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Società Italiana di Mutagenesi Ambientale e Genomica

MEETING – Italian Society of Environmental Mutagenesis and Genomics (SIMAG)

"Genomic Integrity and Environmental Challenges: From Mechanisms to Public Health"

25-27 June 2025

Historic Auditorium of Sapienza

Pisa (Italy)

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ORAL PRESENTATIONS

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Società Italiana di Mutagenesi Ambientale e Genomica

Annual Meeting 2025

Minisymposium n. 1: Environmental mutagenesis and genomics: methods and models

LECTURE

The zebrafish model: applications and future perspectives between science and policy

Mario Carere & Ines Lacchetti
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Zebrafish (*Danio rerio*) model it is used and applied in several research fields. In particular it has wide popularity due to the small size, ease of maintenance, low cost, rapid growth rate, high fecundity rate, external fertilization, optical transparency of the embryo. For all these reasons, zebrafish has been successfully employed in the recent years for various applications, as in cancer research, drug toxicity assessment and drug discovery. In the environmental health field New Approach Methodologies (NAMs) are needed to understand the different effects (genotoxicity, neurotoxicity, embryotoxicity, etc) that are caused by chemicals and mixtures in the ecosystems. The zebrafish embryo is in particular a relevant model in ecotoxicology for the study of emerging chemicals (e.g. pharmaceuticals, PFAS, personal care products) and also it has been included in the legislation in particular as a tool for water monitoring. Embryos are also in compliance with 3Rs (Replacement, Reduction, Refinement) principles for the ethical use of animals. Replacement is achieved by the use of zebrafish larvae instead of adult fishes and in some cases mice in toxicology. In addition, methods of animal care have been refined to enhance the welfare of adult fish and collect embryos non-invasively from the bottom of the tank. Recently zebrafish, due to the scientific potential in different fields, have been also considered as a key model for One Health projects and strategies, in Italy a specific network of laboratories has been established. In conclusion zebrafish, for his peculiar characteristics represents an excellent multidisciplinary research model that should be considered in order to protect our ecosystems and human health.

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Oral presentation

Using salivary lymphocytes in the Comet assay to assess exposure

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Several cell types have been employed in human biomonitoring studies to assess exposure to occupational and/or environmental genotoxic xenobiotics (e.g. buccal, nasal, sperm cells, etc.). However, most human studies have used whole blood and/or isolated peripheral blood mononuclear cells (PBMCs). PBMCs are often used as surrogate cells because they circulate throughout the body and have a long lifespan, allowing them to provide information about exposure levels and potential health risks. Concurrently, the use of whole blood is also drawing attention, as the procedure requires a smaller sample (a few microliters) and a shorter time, and it seems to reduce baseline additional damage produced during the cell isolation process. However, the standard procedure for obtaining blood samples could sometimes be perceived as invasive. Venous blood collection from the median cubital or antebrachial veins is usually carried out to obtain PBMCs. Capillary blood samples are typically collected by a lancet from the side of a finger. These sampling procedures could be cumbersome in children and/or poorly accepted by other subjects without risk perception. For these reasons, focusing on the collection of leukocytes from alternative specimens, as well as developing and optimizing non-invasive sampling procedures, is a crucial issue.
Sampling of buccal mononuclear leukocytes (BMLs) from saliva is less invasive and more widely accepted by participants. Using BMLs—lymphocytes represent 75% of the total mononuclear fraction, and monocytes about 25%— isolated by density gradient centrifugation represents a potential strategy in human biomonitoring studies using the comet assay to assess DNA damage.

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Oral presentation

Intestine-on-Chip platforms for testing toxic or protective properties of ingested food matrices and for evaluating food-delivered drugs

Antonella Prantera[1], Fabiola Troisi[1], Martina Acciari[1], Chiara Ballestracci[1], Beatrice Guerrucci[1], Federica Narra[2], Costanza Ceccanti[2,3], Eugenia Piragine[3,4], Federica Gemignani[1], Stefano Landi[1], Andrea Serra[2,3], Alma Martelli[3,4,5], Lucia Guidi[2,3] & Roberto Giovannoni[1,3,5]

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The intestine-on-chip technology offer a promising approach to study toxic or protective properties of compounds from food matrices, as well as therapeutic peptides from engineered systems. Such platforms allow even complex evaluations and integrated measures and represent a valid alternative to test intestinal absorption in animal models. We developed an intestine-on-chip platform with an associated and optimized in vitro gastric and intestinal digestion mimicking system. Two microfluidic systems, OrganoPlate® (Mimetas) and LiveBox/LiveFlow® (IVTech), were compared with traditional collagen-coated Caco-2 cultures. Caco-2 differentiation was monitored over 21 days via qRT-PCR and immunostaining. Food matrices were digested using a standard INFOGEST protocol with a downstream processing and storage of digesta calibrated and optimized for cell-culture exposure. Different concentrations of digesta were tested for cytocompatibility and barrier integrity (TEER, morphology, LDH assay). Cells cultured on intestine-on-chips showed stable differentiation and enhanced tight junctions, especially in presence of collagen. Exposure to up to 30% of intestinal INFOGEST in cell medium was well tolerated. Exposure of up to 50% of gastric INFOGEST in cell medium was well tolerated. This study supports intestine-on-chip models as effective tools for studying toxic effects and intestinal cell response to digesta from different sources, the platform represents a versatile and reliable model to test toxicity and tolerability of digested compounds, including those pollutants contaminating foods.

