capacity. Oxidative stress biomarkers revealed increased superoxide dismutase (SOD) activity and elevated levels of oxidatively modified proteins (OMP) in gill tissues exposed to both PVA concentrations, with significance at PVA2 ($p<0.05$). In contrast, digestive gland tissues showed no signs of oxidative stress alterations. Byssal plaque production was assessed as a proxy for energy allocation[1]. Since byssus formation is energetically demanding, environmental stressors can influence its production and morphology. While the number of plaques remained unchanged, mussels exposed to PVA2 produced significantly ($p<0.01$) longer plaques, possibly as a compensatory mechanism to enhance adhesion. These findings show PVA's significant impact on marine organisms, potentially affecting aquatic ecosystems. As PVA production rises, understanding its effects is crucial for marine health and ecosystem integrity. Future studies should assess whether longer exposures or combined stressors amplify these effects.

REFERENCE

1. Impellitteri, F., Yunko, K., Calabrese, G., Porretti, M., Martyniuk, V., Gnatyshyna, L., ... & Faggio, C. (2024). Chlorpromazine's impact on *Mytilus galloprovincialis*: a multi-faceted investigation. *Chemosphere*, *350*, 141079.

ANTHROPOGENIC IMPACT IN THE NORTH IONIAN ITALIAN COAST: STRESS BIOMARKERS IN THE SENTINEL *MYTILUS GALLOPROVINCIALIS*

Maria Assunta IOVINE, Alessia CAFERRO, Mariacristina FILICE, Alfonsina GATTUSO, Sandra IMBROGNO, Maria Carmela CERRA
Department of Biology, Ecology and Earth Science, University of Calabria, Arcavacata di Rende (CS), Italy

Coastal marine environments are increasingly exposed to anthropogenic pressures, particularly in regions with high tourist activity. The coastal area of Amendolara, in the North-East part of Calabria, has been recently recognized as a Site of Community Importance (SCI) and included in the list of Regional Marine Parks of Calabria Region, making its preservation a priority. By using a biomonitoring approach, this study aimed to assess the potential impact of seasonal tourism on the health status of aquatic organisms within the Amendolara SCI. The mussel, *Mytilus galloprovincialis,* a sentinel organism widely used to study the impact of environmental stress on animal health, was chosen as a model. Mussels from a local farm (control, CTRL) were transplanted to Amendolara nearshore waters and sampled at two time points: one week after transplantation in July (pre-touristic impact, PreI), and at the end of the tourist season in September (post-touristic impact, PostI). Anthropogenic impact was assessed by evaluating the levels of oxidative stress biomarkers [*i.e.*, catalase (CAT), superoxide dismutase (SOD), lipid peroxidation (LPO), and protein carbonylation (OMP)], as well as the activity of acetylcholinesterase (AChE), a biomarker of pollutant exposure, in gills (G) and digestive gland (DG). Results indicated a time- and, in some cases, tissues-dependent modulation of the oxidative response. Compared to the control, CAT activity decreased in the PreI group and increased in the PostI in both tissues. SOD activity increased in the gills, but decreased in the DG of the PostI, while unchanged in the PreI group. LPO resulted significantly enhanced in both tissues after the tourist season, while OMP displayed an opposite trend, decreasing in the gills but increasing in the DG of the PostI group. Finally, in both tissues, AChE activity appeared reduced after the touristic season. These findings highlight the importance of using well assed biomarkers (*i.e.* oxidative stress indicators) to evaluate the impact deriving from anthropogenic activities, including seasonal tourism, on the health status of sentinel organisms, such as *M. galloprovincialis*. They also underscore the need to improve environmental biomonitoring to support management strategies to mitigate stress in coastal areas.

PRELIMINARY STRESS ASSESSMENT IN *CARETTA CARETTA* DURING THE REHABILITATION PERIOD

Chiara LOMONACO[1], Giorgia SCHIRÒ[1,2], Paola GALLUZZO[2], Rosaria DISCLAFANI[2,3], Irene VAZZANA[2], Gioacchino PATITÒ[2], Vincenzo MONTEVERDE[2], Claudia GIANNETTO[1], Salvatore DARA[2]

[1]Dipartimento di Scienze Veterinarie, Polo Universitario dell'Annunziata, University of Messina; [2]Centro Referenza Nazionale Tartarughe Marine (C.Re.Ta.M), Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Palermo; [3]Centro di Sostenibilità e Transizione Ecologica, University of Palermo, Italy

Loggerhead sea turtle (*Caretta caretta)* is a long-living omnivorous animal and the most abundant species in the Mediterranean Sea [1]. It's considered endangered, included in the IUCN (International Union for Conservation of Nature) Red List [2] and threatened both by infectious diseases and anthropogenetic activities (chemical pollution, plastic ingestion, entanglement) [3]. Therefore, many subjects recovered at rescue centers, are hospitalized for a long time. Aim of this study was to evaluate the stress indicators through blood samples analyses. In particular, Heterophile Lymphocyte ratio (H/L), Creatine Kinase (CK) and Glucose as markers of preliminary stress assessment in *C. caretta* hospitalized at sea turtle rescue center C.Re.Ta.M (IZS Sicilia, Palermo, Italy), were investigated. Twenty-five subjects of *C. caretta* with different clinical situations (injuries, ingestion of plastic, cold stunning) were monitored. For each subject carapace length (CCL) and weight (Kg) were measured; subjects were classified in juveniles (n. 16, CCL: 32±5,92), sub-adults (n. 8 CCL: 52,6±7) and adults (n. 1 CCL 72 cm). Blood samples were collected from the dorsal cervical sinus in litio-heparine and serum tubes in three different time: at the moment of recovering (T0), a month later (T1) and two months later (T2); blood smears were also performed and stained with Diff-Quick stain. Two hundred Leucocytes were counted and classified in Lymphocytes, Heterophils, Eosinophils, Basophils, Monocytes and H/L ratio was calculated. Glucose and Creatine Kinase were analysed from serum by the multiparametric chemistry analyzer BS-480 Mindray. The three analysed time were compared by GraphPad Prism 5: cause of the limited number of adult subjects it was analysed with sub-adult subjects. The results showed a decrease of H/L ratio (*p-value*: 0.0031) in Juveniles from T0 to T2 and a decrease of H/L ratio (*p-value*: 0,0254) and Glu (*p-value*: 0,0001) in Sub-adults from T0 to T2. During the rehabilitation period turtles are subjected to potential stressor as periodical manipulation, life in tanks, limited movement and artificial feeding; by the way, these factors are necessary to readapt the subjects to the wildlife, as to sustain their ability to survive and reproduce, maximizing the numbers [4]. This study suggests that the stress level could be influence by the injury/pathology of subjects [1,3,4,5] but is not influenced by the duration of rehabilitation period. However, further studies are needed to define other causes of variations in stress levels in rehabilitating *C. caretta*.

REFERENCES

1. Caliani *et al.*, Veterinary Immunology and Immunopathology, 2019, doi:10.1016/j.vetimm.2018.11.013.
2. Casal & Oros, Veterinary Science 2007, doi:10.1016/j.rvsc. 2006. 07.017.
3. Flower *et al.*, Journal of Zoo and Wildlife Medicine, 2018, doi:10.1638/2017-0051R1.1.
4. Flint *et al.*, PeerJ, 2017, DOI 10.7717/peerj.3132.
5. Melvin *et al.*, Biological conservation, 2021, doi:10.1016/j.biocon.2020.108943.

TURNING PROBLEM INTO PROFIT BY THE VALORIZATION OF BY-PRODUCTS: THE CASE STUDY OF BIOACTIVE MOLECULES FROM THE INVASIVE BLUE CRAB

Michelle MARCHAN GONZALEZ[1], Rosaria ARENA[1], Simona MANUGUERRA[1], Giuseppe RENDA[2], Andrea SANTULLI[1], Concetta Maria MESSINA[1,2]
[1]Laboratory of Marine Biochemistry and Ecotoxicology, Department of Earth and Marine Sciences, DiSTeM, University of Palermo, Trapani; [2]Istituto di Biologia Marina, Consorzio Universitario della Provincia di Trapani, Italy

The growing interest in marine-derived bioactive compounds has become a prominent area of research in recent years. Fishery supply chain by-products provide a sustainable and easily available source to produce high value-added products such as omega-3 fatty acids, peptides, and antioxidants, which have potential health benefits and applications in the nutraceutical industry. The valorization of fishery supply chain by-products to extract bioactive compounds offers a significant opportunity to minimize waste within the fishing industry, aligning with circular economy principles. This study focuses on the blue crab (*Callinectes sapidus*), an invasive species whose population increase has increased negatively, which has impacted local ecosystems and fisheries. Like other crustaceans, blue crab by-products are rich in bioactive molecules, that which suggest suggests its valorization. In this study, blue crab by-products (BCbp) were exploited for production of protein hydrolysates (PH) by enzymatic hydrolysis, an environmentally friendly process that uses enzymes to break down proteins into peptides and amino acids. The antioxidant properties of blue crab by-product hydrolysates were then evaluated *in vitro*. The results demonstrated significant antioxidant power in the BCbp hydrolysates, along with a protective effect against oxidative damage in human fibroblasts (HS-68). Specifically, the PHs acted as free radical scavengers and hydrogen donors, protecting against oxidative stress. This study demonstrated the effective use of blue crab by-products through environmentally friendly extraction methods. This promising strategy offers a valid approach to the valorization of fishery by-products, producing active biomolecules with potential applications in the food and nutraceutical industry, as sources of essential amino acids, bioactive peptides and components of edible films.

NOT ALL LIKE IT HOT: THE CONTRASTING EFFECTS OF TWO GLOBAL WARMING CONDITIONS ON KEY MEDITERRANEAN SEA URCHIN SPECIES EXPOSED TO POLLUTION

Chiara MARTINO[1,2], Roberto CHIARELLI[1], Dario SAVOCA[1,2], Manuela MAURO[1], Maria BYRNE[3], Thorsten HÜFFER[4], Rosario BADALAMENTI[1],

Antonella MACCOTTA[1,2], Vincenzo ARIZZA[1,2], Mirella VAZZANA[1,2]
[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Italy; [2]NBFC, National Biodiversity Future Center, Palermo, Italy; [3]School of Life and Environmental Sciences and Marine Studies Institute, The University of Sydney, New South Wales, Australia; [4]Department of Environmental Geosciences, Center for Microbiology and Environmental Systems Science, University of Vienna, Austria

Gradual ocean warming and marine heatwaves represent major threats for marine organisms already facing other anthropogenic-derived hazards, such as chemical contamination in coastal areas. Sea urchin larvae are a common model system in marine ecotoxicological studies to assess the impacts of climate change and pollution. Here I report the combined effects of thermal stress and exposure to three different pollutants of the marine environment (gadolinium, vanadium and phthalates) on embryos of two common Mediterranean sea urchin species with predicted opposite responses to warming, the temperate *Paracentrotus lividus* and the sub-tropical *Arbacia lixula*. These species are among the most abundant echinoids living in shallow rocky reefs of the southwestern Mediterranean and are ecologically important because their herbivorous grazing impacts macroalgae and leads to the formation of barrens habitats. Embryos were exposed to several treatments of three temperatures (18°C, 21°C, 24°C) and different concentrations of the three pollutants (from environmentally relevant to cytotoxic). We tested the single and combined effects to thermal stress and pollutants at three functional levels: i) exposure–response relationships, ii) morphological, analyzing impacts of treatments on larval phenotypes and morphometric traits of larval growth and biomineralization; iii) biochemical/cellular, investigating the effects on activity of enzyme biomarkers, protein expression and the activation of the cellular stress response. With respect to developmental progression, elevated temperatures at near-future projections (+3°C, 21°C) accelerated development and achievement of the larval stage, while extreme warming at present-day marine heatwave conditions (+6°C, 24°C) breached the thermotolerance threshold of both species with a high proportion of abnormal larvae (30%). We found a fascinating double side effect of increased temperature combined to pollution: a mild temperature increase (+3°C) reduced the negative effects of pollutants on development with a lower percentage of abnormality and improved skeleton growth, while combined heatwave conditions (+6°C) and pollution resulted in a lower proportion of embryos reaching the advanced larval stages. Our results indicate that the negative effects of pollutants-exposure on *P. lividus* and *A. lixula* larval development and biomineralization will be mitigated by a near-future ocean warming, up to a thermotolerance threshold when negative synergistic effects were evident. Our data highlight the use of biomarkers as sensitive tools to detect environmental impacts as well as the need for a better understanding of the interactions between the multiple stressors faced by marine species in coastal environments.

GENETIC ADAPTATION ENHANCES YEAST SURVIVAL IN ANTARCTIC COASTAL LAKES WITH VARIABLE IRON LEVELS

Domenico GIOSA[1], Alessandro Ciro RAPPAZZO[2,3], Alessia MARCHETTA[1], Filomena DE LEO[1], Gabriele RIGANO[4,5], Carmen RIZZO[2,6], Angelina LO GIUDICE[2], Maurizio AZZARO[2], Maria PAPALE[2]
[1]University of Messina; [2]Institute of Polar Sciences, (CNR-ISP), Messina; [3]Campus Scientifico, Ca' Foscari University of Venice; [4]University of Rome Tor Vergata, Rome; [5]Department of Physics, University of Trento; [6]Stazione Zoologica "Anton Dohrn", Sicily Marine Centre, Messina, Italy

Microbial communities in iron-limited environments depend on complexation with ligands to improve iron (Fe) availability. Understanding the origins and distribution of Fe in these environments is crucial for determining factors that influence their productivity. Antarctic coastal marine environments, which support highly productive ecosystems, receive Fe from various sources. Microorganisms such as fungi, algae, and bacteria employ strategies like biosorption to regulate metal concentration and distribution. Fe enrichment experiments using water and sediment from Antarctic lakes (Deception Island, Antarctic Peninsula) were conducted. Five fungal strains survived at 5000 ppm Fe. The yeast *Goffeauzyma gilvescens* (Y-AZA2), known for its stress resistance, was selected for RNA sequencing to evaluate differential gene expression between wild and stressed conditions and reconstruct its transcriptome. The fungal strain was grown in triplicate into 45 mL of 10% YM and 45 mL of 10% Yeast Malt (YM) supplemented with $FeSO_4$ at a final concentration of 4000 ppm and incubated aerobically at 4°C with shaking at 175 rpm for 15 days. Total RNA was extracted using the Quick-RNATM Miniprep Kit (Zymo), and transcriptome sequencing was performed using Illumina NovaSeqX technology. After trimming, over 218 million paired-end reads were obtained, averaging 36.39 million high-quality reads *per* sample. A *de novo* transcriptome assembly was generated with Trinity v2.15, reconstructing over 40,156 transcripts and 26,000 genes. Annotation and gene expression evaluation were conducted using the *G. gilvescens* reference genome from NCBI (Accession: GCA_025758705.1) within the funannotate pipeline. *De novo* and reference-based transcripts were reconstructed with Trinity and StringTie assemblers and aligned against the UniProt database. Coding probability was assessed using TranSuite and CPC2 software. Gene expression levels were quantified with Salmon v.1.10.2. Differential gene expression analysis was performed with edgeR. The *G. gilvescens* genome was predicted to contain 17,152 genes, comprising 9,486 protein-coding genes, 9,895 long non-coding RNAs (lncRNAs), 184 non-coding RNAs (ncRNAs), 125 tRNAs, and a single rRNA. Under the studied conditions, 1,075 genes exhibited differential expression, with 412 upregulated and 662 downregulated. Gene set analysis revealed enrichment in peroxisome, fatty acid degradation, and Fe metabolism pathways under iron-

enriched conditions, while ribosome biogenesis and RNA processing pathways were uniquely enriched in the absence of iron. This study identified promising fungal strains capable of surviving in high Fe concentrations, such as *Goffeauzyma gilvescens*. The molecular responses of this yeast to high Fe concentration were characterized, particularly in relation to fatty acid metabolism, which plays a role in iron absorption and may influence iron availability an important factor in Fe-limited environments. These findings provide new insights into microbial responses to environmental stressors and offer potential applications for bioremediation and environmental management strategies.

UNDERSTANDING THE EFFECTS OF MARINE NOISE POLLUTION ON ECHINODERM PHYSIOLOGY: A STUDY WITH THE TROPICAL SEA URCHIN *ECHINOMETRA LUCUNTER* (LINNAEUS, 1758)

Vinicius QUEIROZ[1,2,*], Linilson R. PADOVESE[3], Márcio R. CUSTÓDIO[1], Mirella VAZZANA[2], Vincenzo ARIZZA[2], Manuela MAURO[2], Francesco LONGO[2], Renata G.M. WHITTON[1]

[1]Departamento de Fisiologia. Instituto de Biociências. Universidade de São Paulo, Brasil; [2]Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF), Università di Palermo, Italy; [3]Centro de Biologia Marinha, Universidade de São Paulo, Pitangueiras, São Sebastiao, Brasil

Anthropogenic noise is a global threat to marine organisms, and its effects have been observed in vertebrates and invertebrates. For the latter, mollusks and crustaceans have been the most studied models, while Echinodermata – deuterostomes closely related to Chordata – have been overlooked. In this context, the present study aims to understand how exposure to different sound frequencies affects the physiological responses of the sea urchin *Echinometra lucunter* (Lamarck, 1816). Sixty specimens of *E. lucunter* were collected at the São Sebastião channel (São Paulo state - Brazil) and acclimated for 48-72h in 500-L tanks containing circulating natural seawater (24±1°C; 34±1 PSU) at the Centro de Biologia Marinha of the University of São Paulo (CEBIMar-USP). Five control and five experimental individuals were used to evaluate the effects of each frequency (250, 1,000, and 2,000 Hz). The experimental specimens were exposed for 3h in a 400 L polypropylene tank to each specific frequency emitted at a sound pressure level (SPL) of 130 dB. The assays were performed twice, totaling 10 replicates for control and experimental groups for each frequency. After exposure, total protein concentration and esterase, alkaline phosphatase, and peroxidase activities from the cell-free coelomic fluid were analyzed and compared to a control group consisting of animals maintained at the same conditions but with no sound stress and at a natural SPL (90 dB). A One-way ANOVA, followed by a Turkey post-hoc test, was performed to check the significance of the differences ($p < 0.05$). After 3h of exposure, total protein concentrations significantly increased in all frequencies compared to the control but did not differ among them. Esterase and alkaline phosphatase activities showed a similar pattern, significantly increasing in all frequencies compared to their respective controls and reaching the highest values in individuals exposed to 2,000 Hz, which also differed from 250 and 1,000 Hz for both enzymes. Lastly, peroxidase activity increased significantly only in animals exposed to 2,000 Hz, while 250 and 1,000 Hz did not differ from the control. Our results highlight three essential aspects: (1) although poorly studied, echinoderms responded to acoustic stress and seem to be good models for accessing how noise pollution affects animal life; (2) the frequencies used here are in the band of noise produced by vessel activity, the most important source of marine noise pollution, which may bring insights on how vessel affects invertebrates; (3) the parameters analyzed were, in general, responsive in all frequencies, showing that they are helpful to understand echinoderm physiology under acoustic stress.

This work was supported by Fundação de Amparo a Pesquisa do estado de São Paulo-FAPESP-Brazil (Grant number: 2021/10161-8; 2024/05857-1).

EFFECT OF EXPERIMENTAL DIETS ON ABALONE, *HALIOTIS TUBERCULATA*: A FIRST PILOT CASE STUDY IN SICILY

Maxime RANNOU[1], Simona MANUGUERRA[1], Eleonora CURCURACI[1], Rosaria ARENA[1], Andrea SANTULLI[1], Concetta Maria MESSINA[1,2]

[1]Laboratory of Marine Biochemistry and Ecotoxicology, Department of Earth and Marine Sciences, DiSTeM, University of Palermo, Trapani; [2]Istituto di Biologia Marina, Consorzio Universitario della Provincia di Trapani, Italy

The sector of aquaculture needs innovation to meet competitiveness and sustainability standards. Diversification of farmed species is a strategy to innovate the production, however, this solution needs to setup the best farming condition able to combine productivity and welfare with sustainability and quality. Diet composition is one of the most important factors affecting the performance and quality of farmed species. This study reports one of the first experiment of culturing the mollusk abalone in Sicily, focusing also on quality of diet and quality of the product. Abalone (*Haliotis tuberculata*) is an high-value herbivorous mollusk species that feed mainly on fresh algae or, in aquaculture, on formulated dry pellet. Several studies have shown that the diet has a significant effect on growth performance, biochemical composition, taste, texture and colour, determining the overall quality of the final product. In this study, an experiment with *H. tuberculata* specimens was setup in a facility located on the East coast of Sicily. Abalones were fed two commercial diets with different protein levels (high, HP, and low, LP) to evaluate their growth performance and nutritional composition, compared to a control group fed on standard algae based diet. Obtained results on the protein and lipid content and fatty acid profile, showed that all three diets adequately met the nutritional requirements of the

reared specimens. However, a significant increase in growth performance was observed in abalones fed with the two commercial diets (HP and LP) compared to those fed the standard diet. This experiment was part of a research project aimed to setup the best conditions for abalone aquaculture and these preliminary results were useful for a first selection of the artificial diets able to guarantee the best growth performances and quality of the products. More results are needed to strengthen the knowledge about the mechanisms of adaptation and responses of this species to all the culture conditions and to define the final protocol for farming abalone in Sicily, contributing to improve the diversification of local aquaculture.

PRELIMINARY OBSERVATIONS ON BACTERIAL COMMUNITIES FROM GLACIERS TO FJORDS IN NY-ÅLESUND (SVALBARD, NORTHERN NORWAY)

Alessio LENA[1,2], Alessandro Ciro RAPPAZZO[1,3*], Maria PAPALE[1], Maurizio AZZARO[1], Ilaria BANESCHI[4], Marco DOVERI[4,5], Angelina LO GIUDICE[1]
[1]Institute of Polar Sciences - CNR, Messina; [2]University of Messina; [3]Cà Foscari University of Venice; [4]Institute of Geosciences and Earth Resources - CNR, Pisa; [5] University of Pisa, Italy

Cold-adapted bacteria have evolved different mechanisms to survive in extremely cold conditions, such as cell membrane stability, up-regulation of biosynthesis, increased production of extracellular polymeric substances, and presence of membrane pigments and antifreeze proteins. In recent years, there has been increasing interest in using cold-adapted or bacterial enzymes for potential use in biotechnological applications, like bioremediation at low temperatures. As part of the ICEtoFLUX project (grant PRA2021-0027), four field campaigns (spring and summer in both 2022 and 2023) were carried out in the Bayelva catchment (79°N 12°E, Svalbard Islands, Norway). Glacier snowpack, glacial meltwater, Bayelva River water, and Kongsfjorden seawater were collected to obtain bacterial isolates that could grow in the presence of organic pollutants. Aliquots of each sample were spread-plated on agar media to evaluate bacterial viable counts (BVCs, expressed as colony-forming units per mL, CFU/mL). The same agar plates were then used for bacterial isolation. BVCs were on average 10^2 CFU/ml, with the lowest values determined in snow samples and the highest ones in the river and fjord waters. A total of 107 and 99 isolates from samples of the first and second campaign, respectively, were screened for growth in the presence of pesticides (*i.e.*, DDT and DDE), hydrocarbons (both aliphatic and aromatic), and polychlorobiphenyls (PCBs) as the sole carbon and energy source. Results revealed a 14.8% positivity for PCB oxidation in 2022, compared to 12.5% in 2023. For DDT, positivity reached 31.1% in 2022, while no strains grew in the presence of DDE. Strains capable of growing on petroleum and diesel oil were 17.9 and 20%, respectively, in 2022, maintaining similar percentages in 2023. Growth on alkanes (*i.e.*, hexane, octane, dodecane) was more frequent on octane (35.3% of total isolates), followed by hexane (10%). In 2022, 13 strains (12.14%) grew in the presence of naphthalene (this percentage was lower in 2023). These findings suggest a possible role played by microbial communities in organic pollutant removal along the meltwater pathway, emphasizing their ecological importance in Arctic environments undergoing rapid climate change. Future analyses will integrate metagenomics to explore microbial functional potential and adaptation mechanisms.

PHYSIOLOGICAL RESPONSES OF DIGESTIVE GLAND CELLS IN *MYTILUS GALLOPROVICIALIS*: STUDY OF THE IMPACT OF 2-METHYL-4-ISOTHIAZOLIN-3-ONE

Giuseppe STRACUZZI[1], Cristiana Roberta MULTISANTI[2], Federica IMPELLITTERI[2], Giorgia ZICARELLI[1], Caterina FAGGIO[1,3]
[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina; [2]Department of Veterinary Sciences, University of Messina; [3]Department of Eco-sustainable Marine Biotechnology, Stazione Zoologica Anton Dohrn, Naples, Italy

MIT (2-Methyl-4-isothiazolin-3-one) is a biocide commonly used in the formulations of several daily used products, such as detergents, disinfectants, and biodiesel to control microbial growth and to enhnce their performances, including maintain their integrity. The presence of MIT in these products implies its unavoidable releasing into natural environments. Therefore, the need to carry out on further studies to investigate its impact on animals, environment, and also human health, is imperative. For this reason, to obtain the present preliminary data, the model organism *Mytilus galloprovincialis* has been employed to assess the impact of this xenobiotic. *M. galloprovincialis* was selected due to its suitability as sentinel organism, being able in detecting and resisting to the stress of the surrounding environment. Specimens of *M. galloprovincialis* were exposed to two different concentrations of MIT (E1: 200 µg/L and E2: 400 µg/L), for fourteen days. The digestive gland (DG) has been choosen for analyses as it represents one of the main target organ when *M. galloprovincialis* is subjected to stress due to xenobiotics exposure. After isolated according to the protocol by Torre *et al.* (2013) [1], viability of DG cells, through two colorimetric methods (Neutral red retention assay and Trypan blue exclusion test), has been evalutated. Moreover, the ability of the DG cells to regulate their volume (RVD assay) in response to osmolarity variations has been assessed through a video-metric method [2]. Results showed a significant reduction in cell viability in the experimental groups exposed to the lower concentration (E1) compared to the control groups. A significant difference has been also observed between E1 and E2. In addition, no signficant differences were observed between E2 compared to the control groups, probably suggesting an adaptation of the organism to higher concentration of MIT. Although not significant, a reduction in cell ability to regulate their volume has been observed in the experimental

groups exposed to the highest concentration (E2), suggesting the impairment of cellular transports. In conclusion, these results suggest a physiological damage induced by MIT on the physiological performance of the DG in *Mytilus galloprovincialis*, which may be a relevant starting point for future research.

ACKNOWLEDGEMENTS. This work has been funded by the European Union – NextGenerationEU, as part of the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.1 (PRIN) under Grant Assignment Decree n. 1375 adopted on 01-09-2023 by the Italian Ministry of University and Research (MUR) - Project PRIN 2022 PNRR Title: *Investigations of emerging pollutant: ecological and human health impacts of isothiazolinone biocides* – Project ministerial code: P2022BH28Y_03- CUP: C53D23009240001 – Beneficiary: University of Teramo (UniTE).

REFERENCES

1. Torre, A., Trischitta, F., Corsaro, C., Mallamace, D. and Faggio, C., 2013. Digestive cells from *Mytilus galloprovincialis* show a partial regulatory volume decrease following acute hypotonic stress through mechanisms involving inorganic ions. Cell Biochem Funct, 31.
2. Impellitteri F., Riolo K., Multisanti C. R., Zicarelli G., Piccione G., Faggio C., Giannetto A., 2024. Evaluating quaternium-15 effects on *Mytilus galloprovincialis*: New insights on physiological and cellular responses, Science of The Total Environment, Volume 918, 170568, ISSN 0048-9697.

BIOTECHNOLOGY AND BIOENGINEERING

BIOLOGICAL RESPONSE AND MORPHOLOGICAL ASSESSMENT OF HUMAN THP-1 MACROPHAGES TO RIGID MULTIWALLED CARBON NANOTUBES TRAPPED IN ECM-MIMETIC GELATIN

Naym BLAL[1,2], Marianna ROCA[1,2], Claudia ORLANNO[1], Edmondo BATTISTA[2,3], Valentina MOLLO[2], Dario GRECO[4], Daniela GUARNIERI[1,2]

[1]Department of Chemistry and Biology, "Adolfo Zambelli", University of Salerno, Italy; [2]Center for Advanced Biomaterials for Healthcare@CRIB, Istituto Italiano di Tecnologia (IIT), Napoli, Italy; [3]Department of Innovative Technologies in Medicine & Dentistry, University "G. D'Annunzio", Chieti, Italy; [4]Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland

Carbon nanotubes (CNTs) are nanomaterials of particular interest due to their distinctive physicochemical properties that make them promising for technological and industrial development. Their application in industry is continuously growing, which could lead to accumulation in the environment and a consequent impact on both humans and ecosystems. Exposure to CNTs, especially in the occupational context, occurs mainly by inhalation. However, little is known about the possible effects of CNTs on lung diseases and cancer. Since the extracellular matrix (ECM) modulates the behavior of nanomaterials entrapped in it and the immune response, the study of interactions between immune cell and carbon nanotubes should take into account the extracellular microenvironment in which these interactions occur. In the present study, funded by the PRIN 2022 PNRR program, we evaluated the impact of multiwalled carbon nanotubes (MWCNTs) embedded in gelatin as ECM mimic, on THP-1 macrophages; mainly, studies focused on morphological characterization and biological responses. Macrophage-like cells derived from the human monocytic cell line THP-1 were cultured on ECM-like substrates for up to 5 days. Preliminary experiments demonstrated that macrophages adhered to the substrates and that CNT treatments did not affect cell viability 24 h after cell seeding. However, cell morphology changed at higher CNT concentrations and culture times, showing an increase in filopodia and a decrease in the spreading area that assumed a round shape. Macrophages remodelled ECM-like substrates, making CNTs bioavailable for internalization as indicated by confocal and electron imaging. Interestingly, 24 h after macrophage seeding, ROS generation increased in the presence of CNTs. Since among the numerous immunosuppressive cells in the tumor microenvironment, macrophages play an important role in tumor development, the ability of MWCNT-stimulated macrophages to shape the tumor microenvironment was evaluated. Surprisingly, it was observed that conditioned media of THP-1 cells cultured in the presence of CNTs for 24 h facilitated the migration of endothelial cells in the wound healing assay and reduced the growth of 3D glioblastoma spheroids independently from conditioned media of control cells. Overall, MWCNTs, although entrapped in ECM-like gelatin, are able to stimulate macrophage activity, in a concentration-dependent manner, making them in turn able to remodel the tumor microenvironment.

ACKNOWLEDGMENTS. The authors acknowledge financial support under the National Recovery and Resilience Plan (NRRP), Mission 4, Component 2, Investment 1.1, "Fund for the National Research Program and for Projects of National Interest (NRP)" by the Italian Ministry of University and Research (MUR), funded by the European Union – NextGenerationEU.

Project title: Role of exTRAcellular MAtrix in controlling macrophage cell response to nanomaterials (TRAMA) – CUP D53D23021660001.

EXPLORING SEVERE ASTHMA AND COPD THROUGH A NOVEL 3D *EX VIVO* RESPIRATORY MUCOSA MODEL: BRIDGING THE GAP BETWEEN LABORATORY RESEARCH AND CLINICAL PRACTICE

Stefano BURGIO[1,2], Alberto FUCARINO[3], Alessandro PITRUZZELLA[4], Giorgia INTILI[4], Olga Maria MANNA[5], Domenico Michele MODICA[4,6], Salvatore POMA[6], Alida BENFANTE[7], Alessandra TOMASELLO[7], Nicola SCICHILONE[7], Fabio BUCCHIERI[4]

[1]Department of Medicine and Surgery, Kore University of Enna; [2]Euro-Mediterranean Institute of Science and Technology (IEMEST), Palermo; [3]Department of Theoretical and Applied Sciences, eCampus University, Novedrate (CO); [4]Department of Biomedicine, Neurosciences and Advanced Diagnostic (BIND), Institute of Human Anatomy and Histology, University of Palermo; [5]Department of Sciences for Promotion of Health and Mother and Child Care, Anatomic Pathology, University of Palermo; [6]Department of Otorhinolaryngology, Villa Sofia-Cervello Hospital, Palermo; [7]Department of Health Promotion Sciences, Maternal and Infant Care, Internal Medicine and Medical Specialties (PROMISE), Division of Respiratory Diseases, University of Palermo, Italy

The development of advanced experimental models is pivotal for unraveling the complexities of severe asthma and chronic obstructive pulmonary disease (COPD), two debilitating respiratory conditions with significant unmet clinical needs. While biologic drugs, particularly monoclonal antibodies targeting specific inflammatory pathways, have revolutionized their treatment, alternative mechanisms underlying these diseases remain largely underexplored. This narrative underscores the importance of innovative methodologies capable of bridging the divide between traditional *in vivo* or *in vitro* studies and real-world clinical scenarios. We relized a sophisticated 3D *ex vivo* model of the human respiratory mucosa designed to replicate key features of asthma and COPD pathophysiology. By mimicking the intricate interplay of inflammatory cells, cytokine production, and structural alterations characteristic of these diseases, our model provides an unparalleled platform for investigating disease processes at a granular level. Unlike conventional approaches, this system

allows for dynamic assessment of disease responses to various triggers, thereby enabling rigorous testing of novel therapeutic strategies, including personalized medicine interventions. Our findings demonstrate that the proposed model faithfully recapitulates critical aspects of asthma and COPD, offering valuable insights into both fundamental biology and translational applications. Specifically, it facilitates the evaluation of monoclonal antibody efficacy while also paving the way for exploring alternative mechanisms of action. Looking ahead, this technology holds promise as a cornerstone for advancing precision medicine in respiratory care, facilitating the identification of patient-specific biomarkers and tailoring treatments accordingly. Beyond its immediate applications in asthma and COPD research, this model holds promise for advancing personalized medicine. Its ability to replicate patient-specific disease characteristics could facilitate the development of tailored therapeutic approaches, improving outcomes for individuals with severe respiratory conditions. Furthermore, the adaptability of this platform opens avenues for investigating other respiratory diseases, environmental triggers, and emerging pathogens, positioning it as a versatile tool for future research.

REFERENCES

Sun W, Yuan Y, Qiu L, Zeng Q, Jia J, Xiang X, Jia A, Ma L, Liu S, Xiao B, *et al.* Increased proportion of dual-positive Th2-Th17 cells promotes a more severe subtype of asthma. Can Respir J. 2021;2021:9999122-9999128.

Hiemstra PS, Grootaers G, van der Does AM, Krul CAM, Kooter IM. Human lung epithelial cell cultures for analysis of inhaled toxicants: lessons learned and future directions. Toxicol *In Vitro*. 2018;47:137-146.

Boonpiyathad T, Sözener ZC, Satitsuksanoa P, Akdis CA. Immunologic mechanisms in asthma. Semin Immunol. 2019;46: 101333.

Osei ET, Booth S, Hackett TL. What have *in vitro* co-culture models taught us about the contribution of epithelial-mesenchymal interactions to airway inflammation and remodeling in asthma? Cells. 2020;9(7):1694.

Shrestha J, Razavi Bazaz S, Aboulkheyr Es H, Yaghobian Azari D, Thierry B, Ebrahimi Warkiani M, Ghadiri M. Lung-on-a-chip: the future of respiratory disease models and pharmacological studies. Crit Rev Biotechnol. 2020;40(2):213-230.

RECENT CHALLENGES AND EMERGING STRATEGIES IN BONE TISSUE ENGINEERING: FROM BENCH-TO-BEDSIDE

Giovanna CALABRESE

Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy

The worldwide incidence of bone disorders and diseases has increased dramatically in recent years, particularly in populations where aging is associated with a sedentary lifestyle, limited physical activity and increased obesity. Bone tissue engineering is one of the most promising strategies for the restoration of large bone defects, offering a new alternative to conventional bone grafts [1]. This approach harnesses biomimetic materials, stem cells and growth factors, aiming to develop scaffolds able to mimic the native bone tissue and induce new functional bone regeneration. Biomimetic substitutes must provide a provisional matrix that offers a specific environment and architecture to bone cells for three-dimensional (3D) tissue formation [2]. Furthermore, ideal scaffolds for BTE applications must possess several biological, chemical and structural requirements, including good biocompatibility, excellent biodegradability, high bio functionality, appropriate porosity, adequate tensile and compressive strength and roughness. In the last decades, different types of biomaterials including: (i) bioceramics (hydroxyapatite or calcium phosphates) exhibiting good osteointegration, osteoconductivity and compressive strength; (ii) natural polymers (collagens, hyaluronic acid, and fibrin) which show high osteoconductivity and biocompatibility; (iii) synthetic polymers (polyethylene glycol (PEG), polycaprolactone (PCL), and polylactic acid (PLA)) displaying good biocompatibility and mechanical strength; (iv) hydrogels, which support cell adhesion and migration and facilitate the incorporation and targeted release of growth factors; and (v) 3D printed composite scaffolds, which offer structures adaptable to the site being regenerated, have been used in the BTE field. However, although considerable efforts have been made to develop smart biomaterials capable of meeting all clinical needs, and although stem cell technology and 3D printing have significantly contributed to the development of innovative bioengineered tissues with great potential in the orthopedic field, to date there are still many crucial obstacles to overcome, mainly due to the complexity of bone physiology and the need for scaffolds capable of adequately mimicking biological functions, for BTE to become a true clinical reality [3].

REFERENCES

1. Bauso LV, La Fauci V, Longo C, Calabrese G. Bone Tissue Engineering and Nanotechnology: A Promising Combination for Bone Regeneration. Biology (Basel). 2024 Apr 2;13(4):237. doi: 10.3390/biology13040237.
2. Rizzo MG, Palermo N, Alibrandi P, Sciuto EL, Del Gaudio C, Filardi V, Fazio B, Caccamo A, Oddo S, Calabrese G, and Conoci S. Physiologic Response Evaluation of Human Foetal Osteoblast Cells within Engineered 3D-Printed Polylactic Acid Scaffolds. Biology 2023,12,424. https://doi.org/ 10.3390/biology12030424.
3. Amini AR, Laurencin CT, Nukavarapu SP. Bone tissue engineering: recent advances and challenges. Crit Rev Biomed Eng. 2012;40(5):363-408. doi: 10.1615/critrevbiomedeng.v40.i5.10.

EFFECTS OF CSE AND THC ON RESPIRATORY MUCOSA: INNOVATIONS WITH ELECTROSPUN MEMBRANES

Adelaide CARISTA[1], Alessandro PITRUZZELLA[1], Stefano BURGIO[2], Alberto FUCARINO[3], Francesco LOPRESTI[4], Chiara DI MARCO[4], Olga Maria MANNA[5], Domiziana PICONE[1], Antonella MARINO GAMMAZZA[1], Francesco CAPPELLO[1,6], Fabio BUCCHIERI[1]

[1]*Department of Biomedicine, Neuroscience and Advanced*

Diagnostics, Institute of Human Anatomy and Histology, University of Palermo; [2]Department of Medicine and Surgery, Kore University of Enna; [3]Department of Theoretical and Applied Sciences, eCampus University, Novedrate (CO); [4]Department of Engineering, University of Palermo; [5]Department of Sciences for Promotion of Health and Mother and Child Care, Surgical Pathology Unit, University of Palermo; [6]Euro-Mediterranean Institute of Science and Technology (IEMEST), Palermo, Italy

Tobacco smoking is widely recognised as a primary risk factor for respiratory diseases, including Chronic Obstructive Pulmonary Disease (COPD). [1] Recent studies have investigated the effect of cannabis smoke on human respiratory epithelial cells *in vitro*, revealing significant cellular changes. [2] Outside inhalation, the effects of tetrahydrocannabinol (THC) and cannabidiol (CBD) molecules remain poorly explored. The interaction of THC with the cannabinoid receptors CB1 and CB2 has the potential to influence bronchodilation and modulate inflammatory and immune responses within the respiratory system. In contrast, CBD is recognised for its anti-inflammatory and analgesic properties, also mediated through these receptors. [3] In recent years, scientific interest has shifted towards the use of electrospun membranes as delivery vehicles for therapeutic molecules due to their biocompatibility. This study aims to evaluate the effects of cigarette smoke extract (CSE) and THC in an *ex vivo* three-dimensional culture model of human respiratory mucosa, focusing on alterations in the mucociliary epithelium and remodeling of the extracellular matrix. The cultures were treated with CSE dissolved in the culture medium, while THC was administered via electrospun membranes. Preliminary immuno-morphological analyses, concentrating on molecules involved in the epithelial-mesenchymal transition (EMT) such as Sparc, Tight Junction 1, and Hsp90, showed changes in the respiratory mucosa. However, no significant differences were observed between the effects of CSE and THC. These preliminary results indicate the need for further studies to evaluate the anti-inflammatory action of CBD, particularly using electrospun membranes as a delivery system.

REFERENCES

1. Cui Y. The Science of the Total Environment. 2021; 764: 142880.
2. Aguiar JA. Physiol Rep. ottobre 2019;7(20):e14249.
3. Fantauzzi MF. ERJ Open Res. ottobre 2020;6(4):00128-2020.

SYNDROMIC PANELS IN EMERGENCY DIAGNOSTICS

Antonio COSTA[1], Ildebrando PATAMIA[1], Stefania STEFANI[1], Giuseppe MANGANO[1], Davide CAMPAGNA[2], Giuseppe CARPINTERI[2], Guido SCALIA[1]

[1]Laboratorio Analisi, AOU Policlinico di Catania, Dipartimento di Scienze Biomediche e Biotecnologiche, Università degli Studi di Catania; [2]UOC MCAU, AOU Policlinico di Catania, Dipartimento di Medicina Clinica e Sperimentale, Università degli Studi di Catania, Italy

Syndromic panels are molecular biology assays capable of quickly detecting, starting from a primary sample or from positive blood cultures, the presence of the most frequent etiological agents relating to each syndrome, also evaluating some of them determinants of antibiotic resistance. Syndromic panels therefore represent a microbiological diagnosis tool based on an approach that responds to criteria of operational pragmatism rather than a fine etiopathogenetic classification. They are defined as "syndromic" as they allow us to evaluate the set of potential pathogenic microorganisms starting from the initial diagnosis of a defined clinical syndrome of probable or suspected infectious nature. Among the main advantages related to the use of this type of test must certainly be included the significant reduction in response time compared to traditional methods. This aspect is of particular relevance, at least for syndromic panels of bacteriological expertise, from the perspective of antimicrobial stewardship (AMS) and infection prevention and control (IPC), especially in epidemiological contexts characterized by the significant diffusion of multi-resistant microorganisms. All Biofire filmarray samples performed at the centralized analysis laboratory of the "G. Rodolico" university hospital in Catania from 01/01/2022 to 14/08/2024 were included in the study. From 01/01/2022 to 12/31/2022, 448 filmarray samples were examined. From 01/01/2023 to 12/31/2023, 830 filmarray samples were examined. From 01/01/2024 to 08/14/2024, 909 filmarray samples were examined. The filmarray system contains dried reagents for all the steps necessary for extraction, PCR amplification, and provides a negative or positive result with the indication of the pathogen detected and the possible presence of epidemiologically more antibiotic resistance mechanisms significant. Analyzing the clinical data taken into consideration highlights how Biofire positivity has an advantageous effect on hospitalized patients. The data shows how in all the years taken into consideration (2022-2024), BIO+ patients have better outcomes than BIO- in terms of discharge to home and transfer to low intensity and failure to transfer to high intensity. BIO+ patients have a high rate of discharge and low intensity transfer when compared to BIO-. This means that having started an etiological therapy early, even if waiting for confirmation of the gold standard, favored the restitutio ad integrum of the patients so much so that they could be discharged directly from the Sub Intensive department or in any case allowed them to be transferred to low intensity where deemed necessary. The next step in our study is to propose a PDTA for the correct use of the biofire from the patient's first admission to hospital using validated clinical scores such as SOFA and NEWS, in order to guarantee infected patients the best etiological therapy as early as possible.

OPTIMIZATION OF A 3D BIOPRINTED VASCULAR MODEL: DEVELOPMENT OF CELLULAR READOUT TECHNIQUES FOR PROCESS VALIDATION

Elena DELGROSSO[1], Franca SCOCOZZA[1], Federica RIVA[2], Michele CONTI[1,3]

[1]Department of Civil Engineering and Architecture,

University of Pavia; [2]Department of Public Health, Experimental and Forensic Medicine, Histology and Embryology Unit, University of Pavia; [3]3D and Computer Simulation Laboratory, IRCCS Policlinico San Donato, San Donato Milanese (MI), Italy

3D bioprinting has become a powerful tool for fabricating tissue-engineered constructs with complex architectures and advanced structural properties. Among the various techniques, Freeform Reversible Embedding of Suspended Hydrogels (FRESH) enables the printing of soft biomaterials with high shape fidelity by utilizing a temporary support bath. Despite these advancements, several challenges remain, including the assessment of cellular viability and distribution within 3D bioprinted constructs and the reproducibility of a tissue that closely mimics *in vivo* conditions. Standard readout techniques often rely on 2D analyses or destructive sample processing, limiting their applicability for long-term studies and dynamic tissue maturation. Therefore, developing optimized, non-destructive cellular readout methodologies is crucial for validating 3D bioprinted vascular models. This study aims to optimize a FRESH-printed 3D vascular model and develop robust cellular assessment techniques for process validation. Fibroblasts were selected as the cell type due to their key role in extracellular matrix production within the vascular wall. Cylindrical vascular structures were fabricated using a sodium alginate (SA) and gelatin (GEL) bioink, crosslinked with calcium chloride ($CaCl_2$), and printed via extrusion-based bioprinting. Constructs were cultured for up to 21 days and analyzed at multiple time points (24 hours, 7, 14, and 21 days) using Live/Dead staining and fluorescence microscopy to assess cell viability. Additionally, an optimized fixation protocol for histological analysis is being developed to address challenges arising from the bioink's high hydrophilic, which can lead to tissue loss and matrix disruption. Hematoxylin and Eosin (H&E) staining will be employed to investigate cell morphology and distribution within the construct, ensuring adequate preservation of structural integrity. Results demonstrated high initial fibroblast viability (>80%), followed by a gradual decline over time, likely due to limited nutrient diffusion and cell migration. Histological analysis further confirmed the successful encapsulation of fibroblasts, highlighting a uniform distribution but limited extracellular matrix deposition, potentially due to the bioink composition and the absence of additional cell types supporting matrix remodeling. Future efforts will focus on the integration of endothelial and smooth muscle cells, refinement of bioactive hydrogel compositions, and implementation of perfusion systems to better replicate native vascular environments. This study underscores the importance of combining advanced bioprinting techniques with optimized cellular readouts to improve the development of preclinical vascular models.

This work was supported by the ERC (EPEIUS, 101125466) and by Italian MIUR and the University of Pavia within the program "Dipartimenti di Eccellenza 2023.2027".

REFERENCE

Xu, Lei *et al.* "Bioprinting small diameter blood vessel constructs with an endothelial and smooth muscle cell bilayer in a single step." *Biofabrication* vol. 12,4 045012. 29 Jul. 2020, doi:10.1088/ 1758-5090/aba2b6.

PROMISING ANTI-INFLAMMATORY POTENTIAL OF MARINE BENTHIC DIATOMS

Roberta ESPOSITO[1*], Mauro DISTASI[2*], Paola NIERI[2], Maria COSTANTINI[1], Valerio ZUPO[3]

[1]Department of Ecosustainable Marine Biotechnology, Stazione Zoologica Anton Dohrn, Villa Comunale, Napoli; [2]Department of Pharmacy, University of Pisa; [3]Stazione Zoologica Anton Dohrn, Department of Ecosustainable Marine Biotechnology, Ischia Marine Centre, Ischia, Italy

[*]These authors contributed equally to this work.

Inflammation is considered part of the body's non-specific defensive reaction to harmful stimuli, being able to protect tissues by removing dead cells and stimulating tissue repair processes. As a matter of fact, an enormous variety of bioactive compounds with noteworthy anti-inflammatory potential are originated from marine species. In this respect, microalgae, and mainly planktonic diatoms, are potentially recognized as an abundant resource of bioactive compounds, being rich in lipids, omega-3 fatty acids, carotenoids, pigments, vitamins and polysaccharides with applications in pharmacological, nutraceutical and cosmeceutical fields (Ruocco *et al.*, 2020; Esposito *et al.*, 2022; Nieri *et al.*, 2023). On the contrary, benthic diatoms are still unexplored because they are very difficult to sample, cultivate and quantify. In the present study three benthic diatoms, *Cocconeis scutellum scutellum*, *Cocconeis scutellum parva* and *Diploneis* sp., isolated as epiphytes on *Posidonia oceanica* leaves collected in Ischia (Gulf of Naples, Italy) were analyzed for their potential anti-inflammatory effect. Starting from monoclonal cultures, semi-massive and massive cultures were produced for the three diatoms to obtain biomass for chemical extraction. Crude extracts were obtained using 600 mg of lyophilized diatoms by acetonitrile and water (8:2), using the lyophilized powder (mg)/solvents (mL) ratio of 10:1. Firstly, cytotoxicity of the three diatom-derived extracts were evaluated on HaCat cells, spontaneously immortalized human keratinocyte line, in the concentration range 300-0.1 µg/mL. Cells were then treated for 1 hour with the extracts at the non-cytotoxic concentrations of 30, 10, 3 and 1 µg/mL. Subsequently, lipopolysaccharide (LPS) was added to induce inflammation at the concentration of 10 µg/mL for 16 hours. Molecular markers were also tested to detect the induced inflammation. The results showed an anti-inflammatory effect of these three diatom extracts, suggesting the presence of compounds (to be further chemically characterized) able to modulate inflammatory pathways and molecular targets effectively. In conclusion, these findings provided valuable insights for researchers in the field of marine anti-inflammatory pharmacology emphasizing the need for further research to harness the pharmacological benefits of benthic diatom compounds as potential alternative candidates to pharmaceutical compounds for the management of inflammatory disorders.

ACKNOWLEDGMENTS. This work was performed in the

framework of the PRIN 2022 project "Mediterranean marine organisms as sources of new anti-inflammatory and pro-resolving compounds".

REFERENCES

Esposito R. *et al.* (2022) International Journal of Molecular Sciences 23: 10680.

Nappo M. *et al.* (2012) Pharmaceutical Biology 50.4: 529-535.

Nieri P. *et al.* (2023) Nutrients 15.2 (2023): 464.

Ruocco N. *et al.* (2020) Marine Drugs 18: 342.

PHYTOMELATONIN-RICH MEDICINAL-AROMATIC PLANTS (MAPs): POTENTIAL FOR DIVERSE APPLICATIONS FROM PLANT-BASED PRODUCTS

Manuela GIRALDO-ACOSTA, Antonio CANO, Josefa HERNÁNDEZ-RUIZ, Marino B. ARNAO

Phytohormones and Plant Development Lab, Department of Plant Biology (Plant Physiology), Faculty of Biology, University of Murcia, Spain

Melatonin (*N*-acetyl-5-methoxytryptamine) is a biological molecule synthesized in the pineal gland of animals as well as in various plant tissues. In plants, it plays a crucial role as a main messenger in regulating plant responses to abiotic and biotic stresses. In addition to its well-documented function as an antioxidant, melatonin has been shown to be beneficial in postharvest technology by improving the shelf life of fruits and vegetables. The exogenous application of melatonin reduces oxidative stress and mitigates cell damage by counteracting the harmful effects of reactive oxygen species (ROS) and facilitating membrane repair. Furthermore, its involvement in the regulation of stress-responsive genes and the activation of pathogenesis-related proteins, as well as antioxidant enzyme genes, underscores its adaptability to diverse environmental challenges. Moreover, the ability of melatonin to interact with other phytohormones broadens its potential applications, including its possible role in protecting plants against stress conditions that have not been extensively studied, such as viral infections and nematode infestations. In addition to its exogenous application, research has also focused on strategies to enhance endogenous melatonin production to strengthen plant resilience and maintain physiological homeostasis under adverse conditions (1). These findings highlight the significance of melatonin as a multifaceted molecule with promising implications for plant stress management and postharvest preservation (2). Due to the wide variety of functions and applications of this molecule, the presence of melatonin in plants, called phytomelatonin, has gained great interest in recent years. Our research group has focused on the screening of medicinal-aromatic plants (MAPs) with a high endogenous level of phytomelatonin, in order to identify and cultivate them, being able to use these plants to obtain phytomelatonin-rich extracts. These extracts can be used for the design of various plant-based products rich in phytomelatonin, with application in the animal and human nutrition such as nutraceuticals, food dietary, cosmetics, and also in agricultural and biotechnological industries (3). This has allowed us to create PbS-Phytomel Co., a spin-off company of the University of Murcia (UMU) led by Prof. Marino B. Arnao, dedicated to cultivating and providing the highest quality phytomelatonin-rich plants. PbS-Phytomel's mission is to harness the natural benefits of these plants to promote health and well-being. With a focus on sustainability and innovation, taking pride in offering a wide range of phytomelatonin-rich products that cater to various needs (www.pbs-phytomel.com).

ACKNOWLEDGEMENTS. This study forms part of the AgroAlNext program and was supported by MCIU with funding from European Union NextGenerationEU (PRTR-C17.I1) and by Comunidad Autónoma de la Región de Murcia - Fundación Séneca. M. Giraldo-Acosta has a Predoctoral Contract UM-23 from the Univesrsity of Murcia, Spain.

REFERENCES

1. M. B. Arnao *et al.* Melatonin from microorganisms, algae, and plants as possible alternatives to synthetic melatonin. Metabolites, 2023, 13(1), 72; https://doi.org/10.3390/metabo13010072.
2. M. Giraldo-Acosta & M.B. Arnao. Melatonin, a promising natural crop safener. Outlooks Pest Manag. (2024) 35, 115-119. DOI: https://doi.org/10.1564/v35_jun_06.
3. A. Cano *et al.* Current state of the natural melatonin: The phytomelatonin market. Melatonin Res. 2024, 7, 242-248; DOI: https:// doi.org/10.32794/mr112500179.

PRE-CLINICAL DEVELOPMENT OF A DRUG INHIBITING THE CHROMATIN REMODELING PROTEIN WDR5 IN FSHD MUSCULAR DYSTROPHY

Frida KARAKASHI, Emanuele MOCCIARO, Davide GABELLINI

Gene Expression Regulation Unit, Division of Genetics and Cellular Biology, IRCCS Ospedale San Raffaele, Milan, Italy

Facioscapulohumeral muscular dystrophy (FSHD) is one of the most prevalent neuromuscular disorders. Weakness is slowly progressive with high variability among patients. The disease is caused by the aberrant expression of the transcription factor DUX4, which is normally confined to early embryonic development. In FSHD, DUX4 mis-expression activates a pro-apoptotic transcriptional program leading to block of differentiation and muscle wasting[1]. Given its pivotal role in FSHD, blocking DUX4 expression with small molecule drugs is an attractive solution. Previously, by combining proteomics with genetic and pharmacological targeting, we identified the chromatin remodeling protein WDR5 as a key activator of DUX4 expression in FSHD[2]. By testing various compounds, we identified a novel WDR5 inhibitor (WDR5i) showing higher potency and better pharmacological properties, which are important for pre-clinical testing. To this aim, we evaluated WDR5i safety and efficacy in preclinical models of FSHD. We confirmed WDR5i ability to inhibit DUX4 expression using muscle cells isolated from multiple

FSHD patients. We also found that an intermittent WDR5i treatment if sufficient to obtain long-term DUX4 repression. Importantly, long-term WDR5i treatment does not significantly affects muscle cells proliferation or differentiation. To perform *in vivo* tests, we set up a humanized animal model of FSHD that will allow us to evaluate WDR5i safety and efficacy in a relevant setting. Results from our work could provide a novel therapeutic opportunity for FSHD patients that, up to now, have no cure.

REFERENCES

1. Mocciaro E, Runfola V, Ghezzi P, Pannese M, Gabellini D. DUX4 Role in Normal Physiology and in FSHD Muscular Dystrophy. Cells 2021; 10: 3322.
2. Mocciaro E, Giambruno R, Micheloni S, *et al.* WDR5 is required for DUX4 expression and its pathological effects in FSHD muscular dystrophy. Nucleic Acids Res 2023; 51: 5144-61.

IDENTIFICATION OF SPONGE-ASSOCIATED BACTERIA FROM ANTARCTICA AND THEIR POTENTIAL BIOTECHNOLOGICAL APPLICATIONS

Rosamaria SOLDANO[1,2], Maria PAPALE[1], Carmen RIZZO[3], Alessandro Ciro RAPPAZZO[1,4], Filomena DE LEO[2], Alessio LENA[1,2], Maurizio AZZARO[1], Angelina LO GIUDICE[1,5*]

[1]Institute of Polar Sciences, National Research Council, Messina; [2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina; [3]Stazione Zoologica Anton Dohrn, Sicily Marine Centre, Department Ecosustainable Marine Biotechnology, Messina; [4]Ca' Foscari University of Venice, Department of Environmental Sciences, Mestre (VE); [5]Italian Collection of Antarctic Bacteria, National Antarctic Museum (CIBAN-MNA), Messina, Italy

Sponges (Phylum Porifera) are among the most ancient animals establishing close, complex associations with diverse microbial consortia, including bacteria, archaea, fungi and microalgae. Sponge-associated microorganisms can comprise approximately 40% of the host biomass and play various roles, such as photosynthesis, nitrogen fixation, sulfate reduction and secondary active metabolite production. Thus, these benthic invertebrates represent a hidden treasure trove of novel metabolites with biotechnological potential. To date, more than 5,000 compounds have been isolated from about 500 sponge species; as a result, interest in investigating and cultivating sponge-associated microbiomes has increased over the years, as they are considered the potential true source of these natural products. This aspect remains largely underinvestigated in the case of sponges that inhabit Antarctic waters. Due to the extreme nature of Antarctica, the sponge inhabiting the region may harbor unique microbes with unique functions reflecting their metabolic specialization. In this study, 133 bacterial isolates were isolated from the sponge species *Microxina sarai*, *Dendrilla antarctica*, *Mycale acerata*, *Lissodendoryx flabellata*, *Myxodoryx hanitschi*, *Myxilla elongata*, and *Isodictya erinacea* from the Thetys Bay and Adelie Cove areas (Terra Nova Bay Ross Sea). Isolates were screened for the hydrolysis of Tween 80 and tributyrin (to test the production of esterases and lipases), chitin (for chitinase), agar (for agarase), skim milk (for proteases), gelatin (for gelatinase), and keratin (for keratinase) at low temperature. Bacterial strains were further tested for the utilization of xenobiotics (*i.e.*, polychlorinated biphenyls, DDT and DDE) as the sole carbon and energy source. Most promising isolates were identified by the 16S rRNA sequencing. They were mainly affiliated to the Gammaproteobacteria (55.8% of total isolates) and Actinomycetota (38.6%), followed by Alphaproteobacteria and Bacillota (2.8% each). *Psychrobacter* (29 isolates) and *Salinibacterium* (20 isolates) prevailed among retrieved genera. Overall, the production of extracellular enzymes was observed in the majority of the isolates in the order: esterases> gelatinase/lipases > agarase> protease> chitinase/keratinase. A number of bacterial strains (mainly from the sponge *L. flabellata*) were able to produce multiple enzymes among those tested. Growth in the presence of DDT and DDE was observed in the 6.8 and 5.7% of tested bacterial isolates, respectively (mainly from the sponges *M. hanitschi* and *D. antarctica*). Finally, four bacterial isolates from *I. erinacea* and *L. flabellata* were able to grow in the presence of Aroclor 1242 (a polychlorobiphenyl mixture). Our observations contribute in shedding light on the diversity and biotechnological potentials of sponges-associated cultivable bacteria from cold habitats.

Funding: Programma Nazionale di Ricerche in Antartide (PNRA-MUR).

INVESTIGATION OF RESPIRATORY EPITHELIAL CELL DIFFERENTIATION AND STRESS RESPONSES IN MICROGRAVITY USING A 3D *EX VIVO* MODEL: ENGINEERING AND BIOLOGICAL INSIGHTS FROM ISS EXPERIMENTS

Olga Maria MANNA[1,2], Domiziana PICONE[3], Adelaide CARISTA[3], Giuseppa D'AMICO[3], Alberto FUCARINO[4], Stefano BURGIO[2,5], Alessandro PITRUZZELLA[2,3,5], Domenico Michele MODICA[3], Francesco CAPPELLO[2,3], Fabio BUCCHIERI[3]

[1]Department of Sciences for Promotion of Health and Mother and Child Care, Anatomic Pathology, University of Palermo; [2]Euro-Mediterranean Institute of Science and Technology (IEMEST), Palermo; [3]Department of Biomedicine, Neuroscience and Advanced Diagnostics (BiND), University of Palermo; [4]Department of Theoretical and Applied Sciences, eCampus University, Novedrate (CO); [5]Department of Medicine and Surgery, Kore University of Enna; [6]Polo Territoriale Universitario di Caltanissetta, Consorzio Universitario di Caltanissetta, Italy

Human space exploration provides valuable insights into the effects of microgravity on the respiratory system. However, the scope of these experiments is inherently restricted by the limitations of existing flight hardware and culture models. As the frequency and complexity of space-based experi-

ments are projected to increase, there is an evident imperative to enhance both the underlying technologies and cellular culture models employed in these studies. This study aims to investigate the differentiation of ciliated and goblet cells under microgravity conditions, focusing on cilia structure and function, mucus production, tissue stress responses and antimicrobial peptide production. For this research, we used a 3D *ex vivo* culture model that emulates the normal human respiratory mucosa, a model extensively validated by our research team for its resilience and adaptability under various stress conditions. The scientific experiment is subdivided into phases: pre-launch (on the ground), launch, in-flight (aboard the ISS, lasting 10 weeks), re-entry on Earth, evaluation and analysis. This encompasses the maintenance of the 3D culture model under the microgravity environment on the ISS for the specified duration, followed by subsequent fixation for post-flight analyses. Therefore, we preliminarily evaluated the adaptation of the 3D growth model with the automated bioreactor (developed by the two project partners, OHB and CIRiS), which will be launched and installed in the Biolab incubator upon arrival on the ISS and will allow to sustain the cultures throughout the experiment. The main tests that our team conducted were: biocompatibility tests, tests to evaluate the oxygen concentration inside the bioreactor culture chambers, and a short-term simulation of the in-flight science experiment. Morphological (via hematoxylin-eosin staining and immunohistochemistry) and biomolecular analyses (including protein stress studies and pro-inflammatory cytokine assessments through Magpix analysis) were performed on the cell cultures. Results showed that the outgrowths had a well-differentiated epithelial cell population, maintaining mucosal integrity. Test samples were comparable to controls in cellular development, though some cultures displayed stress signs due to engineering challenges like non-standard culture media and variations in injection speed and pressure. We propose that structural and functional alterations in the respiratory epithelium may result from prolonged exposure to altered gravity conditions. Our specific post-flight objectives are to evaluate changes in the development and performance of the pulmonary barrier, as well as to investigate ciliogenesis and the interactions between cells and the extracellular matrix (ECM).

PRELIMINARY STUDIES ON 2D AND 3D SKIN MODELS FOR EVALUATING THE BIOLOGICAL EFFECTS OF RADIATION IN BORON NEUTRON CAPTURE THERAPY

Stefania RICCI[1], Ian POSTUMA[2], Claretta GUIDI[3], Patrizia SOMMI[2,4], Agostina VITALI[2,4], Cinzia FERRARI[2,5], Laura CANSOLINO[2,5], Silva BORTOLUSSI[2,3], Federica RIVA[1]

[1]Department of Experimental Medicine, Histology and Embryology Unit, University of Pavia; [2]National Institute of Nuclear Physics (INFN), Unit of Pavia; [3]Department of Physics, University of Pavia; [4]Department of Molecular Medicine, Human Physiology Unit, University of Pavia; [5]Laboratory of Experimental Surgery, Department of Clinical-Surgical, Diagnostic and Paediatric Sciences, University of Pavia, Italy

Boron Neutron Capture Therapy (BNCT) is an innovative radiotherapy to treat solid tumors unresponsive to conventional radiotherapy with photons: a pre-treatment with a non-radioactive boron compound is followed by irradiation with low-energy neutrons. The neutron beam penetrates the skin, making it a limiting tissue: the aim is to analyse the dose-effect relation in healthy skin, as no studies investigates the radiobiological effects of BNCT on healthy epidermal tissue. In this study, 2D and 3D skin models were irradiated with three different radiations (photon, proton and neutron beam) to obtain a more comprehensive assessment of the radiobiological effects. For the aim of this study, experiments were performed on three models: 1) an *in-vitro* reconstructed human 3D epidermal model (SkinEthic^{TM}) maintained in static culture. Samples were irradiated with photons and neutrons, with and without boronphenilalanine (BPA); 2) a different human-derived 3D epidermal model (EpiDerm^{TM}) maintained in a dynamic culture after irradiation with photons and protons; The high variability in results highlights the need for a larger sample size to obtain more robust statistical data. 3) HaCaT cells, human normal keratinocytes exposed to photon beam and maintained in a 2D culture to evaluate the proliferation rate both of the basal epidermal cells and of the same keratinocytes inducted to the suprabasal differentiation. On the SkinEthic model, morphological and immunohistochemical analyses showed progressive degradation of the constructs over time and across different radiation doses. The Bromo-Deoxy-Uridine incorporation assay (BrdU) showed variable cell proliferation rate with BNCT-treated samples, with a lower positivity on the second observation day; the expression of Proliferation-Cell Nuclear Antigen (PCNA) has fluctuating trends, increasing on the second day in culture. On the 2nd day after BNCT treatment, reduced proliferative activity is linked to a high presence of proteins involved in reparative mechanisms, consistent with previous *in-vitro* studies. Moreover, a decrease of some differentiation proteins such as filaggrin, involucrin, and pan-cytokeratin is visible after the exposure to the radiation. The EpiDerm model allowed to increase the number of irradiated samples, improving statistical robustness. Furthermore, maintaining the samples in dynamic culture, better mimics the physiological environment. This approach is still being optimized. The clonogenic assay on HaCaT cells exposed to photon beams showed a reduction in plating efficiency as radiation dose increases. Immunohistochemical analyses were performed on irradiated HaCaT cells, both in proliferating conditions and following induction to suprabasal differentiation. These preliminary experiments in terms of proliferation and differentiation-associated proteins aim to optimize and improve the characterization of healthy epidermal tissue in response to different types of radiation, especially for BNCT. Samples irradiated at low doses allow to refine the understanding of dose-effect relationships and lead to a more comprehensive evaluation of radiobiological effects.

CELL STRESS

EXTRACTS FROM GREEN LEAVES AND RHIZOMES OF *POSIDONIA OCEANICA* (L.) DELILE AND IL1B-INFLAMED HUMAN ASTROCYTES: PRELIMINARY DATA ON ANTIOXIDANT AND ANTI-INFLAMMATORY EFFECTS

Giulia ABRUSCATO[1], Claudia GUERRIERO[3], Manuela MAURO[1], Mirella VAZZANA[1,2] Vincenzo ARIZZA[1,2], Ada Maria TATA[3], Claudio LUPARELLO[1,2]
[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STeBICeF), University of Palermo; [2]National Biodiversity Future Center (NBFC), Palermo; [3]Department of Biology and Biotechnologies Charles Darwin, Sapienza-University of Rome, Italy

Natural marine bioactives have many benefits for human health, including anti-inflammatory and antioxidant effects[1]. Furthermore, in recent years interest in bioactive compounds has increased, due to their neuroprotective effects and ability to support blood-brain barrier's health[2]. Our previous studies have demonstrated the abundant presence of polyphenols in the extracts obtained from leaves (GLE) and rhizomes (RE) of the seagrass *P. oceanica*, and their potential anti-inflammatory effect on TNFα-inflamed brain pericytes belonging to an *in vitro* model of human blood-brain barrier[3]. Thus, to further investigate and confirm this property, the aim of the present study was to test the potential anti-inflammatory and antioxidant effect of GLE and RE on primary human astrocytes inflamed by IL1β. No cytotoxic effect was observed after 24-hours treatment of cells with GLE and RE at different concentrations. The investigation of cells' redox state showed that both extracts induced a significant down-regulation of reactive oxygen species. Real-time PCR analyses on the pro-inflammatory cytokines IL-6 and TNFα showed that both extracts reduced their expression. At the protein expression level, IL-6 was downregulated to a higher extent by co-treatment with GLE than RE, differently from TNFα whose downregulation was more pronounced after co-treatment with RE. These promising results encourage us to evaluate the potential antioxidant and anti-inflammatory effect of the extracts in more detail, with the aim of elucidating the underlying molecular mechanism.

REFERENCES

1. Abruscato *et al. In vitro* cytotoxic effect of aqueous extracts from leaves and rhizomes of the seagrass Posidonia oceanica (L.) Delile on HepG2 liver cancer cells: focus on autophagy and apoptosis. Biology (Basel). 2023 18;12(4): 616.
2. Wen *et al.* Unraveling the impact of Omega-3 polyunsaturated fatty acids on blood-brain barrier (BBB) integrity and glymphatic function. Brain, Behavior, and Immunity. 2024.115, 335–355.
3. Abruscato *et al.* Effect of aqueous extracts from Posidonia oceanica seagrass on mouse macrophages and human blood brain barrier. Journal of Biological Research 2024. 97 Suppl.1, 44-44.

EFFECTS OF HEAT STRESS EXPOSURE ON PECTORAL SKELETAL MUSCLE OF THE ANTARCTIC HAEMOGLOBINLESS *CHIONODRACO HAMATUS* AND THE RED-BLOODED *TREMATOMUS BERNACCHII*

Daniela AMELIO[1], Gianfranco SANTOVITO[2], Filippo GAROFALO[1]
[1]Department of Biology, Ecology and Earth Science, University of Calabria, Arcavacata di Rende (CS); [2]Department of Biology, University of Padova, Italy

Antarctic fish are extreme stenotherm animals, adapted to live at temperatures close to the freezing point of ocean water (−1.9°C). They experience stable annual temperature fluctuations less than 1°C. Due to climate changes and the consequent increment in water temperature, Antarctic species are exposed to stressful conditions. The effects of temperature rise on morpho-functional traits of Antarctic marine species are under attention. In this study we evaluated the effects of acute heat stress on the pectoral skeletal muscle of both the Antarctic red-blooded *Trematomus bernacchii* and the haemoglobinless *Chionodraco hamatus* at morphological and molecular level (cell death, NOS/NO system, heat shock response). Preliminary results revealed structural changes after heat stress exposure in both species. Moreover, by immunofluorescence the presence of factors involved in control of vascular tone and cell survival has been evidenced. On the whole, our results suggest a specie-specific morpho-functional response to heat stress in the skeletal muscle of both Antarctic teleost. In particular the absence of Hb seems to make skeletal muscle more sensitive to heat stress, then icefishes should be taken into account as sentinel species for climate change.

PERIPHERAL CONCENTRATIONS OF THE NEUROTRANSMITTERS NORADRENALINE, DOPAMINE AND SEROTONIN AND OXIDANT/ANTIOXIDANT EQUILIBRIUM IN HORSE, CAMELIDS, ELEPHANTS, TIGERS AND LIONS FROM ITALIAN CIRCUS

Federica ARRIGO[1], Raffaela COCCO[2], Sara SECHI[2], Giuseppe PICCIONE[1], Caterina FAGGIO[3], Francesca ARFUSO[1]
[1]Department of Veterinary Sciences, University of Messina; [2]Department of Veterinary Medicine, University of Sassari; [3]Department of Chemical, Biological, Pharmaceutical, and Environmental Sciences, University Messina, Italy

The condition of animals in captivity can cause many concerns and must be evaluated carefully. These can be measured through physical/functional and mental domains. In particular, these are nutrition, environment, physical health, behavior and finally affective states. In circus activities, animals are used for different purposes, but their physiological peculiarities differ and it is often not possible to respect their needs. The present study aimed to analyze the emotional state and oxidant/antioxidant balance of camels, dromedaries, llamas,

elephants, horses, lions and tigers. From 5 animals of each species blood samples were collected to assess the serum concentrations of noradrenaline (NORA), dopamine (DOPA), serotonin (SERO), and the plasma levels of reactive oxygen metabolites (d-Roms) and biological antioxidant potential (BAP). All the data collected were shown to be equally distributed ($P>0.05$). One-way analysis of variance (ANOVA) was used to investigate differences, the concentration of the analyzed parameters changed statistically between species ($P<0.05$). The results showed a higher concentration of d-Roms in elephants compared to all other species investigated, in horses compared to llamas and camels, in dromedaries compared to camels and in lions compared to dromedaries. A higher concentration of BAP was observed in elephants and tigers than in dromedaries, horses, lions and llamas. Higher levels of BAP were found in lions compared to dromedaries, and in camels compared to the other camelids and horses. Higher concentrations of NORA were found in elephants than in camels, llamas, horses and lions ($P<0.05$). Llamas showed higher concentrations of SERO than camels, elephants, dromedaries, tigers and lions ($P<0.05$). Results showed higher DOPA concentrations in dromedaries compared to camels, llamas and elephants ($P<0.05$). Higher DOPA levels were also found in horses and lions compared to camels and llamas ($P<0.05$). To the best of the authors' knowledge, this is the first study investigating the oxidative balance and emotional state of animals enrolled in circus activities. All the investigated animals did not present behavioral atypia and they proved to be calm during the blood sampling procedure without requiring any containment practice. The differences found in the levels of investigated parameters among species highlighted the need of species-specific reference range for the neurotransmitters indicated the emotional state of animal as well as for the oxidant/antioxidant balance. As a matter of fact, the situation in circuses is often complex and animals are forced to perform difficult tricks and are often threatened and coerced. Therefore, the assessment of health and welfare of circus animals should be representing a goal for the veterinarian who, however, should have available reference ranges of parameters indicating the emotional state of the animal that are specific to the study species. Additional prospective studies should be performed to collect more objective, evidence-based data in a greater number of animals managed in circus by including also further indicators of good emotional and behavioral state to provide a more holistic view of their welfare state.

INFLUENCE OF EXTERNAL MICROENVIRONMENT ON ADIPOSE-DERIVED STEM CELL BEHAVIOR

Sara CRUCIANI[1], Giuseppe GARRONI[1], Carlo VENTURA[2], Margherita MAIOLI[1]
[1]Department of Biomedical Sciences, University of Sassari; [2]Laboratory of Molecular Biology and Stem Cell Engineering, Istituto Nazionale Biostrutture e Biosistemi (INBB)-Eldor Lab, Bologna, Italy

Adipose-derived stem cells (ADSCs) are promising in regenerative medicine due to their ability to maintain tissue homeostasis and regeneration[1]. Their proliferation, survival and activation are influenced by specific signals within their microenvironment, also known as niche. The organization of the niche provides anatomical and functional interactions that contribute to the maintenance of stemness and modulate the final fate of these cells. Moreover, ADSCs secrete growth factors and pro-inflammatory cytokines, able to trigger several metabolic complications when tissue physiology is compromised[2]. At the same time, a tumor microenvironment can influence stem cell behavior, modulating proliferation and their ability to differentiate into a specific phenotype. Whitin this context, we exposed ADSCs to plasma samples derived from human patients diagnosed with prostate cancer (PC), or precancerous lesions (PL), or benign prostatic hyperplasia (BPH) for a total of 4, 7 or 10 days, in the attempt to investigate how tumor microenvironment can affect their proliferation and cell-cell interactions[3]. We then analyzed the expression of main stemness-related markers and cell-cycle regulators and measured cytokine production. Cell morphology and collagen production by confocal microscopy were then investigated. Differential expression of microRNAs is a critical epigenetic mechanism involved in the development and progression of human cancer. For this reason, miR-145, miR-148, and miR-185 expression was analyzed by qPCR. The results obtained from this study show significant changes in the morphology of ADSCs exposed to plasma samples, especially in the presence of prostate cancer plasma, suggesting that the tumor microenvironment can influence ADSC behavior, promoting their proliferation. These findings could have significant implications for the development of treatments of these diseases, including the use of ADSCs for cell-based tissue engineering, regenerative medicine, and autologous transplantations.

REFERENCES

1. Frese L *et al.* Transfus Med Hemother. 2016 Jul;43(4):268-274. doi:10.1159/000448180.
2. Rochette L, *et al.* Int J Mol Sci. 2020 Dec 4;21(23):9262. doi:10.3390/ijms21239262.
3. Cruciani S. *et al.* Sci Rep. 2024 Jun 26;14(1):14765. doi: 10.1038/s41598-024-64625-0.

EFFECT OF ADIPOSE-DERIVED STEM CELL-CONDITIONED MEDIUM ON MCF-7 CELL GROWTH AND BEHAVIOR

Giuseppe GARRONI[1], Diletta SERRA[1], Sara CRUCIANI[1], Margherita MAIOLI[1,2]
[1]Department of Biomedical Sciences, University of Sassari; [2]Center for Developmental Biology and Reprogramming—CEDEBIOR, Department of Biomedical Sciences, University of Sassari, Italy

MCF-7 cells are a breast cancer cell line widely used in cell biology studies due to their ability to respond to hormones and chemotherapeutic drugs. The tumor microenvironment is a dynamic and complex ecosystem where cancer cells interact with surrounding stromal components, including

adipose tissue and its resident stem cells, which can influence tumor progression and response to therapy. In fact, increasing evidence has shown that mesenchymal stem cells (MSCs) can secrete various cytokines and chemokines that affect cell proliferation. In this study, we exposed MCF-7 cells for three days to a medium obtained from mesenchymal stem cells isolated from adipose tissue and compared it with a medium derived from stem cells isolated from lipofilling procedure, both recovered after 3 days in culture. Additionally, we assessed cytotoxicity assay and evaluated cell proliferation. Furthermore, we analyzed apoptosis and the expression of stress-related genes to better understand the underlying mechanisms. The results obtained showed an increased cell proliferation in both treatment groups, with a more pronounced effect in cells exposed to the medium derived from adipose-derived stem cells. These findings suggest that the microenvironment conditioned by adipose stem cells may significantly influence cell growth and behavior. These results highlight the potential role of adipose stem cell-derived factors in modulating cellular processes and emphasize the importance of the microenvironment in regulating cellular mechanisms.

MULTIFACTORIAL NEUROPROTECTIVE MECHANISMS OF BOSWELLIA SACRA IN ASTROCYTES UNDER OXIDATIVE AND NUTRITIONAL STRESS

Alessia VERMIGLIO[1], Lorenza LA ROSA[1], Roberto CHIARELLI[1], Graziella SERIO[1], Alberto ASTEGGIANO[2], Andrea OCCHIPINTI[2], Italia DI LIEGRO[1], Gabriella SCHIERA[1], Carla GENTILE[1]
[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo; [2]Abel Nutraceuticals S.R.L., Turin, Italy

Astrocytes play an essential role in maintaining neuronal homeostasis. They contribute to the integrity of the blood-brain barrier by interacting with cerebral endothelial cells and regulating its selective permeability. Furthermore, astrocytes support neuronal function by supplying specific metabolites they produce to neurons. *In vitro* studies on neuron-astrocyte co-culture systems have suggested that astrocyte viability is closely linked to neuronal health, especially under stress conditions, including oxidative stress. These findings indicate that neuroprotective strategies targeting astrocytes may offer a promising approach for treating neuronal disorders. Boswellia plants, members of the *Burseraceae* family, are renowned for producing frankincense, a resin extracted from the sap that oozes from the tree's bark. Frankincense has been used for centuries in religious rituals and is also valued for its traditional medicinal properties. The primary bioactive components of Boswellia frankincense are boswellic acids, a group of pentacyclic terpenes, and cembrenes, a class of diterpenic compounds, which are believed to contribute to its protective effects. This *in vitro* study examines the impact of an ethanolic extract of Boswellia sacra resin (BSE), produced by Abel Nutraceuticals, on the viability and function of primary murine astrocytes exposed to nutrient deprivation (serum-free medium) combined with oxidative stress induced by H_2O_2. These conditions simulate ischemic-like scenarios and complex neurotoxic environments where multiple stressors act simultaneously, potentially leading to neuronal damage. MTT assay results reveal that BSE, in a dose-dependent manner, enhances astrocyte survival under oxidative stress condition combined with nutrient deprivation. The extract exerts cytoprotective effects by inhibiting apoptosis (evidenced by the TUNEL test and reduced Caspase-7 levels), alleviating inflammation (through downregulation of iNOS, COX-2, and reduced phosphorylated IκB-α levels), and activating cellular antioxidant response (through increased Nrf2 expression and ROS reduction). Additionally, BSE reduces autophagic flux as demonstrated by decreasing LC3-II and p62 expression, thereby supporting cellular homeostasis. These findings indicate that BSE treatment safeguards primary astrocytes from nutritional and oxidative stress via a multifactorial mechanism, highlighting its potential for further investigation in promotion of neuronal health.

REFERENCES

Schiera G *et al.* Involvement of Astrocytes in the Formation, Maintenance, and Function of the Blood-Brain Barrier. Cells, 2024.

Schiera *et al.* Synergistic effects of neurons and astrocytes on the differentiation of brain capillary endothelial cells in culture. J Cell Mol Med, 2003.

THE HYDROALCOHOLIC EXTRACT OF OLIVE LEAVES ALLEVIATES NON-ALCOHOLIC FATTY LIVER DISEASE

Maria Rosaria MIRANDA[1,2], Vincenzo VESTUTO[1], Manuela Giovanna BASILICATA[3], Giovanna Aquino[1], Giacomo PEPE[1,2], Pietro CAMPIGLIA[1]
[1]Department of Pharmacy, University of Salerno, Fisciano (SA); [2]National Biodiversity Future Center (NBFC), Palermo; [3]Department of Pharmacy, University of Naples Federico II, Naples, Italy

The alteration of lipid metabolism is closely associated with the onset of non-alcoholic fatty liver disease (NAFLD), which is the leading cause of morbidity and mortality associated with the liver. Therefore, the effort to understand new agents capable of preventing the pathogenesis of non-alcoholic steatosis is fundamental. Our aim was to verify if an extract of olive leaves (OE) could improve NAFLD in a cellular steatosis model elucidating its underlying molecular mechanisms. $HepG_2$ cells exposed only to free fatty acids (FFA) showed intracellular lipid accumulation, endoplasmic reticulum (ER) stress, and disrupted expression of proteins, lipids, and lipid synthesis-related genes. It is known that excessive ER stress can destroy cellular homeostasis, causing cellular damage and dysfunction. Growing evidence suggests that Sirtuin1 (SIRT1) plays a positive role in various ER stress-induced organ damage through inhibition of the UPR pathway. Therefore, we evaluated whether OE had a protective action by acting on this target. Our results suggest that the treatment with

OE attenuated and reversed the expression of both ER stress and lipid steatosis markers, acting upstream SIRT1 and partially restoring cell homeostasis.

Funder: Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU.

Award Number: Project code CN_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP: D43C22001260001, Project title "National Biodiversity Future Center - NBFC".

STUDY OF THE EFFECTS OF OXIDATIVE STRESS ON CCT CHAPERONIN AND APOPTOSIS MARKERS IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS (HUVECs) AND THE PRELIMINARY DETECTION OF THE CCT5 SUBUNIT IN MESENCHYMAL STEM CELLS DERIVED EXTRACELLULAR VESICLES (MSCs-EVs) TO EXPLORE ITS THERAPEUTIC POTENTIAL

Leila NOORI, Francesco CAPPELLO, Federica SCALIA
Department of Biomedicine, Neuroscience and Advanced Diagnostics, University of Palermo, Italy

Endothelial cells (ECs) play critical role in neuromuscular system health forming an interface between the blood vessels and the surrounding neural and muscular tissues according to their involvement in the blood-brain barrier and blood flow modulation (1). Reactive oxygen species (ROS), act as signaling molecules at low levels but cause oxidative stress (OS) when generated excessively. ECs, highly exposed to blood flow and metabolic activity, are vulnerable to OS leading to cellular damages (2). OS also alters mitochondrial membrane permeability and triggers apoptosis by activating the pro-apoptotic protein Bax.The function of chaperone system to maintain protein homeostasis can be disrupted by OS which may trigger the overexpression of chaperonins like CCT (3). Mesenchymal stem cells derived extracellular vesicles (MSCs-EVs) showed potential for combating OS (4). We aimed to explore the behaviour of CCT5, actin and apoptosis markers in OS-induced HUVEC, offering insights into therapeutic strategies for OS. Applying different concentrations (50, 100, 200, 500, and 1000 µM) of H2O2 for 4 hours on HUVEC, significant cell viability and lower cytotoxic effects was detected in 50 µM. A high level of ROS was observed in 50 µM, and cells showed normal morphology and confluency. A slight increase in the expression of actin, CCT5, Bax, and Bcl2 was observed in the HUVEC after OS induction for 4 hours. A considerable increase in the RNA expression of the interested genes of CCT5 and HSF1 was observed in OS-induced HUVEC. A slight increase in CCT5 protein was observed in OS-induced HUVEC. EVs were collected in the fractions 3-8 based on their size and confirmed using CD9 and CD63 as positive markers. CCT5 protein was detected in MSCs-EVs and MSCs. This research highlights the critical impact of OS on molecular chaperones and their significant roles in cellular processes. It underscores the need for comprehensive studies to identify molecular chaperones as biomarkers and therapeutic targets, which could lead to early disease detection and the development of innovative treatments for diseases. Future research can work on MSC-EVs potential to restore oxidative balance and protein folding.

REFERENCES

1. Banks WA. From blood–brain barrier to blood–brain interface: new opportunities for CNS drug delivery. Nat Rev Drug Discov. 2016 Apr 22;15(4):275-92.
2. Incalza MA, D'Oria R, Natalicchio A, Perrini S, Laviola L, Giorgino F. Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. Vascul Pharmacol. 2018 Jan;100:1-19.
3. Temiz E, Koyuncu İ, Sahin E. CCT3 suppression prompts apoptotic machinery through oxidative stress and energy deprivation in breast and prostate cancers. Free Radic Biol Med. 2021 Mar;165:88-99.
4. Gao Y, Huang X, Lin H, Zhao M, Liu W, Li W, *et al.* Adipose mesenchymal stem cell-derived antioxidative extracellular vesicles exhibit anti-oxidative stress and immunomodulatory effects under PM2.5 exposure. Toxicology. 2021 Jan;447:152627.

ANALYSIS OF THE EFFECTS OF METHYL GALLATE, A GALLIC ACID DERIVATIVE, IN A BREAST CANCER MODEL

Chiara OCCHIPINTI[1], Giovanni PRATELLI[2], Antonietta NOTARO[1], Federica AFFRANCHI[1], Marzia FRANZÒ[2], Chiara ZICHITTELLA[2], Anna DE BLASIO[1], Diana DI LIBERTO[2], Sonia EMANUELE[2], Michela GIULIANO[1], Antonella D'ANNEO[1]
[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (Stebicef), Laboratory of Biochemistry, University of Palermo; [2]Department of Biomedicine, Neurosciences and Advanced Diagnostics (Bind), Institute of Biochemistry, University of Palermo, Italy

Breast cancer is the most common malignancy among women in the United States and it is second only to lung cancer as a cause of cancer death [1]. Among the different molecular subtypes, triple-negative breast cancer (TNBC) exhibits high metastatic potential and drug resistance. For this reason, it is important to find new therapeutic strategies that can overcome these obstacles and provide targeted treatments for patients [2]. Nowadays, natural products, with their chemical diversity, target affinity and different biological activity, offer great prospects for the development of modern lead compounds [3]. In particular, it was reported that methyl gallate (MG) exhibits different bioactivities, such as antitumor, antimicrobial, anti-inflammatory, neuroprotective and chemopreventive effects [4]. Our previous investigations have shown that MG has promising anti-cancer properties in colon cancer [5]. In the present study our focus was to explore the mechanism of action of this natural compound in breast cancer MDA-MB-231 cells, a highly

aggressive, invasive and poorly differentiated triple negative breast cancer model. In particular, MG reduced cell viability of MDA-MB-231 model in a dose- and time- dependent manner. Moreover, the addition of N-acetylcisteine (NAC), as antioxidant agent, was able to counteract MG cytotoxic effect, suggesting that the action of this phytocompound may be ascribed to the induction of oxidative events. Indeed, MG treatment induced a remarkable increase in intracellular ROS content already after short periods of incubation. Such an effect was associated with the activation of the molecular pathway of Keap-1/Nrf2/HO-1. Interestingly, the pre-incubation with ferrostatin-1 (FRS), a known inhibitor of the ferroptosis process, counteracted the cytotoxic effect induced by MG, thus suggesting the involvement of an iron-dependent cell death mechanism in MG-treated cells. Such conclusions were sustained by western analysis of Ferritin Heavy Chain (FTH-1) and Glutathione peroxidase 4 (GPX-4), two factors involved in ferroptotic process. Indeed, MG decreased the level of both these proteins, an effect that was counteracted by either FRS or NAC addition. Furthermore, data showed that MG induced mitochondrial impairment, with loss of mitochondrial potential membrane and lipid peroxidation, two key events in the process of ferroptosis. To summarize, for the first time, we found that MG-induced cell death was iron-dependent in breast cancer MDA-MB-231 cells. These investigations provide new insights on the potential application natural compounds as agents that can support current therapies in the fight against cancer.

REFERENCES

1. Gradishar W.J, Breast Cancer, Version 3.2024, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. (2024).
2. Hu Y., *et al.* The treatment landscape of triple-negative breast cancer. Med Oncol 41, 236 (2024).
3. Zhang J., *et al.* Natural products and derivatives for breast cancer treatment: From drug discovery to molecular mechanism. Phytomedicine 129, 155600 (2024).
4. Al Zahrani NA., Recent developments of gallic acid derivatives and their hybrids in medicinal chemistry: A review. Eur J Med Chem. 204:112609, (2020).
5. Notaro, A.; Lauricella, M.; Di Liberto, D.; Emanuele, S.; Giuliano, M.; Attanzio, A.; Tesoriere, L.; Carlisi, D.; Allegra, M.; De Blasio, A.; *et al.* A Deadly Liaison between Oxidative Injury and p53 Drives Methyl-Gallate-Induced Autophagy and Apoptosis in HCT116 Colon Cancer Cells. Antioxidants 12, 1292 (2023).

ENO1 - Hsp70 INTERACTION: UNLOCKING A POTENTIAL TARGET TO COUNTERACT METASTATIC CANCER PROGRESSION

Giovanni PERCONTI[1], Maria Rita GULOTTA[2], Ugo PERRICONE[2], Danilo CILLUFFO[3], Angela BONURA[1], Patrizia RUBINO[1], Agata GIALLONGO[3]

[1]Institute of Translational Pharmacology (IFT), CNR, Palermo; [2]Molecular Informatics Unit, Fondazione Ri.MED, Palermo; [3]Institute of Biomedical Research and Innovation (IRIB), CNR, Palermo Italy

Alpha-enolase (ENO1) is a multifunctional protein involved in both glycolysis and tumor progression. In addition to its metabolic role, ENO1 is overexpressed in several cancers where also functions as a plasminogen receptor on the cell surface, facilitating extracellular matrix degradation and promoting cancer cell invasion and metastasis [1]. Our previous studies identified Hsp70 as an ENO1-interacting protein that increases ENO1 surface localization and, accordingly, the migratory capacity of cancer cells, thus revealing a potential therapeutic target to counteract tumor dissemination [2]. To characterize the molecular interface of the ENO1-Hsp70 interaction, we integrated biochemical and computational approaches. Co-immunoprecipitation assays using ENO1 deletion mutants allowed the mapping of critical interaction domains, while molecular mechanics simulations provided structural insights [3]. Additionally, a focused small-molecule library was virtually screened to identify compounds capable of affecting this protein-protein interaction. Promising hits were then tested in cancer cell models, assessing their impact on ENO1-Hsp70 binding through co-immunoprecipitation. To evaluate *in vitro* the functional consequences of ENO1-Hsp70 interaction destabilization, changes in ENO1 surface localization were measured using On-Cell Western assays and cell motility was assessed by transwell migration and invasion assays in breast cancer cells. Furthermore, the effects of the selected compounds on cell viability and ENO1-dependent glycolytic activity were examined. The results demonstrated the effective inhibition of ENO1-Hsp70 interaction by a few small molecules, leading to a significant reduction in ENO1 surface expression and the inhibition of cell migration and invasion. As metastatic dissemination remains the leading cause of cancer-related mortality, the identification of novel therapeutic strategies targeting key regulators of tumor cell invasion is of critical importance. The present study demonstrates that small-molecule inhibitors of the ENO1-Hsp70 interaction can modulate ENO1 surface localization and impair metastatic potential in breast cancer cells, supporting further investigation into their therapeutic potential.

REFERENCES

1. Li Y. *et al. J Transl Med*. 2024 (doi: 10.1186/s12967-024-05847-8).
2. Perconti G. *et al. Sci Rep*. 2017 (doi: 10.1038/s41598-017-04185-8).
3. Gulotta MR. *et al. ACS Omega*. 2025 (doi: 10.1021/acsomega.4c10808).

AÇAÌ BERRY EXTRACT PRESERVES HUMAN ERYTHROCYTE INTEGRITY FROM AGING-INDUCED OXIDATIVE STRESS: ROLE OF ANION EXCHANGER 1

Sara SPINELLI[1], Silvia DOSSENA[2], Angela MARINO[1], Elisabetta STRAFACE[3], Rossana MORABITO[1], Alessia REMIGANTE[4]

[1]*Department of Chemical, Biological, Pharmaceutical and Environmental Sciences University of Messina, Italy;* [2]*Institute of Pharmacology and Toxicology, Research and Innovation Center Regenerative Medicine & Novel Therapies, Paracelsus Medical University, Salzburg, Austria;* [3]*Biomarkers Unit, Center for Gender-Specific Medicine, Istituto Superiore di Sanità, Rome, Italy;* [4] *Department of Biomedical, Dental and Morphological and Functional Imaging University of Messina, Italy*

Aging is a process characterized by a general decline in physiological functions. The high bioavailability of reactive oxygen species plays an important role in the aging rate. Due to the close relationship between aging and oxidative stress, functional foods rich in flavonoids are excellent candidates to counteract age-related changes. The present study aimed to verify the protective role of Açai extract in a D-Galactose (D-Gal)-induced model of aging in human red blood cells. Our results showed that pre-treatment with Açai berry extract (10 µg/mL) avoided the formation of leptocytes, prevented increases in oxidative stress, and restored alterations in the distribution of band 3 and CD47 proteins in red blood cells exposed to 100 mM D-Gal. Moreover, the significant decrease in membrane red blood cell deformability associated with D-Gal treatment was alleviated by Açai extract. The extract completely restored band 3 protein hyper-phosphorylation and Syk kinase levels, and partially reverted the alterations in the distribution of spectrin, ankyrin, and protein 4.1 observed after exposure to 100 mM D-Gal. Interestingly, D-Gal exposure was also associated with an acceleration of the rate constant of SO_4^{2-} uptake through band 3 protein, as well as glycated hemoglobin formation. Both alterations were attenuated by pre-treatment with the Açai extract. These findings contribute to clarifying the aging mechanisms in human red blood cells and propose flavonoid-rich functional foods as natural antioxidants for the treatment and prevention of oxidative stress-related diseases.

BSF PROTEIN HYDROLYSATES PLAY ANTIOXIDANT AND ANTINFLAMMATORY ACTIVITIES IN MAMMALIAN CELLS

Kristian RIOLO[1], Sabrina OLIVA[1], Vincenzo PARRINO[1], Domenico SAVASTANO[2], Gianluca Antonio FRANCO[3], Annamaria FERRERI[4], Enrico GUGLIANDOLO[3], Alessia GIANNETTO[1]
[1]*Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina;* [2]*Via Eritrea, Progetto Hermetia, Gioia Tauro (RC);* [3]*Department of Veterinary Sciences, University of Messina;* [4]*Department of Theoretical and Applied Sciences, Università degli Studi eCampus, Italy*

Protein from insects are recognized as a valuable alternative source in animal feed and more recently are emerging as a source of compounds with biological activity to be used as health-promoter agents [1]. *Hermetia illucens*, also known as Black Soldier Fly (BSF) is a saprophagous dipteran insect, whose larval biomass can be used to produce protein hydrolysates by enzymatic hydrolysis [2]. In this study the cytoprotective potential of protein hydrolysates from BSF (BPH 0.1–0.5 mg/mL) against inflammation and oxidative stress induced by challenging murine fibroblast L-929 cells with 10 µg/mL lipopolysaccharides (LPS) was assessed *in vitro*. Our results provide compelling evidence that BPH can effectively induce a dose-dependent decrease in both LPS-induced ROS and nitrite production as determined by carboxy-H2DCFDA assay and Griess reaction, respectively. Furthermore, BPH decreased protein and mRNA levels of key inflammatory markers, including *TNF-α, IL-6, IL-1α,* and *IL-1β*, while increasing the transcript levels of selected antioxidant genes (*Cu/ZnSod, MnSod, Gpx, HO-1*), as evaluated by ELISA and qPCR. Overall, BPH's antioxidant and anti-inflammatory effects involved both Nrf2-mediated induction of antioxidant genes and inhibition of NF-κB activation a key player in inflammation. The nuclear translocation and activation of NF-kB evaluated by immunolocalization showed that BPH treatment decreases the immune-positive cells compared to LPS treatment confirming that BPH can act as an attenuator of the inflammatory cascade. These findings indicate that BPH are effective in mitigating oxidative stress and inflammation *in vitro*, thus posing the basis for further investigation before their use as novel drugs.

REFERENCES

1. Riolo *et al.*, 2023. *Antioxidants 12*(2), 519. doi:10.3390/antiox12020519.
2. Firmansyah and Abduh 2019. *Heliyon 5* (6). doi:10.1016/j.heliyon.2019.e02005.

DOXORUBICIN-INDUCED SENESCENCE IN OVARIAN CELLS: INVESTIGATING THE ROLE OF FISETIN

Benedetta RIZZA[1], Osama ELSALLABI[1], Tiziano LUPI[1], Marialucia GALLORINI[2], Paola LANUTI[1], Cecilia PAOLINI[3], Fani KONSTANTINIDOU[3], Valentina GATTA[3], Antonia PATRUNO[1]
[1]*Department of Medicine and Science of Aging, University "G. d'Annunzio" of Chieti Pescara, Chieti;* [2]*Department of Pharmacy, University G. d'Annunzio, Chieti;* [3]*Department of Neuroscience, Imaging and Clinical Sciences, University G. d'Annunzio of Chieti-Pescara, Chieti, Italy*

The ovary is the first organ to age in the human body, which has a significant impact on both women's overall health and fertility. This aging phenomenon occurs due to the process of cellular senescence, a state of irreversible growth arrest accompanied by specific phenotypic and molecular changes in cells. As a result, there can be an accumulation of senescent cells during aging, contributing to the development of age-related diseases. To date, the precise role of cellular senescence in ovarian aging remains only partially understood. This study focused on the induction of senescence in the human ovarian granulosa tumor cell line (KGN cells) using doxorubicin (Gao Y *et al.*, 2023) and the potential application of senolytic treatments. Specifically, the involvement of fisetin (Kashyap D *et al.*, 2019) in mitigating doxorubicin-

induced senescence in KGN cells was analyzed. Results showed that treating KGN cells with doxorubicin led to a senescent state, accompanied by an increase in senescence biomarkers, such as -galactosidase activity (SA-β-gal), the expression levels of CDKN1A/p21, altered gene expression profiles of senescence-associated secretory phenotype (SASP) factors (IL-6 and IL-1α), and programmed cell death pathways. Fisetin treatment exhibited senomorphic properties by suppressing SASP factors specific to senescent cells. Transmission electron microscopy (TEM) analysis revealed decrease of cytoplasmic vacuolization. Furthermore, the results showed that fisetin also affects DNA repair mechanisms and cell cycle arrest, reducing apoptosis levels and release of extracellular vescicles. Overall, understanding the mechanisms driving doxorubicin-induced senescence in KGN cells, along with exploring the potential of senolytic/senomorphic treatment such as fisetin, could provide key insights into strategies aimed at alleviating the harmful effects of chemotherapy on reproductive health.

REFERENCES

Gao Y, Wu T, Tang X, Wen J, Zhang Y, Zhang J, Wang S. Increased cellular senescence in doxorubicin-induced murine ovarian injury: effect of senolytics. Geroscience. 2023 Jun;45(3):1775-1790. doi: 10.1007/s11357-023-00728-2. Epub 2023 Jan 17.

Kashyap D, Garg VK, Tuli HS, Yerer MB, Sak K, Sharma AK, Kumar M, Aggarwal V, Sandhu SS. Fisetin and Quercetin: Promising Flavonoids with Chemopreventive Potential. Biomolecules. 2019 May 6;9(5):174. doi: 10.3390/biom 9050174.

DYNAMIC WOUND HEALING MODEL: HYDROLATE OF HELICHRYSUM ITALICUM PROMOTES COLLAGEN DEPOSITION DURING SKIN REPAIR PROCESS

Diletta SERRA[1], Sara CRUCIANI[1], Giuseppe GARRONI[1], Margherita MAIOLI[1,2]

[1]Department of Biomedical Sciences, University of Sassari; [2]Center for Developmental Biology and Reprogramming—CEDEBIOR, Department of Biomedical Sciences, University of Sassari, Italy

Natural bioactive compounds play a crucial role in skin regeneration while minimizing adverse effects. Their therapeutic potential offers a promising opportunity for the development of innovative and effective wound care strategies. In this context, we investigated the wound-healing potential of a waste product (Hydrolate) derived from Helichrysum italicum (*HH*), on skin stem cells (SSCs) and fibroblasts (HFF1) using a dynamic culture system. Indeed, we employed a bioreactor using fluidic cellular crosstalk that facilitates communication between SSCs and HFF1, miming the complexity of skin microenvironment. After scratch assay, both cell types were treated for 48h with two different concentrations of *HH* and compared to untreated controls. We examined the impact of *HH* on collagen I deposition during tissue repair using confocal microscopy under both static and dynamic conditions. Furthermore, gene expression analysis revealed that *HH* treatment activated a stemness-associated molecular program in SSCs. Our results highlight the potential translational application of *HH* in improving skin regeneration and contributing to the development of an advanced dynamic model for studying wound healing.