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LECTURE

Application of Error-Corrected Sequencing to Advance in vivo Regulatory Mutagenicity Testing

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Regulatory genetic toxicology plays a critical role in safeguarding public health by identifying mutagenic hazards. However, current testing strategies are constrained by important limitations, including reliance on genetically modified organisms, focus on single reporter genes that may not reflect broader biological outcomes, and often provide little mechanistic insight or relevance to human cancer risk. Addressing this gap requires tools that are more versatile, mechanistically informative, and predictive of human health effects. Error-corrected sequencing, such as Duplex Sequencing (DS), represents a transformative advance in the detection of chemically induced mutations. This high-resolution approach enables direct, quantification of mutation frequencies in the mammalian genome while precisely identifying their location and molecular signatures. We conducted in a series of dose- and time-response experiments using DS in MutaMouse males exposed to various mutagens to: (i) demonstrate that DS generates mutation data in somatic tissues and germ cells that are both qualitatively and quantitatively comparable to those from traditional transgenic rodent assays; (ii) reveal chemical-specific mutational signatures consistent with each agent's known mechanism of action; and (iii) highlight a protective role for transcription-coupled repair that resulted in lower mutation frequencies in genic versus intergenic regions. Collectively, our findings position DS as a powerful, mechanism-rich tool for characterizing in vivo chemical mutagenesis. Its integration into regulatory testing frameworks could dramatically enhance our ability to evaluate the mutagenic potential of chemicals with greater biological relevance, ultimately improving chemical safety assessments and protecting human health.

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Minisymposium n. 2: Chemical & Physical Mutagenesis-Implications for Public Health

LECTURE

Genotoxicity assessment in Occupational Biomonitoring: Focus on the Micronucleus Cytome assays

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Several assays are available to detect genotoxicity in human studies, among which the micronucleus (MN) assay is generally considered the most informative. This is due to its well-established association with an increased risk of cancer and other diseases—supported by both Adverse Outcome Pathways and prospective cohort studies showing significant association between elevated MN frequency and overall cancer incidence in healthy individuals. While most evidence to date is based on the CBMN (cytokinesis-block micronucleus) cytome assay in peripheral blood lymphocytes, recent years have seen a growing shift toward the buccal cytome assay. This method is increasingly preferred in genotoxicity studies due to its non-invasive nature and promising results. Although the full validation of MN frequency as a predictive biomarker in medical and occupational/environmental settings is still ongoing, recent developments mark significant progress. Notably, the OECD has identified the MN assay as a flagship case study in its document *Guiding Principles to Advance Occupational Exposure Assessment with Effect Biomarkers*. This initiative outlines concrete proposals to define regulatory triggers for occupational safety based on biomarker evidence. This presentation will begin by reviewing the association between MN frequency and disease risk, outlining the remaining steps needed for biomarker validation. It will then address the ongoing transition from lymphocyte-based to buccal cell-based MN assays. In the second part, we will discuss the OECD project in detail and explore future perspectives for integrating effect biomarkers into regulatory frameworks.

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Minisymposium n. 2: Chemical & Physical Mutagenesis-Implications for Public Health

Oral presentation

Biosafety assessment of zirconia nanoparticles for drug-resistant chronic bacterial lung infections

Martina Leoncini, Saman Habibi Anjedani, Francesco Ghiglioni, Stefano Cagnin, Francesca Moret, Gabriele Sales, Elena Reddi & Maddalena Mognato

Department of Biology, University of Padova

Antimicrobial resistance in the treatment of chronic lung infections is the leading cause of morbidity and mortality in patients with diseases such as cystic fibrosis and hospital-acquired lung infections. The present work has been carried out in the Light4Lungs European project, whose aim is the set-up of a new therapeutic scheme, replacing antibiotics with inhalable zirconia nanoparticles (NPs) properly formulated to fight the pathogenic bacteria. To assure the safety of NPs towards human lung cells, we investigated NP biocompatibility/toxicity in normal and tumoral cell lines of the respiratory epithelium. We verified i) the viability of cells incubated for short and long periods with NPs; ii) the cellular uptake of NPs; ii) if NPs are phagocyted by macrophages since the clearance of NPs is of outmost importance to avoid persistent retention of particles in the treated tissues; iv) the genotoxic potential of NPs towards human cells of respiratory tract. Our results show that zirconia NPs are internalized by cells without inducing significant toxic effects against lung cells of the respiratory tract.