OXIDATIVE STRESS-INDUCED KV3.1/KCNC1 CHANNEL DYSFUNCTION: IMPLICATIONS IN AGE-RELATED HEARING LOSS

Sara SPINELLI[1], Alessia REMIGANTE[1], Angela MARINO[1], Silvia DOSSENA[2], Rossana MORABITO[1]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy; [2]Institute of Pharmacology and Toxicology, Paracelsus Medical University, Salzburg, Austria

Kv3.1/KCNC1 is a Shaw-type delayed rectifier potassium channel prominently expressed in high-frequency firing neurons and GABAergic inhibitory interneurons throughout the ascending auditory pathway and in several brain districts [1]. Its high activation threshold and rapid activation and deactivation in response to voltage changes reduce the action potential duration while simultaneously maximizing the firing frequency, thus contributing to the temporal accuracy of sound processing [2]. Since oxidative stress plays a key role in the pathogenesis of age-related hearing loss [3], we hypothesized that an oxidative imbalance may impair the function of this channel. To verify this hypothesis, an already validated *in vitro* model of oxidative stress-related aging, *i.e.* treatment of cell lines with 100 mM D-Galactose, was used to determine both activity and expression of endogenous and ectopic Kv3.1. D-Galactose treatment of different cell lines showed a dysregulation in intracellular reactive oxygen species levels, thiobarbituric acid reactive substances levels, cellular protein sulfhydryl groups content, catalase and superoxide dismutase activity, along with a significant reduction in Kv3.1 the current density, all reverted by cell exposure to 100 µM melatonin, chosen for its antioxidant properties. With regard to Kv3.1 current density, its alteration was associated not only with a reduced trafficking to the cell surface linked to Src phosphorylation, but also with metabolic and endoplasmic reticulum stress. The obtained data revealed: i) a novel oxidative stress-dependent modulation of the Kv3.1 channel, whose dysfunction may contribute to age-related hearing loss; ii) the inhibition of the Kv3.1 ion current was not a consequence of a direct oxidation of the channel protein but rather involved altered trafficking to the plasma membrane. In this context, the antioxidant melatonin showed a protective effect against the loss of Kv3.1 function.

REFERENCES

1. Parameshwaran-Iyer, Suchitra *et al.* "Localization of KCNC1 (Kv3.1) potassium channel subunits in the avian auditory nucleus magnocellularis and nucleus laminaris during development." Journal of neurobiology vol. 55,2 (2003): 165-78. doi:10.1002/neu. 10198.
2. Peixoto Pinheiro, Barbara *et al.* "Age-related hearing loss per-

taining to potassium ion channels in the cochlea and auditory pathway." Pflugers Archiv : European journal of physiology vol. 473,5 (2021): 823-840. doi:10.1007/s00424-020-02496-w.
3. Fujimoto, Chisato, and Tatsuya Yamasoba. "Oxidative stresses and mitochondrial dysfunction in age-related hearing loss." Oxidative medicine and cellular longevity vol. 2014 (2014): 582849. doi:10.1155/2014/582849.

MELATONIN'S EFFECTS ON MICROBIOTA-GUT-BRAIN AXIS IN AUTISM SPECTRUM DISORDER MICE

Francesca SULAS[1], Giorgia COMINELLI[1], Daniela PINTO[3,4], Fabio RINALDI[3,4], Rita REZZANI[1,2,4], Gaia FAVERO[1,4]
[1]Anatomy and Physiopathology Division, Department of Clinical and Experimental Sciences, University of Brescia; [2]Italian Society for the Study of Orofacial Pain (Societa` Italiana Studio Dolore Orofacciale - SISDO), Brescia; [3]Human Microbiome Advanced Project Institute, Milan; [4]Interdepartmental University Center of Research Adaption and Regeneration of Tissues and Organs-(ARTO), University of Brescia, Italy

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition characterized by social impairments and repetitive behaviors, with an unclear etiology. Growing evidence has highlighted alterations in gut microbiota and dysfunctions in the intestinal barrier in ASD patients, leading to the pathological phenomenon of "leaky gut". This condition may be linked to impairments in the blood-brain barrier, which could additionally affect brain development and behavior. In this context, the concept of the microbiota-gut-brain axis has gained increasing attention, highlighting the interplay between the gut microbiota and the central nervous system. The present study aimed to explore the mechanisms underlying intestinal barrier dysfunction in the BTBR T+Itpr3^{tf}/J mouse model of ASD, focusing on the effects of melatonin treatment. Melatonin, known for its role in regulating circadian rhythms, also possesses well-documented anti-inflammatory and antioxidant properties. The goal was to investigate how melatonin could influence intestinal morphology and the expression of tight junction proteins (such as zonulin-1, claudin-1, and claudin-2), which are essential for regulating intestinal permeability. Morphological analyses were performed to assess potential alterations in intestinal villus architecture and the intestinal epithelium, while immunohistochemical and biochemical analyses were used to evaluate the expression of tight junction proteins. The ASD mice exhibited longer intestinal villi and changes in the epithelial surface compared to control animals (C57BL6J mice), which could increase intestinal permeability and alter gut microbiota composition. The melatonin-treated ASD mice showed a reduction in intestinal permeability, linked to the modulation of the expression of key tight junction proteins. These findings support the involvement of the microbiota-gut-brain axis in ASD pathology and suggest that melatonin may provide a potential therapeutic strategy to alleviate some ASD symptoms and comorbidities.

ACKNOWLEDGMENTS: The authors would like to thank Giuliani S.p.A. (Milan, Italy) for the valuable contribution to this research project. Moreover, the authors sincerely thanks also Franchini Acciai S.p.A. (Mairano, Italy) for the precious financial support.

REVOLUTIONIZING ANTIBACTERIAL DEFENSE: HEMOCOMPATIBLE MESOPOROUS NANOPARTICLES LOADED WITH ARTEMISIA ABSINTHIUM EXTRACT FOR ENHANCED ROS MODULATION

Ioannis TSAMESIDIS[1,2], Georgia K. POUROUTZIDOU[1], Athanasios CHRISTODOULOU[1], Dionysia AMANATIDOU[2], Phaedra ELEFTHERIOU[2], Stiliani AXIPOLITOU[1], Sotirios KALFAS[1], Eleana KONTONASAKI[1]
[1]Department of Prosthodontics, School of Dentistry, Faculty of Health Sciences, Aristotle University of Thessaloniki; [2]Department of Biomedical Sciences, International Hellenic University, Thessaloniki, Greece

The escalating global threat of antimicrobial resistance necessitates innovative strategies to enhance antibacterial defense mechanisms. In this context, green chemistry approaches have emerged as environmentally sustainable solutions for extracting bioactive compounds with pharmaceutical applications. *Artemisia absinthium* (commonly known as wormwood) is a perennial plant celebrated for its extensive medicinal properties, including antibacterial, antioxidant, and anti-inflammatory effects. Beyond the well-established antimalarial activity of its active compound, artemisinin (ART), *A. absinthium* exhibits a broader therapeutic profile, particularly in antimicrobial and regenerative applications. This study presents the development of hemocompatible mesoporous nanoparticles loaded with *A. absinthium* extract to harness its therapeutic potential while enhancing reactive oxygen species (ROS) modulation. High-performance liquid chromatography (HPLC) analysis quantified the artemisinin concentration in the extract at 25 µg/ml (89 µM), complemented by a total phenolic content of 1.07 ± 0.02 mmol or 182 ± 3.6 mg GAE per 100 g of dry plant material, underscoring its bioactive richness. The extract-loaded mesoporous nanoparticles were evaluated for their antibacterial efficacy against *Staphylococcus aureus* and *Escherichia coli*, demonstrating significant antimicrobial activity. Furthermore, the nanoparticles exhibited superior ROS modulation, which is critical in managing oxidative stress and promoting tissue regeneration. Hemocompatibility studies confirmed the biocompatibility of the nanoparticle formulation, ensuring safe application in biomedical settings. The findings reveal that encapsulating *A. absinthium* extract in mesoporous nanoparticles amplifies its antibacterial properties and enhances its ROS-regulating capabilities, making it a promising candidate for innovative antibacterial therapies and regenerative medicine. This approach not only underscores the therapeutic versatility of *A. absinthium* but also highlights the transformative potential of mesoporous nanoparticles in revolutionizing antibacterial defense strategies.

POTENTIAL ROLE OF CHAPERONES HSP 90 AND HSP 60 IN THE PATHOGENESIS OF GRAVES' DISEASE: MECHANISM AND THERAPEUTIC IMPLICATION

Giuseppe VERGILIO[1], Giusy VULTAGGIO[1], Giuseppa GRACEFFA[2], Giuseppe D'AMATO[2], Celeste CARUSO BAVISOTTO[1,3], Francesco CAPPELLO[1,3], Francesca RAPPA[1,4]

[1]Department of Biomedicine, Neurosciences and Advanced Diagnostics (BIND), Institute of Human Anatomy and Histology, University of Palermo; [2]Department of Precision Medicine in Medical, Surgical and Critical Care, University of Palermo; [3]Euro-Mediterranean Institute of Science and Technology (IEMEST), Palermo; [4]The Institute of Translational Pharmacology, National Research Council of Italy (CNR), Palermo, Italy

Graves' disease is an autoimmune disorder that affects thyroid gland, resulting in hyperthyroidism. This condition is characterised by the presence of circulating autoantibodies that bind to the thyroid hormone receptor (TSHR), which leads to hyperthyroidism accompanied by hypertrophy and hyperplasia of thyrocytes. Heat shock proteins (Hsps) serve as molecular chaperones, playing a crucial role in protecting cells from stress and mediating immune system responses [1,2]. Hsps are implicated in the pathogenesis of several autoimmune diseases, such as rheumatoid arthritis and Hashimoto's thyroiditis [3,4]. Based on this evidence, we hypothesized a potential role for these molecular chaperones in Graves' disease. To investigate this hypothesis, we first assessed the presence and distribution of Hsp90 and Hsp60 in paraffin-embedded tissues using immunohistochemistry techniques. This analysis was further validated through western blotting, which examined the expression levels of Hsp90 and Hsp60 in surgically resected tissue from Graves' disease patients in comparison to benign goiter tissues. Our results demonstrated a significant increase in these proteins in samples from Graves' disease, indicating their potential involvement in the disease's pathogenic processes. Moreover, it has been widely discussed that Hsp90 and Hsp60 can be released into the extracellular environment via extracellular vesicles (EVs). This release contributes to pathological intercellular communication and may promote the continuation of the disease [5,6,7]. To explore the roles of Hsp90 and Hsp60 further, we conducted western blotting to measure the expression levels of these proteins isolated from plasma-derived EVs. The analysis of EVs from patients with Graves' disease yielded valuable data on the levels of extracellular Hsp90 and Hsp60, both before and after ablative surgery. Hsp90 and Hsp60 may regulate biochemical pathways that control the proliferation and activation of immune system cells. Therefore, understanding how their overexpression influences these processes could pave the way for the development of targeted therapies for Graves' disease and other autoimmune conditions.

REFERENCES

1. Zininga T. *et al.*, 2018.
2. Collier MP. *Et al.*, 2020.
3. Fouani M. *et al.*, 2022.
4. Marino Gammazza A. *et al.*, 2014.
5. D'Amico G. *et al.*, 2024.
6. Caruso Bavisotto C. *et al.*, 2019.
7. Cui X. *Et al.*, 2021.

ENVIRONMENT AND HEALTH

EFFECT OF LOW-DOSE POLLUTANT MIXTURES ON IMMUNE CELL LINE MODEL

Noemi ALOI[1,2], Alessia Maria SAMPINO[2], Alessia LI VIGNI[2], Valeria LONGO[2], Giuseppa AUGELLO[2], Melchiorre CERVELLO[2], Rosa Maria SERIO[1], Maria Grazia ZIZZO[1], Paolo COLOMBO[2]

[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), Università degli Studi di Palermo; [2]Institute for Biomedical Research and Innovation, National Research Council of Italy (IRIB-CNR), Palermo, Italy

The global economy and industrial production have led to widespread contaminant release into the environment, increasing the recognized risk of immune alterations and non-communicable diseases (NCDs), like inflammatory bowel disease (IBD)[1]. BDE-47[2], a flame retardant and immune-endocrine disruptor, and Estrone[3], an estrogen with harmful effects at high concentrations, are chemicals of particular concern found in Sicilian area with relevant anthropogenic activity. The aim of this research is to study the chronic exposure to low doses of mixtures of these chemicals, mimicking real-life scenarios in *in vitro* immune model. *In vitro* assays were performed in murine macrophage cell line RAW 264.7 to study the potential immunotoxic and immunomodulatory effects induced by a wide range doses of pollutant mixtures. For this aim, cell viability assay, reactive oxygen species (ROS) production, transcriptional and epigenetic analysis were performed. To identify the sublethal doses to use in functional assays, the cytotoxic effects of single pollutants and their mixtures were evaluated by MTS assays on RAW 264.7 macrophages. Specifically, murine macrophages were treated with increasing concentrations of BDE-47, estrone E1 or BDE+E1 mixtures (3, 30, 300 nM of BDE-47 and 1, 10 nM of E1). No significant alterations in cell viability were observed for each tested concentration compared to relative controls (DMSO vehicle concentrations). To assess the ability of different BDE-47+E1 mixtures in ROS induction, DCF-DA assays were performed on treated RAW 264.7 cells. All the analyzed mix concentrations did not induce significant alteration in ROS production compared to the controls. The immuno-modulatory effects of BDE-47 and E1 mixtures were investigated analyzing the IL-6, TNF-α, NOS2 and IL-10 gene expression by qPCR. The data obtained highlighted that the mixture containing 3nM BDE-47+1nM E1 reduces the basal expression of IL-6 in treated macrophages, instead, the mixture containing 300nM BDE-47+1nM E1 induces the expression of the pro-inflammatory gene TNF-α. No modulations of all analyzed gene were observed in cells treated with 30nM BDE-47+ 1nM E1 mixture. Starting from the transcriptional results, the mixtures containing 3nM BDE-47+1nM E1 and 300nM BDE-47+1nM E1, were selected to evaluate the possible epigenetic alteration induced in RAW 264.7 cells. A set of 84 miRNAs was analysed. No significant differences in miRNA expression were observed in macrophage treated with 3nM BDE+1nM E1, instead the mix 300nM BDE-47+1nM E1 significantly modulated miRNA profile, down-regulating Let-7a-5p, Let-7c-5p, miR-423-5p, and miR-128-3p. These results show that while the MTS and DCF-DA assays showed no significant changes, functional data revealed differences in immunomodulatory activities and epigenetic effects of the mixtures. These results will drive the design for the application of these mixtures *in vivo*, in a rat model of experimental colitis. These results underscore the dose-dependent effects of pollutants, where low doses may trigger immunomodulatory responses, potentially reflecting a hormetic effect.

REFERENCES

1. Wang, F., *et al.*, *Emerging contaminants: A One Health perspective.* Innovation (Camb), 2024. 5(4): p. 100612.
2. Klinčić, D., *et al.* Levels and distribution of polybrominated diphenyl ethers in humans and environmental compartments: a comprehensive review of the last five years of research. *Environ Sci Pollut Res* 27, 5744–5758 (2020).
3. Harding AT, Heaton NS. *The Impact of Estrogens and Their Receptors on Immunity and Inflammation during Infection*. Cancers (Basel). 2022;14(4):909.

2-METHYL-4-ISOTHIAZOLIN-3-ONE INFLUENCE IN THE ALTERATION OF IMMUNE RESPONSES IN *MYTILUS GALLOPROVINCIALIS*

Emanuela CASTRONOVO[1], Federica IMPELLITTERI[2], Cristiana Roberta MULTISANTI[2], Giorgia ZICARELLI[2], Caterina FAGGIO[1,3]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina; [2]Department of Veterinary Sciences, University of Messina; [3]Department of Eco-sustainable Marine Biotechnology, Stazione Zoologica Anton Dohrn, Naples, Italy

Methylisothiazolinone (MIT), also known as 2-Methyl-4-Isothiazolin-3-one, is a preservative added in the formulation of paints, coatings, and cosmetics products. Being a biocide, its primary role is to prevent the bacterial and fungal growth. Due to its high efficacy even at low concentrations, MIT is active against a wide range of microorganisms. However, it has been found to be able to cause allergic reactions in humans, and this is the reason why its use has been subject to regulations in several countries [1]. Therefore, the aim of the present preliminary data has been to examine the effects of MIT on *Mytilus galloprovincialis*. Specimens were exposed to two different concentrations of the substance, respectively with 200 µg/L (E1) and 400 µg/L (E2) for a period of fourteen days. Haemocyte viability has been evaluated through the employing of two colourimetric methods: the Trypan blue exclusion test and Neutral red retention assay. In addition, the immune response performed by haemocytes has been assessed through the measurement in percentage of phagocytic cell, using the yeast *Saccharomyces cerevisiae* at a concentration of $1x10^7$ ml^{-1}. Data revealed that cell viability underwent a significant reduction in both analyses: concerning the Trypan blue exclusion test at the lower concentration (E1), for the Neutral red retention assay at both concentrations (E1 and E2). Moreover, treated groups exhibited a significant reduction in phagocytic capacity (particularly in cells exposed to

E2) compared to the control group. These preliminary results highlight a significant compromised immulogical function in *M. galloprovincialis* due to exposure to MIT, suggesting that this xenobiotics may compromise the overall health of the organisms, with potential ecotoxicological implications. These preliminary data, represent a starting point for further investigation on the impact of MIT. Further studies need to be carried out on to assess ecotoxicological impact of MIT for a broader range of biological activities and for the overall environmental health.

ACKNOWLEDGEMENTS. This work has been funded by the European Union – NextGenerationEU, as part of the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.1 (PRIN) under Grant Assignment Decree n. 1375 adopted on 01-09-2023 by the Italian Ministry of University and Research (MUR) - Project PRIN 2022 PNRR Title: *Investigations of emerging pollutant: ecological and human health impacts of isothiazolinone biocides* – Project ministerial code: P2022BH28Y_03- CUP: C53D23009240001 – Beneficiary: University of Teramo (UniTE).

REFERENCE

1. Goodier, M. C., Ljungberg, L., Persson, C., Engfeldt, M., Bruze, M., & Warshaw, E. M. (2017). Allergic contact dermatitis from methylisothiazolinone in residential wall paint. *Dermatitis*, *28*(4), 284-287.

STUDY OF NEW BIO-SUSTAINABLE PACKAGING TECHNOLOGIES FOR FOOD TO PREVENT CHRONIC INFLAMMATORY BOWEL DISEASES

Chiara PORRO[1], Francesca Martina FILANNINO[1], Maria Ida DE STEFANO[1], Federico ZOILA[1,2], Dario Domenico LOFRUMENTO[3], Maria Antonietta PANARO[4], Antonia CIANCIULLI[4], Melania RUGGIERO[4]

[1]Department of Clinical and Experimental Medicine, University of Foggia; [2]Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona; [3]Department of Biological and Environmental Sciences and Technologies, Section of Human Anatomy, University of Salento, Lecce; [4]Department of Biosciences, Biotechnologies and Environment, University of Bari, Italy

Chronic inflammatory bowel diseases, including ulcerative colitis and Crohn's disease, are chronic relapsing-remitting disorders of multifactorial aetiology, mainly affecting the gastrointestinal tract. A growing body of experimental evidence shows that constant exposure of the gastrointestinal tract to risk factors generates oxidative stress, that is an overproduction of reactive oxygen species (ROS) and free radicals. These interact with each other and are triggered and maintained by the overproduced ROS within the pro-inflammatory microenvironment. This results in a vicious cycle that amplifies and sustains inflammation, promoting the progression of intestinal diseases and also predisposing to cancer. The goal of our *in vitro* study is to investigate how the utilize of bioplastic may reduce gastrointestinal pathologies related to microplastic impact, with consequent contingent socio-economic and health costs, and to identify factors that reduce the toxic/tumour effects of food contact packaging. In this research we investigate the effects of different bioplastics composed of plant-extracted microgranules on two cell types, CaCo-2 and HT29, which are human-derived colorectal adenocarcinoma cell culture lines. In this comparative study, we analyse the anti-inflammatory and antioxidant properties of the different types of bioplastics in order to define risk factors in the food/packaging paradigm affecting upstream food industry packaging systems and downstream welfare.

HUMAN HEALTH IMPLICATIONS FOLLOWIN EXPOSURE TO ISOTHIAZOLINONES

Marika CORDARO[1], Francesca INFERRERA[2], Federica IMPELLITTERI[3], Caterina FAGGIO[2], Rosanna DI PAOLA[3]

[1]Department of Biomedical, Dental and Morphological and Functional Imaging University of Messina; [2]Department of Chemical, Biological, Pharmaceutical, and Environmental Sciences, University of Messina; [3] Department of Veterinary Sciences, Messina, Italy

With over 100,000 different substances produced and used by industries, chemicals are an integral part of everyday life. The global market of chemicals has an enormous impact on employment and economic growth. Pesticides, hand sanitizing gels, detergents and personal care and baby products are just some of the products all accumulated by the presence in their constituents of a family of molecules called "isothiazolinone" (IS). Despite their effectiveness as biocides, IS are strong sensitizers, producing skin irritations and allergies and may pose ecotoxicological hazards. The lack of scientific data on the effects of these IS poses a risk to ecological and human health because they produce reactive oxygen species, increase cytokines release and induce pro-inflammatory response that led to cell death in mammals and fishes. Due to the increasing use and impact of IS in human and the release it in the environmental, mainly for their presence in medical devices used for the spread of Covid-19, the identification of their mechanism of action, short and long term exposure and human and ecotoxicological effects need to be investigated. In particular the biocides 5-chloro-2-methyl-2h-isothiazolin-3-one and 2-methyl-2h-isothiazolin-3-one (CMIT/MIT) are one of the most used and can be found in many different types of water-soluble consumer products, such as shampoo, dentifrice, and germicide. Therefore, the aim of this study was to examine the impact of CMIT/MIT in SH-SY5Y human neuroblastoma cells. SHSY-5Y cells were exposed to different concentration (0, 12.5, 25 and 50 μM) of CMIT/MIT for 24 h. Cellular proliferation was considerably reduced in the MTT assay after CMIT/MIT exposure. Additionally, the results showed an increase in LDH release and lipid peroxidation and a decrease in physiological antioxidant defense. We also observed an activation of Nrf-2/HO-1 signaling pathway by western blot and qRT-PCR. Exposure to CMIT/MIT also increased the release of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α. Furthermore, in SHSY-5Y, CMIT/MIT raised the levels of

phosphorylated ERK1/2, phosphorylated p38, and phosphorylated JNK1/2 proteins. The activation of these pathways was strongly connected with the cell cycle-related genes p53 and p21 and the activation of apoptotic cascade. These results imply that the Nrf-2/HO-1, p38-JNK1/2-ERK1/2, Bax/Bcl-2 signaling pathways are responsible for inducing cellular damage and accelerating neuronal aging in response to CMIT/MIT exposure.

This work was in part supported by Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) PRIN: PROGETTI DI RICERCA DI RILEVANTE INTERESSE NAZIONALE – Bando 2022 PNRR Prot. P2022BH28Y.

THE DARK SIDE OF PLASTICS: ECOPHYSIOLOGICAL IMPACTS ON ANIMALS

Serafina FERRARA[1], Cristiana Roberta MULTISANTI[2], Irene VAZZANA[3], Caterina FAGGIO[1,4]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina; [2]Department of Veterinary Sciences, University of Messina; [3]Zooprophylactic Institute of Sicily, Palermo; [4]Department of Eco-sustainable Marine Biotechnology, Stazione Zoologica Anton Dohrn, Naples, Italy

Nowadays, plastic pollution is one of the most significant issues affecting the environment, posing a serious threat to marine biodiversity, ecosystem stability, and human health [1]. Millions of tons of plastic waste enter the oceans every year, and these products are continuously subjected to chemical and biological degradation, resulting in the formation of micro- and nano-plastics (MPs and NPs) whose dimension are, respectively, in the ranges of 0.1 µm to 5000 µm (MPs) and 1 nm to 100 nm (NPs). The impact of these smaller particles is much more worrying than visible contamination. The presence of these tiny particles puts a strain on ecological dynamics, causing a significant impact on the health of aquatic organisms and also affecting human health due to the interconnection existing with the environment and the food chain [2]. This work examines the different ways in which MPs can interact with aquatic life, the mechanisms that drive this pollution, and the cascading consequences for the health of organisms and ecosystems. It also highlights the critical links between plastic pollution and human health. Also, it underlines the urgency of a global and coordinated approach to address this growing crisis. Only through deeper understanding, increased awareness, and collective action it will be possible to hope for mitigating the significant impact of plastic pollution and ensure a sustainable future for oceans and our planet.

REFERENCES

1. Banaee, M., Multisanti, C. R., Impellitteri, F., Piccione, G., & Faggio, C. (2025). Environmental toxicology of microplastic particles on fish: A review. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 110042.
2. Multisanti, C. R., Merola, C., Perugini, M., Aliko, V., & Faggio, C. (2022). Sentinel species selection for monitoring microplastic pollution: A review on one health approach. *Ecological Indicators*, *145*, 109587.

VALORIZATION OF SICILIAN WHITE AND RED GRAPE SEED OILS IN THE CONTROL OF GLUCOSE UPTAKE AND CONSUMPTION *IN VITRO*

Daniela GANCI[1], Federica BELLISTRÌ[1,2], Roberto CHIARELLI[1], Manuela MAURO[1], Vincenzo ARIZZA[1,2], Mirella VAZZANA[1,2], Claudio LUPARELLO[1,2]

[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo; [2]NFBC, National Biodiversity Future Center, Palermo, Italy

Grape seed oil, a by-product of wine production, is rich in polyunsaturated fatty acids, particularly linoleic acid, and abundant in antioxidants like polyphenols, which contribute to its notable health-promoting properties[1]. These bioactive compounds provide anti-inflammatory, antioxidant, and antidiabetic effects, making grape seed oil a promising natural agent for the management of metabolic disorders. This study aimed to evaluate the impact of 24-hexposure to the maximum non-inhibitory concentration of grape seed oils, obtained from Sicilian white (WGSO) or red grapes (RGSO), on glucose consumption and uptake in HepG2 liver cancer cells, which retain several differentiated hepatic functions and are a suitable *in vitro* model to study liver metabolism[2]. PAS reaction and glucose uptake assays[3] were performed and the results revealed that both oils induced an increase in the storage of intracellular glycogen and in the uptake of 2-NBDG, a fluorescently labeled deoxyglucose analogue, indicating an improved glucose utilization. Additionally, a decrease in glucose levels in the cell medium was observed, similar to that obtained after treatment with insulin. Furthermore, Western blot analysis was performed to evaluate the expression of key proteins involved in glucose uptake and metabolic regulation, that is, GLUT-2, GLUT-4, AKT-2, pAKT-2 and HNF-1α, in HepG2 cells under the different experimental conditions. The results obtained demonstrated that different molecular mechanisms were involved in the similar glucose-lowering effects of WGSO and RGSO. In fact, exposure to WGSO determined the increase of the sole GLUT-4 and of pAKT-2/AKT-2 ratio, involved in the GLUT-4 expression pathway, whereas treatment with RGSO induced the up-regulation of both GLUT-2 and -4 and of HNF1α, a GLUT-2 transcription factor. Our results highlight the potential of grape seed oil as a modulator of glucose metabolism, particularly in conditions of insulin resistance or metabolic dysfunction, underscoring its relevance in the management of metabolic disorders such as diabetes. Given that grape seed oil is a by-product of wine production, its reuse not only adds value to an otherwise discarded resource but also promotes sustainability, further supporting the need for continued research into its therapeutic applications.

REFERENCES

1. Garavaglia J, *et al.*, Nutrition and Metabolic Insights 2016; 9: 59-64.
2. Donato MT, *et al.*, Methods Mol Biol 2015; 1250:77-93.
3. Abruscato G, *et al.*, Biology 2024; 13(6), 378.

ANALIYSIS OF PESTICIDE RESIDUES AND CHRONIC RISK ASSESSMENT

Maria Chiara GURRERI[1], Gianluigi Maria LO DICO[2], Andrea MACALUSO[2], Giovanni LO CASCIO[3], Fabio VENTURELLA[4]

[1]Independent researcher, Agrigento; [2]Department of Food Chemistry and Food Technologies Area IZS, Palermo; [3]Department of Chemistry IZS, Palermo; [4]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, Palermo, Italy

INTRODUCTION: In modern agriculture, the use of pesticides is essential to protect crops from pests and diseases, ensuring high yields and product quality. However, intensive use of pesticides can leave residues in food products, raising concerns about human health. This study aims to determine pesticide residues in vegetables and fruits using advanced analytical techniques to ensure consumer safety. METHODOLOGY: A total of 72 vegetable samples and 185 fruit samples were collected from various agricultural areas. Analyses were performed using gas chromatography-tandem mass spectrometry (GC-MS/MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). These techniques, in fact, offer high sensitivity and specificity, allowing the detection and quantification of various classes of pesticides, including organophosphates, pyrethroids, carbamates, neonicotinoids, and fungicides. The QuEChERS technique was used for sample preparation. This methodology involves an extraction phase with acetonitrile, followed by a cleanup phase with MgSO4 and NaCl salts, obtaining clean extracts ready for instrumental analysis. RESULTS: The analyses revealed the presence of various classes of pesticides, including organophosphates, pyrethroids, carbamates, neonicotinoids, and fungicides. To assess the chronic risk associated with the consumption of these samples, the chronic Hazard Quotient (cHQ) was calculated for each detected substance. The cHQ is calculated as the ratio of the estimated daily intake (EDI) to the acceptable daily intake (ADI), based on daily consumption data in Sicily. The cHQ calculations for these substances showed low values. This result is due to the level of exposure, the duration of exposure, and the toxicity of the substance. Out of 257 samples analyzed, only 6 samples exceeded the maximum residue limits (MRL) set by EFSA. Three of these samples contained levels of imidacloprid (0.97 mg/kg, 0.11 mg/kg, 0.17 mg/kg, MRL 0.01 mg/kg), while the other three exceeded the MRL for dimethoate (0.089 mg/kg, MRL 0.01 mg/kg), 2-phenylphenol (0.606 mg/kg, MRL 0.01 mg/kg), and phosmet (0.092 mg/kg, MRL 0.005 mg/kg). These substances are highly neurotoxic agents, and the rigorous controls carried out allow the immediate blocking of contaminated batches, thus preventing the onset of toxic effects. Additionally, two unapproved substances were detected: iprodione (Regulation (EU) 2017/2091) and quinoxyfen (Regulation (EU) 2024/1355). To ensure safety, these samples were removed from the food chain, thus preventing the onset of toxic effects. The results indicate that most of the analyzed substances did not exceed the maximum residue limits (MRLs) set by EFSA, ensuring that the detected levels comply with the established toxic doses and that the risk of chronic toxicity is low. CONCLUSIONS: The GC-MS/MS and LC-MS/MS triple quadrupole techniques demonstrated high sensitivity and specificity, allowing precise quantification of pesticide residues. The results highlight the need for continuous monitoring to ensure consumer safety and confirm the effectiveness of EFSA regulations in protecting public health. The QuEChERS technique proved to be effective and reliable for sample preparation, contributing to the quality and accuracy of the obtained data. Human safety was placed at the center of this study, ensuring that residue limits comply with the toxic doses established by EFSA, thus guaranteeing that food products are safe for long-term consumption.

POTENTIAL TOXICOLOGICAL EFFECTS OF FUNGICIDES MIXTURES IN ZEBRAFISH EARLY LIFE STAGE

Annamaria IANNETTA[1], Tommaso SILVESTRINI[2], Giovanni ANGELOZZI[1], Sabrina TAIT[3], Irene MASCIOLA[3], Gabriele LORI[3], Tyrone LUCON-XICCATO[4], Cristiano BERTOLUCCI[4], Monia PERUGINI[1]

[1]Department of Bioscience and Agro-Food and Environmental Technology, University of Teramo; [2]Advanced Study "Theoretical and Applied Neuroscience", Preclinical clinical and Translational Neuroscience, University of Camerino (TO); [3]Center for Gender-Specific Medicine, Istituto Superiore di Sanità, Rome; [4] Department of Life Sciences and Biotechnology, University of Ferrara, Italy

Folpet (F), penconazole (P) and metrafenone (M) are fungicides that act through different mechanisms and are commonly used in agriculture, often in mixtures, to combat fungal pathogens. The application of fungicides on agricultural soils is not without risks, as they can persist in environmental matrices and pose a threat to the entire ecosystem. Given that the synergistic or combined effects of mixed compounds remain largely unexplored, this study evaluated the potential toxicological effects of the individual compounds F, P and M as well as their binary and ternary mixtures using the zebrafish (*Danio rerio*) model. Initially, the acute toxicity of the single compounds was assessed through the Fish Embryo Acute Toxicity Test (FET) to calculate toxicological endpoints and evaluate developmental alterations. Based on the lethal concentration 50 (LC_{50}) of each fungicide, three sublethal concentrations (0.25 mg/L, 0.5 mg/L, and 1 mg/L) were selected to prepare binary and ternary mixtures. Combined treatments resulted in greater effects than individual compounds. The SynergyFinder+ algorithm, used to calculate synergy scores of the mixtures, confirmed a high degree of synergism, particularly in the neurological phenotype, with specimens exhibiting deformed heads. Based on these findings, further analyses were conducted to investigate potential neurotoxicity, including the evaluation of gene expression involved in apoptosis mechanisms (*bcl2a, baxa, casp3a, tp53*) and in craniofacial and nervous system

development (*bdnf, dlx5a, sphk1, col11a1a, col2a1a*). Craniofacial development was also assessed using Alcian blue staining. The results revealed a global dysregulation of the analyzed genes, with *dlx5a* and *col11a1a* alterations particularly confirming the observed craniofacial deformations. Supporting these findings, neurotoxicity evaluation using the transgenic GFP-*Neurod* model further confirmed the neurological impact of the mixtures, as specimens exhibited increased fluorescence emission, indicating a failure in neurogenesis leading to neuronal apoptosis. This aligns with the observed dysregulation of *bcl2a*, *baxa*, *casp3a* and *tp53*. Finally, to further validate these results, behavioral functional studies highlighted behavioral changes such as hyperactivity, lower thigmotaxis and reduced habituation time. These effects, resulting from the neurotoxicity of the mixtures, can be explained by the altered expression of *bdnf* and *neuroD*. In conclusion, this study demonstrated that the synergistic interactions observed in the mixtures significantly amplified the neurological effects compared to single exposures. These findings highlight the critical importance of assessing the combined effects of pesticides to improve environmental and human risk assessment strategies.

CELLULAR AND METABOLIC IMPACTS OF POLYLACTIC ACID NANOPARTICLES IN INTESTINAL-DERIVED CELL LINES: A PRELIMINARY INVESTIGATION

Martina LOMBARDI[1,2,3], Raffaella D'AURIA[1], Marianna MARINO[1], Erwin Pavel LAMPARELLI[1], Giovanna DELLA PORTA[1], Jacopo TROISI[1,2,3,4], Andrea VIGGIANO[1], Antonietta SANTORO[1]
[1]Department of Medicine, Surgery and Dentistry, "Scuola Medica Salernitana", University of Salerno; [2]Theoreo Srl, Montecorvino Pugliano (SA); [3]European Institute of Metabolomics - Fondazione EIM, Baronissi (SA); [4]Department of Chemistry and Biology, "A. Zambelli", University of Salerno, Fisciano (SA), Italy

Polylactic acid (PLA) has garnered significant attention as a sustainable alternative to traditional petroleum-based plastics due to its biodegradability and eco-friendly origins. Widely used in applications such as food packaging and medical devices, PLA is increasingly becoming a cornerstone of green technology. However, as it undergoes degradation over time, PLA fragments into micro and nanoparticles (MPs/NPs), which raise growing concerns about their potential effects on human health (1). Despite its widespread use, the cellular and metabolic impacts of PLA-NPs remain insufficiently explored. This study investigates the cellular and metabolic effects of PLA nanoparticles (PLA-NPs) on HT-29 and Caco-2 intestinal-derived cell lines. PLA-NPs were synthesized in-house using a microfluidic-assisted nanoprecipitation method, with the particles conjugated with Rhodamine for easier detection (2). Both cell lines were exposed to PLA-NPs at a concentration of 100 µg/mL for 24, 48, and 72 hours. Cellular uptake was assessed through flow cytometry, revealing 100% internalization of PLA-NPs, a finding further confirmed by confocal microscopy, which showed perinuclear and cytoplasmic localization of the particles. We also investigated potential effects on cell proliferation using the BrdU assay and assessed pro-inflammatory cytokine release, such as IL-8. No significant changes were observed in cell proliferation, while inflammatory response was detected only in HT-29 cells. On the other hand, untargeted metabolomics using Gas Chromatography-Mass Spectrometry (GC-MS) revealed cell-specific metabolic shifts in response to PLA exposure, including alterations in amino-acids metabolism, disruptions in carbohydrate processing and oxidative stress-related pathways. These findings suggest that PLA-NPs may affect cellular metabolism without inducing overt cytotoxicity. Our findings emphasize the ability of PLA-NPs to penetrate cellular membranes and elicit subtle metabolic changes, offering valuable insights into their potential biological impacts and implications for human health.

ACKNOWLEDGMENTS AND FUNDING: *This work is under the support of the National Recovery and Resilience Plan (NRRP), Mission 4, Component 2, Investment 1.1, "Fund for the National Research Program and for Projects of National Interest (NRP)" by the Italian Ministry of University and Research (MUR), funded by the European Union – NextGenerationEU. Project title: "Plastic Contamination by Poly(Lactic Acid) (PLASTAMINATION): organ injuries and underlying molecular mechanisms", MUR, PRIN-PNRR2022 CODE NUMBER: P2022AA47Y- CUP D53D23021910001*

REFERENCES

1. Ainali, N. M. *et al.* Science of The Total Environment 832, 155014 (2022).
2. Lamparelli, E. P. *et al.* International Journal of Pharmaceutics 667, 124934 (2024).

FOMES INZENGAE EXTRACT LIMITS PROLIFERATION AND MIGRATION OF HEPATOCELLULAR CARCINOMA CELLS THROUGH MODULATION OF THE PTEN/PI3K/AKT PATHWAY

Alberto MASSIMI, Lara LIZZI, Mara MASSIMI, Mirco IOTTI, Marco LEONARDI
Dipartimento di Mina Clinica, Sanità Pubblica Scienze della Vita e dell'Ambiente, Università dell'Aquila, Italy

Fomes inzengae, a sister species of *F. fomentarius*, is a lignicolous fungus parasitic on deciduous trees and widely distributed across Europe. While other species within the genus *Fomes* are well-documented for their production of bioactive compounds, including polysaccharides, triterpenes and their derivatives, lipids, and secondary metabolites, the properties of *F. inzengae* remain largely unexplored, highlighting the need for further research. These compounds are known for their diverse pharmacological activities, including anticancer, immunomodulatory, anti-inflammatory, and antiviral effects [1]. This study investigates the bioactive properties and biological effects of *Fomes inzengae* ethanol extract on two hepatocarcinoma (HCC) cell lines, HepG2

and Huh7. Firstly, we assessed its cytotoxic potential and antiproliferative activity using various methods, including the Trypan Blue Exclusion Test, Lactate Dehydrogenase (LDH) test, and Live/Dead assays. The results demonstrated that *F. inzengae* extract significantly reduced the number of both HepG2 and Huh7 cells in a time- and dose-dependent manner. Based on these preliminary experiments, we identified 100 µg/mL as the optimal concentration for evaluating both cytotoxic and biological effects. At this concentration, treatment with *F. inzengae* extract led to an increase in the levels of tumor suppressor proteins p53, p21, and p27, suggesting a slowdown of the cell cycle. Additionally, we observed a decrease in the expression of the anti-apoptotic protein Bcl-2, alongside an increase in pro-apoptotic proteins Bax, cleaved caspase-9, and caspase-3. Furthermore, the extract impaired the wound-healing ability of Huh7 cells, which are highly migratory, suggesting its potential role in limiting cancer cell dissemination. This was further supported by the upregulation of E-cadherin and downregulation of Twist, as confirmed by Western blot analysis. Additionally, to explore the molecular mechanisms underlying these effects, we investigated potential signaling pathways that could be involved. Given its well-established role in regulating cell survival, proliferation, migration, and metabolism, we focused on the PTEN/PI3K/AKT pathway [2]. PTEN, a tumor suppressor, dephosphorylates PIP3, thereby inhibiting the activity of Phosphoinositide 3-Kinase (PI3K) and subsequently Protein Kinase B (AKT), which are key mediators of cell growth and survival. Western blot analysis of Huh7 cells revealed an increase in active (dephosphorylated) PTEN levels and a decrease in the phosphorylated (active) forms of PI3K and AKT, aligning with the observed biological effects. Although further research is needed to fully characterize the bioactive components and precise molecular targets of *F. inzengae* ethanol extract, our findings indicate that this polypore fungus exerts antiproliferative, proapoptotic, and antimigratory effects, at least in part, through modulation of the PTEN/PI3K/AKT signaling pathway.

REFERENCES

1. Diego Morales, Fomes fomentarius: An underexplored mushroom as source of bioactive compounds, Food Bioscience, Volume 61,2024,104781,ISSN 2212-4292.
2. Papa A, The PTEN-PI3K Axis in Cancer. Biomolecules. 2019 Apr 17;9(4):153. doi: 10.3390/biom9040153. PMID: 30999672; PMCID: PMC6523724.

POTENTIAL OF NATURAL-DERIVED COMPOUNDS IN MITIGATING TOXICITY OF EMERGING CONTAMINANTS: EVALUATION OF CELLULAR AND PHYSIOLOGICAL RESPONSES IN SENTINEL ORGANISMS

Cristiana Roberta MULTISANTI[1], Federica IMPELLITTERI[1], Kristian RIOLO[2], Giuseppe PICCIONE[1], Marina RUSSO[2], Alessia GIANNETTO[2], Caterina FAGGIO[2,3]

[1]Department of Veterinary Sciences, University of Messina; [2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina; [3]Department of Eco-sustainable Marine Biotechnology, Stazione Zoologica Anton Dohrn, Naples, Italy

The COVID-19 pandemic significantly increased the demand for detergents, cleaning products, and foaming agents for medical or industrial purposes to maintain hygiene and prevent microbial growth. These products contain several compounds in their formulations that can readily reach and threaten aquatic ecosystems. Among these are surfactants, recognized as common components in detergents because of their surface-active properties and are, also, classified as emerging contaminants. The most prevalent surfactant is sodium lauryl sulphate (SLS), an anionic detergent with antimicrobial properties, which is extensively used as a synthetic cleansing agent. Although SLS biodegradation ranges from 45% to 95% within 24 hours, its continuous introduction into the environment results in the maintenance of elevated levels in aquatic ecosystems [1]. Anionic detergents, including SLS, exhibit a pronounced affinity for interacting with the lipids of cellular membranes and, at high concentrations, can lead to significant alterations [2]. For this reason, the present investigation aimed to obtain novel insights into the potential mitigation of bergamot peel extract on cellular and physiological performances of *Mytilus galloprovincialis* exposed to SLS. Bioactive molecules were obtained by solvent extraction of bergamot peels according to the procedure by Russo *et al.* [3], and the extract was then subjected to HPLC analysis for characterisation. Specimens were treated with SLS (0.01 mg L^{-1}), bergamot peel extract (BRG: 5 mg L^{-1}), and their mixture (SLS+BRG) for fourteen days. The immune response was assessed through the ability of haemocyte cells to perform phagocytosis, along with the gene expression of *γ-actin,* which plays a key role in the cytoskeleton rearrangement. The Neutral red retention assay and the Trypan blue exclusion test were employed to evaluate cytotoxicity on haemocytes and digestive gland (DG) cells. The physiological response was video-metrically assessed by cell volume regulation analysis (RVD assay) in DG cells, and the energy efficiency of organisms was assessed through byssus analysis. The expression of genes involved in antioxidant activity (*Cu/ZnSOD, MnSOD, Hsp70, and CYP4Y1*) was also evaluated. Results showed a consistent trend between the CTR and BRG groups, with significant differences compared to the SLS-treated groups. Meanwhile, the groups exposed to the combination (SLS+BRG) exhibited a recovery. These results support and provide novel insights into the potential of bergamot's bioactive compounds in mitigating pollutant-induced toxicity, proposing new alternatives to reduce the ecological impact of chemical pollutants in aquatic systems.

REFERENCES

1. Cserháti, T. *et al.* (2002). Biological activity and environmental impact of anionic surfactants. *Environment international*, *28*(5), 337-348.

2. Brunelli, E. *et al.* (2008). Effects of a sublethal concentration of sodium lauryl sulphate on the morphology and Na+/K+ ATPase activity in the gill of the ornate wrasse (*Thalassoma pavo*). *Ecotoxicology and Environmental Safety*, *71*(2), 436-445.
3. Russo, M. *et al.* (2015). Multidimensional preparative liquid chromatography to isolate flavonoids from bergamot juice and evaluation of their anti-inflammatory potential. *Journal of Separation Science 38*, 4196-4203.

IMATINIB AS A NOVEL ANTIMALARIAL THERAPY: FROM MECHANISTIC INSIGHTS TO CLINICAL APPLICATION

Antonella PANTALEO[1], Chien Huynh DINH[2], Philip S. LOW[3,4], Francesco M. TURRINI[5]
[1]Department of Biomedical Science, University of Sassari, Italy; [2]College of Health Science, Vin University, Hanoi, Vietnam; [3]Department of Chemistry, Purdue University, West Lafayette, IN, USA; [4]Institute for Drug Discovery, Purdue University, West Lafayette, IN, USA; [5]Department of Oncology, University of Turin, Italy

Malaria continues to pose a significant global health challenge, underscoring the need for innovative therapeutic approaches. Our research investigates the repurposing of imatinib, a known tyrosine kinase inhibitor, as a novel antimalarial drug targeting erythrocyte membrane stability. We have identified that imatinib effectively inhibits spleen tyrosine kinase (Syk), a key enzyme involved in the phosphorylation of erythrocyte membrane protein band 3 during *Plasmodium falciparum* infection. This phosphorylation event is crucial for the parasite's egress from host erythrocytes. *In vitro* studies demonstrated that treatment with imatinib prevents band 3 phosphorylation, thereby inhibiting parasite egress and terminating parasitemia. Further analysis revealed that imatinib-induced inhibition of Syk kinase activity leads to the stabilization of the erythrocyte membrane, preventing the formation of membrane aggregates and subsequent vesiculation. This mechanism disrupts the parasite's life cycle by hindering its ability to exit the host cell, offering a novel therapeutic strategy against malaria. Building on these promising *in vitro* results, we conducted a Phase 2 clinical trial in Vietnam, where delayed parasite clearance (DPC) is a frequent challenge. Patients with uncomplicated *P. falciparum* malaria were treated for three days with either standard-of-care (SOC) therapy (dihydroartemisinin 40 mg + piperaquine 320 mg/day) or SOC + imatinib (400 mg/day). The combination therapy significantly accelerated parasite clearance, with 90% of patients achieving clearance within 48 hours and 100% within 72 hours, eliminating cases of DPC. Pyrexia resolution was also significantly faster in the imatinib-treated group, with no increase in adverse events (DOI: 10.1084/jem.20210724). These findings confirm that imatinib enhances SOC malaria treatment by targeting host erythrocyte membrane stability. Given its established clinical use, imatinib represents a promising adjunctive therapy for malaria, warranting further evaluation for widespread implementation.

FIRST INSIGHTS ON DIVERSITY AND BIOTECHNOLOGICAL POTENTIAL OF ARCTIC BIOAEROSOL CULTIVABLE BACTERIA

Carmen RIZZO[1,2], Maria PAPALE[2], Alessandro Ciro RAPPAZZO[2], Vittoria DIAS[1], Matteo FELTRACCO[3], Maya PETRICCIUOLO[4], Ermanno FEDERICI[4], Angelina LO GIUDICE[2], Vito VITALE[5], Maurizio AZZARO[2]
[1]Stazione Zoologica Anton Dohrn, Sicily Marine Centre, Messina; [2]Institute of Polar Sciences, National Research Council (CNR-ISP), Messina; [3]Ca' Foscari University of Venice; [4]University of Perugia; [5]Institute of Polar Sciences, National Research Council (CNR-ISP), Bologna, Italy

The advancement of aerobiology as a discipline should aid in determining whether microbes interact and evolve within the atmosphere at the ecological level. Bioaerosol microbial communities represent a dynamic and crucial ecosystem component, which has captured increasing interest in the last decades. In the Arctic region, airborne microbes establish interactions with snow, ice, and water, where they become aerosolized by natural processes such as wind, ocean currents, and melting ice. They are highly specialized for survival in sub-zero temperatures and low-nutrient environments and actively contribute to biogeochemical processes such as the degradation of organic matter, nitrogen cycling, and the modulation of atmospheric trace gases. Furthermore, they influence the climate system by producing ice-nucleating proteins affecting cloud formation and precipitation patterns. Studies of Arctic bioaerosols have identified several key bacterial taxa which possess special adaptations such as the production of cold-active enzymes and resistance to desiccation. Bioaerosol samples were collected during an oceanographic cruise onboard the N/R Laura Bassi within the context of the project Cassandra (*AdvanCing knowledge on the present Arctic Ocean by chemical-phySical, biogeochemical, and biological obServAtioNs to preDict the futuRe changes*) in the subarctic area including the eastern Fram Strait and the Greenland, Norwegian and North Seas. Bacterial strains were isolated and investigated for their biotechnological potential. Viable counts were determined and based on the morphology and color of the colonies, a total of twenty strains were isolated on Nutrient Agar. The resistance to UV radiation was estimated through direct exposure for 1, 4 and 6 hours. The sensitivity to amoxicillin, ciprofloxacin and penicillin at different concentrations was assessed. Finally, desiccation resistance and ice nucleation protein production were investigated in all isolates. Bioaerosol isolates showed promising results in terms of metabolic resistance, by maintaining viability after exposure to UV for up to six hours and growing in the presence of all tested antibiotics up to a final concentration of 200 ppm. 16S rRNA Sanger sequencing showed affiliation of the strains mainly to *Delftia*, *Rhodococcus*, *Micrococcus* and *Sphingomonas* spp. As the Arctic continues to experience rapid environmental changes, understanding the dynamics of airborne microbial communities will be essential for predicting future shifts in Arctic ecosystems. Future research will be focused on understanding the mech-

anisms of bioaerosol formation, ecological implications, and interactions with a changing climate.
Funding: Italian Ministry of University and Research through the Italian Arctic Research Programme

NANOPLASTICS AND IMMUNE SYSTEM: EXPLORING THE ECM'S ROLE IN MACROPHAGE RESPONSE TO POLYSTYRENE NANOPARTICLES

Marianna ROCA[1], Naym BLAL[1], Claudia ORLANNO[1], Zeynep RENKLER[2], Vincenzo GUARINO[2], Antonia DI MOLA[1], Antonio MASSA[1], Daniela GUARNIERI[1]
[1]Department of Chemistry and Biology, "Adolfo Zambelli", University of Salerno; [2]Institute of Polymers, Composites and Biomaterials, National Research Council of Italy, Pozzuoli (NA), Italy

Plastics are widely used globally, but their limited biodegradability leads to significant environmental pollution. They are classified by size: nanoplastics (NPs), ranging from 1 nm to 1000 nm, and microplastics, from 1 µm to 5 mm. Nano- and microplastics can enter the human body through inhalation, ingestion of contaminated food and water, or skin exposure via cosmetics and clothing. Once inside, plastics can accumulate and potentially cause health problems such as respiratory disorders (*e.g.*, lung cancer, asthma), neurological symptoms (*e.g.*, fatigue), and inflammatory bowel disease. In most *in vitro* studies, cells are exposed to NPs suspended in culture medium, which has shown that nano- and microplastics can induce apoptosis and have genotoxic and cytotoxic effects. In human tissues, NPs are not only diffusible in biological fluids but can also be trapped or adsorbed by ECM components. This interaction could alter the ECM properties and how cells interact with NPs. Controlled exposure to NPs via the ECM could affect cell behavior and their response to NPs. In this study, funded by the PRIN 2022 PNRR program, we aim to explore the role of the microenvironment in macrophage response to nanoplastics embedded in the ECM. We exposed macrophage-like cells from the human monocytic THP-1 cell line to ECM-like substrates pre-loaded with polystyrene NPs. These substrates, resembling the structural, chemical, and physical properties of native ECM, were made from polymer and gelatin and hyaluronic acid (HA) using electrospinning. Preliminary experiments showed macrophages adhered to all substrates, particularly those with gelatin, without affecting cell viability up to 48 hours after seeding. Furthermore, gelatin matrices adsorbed more NPs compared to controls without gelatin. By using fluorescence microscopy, it was confirmed that the NPs embedded in these ECM-like substrates are bioavailable and can be internalized by macrophages. This research highlights the need for further studies to fully understand the cellular and molecular mechanisms of immune cell response to nanoplastics, which may provide insight into the impact of plastics on human health.
ACKNOWLEDGMENTS. The authors acknowledge financial support under the National Recovery and Resilience Plan (NRRP), Mission 4, Component 2, Investment 1.1, "Fund for the National Research Program and for Projects of National Interest (NRP)" by the Italian Ministry of University and Research (MUR), funded by the European Union – NextGenerationEU.
Project Title: Role of exTRAcellular MAtrix in controlling macrophage cell response to nanomaterials (TRAMA) – CUP D53D23021660001.