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Minisymposium n. 2: Chemical & Physical Mutagenesis-Implications for Public Health

Oral presentation

Title: In vitro assessment of the aneugenic activity of Neodecanoid Acid

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Neodecanoic acid (NDA) is an industrially manufactured chemical used in food contact materials (FCMs). FCMs may release harmful substances into food and, consequently, their toxicological risk assessment is necessary, particularly the evaluation of the genotoxic activity, to determine whether the use may pose concern for public health. The present study was conducted to investigate in vitro the genotoxicity of NDA. No induction of gene mutations was observed in bacterial cells (Ames test) as well as no increase in structural chromosomal damage in Chinese Hamster Ovary (CHO) cells. Conversely, a statistically significant, concentration-dependent increase in the frequency of micronuclei (MN) was observed in CHO cells. These data, suggesting an aneugenic mode of action, were confirmed by the characterisation of the MN content using CREST staining. A higher frequency of centromere-positive micronuclei was detected in NDA-treated samples associated with high frequency of multipolar mitotic spindles. Further studies were conducted to investigate membrane fluidity and spindle apparatus structure searching for possible cellular target(s) of NDA. Immunofluorescence highlighted mitotic spindle defects and cytoskeleton disruption suggesting microtubules and actin filament as targets. Since the cytoskeleton is tightly connected the nucleoskeleton, the organization of nuclear lamina was investigated and lamina defects associated with irregular and deformed nuclei were observed after NDA treatment. These findings agree with literature data showing that alterations in nuclear lamina are related to genomic instability. In conclusion, this study provided evidence showing a thresholded genotoxic activity for NDA possibly resulting from its property of metal ion chelator affecting microtubule and microfilament network.

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Minisymposium n. 2: Chemical & Physical Mutagenesis-Implications for Public Health

Oral presentation

Effects of mild mutagenic $PM_{2.5}$ organic extracts in the atherosclerosis context: inflammatory response and possible cholesterol involvement

Elena Riva[1], Alessandra Fanelli[1], Serena Montalbano[1], Alexa Torri[1] & Annamaria Buschini[1]

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Air pollution represents a critical environmental health risk, with primary sources including industrial emissions, vehicular traffic, agricultural activities, and fossil fuel use. A major component of air pollution is atmospheric particulate matter (PM). $PM_{2.5}$ has an impact on numerous cardiovascular diseases and contributes to the development of atherosclerosis process, a chronic inflammatory condition linked to dysregulated lipid metabolism and maladaptive inflammatory responses. Atherosclerotic plaque development involves key events such as foam cell formation, a process in which macrophages accumulate lipids. Exposure to PM may compromise the function of high-density lipoproteins (HDL), which play a protective role by promoting cholesterol efflux from macrophages and thereby limiting foam cell formation. To bridge gaps in understanding individual exposure levels and their relevance to atherosclerosis, we employed mild mutagenic organic extracts of $PM_{2.5}$ derived from two distinct areas in Emilia-Romagna. We evaluated the expression of cytokines linked to PM exposure, intracellular reactive oxygen species (ROS), and genes probably implicated in foam cells formation, such as ABCA1 (ATP-binding cassette transporter A1), ABCG1 (ATP-binding cassette transporter G1), and CD36 (transmembrane protein, receptor B2). Our findings demonstrated that the $PM_{2.5}$ organic extracts triggered a significant release of ROS in macrophages already after 30 minutes of treatment and the ELISA assay showed a different release of investigated cytokines (IL-1β, TNF-α and IL-6) following treatments. Furthermore, qRT-PCR analyses showed upregulation of critical genes involved in cholesterol influx and efflux, highlighting the ability of these organic extracts to modulate key molecular pathways related to lipid metabolism and atherosclerosis development.

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Minisymposium n. 2: Chemical & Physical Mutagenesis-Implications for Public Health

Oral presentation

Genomic instability induced by BPA alternatives

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Bisphenol A (BPA) is a synthetic chemical used in the production of consumer products ranging from inner coatings for beverage and food cans to medical devices. BPA has harmful health effects, including metabolic, immune, reproductive, neurodevelopmental diseases, and its use has been restricted in EU. Consequently, industrial production has shifted to BPA alternatives for most of which, however, toxicological studies are still necessary to assess their safety for human health. On this basis, the genotoxic potential of BPE and BPP, two regulatory relevant BPA-alternatives, was evaluated in the present study. I*n vitro* genotoxicity tests were carried out in human lymphocytes to analyze chromosomal damage both in interphase, through the micronucleus test and characterization of micronucleus content, and in anaphase, by the analysis of anaphase morphology.
BPE and BPP induced a statistically significant increase in the frequency of micronuclei through an aneugenic mode of action, as identified by centromere-specific staining via immunofluorescence with anti-kinetochore antibodies (CREST). The analysis of anaphase morphology showed an increase in the percentage of abnormal anaphases, supporting the possible interference of these substances with chromosome segregation. Overall, these data indicate that BPE and BPP may raise concern for genotoxicity and pose doubt on their use as alternatives to BPA.

Acknowledgment
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Minisymposium n. 3: DNA damage and repair: new insights

Oral presentation

SMC1A downregulation as therapeutic target for colon cancer

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It has been hypothesized that mutations in cohesin genes trigger genome instability and cancer progression. Recently, we showed that colorectal tissue acquires extra-copies of the cohesin *SMC1A* gene, and its expression was significantly more robust during colorectal cancer (CRC) tumorigenesis. These findings suggest that overexpression of *SMC1A* plays a role in cancer pathogenesis and have important clinical applications because *SMC1A* could serve as a potential target for developing new therapies in CRC. To test the use of *SMC1A* knockdown as a therapeutic approach for CRC, we used short hairpin RNA (shRNA) against *SMC1A* in *in vivo* CRC models. In addition, mice have been treated with Bevacizumab, a monoclonal antibody capable of blocking the biomolecular activity of all isoforms of the circulating Vascular Endothelial Growth Factor A. Results showed that shRNA treatments against *SMC1A* (± Bevacizumab) significantly reduced the volume of nodules and increased the survival at 60 days as evaluated by Kaplan-Meir test. In addition, RNA-seq analyses allowed us to identify biochemical pathways involved in cancer phenotype rescue. These results support the notion that the shRNA against *SMC1A* approach can serve as a promising therapeutic strategy for CRC.

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Minisymposium n. 3: DNA damage and repair: new insights

Oral presentation

Mechanisms of cell death and cellular stress response following enzymatic depletion of methionine in a colon adenocarcinoma cell model

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Background: One of the distinctive metabolic features of cancer cells is their dependency on the essential amino acid methionine for proliferation and survival and this pronounced reliance represents a compelling metabolic weakness and a promising therapeutic target. This has led to extensive investigation of methionine γ-lyase (MGL), a bacterial, pyridoxal 5′-phosphate-dependent enzyme that degrades methionine, as a potential therapeutic strategy to inhibit cancer cell growth via methionine depletion. Understanding the still unclear mechanistic basis of this cancer-specific metabolic vulnerability is crucial to advancing the therapeutic application of MGL.
Aims: This study aimed to explore the cellular responses to MGL-induced methionine depletion against an in vitro colorectal adenocarcinoma model, with a particular focus on mechanisms of cell death and stress response pathway.
Methods: Cells were treated with MGL at its IC_{50} concentration for various time points. A range of analytical techniques, including cell viability assays, flow cytometry, RT-qPCR, proteomic profiling, and alkaline Comet assay, was used to assess cell cycle progression, activation of apoptotic and non-apoptotic cell death pathways, oxidative stress responses, DNA damage and histone post-translational modifications.
Results: MGL-mediated methionine depletion triggered a complex, time-dependent cascade of molecular events. This included an early onset of redox imbalance, followed by a cell cycle arrest and the activation of autophagy, without the induction of DNA damage. Prolonged metabolic stress ultimately led to the activation of ferroptosis, while apoptotic signaling pathways were only minimally involved.
Conclusion: Our findings provide new mechanistic insights into the cellular response to methionine starvation induced by MGL.

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Minisymposium n. 4: Gene/environment interaction in complex diseases

LECTURE

Epigenetic Editing for Targeted Gene Silencing: A Potential Pre-Therapeutic Strategy

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Epigenetics studies the mechanisms and inheritable epigenetic factors that regulate genetic expression without changing the DNA sequence. These processes orchestrate the cell type-specific use of the genetic information essential for normal development and for maintaining the overall integrity of the genome. Due to the highly plastic nature of the epigenome, deregulation of epigenetic processes is at the root of several complex disorders, such as diabetes and cancer. While there is a lack of fully effective preventive or therapeutic strategies due to the complex etiologies and phenotypes of these diseases, the epigenetic malleability of the genome renders it open to therapeutic drug targeting. ***DNA methylation*** is probably the most extensively studied epigenetic mark and plays an important role in the regulation of gene expression. The alteration of DNA methylation patterns by hyperglycaemia, oxidative stress and inflammation may have potential epigenetic impacts on gene regulation in diabetic individuals. ***Our main goal is to establish proof of concept of targeted epigenetic therapy for the generation of new pancreatic beta cells by epigenetic rewiring***. We are offering a novel synthetic epigenetic tool, Epi-CRISPRs, in the form of stably integrating and inducible vectors, for targeted editing of epigenetic signals at preferred genomic regions that can be seamlessly removed from the genome. We are using Epi-CRISPR to reprogram pancreatic alpha to insulin-producing cells by modifying the epigenetic and transcriptional states of the master regulator in pancreatic cell development, Arx genes. Our study will provide deeper understanding of the epigenetic maintenance mechanisms that define the pancreatic alpha and beta phenotypes. Finally, any methodological success in increasing the number of insulin-producing cells is a promising therapeutic avenue for curing diabetes. Furthermore, we are trying ***to address the potential use of Epi-CRISPR epigenetic editing tool for BRCAness phenotype induction via targeted DNA methylation and subsequent suppression of the BRCA1 promoter as a pre-therapeutic approach in the triple negative breast cancer cells (TNBCs) with unknown mutational signature of BRCA1.*** We are examining the interconnection of a BRCA1 promoter methylation state (EPIC array) with different active (H3K4) or repressive (H3K9 and H3K27) histone methylation marks, as well as the regulatory role of histone H1 in connecting the two methylation systems, in TNBC cell line using ACT-seq. Using BRCA1 methylation (BRCAness) as a predictor for therapeutic response to PARP inhibitors and other therapeutics, will allow direct TNBC treatment without previous screening for BRCA1 mutations. This procedural setup will facilitate the faster decision toward the use of newest medicaments to increase cells' susceptibility to apoptosis and cancer cell diminishment.