OCTYLISOTHIAZOLINONE IMPAIRS ZEBRAFISH MELANOGENESIS AND MELANIN SYNTHESIS

Ludovica SULCANESE[1], Annamaria IANNETTA[1], Tommaso SILVESTRINI[1,2], Giordano ZIPPILLI[1], Vanessa D'ALTILIA[1], Valentina IEZZI[3], Elisabetta BENEDETTI[3], Monia PERUGINI[1]
[1]Department of Biosciences and Agro-Food and Environmental Technologies, University of Teramo; [2]Advanced Study "Theoretical and Applied Neuroscience", Preclinical Clinical and Translational Neuroscience, University of Camerino (TO); [3]Department of Life, Health and Environmental Sciences, University of L'Aquila, Italy

2-octyl-2H-isothiazol-3-one (Octylisothiazolinone, OIT) is a biocide belonging to the isothiazolinone family. Isothiazolinone biocides are antibacterial agents commonly used as disinfectants or preservatives. According to the European Chemical Agency (ECHA), OIT is employed as a bacterial and fungal biocide for wood preservation, while its use in products such as leather, cleaning agents, glue and paints is under review in the EEA and Switzerland. Despite its widespread industrial application, OIT is potentially hazardous, but limited studies have been conducted to assess its effects. This study aimed to analyse and characterize the effects of OIT on the early-life stages of zebrafish (*Danio rerio*), a well-established toxicological model. To evaluate the lethal and sublethal effects of OIT, zebrafish embryos/larvae were exposed to concentrations of 0.01, 0.05, 0.1, 0.2 and 0.4 mg/L for up to 96 hours post-fertilization (hpf) following the Fish Embryo Acute Toxicity (FET) test protocol. The median lethal concentration (LC_{50}) was determined to be 0.151 mg/L while 0.05 mg/L and 0.03 mg/L were the values of LC_{20} and LC_{10}, respectively. Zebrafish embryos and larvae were observed using an inverted phase light microscope, and the sublethal alterations recorded included pericardial and yolk oedema, as well as blood stasis. Notably, depigmentation was the primary sublethal effect observed at concentrations of 0.1 and 0.2 mg/L at 72 hpf, suggesting interference with melanophore development. In zebrafish, melanophores are pigmented chromatophores containing melanosomes—organelles where tyrosine-derived melanin is deposited. Building on these findings, the research focused on investigating OIT effects on melanophore differentiation at the molecular level. The expression of key melanophore regulatory genes, including *microphthalmia-related transcription factor a* (*mitfa*) and *mitfb*, as well as *tyrosinase* (*tyr*), the enzyme responsible for melanin synthesis, was analysed using qPCR. The results revealed a significant upregulation of *tyr* ($p<0.01$) at 0.1 mg/L, while *mitfa* and *mitfb* expression remained unchanged. Moreover, melanin quantifica-

tion confirmed a significant reduction of melanin production, particularly at 0.2 mg/L. Subsequently, the enzymatic activity of tyrosinase was evaluated, confirming the reduction of more than 50% of its activity at both tested concentrations compared to the control group. Further replications will be carried out to confirm the reduced enzymatic activity, and melanophores count will be performed to assess impaired development and migration of these cells. Overall, this study provides valuable insights into the toxic mechanisms of OIT. The dysregulation of melanin production and melanophore differentiation observed is well-known to be closely associated with thyroid dysfunction in zebrafish, suggesting that OIT may act as an endocrine-disrupting substance. These findings highlight the potential of OIT to be classified as a thyroid-disrupting chemical. However, further targeted studies are needed to confirm its effects on thyroid function, including potential gender-specific responses.
ACKNOWLEDGMENTS. Finanziato dall'Unione europea-Next Generation EU, Missione 4 Componente 2 INVESTIMENTO 1.1 – Fondo per il Programma Nazionale di Ricerca e Progetti di Rilevante Interesse Nazionale (PRIN), CUP C53D23009240001.

SURFACTANTS IN PERSONAL CARE PRODUCTS: DETRIMENTAL EFFECTS OF SODIUM LAURYL SULFATE ON FRESHWATER FISH AND AMPHIBIAN EMBRYOS

Giorgia ZICARELLI[1], Federica IMPELLITTERI[2], Pavla LAKDAWALA[3], Jana BLAHOVA[3], Zdenka SVOBODOVA[3], Renata HESOVA[3], Barbora RIESOVA[3], Michaela Frederika VARGOVA[3], Giuseppe PICCIONE[2], Cristiana Roberta MULTISANTI[2], Caterina FAGGIO[1,4]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy; [2]Department of Veterinary Sciences, University of Messina, Italy; [3]Department of Animal Protection and Welfare & Veterinary Public Health, Faculty of Veterinary Hygiene and Ecology, University of Veterinary Sciences Brno, Czech Republic; [4]Department of Eco-Sustainable Marine Biotechnology, Stazione Zoologica Anton Dohrn, Naples, Italy

In Personal Care Products (PCPs), sodium lauryl sulfate (SLS) is one of the main surfactants used in their formulation as an emulsifying cleaning agent. Introduced in the second half of the 20th century, SLS increased its presence in PCPs after the COVID-19 pandemic with a demand of over $28.8 billion in 2023. The rising demand caused an increase in the accumulation of SLS in the aquatic environment leading it to be regarded as a pseudo-persistent agent with an amount detected in freshwater bodies between 1.15 and 24 mg/L that may cause adverse effects on the communities. Even though the SLS-based products of daily personal use (such as toothpaste, cosmetics, shampoos, detergents, and many others) are still frequently used, most studies on the risk to ecosystems and non-target species were conducted before 2010. Therefore, the present study aimed to investigate the effects of SLS on three freshwater vertebrates. Applying embryotoxicity tests to the embryos of two fish (*Cyprinus carpio*, *Danio rerio*) and one amphibian species (*Xenopus laevis*), the possible harm caused by SLS exposure and the differences in action on different model organisms were evaluated. Embryos were exposed to six concentrations of SLS (0.1, 0.5, 1, 5, 10, and 15 mg/L) and one control group for 96h. During the experimental phase, the embryotoxicity endpoints of mortality, hatching rate, and occurrence of malformations were monitored every 24h. Additionally, the heartbeat rate alteration was evaluated after 48h of exposure for the fish organisms and after 52h for the frogs due to the transparency of the eggs. The results obtained from the analyses highlight a high sensitivity of *D. rerio* and *X. laevis* when exposed to environmental concentrations of SLS. Specifically, the results showed significant mortality of these two species in higher concentrations, alteration in hatching rate, and presence of many malformations on the body of the animals. Such effects were, however, not detected, in *C. carpio* embryos. In contrast, the results obtained for the heartbeat rate analyses showed significant differences in *C. carpio* and *D. rerio* embryos compared to the control. Such changes were not observed in *X. laevis* embryos. Such outcomes draw attention to the importance of conducting ecotoxicological analyses on multiple model organisms better to understand the real effects on the aquatic environment. Continued investigation will allow for a more comprehensive understanding of SLS's mechanisms of action and its interaction with biological systems, ultimately informing the development of safer formulations and mitigation techniques.

EXTRACELLULAR VESICLES

HORIZONTAL TRANSFER OF LONG NON-CODING RNA H19 VIA COLORECTAL CANCER-DERIVED EXTRACELLULAR VESICLES INDUCES EPITHELIAL-TO-MESENCHYMAL TRANSITION IN HEALTHY HEPATOCYTES

Marco LORIA[1], Denise ANELLO[1], Chiara ZICHITTELLA[1], Aurora CORDARO[1], Maria Magdalena BARRECA[1], Simona FONTANA[1], Riccardo ALESSANDRO[1,2], Alice CONIGLIARO[1]

[1]Department of Biomedicine Neuroscience and Advanced Diagnostic, University of Palermo; [2]Institute for Biomedical Research and Innovation (IRIB), National Research Council (CNR), Palermo, Italy

Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer-related death, with liver metastases accounting for most fatalities. Despite significant therapeutic advances, there is still a critical need to identify novel therapeutic targets to block tumor progression and metastasis. The long non-coding RNA H19 (lncH19) is overexpressed in CRC and is widely associated with tumor progression. We identified lncH19 inside CRC-derived small extracellular vesicles (CRC-sEVs), which are known to promote metastatic processes by acting on both surrounding tumor cells and distant sites. In particular, CRC-sEVs have been reported to induce epithelial-to-mesenchymal transition (EMT) and increase tumor cell invasion in secondary sites, such as the liver, thereby contributing to the formation of the pre-metastatic niche (PMN) [1]. However, the role of lncH19 carried by CRC-sEVs in hepatic PMN formation remains unclear. We recently demonstrated that in CRC cells lncH19 interacts with splicing factors such as RBFOX2, regulating alternative splicing (AS), a process linked to tumor progression, metastasis, and drug resistance. Specifically, we found that lncH19 recruits splicing factors to RAC1 mRNA, promoting the formation of RAC1B, a constitutively active isoform of the GTPase associated with aggressive CRC phenotypes [2]. In this study, we explored the role of lncH19 carried by CRC-sEVs in liver metastasis, hypothesizing that it functions both as a "shuttle" for multiprotein complexes, including splicing factors, and as a competing endogenous RNA (ceRNA). CRC-sEVs were isolated from the conditioned medium of CRC cells (SW620 and HCT-116) by ultracentrifugation and were resuspended in PBS, RNA lysis buffer, or RIPA buffer. RNA and protein content were assessed by qRT-PCR and Western Blot, respectively. A healthy hepatocyte cell line (THLE-2) was treated with CRC-sEVs, and the biological effects were investigated by qRT-PCR, immunofluorescence, and RNA-ISH. Our data demonstrated that CRC-sEVs transport both lncH19 and RBFOX2. Moreover, the treatment of human hepatocytes with CRC-sEVs induces AS of RBFOX2 target mRNAs involved in EMT, suggesting the horizontal transfer of multimolecular complex. Preliminary data also suggest that internalized lncH19 can function as a ceRNA, sequestering miRNAs involved in EMT, thus highlighting its multifunctional role in tumor progression.

REFERENCES

1. Pucci, Marzia *et al.* "Colorectal cancer-derived small extracellular vesicles induce TGFβ1-mediated epithelial to mesenchymal transition of hepatocytes.", Cancer cell international, 2023.
[2. Cordaro, Aurora *et al.*, "Regulatory role of lncH19 in RAC1 alternative splicing: implication for RAC1B expression in colorectal cancer.", Journal of experimental & clinical cancer research, 2024.

HUMAN HEALTHY LIVER SPHEROID AS MODEL TO STUDY THE ROLE OF COLORECTAL CANCER DERIVED SMALL EXTRACELLULAR VESICLES IN PRIMING HEPATIC PRE-METASTATIC NICHE

Gabriele ANTONUCCIO, Elisa COSTANZO, Ornella URZÌ, Marzia PUCCI, Simona FONTANA, Riccardo ALESSANDRO

Department of biomedicine, neuroscience and advanced diagnostic (B.I.N.D), University of Palermo, Italy

The majority of colorectal cancer related deaths is caused by liver metastases (LM). It is well established that the primary CRC can prepare a supporting micro-environment for tumor cell invasion in the liver, called hepatic pre-metastatic niche (h-PMN). This occurs through the release of several factors including small extracellular vesicles (SEVs). Our research group focuses on investigating the role of CRC derived-SEVs (CRC-SEVs) in affecting hepatocytes behaviour, thereby impacting h-PMN. Our previous study highlighted the ability of CRC-SEVs to induce TGFβ1 mediated epithelial to mesenchimal transition (EMT) in hepatocytes. As the EMT is preliminary onpoint fibrosis, our aim is to evaluate the role of CRC-SEVs in liver fibrosis establishment. However, fibrosis is the result of complex and highly dynamic processes that cannot be investigated using a canonical bidimensional model. Therefore, we established a tridimensional *in vitro* model of hepatic spheroid (HEP-3D) where to analyse the CRC-SEVs' pro-fibrotic role in h-PMN. The HEP-3D model has been developed by seeding in ultra-low attachment 96 wells the human healthy hepatocytes THLE-2 cell line, which were subsequently treated with SEVs isolated from CRC cell line SW480 and from non-metastatic CRC patients' plasma. The first evaluation of CRC-SEVs effect on HEP-3D confirmed the results obtained in our previous study, by revealing a decreased level of the hepatocytes functional markers Albumin and HNF4 and of the epithelial markers Cytokeratin 8/18 and E-Cadherin as well as an increased expression of the mesenchymal markers Vimentin and N-Cadherin. Moreover, we found that CRC-SEVs exposure to HEP-3D caused an amplified release of the fibrosis mediators Interleukin-6, Transforming Growth Factor β, and Growth Arrest Specific-6 together with an increased deposition of the extracellular matrix protein Collagen-IV, Tenascin C, and Fibronectin. Taken together these results show that HEP-3D model is responsive to CRC-SEVs and represents a suitable system to

study liver fibrosis, providing insight into the important role of the hepatocytes in the h-PMN shaping.

DIAMETERS AND FLUORESCENCE CALIBRATION FOR EXTRACELLULAR VESICLE ANALYSES BY FLOW CYTOMETRY

Arianna AQUILINI-MUMMOLO[1,2], Francesca D'ASCANIO[1,2,3], Giulia COLASANTE[1,2], Domenico DE BELLIS[1,2], Pasquale SIMEONE[1,2], Sabino PORRO[1,2], Tamer Shibl Ali ESMAIL[1,2], Ayesha YOUNAS[1,2], Paola LANUTI[1,2]
[1]Department of Medicine and Aging Sciences, University "G. d'Annunzio", Chieti-Pescara, Chieti; [2]Center for Advanced Studies and Technology (CAST), University "G. d'Annunzio", Chieti-Pescara; [3]Department of Humanities, Law and Economics, "Leonardo da Vinci" University, Torrevecchia Teatina (CH), Italy

Extracellular vesicles (EVs) play a crucial role in intercellular communication and have emerged as promising biomarkers and therapeutic tools. Among them, mesenchymal stem cell-derived EVs (MSC-EVs) have demonstrated regenerative and anti-inflammatory properties, making them potential candidates for clinical applications. However, the standardization of EV analysis remains a challenge, particularly in flow cytometry (FC), where variability in protocols has led to inconsistent results. Conventional FC approaches, which rely on scatter-based gating strategies, fail to accurately detect the full EV population, often missing smaller vesicles. In this study, we present an optimized FC methodology for EV characterization and quantification. By employing a fluorescence-based instead of a side scatter (SSC) threshold, the detection of MSC-EVs has been significantly improved. The proposed approach was validated using three different flow cytometers with high reproducibility (CV <20%) and sensitivity in detecting EVs with diameters in the range of 100–1000 nm. Furthermore, we used Rosetta Calibration beads for precise EV size discrimination, ensuring a robust and standardized analysis. Our findings highlight the importance of fluorescence-triggered flow cytometry for reliable EV quantification, paving the way for its clinical application in biomarker discovery and regenerative medicine. This standardized approach may provide a valuable tool for future studies focusing on EV-based diagnostics and therapeutics.

GENERATION OF A ZEBRAFISH LARVAL XENOGRAFT MODEL TO STUDY THE ROLE OF COLORECTAL CANCER-DERIVED EXTRACELLULAR VESICLES IN TUMOR PROGRESSION

Elisa COSTANZO[1], Bárbara ADEM[2], Marzia PUCCI[1], Simona FONTANA[1], Frederik Johannes VERWEIJ[2]
[1]Department of Biomedicine, Neuroscience, and Advanced Diagnostics, University of Palermo, Italy; [2]Department of Cell Biology, Neurobiology and Biophysics, Univerisity of Utrecht, The Netherlands

Growing evidence supports a key role of small extracellular vesicles (EVs) in favoring metastasis establishment in distant organs by preparing a supportive and receptive microenvironment for cancer cell colonization, defined as "pre-metastatic niche". *In vivo* studies investigating the role of EVs in cancer development are currently conducted in murine tumor xenografts. However, the application of these models is hampered by cost, time, and ethical implications. During recent years, zebrafish (*Danio rerio*) larvae have emerged as versatile and rapid *in vivo* systems suitable for EVs-based studies. Therefore, in this study we aim to develop zebrafish larval xenografts as an *in vivo* model to investigate the role of tumor derived EVs in cancer progression. To reach this goal, the transgenic zebrafish line Tg(*kdrl*:mCherry-CAAX) exhibiting mCherry-labeled endothelial cells was used to engraft the human colorectal cancer (CRC) cell line SW480. To follow SW480 cells and SW480-derived EVs distribution in zebrafish larvae, SW480 stable lines (dually) expressing cytosolic cerulean and/or EV-marker CD63-pHluorin were generated. Zebrafish larvae were injected at 2 days post fertilization (2dpf) in the Duct of Cuvier with SW480-CD63-pHluorin-EVs. The imaging, performed 1h after injection, showed the presence of SW480-CD63-pHluorin-EVs in zebrafish vasculature and their uptake by endothelial cells and macrophages, revealing the suitability of the model to investigate the distribution of CRC-derived EVs. CRC-xenografts were then generated by injecting zebrafish larvae at 2dpf in the perivitelline space (PVS) with SW480-cerulean CD63-pHluorin cells. The imaging at 3 days post injection revealed the presence in the PVS of a dense tumor mass and SW480-CD63-pHluorin-EVs were once again detected in zebrafish vasculature. To unravel the role of CRC-EVs in cancer development, we aim to analyze in depth CD63-pHluorin EV transfer to the liver, being the most common site of CRC-derived metastases. To do so, the transgenic line Tg(*fabp10*:nls-mCherry) with mCherry tagged hepatocytes was produced and will be used to investigate the distribution of CRC-EVs in liver and their role in favoring CRC-metastatic cells colonization in the organ. Taken together these findings portray zebrafish larval xenograft as a suitable system to study the distribution of engrafted cancer cells-derived EVs and a promising *in vivo* model to investigate the role of cancer-derived EVs in tumor progression.

EVALUATION OF TWO- AND THREE-DIMENSIONAL CULTURE MODELS TO STUDY EXTRACELLULAR VESICLES AS POTENTIAL BIOMARKERS IN OVARIAN CANCER

Giulia DI FAZIO[1], Giuseppina POPPA[1], Giuseppe CAMMARATA[2], Sandra D'ASCENZO[1], Angela D'ALFONSO[1], Vincenza DOLO[1], Simona TAVERNA[2], Ilaria GIUSTI[1]
[1]Department of Life, Health and Environmental Sciences, University of L'Aquila; [2]Institute of Translational Pharmacology (IFT), National Research Council of Italy (CNR), Palermo, Italy

Ovarian cancer (OC) is a gynaecologic malignancy with the

highest mortality rate, it remains one of the leading causes of cancer-related deaths among women worldwide. A favourable prognosis significantly depends on the timeliness of diagnosis and the stage of the disease. Ovarian cancer is often diagnosed at an advanced stage, as in the early stages it tends not to show obvious symptoms[1]. The identification of biomarkers for early diagnosis is therefore an urgent challenge. Extracellular Vesicles (EVs), and their molecular cargo, are considered potential and promising biomarkers for this purpose in liquid biopsy. The identification of valid biomarkers, however, requires appropriate preliminary *in vitro* studies. Although most preliminary studies on EVs have been conducted in two-dimensional (2D) cell culture models, in recent years there has been a focus on three-dimensional (3D) models, such as spheroids, that may better mirror the *in vivo* cancer status. Understanding the biology, the architecture of the OC, and communication between cells and tumour microenvironment is critical to exploring disease mechanisms more accurately[2]. The study aimed to devise a protocol for the formation of spheroids and the collection and isolation of EVs, in an approach more like *in vivo* conditions. Therefore, a protocol was optimized to obtain 3D, rounded spheroids of clinically relevant size, while also ensuring an optimal yield of EVs; to this purpose, a protocol for collection and isolation was also designed. EVs were isolated by ES-2 cells by differential centrifugation and characterized according to MISEV 2023 guidelines[3]. To understand whether their molecular composition could be influenced by the culture model, functional assays (such as proliferation and wound healing) were conducted to examine their biological and molecular activity; HUVECs were chosen as target cells. 2D and 3D EVs appear not to stimulate the proliferation of HUVECs, but they differentially induced their motility, depending on the dose. In conclusion, studies on the EVs released from the two culture models showed some differences in their biological activity, leading to hypothesize that the choice of *in vitro* model should be carefully evaluated.

This work was supported by the Funding Program PRIN 2022 under grant agreement N°20224XB79P, funded by European Union, Next-generation EU, PNRR, M.4-C2-1.1.

REFERENCES

1. Radu M.R., *et al.*, *Biomedicines* 2021, *9*, 693.
2. Giusti, I.; *et al.*, *Int. J. Mol. Sci.* 2022, *23*, 11782.
3. Welsh JA, *et al.*, *J Extracell Vesicles*. 2024 Feb;13(2): e12404.

ISOLATION AND CHARACTERIZATION OF GRAPE-DERIVED EXTRACELLULAR VESICLES FOR THERAPEUTIC APPLICATIONS

Tamer Shibl Ali ESMAIL[1,2], Sabino PORRO[1,2], Francesca D'ASCANIO[1,2,3], Giulia COLASANTE[1,2], Arianna AQUILINI-MUMMOLO[1,2], Domenico DE BELLIS[1,2], Pasquale SIMEONE[1,2], Angelo CICHELLI[3], Paola LANUTI[1,2] Federica FLAMMINI[3]

[1]Department of Medicine and Aging Sciences, D'Annunzio University of Chieti-Pescara; [2]Center for Advanced Studies and Technology (CAST), University "G. d'Annunzio", Chieti-Pescara; [3]Department of Innovative Technologies in Medicine & Dentistry, D'Annunzio University of Chieti-Pescara, Italy

Extracellular vesicles (EVs) are small, non-living, heterogeneous membranous structures naturally released by plant and animal cells. These vesicles carry various bioactive molecules, such as miRNA, lipids, proteins, and small biomolecules, and serve crucial roles in cell-to-cell communication and the transport of biomolecules. Grapes, along with other plant species, produce EVs that have gained considerable attention in recent years due to their potential applications in food science, nutrition, and health. Specifically, grape-derived EVs (GDEVs) have shown antioxidant, anti-inflammatory, and anticancer properties, making them promising candidates for therapeutic and nutraceutical applications. The objective of this study was to isolate grape-derived nanovesicles (GDNVs) and evaluate their potential for therapeutic applications. GDNVs were isolated from raw grape fruits using ultracentrifugation. The EVs obtained from grape juice were characterized by multiple techniques, such as nanoparticle tracking analysis, atomic force microscopy (AFM) and transmission electron microcopy (TEM). The quantification of EVs, and their phenotypes were analyzed by flow cytometry. In addition, EV sorting was carried out using the BD FACSARIA system for further analysis. The grape pomace samples were isolated by Tangential Flow Filtration. EVs isolated both by grape juice and grape pomace samples demonstrated diameters in the range of small EVs (<200nm), as demonstrated by NTA, AFM and TEM. When ultracentrifugation and tangential flow filtration isolation procedures were paralleled, we demonstrated tangential flow filtration yielded a remarkable five-fold increase in the amount of EVs compared to the ultracentrifugation. This enhanced efficiency demonstrated the potential of this approach to significantly boost the grape-derived EV production, making it a highly effective technique for isolating large quantities of EVs for further analysis and therapeutic applications. Following isolation, the GDNVs were again characterized using flow cytometry, and the storage of EVs was tested under various temperatures (2°C, -20°C, and -80°C) to examine the influence of temperature stress on vesicle morphology and concentrations. Results suggest that grape-derived EVs possess favorable properties, including their stability under varying storage conditions, making them suitable candidates for future applications in therapeutic delivery systems. This study provides an in-depth understanding of the isolation, characterization, and storage conditions of GDNVs and their potential for therapeutic applications.

CHRYSIN LOADED EXTRACELLULAR VESICLES REDUCES NEUROINFLAMMATION *IN VITRO* MODEL

Francesca Martina FILANNINO[1], Chiara PORRO[1], Maria Ida DE STEFANO[1], Federico ZOILA[1,2], Dario Domenico LOFRUMENTO[3],

Maria Antonietta PANARO[4], Rosa CALVELLO[4], Melania RUGGIERO[4]
[1]Department of Clinical and Experimental Medicine, University of Foggia; [2]Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona; [3]Department of Biological and Environmental Sciences and Technologies, Section of Human Anatomy, University of Salento, (LE); [4]Department of Biosciences, Biotechnologies and Environment, University of Bari, Italy

Neuroinflammation is a protective response of the central nervous system (CNS) to tissue damage or viral insults. Scientific studies have demonstrated that sustained inflammation can contribute to the progression of neurodegenerative diseases. This process is characterised by the activation of glial cells, including astrocytes and microglia. Extracellular vesicles are membrane-bound particles released by cells that are essential for intercellular communication. Extensive research has demonstrated the involvement of these vesicles in various physiological and pathological processes, including immune responses, angiogenesis and cell-cell communication. The transfer of proteins, lipids and nucleic acids to recipient cells is a primary function of these vesicles. Furthermore, the transfer of various factors, including cytokines and miRNAs, via extracellular vesicles can significantly modulate cell polarization and influence glial behaviour. Chrysin is a flavone, a polyphenolic compound with a 15-carbon skeleton. It is present in fruit, honey and propolis. Recent studies have reported the ability of chrysin to inhibit neuroinflammation *in vitro* and *in vivo*. This study examined the effects of vesicles derived from BV2 microglia cell cultures, stimulated with chrysin for 24 hours. These vesicles were then used to pre-incubate BV2 cells before the addition of the inflammatory stimulus, LPS. The results demonstrated that vesicles have neuroprotective effects on LPS-induced microglial activation, preserving the typical ramified morphology of microglia in a physiological state, reducing the migratory capacity, and the production of pro-inflammatory cytokines, thus reducing the progressive increase in neuroinflammation typical of neurodegenerative diseases. Further research is needed to fully understand these results and their implications. Nevertheless, these results pave the way for the future use of vesicles as carriers to provide enhanced neuroprotection *in vivo* and help prevent neurodegenerative diseases.

EXTRACELLULAR VESICLES DERIVED FROM B7-H3 CAR-T CELLS: A PROMISING STRATEGY FOR PANCREATIC CANCER TREATMENT

Rosalba FLORIO[1], Sara PAGOTTO[2,3], Davide BROCCO[3], Pasquale SIMEONE[2,4], Anna PIRO[1], Francesca D'ASCANIO[2,4,5], Serena VESCHI[1], Serena PILATO[1], Laura DE LELLIS[1], Michela BATTISTELLI[6], Rosa VISONE[2,3], Manuela IEZZI[2,7], Assunta PANDOLFI[2,3], Nicola TINARI[2,3], Paola LANUTI[2,4], Mauro DI IANNI[2,4], Alessandro CAMA[1]
[1]Department of Pharmacy, G. d'Annunzio University of Chieti-Pescara, Chieti; [2]Center for Advanced Studies and Technology (CAST), G. d'Annunzio University of Chieti-Pescara, Chieti; [3]Department of Medical, Oral and Biotechnological Sciences, G. d'Annunzio University of Chieti-Pescara, Chieti; [4]Department of Medicine and Aging Sciences, G. d'Annunzio University of Chieti-Pescara, Chieti; [5]Department of Humanities, Law and Economics, Leonardo da Vinci University, Torrevecchia Teatina (CH); [6]Department of Earth, Life and Environmental Sciences, University of Urbino Carlo Bo, Urbino; [7]Department of Neuroscience, Imaging and Clinical Sciences, G. d'Annunzio University of Chieti-Pescara, Chieti, Italy

Pancreatic cancer (PC) is associated with a poor prognosis and exhibits resistance to conventional immunotherapy. Chimeric antigen receptor (CAR)-T cells emerged as a promising approach to cell-based immunotherapy, particularly in hematological malignancies. However, their effectiveness in treating solid tumors remains limited. Extracellular vesicles (EVs) derived from CAR-T cells represent a potential advancement in CAR-T immunotherapy. In this study, we investigated the antitumor activity of EVs derived from CAR-T cells targeting B7-H3, a protein overexpressed in PC cells that demonstrated safety in clinical trials. B7-H3 CAR-T and control T cells (CTRL) were sorted and co-coltured with B7-H3-positive PC cell lines to assess the cytotoxicity of the engineered T cells. EVs from both B7-H3 CAR-T and CTRL-T cells were isolated by cell sorting using a flow cytometry method patented by our laboratory. EVs were further characterized using atomic force microscopy (AFM), western blot (WB), nanoparticle tracking analysis (NTA), flow cytometry and transmission electron microscopy (TEM). The antitumor activity of EVs derived from B7-H3 CAR-T cells was assessed by both MTT and flow cytometry killing assays. EVs derived from B7-H3 CAR-T cells exhibited a globular shape and an average size of 140 nanometers. Furthermore, B7-H3 CAR-T-derived EVs expressed typical EV markers, such as CD63 and Flotillin-1, and were negative for cytochrome C. Co-culture experiments demonstrated that B7-H3 CAR-T cells induced 36% (**$p<0.01$) killing of L3.6pl PC cells after 24 hours. Moreover, EVs derived from B7-H3 CAR-T cells displayed a time-dependent cytotoxic activity against L3.6pl cells. Specifically, the killing percentage of L3.6pl cells was 10% ($p=0.05$) and 22% (*$p<0.05$) after 24- and 48-hour treatments with 150 µg of B7-H3 CAR-T-derived EVs, respectively. In line with the killing assay results, B7-H3 CAR-T-derived EVs significantly reduced L3.6pl cell viability compared to control. Our preliminary results demonstrated promising antitumor activity of B7-H3 CAR-T-derived EVs against PC cells *in vitro*, providing a basis for further studies to assess their potential application in PC therapy.

CITRUS-DERIVED NANOVESICLES ARE A NATURAL ALLY IN ENHANCING MACROPHAGE ANTIBACTERIAL ACTIVITY

Roberta GASPARRO[1], Christian SANCHEZ-LÓPEZ[2],

Vincenza TINNIRELLO[1], Giulia DUCA[1], Mari Cruz MANZANEQUE-LÓPEZ[2], Riccardo ALESSANDRO[1], Antonio MARCILLA[2], Stefania RAIMONDO[1]

[1]Department of Biomedicine, Neurosciences and Advanced Diagnostics (Bi.N.D), Section of Biology, University of Palermo, Italy; [2]Departament de Farmàcia i Tecnologia Farmacèutica i Parasitologia, Universitat de València, Burjassot, Valencia, Spain

Plant-derived nanovesicles (PDNVs) have recently garnered significant interest within the scientific community due to their functional properties. PDNVs carry lipids, proteins, nucleic acids, and metabolites, playing roles in cross-kingdom communication and influencing mammalian cells. Citrus-derived nanovesicles, extensively studied by our research group, show promising potential with demonstrated anti-inflammatory [1] and antioxidant [2] effects. Additionally, various citrus compounds, such as Citral and polysaccharides, are known to activate immune cells, potentially enhancing bacterial recognition [3]. For the first time, in this study, we aim to explore the ability of Citrus-derived nanovesicles to enhance the action of macrophages against bacteria. Lemon (LNVs) and Tangerine (TNVs)-derived nanovesicles were isolated from the juice through Tangential flow filtration and Size Exclusion Chromatography. We characterized LNVs and TNVs, confirming their integrity and size distribution, by Transmission Electron Microscopy and Nanoparticle Tracking Analysis and we investigated the positivity for HSP70 and TET8 markers by Immunogold. Furthermore, we established a protocol for evaluating the effect of human monocytes differentiated into macrophages (THP-1 M0) against *Enterococcus faecalis* or *Escherichia coli*. The THP-1 M0 cells were first pre-treated for 24h with LNVs/TNVs and then subjected for 3h to bacteria. THP-1 M0 cells and bacteria were co-plated in LB Agar and colony-forming units (CFU) were counted. To be more specific, after evaluating LNVs and TNVs internalization in THP-1 M0 cells, we demonstrated that both types of nanovesicles enhance macrophage activity, essential for pathogen detection and elimination. We observed that the pre-treatment with nanovesicles influences bacterial growth. In particular, THP-1 M0 cells pre-treated with LNVs/TNVs significantly reduced the bacteria's CFU compared to untreated cells. We validated the result related to *Escherichia coli* also on monocytes isolated from blood samples of healthy donors and differentiated into macrophages. In conclusion, these results represent an interesting starting point for understanding how the LNVs and TNVs can modulate the activity of the macrophages and improve their response against the bacteria.

This work supported by Eurostart and FFR 2024 (University of Palermo, Italy) to Stefania Raimondo. R.G. is the recipient of a Postdoctoral fellowship funded by Fondazione Umberto Veronesi.

REFERENCES

1. Raimondo S *et al.*, Anti-inflammatory properties of lemon-derived extracellular vesicles are achieved through the inhibition of ERK/NF-κB signalling pathways. J Cell Mol Med. 2022 Aug;26(15):4195-4209. doi: 10.1111/jcmm.17404.
2. Gasparro R *et al.*, Protective effects of lemon nanovesicles: evidence of the Nrf2/HO-1 pathway contribution from *in vitro* hepatocytes and *in vivo* high-fat diet-fed rats. Biomed Pharmacother. 2024 Nov;180:117532. doi: 10.1016/j.biopha.2024.117532.
3. Oliveira HBM *et al.*, Citral modulates human monocyte responses to Staphylococcus aureus infection. Sci Rep. 2021 Nov 11;11(1):22029. doi: 10.1038/s41598-021-01536-4.

THE BEHAVIOR OF MACROPHAGES IS AFFECTED BY EXTRACELLULAR VESICLES ORIGINATING FROM MOUSE MESOANGIOBLASTS

Roberto CHIARELLI, Fabiana GERACI

Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Italy

Mouse-derived mesoangioblasts represent multipotent progenitor stem cells linked to blood vessels, which can differentiate into a range of mesodermal cell types and release membrane vesicles (EVs) into the extracellular milieu. EVs contain several molecules such as MMP2/9 and HSP70 as a transmembrane protein. This study aimed to investigate the impact of C57 EVs on murine macrophages (Raw264.7), which are essential players throughout the entire inflammatory process. In response to tissue damage, macrophages migrate to the site to perform their critical function of detecting and eliminating dead cells, debris, and foreign particles through the process of phagocytosis. We explored the effects of C57 extracellular vesicles on the immune modulation of Raw264.7 cells. Our study has established that EVs interact with Raw264.7 cells, negatively impacting their proliferation while positively affecting their migratory functions. Our study confirms that the enhanced migration process is influenced by a rise in both the expression and activity of MMP2/9. Additionally, the calculation of the *in vitro* phagocytosis index demonstrated that treatment with EV enhances the phagocytic capacity of Raw264.7 cells, a crucial factor during the initial phases of tissue repair. Through the application of neutralizing antibodies like anti-Hsp70, anti-TLR2, and anti-TLR4, it was demonstrated that C57-EVs enhance the phagocytic function of Raw264.7 cells by engaging Hsp70 and its surface receptor pathways. To determine the effect of EVs on macrophage phenotype, we assessed the levels of M1/M2 markers, including iNOS and arginase, as well as the production of nitric oxide. In the early phase following EV treatment, Raw264.7 produced both iNOS and nitric oxide, while arginase mRNA remained undetectable. These data suggest an M1 phenotype. After undergoing EV treatment and subsequent recovery, there was a significant rise in the release of anti-inflammatory cytokines, which are indicative of M2 macrophage activity. These results propose that C57-EVs might support tissue restoration through the alteration of macrophage dynamics.

UNRAVELING THE ROLE OF EXTRACELLULAR VESICLES IN MELANOMA STROMA

Gaia GIANNITTI[1], Alyssa Julia Jennifer PAGANONI[1], Sara MARCHESI[1], Riccardo GARAVAGLIA[1], Ivan PREOSTO[1], Emanuela CAROLLO[2], Patrizia SARTORI[3], Fabrizio FONTANA[1]
[1]Department of Pharmacological and Biomolecular Sciences "Rodolfo Paoletti", University of Milan, Italy; [2]Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, UK; [3]Department of Biomedical Sciences for Health, University of Milan, Italy

Melanoma is an aggressive cancer characterized by a rapid metastatic process. Thus, understanding the mechanisms underlying its progression is urgently needed to improve patient outcomes. In this regard, there is consistent evidence of a tumor-sustaining crosstalk between melanoma and subcutaneous adipose tissue; however, the role of extracellular vesicles (EVs) in this communication still needs to be clarified. We demonstrated that the EVs derived from adipocytes did not alter melanoma cell proliferation but significantly promoted tumor cell migration and invasion by determining an enrichment in mesenchymal markers, such as N-cadherin and vimentin. In particular, these changes were accompanied by the transition towards a stem-like phenotype, characterized by enhanced spherogenic ability and ABCG2 upregulation. Interestingly, this led to a reduced response to vemurafenib, with diminished apoptotic rates and decreased caspase 3 and PARP cleavage. Mechanistically, an increase in PGC-1α expression was found, resulting in higher mitochondrial mass and activity and ROS overproduction; of note, treatment of melanoma cells with XCT790 and SR-18292, two specific inhibitors of mitochondrial biogenesis, successfully counteracted the above EV-related effects, suggesting that this process could be targeted to suppress the EV-mediated interactions between subcutaneous adipocytes and melanoma. Taken together, these results highlight the crucial role played by EVs in melanoma stroma, highlighting the ability of adipocyte-derived vesicles to sustain melanoma cell aggressiveness via PGC-1α activation.

EVALUATION OF SURFACE GLYCANS OF EXTRACELLULAR VESICLES IN 2D AND 3D CULTURES

Giuseppina POPPA, Giulia DI FAZIO, Sandra D'ASCENZO, Vincenza DOLO, Ilaria GIUSTI
Department of Life, Health and Environmental Sciences, University of L'Aquila, Italy

Glycosylation represents the most common post-translational modification observed in proteins and plays an intricate role in many pathophysiological processes. Its importance is emphasized in the context of complex diseases, particularly cancer. Indeed, cancer cells exhibit a plethora of glycosylation alterations, related to the accumulation of aberrant glycan structures, that disrupt essential cellular mechanisms and alter the tumor microenvironment, thereby facilitating cancer progression and adversely affecting patient prognosis[1]. Extracellular vesicles (EVs) are heterogeneous particles released by all cells and detectable in all biofluids. EVs display distinct characteristics depending on their cellular origin; among these features, the expression of surface glycans stands out as a key point; therefore, the accumulation of aberrant glycans detectable in tumor-derived EVs could represent a source for the discovery of biomarkers associated with EVs[2,3]. In this scenario, *in vitro* preclinical research for the evaluation of EVs-associated glycans is still in its early stages, and most studies have been performed on monolayer cultures (2D); fewer studies have been conducted on 3D cultures, aimed at assessing the impact of 3D architecture on the packing of glycans into EVs. This study delves into the comparative analysis of glycan patterns of EVs derived from two human breast cancer cell lines- MDA-MB-231 and MCF-7- cultured in both conventional 2D culture and 3D culture models, recognizing the latter's potential to more accurately mimic the *in vivo* tumor microenvironment. For the 3D cultures, the two cell lines have been grown as spheroids, generated using the *hanging drop* method for the MDA-MB-231 cell line and ultra-low attachment plates for the MCF-7 one. The isolation of EVs was performed from both cell culture models by differential centrifugation; then, the analysis of EV-glycans was performed by an ELISA test lab-made, based on the use of lectins (proteins that specifically bind glycan structures). The results showed for both cell lines a different expression of some EV-glycans from 2D and 3D cultures, particularly related to galactose, fucose, and N-acetylglucosamine levels. These findings highlight the importance of tumor cellular architecture when assessing the biomolecules associated with EVs- in our case glycans- for cancer biomarkers research, leading us to carefully choose the right *in vitro* model for these studies.

REFERENCES

1. Pinho SS, Reis CA. Glycosylation in cancer: mechanisms and clinical implications. Nat Rev Cancer. 2015 Sep;15(9):540-55. doi: 10.1038/nrc3982. Epub 2015 Aug 20. PMID: 26289314.
2. Welsh *et al.* Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches. J Extracell Vesicles. 2024 Feb;13(2):e12404. doi: 10.1002/jev2.12404. Erratum in: J Extracell Vesicles. 2024 May;13(5):e12451
3. Shimoda A, Akiyoshi K. Surface Glycan Profiling of Extracellular Vesicles by Lectin Microarray and Glycoengineering for Control of Cellular Interactions. Pharm Res. 2023 Apr;40(4):795-800. doi: 10.1007/s11095-023-03511-2. Epub 2023 Apr 10. PMID: 37038008.

FIGHT AGAINST CANCER

ARTEMISININ AND ITS DERIVATIVES AS POTENTIAL ANTIDIABETIC AND ANTICANCER AGENTS TARGETING PTP1B

Dionysia AMANATIDOU[1], Ioannis TSAMESIDIS[1,2], Antonella PANTALEO[3], Ilias PESSACH[1], Phaedra ELEFTHERIOU[1]

[1]Department of Biomedical Sciences, International Hellenic University, Thessaloniki, Greece; [2]Department of Prosthodontics, School of Dentistry, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece; [3]Department of Biomedical Science, University of Sassari, Italy

Protein Tyrosine Phosphatase 1B (PTP1B) is a well-characterized enzyme extensively studied as a potential therapeutic target for diabetes mellitus. It dephosphorylates insulin and leptin receptors, thereby reducing glucose uptake in cells and leading to elevated glucose levels in circulation. Additionally, PTP1B has emerged as a promising therapeutic target in various types of cancer. It acts as a negative regulator of interleukin-13 receptor alpha 2 (IL-13Rα2), whose overexpression or hyperactivation has been associated with multiple cancer types. However, in some cases, excessive stimulation can facilitate metastasis. Furthermore, aberrant IL-13 activity has been linked to other cancer types[1]. Numerous PTP1B inhibitors have been proposed, including natural, synthetic, and semi-synthetic compounds. Structurally, terpenoids[2] and lactones[3] have been reported as effective inhibitors. In this study, we investigated the inhibitory action of artemisinin, a naturally derived compound, along with several synthetic and semi-synthetic derivatives, against PTP1B. An *in silico* analysis was conducted to explore the formation of stable complexes between potential inhibitors and PTP1B, aiming to predict potent inhibitors and elucidate possible binding modes. Subsequently, the PTP1B inhibitory action of the compounds was evaluated *in vitro*, with some demonstrating IC_{50} values in the micromolar and sub-micromolar range.

REFERENCES

1. Kołodziej-Sobczak D *et al.* Protein Tyrosine Phosphatase 1B (PTP1B): A Comprehensive Review of Its Role in Pathogenesis of Human Diseases. Int J Mol Sci. 2024.
2. Chen C *et al.* Lanostane triterpenoids from Ganoderma calidophilum exhibit potent anti-tumor activity by inhibiting PTP1B. Chem Biol Interact. 2024.
3. Ren YM *et al.* Macrocephatriolides A and B: Two Guaianolide Trimers from Ainsliaea macrocephala as PTP1B Inhibitors and Insulin Sensitizers. J Org Chem. 2021.

INTEGRATING EXPERIMENTAL AND COMPUTATIONAL APPROACHES TO QUANTIFY DRUG AND NANOCARRIER INTERACTIONS WITH PLASMA PROTEINS AND DNA

Gabriele CAVALIERI, Erik LAURINI, Domenico MARSON, Sabrina PRICL

Molecular Biology and Nanotechnology Laboratory (MolBNL@UniTs), Department of Engineering and Architecture (DEA), University of Trieste, Italy

In the fight against cancer, understanding the interaction between key plasma proteins like human serum albumin (HSA) or immunoglobulin G (IgG) and both small drugs and dendrimers is crucial for drug pharmacokinetics and the formation of the protein corona in nanomaterials, influencing the ADME properties of the drug system. This study presents a comprehensive approach that integrates experimental and computational techniques to quantify these interactions across different molecular systems. Fluorescence and UV spectroscopy, isothermal titration calorimetry (ITC), circular dichroism (CD), and molecular simulations are systematically employed to investigate binding affinities, thermodynamic parameters, and structural effects of various compounds interacting with the most important plasma proteins. The interaction between HSA or IgG, forming protein corona, with a self-assembling amphiphilic dendrimer (AD) was analyzed, revealing moderate binding driven by electrostatic interactions and hydrogen bonding. Structural analysis confirmed that HSA undergoes minimal conformational changes upon complexation, supported by molecular simulations demonstrating stable interactions at the atomistic level. These findings provide insights into how dendrimers engage with plasma proteins, a key factor in their biomedical application. In parallel, the study explored the binding of small-molecule inhibitors, particularly BRAF and MEK inhibitors used in melanoma therapy, to HSA, a protein directly involved in pharmacokinetics. The combined application of fluorescence quenching, ITC, and molecular modeling revealed moderate but distinct binding mechanisms. Encorafenib interacts with HSA primarily through enthalpic contributions, whereas Binimetinib is stabilized by entropy-driven interactions. Both inhibitors bind within the same protein binding site without significantly altering the protein's secondary structure. Similarly, the binding of Dabrafenib and Vemurafenib was examined, showing spontaneous complex formation, with vemurafenib exhibiting a slightly higher residence time. This integrative approach also extends beyond protein interactions, as demonstrated by the study of Vemurafenib binding to calf thymus DNA (ctDNA), one of its potential off-targets. Spectroscopic and calorimetric data, coupled with molecular dynamics simulations, confirmed a minor groove-binding mechanism with minimal structural perturbation of the DNA double helix. Overall, these studies highlight the importance of quantifying drug-protein interactions through a multidisciplinary methodology. By systematically applying experimental and computational techniques, it establishes a robust framework for evaluating the behavior of both nanocarriers and small-molecule drugs in biological environments, enhancing our understanding of drug distribution, stability, and efficacy.

REFERENCES

Cavalieri, G. Pharmaceutics, 2024, 16, 533.

Cavalieri, G. European Journal of Pharmaceutical Sciences, 2023, Volume 189, 106550.

Cavalieri, G. Mol. Pharmaceutics, 2022, 19, 5, 1619-1634.

Cavalieri, G. Appl. Biosci. 2024, 3(4), 468-483.

MUTANT-p53 AS A KEY PLAYER IN FERROPTOSIS RESISTANCE IN PANCREATIC DUCTAL ADENOCARCINOMA

Adriana CELESIA[1], Filippo PICCOLI[1], Raffaella PACCHIANA[1], Federica DANZI[1], Maria POLES[1], Antonino APARO[2], Maria Teresa SCUPOLI[2], Barbara CISTERNA[1], Massimo DONADELLI[1], Alessandra FIORE[1]

[1]Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona; [2]Department of Engineering for Innovation Medicine, University of Verona, Italy

Pancreatic ductal adenocarcinoma (PDAC) is the most prevalent form of pancreatic cancer. Known for its high lethality, PDAC is projected to become the second leading cause of cancer-related death in Western societies within the next decade. This underscores the urgent need for novel therapeutic targets to fight this disease. In recent years, ferroptosis —an iron-dependent cell death— has gained significant attention for its potential therapeutic value in oncology. Notably, the tumor suppressor protein p53 has been found to be closely linked to ferroptosis. In pancreatic ductal adenocarcinoma (PDAC), approximately 70% of patients carry missense mutations in the TP53 gene, resulting in mutant p53 (mutp53) isoforms with novel gain-of-function (GOF) properties. In this project, we initially focused on the human PDAC cell line Panc-1, which carries the p53-GOF mutation R273H. Using CRISPR-Cas9, we generated mutp53 Knock-Out (KO) cells and, for the first time, demonstrated that PDAC cells harboring mutant p53 are more resistant to the effects of various ferroptosis inducers compared to their p53-KO counterparts. This finding reveals a novel role for mutant p53 in promoting anti-ferroptotic mechanisms. To corroborate these data, we transfected the p53-null human PDAC cell line AsPC-1 with six different mutp53 isoforms commonly found in PDAC patients. Our results showed that AsPC-1 cells transfected with various mutp53 isoforms exhibited greater resistance to three distinct ferroptosis inducers compared to mock-transfected cells. This suggests that mutp53 plays a key role in ferroptosis resistance and indicates that this mechanism is not specific to any particular mutp53 isoform. We also found that ferroptosis onset is associated with a higher accumulation of reactive oxygen species (ROS) and lipid peroxidation in p53-KO cells compared to parental Panc-1 cells. In addition, Transmission Electron Microscopy (TEM) analysis revealed that mitochondria, Rough Endoplasmic Reticulum (RER), and Golgi apparatus are more severely affected in p53-KO cells following ferroptosis induction. Further analyses showed that ferroptosis induction leads to a disruption of mitochondrial metabolism, particularly in p53-KO cells, indicating a heightened sensitivity to ferroptosis in the absence of mutp53. To explore the anti-ferroptosis mechanism induced by mutp53, we performed an RNA-seq analysis following treatment with various ferroptosis inducers. This approach enabled us to identify a set of genes modulated by ferroptosis and regulated by mutp53, which could serve as potential novel molecular targets. Notably, these genes were upregulated in Panc-1 cells but not in the p53-KO counterpart, highlighting their relevance in the context of mutp53-driven ferroptosis resistance. Taken together, these data strongly support the hypothesis that mutant p53 has a pivotal role in ferroptosis regulation. This provides a foundation for identifying novel networks of ferroptosis regulators and biomarkers that could predict PDAC patients' responses to therapy, ultimately paving the way for more targeted and effective treatments.

LOW PHOSPHATIDYLSERINE+ LEVELS AND PERCENTAGES OF CELLS WITHIN THE CD34+/CD45dim/CD117(c-kit)+ SUBPOPULATION ARE ASSOCIATED WITH POOR OUTCOMES IN METASTATIC COLORECTAL CANCER

Giulia COLASANTE[1,2], Davide BROCCO[1,2,3], Francesca D'ASCANIO[1,2,4], Arianna AQUILINI-MUMMOLO[1,2], Sabino PORRO[1,2], Tamer Shibl Ali ESMAIL[1,2], Ayesha YOUNAS[1,2], Domenico DE BELLIS[1,2], Rosalba FLORIO[5], Pasquale SIMEONE[1,2], Alessandro CAMA[5], Paola LANUTI[1,2]

[1]Department of Medicine and Aging Sciences, University "G. d'Annunzio" of Chieti-Pescara, Chieti; [2]Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University of Chieti-Pescara, Chieti; [3]Department of Medical, Oral & Biotechnological Sciences, University "G. d'Annunzio", Chieti; [4]Department of Humanities, Law and Economics, "Leonardo da Vinci" University, Torrevecchia Teatina (CH); [5]Department of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy

Colorectal cancer (CRC) is one of the leading causes of cancer-related mortality worldwide, with metastatic colorectal cancer (mCRC) posing a significant therapeutic challenge. Antiangiogenic therapies have improved outcomes in mCRC. However, reliable biomarkers are needed to optimize treatment strategies. This study aimed to investigate the prognostic and predictive role of circulating endothelial progenitor cells (CEPCs) and their subsets in mCRC patients undergoing systemic therapy. Using an optimized flow cytometry (FC) protocol, we analyzed the blood of 40 mCRC patients, distinguishing endothelial cell populations based on CD34+/CD45dim/CD117+ expression and phosphatidylserine exposure (Annexin V staining). Our results highlighted that a high percentage of Annexin V-negative cells within the CD34+/CD45dim/CD117+ subset correlates with worse overall survival (log-rank $p = 0.03$) and reduced response to systemic treatments ($p = 0.015$). Furthermore, elevated levels of CD34+/CD45dim/CD117+/Annexin V- cells were associated with a higher number of metastatic sites ($p = 0.03$). These findings suggest that this specific blood progenitor cell subset may serve as a potential biomarker for disease progression and therapeutic response in mCRC patients. Further studies on larger cohorts are warranted to validate these observations and explore their clinical implications.

HALOPERIDOL DRUG REPURPOSING AS ANTICANCER AGENT ACTIVATES FERROPTOSIS AND HEME-OXYGENASE-1

Valeria CONSOLI[1], Valeria SORRENTI[1], Salvatore SACCONE[2], Concetta FEDERICO[2], Francesca BRUNO[3], Domenico Andrea CRISTALDI[4], Valeria PITTALÀ[1], Sebastiano INTAGLIATA[1], Luca VANELLA[1]

[1]Department of Drug and Health Sciences, University of Catania; [2]Department of Biological, Geological and Environmental Sciences, University of Catania; [3]Department of Medicine and Surgery, University of Enna "Kore", Enna; [4]Cell Dynamics isrl, Bologna, Italy

Breast cancer (BC) remains the leading cause of cancer-related mortality among women, with disease progression often attributed to the development of resistance to apoptosis following conventional drug treatments. In response, recent research has focused on identifying novel therapeutic strategies capable of overcoming this resistance. The discovery of ferroptosis and its underlying molecular mechanisms has opened new avenues for drug development, highlighting ferroptosis-inducing compounds as potential anticancer agents. However, despite ongoing efforts, only a limited number of synthetic ferroptosis inducers have been identified, and their application remains confined to preclinical studies. This underscores the importance of investigating already-approved drugs with potential ferroptotic activity. In this context, haloperidol (HALO), a widely used first-generation antipsychotic, emerges as a promising candidate. This study aimed to assess HALO's ability to induce ferroptosis in two distinct BC cell lines (MDA-MB-231 and MCF-7) by evaluating ferroptotic markers through western blot analysis, measuring ROS and lipid hydroperoxide levels, and assessing mitochondrial dysfunction and glutathione depletion. Preliminary investigations were also conducted on 3D cultures. Results indicated that HALO selectively triggered ferroptosis in MCF-7 cells, as evidenced by increased ROS and lipid hydroperoxide levels accompanied by a significant reduction in intracellular glutathione. Notably, the analysis of NCOA4, ferritin, and iron levels suggested a possible involvement of ferritinophagic processes in HALO-induced ferroptosis. Furthermore, HALO significantly upregulated heme oxygenase 1 (HO-1) expression and enzymatic activity, which was linked to an intriguing subcellular localization pattern observed via confocal microscopy. Overall, these findings suggest that haloperidol exerts pro-ferroptotic effects by modulating multiple iron-related proteins, including the HO system, underscoring its potential repurposing as an anticancer agent.

DECIPHERING THE ROLE OF LIPID DROPLETS IN MELANOMA STEM CELLS

Gaia GIANNITTI, Alyssa Julia Jennifer PAGANONI, Sara MARCHESI, Riccardo GARAVAGLIA, Ivan PREOSTO, Fabrizio FONTANA

Department of Pharmacological and Biomolecular Sciences "Rodolfo Paoletti", University of Milan, Italy

Little is known about the metabolic regulation of cancer stem cells (CSCs) in melanoma. Here, we used A375 and WM115 cell lines to dissect the role of lipid droplets (LDs) in conferring CSC traits. Notably, we observed that A375 and WM115 melanospheres, known to be enriched in ABCG2$^+$ CSCs, showed higher LD content compared with their adherent counterpart. In particular, they displayed increased protein levels of DGAT1, an enzyme responsible for LD assembly. Interestingly, DGAT1 silencing resulted in the suppression of CSC features, including clonogenic ability, migration, spheroid formation, ABCG2 enrichment and vemurafenib tolerance. Similarly, A922500, a DGAT1 pharmacological inhibitor, was able not only to reduce melanoma tumorigenicity and invasion but also to block melanosphere growth and propagation and ABCG2$^+$ cell proliferation. More importantly, this drug was found to synergize with vemurafenib, sensitizing melanoma SCs to targeted therapies. In conclusion, DGAT1-driven LD formation is associated with a stem-like phenotype in melanoma, and therapeutically targeting the LD-enriched CSC subpopulation might overcome tumor progression.

ZIKA VIRUS ONCOLYTIC ACTIVITY FOR CANCER THERAPY: POTENTIAL APPLICATIONS FOR GLIOBLASTOMA TREATMENT AND PERSPECTIVES FOR SOLID TUMORS

Gioacchin IANNOLO[1], Martina Maria COCCO[1], Alessia ZIMONE[1], Simona DI STEFANI[1], Claudia CARCIONE[2], Rosaria TINNIRELLO[1], Cinzia Maria CHINNICI[1,2], Vitale MICELI[1], Pier Giulio CONALDI[1], Massimo PINZANI[1]

[1]Department of Research, IRCCS ISMETT (Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione), Palermo; [2]Fondazione Ri.MED, Palermo, Italy

The recent discovery that Zika virus (ZIKV) has an oncolytic action against GBM has brought hope for the development of new therapeutic approaches against Glioblastoma (GBM), the most aggressive among the neurological tumors. ZIKV is an arbovirus belonging to the Flaviviridae family, and its infection during development has been associated with central nervous system (CNS) malformations, including microcephaly, through the targeting of neural stem/progenitor cells (NSCs/NPCs)[1]. The peculiar features of this virus offer the opportunity to use an agent already tested *in vivo* through natural transmission, with minimal effects on adults where ZIKV infection is typically asymptomatic. The activity of ZIKV in Glioblastoma tumors has been widely tested through *in vitro* an *in vivo* models[1]. Moreover, it has been shown that ZIKV enters NSCs through the neural cell adhesion molecule (NCAM1) receptor, suggesting that its specific neurotropism may be related to the expression of this receptor. Given ZIKV neural specificity we sought to evaluate whether its oncolytic effect could be effectively used as treatment for neuroendocrine tumors (NETs)[2]. We found that ZIKV exerts its oncolytic activity by specifically infecting NET cells, leading to growth inhibition and cell death. We also assessed whether

the oncolytic action could be extended to pancreatic tumors different from neuroendocrine tumors (NETs). However, as expected, the viral specificity is limited to NETs and is not applicable to adenocarcinoma tumors, indicating a narrow spectrum of action for this virus. These findings support the potential use of ZIKV in therapeutic approaches not only in glioblastoma, but also against other tumors, such as neuroendocrine pancreatic tumors. ZIKV could well represent a unique tool for tumor targeting, nevertheless, at present, its potential role in cancer clinical trials or compassionate studies remains unexplored.

REFERENCES

1 R. Tinnirello, C.M. Chinnici, V. Miceli, R. Busa, M. Bulati, A. Gallo, G. Zito, P. G. Conaldi, G. Iannolo, *Int J Mol Sci* 2023, *24*, 10.3390/ijms241713550.

2 M.M. Cocco, C. Carcione, V. Miceli, R. Tinnirello, C.M. Chinnici, C. Carbone, G. Zito, P. G. Conaldi, G. Iannolo, *Int J Mol Sci* 2023, *24*, 10.3390/ijms242417271.

INVESTIGATION OF THE INTERACTION BETWEEN HUMAN SERUM ALBUMIN AND A SELF-ASSEMBLING NANOCARRIER WITH GALLIUM C18 - NOTA FOR PET IMAGING USING SPECTROSCOPIC AND CALORIMETRIC METHODS

Anna Laura LA MONACA, Gabriele CAVALIERI, Erik LAURINI, Sabrina PRICL
Molecular Biology and Nanotechnology Laboratory (MolBNL@UniTs), Department of Engineerig and Architecture (DEA), University of Trieste, Italy

Medical imaging plays a fundamental role in the diagnosis and treatment of cancer. However, challenges related to sensitivity and specificity remain significant. Nanotechnology-based imaging agents represent a promising approach to overcoming these limitations, leveraging the Enhanced Permeation and Retention (EPR) effect to enable selective tumour accumulation and enhance imaging resolution. This study investigates an innovative nanosystem designed for positron emission tomography (PET) imaging, based on a self-assembling amphiphilic dendrimer functionalized with Gallium-68. This dendrimer spontaneously forms uniform nanomicelles that preferentially accumulate in tumours, significantly improving PET signal sensitivity and specificity. The research primarily focuses on characterizing the behaviour of this system in the bloodstream, with particular attention to its interaction with human serum albumin (HSA), the most prevalent plasma protein. The study explores the formation of the protein corona (PC) on the nanocarrier's surface, a phenomenon that can significantly alter its physicochemical properties and interactions with biological systems. A comprehensive analysis was conducted using spectroscopic and calorimetric techniques, including Circular Dichroism (CD), UV-Vis Spectroscopy, Fluorescence Spectroscopy, and Isothermal Titration Calorimetry (ITC), to elucidate the interaction between the nanocarrier and HSA and to characterise the resulting PC. The findings indicate a substantial interaction between the dendrimeric system and HSA, leading to the formation of a stable PC. These results provide valuable insights into the pharmacokinetics of the nanocarrier and its potential as a PET imaging agent. A thorough understanding of these interactions is crucial for optimizing nanomedicine-based imaging systems and improving targeted cancer diagnostics.

PROTEOMIC SCREENING IDENTIFIES PDPN AS A DRIVER OF COLON ADENOCARCINOMA CELL GROWTH

Davide LA PADULA[1], Caterina GABRIELE[2], Marco GASPARI[2], Vittoria RAGO[3], Giovanni CUDA[2], Elvira PARROTTA[4], Michele AMMENDOLA[2*], Vittorio ABBONANTE[1*], Silvia DI AGOSTINO[1*]
[1]Department of Health Sciences, Magna Graecia University, Catanzaro; [2]Department of Experimental and Clinical Medicine, Magna Graecia University, Catanzaro; [3]Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Rende (CS); [4]Department of Medical and Surgical Sciences, Magna Graecia University, Catanzaro, Italy
[*]Equal contribution.

Colorectal cancer (CRC), the second most deadly cancer, arises from the abnormal proliferation of glandular epithelial cells lining the colon or rectum. CRC is a highly heterogeneous and molecularly complex disease. One commonly used classification system for CRC is the Consensus Molecular Subtypes (CMS), which categorizes tumors based on gene expression patterns. Through CMS classification, CRC patients can be divided into four molecular subtypes. The mesenchymal CMS4 subtype, characterized by high expression of genes associated with epithelial-to-mesenchymal transition (EMT), transforming growth factor beta (TGF-β) signaling, and extracellular matrix (ECM) remodeling, is linked to worse overall survival and relapse-free survival. The tumor microenvironment (TME) plays a key role in CRC tumorigenesis, progression, and therapeutic response. Among the most abundant stromal cells in the TME are cancer-associated fibroblasts (CAFs), which promote neoplastic progression by secreting cytokines, growth factors, and ECM proteins that enhance tumor cell proliferation, migration, and invasion. Podoplanin (PDPN), a small cell surface glycoprotein, has recently gained attention as an emerging CAF marker in various tumor types. PDPN regulates cell proliferation, contractility, migration, epithelial–mesenchymal transition, and ECM remodeling through interactions with proteins within the same or adjacent cells. In most cancers, high PDPN expression in CAFs is associated with poor prognosis, lymph node metastasis, and shorter overall survival. In this study, we performed mass spectrometry-based proteomics (LC-ESI-MS/MS) to assess alterations in signaling pathways and protein abundance in six patient-matched tumor-distant normal tissues and primary CRCs. Our analysis identified 7,321 proteins, of which 89 (41 upregulated and 48 downregulated) showed significant changes in primary carcinomas compared to tumor-distant normal tissues. Through bioinformatic analysis, we analyzed the label-free proteomic results to predict biochemical pathways and functional biological processes

associated with differentially expressed proteins. Upregulated proteins in CRC were involved in pathways such as EMT, TGF-β1 signaling, innate immune response, inflammation, and ECM organization. Our proteomic analysis identified PDPN as one of the most upregulated proteins in primary carcinoma compared to tumor-distant normal tissue, with a >3-fold median increase. RT-qPCR and western blot analyses of PDPN gene and protein expression in matched tumor-distant normal tissues and primary carcinomas confirmed the proteomic data. Immunohistochemical analysis of CRC patient tissues revealed that CAFs in the tumor stroma are the primary source of PDPN. Interrogation of public datasets showed that PDPN expression is significantly higher in CMS4 CRCs than in other subtypes and positively correlates with tumor size. Furthermore, using direct co-culture systems, we demonstrated that reducing PDPN expression via siRNA in mesenchymal stromal cells diminishes their capacity to support colorectal adenocarcinoma cell growth.

EXPLORING THE LINK BETWEEN PDE5A AND HYPOXIA IN HEPATOCARCINOMA CELL LINES

Lara LIZZI, Maria Giovanna ARMILLOTTA, Martina DANIEL, Mara MASSIMI
Department of Life, Health and Environmental Sciences, University of L'Aquila, Italy

Hypoxia is a hallmark of solid tumours and plays a crucial role in tumour progression by stabilizing hypoxia-inducible factors (HIFs), which orchestrate metabolic and transcriptional adaptations that promote survival, angiogenesis, and invasiveness. Among the genes influenced by hypoxia, phosphodiesterase-5 (PDE5), a key regulator of intracellular cyclic GMP (cGMP) levels, is emerging as a potential player in cancer biology. Under physiological conditions, PDE5A expression in the liver is restricted to the centrilobular zone (zone 3), a naturally hypoxic region surrounding the central vein, suggesting a functional link between hypoxia and PDE5A regulation. However, in hepatocellular carcinoma (HCC), PDE5A expression is markedly upregulated, losing its zonal specificity and becoming widespread throughout cancerous tissue. Previous studies in yeast models using transfected mammalian PDE5 isoforms have demonstrated distinct subcellular localizations: PDE5A1 and PDE5A3 predominantly localize in the nucleus and cytoplasm, where they appear to be associated with a shift toward fermentative metabolism, whereas PDE5A2 is preferentially found in mitochondria, where it seems to influence cyclic nucleotide balance and redox homeostasis. These findings suggest that distinct PDE5 isoforms may actively contribute to metabolic adaptation by modulating cellular energy metabolism and redox balance, a mechanism that could be particularly relevant under the hypoxic conditions commonly encountered in tumours. Based on these observations, we analysed PDE5 activity and protein expression under hypoxic conditions (1% O_2) in three different HCC cell lines (HepaRG, HepG2, and Huh7). Hypoxia was confirmed via lactate dehydrogenase (LDH) activity and HIF-1α protein induction. PDE5 activity was determined by measuring the difference between basal activity and that inhibited by sildenafil. Activity and protein levels varied among the HCC cell lines of different origins, with the most aggressive cells exhibiting the highest PDE5A activity and expression. To further explore the relationship between PDE5A and hypoxia, we also analysed its expression in HepG2 and Huh7 cells under simulated hypoxic conditions (using $CoCl_2$ and deferoxamine mesylate) and oxidative stress induced by exogenous hydrogen peroxide (H_2O_2). Our results indicate that both real and simulated hypoxia significantly induce PDE5A expression. Moreover, oxidative stress induced by mitochondrial ROS enhances PDE5A levels, suggesting a dual regulatory mechanism in which HIF activation and oxidative stress cooperate to modulate PDE5A expression. These findings, together with previous evidence of isoform-specific subcellular localization, support the hypothesis that PDE5A contributes to metabolic adaptation in tumour hypoxia, potentially linking cyclic nucleotide signalling to redox homeostasis and metabolic reprogramming in HCC. However, further investigations are warranted to elucidate the precise molecular mechanisms underlying the interplay between PDE5A, hypoxia, and tumour metabolism, with the goal of identifying novel therapeutic targets in HCC.

PRIMARY TUMOR CELLS AND MICROVASCULAR FRAGMENT INTERACTIONS IN 3D MODELS OF CANCER ANGIOGENESIS

Gabriele LO BUGLIO[1,3], Francesco LA MONICA[1], Simona CAMPORA[1], Selene SAMMATARO[1], Giulio GHERSI[1,4], Alessandra LO CICERO[1]
[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Italy; [2]Department of Engineering, University of Palermo, Italy; [3]Department of Pharmacy, University of Copenhagen, Universitetsparken 2, Copenhagen, Denmark; [4]Abiel srl, Palermo, Italy

Angiogenesis plays a pivotal role in cancer progression, influencing tumor growth, invasion, and metastasis. To investigate the complex interactions within the tumor mass and the blood vessels, we developed a three-dimensional (3D) tumor spheroid model by combining primary tumor cells (PTCs) and microvascular fragments (MVFs), respectively extracted from rat tumor mass and adipose tissue. The presence of MVFs, including cellular compounds like endothelial cells and pericytes, better mimics the vascular components of the tumor microenvironment (TME). Our study aimed to investigate the crosstalk between MVFs and PTCs within 3D spheroids, analyzing morphology, angiogenesis effects, and matrix metalloproteinases (MMPs) activity. Spheroids with MVFs and PTCs present a well-organized 3D structure with an enhanced angiogenic process, as suggested by the typical sprouting and branching of new blood vessels, along with enhanced MMP9 activity. In contrast, PTC spheroids without MVFs did not exhibit significant sprouting or vessel formation, highlighting the importance of incorporating vascular components to mimic tumor angiogenesis. Moreover, conditioned medium from PTCs stimulated increased sprouting in MVF spheroids, suggesting that tumor cells actively influence the angiogenic process. These findings demonstrate a promising *in vitro* model for studying the complex dynamics

of tumor angiogenesis, providing valuable insights into the crosstalk between tumor cells and the vasculature within the TME. This model holds potential for investigating therapeutic strategies targeting tumor angiogenesis and enhancing drug delivery to solid tumors.

ACKNOWLEDGMENTS. The work was carried out in the frame of the "SiciliAn MicronanOTecH Research And Innovation CEnter "SAMOTHRACE" (MUR, PNRR-M4C2, ECS_00000022), spoke 3 - Università degli Studi di Palermo "S2-COMMs - Micro and Nanotechnologies for Smart & Sustainable Communities".

AN APPROACH AGAINST CANCER: TRANSLATIONAL READTHROUGH INDUCING DRUGS (TRIDS) FOR RESTORING P53 EXPRESSION IN STOP MUTATED CELLS

Michele MENDITTO, Davide RICCI, Elisa CIULLA, Ivana PIBIRI, Andrea PACE, Laura LENTINI
Università degli Studi di Palermo, STEBICEF, Palermo, Italy

Stop mutations are gene mutations characterized by the substitution of a single nucleotide in the coding sequence of a gene, which causes the onset of a premature stop codon (PTC) within the reading frame of the mRNA, resulting in the formation of a truncated and non-functional protein. This type of mutation accounts for approximately 11% of genetic diseases, including conditions such as Cystic Fibrosis, Duchenne Muscular Dystrophy, Choroideremia, Schwachman-Diamond syndrome, and certain types of hereditary cancers involving mutations in the TP53 gene. About 10% of TP53 mutations are stop mutations [1, 2]. TP53 encodes a protein made up of 393 amino acid residues called p53, which acts mainly as a transcription factor, regulating numerous pathways such as the cell cycle arrest, DNA damage repair, apoptosis, autophagy, and metabolism when cells are under certain stress conditions. TP53 mutations create a favorable environment for tumor formation, and mutant p53 may exhibit loss of function, dominant-negative repression, or gain of oncogenic function, contributing to tumor stability and progression [2]. Today there is no therapy for the pathologies caused by this type of mutation, but an approach that has proven to be particularly effective is represented by molecules with readthrough activity (TRIDs; Translational Readthrough Inducing Drugs) which intervene on the ribosome allowing the overcoming of the PTC and the restoration of the synthesis and subsequent functionality of the protein [3]. In this work, we investigate the effects of TRID molecules with readthrough activity on the TP53 gene in tumor cells, which harbors the PTC R213X, the most common TP53 stop mutation, that generates a truncated and non-functional p53. We analyzed the restoration of p53 protein expression before and after induction of DNA damage by Western blot, its nuclear localization with fluorescence microscopy, the mRNA expression of p53, and its targets p21 and GADD45 to evaluate the functionality of the protein by Real-Time RT PCR. After 24 hours of treatment with TRIDs, we observed a partial nuclear localization of p53, an increase in mRNA expression of its targets and a restoration of protein expression after the induction of DNA damage. These results represent a promising path for developing targeted cancer therapies against stop mutations, a new approach to impede tumor proliferation, and a solid foundation for the formulation of novel personalized therapy modalities not only against cancer.

REFERENCES

1. Martins-Dias P. Nonsense suppression therapies in human genetic diseases. Cell Mol Life Sci. 2021 May. doi: 10.1007/s00018-021-03809-7.
2. Wang H. Targeting p53 pathways: mechanisms, structures, and advances in therapy. Signal Transduct Target Ther. 2023 Mar 1. doi: 10.1038/s41392-023-01347-1.
3. Fiduccia I. Promoting readthrough of nonsense mutations in CF mouse model: Biodistribution and efficacy of NV848 in rescuing CFTR protein expression. Mol Ther. 2024 Dec 4. doi: 10.1016/j.ymthe.2024.10.028.

NEURON-TUMOR CROSSTALK *IN VITRO*: INVESTIGATING CO-CULTURE SYSTEMS AND MORPHOLOGICAL CHARACTERIZATION

Ersilia NICORVO, Alessia FERRARO, Marina BOIDO, Ferdinando DI CUNTO, Alessandro VERCELLI
Department of Neuroscience "Rita Levi Montalcini", Neuroscience Institute Cavalieri Ottolenghi, University of Turin, Italy

The tumor microenvironment (TME) is a highly intricate and dynamic network of cellular and non-cellular elements that envelop and interact with cancer cells, and can influence tumor development and metastasis formation. Notably, neuron-tumor interactions are increasingly recognized as key drivers of cancer progression and aggressiveness, particularly in tumors affecting the nervous system. Emerging evidence suggests that these interactions involve reciprocal communication between neurons and cancer cells. To investigate the underlying mechanisms of neuron-tumor crosstalk, we employed various *in vitro* models to establish and optimize innovative co-culture systems. We utilized both primary cortical neurons from C57BL/6 mice and glutamatergic neurons derived from human induced pluripotent stem cells (hiPSCs), alongside the U87 glioblastoma cell line. This allowed us to evaluate the reciprocal influences between neurons and U87 cells, by using both conditioned media (CM) and transwell co-culture systems, in order to explore the effects of secreted factors on neuron-tumor dynamics. A primary focus of our research was on evaluating morphological changes in neurons using the Incucyte live-cell imaging system, which enabled real-time monitoring of neurite length and cell mortality. Quantitative analysis revealed a significant reduction in neurite length following exposure to CM: primary cortical neurons treated with CM exhibited a 36% decrease in neurite length compared to control ($p=0.0055$). In addition, CM treatments resulted in a marked reduction in neuronal cell number compared to control (33.4 %; $p=0.0024$) and in a significant increase in cytotoxicity (196,2%; $p=0.0116$) after 48 hours of CM exposure. These findings suggest that glioblastoma-secreted factors can strongly affect neuronal morphology, increase cytotoxicity and reduce neuronal survival, highlight-

ing potential neurotoxic effects. In parallel, preliminary Western Blot analyses revealed a reduction in tubulin and synapsin protein levels, suggesting CM-induced structural and functional alterations in neurons. Specifically, tubulin levels in neurons treated with CM exhibited a 60% decrease, indicating a progressive loss of neuronal integrity over time. Similarly, synapsin levels showed a substantial 80% reduction, further supporting the observation of synaptic dysfunctions and neuronal degradation following CM exposure. We are currently optimizing the experimental setup to improve co-culture conditions and extend these first findings also to human-derived neurons. In this regard, we aim to implement more advanced and physiologically relevant co-culture systems, such as microfluidic devices, which allow for precise spatial and temporal control of cellular interactions. By integrating both direct and indirect co-culture systems, we aim to capture a comprehensive view of the interactions occurring at the neuron-tumor interface and identify new potential therapeutic targets.

EXPLORING THE COMBINED EFFECTS OF CAFFEIC ACID AND PARTHENOLIDE IN TRIPLE-NEGATIVE BREAST CANCER: INVESTIGATING POTENTIAL CORRELATIONS WITH LIPID DROPLETS

Roberta OLIVERI[1], Marianna LAURICELLA[1], Diana DI LIBERTO[1], Antonella D'ANNEO[2], Anna DE BLASIO[2], Michela GIULIANO[2], Chiara OCCHIPINTI[2], Marzia FRANZÒ[1], Antonietta NOTARO[2], Federica AFFRANCHI[2], Giovanni PRATELLI[1], Daniela CARLISI[1]

[1]Department of Biomedicine, Neurosciences and Advanced Diagnostics (BiND), Institute of Biochemistry, University of Palermo; [2]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), Laboratory of Biochemistry, University of Palermo, Italy

Lipid droplets (LDs) are pivotal organelles derived from the endoplasmic reticulum (ER), crucial for lipid storage and metabolism regulation. Regulated by perilipin (PLIN) proteins, LDs dynamics profoundly influence cellular homeostasis and disease pathogenesis. In cancer, they promote tumor growth, metastasis, and chemoresistance. [1] Triple-negative breast cancer (TNBC) accounts for approximately 10-15% of diagnosed breast cancers and is characterized by high heterogeneity, aggressiveness, and poor prognosis [1]. Increasing evidence suggests that a high lipid droplet (LD) content is linked to enhanced TNBC malignancy [2]. Our study on MDA-MB-231 TNBC cells reveals that the combined treatment with the polyphenol caffeic acid (CA) [3] and the sesquiterpene lactone parthenolide (PN) [4] synergistically reduces cell viability. Oil-red O staining demonstrates that this cytotoxic effect is accompanied by a significant accumulation of LDs and Perilipin-1 (PLIN-1) compared to untreated cells. Mechanistically, CA/PN co-treatment promotes Bax oligomerization while downregulating the anti-apoptotic proteins Bcl-xL and Bcl-2. Additionally, the combined treatment disrupts mitochondrial function and cellular energy balance. Notably, the cytotoxic effects of CA/PN are partially reversed by Bafilomycin A, an autophagy inhibitor, pointing toward the involvement of lipophagy, a selective form of autophagy. Further investigations are required to elucidate the molecular mechanisms underlying the synergy between CA and PN, as well as the precise role of lipophagy in this process.

REFERENCES

1. Butler *et al.*, Adv. Drug Deliv. (2020), 159:245-293.
2. Pizato, N. *et al.* Nutrients. (2019), 11(6):1199.
3. Shyu, P.Jr. *et al.*. Biosci Rep. (2018), 38(5): BSR20180764.
4. Lim SC, *et al.* Anticancer Res. 2024 Jun;44(6):2587-2595.
5. Carlisi D, *et al.* Biomedicines. 2022;10(2):514.

NEXT-GENERATION CANCER IMMUNOTHERAPY: ANTI-LEUKEMIC ACTIVITY THROUGH EVS DERIVED FROM CD19 CAR-T CELLS

Pasquale SIMEONE[1,2], Domenico DE BELLIS[1,2], Francesca D'ASCANIO[1,2], Giulia COLASANTE[1,2], Arianna AQUILINI-MUMMOLO[1,2], Sabino PORRO[1,2], Francesco GUARDALUPI[1,2], Giulia CORRADI[1,2], Tamer Shibl Ali ESMAIL[1,2], Rosalba FLORIO[3], Serena VESCHI[3], Anna PIRO[3], Alice DI SEBASTIANO[2,4], Maria Concetta CUFARO[2,5], Damiana PIERAGOSTINO[2,5], Piero DEL BOCCIO[2,3], Alessandro CAMA[3], Antonella FONTANA[3], Serena PILATO[3], Mauro DI IANNI[1,2], Paola LANUTI[1,2]

[1]Department of Medicine and Aging Sciences, University "G. d'Annunzio" of Chieti-Pescara, Chieti; [2]Center for Advanced Studies and Technology (CAST), "G. d'Annunzio" University of Chieti-Pescara, Chieti; [3]Department of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara, Chieti; [4]Department of Medical, Oral and Biotechnological Sciences, G. d'Annunzio University, Chieti; [5]Department of Innovative Technologies in Medicine and Dentistry, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy

Chimeric antigen receptor (CAR)-T cell therapy has revolutionized the treatment of relapsed or refractory haematological malignancies, yielding remarkable clinical responses. Nevertheless, its broader application is hindered by severe adverse effects, including cytokine release syndrome (CRS) and neurotoxicity. Extracellular vesicles (EVs) have recently emerged as a promising alternative, given their capacity to transport functional biomolecules (*e.g.*, proteins, mRNA, and microRNAs) while potentially reducing cell-mediated toxicity. Here, we investigated the use of CAR-derived EVs as circulating biomarkers and novel therapeutics in a cohort of 22 patients who received CD19.CAR-T cells. Circulating CAR-T cells and their EVs were monitored by flow cytometry and ddPCR. CD19.CAR-T cells were isolated from both pre-infusion bags and patient peripheral blood samples by fluorescence-activated cell sorting, then expanded *in vitro*. The conditioned medium was concentrated using tangential flow filtration (TFF), and EVs were subsequently isolated using a new flow cytometry-based method (patent EP3546948A1). Following the latest MISEV guidelines, comprehensive characterization *via* transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), and atomic force microscopy (AFM) confirmed that

CD19.CAR+ EVs ranged from 50 to 150 nm and had a typical spherical morphology. Proteomic analysis identified canonical EV markers (CD63, Flotillin-1) alongside cytotoxic effectors (perforin, granzyme B), while contaminants (*e.g.*, Cytochrome c) were absent. Notably, CD19.CAR+ EVs remained detectable in patient peripheral blood for up to two years post-infusion, correlating with the long-term persistence of their parental CAR-T cells. Functionally, these EVs exhibited significant cytotoxicity against CD19-positive cell lines (Raji and SUP-B15) *in vitro* compared to controls ($p < 0.05$). Overall, our findings suggest that CD19.CAR+ EVs serve as dynamic biomarkers reflecting CAR-T cell activity and possess intrinsic anti-leukemic properties. These results highlight the potential of CAR-derived EVs to function as standalone therapeutic products, expanding the scope of cell-based immunotherapies.

POLYPHENOLS AS A POTENTIAL STRATEGY IN OVERCOMING THERAPY RESISTANCE IN ANAPLASTIC THYROID CANCER THROUGH THE SOX2-SOX17 MOLECULAR SWITCH

Fabiola VAGLICA[1], Laura TOMASELLO[1], Mattia BIONDO[2], Giuseppe SIRAGUSA[2], Giorgio ARNALDI[1], Giuseppe PIZZOLANTI[1,3]
[1]Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE), University of Palermo; [2]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo; [3]ATeN Center, Palermo, Italy

Anaplastic thyroid cancer (ATC) is a rare and undifferentiated endocrine tumor, without reliable therapies and poor prognosis. Due to severe early metastasis and the rapid fatal course, surgery is rarely performed, while radio and chemotherapy exhibit low efficacy. Therapy resistance is largely attributed to a loss of thyroid cell function due to the downregulation of thyroid terminal differentiation genes, among them sodium-iodide symporter (NIS), transcription termination factor (TTF-1), and thyroid peroxidase (TPO).[1] Disrupting the circuit that sustains the acquisition of a dedifferentiated state in ATC, remains the main challenge in order to resensitize cells to conventional or novel therapies. We previously identified a cancer stem cell (CSC) subpopulation derived from ATC, characterized by high expression of several stem cell markers, such as SOX2, OCT4, and NANOG. We proposed a potential upstream role of SOX2 in regulating cell proliferation and tumor progression.[2] In contrast, SOX17 displayed anti-tumoral properties in papillary thyroid cancer (PTC). Its low expression positively correlates with increased PTC cell migration and invasion. However, the impact of the SOX family on thyroid cancer is unclear. Our previous *in vitro* studies shown that resveratrol (RSV), a natural polyphenol, could affect the stem cell features, promoting the differentiation towards the epithelial lineage by modulation of the SOX2/SOX17 balance in limbal primary mesenchymal stem cells.[3] In this regard, two ATC cell lines, SW1736 and 8505c, were used to investigate the effects of RSV and two natural analogues, 3,4',5-trans-trimethoxystilbene (3-MET-OX) and isorhapontigenin (ISOR-H-PG). Polyphenol treatments did not induce apoptosis, whereas a slowdown in the cell cycle in anaplastic thyroid cells compared with normal thyroid cells (Nthy-ori) was observed. To better mimic tumor microenvironment, 3D cell culture systems were employed along the traditional monolayer cultures. In 3D cultures, after 14 days of polyphenol treatments a gradual loss of spheroidal structure was observed, suggesting reduced self-renewal and enhanced differentiation. Moreover, at a molecular level, treatments resulted in a significant up-regulation of SOX17, along with the re-expression of specific thyroid markers TTF-1, TPO, and NIS, indicating a possible function restoration. In conclusion, our work aims to provide new insight into upstream regulatory mechanisms in ATC dedifferentiation molecular processes by implementing a 3D cell culture model that more closely resembles the *in vivo* tumor environment. Our data highlight the potential antitumor effect of polyphenols as differentiation-inducing agent and a promising therapeutic agent or coadjutant component to existing treatments to overcome ATC drug resistance. Further investigation will focus on exploring the molecular upstream pathways involved in ATC dedifferentiation, as well as cytoskeletal remodeling patterns, which play a key role in the epithelial-mesenchymal transition (EMT) during the metastatic progression.

Supported by the PNRR Project M4C2I1.3 Samothrace; ID: PRJ-1103.

REFERENCES

1. Molinaro, E. *Nat Rev Endocrinol.*, 2017.
2. Carina, V. *Thyroid*, 2013.
3. Tomasello, L. *J Cell Mol Med*, 2019.

EXPLORING OXIDATIVE STRESS BIOMARKERS IN LARYNGEAL SQUAMOUS CELL CARCINOMA: INSIGHTS INTO PATHOGENESIS AND CLINICAL RELEVANCE

Barbara VERRO[1], Diana DI LIBERTO[2], Giovanni PRATELLI[2], Daniela CARLISI[2], Sonia EMANUELE[2], Chiara ZICHITTELLA[2], Carmelo SARANITI[1], Marianna LAURICELLA[2]
[1]Division of Otorhinolaryngology, Department of Biomedicine, Neuroscience and Advanced Diagnostic, University of Palermo; [2]Section of Biochemistry, Department of Biomedicine, Neuroscience and Advanced Diagnostic, University of Palermo, Italy

Laryngeal squamous cell carcinoma is one of the most common head and neck cancers, with a five-year survival that, despite diagnostic and therapeutic advances, has not shown significant improvements in recent decades. Cellular oxidation and oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) production and cellular antioxidant systems, are recognized as central mechanisms in the pathogenesis of LSCC. However, the specific role of oxidative stress biomarkers in tumor progression remains unclear. This prospective case-control observational study analyzed the expression of six antioxidant proteins (superoxide dismutase - SOD, catalase - CAT, heme oxygenase 1 - HO-1, vimentin - VIM, metallothionein - MT and

nuclear factor erythroid-2 related-2 - NRF2) in the tumor tissues of 15 patients with laryngeal squamous cell carcinoma, comparing them with adjacent healthy tissues. In addition, correlations between the expression levels of these biomarkers and clinical characteristics of patients including age, sex, smoking habits and degree of tumor differentiation were evaluated. The results show a statistically significant overexpression of all proteins analyzed in cancer tissues compared to healthy tissues (controls), suggesting a strong involvement of oxidative stress in the progression of laryngeal squamous cell carcinoma. In particular, superoxide dismutase showed significant correlations with sex and smoking, with a smaller increase in smokers and men. Catalase was found to be correlated with age and C-reactive protein (CRP) levels, indicating a connection between systemic inflammation and oxidative stress. Heme oxygenase 1 and nuclear factor erythroid-2 related-2 showed an inverse correlation with the degree of tumor differentiation, suggesting a reduction in antioxidant capacity in less differentiated tumors. These preliminary results support the hypothesis that oxidative stress biomarkers may play a key role in laryngeal squamous cell carcinoma progression and may represent potential diagnostic and therapeutic targets. However, the limited sample size and complexity of molecular regulation require further studies on larger cohorts to confirm these data and investigate the mechanisms involved. Better understanding the role of oxidative stress in laryngeal carcinogenesis could help develop personalized treatment strategies, improving patients' clinical outcomes and prognosis.

EPIGENETIC AND METABOLIC CONTROL OF CHEMORESISTANCE IN COLON CANCER CELLS: BIOCHEMICAL MECHANISMS AND THE EFFECTS OF THE HDAC INHIBITOR ITF2357

Chiara ZICHITTELLA[1], Marzia FRANZÒ[1], Diana DI LIBERTO[1], Riccardo ALESSANDRO[2], Alice CONIGLIARO[2], Sonia EMANUELE[1]

[1]Department of Biomedicine, Neurosciences and Advanced Diagnostics (Bi.N.D.), Biochemistry Building, University of Palermo, Policlinico, Palermo; [2]Department of Biomedicine, Neurosciences and Advanced Diagnostics (Bi.N.D.), Section of Biology and Genetics, University of Palermo, Italy

Chemoresistance (CR) represents a major problem in conventional anti-tumor therapy. Among the biochemical mechanisms that contribute to the acquisition of CR, epigenetic regulation and metabolic reprogramming are recently emerging as crucial events [1-2]. In the epigenetic context, we have recently found that the epi-drug ITF2357 (Givinostat) induces apoptosis in colon cancer cells with the involvement of the long non-coding RNA H19 (H19) [3]. Here, show that ITF2357 overcomes resistance to 5-Fluorouracil (5-FU) in colon cancer cells. 5-FU resistant cells (5-FU-R) were selected in our laboratory using HCT-116 colon cancer cells. Along the selection process with increasing doses of 5-FU, the cells transiently showed a dramatic vacuolization, accumulated lipids and expressed high H19 levels. Given the dual role of H19 in tumor cells, this study aims to clarify whether H19 and lipid accumulation are involved in the acquisition of 5-FU resistance. Intriguingly, preliminary data indicate that H19 overexpressing cells showed a vacuolated phenotype and accumulated lipids. The observation that ITF2357 further increased H19 expression in 5-FU-R cells prompted us to verify the pro-apoptotic role of H19 in this system. To this purpose, ongoing studies are focused on silencing H19 in 5-FU-R cells. Moreover, to understand the connection between H19 and lipid metabolism, we are using inhibitors of lipogenesis in combination with ITF2357. Finally, to elucidate the mechanism accounting for the ability of ITF2357 to overcome 5-FU resistance, the expression levels of multi-drug resistance (MDR) ABC transporters as well as thymidylate synthetase (TS) were analysed. The results indicated that ABCB5 and TS are involved in the acquisition of 5-FU resistance. Notably, TS expression levels increased in 5-FU-R cells compared to parental HCT-116 cells and were markedly reduced by ITF2357, thereby suggesting that TS may be a target of the epi-drug, and its downregulation contributes to the sensitization mechanism. This was fully confirmed at the protein level. Overall, our data suggest that ITF2357 represents a promising potential antitumor epi-drug and a chemo-sensitizing agent for colon cancer treatment.

REFERENCES

1. Chen X. *et al. Metabolic Reprogramming of Chemoresistant Cancer Cells and the Potential Significance of Metabolic Regulation in the Reversal of Cancer Chemoresistance*. Metabolites. 2020 Jul 16;10(7):289. doi: 10.3390/metabo10070289.
2. Ge T. *et al. Crosstalk between metabolic reprogramming and epigenetics in cancer: updates on mechanisms and therapeutic opportunities*. Cancer Commun (Lond). 2022 Nov;42(11):1049-1082. doi: 10.1002/cac2.12374.
3. Zichittella C. *et al. Long non-coding RNA H19 enhances the pro-apoptotic activity of ITF2357 (a histone deacetylase inhibitor) in colorectal cancer cells*. Front Pharmacol. 2023 Sep 28;14:1275833. doi: 10.3389/fphar.2023.1275833.

MISCELLANEA

THE NATURAL BALANCE IN URBAN AND AGRICULTURAL BIODIVERSITY

Paolo BONIVENTO
Istituto di Studi Europei "Alcide De Gasperi", Roma, Italy

Around 10,000 years ago, Homo sapiens adopted agriculture and animal husbandry, shifting away from nomadism to establish the first sedentary villages. Today, the study of biodiversity's relationship with human management of agricultural and urban areas has identified a series of parameters that, thanks to modern technologies, should be promoted and utilized as indicators of ecosystem health. The journey from scientific research to measurement technologies, through applied and industrial research, is presented using communication methods that bridge various intersecting fields—from fundamental research to technological transfer for industrialization. The focus will be on complex environmental issues (such as microplastics, urban greenery, and invasive species) that vary widely. Validated studies moving from research to application through technology transfer are finally being recognized as foundational steps to managing agricultural and urban biodiversity effectively, emphasizing the need for proactive management before climate change renders the situation unmanageable. Implementing any conservation strategy for agricultural and urban biodiversity relies on effective collaboration across different sectors, communities, and perspectives. Understanding each member's capacities and skills in a coalition is vital for success, allowing for the melding of these elements into an inclusive collective agreement and actionable path. One approach to ease this process is to establish a conceptual architecture that offers guidelines recognizing various forms of expertise and their interrelations. This framework divides agricultural and urban conservation into four key areas. Conservation in Anthropic Spaces focus on preserving and enhancing genetic diversity, physical traits, and living communities to support healthy ecosystems. These efforts are guided by conservation biology principles tailored to urban and peri-urban settings. Research aimed at maintaining genetic connectivity among species or managing relationships between native and non-native species fits within agricultural and urban conservation, employing modern conservation biology tools suitable for city environments. Conservation of Anthropic Spaces considers the organic, social, and built components of urban ecosystems to understand how ecosystem processes—like water systems and nutrient flows—are influenced by social and ecological factors. By concentrating on urban heterogeneity, such as green space distribution and socio-economic dynamics, city conservation aims to improve ecosystem conditions for both species and communities through urban process-driven approaches. Research on social and ecological factors affecting biodiversity, along with social impacts on living and non-living urban processes, combines tools from both natural and social sciences to assess urban ecosystems. Conservation For Anthropic Spaces operationalizes the lessons learned from the components "in" and "of" to establish a model for action. It emphasizes justice-based urban solutions, promoting a collective and unified approach. This strategy applies unconventional conservation practices to tackle complex socio-ecological challenges while integrating environmental justice principles into these practices—favoring nature-centered solutions, sustainable urban planning, equitable urban greening, and biodiversity management that prevents displacement. Conservation with Anthropic Spaces champions a significant change in our research and scientific practices. It highlights the necessity of equitable urban development to preserve urban and agricultural biodiversity and address the global biodiversity crisis. This notion transcends academic boundaries and demands active collaboration, moving beyond traditional research methods and valuing community knowledge. It necessitates fair resource distribution and recognition of all stakeholders involved.

MONITORING OF SUSPECTED ADVERSE REACTIONS FROM DRUGS AND VACCINES. DATA ANALYSIS COLLECTED AT THE UOS PHARMACOVIGILANCE DEPARTMENT OF ASP CALTANISSETTA

Marta CARUANA[1], Maria Teresa RUSSO[2], Fabio VENTURELLA[3]
[1]Independent Researcher, Palermo; [2]Head of UOS Pharmacovigilance and Proper Use of Medicines ASP Caltanissetta; [3]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies University of Palermo, Italy

Pharmacovigilance encompasses post-marketing surveillance activities on medications that aim to identify adverse effects, expanding the safety profile of the drug. All health professionals and citizens can report any suspected adverse reaction by filling out a specific reporting form. This study presents an exploratory analysis of suspected adverse reactions caused by drugs and vaccines reported to the Simple Operational Unit of Pharmacovigilance at the A.S.P. of Caltanissetta. Reports (spontaneous and not) collected from 2018 to 2022 relating to male and female patients aged 0 to 99 years were taken into account. In respect of the privacy of individual patients, data regarding the age and sex of the patient, the drug responsible for the reaction, symptoms, severity of the reaction and the indicator were extrapolated. The data processing was carried out thanks to the use of the RStudio Team software (2020). The main demographic and clinical variables were evaluated, highlighting the number of reports year by year, the sex of the patients, the median age, the type of reporter, the severity of the reactions and the five most frequent reactions. The study then focuses on the drugs most frequently reported by patients, analyzing the top ten drug classes and active ingredients most responsible for severe and non-severe reactions. Finally, the symptoms related to these drugs were evaluated. In the pharmacovigilance reports of the Sicily region for the period from 2018 to 2022, the total reports reported are 16,951, those recorded in the province of Caltanissetta are about 3.3%. The highest number of reactions (40%) was recorded in 2021 (the year of administration of the Covid-19

vaccines for which several reports were recorded). The percentage of men and women affected is the same. The median age of patients is around 56 years. More than half of the reporters are doctors (82%). 83% of reactions were classified as non-severe, with the most frequently reported reaction being "ineffective drug", primarily related to monoclonal antibodies (82%) and immunosuppressants (71%). Discrepancies between the period before the marketing authorization of the COVID-19 vaccines and the two years following (2021-2022) were also highlighted. Specifically, the percentage of patients reporting reactions rose to 26%, and the percentage of patients with severe or permanent disability increased from 5% to 20%. Monitoring of reactions revealed that among the drugs that have reported the highest number of reports were Covid-19 vaccines Comirnaty, Spikevax and Vaxzevria, it should be emphasized that, considering the administration of these vaccines on almost the entire population, it is credible that they are also the first in terms of side effects. The analysis based on the median age showed that, in both the over 56 and the under 56, the highest number of reports, in descending order, were for the COVID-19 vaccines Comirnaty, Vaxzevria, Spikevax, and the monoclonal antibody Adalimumab. The top five drug classes associated with non-severe reactions were vaccines, monoclonal antibodies, immunosuppressors, statins, and antineoplastic drugs. More precisely, the year-by-year analysis showed that the difference between the number of vaccine reactions and that of other drug classes is only greater in 2021. As for the class of drugs that reported the highest number of serious reactions, it was always that of vaccines followed by antineoplastics, monoclonal antibodies, immunosuppressants and antithrombotics. Furthermore, the active ingredients and the most common severe and non-severe symptoms caused by these drug classes were identified. In particular, non-serious reactions caused by vaccines (excluding Covid-19 vaccines) mainly included hyperpyrexia and pain at the vaccination site (45%); while Covid-19 vaccines have, in most cases, caused fever and arthromyalgia (28%). Among the reports, there were also six cases of death.

EMPOWERING YOUNG SCIENTISTS THROUGH ADVANCED GENOMICS TECHNOLOGIES: THE ROLE OF THE NATIONAL FACILITY FOR GENOMICS AT HUMAN TECHNOPOLE

Carola CONCA DIOGUARDI, Niccolò ALFANO, Daniel CARRILLO BAUTISTA, Javier CIBELLA, Paolo FERRARI, Francesca GIANNESE, Luigi Antonio LAMPARELLI, Alessandra PIROLA, Eugenia RICCIARDELLI, Luca ROTTA, Giulia SABBATINELLI, Fabio SIMEONI, Mariateresa DE CESARE, Clelia PEANO
Human Technopole, Milan, Italy

The National Facilities at Human Technopole represent a cornerstone of Italy's research infrastructure, providing access to cutting-edge technologies and scientific expertise in various fields of life sciences. Among these, the National Facility for Genomics provides state-of-the-art and innovative services in genomics. Its core mission is to implement robust experimental and analytical workflows to probe all major domains of genomic exploration, including but not limited to the analysis of DNA, RNA, chromatin and other markers of epigenetic and regulatory activity. These techniques can be applied to diverse areas of biology, with resolution spanning from whole organisms to tissues or individual cells. Overall, NF Genomics aims to empower research in all domains of genomics for the Italian scientific community at large. The NF for Genomics foresees four Infrastructural Units: The High Throughput Sequencing mainly focused on providing state-of-the-art high-throughput sequencing services. The Multi-Omics Technologies specialized in multi-omics technologies. Its focus extends to providing cutting-edge services in single-cell and spatial multi-omics analysis, as well as long-read sequencing. The Technology Development unit stays at the forefront of innovation, this dedicated team is committed to methods and technology development. The Computational Genomics unit dedicated to developing, implementing, and maintaining automated pipelines for the pre-processing and primary data analysis of sequencing data. A key feature of the National Facilities is their commitment to fostering the next generation of scientists, particularly early-career Principal Investigators (young PIs). Access to the Facilities is free of charge for projects deemed scientifically worthy by an external scientific committee, and it is managed through competitive public calls, ensuring transparency and equal opportunities for all applicants. This approach enables researchers to access advanced technologies and specialized expertise necessary to generate high-quality preliminary data, a critical step in launching their scientific careers. In alignment with its mission to support the scientific community, the National Facilites also provide free training opportunities to researchers who successfully obtain access through the competitive calls. These training sessions are designed to enhance the technical and analytical skills of participants, enabling them to maximize the potential of the platform's cutting-edge technologies, fostering a culture of scientific excellence and collaboration.By lowering the barriers to advanced genomic technologies and providing tailored training opportunities, the National Facility for Genomics empowers researchers to pursue ambitious projects that span diverse disciplines, driving innovation and contributing to the advancement of knowledge in the life sciences.