How epigenetic mechanisms regulate genome performance and response to stimuli is a fundamental question in health and disease. Epigenetic editing represents a potentially precise and non-invasive strategy to combat certain diseases. Its advantage is a better safety profile as it is inherently reversible and does not alter the host DNA sequence.

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Minisymposium n. 4: Gene/environment interaction in complex diseases

Oral presentation

Genomic and Epigenomic regulation in Liposarcoma

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Liposarcoma (LPS) account for 17% of soft tissue sarcomas and are heterogeneous rare malignant tumours of mesenchymal origin. Despite recent advances, LPS is an aggressive cancer with low patient survival rate and the five histological LPS subtypes carry complex genetic alterations including chromosomal rearrangements, fusions, amplifications, and deletions. Well- and dedifferentiated LPS subtypes (WDLPS/DDLPS) are characterized by extra giant or ring chromosomes (neochromosomes) carrying chromosome 12q13-15 region amplifications containing key oncogenes *MDM2, HMGA2* and *CDK4*. However, MDM2 and CDK4 antagonists have failed inn LPS patients the clinic. Increasing evidence suggests that tumours can develop and progress as a result of both genetic and epigenetic factors, and the understanding of which epigenetic events that are responsible for driving and sustaining LPS is incomplete. We have mapped and characterized the sequence of neochromosomes in WDLPS and DDLPS patient cell lines by Oxford nanopore and high throughput sequencing and show that the amplifications on chr12q13 are extended to q23. These sequences are highly rearranged into the structural variant class Typhonas with amplified fold-back inversions. Our haplotype analysis suggests that this has occurred by multiple chromothripsis events. We used Fluorescence In situ Hybridization to show that *MDM2*, *CDK4* and *FRS2* are highly amplified, however, *HMGA2* is not. Moreover, we have performed integrated single-cell omics and globally mapped epigenetic marks. Based on our characterisation of LPS we propose that epigenetic regulation of neochromosomes may explain why targeting *MDM2* and *CDK4* are failing LPS clinical trials.

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Minisymposium n. 4: Gene/environment interaction in complex diseases

Oral presentation

Mitochondrial DNA methylation in neurodevelopmental and neurodegenerative disorders

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Mitoepigenetics, the study of epigenetic mechanisms within the mitochondrial genome, is an emerging field that is reshaping our understanding of mitochondrial regulation in physiological and pathological conditions. Among these mechanisms, mitochondrial DNA (mtDNA) methylation, particularly within the displacement loop (D-loop), a key regulatory region for mtDNA replication and transcription, has garnered increasing attention. Several environmental factors, such as exposure to air pollution, heavy metals, and dietary factors, can impact mitoepigenetic mechanisms, potentially contributing to the pathogenesis of a wide range of human diseases. Altered mtDNA methylation has been associated with various complex disorders, including cancer, metabolic disorders, and neurodegenerative and neurodevelopmental diseases, though its functional significance remains debated.

An overview of mtDNA methylation and its role in human pathophysiology, with a focus on current evidence from neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS), as well as neurodevelopmental disorders, will be provided. Data from published studies analyzing D-loop methylation in relation to folate cycle-related gene polymorphisms and circulating biomarkers of one-carbon metabolism, as well as its patterns in the peripheral blood of individuals with AD, PD, and ALS, will be discussed. These findings highlight the complex interplay between genetic and environmental factors in modulating mtDNA epigenetic signatures. Additionally, unpublished data on D-loop methylation in individuals with autism spectrum disorder and Down Syndrome will be presented.

Collectively, these findings underscore the importance of mitoepigenetic regulation at the interface between genetic background, environment, and brain health, opening new avenues for biomarker discovery and therapeutic interventions.