NONSENSE MUTATIONS IN CYSTIC FIBROSIS: INVESTIGATING THE EFFICACY OF *NV* MOLECULES AS TRANSLATIONAL READTHROUGH-INDUCING DRUGS IN THE CFTR$^{G542X/G542X}$ MOUSE MODEL

Ilenia CRUCIATA, Emanuele VITALE, Riccardo VARRICA, Ignazio FIDUCCIA, Davide RICCI, Marco TUTONE, Raffaella MELFI, Ivana PIBIRI, Andrea PACE, Laura LENTINI, Maria Grazia ZIZZO
Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STeBiCeF), University of Palermo, Italy

Cystic fibrosis (CF) is an autosomal genetic disease caused by loss-of-function mutations in the *CFTR* (Cystic Fibrosis Transmembrane Conductance Regulator) gene. Among the about 2500 different mutations related with CF, nonsense mutations (class I *CFTR* mutations) affect 10% of CF population worldwide and 20% of the patients in Italy (Reg. ita. FC 2021-2022). These mutations, also called stop mutations, generate a premature termination codon (PTC) in the mRNA transcript, causing the early termination of protein synthesis and the consequent production of truncated, non-functional polypeptides. In nonsense CF patients, the complete absence of CFTR protein results in more severe symptoms. Currently, a specific treatment for this genetic defect is not available, and developing new drugs targeting specific *CFTR* gene mutations is an important goal of personalized medicine. Pharmacological induction of the translational readthrough of PTCs allows the rescue of the synthesis of a full-length functional protein, and represents a promising therapeutic approach for nonsense genetic disorders. Recently, three new patented Translational Readthrough-Inducing Drugs (TRIDs), called NV848, NV914, and NV930 (together referred as *NV* molecules), have been proposed by our research group, and validated *in vitro* for the rescue of the expression of the CFTR protein [1]. Acute toxicity studies *in vivo* demonstrated that the same molecules are safe and well tolerated in mouse models [2]. Furthermore, we validated the *in vivo* efficacy of NV848 molecule in recovering the CFTR protein in homozygous mice carrying the G542X stop mutation in the *CFTR* gene [3]. In the present study, the same $CFTR^{G542X/G542X}$ mouse model was used to assess the *in vivo* translational readthrough potential of NV914 and NV930 molecules. Chronic administration by oral gavage of NV914 or NV930 at 60 mg/kg for 14 days was performed in $CFTR^{G542X/G542X}$ mice. PTC124 (ataluren) was similarly administrated as readthrough reference compound. Furthermore, untreated CFTR wild-type and $CFTR^{G542X/G542X}$ homozygous mice were used as positive and negative controls, respectively. All the experimental mice were maintained with a liquid diet to avoid intestinal obstructions, displayed in CF mice. Body weight was monitored during the administration period, and at the end of the treatment, the animals were sacrificed, and their organs were collected. We investigated the CFTR expression in mouse lung tissue, showing a partial stabilization of *CFTR* mRNA in NV914-treated mice and a recovery of CFTR protein expression in NV914- and NV930-treated mice, as evidenced by RT-qPCR and Western blot analyses. These results demonstrate the *in vivo* efficacy of *NV* molecules in inducing the translational readthrough of PTCs in the *CFTR* mRNA and the consequent rescue of protein synthesis, highlighting their therapeutic potential for the treatment of CF and other diseases caused by nonsense mutations.

REFERENCES

1. Pibiri *et al.* Int J Mol Sci. 2020. 21(17):6420. doi:10.3390/ijms21176420
2. Corrao *et al.* Biomed Pharmacother. 2022. 156:113886. doi: 10.1016/j.biopha.2022.113886
3. Fiduccia *et al.* Mol Ther. 2024. 32(12):4514-4523. doi: 10.1016/j.ymthe.2024.10.028

STUDY OF THE IMMUNOMODULATORY ACTIVITY OF *HELLEBORUS BOCCONEI* EXTRACTS

Paola GALLUZZO[1,2], Laura PALUMBO[3], Antonella GIRGENTI[3], Pasquale PICONE[3], Santina DI BELLA[1], Sergio MIGLIORE[1], Gigliola BORGONOVO[4], Salvatore PRINCIOTTO[4], Salvatore DARA[1], Domenico NUZZO[3], Rosa ALDUINA[2], Guido Ruggero LORIA[1]

[1]Istituto Zooprofilattico Sperimentale della Sicilia A. Mirri, Palermo; [2]Dipartimento Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche, Viale delle Scienze, University of Palermo; [3]Istituto per la Ricerca e l'Innovazione Biomedica, CNR, Palermo; [4]Department of Food, Environmental and Nutritional Sciences, Section of Chemical and Biomolecular Sciences, University of Milano, Italy

The genus *Helleborus* spp., belonging to *Ranunculaceae*, includes perennial herbs native to Europe and Asia, known for their toxicity (1). Despite this, various species have been used in traditional medicine to treat conditions such as tooth pain, abortion, skin diseases, and joint pain (2). In Italy, species like *H. foetidus* and *H. viridis* have been used in veterinary medicine to treat diseases in livestock, with *H. bocconei* particularly used in Sicily for treating pneumonia and bronchitis in cattle (3). This study aimed to evaluate the anti-inflammatory effects of methanolic (ME), butanolic (BE), and aqueous (AE) extracts from *H. bocconei* roots. For this purpose, the roots were air dried, then extracted with methanol, and partitioned with *n*-butanol (*n*-BuOH) and water (H_2O). All three extracts were used to assess both cytotoxicity on human peripheral blood mononuclear cells (PBMCs) and immunomodulatory activity after bacterial lipopolysaccharides (LPS) stimulation. PBMCs were cultured in 24-well flat-bottom plates in RPMI medium. After 24h, two non-cytotoxic concentrations of each plant extract (0.31 and 0.62 µg/ml for BE, 0.07 and 0.15 µg/ml for ME, and 0.62 and 1.25 µg/ml for AE) were tested to evaluate immunomodulatory activity after cell stimulation with bacterial LPS (1µM). After 24h of LPS stimulation, cells were collected to extract RNA, synthesize cDNA and amplify the IL1β, IL17, TNFα, iNOS, TGFβ 1 and γINF genes. Concentrations higher than 2.5 µg/ml for ME and AE, and 0.62 µg/ml for BE were cytotoxic to human PBMCs. During cell stimulation with LPS, both tested concentrations of BE and AE reduced IL1β and iNOS gene expression. In contrast, the ME induced the transcription of both IL1β and iNOS gene expression. Regarding the other genes, no significant variations were observed. IL-1β overproduction is linked to several diseases, including rheumatoid arthritis, gout, inflammatory bowel disease, and type 2 diabetes (4). Pharmacological blockade of IL-1 signaling has shown to be beneficial in some autoimmune and autoinflammatory diseases, making IL-1β a promising therapeutic target in neuroinflammatory conditions (5). A decrease in iNOS expression suggests a reduction in the

production of nitric oxide, which could indicate suppression of the inflammatory response. In this study, the reduction in IL-1β and iNOS gene expression by the BE and AE suggests that these extracts may have anti-inflammatory effects, potentially mitigating excessive inflammation. Future research should explore the specific bioactive compounds in these extracts and evaluate their safety and efficacy *in vivo*.

REFERENCES

1. Olivieri MF *et al.* J Hist Neurosci. 2017 Apr-Jun;26(2):193-215. doi: 10.1080/0964704X.2016.1211901.
2. Maior M, Dobrotă C. Open Life Sciences. 2013;8(3): 272-285. https://doi.org/10.2478/s11535-013-0129-x.
3. Raimondo FM, Lentini F. Il Naturalista Siciliano, 3/4 (1990), pp. 77-99.
4. Dinarello CA, *et al.* Nat Rev Drug Discov. 2012;11(8):633–652. doi: 10.1038/nrd3800.
5. Mendiola AS, Cardona AE. J Neural Transm (Vienna). 2018 May;125(5):781-795. doi: 10.1007/s00702-017-1732-9.

INVESTIGATING THE PLANTS ADAPTATION MECHANISMS TO URBAN ENVIRONMENT

Noemi GATTI, Chiara DELLAPIANA, Moez MAGHREBI, Matteo CHIALVA, Francesco BERRUTO, Valentina FIORILLI, Luisa LANFRANCO, Alessandra SALVIOLI DI FOSSALUNGA, Gianpiero VIGANI
University of Turin, Department of Life Sciences and Systems Biology, Turin, Italy

The urban environment inherently possesses features that might be hostile to the growth of plants and the development of plant communities. In particular, the soil may exhibit adverse traits such as fluctuating moisture levels, contamination by heavy metals and/or organic compounds, and acidic or alkaline pH that can have negative effects on plants. However, a careful observation of the urban areas reveals how many plant species have successfully adapted to this challenging environment. This phenomenon raises significant questions about the capacity of plants to adapt to various and combined stresses. This study aims to understand how different urban soils, collected from three parks located in the city of Turin (Northern Italy) and holding diverse features, influence plant growth by monitoring biometric parameters, leaf ionomic profile and root-associated microbiome. This approach has been integrated with the sampling and analysis of soil physico-chemical parameters. Six plant species were selected to assess the effects of urban soil on plant growth: *Plantago lanceolata*, *Poa pratensis*, *Lolium perenne*, *Taraxacum officinale*, which naturally occur in all studied parks and *Medicago sativa*, absent in all the parks considered. The growth of these plants has been compared with that of the model and crop species *Solanum lycopersicum*. While the characterization of root-associated microbiota is still under investigation, the morphometric analysis revealed that alkaline soil, severely and differentially affected the growth of the plant species tested. The analysis of biomass values showed that growth of *L. perenne*, *P. lanceolata* and tomato increased in such soil. Therefore, to deeply investigate the impact of high pH, *S. lycopersicum* plants were grown hydroponically and evaluation of morphometric parameters and mineral nutrients concentration analyses using capillary electrophoresis and ICP-MS were carried out. These analyses revealed that tomato plants grown at high pH displayed an increase in sulfate and phosphate content, suggesting that such nutrients might play a role in the acclimation mechanism to alkaline conditions. This hypothesis will be further tested by performing the same analyses on all the other plant species. These results offer insights into the mechanisms that drive plant adaptation strategies in urban environments. Exploring urban plant biodiversity could provide fertile ground for the development of innovative solutions to mitigate the impacts of environmental changes.

BIOACTIVE MOLECULES FROM THE INVASIVE BLUE CRAB *CALLINECTES SAPIDUS* EXOSKELETON: EVALUATION OF REDUCING, RADICAL SCAVENGING, AND ANTITUMOR ACTIVITIES

Francesco LONGO[1], Alessandro ATTANZIO[1], Laura MARRETTA[1], Claudio LUPARELLO[1,2], Serena INDELICATO[1], David BONGIORNO[1], Giampaolo BARONE[1], Luisa TESORIERE[1], Ilenia Concetta GIARDINA[1], Giulia ABRUSCATO[1], Manuela PERLOTTI[1], Lucie Branwen HORNSBY[1], Vincenzo ARIZZA[1,2], Mirella VAZZANA[1,2], Aiti VIZZINI[1,2], Chiara MARTINO[1,2], Angelica LISTRO[1], Vinicius QUEIROZ[3], Antonio FABBRIZIO[4], Paolo Salvatore Francesco CIACCIO[5], Stella Maria CASCIOFERRO[1], Francesca DI GAUDIO[6], Manuela MAURO[1]
[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Italy; [2]National Biodiversity Future Center (NBFC), Palermo, Italy; [3]Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo, Brazil; [4]Department of Theoretical and Applied Sciences (DiSTA), University e Campus, Novedrate (CO), Italy; [5]Independent Researcher, Sciacca (AG), Italy; [6]Department PROMISE, University of Palermo, Italy

In recent years, the Mediterranean Sea has experienced a sharp rise in the spread of alien species. Among them, the Atlantic blue crab (*Callinectes sapidus*) has shown exceptional adaptability, leading to a rapid and uncontrolled expansion along the Italian coasts. This invasive species poses a serious threat to native biodiversity and local economies. Due to the challenges associated with its eradication, current management strategies have shifted toward promoting its use as a food resource. Beyond this approach, the valorization of this invasive species could be further enhanced by utilizing its non-edible parts, mainly consisting of exoskeleton, as a source of valuable bioactive molecules. In this study, the exoskeletons of *C. sapidus* were used to extract bioactive

molecules, specifically chitosan, astaxanthin, and polyphenols. The extraction methods employed yielded high amounts of chitosan and astaxanthin, while, for the first time, the polyphenols of this species were characterized using UPLC-MS analysis. Six bio-phenols were identified above the limit of quantification (LOQ), with mandelic acid being the most abundant compound. The bioactive properties of these molecules were evaluated, focusing on their reducing, radical scavenging, and antitumor activity. The ferric ion reducing antioxidant power (FRAP) and the free radical scavenging activity against radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were significantly higher for chitosan (3.16 ± 0.10 mg AAE/g and 8.1 ± 0.10 µmol TE/g). No significant differences were observed among the tested biomolecules in their activity in scavenging the radical 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). Both polyphenolic compounds and astaxanthin exhibited dose-dependent cytotoxicity on two different tumor cell lines, CaCo-2 (IC_{50} = 12.47 and 18 µg/mL) and HepG2 (IC_{50} = 10.25 and 1.26 µg/mL). Moreover, polyphenolic extracts showed no cytotoxic effects on CaCo-2 cells differentiated into enterocytes up to 20 µg/mL, representing an added value for potential future applications of this extract. These findings highlight the value of blue crab by-products in supporting a circular economy, offering a sustainable approach to managing this invasive species while providing bioactive compounds with promising medical and nutraceutical applications.

BETANIN BREAKDOWN PRODUCTS ON STRESS RESPONSE OF GERMINATING ARABIDOPSIS: PRELIMINARY DATA FOR A NEW BIOSTIMULANT PROTOTYPE

Giuseppe MANNINO[1], Noemi GATTI[1], Graziella SERIO[2], Carla GENTILE[2]
[1]Department of Life Sciences and Systems Biology, Plant Physiology Unit, University of Turin; [2]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Italy

Seed germination is a pivotal stage in plant development, subject to numerous internal and external factors. While mitochondrial activity, ROS balance, and phytohormonal regulation are well-known, recent studies suggest additional involvement of bioactive compounds, such as those found in agricultural byproducts. This study investigates the potential effects of betanin degradation products (BDPs), derived from discarded pitaya fruit processing waste, on *Arabidopsis thaliana* germination and early seedling growth, grown under standard, under salt (100 mM NaCl) or osmotic (100 mM Mannitol) stress condition. The research encompasses various physiological processes, including ROS signaling, proline accumulation, and phytohormonal regulation. Interestingly, lower concentrations of BDPs (0.02 – 0.20 mg mL^{-1}) enhanced seedlings development performances and biomass compared to controls, while higher doses (>1.00 mg mL^{-1}) show adverse effects on morphological traits. Moreover, the beneficial concentrations displayed positive effects also on seedlings grown under abiotic stress conditions. Through assessments using the MTT assay on both seeds and purified organelle fractions, it is confirmed that the distinct compounds within BDPs neither affect mitochondrial activity nor compromise its integrity. Mechanistically, BDPs modulate ROS signaling by reducing free H_2O_2 content through enhancing antioxidant activity and regulating the expression of ROS scavenging genes. Furthermore, BDPs influenced proline accumulation, indicating enhanced stress tolerance. The observed rise in proline content correlates with alterations in its metabolism and catabolism, evidenced by changes in gene expression associated with these pathways. Additionally, BDPs disrupt phytohormone homeostasis, favoring seedling establishment. Particularly, the balance between ABA/ABA-glu, tZea/tZea-rib, and tZea/IAA suggests improved germination performance and seedling development at lower concentration ranges (0.02 – 0.20 mg mL^{-1}) and inhibition at higher doses. The increase in GA4 and GA7 content compared to other gibberellins implies involvement of GA13ox, a crucial enzyme in the biosynthetic switch, supported by gene expression evaluations. In conclusion, the findings of this study underscore the promising role of betanin degradation products as sustainable enhancers of plant growth, particularly in mitigating the impacts of abiotic stress on germination and early seedling development, opening avenues for their application in agriculture.

STANDARDIZATION OF CIRCULATING ENDOTHELIAL CELL AND ENDOTHELIAL PROGENITOR CELL PHENOTYPES AND COUNTS BY FLOW CYTOMETRY: NEW PERSPECTIVES FOR CLINICAL RESEARCH

Sabino PORRO[1,2], Francesca D'ASCANIO[1,2,3], Giulia COLASANTE[1,2], Arianna AQUILINI-MUMMOLO[1,2], Tamer Shibl Ali ESMAIL[1,2], Ayesha YOUNAS[1,2], Domenico DE BELLIS[1,2], Pasquale SIMEONE[1,2], Paola LANUTI[1,2]
[1]Department of Medicine and Aging Sciences, University "G. d'Annunzio", Chieti-Pescara, Chieti; [2]Center for Advanced Studies and Technology (CAST), University "G. d'Annunzio", Chieti-Pescara; [3]Department of Humanities, Law and Economics, "Leonardo da Vinci" University, Torrevecchia Teatina (CH), Italy

Circulating endothelial cells (CEC) and endothelial progenitor cells (EPC) are rare subpopulations found in peripheral, cord, and bone marrow blood, essential for maintaining endothelial homeostasis. In recent years, numerous studies have highlighted the potential of CEC and EPC as biomarkers for cardiovascular, inflammatory, metabolic, and oncological diseases, as well as for monitoring therapeutic responses. However, the lack of standardized protocols for their identification has led to inconsistent results, limiting their clinical application. This study addressed the issue of standardization by applying an optimized polychromatic flow cytometry protocol for the characterization of CEC and EPC in peripheral, cord, and bone marrow blood samples. The results confirmed the presence of CEC, defined as

CD45neg/CD34bright/CD146pos cells, in peripheral blood, with consistent counts across different laboratories due to the use of a highly reproducible method. Thess findings reinforce the value of these cells as a potential biomarker for monitoring endothelial dysfunctions. In parallel, the analysis of EPC challenged the commonly accepted definition of these cells. Traditionally, EPC are identified by the co-expression of VEGFR2 and CD133 on CD34pos/CD45dim cells. However, our study did not detect any population with this phenotype in the peripheral or cord blood of healthy individuals, suggesting that EPC may primarily reside in the bone marrow and be mobilized only under pathological conditions or specific physiological stimuli. Furthermore, it emerged that the conventional definition of EPC might overlap with the antigenic profile of hematopoietic stem cells, raising concerns about the specificity of the markers used so far. These findings emphasize the need to redefine EPC identification criteria and promote the adoption of standardized protocols for CEC quantification. Their implementation could enhance the understanding of vascular damage and repair processes and facilitate the development of new diagnostic and therapeutic strategies.

SYSTEMIC INFLAMMATION, COAGULATION, AND ENDOTHELIAL HEALTH IN TAVI PATIENTS: A MULTIMODAL DIAGNOSTIC APPROACH

Vahid SAQAGANDOMABADI[1,2], Adelaide CARISTA[1], Francesco CAPPELLO[1,2], Valentina DI FELICE[1]
[1]Department of Biomedicine, Neuroscience and Advanced Diagnostics (BIND), University of Palermo; [2]Euro-Mediterranean Institute of Science and Technology (IEMEST), Palermo, Italy

Chronic heart failure patients exhibit systemic blood coagulation induction, potentially due to inflammatory activation, increasing vulnerability to thromboembolic complications and poor prognosis, affecting coagulation indicators like VIII–von Willebrand factor (VWF)(1). Our project introduces the EndoTAVI System 1.0, an integrated solution designed for hybrid hospital environments to perform transcatheter aortic valve implantation (TAVI), while simultaneously evaluating endothelial function through advanced diagnostics. This multifunctional system allows conventional surgery, percutaneous approaches, and extensive endothelial assessments, including flow-mediated dilation (FMD) and molecular markers. A prospective study with 150 patients undergoing TAVI will evaluate these markers immediately post-procedure and during follow-up. Preliminary results suggest significant insights into the comparative impacts of these treatments on endothelial health. The project's originality lies in its comprehensive approach, combining advanced imaging, molecular diagnostics, and hybrid procedural capabilities. This innovative system represents a significant advancement in managing aortic stenosis, with potential for broader applications in cardiovascular medicine. In the ENDOTavi project, we examined various clinical elements among 77 patients and biological micro-markers among 45 patients; They are involved with the patients who are candidates for Transcatheter Aortic Valve Implantation. It has been evaluated the biological results and clinical tests before the surgery, post-procedure, and follow-up. Based on the results obtained, patients with elevated vWF levels (>30 ng/mL) in their serum show lower left ventricular ejection fraction (LVEF) (mean 38.9%) and it depicts the inverted relation between these elements, at the same time they manifest higher levels of pro B-type Natriuretic Peptide (pro-BNP) hormone (mean 6989 pg/mL) which is a manifestation on the stress on the cardiac wall and endothelial dysfunction and Troponin T levels (mean 155.687 pg/mL) that reflecting platelet activation and endothelial injury, in compare to normal patients (mean LVEF 59.32%; pro-BNP 742 pg/mL, Troponin T 16.15 pg/mL). The hypertension makes vWF to play a crucial role in platelet adhesion which is significantly higher in the areas with endothelial shear stress, in our study 81% of patients have hypertension that together with other elements shows an increase in the level of vWF. In addition, hypercholesterolemia leads to atherosclerosis (65.5% of the patients who went under biological examinations), it is a situation to distinguish endothelial dysfunction with leads to an increase in vWF levels. Furthermore, it has been depicted that higher levels of IL-6 and IL-8 are associated with higher levels are Troponin T. Respect to this evidence, in our project we observed that patients with elevated vWF levels (>30 ng/mL), they have higher levels of IL-6 with a mean of 22.296 pg/ml in compare to patients who are considered as normal patients with 7.105 pg/ml and for IL-8 is 152.962 pg/ml and the patients with normal range of troponin T was 14.99 pg/ml. it is worthy to mention that the data for IL-8 in our experiment is not complete and massive. An increase in pro BNP hormone is linked to systematic inflammation, for elevated IL-6 (mean 33.96 pg/ml) is evaluated with a mean of 14002 pg/ml and for patients categorized normal is 744.833 pg/ml with the level of IL-6 with a mean of 6.513 pg/ml. and this data shows the amount for increased IL-8 is 104.955 pg/ml compared to 9.735 pg/ml for normal patients.

REFERENCE

1. Mongirdienė A, Kuršvietienė L, Kašauskas A. The coagulation system changes in patients with chronic heart failure. Medicina. 2010;46(9):642.

PRECLINICAL EVALUATION OF NV848 MOLECULE IN DUCHENNE MUSCULAR DYSTROPHY: TRANSLATIONAL READTHROUGH EFFICACY AND FUNCTIONAL OUTCOMES

Fazal SUBHAN[1], Ilenia CRUCIATA[1], Sefora MARINO[1], Riccardo VARRICA[1], Davide RICCI[1], Simona MERIGHI[2], Beatrice BELMONTE[2], Marco TUTONE[1], Ivana PIBIRI[1], Andrea PACE[1], Laura LENTINI[1], Maria Grazia ZIZZO[1]
[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STeBiCeF), University of Palermo; [2]Department of Health Promotion, Maternal-Child, Internal Medicine and Specialist Excellence (ProMISE) "G. D'Alessandro", University of Palermo, Italy

Duchenne muscular dystrophy (DMD) is a severe, neuromuscular, genetic disorder with X-linked inheritance, caused by mutations in the *DMD* gene, encoding dystrophin protein. The absence of a functional dystrophin in muscle leads to progressive muscle wasting, physical inactivity, respiratory depression, cardiac insufficiency, and premature mortality. A substantial proportion of *DMD* gene mutations (~13%) are nonsense mutations that generate premature termination codons (PTCs) in the mRNA sequence, resulting in premature termination of translation and the consequent production of a truncated, non-functional protein. Molecules that induce ribosomal readthrough of PTCs (Translational Readthrough-Inducing Drugs, TRIDs), allowing the rescue of the synthesis of a full-length functional protein, hold great therapeutic potential for treating genetic nonsense disorders, including DMD. Published results from our lab revealed that a new TRID compound, NV848 (N-(5-methyl-1,2,4-oxadiazol-3-yl) acetamide), was able to rescue target protein expression in nonsense-related *in vitro* assays and Cystic Fibrosis nonsense murine model [1,2]. The same compound demonstrated a favourable safety profile, with acute toxicity studies conducted in mouse model indicating no mortality and no significant tissue damage at 300 mg/kg, categorizing it as low risk for heath (GHS category 4) [3]. Pharmacokinetic investigations illustrated fast absorption, with peak plasma concentration reached in 45 minutes, and organ-specific distribution, with clearance within 120 minutes. Furthermore, metabolic stability evaluations suggested lower degradation rates compared to PTC124 (ataluren), indicating improved bioavailability [2]. The current study investigates the translational readthrough potential of NV848 in restoring the expression of dystrophin protein and in improving skeletal muscle functionality in mdx mice, the most common murine model of DMD, carrying a spontaneous nonsense mutation in the *DMD* gene. Mdx mice were administered NV848 or the readthrough reference drug PTC124 orally by gastric gavage at a dosage of 60 mg/kg for 14 consecutive days. The forelimb grip strength test was performed to assess muscle function in wild-type and mdx mice before, during, and after the administration period. NV848-treated mice exhibited significantly improved grip strength compared to untreated controls, and similar to the PTC124-treated group. Immunohistochemistry assessment performed on skeletal muscle sections of mdx mice demonstrated that NV848 treatment reinstated dystrophin protein localization, showing staining patterns like wild-type controls. Conversely, untreated mdx mice showed little to no observable changes. Real-time RT-PCR assessment additionally corroborated these results, showing a partial rise in *DMD* mRNA levels in NV848-treated mice compared to untreated controls. Our study contributes to the development of targeted approaches for DMD by offering important insights into the therapeutic efficacy of the novel TRID compound NV848, and by making preclinical data available for future drug development.

REFERENCES

1. Pibiri *et al.* Int J Mol Sci. 2020. 21(17):6420. doi:10.3390/ijms21176420
2. Fiduccia *et al.* Mol Ther. 2024. 32(12):4514-4523. doi: 10.1016/j.ymthe.2024.10.028
3. Corrao *et al.* Biomed Pharmacother. 2022. 156:113886. doi: 10.1016/j.biopha.2022.113886

RESTORING MMACHC EXPRESSION: TRIDS AS A PROMISING APPROACH FOR CBLC DEFICIENCY TREATMENT

Riccardo VARRICA[1], Silvia VILASI[2], Rosa PASSANTINO[2], Lisa LONGO[1,2], Anita SAMMARCO[1], Marco TUTONE[1], Sefora MARINO[1], Ivana PIBIRI[1], Laura LENTINI[1]
[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STeBiCeF), University of Palermo; [2]Biophysics Istitute, National Research Council (CNR), Palermo, Italy

Rare genetic diseases, often defined as ‘’orphan diseases’’ due to their low influence and limited therapeutic options, have always represented a demanding challenge for research and medicine. Recent attention has focused on stop mutations, one of the different classes of mutations responsible for these pathologies. This gene defect, considered among the most harmful, determines the formation of premature termination codons (PTCs), whose presence on the coding mRNA results in the synthesis of a non-functional truncated protein or with alterations that can compromise its functioning biological activity. One of the most promising approaches to counteracting these mutations is based on the use of a new class of molecules, known as Translational Readthrough-Inducing Drugs (TRIDs), which interfere with protein translation, allowing the synthesis of a complete and potentially functional protein. This study aimed to evaluate the readthrough activity of 3 new TRID molecules (NV848, NV914, and NV930) to rescue the expression of the cobalamin chaperone MMACHC protein associated with the metabolism of vitamin B12. We engineered HCT116 cells with two vectors harboring the R132X and Y222X mutations in the MMACHC cDNA and treated them with TRIDs at different time points. After 24, 48, and 72 hours of TRID treatments, *Real-Time RT-PCR* analysis revealed an increase in the expression levels of mutated *MMACHC* mRNA, suggesting a possible transcript stabilization. Immunofluorescence analyses further supported these data, confirming the protein’s partial rescue. These results suggest that the analyzed TRIDs could represent a promising therapeutic option for the treatment of cobalamin C (cblC) deficiency disease. Furthermore, such evidence paves the way for developing personalized therapeutic strategies based on the readthrough of premature stop codons, offering an innovative perspective for treating numerous rare genetic diseases currently without effective treatments.

IMPACT OF DECALCIFICATION PROTOCOLS IN IMMUNOSTAINING OF BONE-CONTAINING EXPERIMENTAL MODELS

Luca VENTURA[1,2], Veronica FORTE[1], Laura CIRELLA[1], Emiliana GIANCARLI[1], Claudio FESTUCCIA[2], Giovanni Luca GRAVINA[2]

[1]*UOC Anatomia Patologica, Ospedale S. Salvatore, L'Aquila;* [2]*Dipartimento DISCAB, Università dell'Aquila, Italy*

Histological analysis of calcified tissues - such as bone - or pathologically calcified tissues, presents unique challenges due to their mineralized matrix. Traditional approaches often involve decalcification, which can compromise antigen integrity, adversely affecting immunohistochemical results. Experimental models, including murine and xenograft systems, rely on such analyses to evaluate prognostic markers and therapeutic targets. However, the impact of decalcification techniques on antigen preservation remains underexplored. This study aims to compare the effects of different decalcification protocols on tissue morphology and antigen preservation, with a focus on standardizing reliable methods for experimental research. The study utilized murine limb samples and xenograft glioblastoma tissues. Murine tissues were harvested from C57BL/6 mice, while glioblastoma samples originated from xenograft models. Samples were fixed in 10% neutral buffered formalin and divided into four experimental groups: • F AC: 3 days decalcification with 5% HCl prior to paraffin embedding. • F CH: 6 days decalcification with 14% EDTA prior to paraffin embedding. • S AC: 30 minutes surface decalcification of paraffin-embedded tissues with 5% HCl. • S CH: 30 minutes surface decalcification of paraffin-embedded tissues with 14% EDTA. Tissue sections were stained with hematoxylin-eosin (H&E) and subjected to immunohistochemical staining for the following markers: alpha-smooth muscle actin, cytokeratin AE1/AE3, CD31, CD34, desmin, GFAP, IDH-1, Ki-67, MGMT, myosin, p53, S100 protein, synaptophysin, and vimentin. Semi-quantitative analysis evaluated antigen expression intensity and morphologic preservation of the tissues. Surface decalcification with EDTA (S CH) preserved tissue morphology better than post-fixation protocols (F AC, F CH) resulted in more complete and intact tissue sections. Samples treated with HCl post-fixation (F AC) exhibited tissue staining artifacts, while surface decalcification minimized these effects. EDTA-treated samples (F CH and S CH) showed less severe basophilic staining loss compared to HCl-treated groups. In murine limbs samples, markers as desmin, myosin, CD34, Ki-67, p53, and vimentin were significantly better preserved in surface-decalcified tissues. Xenograft glioblastoma samples demonstrated higher nuclear staining intensity for Ki-67 in S CH-treated tissues compared to F AC or F CH groups. Similarly, endothelial markers like CD34 exhibited superior membrane staining in surface-decalcified samples. Our findings confirm that decalcification methods significantly impact tissue integrity and antigen preservation. Surface decalcification using EDTA combines effective decalcification with minimal antigen degradation, making it a superior choice for experimental studies. These results align with recent advancements advocating for chelating agents over strong acids for histopathological applications. This study demonstrates that surface decalcification of paraffin-embedded tissues with 14% EDTA is an optimal method for preserving antigen integrity in histological and immunohistochemical analyses. Adoption of this technique can improve the reliability of experimental results and support translational applications in oncology and bone research.

PERSONALIZED MEDICINE FIGHTING NONSENSE DISEASES: EXPLORATION OF NV MOLECULES MECHANISM OF ACTION IN PURE-LITE SYSTEM AND THEIR READTHROUGH ACTIVITY IN CHOROIDEREMIA NONSENSE MODEL

Emanuele VITALE[1], Davide RICCI[1], Raffaella MELFI[1], Andrea PACE[1], Ivana PIBIRI[1], Barry S. COOPERMAN[2], Laura LENTINI[1]

[1]*Department of Biological, Chemical, and Pharmaceutical Sciences and Technologies (STeBiCeF), University of Palermo, Italy;* [2]*Department of Chemistry, University of Pennsylvania, Philadelphia, USA*

Precision medicine represents a new approach in genetic medicine for treating a patient's mutation profile. Nonsense mutations cause 11% of inherited genetic diseases, including Choroideremia (CHM), Cystic fibrosis (CF), Duchenne Muscular Dystrophy (DMD), and some Cancers. Choroideremia is an X-linked disease associated with many retinal disorders. This retinal dystrophy causes the progressive degeneration of the choriocapillaris, photoreceptors, and retinal pigment epithelium. The CHM gene codifies for a 95 kDa, known as rab escort protein 1 (Rep1), involved in the intracellular trafficking and prenylation of polypeptides, a post-translational modification fundamental for the correct functionality of specific proteins. Nonsense mutations represent about 34% of mutations in the CHM gene. These mutations prematurely introduce a premature termination codon (PTC: TGA, TAG, and TAA) in the mRNA frame, causing a truncated and non-functional protein that will be eliminated from intracellular surveillance pathways. Nowadays there is no cure for diseases caused by nonsense mutations, but in the last decades, promising results have come from a pharmacological approach, called suppression therapy. This approach uses specific drugs having readthrough activity, known as TRIDs (Translational Readthrough Inducing Drugs), which permit the insertion of a near-cognate-tRNA in correspondence with a PTC during protein translation, allowing the correct development of a functional full-length protein. Our study is focused on investigating three molecules, NV848, NV914 and NV930, patented by Pibiri-Lentini group, that have shown readthrough activity in different nonsense model diseases. Specifically, elucidating their Mechanism of Action in the PURE-LITE system and exploring their readthrough activity in a choroideremia nonsense model system have been the goals of our efforts. PURE-LITE system is a highly purified, eukaryotic cell-free protein synthesis system, that allows the investigation of both the termination of protein synthesis in the P-site catalyzed by the RF complex (eRF1/eRF3/ribosome) and the readthrough via mispairing at the termination codon by a near-cognate tRNA when a PTC enter into the 40S A-site ribosome subunit. Simultaneously, chronic treatment and protein expression analyses was performed on Choroideremia nonsense cell model system to study their readthrough activity. Our results have shown both that the

mechanism of action of these molecules is different by their precursor Ataluren, and in addition their readthrough activity with the rescue of Rep1 protein.

REFERENCES

1. Pibiri I. Targeting Nonsense: Optimization of 1,2,4-Oxadiazole TRIDs to Rescue CFTR Expression and Functionality in Cystic Fibrosis Cell Model Systems. Int J Mol Sci. 2020 doi: 10.3390/ijms21176420.
2. Bhattacharya A. "Ataluren binds to multiple protein synthesis apparatus sites and competitively inhibits release factor-dependent termination. Nat Commun, May 2022. doi: 10.1038/s41467-022-30080-6.

EFFECT OF L-CARNITINE SUPPLEMENTATION ON THE LIVER MORPHOFUNCTIONALITY OF PCOS MICE

Silvia ZACCARDI, Domenica COCCIOLONE, Carla TATONE, Giovanna DI EMIDIO, Guido MACCHIARELLI, Maria Grazia PALMERINI
Department of Life, Health and Environmental Sciences, University of L'Aquila, Italy

Polycystic ovary syndrome (PCOS) is an endocrine disorder, affecting females of reproductive age. This syndrome leads to infertility, insulin resistance, obesity, and cardiovascular problems. PCOS is a polygenic, multifactorial, systemic, inflammatory, and dysregulated steroid state [1]. The etiology of this syndrome remains largely unknown. Many studies have suggested a central role for oxidative and glycative stress in the pathogenesis of the PCOS and a decreased antioxidant capacity in patients with PCOS. In search for effective antioxidants to employ as a complementary therapeutic approach to improve the prognosis of PCOS patients, L-carnitine (LC) supplementation has emerged. The endogenous carnitine pool is formed by carnitine esters acetyl-L-carnitine (ALC) and propionyl-L-carnitine (PLC) [2]. Being a systemic and multifactorial pathology, PCOS affects not only ovaries, uterus and oviducts; androgen excess may be, in fact, responsible for an increased risk of liver fibrosis and steatosis. We, here, investigated morpho-functional alterations in the PCOS liver. PCOS was induces in CD-1 mice by a subcutaneous administration of DHEA (Dehydroepiandrosterone) (6 mg/100 g body weight) for 20 days; two groups of mice concomitantly received orally 1) L-carnitine (LC) and acetyl-L-carnitine (ALC) (DHEA/LC-ALC group) or 2) LC, ALC and propionyl-L-carnitine (PLC) (DHEA/LC-ALC-PLC group). Control animals were untreated. At the end of the treatments, livers were collected and subjected to protocols for histology and immunohistochemistry to evaluate morphology, collagen deposition, inflammation, glycative and oxidative stress. Liver histology, analysed by haematoxylin-eosin (H&E) and Azan Mallory staining revealed that PCOS mice exhibited a higher number of balloon hepatocytes with hypertrophic forms, compared to control mice; additionally, liver fibrosis was observed. The treatment with both carnitine formulations (DHEA/LC-ALC and DHEA/LC-ALC-PLC groups) had a beneficial effect on hepatocyte morphology. Androgen overexposure in PCOS mice exacerbated liver inflammation, as evidenced by increased IL-1β expression. IHC analysis confirmed IL-1β presence in mouse PCOS hepatocytes, with reduced levels in both carnitine-treated groups. MG-AGE immunoreactivity indicated an extensive glycation damage, which was mitigated by carnitine supplementation. Additionally, the marker of oxidative stress HNE was predominantly observed in PCOS hepatic lobules; the supplementation with LC-ALC showed the strongest protective effect against oxidative stress. To further investigate ultrastructural changes in liver related to PCOS, TEM studies are currently being conducted. In conclusion, our data suggest that L-carnitine supplementation may have protective effects on the morphofunctionality of organs affected by androgen excess in the PCOS, such as the liver.

REFERENCES

1. Patel S. Polycystic ovary syndrome (PCOS), an inflammatory, systemic, lifestyle endocrinopathy. J Steroid Biochem Mol Biol. 2018;182:27-36.
2. Di Emidio G, Rea F, Placidi M, Rossi G, Cocciolone D, Virmani A, Macchiarelli G, Palmerini MG, D'Alessandro AM, Artini PG, Tatone C. Regulatory Functions of L-Carnitine, Acetyl, and Propionyl L-Carnitine in a PCOS Mouse Model: Focus on Antioxidant/Antiglycative Molecular Pathways in the Ovarian Microenvironment. Antioxidants (Basel). 2020;15;9(9):867.

MOVEMENT AND NUTRITION SCIENCES

THE EFFECTS OF PHYSICAL ACTIVITY SUPPORTED BY TECHNOLOGICAL DEVICES ON GAIT ANALYSIS IN ADULTS WITH LOW BAC PAIN: A PILOT STUDY

Anna ALIOTO[1,2], Antonino PATTI[1], Giacomo BELMONTE[1], Andrea PAGLIARO[1], Alessia BOATTA[3], Domenico NUZZO[4], Antonino BIANCO[1], Patrizia PROIA[1]

[1]Sport and Exercise Sciences Research Unit, Department of Psychology, Educational Science and Human Movement, University of Palermo; [2]Physiotherapy Medical Center "Villa Sarina", Alcamo (TP); [3]Department of Human Sciences and Promotion of Quality of Life, San Raffaele, Roma; [4]Institute for Biomedical Research and Innovation, National Research Council, Palermo, Italy

Gait and balance directly impact the autonomy and quality of life of subjects with low back pain. In this context, TecnoBody® robotic devices play an active and complementary role in assessing and programming physical activity adapted to the subject's functional limitations [1]. The purpose of this study was to evaluate the effectiveness of a physical activity protocol administered and monitored through digital technology devices on gait analysis in Low back pain subjects. In total, 19 participants affected by Low Back Pain, with an average age of di 62.26±16.45, joined the study. All subjects presented impaired balance and walking at the first assessment. For each, an initial (T0) and final (T1) gait analysis was performed using the Walker view (Wv) device from Tecnobody®[2]. The integrated use of the 3D video camera allowed obtaining data inherent to the RoM of the trunk, such as anteroposterior oscillations and lateromedial tilts, the RoM of hips and knees. Flexion-extension and prone-supination movements of the foot were detected using inertial sensors that were inserted inside special socks and placed at the level of the metatarsal. Participants took part in a 4-week training protocol with a frequency of 3 times a week, lasting 45' structured as follows: 5' warm-up on Wv followed by 35' with free-body and small apparatus exercises and 5' of cool down with stretching exercises. Across a paired-sample t-test, we found statistical significance in the mean value of trunk flexion-extension ($p=0.009$); RoM of trunk lateral flexion ($p=0.010$); RoM of right foot ($p<0.01$) and left foot ($p=0.004$). In conclusion, these devices allow continuous and personalized monitoring of the subject's gait movement and posture, inducing adaptations in subjects with Low Back Pain. Therefore, the combination of physical activity and the use of hi-tech devices, such as wearable sensors, movement monitoring apps, and real-time feedback systems, offers new opportunities for the management of Low Back Pain. However, further long-term investigation is needed to assess their sustainable effects and integration into traditional exercise programs.

REFERENCES

1. Diego, P., *et al.*, *Devices for Gait and Balance Rehabilitation: General Classification and a Narrative Review of End Effector-Based Manipulators.* Applied Sciences, 2024. 14(10): p. 4147.
2. Roggio, F., *et al.*, *Technological advancements in the analysis of human motion and posture management through digital devices.* World J Orthop, 2021. 12(7): p. 467-484.

INVOLVEMENT OF GUT MICROBIOTA IN THE BENEFICIAL EFFECTS OF *OPUNTIA FICUS INDICA* FRUIT ON GLUCOSE DYSMETABOLISM IN OBESE MICE

Pasquale CALVI[1], Simona TERZO[1], Antonella AMATO[1], Marta GIARDINA[1], José-Manuel FERNÁNDEZ-REAL[2], Mario ALLEGRA[1], Flavia MULÈ[1]

[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Italy; [2]Department of Diabetes, Endocrinology and Nutrition, Institut d'Investigació Biomèdica de Girona (IDIBGI), Girona, Spain

The current epidemic of obesity and diabetes has led to a search for functional foods for the prevention of these pathologies. It is well known that fruit and vegetables, rich in phytochemicals and fibers, exert significant anti-inflammatory, anti-oxidative and probiotic effects able to ameliorate dysmetabolic conditions. Among these, *Opuntia ficus-indica* (OFI) cladodes and vegetative parts are known from a long time for health promoting properties, but OFI fruit (OFIF), that has a completely different bioactive fingerprint, has not been explored yet. The present research has been addressed to evaluate the efficacy of OFIF supplementation to counteract the obesity-induced glucose dysmetabolism in high fat diet (HFD)-fed mice. For this purpose, 16 mice were fed a HFD for 10 weeks to induce obesity and then separated into: HFD control group fed HFD and HFD+OFIF group fed HFD supplemented with OFIF, for further 10 weeks; 8 lean mice were fed standard diet (STD) for 14 weeks and they represent the negative control group. We measured body weight, food intake, fasting glycaemia and plasma insulin concentrations, glucose tolerance, insulin sensitivity, and insulin receptor protein expression in adipose tissue and liver. Moreover, a microbiome 16s rRNA analysis was performed on fecal samples collected at the end of the experimental period to characterize composition and abundance of gut microbiota in the different animal groups. The changes in bacterial genera were related to the glucose metabolism parameters by Spearman's correlation analysis. We found that in HFD+OFIF mice the fasting basal glycaemia, the glucose and insulin tolerance and HOMA-IR, index of insulin resistance were decreased, while the hepatic and adipose tissue insulin receptor expression was increased in comparison with HFD mice, suggesting that OFIF supplementation improved glucose dysmetabolism. Moreover, analysis of Microbiome Compositions with Bias Correction (ANCOM-BC) through its R packages revealed numerous bacterial genera differentially abundant between STD mice and HFD mice. However, in HFD mice, OFIF supplementation increased the presence of some bacterial genera, such as *Alloprevotella, Lachnospiraceae NK4A136 group*, and *NK4A214_group*, that our Spearman's correlation

analysis, demonstrated to be negatively correlated to the glucose dysmetabolism parameters. On the contrary, OFIF supplementation reduced the abundance of some bacterial genera (*Staphylococcus, Romboutsia, Sporosarcina, Lachnoclostridium and Enterohabdus*), correlated positively to glucose dysmetabolism. In conclusion, our research demonstrates for the first time that *Opuntia ficus indica* fruit has an euglycemic potential, counteracting the obesity-induced insulin resistance. This beneficial effect appears linked to the positive modulation of gut microbiota, because OFIF increases abundance of bacterial genera associated to beneficial effects and it decreases bacterial genera associated to harmful actions.

This work was funded by the Ministery of University and Research (MUR), project code: IRIS PRJ-1658.

A 200-DAY FOLLOW-UP STUDY OF GLUTEN DEPRIVATION ON GUT MICROBIOTA AND SYMPTOMS IN HEALTHY SUBJECTS, CELIAC DISEASE AND NON-CELIAC GLUTEN/WHEAT SENSITIVITY PATIENTS

Anna COSTANZINI[1], Lisa LUNGARO[1], Francesca MANZA[1], Michal PUNCOCHÁR[2], Federica PINTO[2], Francesco ASNICAR[2], Federica ARMANINI[2], Aurelio SEIDITA[3], Antonio CARROCCIO[3], Pasquale MANSUETO[3], Antonio CALABRÒ[4], Duccio CAVALIERI[5], Nicola SEGATA[2], Umberto VOLTA[6], Giacomo CAIO[1], Roberto DE GIORGIO[1]

[1]Department of Translational Medicine, University of Ferrara; [2]CIBIO, University of Trento; [3]Department of Health Promotion Sciences, Maternal and Infant Care, Internal Medicine and Medical Specialties (PROMISE), University of Palermo; [4]Department of Experimental and Clinical Biomedical Sciences "Mario Serio", University of Florence; [5]Department of Biology, University of Florence; [6]Department of Medical and Surgical Sciences, University of Bologna, Italy

Background and Aims: Gluten-free diet (GFD) is currently the only treatment for Celiac disease (CD) and Non-Celiac Gluten/Wheat Sensitivity (NCG/WS). Self-prescribed GFD among healthy individuals has increased worldwide, based on the unsupported belief that gluten avoidance is healthy. Conversely, evidence indicates that GFD negatively impacts living costs and psychosocial well-being and promotes malnutrition. Additionally, dietary changes can negatively affect gut microbiota, which plays a crucial role in nutrient absorption and metabolism, as well as immune and nervous systems. This study aimed at evaluating the effects of GFD on: i) gut microbiota of healthy individuals (Ctrl), CD and NCG/WS patients; and (ii) on gastrointestinal (GI) and extra-GI symptoms up to 200 days. Population and Methods: Subjects with suspected NCG/WS or CD, and Ctrl, were enrolled on a free diet. Diagnostic evaluations confirmed either NCG/WS or CD. Participants were then instructed to follow a GFD. Stool samples were collected at baseline (t0) and after 3, 7, 30, and 200 days of GFD (t1, t2, t3, t4) for patients, and up to t3 for Ctrl. The severity of symptoms (GI and extra-GI) was assessed by a modified GSRS questionnaire, while bowel habits via the Bristol stool scale. Microbiome analysis from stool was conducted through shotgun sequencing and species profiling (MetaPhlAn v4.1). α-diversity (Shannon index, SI), β-diversity, and relative abundances were assessed. Results: N=37 NCG/WS (37±16 yrs, 25 F), 11 with CD (32±15 yrs, 9 F), and 37 Ctrl (33±14 yrs, 25 F) were enrolled. At baseline (t0), the majority of NCG/WS referred mixed bowel habits (33%, Bristol: 1, 6), while 56% of CD had normal stools (Bristol: 3-5). The severity of GI symptoms was higher in NCG/WS, who also had a greater prevalence of extra-GI symptoms *vs.* CD. At t0, microbiome α-diversity was comparable across groups (p=0.19; NCG/WS: SI=4.0, CD: SI=3.7, Ctrl: SI=4.1), although β-diversity differed (p=0.026). In NCG/WS, GFD significantly improved both GI and extra-GI symptoms in the short term (t1-t3), while no improvements were observed for CD at t1. α-diversity remained stable (p>0.05) in both NCG/WS and CD participants up to t4, and Ctrl at t3. Conversely, the relative abundance of Bifidobacteria significantly decreased in Ctrl, NCG/WS and CD during the GFD. Conclusions: GFD among healthy individuals should be approached with caution. Further research into Bifidobacteria supplementation as a potential co-therapeutic strategy for treating CD and NCG/WS is eagerly awaited.

REFERENCE

Caio G *et al.*, Nutrients 2020; Beghini F *et al.*, Elife 2021; Yao S *et al.* J Immunol Res. 2021.

EVALUATION OF THE POTENTIAL ANTI-INFLAMMATORY EFFECT OF THE KETOGENIC DIET MEDIATED BY THE SYNERGISTIC ACTION OF B-HYDROXYBUTYRATE AND MCT OIL ON CACO-2 CELLS

Maria Ida DE STEFANO[1], Antonietta MONDA[2], Chiara PORRO[1], Francesca Martina FILANNINO[1], Federico ZOILA[1,3], Vincenzo MONDA[4], Antonietta MESSINA[5], Marcellino MONDA[6], Emanuele CAPASSO[7], Mario CHISARI[8], Lucio DI MAURO[9], Rita POLITO[10], Giovanni MESSINA[6]

[1]Department of Clinical and Experimental Medicine, University of Foggia; [2]Department of Human Science and Quality of Life Promotion, San Raffaele Telematic University, Rome; [3]Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona; [4]Department of Economics, Law, Cybersecurity, and Sports Sciences, University of Naples "Parthenope", Naples; [5]Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Naples; [6]Department of Experimental Medicine, Section of Human Physiology, Unit of Dietetics and Sports Medicine, University of Campania "Luigi Vanvitelli", Naples; [7]Department of Advanced Biomedical Sciences,

University of Napoli "Federico II", Naples; [8]Rodolico-San Marco Hospital Santa Sofia Street, Catania; [9]Department of Medical, Surgical and Advanced Technologies "G.F. Ingrassia", University of Catania; [10]Department of Psychology and Health Sciences, Pegaso Telematic University, Naples, Italy

Low-carbohydrate diets containing very high percentage of fat and adequate protein intake are called ketogenic diets (KD) due to their ability to stimulate the hepatic production of the ketone bodies acetoacetate (AcAc) and β-hydroxybutyrate (BHB). Ketone body production has various beneficial effects on bowel health, preferring energy sources for colonocytes, maintaining mucosal integrity, promoting satiety, suppressing inflammation and carcinogenesis. Exogenous MCTs are known to promote ketogenesis due to their rapid absorption through the portal vein and their oxidation to acetyl-CoA in the liver. Medium-chain triglycerides (MCTs) are fats composed by fatty acids with carbon chain lengths ranging from C_6 to C_{12}. MCTs are naturally found in coconut oil, dairy products like butter and goat milk and in human milk. This study aims to evaluate the anti-inflammatory synergic effects of MCT, in particular coconut oil from Cocos Nucifera, and BHB, *in vitro*, on CaCo-2 cells. These are an immortalized cell line of human colorectal adenocarcinoma cells, used primarily as a model of the intestinal epithelial barrier, on which cell viability tests, wound healing tests and ELISA tests are performed. The CaCo-2 cells were treated with 5 mM BHB and 0,5 mM MCT in the presence or absence of LPS (1µg/ml) for 24h and 48h. BHB and MCT oil increase cell viability, the migratory capacity and the production of anti-inflammatory cytokines, but the synergistic effect induces a notable improvement in the result, therefore a strengthening of the anti-inflammatory activity given by the combined action of the two substances is hypothesized. The future research proposal will consist of treating Caco-2 cells with sera from subjects who have undergone the ketogenic protocol enriched with MCT oil.

NEUROPROTECTIVE ROLE OF KUMQUAT CONSUMPTION ON NEURONAL DAMAGE IN A MOUSE MODEL OF DIET-INDUCED OBESITY

Marta GIARDINA[1], Simona TERZO[1], Pasquale CALVI[1], Mario ALLEGRA[1], Alessandro MASSARO[1], Luisa TESORIERE[1], Pasquale PICONE[2], Domenico NUZZO[2], Flavia MULÈ[1], Antonella AMATO[1,2]
[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), Palermo; [2]Istituto per la Ricerca e l'Innovazione Biomedica (IRIB), CNR, Palermo, Italy

Obesity and related metabolic dysfunctions are associated to the onset and progression of neurodegenerative disorders, such as Alzhaimer's Disease. Research on functional foods or bioactive phytochemicals with neuroprotective properties may offer novel strategies to mitigate obesity-related neurodegeneration. Kumquats are small citrus fruits known for their nutritional value and health benefits, being rich in flavonoids, polyphenols, and essential oils, specially limonene. Unlike other citrus fruits, kumquats are typically consumed whole, including the peel, which increases the intake of beneficial phytochemicals. Nevertheless, their potential role in preventing obesity-related neurological alterations remains limited. The present study aimed to investigate the potential neuroprotective effects of kumquat against obesity-induced neurodegeneration. To this purpose, male C57BL6/J mice were randomly divided into three different groups: Lean fed a standard diet (lean control), HFD fed a hyperlipidemic diet (obese control), HFD-K fed the hyperlipidemic diet supplemented with 5% of lyophilized Kumquat. After 24 weeks of different dietary treatments, brain atrophy and neurodegeneration (TUNEL assay), pro- and anti-apoptotic gene expression (RT-PCR), gene expression of Alzheimer's disease markers (RT2 Profiler PCR arrays), and insulin signalling proteins (Western Blotting) were assessed in the brain of differently fed animals. Our investigation showed that, daily Kumquat intake led to a higher brain to body weight ratio (indicating reduced cerebral atrophy), decreased number of apoptotic nuclei in cerebral cortex, a significantly down regulation of *Fas-L, Bim, and P27* expression levels (marker of neuronal apotosis) and an increase of anti-apoptotic factors (*BDNF* and *BCL2*) in comparison with HFD mice. These results highlighted the neuroprotective effects of Kumquat. The Alzheimer disease RT2 Profiler PCR arrays showed that Kumquat consumption positively modulated the expression of genes involved in cell signalling by increasing *Gnb5, Gng 4, Gng5, Gng8, Gng10, Ide, Igf2, and InsR* gene expression. Furthermore, genes associated with β-amyloid plaque generation, acetylcholine degradation and inflammation (*Apba3, Apbb1, Apoe, Gnb2, Prkcd, Bche,* and *Clu*) were downregulated in kumquat-fed mice compared to HFD mice. Additionally, our results also indicated that kumquat consumption mitigated insulin resistance in the brain, as indicated by the improved Ins-R, pAKT, pGSK3B and pSer-IRS1 protein expression levels. In conclusion, the results of the present study demonstrate that kumquat supplementation can protect the brain from HFD-induced neuronal damage. Specifically, kumquat counteracts brain cell death and neurodegeneration in obese mice, thought the reduction of neuronal apoptosis, central insulin resistance and neuroinflammation.

AÇAÍ BERRY AMELIORATES COGNITIVE IMPAIRMENT BY INHIBITING NLRP3/ASC/CASP AXIS IN STZ-INDUCED DIABETIC NEUROPATHY IN MICE

Francesca INFERRERA[1], Marika CORDARO[2], Rosanna DI PAOLA[3]
[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina; [2]Department of Biomedical, Dental and Morphological and Functional Imaging, University of Messina; [3]Department of Veterinary Science, University of Messina, Italy

Diabetic peripheral neuropathy (DPN), a common and debilitating complication of diabetes, contributes significantly to

patient morbidity and mortality. Characterized by nerve damage that causes pain, discomfort, weakness, and sensory deficits, DPN currently lacks fully effective treatments. Specifically DPN can affect different areas of the body, but the lower limbs are affected most frequently, causing muscle weakness, altered sensation and pain. In fact, when nerves in the lower limbs are affected, balance problems and difficulty walking are also encountered. Although some therapeutic agents show promise in targeting neuroinflammation, their use is often limited by adverse side effects. This study investigated the potential beneficial properties of açaí berries, a fruit known for its antioxidant and anti-inflammatory properties, in a mouse model of DPN induced by streptozotocin (STZ) injection. Both diabetic and control mice were orally administered açaí berries (500 mg/kg) daily starting two weeks after STZ injection and for 16 weeks. During the experiment, the designed groups of animals were subjected to behavioral analysis to assess motor changes typical of DPN. At the end of the study, spinal cord, sciatic nerve and urine samples were collected for analysis. Our results demonstrate that daily treatment with açaí berries effectively attenuated several key pathological features of DPN in diabetic mice. In particular, the treatment prevented the development of behavioral changes indicative of DPN. It also attenuated mast cell activation and protected against nerve fiber deterioration. Mechanistically, these protective effects appear to be mediated by regulation of the NLRP3 (NOD-like receptor family pyrin-domain-containing-3) inflammasome, including its apoptosis-associated speck-like protein containing a CARD (ASC) and caspase (CASP) components. Our results suggest that açaí berries may modulate the biochemical processes involved in DPN, particularly through inhibition of the NLRP3/ASC/CASP axis, reduction of oxidative stress, and attenuation of neuroinflammation, thus contributing to neuronal protection and improvement of cognitive and motor function.

MICROBIOTA-GUT-BRAIN AXIS FOR HEALTH: ROLE OF THE DIET

Lisa LUNGARO[1,2]
[1]Department of Translational Medicine, University of Ferrara; [2]Geriatric Unit, P. Giaccone University Hospital, Palermo, Italy

This Minilecture examines the complex interplay among diet, gut microbiota, and brain function. It illustrates how diet shapes microbial composition, influencing neurochemical pathways, immune responses, and overall brain health. Key mechanisms, such as microbial metabolites and neurotransmitter production, link gut health to cognitive function and mood regulation. Emerging research on dietary interventions enhancing gut-brain communication will be discussed, highlighting the potential for microbiota modulation in gastrointestinal disorders and mental health. The gut microbiome comprises various microorganisms (archaea, bacteria, fungi, viruses) colonizing the gastrointestinal tract mainly during childbirth and significantly contribute to human health. The microbiota-gut-brain axis represents a bidirectional cross-talk affecting the gut, its microbiota and the central nervous system. The dynamic balance is influenced by various factors, (*i.e.*, genetics, environmental, drugs and diet) and impacts behavior and emotional health via neural, immune, and endocrine signals. Omics techniques, such as metagenomics, metabolomics, and transcriptomics, enable comprehensive investigations of the disorders of the microbiota-gut-brain axis by analyzing the complex interplay of microbial genes, metabolites, and gene expression, in response to various dietary influences. Nutrition plays a critical role in shaping the microbiota-gut-brain axis. Diet influences both the gut microbiota and neurodevelopment. The microbiota-gut-brain axis is facilitated by neuroendocrine-immune pathways and short-chain fatty acids (SCFAs) from dietary fiber fermentation. These compounds regulate hormones and gut integrity, while gut bacteria produce neuroactive substances, *e.g.* GABA and serotonin, which affect behavior. Furthermore, the lecture will focus on the primary pathological conditions—such as obesity, metabolic syndrome, gastrointestinal disorders, and mental health issues—where the impact of the microbiota-gut-brain axis has been investigated. I will also cover therapeutic interventions and nutritional strategies aimed at modulating the intestinal microbiota. Given individual microbiome variability, it is important to consider dietary composition and specific metabolic potential when studying nutrient effects on behavior. Although research progresses, a complete understanding of gut barrier function linked to the microbiome is ongoing. This knowledge will influence future clinical practices based on personalized dietary interventions and accurate microbiome profiles.

REFERENCES

Schneider E, *et al. Nat Metab*. 2024;6(8):1454-1478.
Ezra-Nevo G, *et al. Curr Opin Neurobiol*. 2020 Jun;62:122-132.

PROTECTIVE EFFECTS OF LEMON NANOVESICLES: EVIDENCE OF THE NRF2/HO-1 PATHWAY CONTRIBUTION FROM *IN VIVO* HIGH-FAT DIET-FED RATS

Nicolò RICCIARDI[1], Roberta GASPARRO[2], Danila DI MAJO[1], Valentina DI LIBERTO[1], Giulia URONE[1], Monica FRINCHI[1], Giuseppe GIGLIA[1], Pierangelo SARDO[1], Giuseppe FERRARO[1], Riccardo ALESSANDRO[2,3,4], Stefania RAIMONDO[2,3], Giuditta GAMBINO[1]
[1]Department of Biomedicine, Neurosciences and Advanced Diagnostics (Bi.N.D), University of Palermo, Section of Human Physiology, Palermo; [2]Department of Biomedicine, Neurosciences and Advanced Diagnostics (Bi.N.D), University of Palermo, Section of Biology and Genetics, Palermo; [3]Navhetec s.r.l., Spinoff of the University of Palermo; [4]Institute for Biomedical Research and Innovation (IRIB), National Research Council (CNR), Palermo, Italy

The interaction between plant-derived nanovesicles (PDNVs) and mammalian cells has been extensively inves-

tigated, highlighting the ability of these natural nanovesicles to modulate various molecular pathways. Furthermore, PDNVs exhibit biological properties that make them promising candidates for treating pathological conditions, such as hepatic diseases. In this study, we investigated the antioxidant properties of lemon-derived nanovesicles isolated at industrial scales (iLNVs) in a rat model of metabolic syndrome (MetS) induced by a high-fat diet (HFD). Following 10 weeks of HFD to induce MetS, rats received daily iLNVs oral gavage supplementation for further 4 weeks until the end of experimental protocol. The effects of iLNVs on MetS-induced alteration were evaluated via biometric, biochemical and oxidative homeostasis assays in plasma samples and via molecular analyses on ex-vivo hepatic tissue. Body weight was monitored throughout the study to detect eventual differences among control, HFD and HFD-iLNVs groups. To assess the impact of nutritional treatment on glucose homeostasis we performed glucose tolerance test. Furthermore, plasma samples were gathered for subsequent analyses for the assessment of lipid homeostasis, oxidative stress parameters, and plasma antioxidant status. Additionally, hepatic samples were collected for ex-vivo evaluations after euthanasia using qRT-PCR and Western Blot. Our results indicate that iLNVs supplementation significantly ameliorates glucose tolerance and improves lipid metabolism, evidenced by reduced triglycerides and low-density lipoprotein levels, along with increased high-density lipoprotein cholesterol levels. In addition, biometric parameters improved, as demonstrated by reduced final body weight and weight gain throughout the study. Importantly, iLNVs enhanced systemic redox balance, as shown by decreased levels of primary lipoperoxides and hydroperoxides, alongside increased antioxidant defenses, including SHp and endogenous anti-ROMs. Furthermore, qRT-PCR and Western Blot analyses have shown that iLNV treatment could modulate the activation of antioxidant pathways, as evidenced by the upregulation of Nrf2/HO-1 signaling in the livers of HFD-fed rats. These findings suggest that iLNVs hold considerable potential as therapeutic agents for managing hepatic and metabolic disorders.

EFFECTS OF URBAN *VS.* RURAL ENVIRONMENT ON BODY MEASURES, STRENGTH AND LIFESTYLE ON UNIVERSITY FRESHMEN STUDENTS

Francesco SGUAIZER[1], Bianca FERRERI[1], Francesco TUFO[2], Anna MULASSO[2], Margherita MICHELETTI CREMASCO[1]

[1]Department of Life Sciences and Systems Biology, University of Torino; [2]NeuroMuscular Function Research Group, Department of Medical Sciences, School of Exercise and Sport Sciences, University of Torino, Italy

In recent years, primary prevention has adopted a multifactorial approach, recognizing that diseases result from a combination of genetic, environmental, and lifestyle factors. Primary prevention could be particularly relevant for young adults (18-25 years old), who are experiencing major life changes that may affect their overall health conditions (Castelao-Naval *et al.*, 2019; Lee & Kim, 2019). The aim of this study was to provide an overview of the physical condition and lifestyle of university freshmen students (UniTo-Wellness4Students project) and to investigate possible differences according to sex and area of residence (urban or rural). The sample consisted of 315 students (F=59%, 18 to 25 years old), including 159 from urban areas (F=62%) and 156 from rural areas (F=57%). Anthropometric characteristics such as height, weight and waist circumference were collected (ISO 7250/2017); body composition (Fat Mass, Fat Free Mass and Muscular Mass) was estimated through Classic Bioelectrical Impedance Vector Analysis (BIVA) and Handgrip Strength (HGS) was measured using a Jamar dynamometer. The quantity of physical activity (PA) was evaluated through the Global Physical Activity Questionnaire and the adherence to the Mediterranean diet (MD) using the Medi-Lite questionnaire. Subjects were categorized on the basis of body mass index (BMI – WHO 1998 cut-off) and on waist-to-height ratio (WHtR - Gibson & Ashwell, 2020 cut-off). Statistical analysis (normality tests, multiple comparisons with Bonferroni post hoc test and correlations) were conducted using IBM SPSS Statistics (version 29) with significance set at $p < 0.05$. As expected, the results highlighted significant differences between the sexes in all anthropometric traits, body composition and HGS. No differences were found between urban and rural females. However, the rural male sample had a significantly lower percentage of Fat Mass and a higher percentage of Fat Free Mass and Muscular Mass than the urban sample. Lower levels of PA were found in the female samples compared to the male samples, but significance was only reached in the rural environment. In the rural samples, BMI was correlated with PA, whereas in the urban groups, BMI was negatively correlated with sedentary lifestyle. PA was also correlated with Muscular Mass in urban males and with HGS in all samples except rural males. Adherence to MD correlated with HGS in urban females and with weight, BMI, waist circumference and WHtR in rural males. There were no differences in BMI and WHtR among groups; the prevalence of overweight and obesity in the total sample (17.5%) was similar to that of Italians aged 16-24 years (Eurostat, 2024), with lower prevalence in the rural groups. In conclusion, this study pointed out significant differences and different correlations among variables between urban and rural groups, especially for males. This finding demonstrates that, in addition to genetic factors, the environment can have a significant influence on an individual's physical condition. Following these results, we are extending the sample to older students who have moved to Turin for more years, in order to observe whether the differences found might change during the years spent in an urban context.

NEUROSCIENCE

THE "GREEN THERAPY" EFFECTS ON DEPRESSION AND ALZHEIMER' DISEASE

Marina BOIDO[1,2], Sveva DALLERE[1,2,3], Gianna PAVARINO[1,2], Claudio BRASSO[1], Anna CARLUCCIO[1,4], Silvia CHASSEUR[1,2], Giulio MENGOZZI[5], Roberta SCHELLINO[1,2], Francesca CIRULLI[6], Paola ROCCA[1,4], Alessandro VERCELLI[4]

[1]Department of Neuroscience Rita Levi Montalcini, University of Turin; [2]Neuroscience Institute Cavalieri Ottolenghi, Orbassano (TO); [3]Scuola Universitaria Superiore, Pavia; [4]Struttura Complessa Psichiatria Universitaria, Department of Neuroscience and Mental Health, Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino; [5]Department of Medical Sciences, University of Turin; [6]Center for Behavioral Sciences and Mental Health, Istituto Superiore di Sanità, Rome, Italy

Nature exposure can provide positive benefits on blood pressure, heart rate and physical stress. Moreover, spending time in natural environments can also improve cognitive functions, brain activity, mental health, and sleep. In particular, volatile organic compounds (terpenes and terpenoids) released by certain plants have been shown to possess therapeutic properties that can promote a number of positive effects, leading to improved overall health. However, the underlying biological mechanisms of action are still elusive. Within this context, we investigated the "green therapy" effects in two highly debilitating neurological diseases, *i.e.* Alzheimer's disease (AD) and Major Depressive Disorder (MDD). AD is the primary cause of dementia in aging individuals. By using both murine neural stem cells (NE-4C cell line) and human induced pluripotent stem cell (hiPSC)-derived cortical neurons, underwent Aβ1-42 monomers or oligomers administration, we investigated the neuroprotective effects of pinene (a diffuse terpene in Italy). We observed that pinene is able to counteract Aβ1-42-related apoptosis, by reducing Cleaved Caspase 3 expression, and also affected acetylcholinesterase activity as demonstrated through Ellman's assay. Concerning MDD, it is one of the most prevalent psychiatric diseases. We evaluated whether green exposure affects depressive symptoms and inflammatory biomarker levels in patients with major depressive episodes (MDE). We examined the association between exposure to green environments (large parks and city gardens) for at least 45 minutes twice a week, depressive symptoms, and inflammatory biomarkers in 31 patients with an ongoing MDE. Our results showed that after 6 weeks, greenness (together with the modification of antidepressant therapy) was associated with improved depressive symptoms, lower levels of inflammatory biomarker interleukin-6, and higher concentrations of adiponectin after six weeks of treatment. We are also evaluating the epigenetic impact of spending time in greenery by analyzing the expression of post-translationally modified histone proteins from patient PBMCs. Overall, these studies are shedding light on the mechanisms underlying the benefits derived from green exposure, thus laying the scientific basis to promote the diffusion of lifestyle interventions to prevent and treat severe neurological diseases, even representing complementary therapeutic strategies. Such information could be relevant to clinicians and urban planners.

NEW *IN VITRO* MICROFLUIDIC DYNAMIC SYSTEM TO UNDERSTAND THE CROSSTALK BETWEEN SECRETOME AND GLIOBLASTOMA CANCER MASS

Agata Grazia D'AMICO[1], Grazia MAUGERI[2], Velia D'AGATA[2]

[1]Department of Drug and Health Sciences, University of Catania; [2]Section of Anatomy, Histology and Movement Sciences, Department of Biomedical and Biotechnological Sciences, University of Catania, Italy

Glioblastoma (GBM) is a highly aggressive and challenging-to-treat brain tumor characterized by a negative prognosis due to therapeutic resistance and relapse after treatment. The therapeutic resistance could depend on innate differences in clonogenic glioblastoma stem cells (GSCs). These cells reside in specific regions of the cancer mass, forming tumor niches, which support their quiescent status and include perivascular, vascular, and hypoxic-necrotic tumor microenvironments. It was reported that the therapeutic resistance of cancer cells is related to the interactions between GSCs and the tumor microenvironment (TME), defined as a "*microenvironment-stem cell unit*". More specifically, the secretome (e.g, proteins, growth factor, and cytokines) released by GSCs in TME contributes to maintaining their pluripotent potential, conferring them self-renewal ability and exerting a pivotal role in invasiveness and intra-brain tumor spreading, as well as in the resistance to chemotherapeutic treatments. The present investigation aimed to set up an innovative *in vitro* microfluidic dynamic system to improve the understanding of secretome and cancer mass interplay. The *in vitro* microfluidic dynamic model was assessed by using the MIVO® single-organ platform to culture GBM immortalized cell lines or patient-derived GBM organoids. In the preliminary step, we have cultured immortalized cells (U87MG) in a compartment physically separated through a porous permeable membrane from the fluid flow compartment to emulate the microcirculation of secreted cells within the TME. The circulating compartment is accessible, allowing us to monitor and quantify the changes occurring in the TME. The closed-loop fluidic circuit pumps the culture medium through the lower chamber at a rate of 2.3 mL/min, simulating the capillary flow rate (0.1 cm/s), and mimicking the circulatory system disseminating the GBM secretome. Preliminary results have shown that secretome composition derived from immortalized GBM cells exposed to a hypoxic mimetic agent, deferoxamine, showed increased levels of vascular endothelial growth factor (VEGF) and interleukin (IL)-1β as compared to the control group cultured without stressor. Although our investigation represents a first step to secretome characterization, the optimization of this dynamic system could elucidate

many significant aspects of GSCs biology, characterizing their interplay with the niches where they reside. Considering that niches undergo dynamic alterations following therapeutic agents' treatment, the validation of this new system able to mirror TME could be useful for deeply understanding the behavior of GSCs during therapeutic intervention. Notably, the optimized system could be useful for performing preliminary screening of therapeutic agents.

This work was supported by European Union - NextGenerationEU, Italian Ministry of University and Research - PNRR M4C2—Action 1.4—Call "Potenziamento strutture di ricerca e creazione di "campioni nazionali di R&S". Project: "National Center for Gene Therapy and Drugs based on RNA Technology" (CN00000041).

INFLUENCE OF HIGH-GROOVE AND EMOTION-RELATED MUSIC ON CORTICOSPINAL AND INTRACORTICAL EXCITABILITY AND INHIBITION OF HUMAN MOTOR CORTEX. A TRANSCRANIAL MAGNETIC STIMULATION STUDY

Fabrizio DI GIOVANNI, Giuditta GAMBINO, Ivan GIARDINA, Pierangelo SARDO, Giuseppe GIGLIA

Università degli Studi di Palermo, Biomedicine, Neuroscience and Advanced Diagnostics Bi.N.D., Palermo, Italy

Neuroaesthetics studies have shown how music can evoke different psychological, behavioural states and even neurophysiological changes in brain activity, influenced by different features of music itself, such as beat for minutes, rhythm, interval, scales, melodic and harmonic characteristics (Stupacher *et al.*, 2012). This study investigates the effects of listening to music on corticospinal and intracortical excitability and inhibition, with the aim of evaluating whether and how different types of musical stimuli can differentially modulate the activity of the motor cortex. To this end, two stimuli were selected: Superstition by Stevie Wonder as high-groove music (Janata *et al.*, 2012) and the Symphony number 6 (first movement) composed by Ludwig van Beethoven as music that evokes the emotion of happiness in the listener (Giovannelli *et al.*, 2013). In a sample of 12 healthy volunteers, non-musicians and non-professional dancers, we measured motor evoked potentials (MEPs) recorded from the abductor pollicis brevis muscle (APB). These MEPs were elicited by single-pulse transcranial magnetic stimulation (spTMS) delivered to the primary motor cortex (M1). We assessed the input-output recruitment curve (IOC) at 100%, 110%, and 120% of the resting motor threshold (rMT) and evaluated the duration of the cortical silent period (CSP) during maximum contraction of the APB muscle. IOC is considered a reliable measure of corticospinal and intracortical excitability, while CSP allows to study intracortical GABAergic inhibitory circuits. IOC and CSP were measured during three different conditions: silence (BASELINE), while listening to high-groove music (GROOVE MUSIC) and classical music evoking happiness (HAPPINESS MUSIC). For the statistical analysis, Repeated Measures Analysis of Variance (rmANOVA) was employed to compare CSP and the IOC recorded during the three conditions. Post hoc Duncan analysis was applied where necessary to highlight potential significant differences among conditions. Our data revealed that GROOVE MUSIC effectively modulates intracortical inhibition, as evidenced by a statistically significant reduction in CSP ($p = .008$), compared to both the BASELINE and the HAPPINESS MUSIC. Since participants were instructed to remain still during the music listening sessions, this reduction in CSP may reflect a covert urge to move elicited by high-groove music, potentially linked to a decreased activity of intracortical GABAergic inhibitory circuits. No statistically significant correlations were found in the IOC evaluations across the three different conditions. These findings suggest that high-groove music can significantly reduce the activity of intracortical GABAergic interneurons, without affecting overall corticospinal and intracortical excitability. This study paves the way for a deeper understanding of the relationships between music and motor cortex excitability, which has been associated with various behavioural and perceptual processes, such as pain modulation and aesthetic experiences.

REFERENCES

Stupacher *et al.*, 2012 "Musical groove modulates motor cortex excitability: a TMS investigation."

Janata *et al.*, 2012 "Sensorimotor coupling in music and the psychology of the groove."

Giovannelli *et al.*, 2013 "The effect of music on corticospinal excitability is related to the perceived emotion: a transcranial magnetic stimulation study."

CEREBRAL ORGANOIDS AS A 3D MODEL FOR FRONTOTEMPORAL DEMENTIA: INSIGHTS INTO DISEASE MECHANISMS

Clelia FERRERO[1,2], Alessandro VERCELLI[1], Marina BOIDO[1]

[1]Department of Neuroscience "Rita Levi Montalcini", Neuroscience Institute Cavalieri Ottolenghi, University of Turin; [2]University School for Advanced Studies IUSS Pavia, Italy

Organoids represent an innovative *in vitro* model for studying the three-dimensional structure and function of developing organs. Derived from human induced pluripotent stem cells (hiPSCs), these self-organizing cultures provide a powerful alternative to traditional animal models, addressing ethical concerns while advancing research in disease modelling, drug discovery, transplantation, and personalized medicine. This study focuses on the development of cerebral organoids to model Frontotemporal Dementia (FTD), a neurodegenerative disorder characterized by progressive neuronal loss in the frontal and temporal lobes, leading to significant changes in personality, behaviour, and language. FTD is a heterogeneous disease with diverse clinical presentations and genetic causes, including mutations in

the *C9ORF72* gene, which is also implicated in amyotrophic lateral sclerosis. Using hiPSCs derived from both healthy donors and FTD patients carrying the *C9ORF72* mutation, we generated cerebral organoids. Preliminary results confirm the correct development of the organoids, with stem cells gradually differentiating into mature neurons during the first 4 weeks of culture. Indeed, the expression of stemness markers (SOX2, PAX6) decreases over time, while the expression of markers associated with mature neurons (MAP2) increases. Moreover, preliminary data suggest that FTD organoids exhibit anticipated neuronal maturation compared to controls. Consistent with this observation, brightfield microscopy-based morphometric analyses of live cultures reveal that FTD organoids are larger in the early stages of maturation but then undergo a significant reduction during the following weeks, compared to controls. Additional analyses to validate the model are still ongoing. In conclusion, by leveraging the potential of organoids to replicate human brain development and disease progression in a physiologically relevant 3D environment, this research aims to deepen our understanding of FTD at both the cellular and molecular levels. Ultimately, this approach could provide a robust platform for studying disease mechanisms and testing novel therapeutic strategies, potentially reducing reliance on animal models.

POTENTIAL ROLE OF ALPHA-SYNUCLEIN LEVELS IN PLATELETS IN GBA-RELATED PARKINSON'S DISEASE: PRELIMINARY RESULTS FROM A SINGLE-CENTRE LARGE COHORT ANALYSIS

Veronica LENTINI[1], Sara LUCAS DEL POZO[2], Giuseppe URAS[1,2], Alessia MANCA[1], Antonella PANTALEO[1], Anthony SCHAPIRA[2]
[1]Department of Biomedical Science, University of Sassari, Italy; [2]Department of Clinical and Movement Neurosciences, Queen Square Institute of Neurology, University college London, United Kingdom

Previous evidence suggests differences in systemic alpha-synuclein levels in glucocerebrosidase (GBA) mutation carriers with Parkinson's disease (PD) could exist, which might contribute to pathogenesis and be a maker for conversion to PD. We are recruiting and sustaining a patient cohort with five health statuses: GBA mutation carriers without PD; idiopathic PD (iPD); GBA-PD; Gaucher disease (GD) without PD; and healthy controls. Alpha-synuclein levels in platelets (Pt A-syn) by ELISA screening were quantified. A preliminary univariate analysis of 180 patients (48 HC, 77 iPD, 20 GBA-carriers, 32 GBA-PD and 3 GD) showed no variable was statistically associated with Pt A-syn; however, glucocerebrosidase activity in platelets (Pt_Gcase) (p=0.08), health statuses GBA-PD (p=0.08) and GD (0.09) displayed a trend towards association. Univariate analysis to predict health status exhibited expected statistical association among several clinical scales (MDS-UPDRS_II, III, Hoen&Yahr, HADS_Anxiety and Depression, RBDSQ, BDI-I) with iPD and GBA-PD. Additionally, MDS-UPDRS I was associated with iPD, GBA-PD and GBA carriers (p=0.04), and SCOPA_AUT showed association not only with iPD and GBA-PD but also with GD (p=0.02). Subsequently, a multivariate analysis to assess whether Pt A-syn was associated with health status adjusted by sex, age, cognitive status and Pt_Gcase found a significant statistical association (p=0.01). Furthermore, we explored the association of Pt A-syn in the group of PD patients (iPD and GBA-PD) with motor severity by MDS-UPDRS III. The analysis revealed a significant association of MDS-UPDRS III with Pt A-syn (p=0.01) when corrected by sex, age, cognitive status, health status, disease duration and levodopa equivalent daily dose. Interestingly, female gender (p=0.03), age (p=0.04), dementia status (p=0.001) and Pt_GCase (p=0.03) were also associated with MDS-UPDRS III. These results show a promising role of Pt A-syn as an easy-access biochemical parameter to evaluate health status and PD severity. Further studies are warranted to assess Pt A-syn role in GBA-PD.

SPACE GRAVITY SIMULATION RECAPITULATES PARKINSON'S DISEASE PATHOLOGY IN NEURAL CELL MODEL

Alessia MANCA[1], Giuseppe URAS[1,2], Veronica LENTINI[1], Amine Mohammed EL FAQIR[1], Antonella PANTALEO[1]
[1]Department of Biomedical Science, University of Sassari, Italy; [2]Department of Clinical and Movement Neurosciences, Queen Square Institute of Neurology, University college London, United Kingdom

Alpha-synuclein (α-syn) is a presynaptic neuronal protein involved in synaptic vesicle trafficking and neurotransmitter release. Its pathological aggregation into insoluble fibrils and Lewy bodies is a hallmark of Parkinson's disease (PD), contributing to neurodegeneration through mechanisms such as protein misfolding and mitochondrial dysfunction. However, the underlying mechanisms driving α-syn aggregation remain unclear, largely due to the late diagnosis of PD and the limitations of conventional *in vitro* models, which require long-term experiments to develop detectable aggregation patterns. To address this, we investigated the effects of simulated microgravity on α-syn aggregation at the cellular level, considering reports of accelerated aging following space travel. We used the SH-SY5Y cell line and a mutant clone that overexpresses an aggregation-prone form of α-syn (3K-SNCA). These cell lines were exposed to simulated microgravity by using a Random Positioning Machine at different time points, after which we quantified α-syn levels and its insoluble forms. Our results demonstrated a time-dependent increase in aggregate formation, peaking at 48 hours. Our findings suggest that microgravity enhances synuclein aggregation in a time-dependent manner. Further investigation is required to unravel the mechanisms involved. These results provide novel insights into the potential of microgravity as a model for studying Parkinson's disease and aging-related conditions, offering a platform for the rapid generation of complex cellular models.

POTENTIAL NEUROTOXICITY OF POLYLACTIC ACID MICRO AND NANOPLASTICS: EVIDENCE ON ASTROCYTE *IN VITRO* MODEL

Marianna MARINO[1], Raffaella D'AURIA[1], Martina LOMBARDI[1,2,3], Erwin Pavel LAMPARELLI[1], Giovanna DELLA PORTA[1], Andrea VIGGIANO[1], Rosaria MECCARIELLO[4], Antonietta SANTORO[1]

[1]Department of Medicine, Surgery and Dentistry, "Scuola Medica Salernitana", University of Salerno; [2]Theoreo Srl, Montecorvino Pugliano (SA); [3]European Institute of Metabolomics - Fondazione EIM, Baronissi (SA); [4]Department of Department of Physical Sciences and Wellness, University of Naples Parthenope, Italy

Microplastics (MPs) and nanoplastics (NPs) are considered emerging pollutants posing risks to both the environment and human health. MPs and NPs can enter the human body by penetrating biological barriers and translocate to the brain through the bloodstream causing histopathological alterations, and impaired neurological function [1-2]. Previous reports have mainly emphasized the evaluation of potential neurotoxicity related to petroleum-based MPs/NPs. Until now, little is known on MPs/NPs derived from biodegradable plastics, such as those based on polylactic acid (PLA). Therefore, the aim of the current study was to evaluate the potential impact of PLA MPs/NPs on astrocytes, a brain cell population highly sensitive to external stimuli. For this purpose, rat glioma C6 cells were used. For the experimental setup, PLA-MPs and NPs conjugated with Rhodamine of an average size of 275 ± 75 nm and 170±58 nm size respectively, were synthesized through a microfluidic-assisted nanoprecipitation technique [3]. Two complementary techniques were used to monitor cellular uptake: flow cytometry and immunofluorescence (IF). Internalization analysis was performed by treating cells at different concentrations (ranging from 10 to 300 μg/mL) and different exposure times (24 – 48hs). Notably, the highest value of cells population positive to PLA-MPs was less than 15 %. In contrast to larger PLA-MPs, C6 cells efficiently internalized PLA-NPs, with an average of 80% of positive cells already at the lowest concentration and time point tested. IF analysis showed mainly a perinuclear and intracytoplasmic distribution for both PLA MPs and NPs, with some extracellular aggregates detected for PLA MPs. To better understand whether the high PLA-NPs uptake could impact cell viability or proliferation, we performed MTT and BrdU assays. Results showed that neither viability nor proliferation were affected. However, a specific ELISA immunoassay demonstrated a reduction in the vascular endothelial growth factor type alpha (VEGF-α), a protein whose down-regulation correlates positively with increased intracellular ROS. To further investigate this issue, we evaluated protein expression levels of protein kinase B (pAKT) and glial fibrillary acidic protein (GFAP). Western blot analysis revealed increased expression of these proteins in treated cells, corroborating a pro-inflammatory response of astrocytes exposed to PLA-NPs. Overall, our findings suggest that PLA-NPs exposure may induce the onset of an inflammatory state in astrocyte highlighting potential neurotoxic effects.

ACKNOWLEDGEMENTS AND FUNDING. This work is under the support of the National Recovery and Resilience Plan (NRRP), Mission 4, Component 2, Investment 1.1, "Fund for the National Research Program and for Projects of National Interest (NRP)" by the Italian Ministry of University and Research (MUR), funded by the European Union – NextGenerationEU. Project title: "Plastic Contamination by Poly(Lactic Acid) (PLASTAMINATION): organ injuries and underlying molecular mechanisms", MUR, PRIN-PNRR2022 CODE NUMBER: P2022AA47Y- CUP D53D23021910001.

REFERENCES

1. Santoro A. *et al.*, Curr Neuropharmacol. 2024; 22(11): 1870–1898 PMID: 38549522.
2. Liu S. *et al.*, J Hazard Mater. 469:134054, 2024 doi: 10.1016/j.jhazmat.2024.134054.
3. Lamparelli EP *et al.*, Int J Pharm. 667(Pt B):124934. 2024 doi: 10.1016/j.ijpharm.2024.124934.

IRON DYSHOMEOSTASIS AND MITOCHONDRIAL FEATURES' ALTERATION IN THE EARLY PHASE OF ALZHEIMER'S DISEASE IN 5XFAD MOUSE MODEL

Mariarosa MEZZANOTTE[1], Javier CHICOTE[1], Noemi SCIMIA[1], Chiara RIGANTI[2], Paolo Ettore PORPORATO[3], Serena STANGA[1]

[1]Neuroscience Institute Cavalieri Ottolenghi, Department of Neuroscience Rita Levi Montalcini, University of Turin; [2]Department of Oncology, Oncological Pharmacology Lab, University of Turin; [3]Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center, University of Turin, Italy

Iron is an essential element that plays a key role in regulating different biological processes, such as oxygen transfer, cell growth, DNA synthesis and repair. It is important for neuronal activity and neurotransmitters' synthesis. Intracellularly, mitochondria are the center of iron utilization for the production of cofactors necessary for redox reactions, energy metabolism, especially for ATP production by the electron transport chain (OXPHOS)[1]. We demonstrated that iron dyshomeostasis occurs during aging[2] and accumulating evidence reported that perturbation in iron homeostasis impacts mitochondrial function in neurodegenerative diseases, including Alzheimer's disease (AD), leading to energy failure and contributing to neuronal death[3]. However, how iron-induced mitochondrial dysfunction participates to AD remains unknown. Here, we investigated if iron homeostasis in 5xFAD mouse model, expressing five familial human AD mutations, is altered during the pre-symptomatic phase of the disease. We found consistent brain iron deposits in 2-month-old 5xFAD mice especially in the subcortical and striatal regions, verified by Prussian blue Perl's staining together with an initial accumulation of intracellular amyloid beta. Being mitochondria an important site for iron trafficking and utilization, we isolated enriched mitochondrial fractions from 5xFAD brains and we observed a significant increase in the

expression of the mitochondrial import receptor subunit TOM20, mitochondrial aconitase and all the complexes of the OXPHOS. Interestingly, we found a significant increase of mitochondrial ferritin, the iron stock within mitochondria. However, the increase of the number of mitochondria is not due to *de novo* biogenesis, verified by Cytochrome b, NADH dehydrogenase 1 and Peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1alpha genes' expression, but to mitochondrial accumulation. Indeed, we observed that the levels of the autophagic marker SQSTM1/p62, a cargo protein present in the membrane of the autophagosome are drastically decreased. We also measured the mitophagosome initiation process by evaluating Serine/threonine-protein kinase (ULK1) and its phosphorylation on Serine 555. Strikingly, our results showed a significant decrease in the ULK1 phosphorylation in the striatal region. Altogether indicating that the autophagic pathway is compromised in 5xFAD. Interestingly, accumulated mitochondria showed a significant reduction in ATP production and increased mitochondrial lipoperoxidation (mt-HNE), DNA (mt-8OH-dG) and proteins oxidated (CO-prot) signs of oxidative stress. Collectively, our data show early alterations in brain iron metabolism together with perturbations in mitochondria turnover and function in 5xFAD mice suggesting mitochondrial brain iron an important target for amyloid pathology.

This work is supported by MIUR project "Dipartimenti di Eccellenza 2023–2027" to Department of Neuroscience "RitaLevi Montalcini", Fondazione CRT "Richieste Ordinarie 2021", UNITO and UNITA grants for Internationalization 2022 to Serena Stanga.

REFERENCES

1. Zhao Y, J Transl Med. 2024, doi: 10.1186/s12967-024-05740-4.
2. Mezzanotte M, Sci Rep. 2022, doi:10.1038/s41598-022-15812-4.
3. Mezzanotte M, Aging Dis. 2024, doi: 10.14336/AD.2024.0094.

THE LANGUAGE OF SPACE: WHERE IS "THIS" AND WHERE IS "THAT"

Umberto QUARTETTI[1], Giuditta GAMBINO[1], Filippo BRIGHINA[1], Fabrizio DI GIOVANNI[1], Giulio MUSOTTO[2], Danila DI MAJO[1], Giuseppe FERRARO[1], Pierangelo SARDO[1], Giuseppe GIGLIA[1]

[1]Department of Biomedicine, Neuroscience and Advanced Diagnostics, Section of Human Physiology, University of Palermo; [2]Bioengineering Unit, Ri.MED Foundation, Palermo, Italy

According to the *Grounded Cognition* theory, it has been hypothesized that a language-cognitive system interaction is involved in the comprehension of terms semantically related to a specific domain. In this study, our aim was to evaluate whether visuospatial coding influences the semantic understanding of space-related words in a dissociated manner for peripersonal and extrapersonal space, and if the right posterior parietal cortex (PPC) has a causative role. To explore this hypothesis, we developed a virtual reality environment using Unity3D and delivered it through an Oculus Rift S VR headset. Three-dimensional images of words pairs were randomly shown in peripersonal (60 cm) or extrapersonal (120 cm) space. Subjects underwent a lexical decision task, with word pairs consisting in a pseudoword and a word semantically related to near (*e.g.* this) or far space (*e.g.* that), in a baseline condition (NO TOOL) or with a tool (TOOL), considering that tool use can modulate the perceived boundaries between peripersonal and extrapersonal space. In a separate session (at least one week apart) subjects underwent the same task, following a cathodal tDCS over right PPC or a dual tDCS stimulation (anodal over left PPC, cathodal over right PPC). Vocal Reaction Times (RTs) were collected and analyzed through rmANOVA to compare far and near semantics, considering: 2 conditions (TOOL *vs* NO TOOL), and 2 positions (far *vs* near) in baseline, cathodal and dual tDCS stimulations. The key findings of our study indicate that, in the baseline condition, RTs were significantly shorter when near-semantically related words were presented in peripersonal space compared to far-semantically related words. Additionally, tool use significantly reduced RTs for near-semantically related words presented in extrapersonal space compared to far-semantically related words. Notably, cathodal tDCS reversed this effect, increasing RTs for near-semantically related words when a tool was used. In contrast, dual tDCS globally reduced RTs, regardless of spatial position, semantic category, or tool use. In conclusion, our findings suggest that visuospatial coding plays a role in processing near- and far-related semantics, both with and without the presence of a virtual tool, and that the right PPC has a causal role in this mechanism. The overall reduction in RTs following dual tDCS may reflect an enhancement in reading performance, likely due to increased excitability of the left PPC and decreased inter-hemispheric inhibition via reduced excitability of the right PPC.

REFERENCES

1. Pulvermüller F. (2021) Biological constraints on neural network models of cognitive function. Nat Rev Neurosci. doi:10.1038/s41583-021-00473-5
2. Giglia G. (2015). Far space remapping by tool use: A rTMS study over the right posterior parietal cortex. Brain Stimul. doi:10.1016/j.brs.2015.01.412.
3. Giglia G. (2011). Neglect-like effects induced by tDCS modulation of posterior parietal cortices in healthy subjects. Brain Stimul. doi:10.1016/j.brs.2011.01.003.

THE STRESS EFFECT ON AMYOTROPHIC LATERAL SCLEROSIS PATHOGENESIS IN DISEASE-PREDISPOSED CONDITIONS

Daniela Maria RASÀ[1,2,3], Ilaria STOPPA[2], Noémie BÉRENGER-CURRIAS[4], Elena PASHO[5], Sorana CIURA[5], Edor KABASHI[5], Cécile MARTINAT[4], Marina BOIDO[1,2]

[1]Department of Neuroscience "Rita Levi Montalcini", University of Turin, Italy; [2]Neuroscience Institute Cavalieri Ottolenghi, University of Turin, Orbassano (TO), Italy;

[3]*University School for Advanced Studies IUSS Pavia, Italy;* [4]*Institut National de la Santé et de la Recherche Médicale (INSERM/UEPS) UMR 861, Université d'Evry/ Paris Saclay, I-Stem, AFM 91100 Corbeil-Essonnes, France;* [5]*Imagine Institute, INSERM UMR 1163, Team Translational Research for Neurological Diseases, Paris Descartes University, Paris, France*

Amyotrophic Lateral Sclerosis (ALS) is a motor neuron (MN) disease due to the progressive degeneration of both upper and lower MNs, with consequent muscle atrophy, weakness and premature death. Its pathogenesis is characterized by several cellular dysfunctions, including mitochondria alterations, protein accumulation, inflammation, aberrant proteostasis, DNA and RNA alterations, and neuronal dysfunctions and death. Here we investigated if a stressful lifestyle might exacerbate the above-mentioned altered mechanisms and affect the disease pathogenesis in ALS-predisposed conditions. To this aim, we exploited different experimental approaches, both *in vivo* and *in vitro*. First, h*SOD1*^{G93A} mice underwent a chronic unpredicted mild stress protocol to study the stress effects *in vivo*. We observed a significantly worsened weight increase and motor behavior of stressed mice, in a gender-specific manner. In addition, by performing RT-qPCR in the motor cortex and spinal cord, we highlighted a significant deregulation of *Col1a1* and *Col1a2* in females, and *Il6* in males. Then, to evaluate the stress impact specifically on MNs, NSC-34 h*SOD1*^{G93A} cells underwent oxygen and glucose deprivation (OGD): upon stress, we observed a reduced capability of MNs to endure and respond to stress, compared to NSC-34 h*SOD1*^{WT}. Then, by performing gene expression, protein-protein interaction, gene ontology and pathway enrichment analyses, we revealed the pivotal roles of the PI3K/Akt and focal adhesion pathways (triggered by the expression of *Gsk3b*, *Il6*, *Igf1* and/or *collagen*) in mediating stress response. Finally, hiPSCs-derived ALS MNs (*TARDBP*^{G298S} mutation) were stressed by OGD and compared to healthy hMNs, providing similar results to those observed in NSC-34 cells. In conclusion, the PI3K/Akt and focal adhesion pathways seem to play a crucial role in stress response in different ALS-predisposed models: the study paves the way for novel therapeutic targets and highlights the relevance of a healthy lifestyle.

BEYOND THE MOTOR NEURON: EXPLORING THE DYNAMICS OF CORTICAL PROJECTION NEURONS IN SMA DISEASE

Roberta SCHELLINO[1,2], Giulia IEZZI[1,2], Anna CARETTO[1,2], Federica LA ROCCA[1,2], Marina BOIDO[1,2], Alessandro VERCELLI[1,2]

[1]*Department of Neuroscience "Rita Levi Montalcini", University of Turin;* [2]*Neuroscience Institute Cavalieri Ottolenghi, University of Turin, Italy*

Spinal Muscular Atrophy (SMA) is a severe neurodegenerative disease of the early childhood, caused by the mutation/deletion of the survival motor neuron (SMN1) gene. The lack of functional SMN protein determine the degeneration of lower motor neurons (MNs) in the spinal cord; however, recent research in animal models and patients revealed that the brain is also impacted by SMN deficiency. Spinal MN degeneration can be influenced by pathological changes in the cortex, thus the involvement of cortical alterations in SMA needs to be clarified. In this work we are investigating the sensorimotor cortex of SMAΔ7 mice, a severe SMA model, to examine the effect of SMN deficiency on cortical projection neuron survival and morphology. We analyzed early (postnatal day 5) and late (P11) symptomatic animals, comparing SMA mice with their wild-type (WT) littermates, and used immunofluorescence to identify projection neuron subtypes and retrograde tracers for morphological analysis. We found that both corticospinal (Ctip2-positive) and callosal (Satb2-positive) neurons are selectively reduced at P11 in SMA cortex, suggesting that SMN reduction affects upper MNs as well. These two projection neuron populations also show alteration in some morphological traits in SMA condition, such as a reduction in soma size and in dendrite length and complexity. Moreover, dendritic spines in SMA projection show a less mature phenotype, with a higher percentage of filopodia and stubby spine types. Interestingly, although the same analyses at P5 suggest that cortical cell death concurrently with spinal MN death, the morphological changes described above already appear at the early stage of the disease, suggesting that specific populations of cortical projection neurons are more sensitive to the absence of SMN from the beginning. Thus, the detection of possible precocious signs of cellular stress and dysfunction in the cerebral cortex, in SMA pathology, can be crucial to provide vital insights into disease progression. Indeed, tracking down precocious alterations can serve as a valuable landmark in assessing cell susceptibility to uncover causes rather than disease's symptoms. Overall, the identification of cellular and molecular alterations in the cortex may lead to the identification of potential therapeutic targets that may mitigate the upstream effects contributing to motor neuron degeneration. Ultimately, a comprehensive understanding of cortical involvement in SMA can pave the way for innovative strategies aimed at preserving MN function and improving patient outcomes.

NEUROPROTECTIVE AND ANTIOXIDANT PROPERTIES OF OXOTREMORINE-M, A NON-SELECTIVE MUSCARINIC ACETYLCHOLINE RECEPTORS AGONIST, IN CELLULAR AND ANIMAL MODELS OF ALZHEIMER DISEASE

Miriana SCORDINO[1], Giulia URONE[1], Monica FRINCHI[1], Domenico NUZZO[2], Marta DI CARLO[2], Natale BELLUARDO[1], Giuseppa MUDÒ[1], Valentina DI LIBERTO[1]

[1]*Department of Biomedicine, Neuroscience and Advanced Diagnostics, University of Palermo;* [2]*Institute for Biomedical Research and Innovation (IRIB), National Research Council (CNR), Palermo, Italy*

Alzheimer disease (AD) is a multifactorial and age-dependent neurodegenerative disorder classically associated with the

formation of senile plaques and neurofibrillary tangles, and characterized by oxidative stress and neuroinflammation chronicization. Since previous investigations demonstrated that Oxotremorine-M (Oxo), a non-selective muscarinic acetylcholine receptors agonist, produces neurotrophic effects in primary neurons and downregulates basal oxidative stress and neuroinflammation in rat brain, in this study we explored Oxo neuroprotective effects in cellular and animal models of AD. In differentiated SH-SY5Y neuroblastoma cells, Oxo treatment upregulates HSP70 expression, which in turn enhances SOD1 expression and cell survival against oxidative stress-driven cell death. When SH-SY5Y cells are exposed to $A\beta_{1-42}$ peptide, Oxo treatment is able to recover cell survival, neurite length and to counteract DNA fragmentation induced by the neurotoxic peptide. An in-depth investigation into the mechanisms involved in Oxo-induced neuroprotection revealed that Oxo treatment blocks ROS production and recovers mitochondrial dysfunction and SOD activity impaired by $A\beta_{1-42}$ peptide. Similarly, in $A\beta_{1-42}$ injected rats, Oxo treatment a) counteracts oxidative stress by lowering ROS and lipids peroxidation amount, and by increasing SOD activity levels; b) counteracts microglia activation, downregulates pro-inflammatory cytokine levels, including IL-1β and IL-6, and upregulates anti-inflammatory IL-10 levels; c) recovers the impairment in cognitive performances.
Altogether these results suggest that Oxo, by modulating cholinergic neurotransmission, survival, oxidative stress response, mitochondria functionality and neuroinflammation, may represent a novel multi-target drug able to achieve a therapeutic synergy in AD.