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Minisymposium n. 4: Gene/environment interaction in complex diseases

Oral presentation

Integration of Multiple Omics to predict pancreatic cancer development in high-risk individuals

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The incidence and the prevalence of intraductal papillary mucinous neoplasm (IPMN) is increasing in these last years, reaching a prevalence rate of 15-40%. However, the real values of these estimates are difficult to obtain since the majority are diagnosed accidentally in asymptomatic patients undergoing imaging. The difficult point in the management of these cysts is to understand if they become invasive carcinoma or not. The IPMN to pancreatic ductal adenocarcinoma (PDAC) progression is a multifactorial and complex process with an annual rate of progression to invasive carcinoma of 1.4-6.9%. The management of IPMNs is challenging and many patients have overtreatment or undertreatment. In order to enhance the sensitivity of our guidelines and to perform a specific patient selection for resection, molecular and genetic markers need to be identified. Considering that carcinogenesis is a complex and multifactorial process the only viable way for predicting the individual risk is to consider multiple markers in a comprehensive approach. In this project we analyzed the exposome, somatic and germline genetic variability, epigenetic and transcriptomic markers. We used nonparametric epidemiologic approaches and explainable machine learning (XAI) algorithms to compute a progression score and to identify the interplay among various Omics. We identified several 30 genetic variants, 5 micro-RNAs and several environmental features that are associated with PDAC development. The XAI models showed good performances, with a very good overall accuracy (0.87) and an excellent recall (0.97).

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Società Italiana di Mutagenesi Ambientale e Genomica

Annual Meeting 2025

Minisymposium n. 4: Gene/environment interaction in complex diseases

Oral presentation

The extended genome impact on the first 1,000 days of life

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The first 1,000 days of life are critical for development and long-term health, with birthweight and neonatal pain being key indicators. While some anthropometric and clinical factors have been linked to these traits, the role of host genetics, neonatal microbiome variability, and their interaction remains unclear. This study integrated genetic epidemiology, pharmacogenetics, and metagenomics to examine how environmental factors, host genetics, and the neonatal microbiome influence birthweight, growth, and response to glucose-based analgesia. Twenty environmental exposome-related variables and 81 single-nucleotide polymorphisms (SNPs) across eight genes were investigated in more than 1300 newborns, while a subset of 380 individuals underwent microbiome profiling. Furthermore, the potential effect of host genetics on microbiome variability was evaluated. Maternal pregravidic weight and height were good predictors of growth, while the *SLC2A1*-rs3820546-G allele was consistently associated with lower weight during the whole period of observation. Three associations were observed between *SLC2A1* genetic variability and treatment response, suggesting gene expression modulation in the brain. Increased microbiome diversity was consistently associated with higher analgesic efficacy, while microbial taxa discriminated between responders and non-responders with an AUC of 0.722. Finally, the *ABO*-rs505922-C allele (non-O blood type) was associated with increased abundance of Bacteroidia-class genera (beta=0.44, 95% CI 0.20-1.00, p_{adj}=0.011), in line with data in the adult. These results underscore the value of a holistic approach, where complex traits are shaped by host genetics, environment, microbiome diversity, and their interaction, and highlight the neonatal microbiome as a potential tool for personalized analgesia to complement the environmental exposome and host genetics.

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Minisymposium n. 4: Gene/environment interaction in complex diseases

Oral presentation

Childhood maltreatment increases psychopathy in genetically vulnerable individuals: an endophenotype/genome-wide based approach

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Psychopathy, characterized by affective, interpersonal, and lifestyle traits, affects up to 25% of incarcerated people and is associated with violent crimes, treatment resistance, and high recidivism rate. Psychopathy is also heritable, and genetically predisposed individuals appear more vulnerable to adverse environmental factors.
Aim of this study was to investigate, in 236 adult White incarcerated males, significant associations among psychopathy, childhood maltreatment and single nucleotide polymorphisms (SNPs), previously associated with psychopathy endophenotypes (e.g., lack of empathy, callousness, impulsivity, aggression) by genome-wide studies.
DNA extracted from the inmates' saliva was genotyped using the Illumina's Infinium Global Diversity Array. Psychopathy was measured by the Psychopathy Checklist-Revised (PCL-R), and childhood maltreatment by the Measure of Parental Style (MOPS) questionnaires. One hundred and forty-seven SNPs associated with psychopathy endophenotypes were selected from the GWAS Catalog and analyzed.
A positive correlation was observed between MOPS and PCL-R scores ($\rho s = 0.184$, $p = 9 \times 10^{-3}$), which became stronger in rs30266 G/G genotype carriers ($\rho s = 0.455$, $p = 8.6 \times 10^{-5}$, $n = 69$). Among G/G carriers, childhood maltreatment explained 19% of psychopathy scores variance, compared to just 4% in the full sample.
rs30266 is located in the long non-coding RNA NIHCOLE that regulates DNA double-strand break repair (3). Stress from negative parenting may generate DNA breaks (4) and the G/G genotype might impair the NIHCOLE's repair function (3). We hypothesize that an accumulation of unrepaired DNA breaks in neurons may contribute to increased vulnerability to the effects of childhood maltreatment, thereby leading to higher psychopathy scores in inmates carrying the G/G genotype.