A ROLE FOR DYSFUNCTIONAL MITOCHONDRIA IN ALS

Serena STANGA[1,2,4], Deepti LALL[2], Frank DIAZ[2,3], Elisabeth DI LULLO[4], Mariarosa MEZZANOTTE[1], Javier CHICOTE[1], Noemi SCIMIA[1], Sergio HERNANDEZ[2], Alba SANSA[2], Alain PROCHIANTZ[4], Clive N. SVENDSEN[2]
[1]Department of Neuroscience "Rita Levi Montalcini", Neuroscience Institute Cavalieri Ottolenghi, University of Torino, Italy; [2]Department of Biomedical Sciences, Regenerative Medicine Institute, Cedars Sinai, Los Angeles (CA), USA; [3]Department of Neurology, Cedars Sinai, Los Angeles (CA), USA; [4]BrainEver and Center for Interdisciplinary Research in Biology (CIRB) UMR 7241 - U1050, Paris, Île-de-France, France

Motor neuron diseases (MNDs) are progressive and multifactorial pathologies defined by loss and death of both upper and lower motor neurons (MNs), with consequent muscle weakness and wasting, loss of skeletal muscle movements, and spastic paralysis. Because of high energy requirements, neuronal and muscle cells are enriched in mitochondria which play a central role in the maintenance of cellular homeostasis. Amyotrophic Lateral Sclerosis (ALS) is the most common adult-onset MND for which there is no effective treatment.
Extensive research on mitochondria is ongoing in the field[1], since mitochondria dysfunctions may be one trigger for the neuronal decline. Indeed, mitochondrial oxidative stress and defective axonal transport of mitochondria are some of the earliest neuropathological features observed in ALS. However, how mitochondrial dysfunctions promote neuronal degeneration is not clear from a mechanistic standpoint. This study aims to improve the knowledge of pathological mechanisms related to mitochondria dysfunctions to facilitate the recognition of early disease manifestations and ultimately identify new therapies. We used biochemical and imaging approaches to study human post-mortem brains as well as cortical and spinal MNs derived from induced pluripotent stem cells of ALS patients and controls. We aim to understand the heterogeneity in ALS to define mitochondria-related mechanisms of neurodegeneration and test the capability of a diverse pharmacological arsenal (homeoproteins, antioxidants, iron supplementation) to recover mitochondrial functionality and mobility. Preliminary data show that the ALS motor cortex is enriched in iron deposits. Further, we show that healthy control iPSC-derived cortical neurons are resistant to high concentrations of iron, while ALS-patient derived MNs show a clear dysfunction at the level of mitochondria. We have also shown that the human recombinant homeoprotein Engrailed 1 is able to promote survival following toxic insult to the MN cultures. Collectively, this data indicates that mitochondria dysfunction is involved with MNDs and represent a promising therapeutic target.
This work is supported by UNITA Grants for Internationalization 2022, IBRO Collaborative Research Grant program 2022 and MUR-Next Generation EU-PRIN2022 Prot. 20225BMTWJ granted to SS; BrainEver funds to DLE and PA; Svendsen Lab.

REFERENCE

1. Stanga S. *et al.*, 2020 IJMS doi: 10.3390/ijms21103719.

MONOCYTES ALPHA-SYNUCLEIN AND LIPIDS AS PREDICTORS OF PARKINSON'S DISEASE

Giuseppe URAS[1,2], Sara LUCAS DEL POZO[2], Veronica LENTINI[1], Alessia MANCA[1], Pierluigi CABONI[3], Cristina MANIS[3], Antonella PANTALEO[1], Anthony SCHAPIRA[2]
[1]Departiment di Scienze Biomediche, Universita' degli Studi di Sassari; [2]Department of Clinical and Movement Neurosciences, Queen Square Institute of Neurology; [3]Dipartimento di Scienze della Vita e dell'Ambiente, Università di Cagliari, Italy

A chronic pro-inflammatory pattern is present in Parkinson's disease (PD) patients' brain and in peripheral blood, with evidence for a crosstalk between central nervous system and peripheral immune cells. Monocytes display elevated alpha-synuclein (A-syn) levels intracellularly. GBA1mutations, the major genetic risk factor for PD, also modify GCase expression in phagocytic cells. To study the role monocytic A-syn and lipids as biomarkers of PD. We recruited a cohort of patients: GBA carriers without PD (GBA-NMC), idiopathic PD (iPD), GBA carriers with PD (GBA-PD) and healthy con-

trols (HC). Monocytes from 241 patients were screened by ELISA and 4-MUG assay to quantify A-syn levels and GCase activity. We also performed untargeted lipidomic by mass spectrometry in 100 monocytes (25 each group). Preliminary univariate analysis did not reveal significant changes between groups for A-syn levels and GCase activity. When analysing presence of PD (iPD + GBA-PD), logistic regression analysis revealed a significant association between levels of A-syn and PD (p=0.025). Subsequent multivariate logistic regression analysis showed a significant association between PD diagnosis and A-syn (P=0.022; AUC=0.69). Regarding lipidomics, a significant increase was found in GluCeramide (GluCer) 16:0 and 22:0 in GBA-PD compared to HC. GluCer 24:1 was increased in iPD only. Concerning GCase substrates, a only positive trend was present in GBA-PD and iPD. Comparison between GBA-PD and GBA-NMC showed a significant increase in GBA-PD of 3 substrates: GluCer 16:0, 20:0, and 22:0. GBA-PD presented a significant increase in Glusphingosine (GluSph) compared to HC. Grouping lipids according to genotype did not identify differences. A regression analysis showed a significant association between GluCer 20:0 and GluSph in GBA-PD *vs* GBA-NMC (p=0.044 and p=0.015, respectively), with an AUC=0.81 for GluCer 20:0 and 0.84 for GluSph. The lipidomic analysis identified differences between GBA-PD and HC, and also major differences in various GCase substrates and GluSph between GBA-PD and GBA-NMC. No differences were found in substrates in PD groups, which is in line with GCase activity results obtained. These preliminary results indicate GluCer 20:0 and GluSph levels could represent a potential PD biomarker in GBA carriers. A-syn and selected GluCer species in monocytes could be exploited as potential marker for PD.

NEUROPROTECTIVE POTENTIAL OF INDOLE-BASED COMPOUNDS: A BIOCHEMICAL STUDY ON ANTIOXIDANT PROPERTIES AND AMYLOID DISAGGREGATION IN NEUROBLASTOMA CELLS

Mariapia VIETRI[1], Tania CIAGLIA[1], Maria Rosaria MIRANDA[1,2], Simone DI MICCO[3], Geraldina SMALDONE[1], Simona MUSELLA[1], Veronica DI SARNO[1], Giulia AURIEMMA[1], Carla SALDO[1], Ornella MOLTEDO[1], Giacomo PEPE[1,2], Giuseppe BIFULCO[1], Carmine OSTACOLO[1], Pietro CAMPIGLIA[1], Michele MANFRA[4], Vincenzo VESTUTO[1], Alessia BERTAMINO[1]

[1]Department of Pharmacy, University of Salerno, Fisciano (SA); [2]NBFC—National Biodiversity Future Center, Palermo; [3]European Biomedical Research Institute of Salerno (EBRIS), Salerno; [4]Department of Health Science, University of Basilicata, Potenza, Italy

Alzheimer's disease (AD) is a complex neurodegenerative disorder driven by multiple interconnected pathological mechanisms, including oxidative stress (OS), mitochondrial dysfunction, neuroinflammation, neuronal loss, and aberrant protein aggregation. Given this complexity, multitarget therapeutic strategies are gaining interest in addressing the various pathways involved in AD progression. In this study, a series of novel indole–phenolic derivatives were synthesized and evaluated for their neuroprotective potential. These compounds demonstrated a combination of antioxidant, metal-chelating, and amyloid-disaggregating properties. The *in vitro* neuroprotective effects were assessed in SH-SY5Y cells subjected to oxidative damage induced by Aβ(25–35) peptide and hydrogen peroxide. The compounds exhibited significant antioxidant and cytoprotective activity, enhancing cell viability by approximately 25% while restoring ROS levels to basal conditions. Their metal-chelating ability was particularly notable for copper ions, with quantitative analyses revealing around 40% chelation efficiency across all tested compounds. Additionally, Thioflavin T fluorescence assays, circular dichroism, and computational modeling confirmed the compounds' ability to destabilize and disaggregate Aβ(25–35) aggregates. Molecular docking and dynamics simulations further supported these findings, demonstrating that the synthesized derivatives interact with Aβ(25–35) via stable hydrogen bonds, salt bridges, and van der Waals interactions. Notably, computational simulations suggested that these interactions persist over a 50 ns period, contributing to structural destabilization of the amyloid fragment. Collectively, these results highlight the potential of indole–phenolic derivatives as promising candidates for the development of multitarget AD therapies. Their combined chelating, antioxidant, and anti-aggregation activities position them as effective neuroprotective agents. However, further *in vivo* investigations and pharmacokinetic analyses are necessary to validate their therapeutic potential, particularly in terms of blood–brain barrier permeability and long-term efficacy.

MITOCHONDRIAL TURNOVER IS IMPAIRED IN SPINAL MUSCULAR ATROPHY MOTONEURONS: MITOCHONDRIAL DYNAMISM STUDY AND PRELIMINARY RESULTS OF A MFN2 EXPRESSION ACTIVATOR DRUG

Francesco Paolo ZUMMO[1], Maria BELTRAN[2], Francesco FEDELI[2], Letizia PALADINO[1], Serena STANGA[3], Fabio BUCCHIERI[1], Rosa Maria SOLER[2], Marina BOIDO[3]

[1]Department of Biomedicine, Neurosciences and Advanced Diagnostics, University of Palermo, Italy; [2]Neuronal Signaling Unit, Experimental Medicine Department, Universitat de Lleida-IRBLleida, Lleida, Spain; [3]Department of Neuroscience "Rita Levi Montalcini", Neuroscience Institute Cavalieri Ottolenghi, University of Turin, Italy

Mitochondria are unique organelles that ancestrally arise from an endosymbiotic event involving an anaerobic bacterial cell and an α-Proteobacterium and contain elements of the respiratory chain. This explains why mitochondria are often referred to as the powerhouse of the cell, and why they can undergo fusion and fission, modifying the network they form within cells. This behavior, known as mitochondrial dynamism, governs both their morphology and function, as the fusion-fission balance is crucial for the mitochondrial

turnover. Fission commits damaged mitochondria to mitophagy, while fusion promotes their biogenesis. Abnormalities in mitochondria have been already described in Spinal Muscular Atrophy (SMA), a severe neuromuscular disorder caused by loss-of-function mutations in the *SMN1* (Survival Motor Neuron 1) gene. Studies on several SMA models have shown a reduced content of fragmented mitochondria, which is associated with impaired mitochondrial respiration and decreased ATP production. In this study we examined the mitochondrial dynamism state in motor neuron (MN) soma and neurites, by using both SMA patients induced pluripotent stem cell (iPSC)-derived MNs, and primary MNs from the SMNΔ7 mouse model. Western blot analysis of two mitochondrial content markers, TOM20 and LONP1, highlighted a significant reduction in mitochondrial mass in both SMA human and mouse MNs, respectively, compared to healthy MNs. Furthermore, immunofluorescence analyses revealed a decreased distribution of TOM20 and LONP1 in the neurites of SMA MNs. Interestingly, western blot analysis of fission (Drp1) and fusion (Mfn2, OPA1) regulators indicated an increased fission state, localized specifically in the neurites by performing an in-silico study of the mitochondrial network (MiNA). Based on these results, we also investigated the effects of a compound acting as a Mitofusin 2 (Mfn2) expression activator to promote mitochondrial fusion. Our preliminary results about the Mfn2 expression activator drug indicated its ability to increase also LONP1 protein levels. This evidence confirms that regulating fusion-fission balance impacts mitochondrial turnover, enhancing mitochondrial mass and biogenesis. Our results suggest that there is a mitochondrial turnover impairment in the neurites of SMA MNs, where enhanced fission may lead to aberrant mitophagy, while stimulating fusion could preserve mitochondrial mass depletion.

REGENERATIVE BIOMEDICINE

CHARACTERIZATION OF HEPATIC MARKERS IN HUMAN HEPATOCYTE-LIKE CELLS DERIVED FROM WHARTON'S JELLY MESENCHYMAL STEM CELLS

Giusi ALBERTI[1], Melania LO IACONO[1], Simona CORRAO[2], Eleonora RUSSO[3], Francesca RAPPA[1,4], Giampiero LA ROCCA[1]

[1]Department of Biomedicine, Neurosciences and Advanced Diagnostics (BiND), University of Palermo; [2]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo; [3]Departmental Faculty of Medicine, Saint Camillus International University of Health Sciences, Rome; [4]The Institute of Translational Pharmacology, National Research Council of Italy (CNR), Palermo, Italy

Liver transplantation is considered the best choice for patients with end-stage liver disease, although the shortage of organ donors and immune rejection after surgery represent major concerns. The human Wharton's Jelly-derived Mesenchymal Stem Cells (hWJ-MSCs) can exert liver tissue repair and regeneration, either through a pre-differentiation step or as naïve cells[1]. Indeed, WJ-MSCs feature key immunomodulatory properties and multipotential differentiation characteristics as shown in *in vitro* and *in vivo* studies[2]. Hepatocyte-like cells (HLCs) derived from WJ-MSCs have gene expression profiles similar to those of hepatocytes and perform several hepatocyte-like functions that may promote the recovery of damaged liver function[3]. In this study, we performed a specific differentiation protocol of WJ-MSCs into HLCs, followed by their characterization using different techniques and functional assays to highlight the features related to liver function, hepatic expression profile, and immunogenic properties. METHOD. Expression analysis of typical mesenchymal stromal cell markers in naïve WJ-MSCs maintained in 2D culture (such as CD29, CD73, CD90, CD105 and others) was assessed by flow cytometry (FC). The expression of liver-specific epithelial cytokeratins, namely CK-8, CK-18, CK-19; of hepatocyte markers such as HNF-4α and albumin; of hepatocyte-specific connexins 32 and 43, and of four CYP450 molecules (*i.e.*, CYP3A4, CYP2B6, CYP3A7 and CYP7A1) was assessed at protein level by both immunocytochemistry and FC. Study of the morpho-functional change of the HLCs compared with control WJ-MSCs was performed through PAS staining. RESULTS. We detected the expression of hepatocyte-specific molecular markers related to the differentiation of naïve WJ-MSCs, namely some liver-specific epithelial cytokeratins (in particular CK-18), of HNF-4α (cytoplasmatic and nuclear staining), albumin, and connexins 32 and 43. Routine microscopy imaging confirmed that HLCs displayed a polygonal shape (similar to that of hepatocytes), while control WJ-MSCs maintained their fibroblast-like morphology in the third and fourth weeks of the differentiation process. PAS staining further confirmed this morphological switch as well as the deposition of intracellular glycogen in HLCs. After treatment with rifampicin, we showed a significant increase in the expression of CYP3A4, CYP3A7 and CYP2B6 in HLCs compared to controls, while the expression of CYP7A1 was not significantly different. CONCLUSIONS. The specific morphofunctional evaluation of the phenotype of HLCs cells allows us to affirm the differentiation potential of WJ-MSCs. This is potentially useful in liver repopulation strategies for specific diseases and, more generally, in regenerative medicine approaches.

REFERENCES

1. Wen, F., *et al.* Stem Cell Res. Ther. 2024, 15, 320.
2. La Rocca, G., *et al.* Curr. Stem Cell Res. Ther. 2013, 8, 100-113.
3. Xie Y., *et al.* Front Cell Dev Biol. 2021, 9, 765980.

SPONTANEOUS NEURONAL DIFFERENTIATION OF SH-SY5Y CELLS USING A CONDUCTIVE POLYMER SUBSTRATE FOR NEURAL TISSUE ENGINEERING APPLICATIONS

Luana Vittoria BAUSO[1], Paola CAMPIONE[2], Grazia Maria Lucia MESSINA[2], Giovanna CALABRESE[1]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina; [2]Department of Chemical Sciences, University of Catania, Italy

Nowadays, neurodegenerative diseases represent the leading cause of physical and cognitive disability, currently affecting approximately 15% of people worldwide. Unfortunately, effective therapeutic options to prevent neuronal loss are still not available. In this context, the emerging field of regenerative medicine represents a promising new approach for addressing neurodegeneration, using implantable neuroprosthetic devices, designed to interface with neural circuits and restore damaged tissues[1]. Specifically, conductive polymer-based systems (CPs) have gained great attention due to their ability to mimic biological tissues providing a platform for electrical stimulation, which is crucial for driving cell differentiation into neurons and glial cells, opening new options for neural regenerative therapies[2,3]. Based on this evidence, in this study we investigated the physiological response of human neuroblastoma SH-SY5Y cells, grown on a semiconductive polymer, poly(3-hexylthiofene) (P3HT)-based substrate, in terms of cell adhesion, proliferation, and neural differentiation, for 15 days in absence of neuronal differentiation factors. For this aim, MTT test and DAPI staining were performed to evaluate the biocompatibility of P3HT substrate, and gene expression and immunofluorescence analyses, with specific early and late neuronal differentiation markers, were assessed to evaluate the neural inductive capability of the biomaterial. Moreover, to further analyze the spontaneous neuronal differentiation of SH-SY5Y cells grown on the conductive polymer substrate we traced and quantified the elongation of neuronal processes through NeuronJ software. Our preliminary *in vitro* results showed an excellent biocompatibility of the conductive polymer substrate, the presence of high amount of β-tubulin III and NFH target proteins, and the expression of specific markers mainly expressed in neurons, suggesting that it is able to induce spontaneous neuronal dif-

ferentiation already after 8 days of culture, even in the absence of retinoic acid. These data suggest that the electroconductive substrate could be considered as a microenvironment for inducing neuroblastoma cell differentiation towards neuronal lineage. Therefore, the development of a P3HT-based system could represent a promising strategy to promote nerve regeneration and functional recovery.

REFERENCES

1. Esmaeili, A., Eteghadi, A., Landi, F. S., Yavari, S. F., & Taghipour, N. (2024). Recent approaches in regenerative medicine in the fight against neurodegenerative disease. *Brain research*, *1825*, 148688. https://doi.org/10.1016/j.brainres.2023.148688.
2. Iyad A Hammam, Ryan Winters, Zhongkui Hong, Advancements in the application of biomaterials in neural tissue engineering: A review, Biomedical Engineering Advances, Volume 8, 2024,100132, https://doi.org/10.1016/j.bea.2024.100132.
3. Yi, H., Patel, R., Patel, K. D., Bouchard, L. S., Jha, A., Perriman, A. W., & Patel, M. (2023). Conducting polymer-based scaffolds for neuronal tissue engineering. *Journal of materials chemistry. B*, *11*(46), 11006–11023. https://doi.org/ 10.1039/d3tb01838e.

USE OF HUMAN MESENCHYMAL STEM CELLS-DERIVED EXTRACELLULAR VESICLES AND BIOCOMPATIBLE GELLAN GUM SCAFFOLDS FOR DIABETIC FOOT ULCERS HEALING

Mattia BIONDO[1], Laura TOMASELLO[2], Giuseppina BISCARI[1], Grazia Laura DI GRADO[2], Luigi DI ROSA[1], Fabio Salvatore PALUMBO[1], Calogero FIORICA[1], Sonya VASTO[1], Antonio Simone LAGANÀ[2], Giovanna PITARRESI[1], Giorgio ARNALDI[2], Giuseppe PIZZOLANTI[2,3]

[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo; [2]Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties (PROMISE), University of Palermo; [3]ATeN Center, Palermo, Italy

Diabetic foot ulcers (DFUs) are common complications of diabetes and a relevant health topic: they are characterized by a high recurrence rate and often lead to the amputation of the interested body segment. Indeed, their treatment is quite difficult because of the impaired wound healing due to the ischemic and nervous alterations of diabetic patients. [1, 2] The use of human mesenchymal stem cells (hMSCs)-derived extracellular vesicles (EVs), along with a biocompatible scaffold, could be part of an innovative strategy to heal DFUs, due to their anti-inflammatory and immunomodulatory properties. Moreover, EVs are not expected to induce an immunogenic response after their *in situ* administration. [3, 4] In this study the hMSCs were obtained from primary cell cultures derived from adipose tissue – from elective abdominoplasties - amniotic membranes and umbilical cords – from elective cesarean sections – of volunteer healthy patients. Normally, they would be wasted without making the most of their hMSCs content. The tissues were processed via mechanical and/or enzymatic methods, then placed in culture flasks and dishes to obtain hMSCs adherent primary cultures. At passage 3-5, the cultures were characterized via flow cytometry and their stemness potential was confirmed inducing an osteogenic and a chondrogenic differentiation. The hMSCs-derived EVs were obtained via a tangential flow filtration process, and the expression of typical surface markers (CD63 and CD81) was evaluated via flow cytometry, then their size (up to 200 nm) was assessed via Dynamic Light Scattering. As a biocompatible scaffold was considered essential to contain EVs and elicit their healing potential in the context of DFUs, a gellan gum modified with ethylenediamine (GG-EDA) scaffold was prepared and recovered via freeze-drying. The GG-EDA scaffolds were rehydrated with 100 µL of cell suspension containing human fibroblasts - cytotypes essential in wound healing processes - then a pro-inflammatory environment reproducing the one of diabetic ulcers was created treating the cells with Tumor Necrosis Factor α (TNF-α) and Interleukin-1β (IL-1β). The protective effects of EVs were tested adding 20 µg of the isolated EVs to some scaffolds with treated fibroblasts. After 48 hours, cell viability was assessed via MTS assay, demonstrating a proliferative advantage in treated fibroblasts seeded in EVs-enriched scaffolds compared to the treated fibroblasts seeded in scaffolds not enriched with EVs. Although further studies and analyses are necessary, these preliminary results suggest that hMSCs-derived EVs have anti-inflammatory properties promoting cell proliferation, thus they could be part of a strategy for DFUs treatment.

Supported by the PNRR Project HEAL ITALIA

REFERENCES

1. McDermott K *et al., Diabetes Care* 2023.
2. IDF Diabetes Atlas, 10th edition, 2021.
3. Zhou C *et al.*, *Stem Cell Res Ther* 2023.
4. Lalebeigi F *et al.*, *Carbohydr Polym* 2024.

STUDY OF THE REGENERATIVE POTENTIAL OF THE ADIPOSE STEM CELL SPHEROID SECRETOME AS A POSSIBLE CELL-FREE THERAPY

Anna Barbara DI STEFANO[1], Marco TRAPANI[1], Francesca TOIA[1,2], Adriana CORDOVA[1,2]

[1]BIOPLAST- Laboratory of BIOlogy and Regenerative Medicine-PLASTic Surgery, University of Palermo, Department of Surgical, Oncological and Stomatological Sciences, Palermo; [2]Plastic and Reconstructive Surgery Unit, University of Palermo, Department of Surgical, Oncological and Stomatological Sciences, Palermo, Italy

Numerous factors can influence the ability to repair and regenerate damaged tissue, and the therapeutic efficacy of mesenchymal stem cell (MSC)-based treatments has already been demonstrated, while studies on cell-free treatments are ongoing (1). Cells need to communicate with each other and

exchange information not only through direct cell-cell interaction, but also through an indirect route such as endocrine, autocrine and paracrine signalling. Paracrine signalling can occur through a pool of soluble factors and/or extracellular vesicles (EVs) secreted by cells and released into the culture medium, called 'conditioned medium'. The pool of factors secreted together with EVs is called the 'secretome'. In the literature, several studies show that the therapeutic efficacy of the secretome is similar to that of MSCs, due to the heterogeneous cargo of proteins, mRNAs and small RNAs. Among treatments with MSCs, we demonstrated that spheroids from adipose stem cells (SASCs) have a high potential for multilineage differentiation and immunomodulatory activity (2). The aim of this work was to characterise the composition of the secretome and exosomes isolated from SASCs and to assess their regenerative potential. Analysis of the secretome and exosomes demonstrated a large production of stemness-related factors such as NANOG and SOX2, immunomodulation and angiogenic factors from SASCs. In addition, exosomes showed a regenerative effect. The secretome of SASCs carries paracrine signals involved in the maintenance of stemness, pro-angiogenic and pro-osteogenic differentiation, immune regulation and regeneration (3). The use of the secretome offers several advantages, both biologically and practically; once isolated from the culture medium of SASCs, the secretome can be more easily standardised and characterised as a 'cell-free' product.

REFERENCES

1. Urrata V. Life Sciences, DOI: 10.1016/j.lfs.2022.120990.
2. Toia F. Life Sciences, DOI: 10.1016/j.lfs.2023.121610.
3. Urrata V. Biomedicines, DOI: 10.3390/biomedicines 12081842.

BIODEGRADABLE NANOFIBER FILMS INCORPORATING REDUCED GRAPHENE OXIDE FOR NERVE REGENERATION

Federica FREGNAN[1], Giulia ZO[1], Yuki SHIROSAKI[2], Elena Raluca SANDU[1], Nariman ABDULLAYEV[1], Stefania RAIMONDO[1]

[1]Department of Clinical and Biological Sciences, University of Turin, Italy; [2]Faculty of Engineering, Kyushu Institute of Technology, Japan

Due to challenges in finding suitable donor nerves, artificial nerve conduits are becoming increasingly necessary for nerve regeneration, offering a viable alternative to the transplantation of fresh nerves. One promising material for this purpose is chitosan (Ch), a cationic biopolymer known for its biocompatibility and biodegradability. Over the past few years, chitosan has gained significant attention in biotechnology due to these valuable properties. Moreover, chitosan can easily interact with carboxymethyl cellulose (CMC), another biocompatible and biodegradable polymer, but with an anionic nature. This interaction allows the formation of complex materials that hold potential for various applications. In addition to these biopolymers, reduced graphene oxide (RGO), a cutting-edge carbon nanomaterial, can be incorporated into the mix to impart electrical properties to the polymer blend. The introduction of electroconductive materials, such as RGO, is particularly beneficial for nerve regeneration, as it has been shown to help modulate neuronal activity and promote the formation of functional neural connections. Here we will report an *in vitro* characterization of chitosan (Ch) and carboxymethyl cellulose (CMC) nanofiber films, with and without the incorporation of the conductant reduced graphene oxide (RGO), as a device to be employed, in a future perspective, to promote the regeneration of the peripheral nervous system. The films were fabricated using a filtration process, resulting in uniform and robust structures where Ch and CMC fibers are bonded through electrostatic interactions. The addition of RGO increased hydrophilicity, indicated by a decrease in contact angles with higher RGO concentrations, which supports better cell adhesion. Biological tests with RT4-D6P2T Schwann cells demonstrated that the films supported cell viability and spreading, with RGO concentrations above 6 wt% promoting enhanced cell adhesion and elongated cell morphology. The results of the analyses with DRG dissociated neurons indicated that 6-10 wt% RGO concentrations were optimal for promoting neuronal growth, while lower concentrations hindered cell differentiation. Overall, Ch-CMC-RGO composite films show promise for nerve tissue engineering, with RGO improving both cell behavior and material conductivity.

THE EFFECTS OF OXYGEN-OZONE MIXTUR E THERAPY ON EPITHELIAL CELL WALLS: MEASUREMENT AND ALGORITHM DEVELOPMENT

Antonio GALOFORO[1], Paolo BONIVENTO[2]

[1]S.I.O.O.T. (Società Scientifica Ossigeno Ozono Terapia), Bergamo; [2]Istituto di Studi Europei "Alcide De Gasperi", Roma, Italy

Modern Raman Spectro microscopy nearly always involves the use of lasers as excitation light sources. The resolution of the spectrum relies on the bandwidth of the laser source used. Generally shorter wavelength lasers give stronger Raman scattering due to the ν4 increase in Raman scattering cross-sections, but issues with sample degradation or fluorescence may result. The approach is based on hyperspectral imaging or chemical imaging, in which thousands of Raman spectra are acquired from all over the field of view by, for example, raster scanning of a focused laser beam through a sample. The data can be used to generate images showing the location and amount of different components. Having the full spectroscopic information available in every measurement spot has the advantage that several components can be mapped at the same time, including chemically similar and even polymorphic forms, which cannot be distinguished by detecting only one single wavenumber. Furthermore, material properties such as stress and strain, crystal orientation, crystallinity and incorporation of foreign ions into crystal lattices (*e.g.*, doping, solid solution series) can be determined from hyperspectral maps. The EpiDerm™ Skin Model (exhibits *in vivo*-like morphological and growth characteristics which are uniform and

highly reproducible. EpiDerm™ consists of organized basal, spinous, granular, and cornified layers analogous to those found *in vivo*. EpiDerm™ is mitotically and metabolically active. RAMAN analysis has revealed the presence of keratohyalin granules, tonofilament bundles, desmosomes, and a multi-layered stratum corneum containing intercellular lamellar lipid layers arranged in patterns characteristic of *in vivo* epidermis. The studies, already in the initial phases, have highlighted a certain dynamism of cell development. It was possible to measure and record the times of cellular reproduction and intensity of cellular activities, both for the "Control" group and the "O3" samples. Raman Spectro microscopy Observations find EpiDerm™ rigid substrate design easier to handle in routine repetitive testing environments and scientists find that they are able to perform discriminating tests due to low background interference. The data tables obtained made it possible to roughly trace the trends of the events and, thanks to the best-fit systems, the first algorithms were sketched. The continuation of the research involves an in-depth study on the effectiveness of autohemotherapy treatments on epidermal tissue and will be aimed at verifying, using RAMAN spectromicroscopy, the dosages suitable for counteracting cellular degradation in order to obtain a series of databases aimed at specific and precise protocol of the dosages applicable for the treatments.

INDUCTION OF OSTEOGENIC DIFFERENTIATION *IN VITRO* BY 3D PRINTED BIOACTIVE METHACRYLATED GELLAN GUM SCAFFOLDS

Serena MUNAÒ[1], Luana Vittoria BAUSO[1], Ugo D'AMORA[2], Maria Grazia RAUCCI[2], Luigi AMBROSIO[2], Giovanna CALABRESE[1]
[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences (ChiBioFarAm), University of Messina; [2]Institute of Polymers, Composites and Biomaterials, National Research Council, Naples, Italy

Natural polymer-based hydrogels, such as polysaccharides, have gained considerable interest in bone tissue engineering (BTE) applications, due to their good biocompatibility, high biodegradability, and good ability to mimic the extracellular matrix, supporting cellular adhesion, proliferation, growth and differentiation[1,2]. Among these, gellan gum (GG) has attracted great attention for the development of three-dimensional (3D) scaffolds in BTE field, due to its favorable physicochemical characteristics and high biocompatibility, although it further requires chemical modifications, such as methacrylation, to improve its reduced mechanical strength, stability, and suitability for 3D printing applications[3]. 3D printed scaffolds offer the possibility to develop structures with biological and chemical properties adaptable to the site to be regenerated. Furthermore, it allows the design of scaffolds capable of accelerating bone regeneration, incorporating nanoparticles (NPs) and bioactive factors into the polymeric matrix, improving their biocompatibility, osteoconductivity and osteoinductivity. Herein, the aim of this study was to deeply evaluate the biological response of osteoprogenitor cells cultured on different types of GGMA scaffolds, in terms of biocompatibility, osteoconductivity and osteoinductivity. Specifically, human adipose-derived mesenchymal stem cells (hADMSCs) were cultured, for 21 days, on the following scaffolds: i) neat GGMA, ii) GGMA functionalized with BSF-eumelanin, and iii) GGMA functionalized with HAp. The cell viability, adhesion, proliferation and osteogenic differentiation were analyzed at several timepoints. For this purpose, MTT and hematoxylin and eosin (H&E) staining were performed to evaluate the biocompatibility and osteoconductivity, while Alizarin Red S (AR S) staining and gene expression profile of specific markers expressed during the different osteo-differentiation phases were analysed to evaluate the osteoinductive potential of GGMA-based scaffolds. Our results highlighted that: 1. all the tested GGMA-based scaffolds are biocompatible with hADMSCs, promoting cell adhesion, growth and proliferation; and 2. the embedding of BSF-Eumel and HAp into GGMA scaffolds significantly increase the hADMSCs osteoinductivity, inducing ECM matrix maturation and mineralization. The overall findings suggest that 3D printed GGMA-based scaffolds functionalized with BSF-Eumel or HAp could represent a promising strategy for the development of innovative and customizable biomaterials for the treatment of severe bone lesions.

REFERENCES

1. Campione P, *et al.* (2025). https:// doi.org/10.3390/jfb16010010
2. Bauso LV, *et al.* (2024). https://doi.org/10.3390/biology 13040237
3. D'Amora U, *et al.* (2023). https://doi.org/10.3390/nano 13040772

DECELLULARIZED EXTRACELLULAR MATRIX HYDROGEL: A CHALLENGING BIOMATERIAL FOR NERVE REGENERATION

Luisa MURATORI[1], Miriam METAFUNE[1], Jesús CIRIZA[2], Estabiliz FERNANDEZ-CARRO[2], Stefania RAIMONDO[1]
[1]Department of Clinical and Biological Sciences, Neuroscience Institute Cavalieri Ottolenghi (NICO), University of Turin, Italy; [2]Tissue Microenvironment Lab, Institute I3A, University of Zaragoza, Spain

Peripheral nerve injuries result in partial or total loss of functions with important consequences and impairment in quality of life of affected patients (Grinsell and Keating 2014). Even peripheral nerve retains an intrinsic ability to regenerate spontaneously, this process is often unsatisfactory especially for severe injury with substance loss (Gordon 2020). To date, the gold standard surgical procedure for injuries with substance loss is the autograft technique even if it presents important limitations (limited availability, two-step surgery time) with side effects causing donor site morbidity (Hoben *et al.* 2018). In order to improve the outcome of such injuries, many attempts have been made to develop a device that can be used to bridge nerve gap and support nerve regeneration. Along with the rapid development of tissue engineering in the past decades, biological scaffolds have attracted significant inter-

est in this field due to their great biocompatibility and bioactivity, and moderate mechanical performances for supporting cells. Among various types of scaffolds, decellularized extracellular matrix (dECM), which refer to biomaterials formed by human or animal organs/tissues with the removal of immunogenic cellular components via decellularized technologies, are under the spotlight (Hinderer, Layland, and Schenke-Layland 2016). The ECM tested in this study is derived from cadaver human skin from frozen tissues and underwent a decellularization protocol to obtain a dECM hydrogel (Fernandez-Carro E, et al 2024). It was tested *in vitro* on neuronal (NSC34) and glial (RT4-D62PT) cell lines and on primary SCs. Proliferation assay was performed on RT4-D62PT cell line, using dECM in solution, while primary SCs have been cultured to analyze its role in promoting migration, with promising results. To study the interactions of neurons with the extracellular molecules and to evaluate neurite orientation and outgrowth, NSC34 cells were cultured on coverslips coated with dECM, differentiated after 3 days of culture in order to quantify neurites number and length. The results showed that this matrix has a significant impact on the proliferation and migration of glial cells, and on axonal sprouting and elongation of motor neurons. Further investigations are underway to deepen the effect of the dECM in the activation of molecular pathways related to peripheral nerve regeneration to investigate a possible *in vivo* application for nerve repair.

REFERENCES

Fernandez-Carro E, *et al.*J. Human Dermal Decellularized ECM Hydrogels as Scaffolds for 3D *In Vitro* Skin Aging Models. Int J Mol Sci. 2024.

Gordon, T. 2020. 'Peripheral Nerve Regeneration and Muscle Reinnervation', *Int J MolSci*,21.

Grinsell, D., and C. P. Keating. 2014. 'Peripheral nerve reconstruction after injury: a review of clinical and experimental therapies', *Biomed Res Int*, 2014: 698256.

Hinderer, S., S. L. Layland, and K. Schenke-Layland. 2016. 'ECM and ECM-like materials - Biomaterials for applications in regenerative medicine and cancer therapy', *Adv Drug Deliv Rev*, 97: 260-9.

Hoben, G. M., X. Ee, L. Schellhardt, Y. Yan, D. A. Hunter, A. M. Moore, A. K. Snyder-Warwick, S. Stewart, S. E. Mackinnon, and M. D. Wood. 2018. 'Increasing Nerve Autograft Length Increases Senescence and Reduces Regeneration', *Plast Reconstr Surg*, 142: 952-61.

BIOFABRICATED 3D GELLAN GUM PATCHES CUSTOMIZED WITH BIOACTIVE COMPOUNDS FOR WOUND HEALING APPLICATION

Domiziana PICONE[1], Alessandra Maria VITALE[1], Ugo D'AMORA[2], Beata KACZMAREK-SZCZEPANSKA[3], Fabio BUCCHIERI[1], Francesco CAPPELLO[1-4], Marta Anna SZYCHLINSKA[5], Federica SCALIA[1]

[1]Department of Biomedicine, Neurosciences and Advanced Diagnostics (BiND), University of Palermo, Italy; [2]Institute of Polymers, Composites and Biomaterials (IPCB), National Research Council (CNR), Naples, Italy; [3]Department of Biomaterials and Cosmetics Chemistry, Faculty of Chemistry, Nicolaus Copernicus University in Torun, Poland; [4]Euro-Mediterranean Institute of Science and Technology (IEMEST), Palermo, Italy; [5]Department of Precision Medicine in Medical, Surgical and Critical Care (Me.Pre.C.C.), University of Palermo, Italy

Skin regeneration and remodeling following injuries, are essential for preserving its integrity and functionality. Rapid and efficient wound healing prevent the risks of infection, the prolonged inflammation, the impaired angiogenesis which can lead to pathological healing and chronic wound formation. In recent years, the advanced biofabrication techniques and the application of customized biomaterials, significantly enhanced these processes by improving cellular response and accelerating healing process. In this context, the use of three-dimensional (3D) bioprinting represents an innovative strategy offering the possibility of creating controlled microarchitectures using highly biocompatible materials and bioactive compounds that closely resemble the characteristics of natural tissue and promote endogenous repair process. Gellan gum (GG) is a linear exopolysaccharide produced by the bacterium *Sphingomonas*, that has been widely used in tissue engineering and regenerative medicine, thanks to its physico-chemical properties, stability, biocompatibility and the ability to retain moisture. Tannic acid (TA) and ascorbic acid (AA) have been reported to possess tissue healing and regenerative activities, mainly thanks to their antioxidant and anti-inflammatory properties; in particular, AA was shown to promote collagen synthesis, while TA demonstrated anti-inflammatory and pro-angiogenic properties. For this reason, in the present study, the potential application of innovative 3D methacrylated gellan gum (GGMA)-based patches, combined with the two bioactive composites, TA and AA, have been investigated for wound dressing application. To this aim, the *in vitro* studies were performed using a human dermal fibroblasts (HDFs) cell line.The cytocompatibility of the patches was demonstrated by direct and indirect tests on HDFs as per ISO 10993. The positive effect of GGMA patches on cell migration was assessed through a wound healing assay, while their antioxidant activity was demonstrated through by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test. Furthermore, the patches demonstrated antimicrobial potential against common pathogens *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* in accordance with ISO 22196:2011. The global obtained results confirmed the biocompatibility of the bio-functionalized GGMA-based patches, along with their suitable mechanical features and antioxidant and antimicrobial potential, suggesting their promising applications in the field of wound dressing.

REFERENCES

1. Scalia, F.; Vitale, A.M.; Picone, D.; De Cesare, N.; Swiontek Brzezinska, M.; Kaczmarek-Szczepanska, B.; Ronca, A.; Zavan, B.; Bucchieri, F.; Szychlinska, M.A.; *et al.* Exploring Methacrylated Gellan Gum 3D Bioprinted Patches Loaded with

Tannic Acid or L-Ascorbic Acid as Potential Platform for Wound Dressing Application. *Gels* 2025, *11*, 40. https://doi.org/10.3390/gels11010040.
2. Downer, M.; Berry, C.E.; Parker, J.B.; Kameni, L.; Griffin, M. Current Biomaterials for Wound Healing. Bioengineering (Basel) 2023, 10, 1378.
3. D'Amora, U.; Ronca, A.; Scialla, S.; Soriente, A.; Manini, P.; Phua, J. W.; Ottenheim, C.; Pezzella, A.; Calabrese, G.; Raucci, M. G.; Ambrosio, L. Bioactive Composite Methacrylated Gellan Gum for 3D-Printed Bone Tissue-Engineered Scaffolds. Nanomaterials (Basel) 2023, 13, 772.
4. Michalak, M.; Pierzak, M.; Kręcisz, B.; Suliga, E. Bioactive Compounds for Skin Health: A Review. Nutrients 2021, 13, 203.

3D CELLULAR MODELS AND BIOACTIVE MOLECULES IN REGENERATIVE MEDICINE: MIMICKING PHYSIOLOGICAL CONDITIONS FOR ORGAN-ON-CHIP SYSTEMS

Maria Giovanna RIZZO[1], Sabrina CONOCI[1,2], Caterina FAGGIO[1,3]
[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina; [2]Department of Chemistry "Giacomo Ciamician", University of Bologna; [3]Department of Eco-sustainable Marine Biotechnology, Stazione Zoologica Anton Dohrn, Naples, Italy

In recent years, the generation of three-dimensional (3D) cellular models is increasing in the biomedical research to overcome the limitations of conventional two-dimensional (2D) cultures in simulating the real microenvironmental cellular conditions. Actually, both spheroids and organoids offer a more physiologically relevant system for studying cell-cell and cell-extracellular matrix (ECM) interactions, enhancing the accuracy of *in vitro* models for tissue architecture and functions [1]. The development of these cellular models can also allow the study of the effects of the microenvironment on the integration of bioactive materials, including functionalized nanomaterials and matrix mimic peptides selected for example through phage display technology. These components mimic tissue-specific proteins or ECM structural properties, promoting cell adhesion, communication and physiological microenvironment that better replicates *in vivo* complexity [2]. In parallel, microfluidic platforms (Organ-on-Chip) have emerged as powerful systems not only for real-time monitoring of microenvironmental dynamics, but also for the standardized development of 3D models. Systems such as MicroOrganoSpheres (MOSs), developed via dual-channel chip architectures, enable controlled self-assembly ensuring reproducibility and biomimetic structural organization [3]. This lecture will present the strategies for the development of 3D cellular models also induced by matrix mimic peptides, highlighting the importance of physiological conditions and the controlled extracellular microenvironment. Various spheroid formation techniques (such as hanging drops, low-attachment plates, spinner flasks, and microfluidic systems) will be discussed and their advantages in achieving self-organized cellular structures that closely resemble native tissue organization and pay the way for Organ-on-Chip development. By addressing the emerging frontiers of 3D model standardization, this session will showcase innovative approaches for dynamic tissue modeling and translational applications, with a strong focus on their relevance to regenerative medicine and advanced *in vitro* systems.

FUNDING AND ACKNOWLEDGEMENT: European Union (Next Generation EU), through the MUR-PNRR Project SAMOTHRACE (ECS00000022).

REFERENCES

1. Sang-Yun Lee, In-Seong Koo, Hyun Ju Hwang, Dong Woo Lee. *In Vitro* three-dimensional (3D) cell culture tools for spheroid and organoid models. SLAS Discovery, Volume 28, Issue 4, 2023, Pages 119-137, ISSN 2472-5552, https://doi.org/10.1016/j.slasd. 2023.03.006.
2. Binrui Cao, Yan Li, Tao Yang, Qing Bao, Mingying Yang, Chuanbin Mao. Bacteriophage-based biomaterials for tissue regeneration. Advanced Drug Delivery Reviews, Volume 145, 2019, Pages 73-95, ISSN 0169-409X, https://doi.org/10.1016/j.addr.2018.11.004.
3. Agnieszka Żuchowska, Patrycja Baranowska, Magdalena Flont, Zbigniew Brzózka, Elżbieta Jastrzębska. Review: 3D cell models for organ-on-a-chip applications. Analytica Chimica Acta, 2024, Volume 1301, 342413, ISSN 0003-2670, https://doi.org/10.1016/ j.aca.2024.342413.

REGENERATIVE AND ANTI-SENESCENCE POTENTIAL OF PEEL, PULP, AND SEED EXTRACTS FROM *DIOSPYROS DIGYNA* JACQ. FRUIT IN AN IN VITRO MODEL OF VASCULAR ENDOTHELIUM

Graziella SERIO[1], Giuseppe MANNINO[2], Sina NASERIAN[3], Sawssen BEN FRAJ[3], Georges UZAN[3], Carla GENTILE[1]
[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Italy; [2]Department of Life Sciences and Systems Biology, Innovation Centre, University of Turin, Italy; [3]Institut National de la Santé Et de la Recherche Médicale (INSERM) UMR-S-MD 1197, Hôpital Paul Brousse, Villejuif, France

Diospyros digyna Jacq. is a tropical fruit tree native to Mexico, and while still relatively unknown in Europe, successful cultivation trials have been conducted in Mediterranean regions, including Sicily. The species produces fruits with a distinctive chocolate-colored pulp, commonly known as "chocolate pudding fruits" or black persimmons. These fruits are rich in redox-active compounds, which are distributed uniquely across different parts, resulting in distinct bioactivity profiles (1). Antioxidants play a crucial role in reducing oxidative stress and supporting vascular health by scavenging reactive oxygen species (ROS), preserving vascular integrity, and modulating key signaling pathways involved in inflammation and angiogenesis (2-4). These properties make antioxidant-based therapies a promising approach for protecting and restoring vascular function under stress conditions. Endothelial colony-forming cells

(ECFCs) are vital for endothelial regeneration and angiogenesis and serve as a useful model for studying endothelial cell diversity across different organs (5). This study evaluated the effects of black persimmon peel, pulp, and seed extracts on human cord blood-derived ECFCs (CB-ECFCs) to investigate how their antioxidant profiles influence cellular functions. The extracts showed no cytotoxic or inflammatory effects, as they did not alter endothelial marker expression, cell proliferation, or nitric oxide production. Functional assays revealed that the seed extract significantly enhanced tubule formation, increasing the number of closed tubular structures by 1.5-fold. All extracts promoted cell migration, with the seed extract showing the most pronounced effect, surpassing even vascular endothelial growth factor (VEGF). Additionally, both before and after oxidative stress induction with H□O□, the seed extract exhibited the strongest reduction in cellular senescence. These findings highlight the potential of black persimmon extracts, particularly from the seed, to enhance the regenerative capacity of CB-ECFCs and reduce cellular senescence while maintaining a normal endothelial phenotype. This makes them strong candidates for developing of endothelial cell therapies and promoting of vascular regeneration.

REFERENCES

1. doi: 10.1016/j.foodchem.2022.132137
2. doi: 10.2174/1871530317666170921142055
3. doi: 10.1007/s00394-014-0760-6
4. doi: 10.3390/ijms20092155
5. doi: 10.1016/j.jocit.2018.09.004

TOMATO AND OLIVE-BASED BIOACTIVE COMPOUNDS IN WOUND HEALING: AN *IN VITRO* AND *IN VIVO* STUDY

Tommaso SILVESTRINI[1,2], Annamaria IANNETTA[2], Ludovica SULCANESE[2], Sara FIORINI[3], Margherita EUFEMI[3], Elisabetta BENEDETTI[4], Silvana ZUGARO[4], Marco MINACORI[3], Monia PERUGINI[2]

[1]Advanced Study "Theoretical and Applied Neuroscience", Preclinical clinical and Translational Neuroscience, University of Camerino (TO); [2]Department of Bioscience and Agro-Food and Environmental Technology, University of Teramo; [3]Department of Biochemical Science "A. Rossi Fanelli", Sapienza University of Rome; [4]Department of Life, Health and Environmental Sciences, University of L'Aquila, Italy

In 2022, the World Health Organization (WHO) reported that approximately 4.4 million people die each year worldwide due to inadequately treated wounds, accounting for nearly 8% of total deaths. A wound can be defined as any damage to tissue caused by physical, chemical, thermal, or biological harm. Wound healing is a complex and dynamic physiological process that involves a series of cellular events contributing to tissue regeneration. The three key phases in tissue regeneration are inflammation, proliferation, and remodeling. In recent years, research has focused on the use of natural bioactive compounds as potential agents to modulate these three phases of tissue regeneration (Akhtari *et al.*, 2024).In this regard, the aim of this study is to evaluate the modulation of the inflammatory response *in vitro* using bronchial epithelial cells (BEAS-2B) and *in vivo* using both, a wild type zebrafish tail-amputated model and the Tg(mpx: GFP), to assess the effects of a food supplement approved by the Italian Ministry of Health (Food Supplement Register Code No. 68843, EU Patent No. EP2851080A1). This supplement the TOBC, (Tomato and Olive Bioactive Compounds) derived from whole tomatoes and olive mill wastewater. The *in vitro* results highlighted the proliferative effects of TOBC, as determined by the WST viability test, with a significant increase in cell viability in the TOBC-treated sample ($p < 0.005$). Additionally, cell cycle analysis via flow cytometry showed that the treated sample's cellular fate was directed toward mitotic progression. To confirm these results, Dot Blot testing revealed an increase in IL-1β and EGF, indicating the activation of inflammatory and proliferative phases typical of tissue repair. Finally, the definitive cell assay for wound healing, the "scratch assay," showed a more significant closure of the scratch in the TOBC-treated sample after 24 hours compared to the untreated sample. Supporting these findings, *in vivo* experiments showed that, in the TOBC-treated specimens, caudal fin regeneration was significantly greater ($p < 0.01$), along with molecular data confirming that TOBC is capable of modulating the inflammatory response through an increase in *IL-1β, irf8*, and a decrease in *tnf-α*. This data was further confirmed by neutrophil counts at the confocal microscopy. In conclusion, these preliminary data provide a good starting point to assess the potential therapeutic benefits of TOBC in modulating inflammation, wound healing, and tissue regeneration.

This work has been funded by the European Union - NextGenerationEU, Mission 4, Component 1, under the Italian Ministry of University and Research (MUR) National Innovation Ecosystem grant ECS00000041 - VITALITY - CUP: C43C22000380007.

REFERENCE

Akhtari, N. *et al.* (2024). Natural agents as wound-healing promoters. Inflammopharmacology, 32(1), 101–125. https://doi.org/10.1007/s10787-023-01318-6

EXPLORING A-PRF+ (ADVANCED PLATELET-RICH FIBRIN+) FOR EQUINE FIBROBLAST REGENERATION: A BIOCHEMICAL PERSPECTIVE

Vincenzo VESTUTO[1], Maria Rosaria MIRANDA[1], Chiara MONTANO[2], Valentina GOLINO[1], Giacomo PEPE[1], Davide DE BIASE[1], Pietro CAMPIGLIA[1], Eduardo SOMMELLA[1], Maria Pia PASOLINI[2]

[1]Department of Pharmacy, University of Salerno, Fisciano (SA); [2]Department of Veterinary Medicine and Animal Production, Unit of Surgery, University of Naples "Federico II", Naples, Italy

Wounds are common in equine practice, and often lead to complications such as infections, delayed healing and hypertrophic scarring. These complications can be costly for owners and are not always easily resolved. Developing affordable and effective treatments has become an increasingly important focus in veterinary research. Equine A-PRF+ (Advanced Platelet-Rich Fibrin plus) demonstrates regenerative properties comparable to its human counterpart, but cellular-level investigations exploring its molecular mechanisms remain limited. Therefore, the aim of this study was to evaluate the *in vitro* regenerative effects of equine A-PRF+ on primary equine fibroblast cultures. A-PRF+ was prepared from the autologous blood of the horses enrolled in the research, from which primary fibroblasts were also isolated. First, the secretome analysis of A-PRF+ revealed a complex protein profile involved in matrix remodelling, cell proliferation, and inflammation. Treating cells with A-PRF+, our results demonstrate stimulation of equine fibroblast proliferation, migration, metabolic activity, and cell cycle re-entry, effects that are accompanied by increased reactive oxygen species (ROS). The ROS-mediated response enhances mitochondrial, lysosomal, and endoplasmic reticulum activities, driving cellular function and promoting collagen synthesis and secretion of growth factors critical for wound healing. To further explore the molecular underpinnings of these effects, q-PCR, ELISA experiments and an intracellular proteomics analysis were employed revealing the cell regenerative pathways. Collectively, our findings underscore the potential of A-PRF+ as a powerful tool in equine wound management and contribute to a deeper understanding of its mechanism of action. This study overall provides new insights into the molecular mechanisms of A-PRF+, highlighting its potential to modulate equine fibroblast activity and promote tissue regeneration. These findings support further exploration of A-PRF+ in regenerative medicine applications.

Index of authors

D

M

N