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Minisymposium n. 5: Genotoxicity and emerging pollutants

LECTURE

Emerging pollutants and new toxicological risks for marine organisms

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Contaminants of Emerging Concern (CECs) represent a vast and dynamic group of anthropogenic compounds—ranging from pharmaceuticals and endocrine disruptors to microplastics and industrial byproducts—that, while often present at trace concentrations, may exert significant sublethal and chronic toxicity in marine organisms. Unlike legacy pollutants, many CECs are currently unregulated within the EU framework, yet they are increasingly detected in environmental matrices. CECs are of particular toxicological relevance due to their complex interactions with biological systems and environmental stressors. These compounds can disrupt endocrine and nervous systems, induce oxidative stress, trigger DNA damage, and compromise reproductive and immune functions, with repercussions at individual, population, and ecosystem levels.

Integrating multiple stressors—including those associated with climate change such as warming, acidification, and salinity shifts—is essential for comprehensive ecological risk assessment, since marine organisms are typically exposed to complex mixtures of low-dose pollutants rather than single compounds. This environmental realism highlights the necessity for multidisciplinary approaches combining chemical analysis, bioaccumulation studies, and mechanistic toxicology through in vivo, ex vivo, and in vitro models.

Among CECs Micro- and Nanoplastics (MNPs) represent both physical and chemical hazards. Their small size enhances their bioavailability and ability to translocate across biological barriers, serving as vectors for other contaminants, including persistent organic pollutants. Field studies and laboratory experiments on marine invertebrates such as *Mytilus galloprovincialis* demonstrate that MNPs induce a range of molecular, biochemical, and histopathological alterations, with the response intensity varying according to particle size, polymer composition, and morphology. Recent data from extensive biomonitoring campaigns along the Italian coastline reveal a widespread occurrence of pharmaceuticals in wild mussels, with up to 90% of samples containing at least one drug and over half containing three or more. Experimental exposures confirmed both bioaccumulation and partial depuration capabilities in mussels, reinforcing their suitability as sentinel species.

The presence of emerging contaminants in marine environments poses a growing threat: their toxicological profiles, environmental persistence, and interactions with other stressors demand an urgent evaluation of current monitoring protocols and regulatory frameworks. Strengthening collaboration between research institutions, industries, and policymakers is vital to mitigate long-term ecotoxicological effects.

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Minisymposium n. 5: Genotoxicity and emerging pollutants

Oral presentation

Cross-talk between Molecular and Metabolic Effects Underlying PFAS Toxicity: a Case-Study with PFOA and its Analogue PFBA.

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Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are synthetic chemicals used in industrial and consumer products. Human exposure to PFASs has been associated with adverse effects, including developmental and immune toxicity, cardiovascular disease, cancer. To protect human health, safe exposure limits should be established based on the identification of the critical adverse health effects; however, this resulted challenging for PFASs, possibly because of uncertainties on the underlying mechanisms of toxicity. New Approach Methodologies (NAMs) have emerged as promising tools in chemical risk assessment offering insights on the molecular mechanisms of toxicity. Thus, in this study, NAMs were applied in search of combined biomarkers of early adverse effects to PFASs. Mice were orally treated with PFOA and its analogous PFBA and multiple analyses were conducted, including metabolomic profiling, gene expression, mitochondrial and telomere dysfunctions, to verify whether structurally similar PFASs with different toxicological properties could be distinguished at biochemical and cellular levels. Indeed, chemical-specific metabolomic and molecular signatures were identified in the liver of treated mice showing statistically significant dose-related changes across most parameters. These results indicate that the selected methodologies were sensitive enough to identify dose-related responses. Statistically significant changes in most of the endpoints were also detected at the lowest dose tested of PFOA, corresponding to the no-observable effect level (NOAEL) in classical toxicity studies, a result highlighting the potential of NAMs in identifying subtle toxic effects at low exposure levels. These findings support the possibility to integrate NAMs in risk assessment.

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Minisymposium n. 5: Genotoxicity and emerging pollutants

Oral presentation

Human Health Hazard Assessment of Micro- and Nanoplastics: In Vitro and In Vivo Methodologies in a One Health Perspective

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The pervasive presence of micro- and nanoplastics (MNPs) in the environment has raised increasing public and scientific concern due to their potential impact on human health. Although current data are limited, MNP exposure has been associated with oxidative stress, inflammation, genotoxicity, and metabolic disturbances. The hazard assessment for human health following oral exposure to MNPs requires reliable information on both actual exposure levels and biological effects. This study aims to support the development and standardization of innovative *New Approach Methodologies* (NAMs), integrating both *in vitro* and *in vivo* approaches, to improve the assessment of health risks associated with MNPs present in the food chain and drinking water. In line with the 3Rs principles—Replacement, Reduction, and Refinement—our approach prioritizes advanced *in vitro* models to minimize animal use, while refining *in vivo* studies to yield more ethical and scientifically relevant outcomes. These efforts contribute to a more sustainable and holistic assessment framework, consistent with the integrative vision of the One Health approach.

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Minisymposium n. 5: Genotoxicity and emerging pollutants

Oral presentation

Industrial Hemp as a Model Plant for the Genotoxic and Epigenotoxic Evaluation of Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS)

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Due to their environmental persistence, widespread application, and toxicity to living organisms, contamination by per- and polyfluoroalkyl substances (PFAS) has become a significant global concern. The ability of industrial hemp (*Cannabis sativa* L.) to accumulate PFAS in its aerial tissues makes it a candidate for PFAS phytoremediation, as well as a suitable biological model for assessing the toxicological effects of PFAS in higher eukaryotes. After *in vitro* culturing of industrial hemp for 7 or 14 days in the presence of 1 mg L^{-1} PFOA, 1 mg L^{-1} PFOS, or 0.5 mg L^{-1} PFOA and 0.5 mg L^{-1} PFOS (co-exposure), geno- and epigenotoxic effects induced by PFAS exposure in leaf tissue cells were assessed, including single- and double-stranded DNA breaks (Alkaline Comet Assay), oxidative DNA damage (8-oxo-deoxyguanosine quantification), and genomic DNA methylation levels (ELISA and Methy-Sens Comet Assay). Early genotoxic effects, including increased DNA fragmentation and adduct formation induced by oxidative damage, were evident already after 7 days of exposure. Furthermore, PFAS exposure appears to induce a decrease in CpG island methylation levels, which could result in increased DNA exposure to oxidative damage and breaks. For the first time, protocols for the Comet Assay and Methy-Sens Comet Assay were developed specifically for *Cannabis sativa* leaves, enabling the assessment of toxicological effects induced by PFOA and PFOS in this plant species.

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Minisymposium n. 5: Genotoxicity and emerging pollutants

Oral presentation

Evaluation of effects of pharmaceuticals and plastic additives on blood of loggerhead sea turtles (*Caretta caretta*) using genotoxicity and biochemical biomarkers

Ilaria Caliani[1], Agata Di Noi[2], Barbara Ingletto[1], Giacomo Limonta[1,4], Cristina Panti[1,4], Giovanni Furii[3], Massimo Del Vecchio[3], Maria Cristina Fossi[1,4] & Silvia Casini[1,4]

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Sea turtles are exposed to complex mixtures of contaminants, including pharmaceuticals and plastic additives, and it is therefore important to study their potential toxic effects. In this study, conducted as part of the PNRR-National Biodiversity Future Center (NBFC), Spoke 2, Activity 1.2 (Zeropollution), we sampled whole blood from forty-four specimens of loggerhead sea turtles in the Rescue Centre of Manfredonia (Puglia, Italy) to perform in parallel an *ex vivo* experiment and an ecotoxicological monitoring. For each specimen part of the blood was exposed to different pharmaceuticals and plastic additives (carbamazepine (CBZ), ibuprofen (IBU), valsartan (VAL), a mix of VAL+CBZ+IBU, bisphenol A (BPA), and a mix of phthalates), at increasing concentrations. After the exposure, we tested comet assay, lysozyme and carboxylesterase. In parallel, a set of biomarkers of oxidative stress (tGSH, GSH, GSSG, LPO), genotoxicity (comet assay; ENA assay), immunotoxicy (lysozyme, differential count of white cells) and neurotoxicity (CaE) were tested in unexposed blood to assess the health status of *Caretta caretta*. The *ex vivo* exposure showed no effect on the immune system. Treatments with IBU, VAL or the mix of phthalates resulted in a significant and dose-related increase in DNA damage. Concerning monitoring results, the immune system resulted more altered in adults than younger animals, while no relevant signs of neurotoxicity were found. Comet and ENA assay suggest a good integrity of the animals' DNA.

POSTER PRESENTATIONS

S.I.M.A.G.
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Annual Meeting 2025

Minisymposium n. 1: Environmental mutagenesis and genomics: methods and models

P. 1.1.

Targeting mutated *NRAS* reduces viability and cell cycle progression in Multiple Myeloma

Maristella Canovai[1], Marianna Vitiello[1], Chiara Bertini[1, 2], Giulia Parri[1], Arianna Tavanti[1], Gabriele Buda[1], Chiara Gabellini[1], Roberto Giovannoni[1], Federica Gemignani[1] & Stefano Landi[1]

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Despite significant advances in the understanding and treatment of multiple myeloma (MM), this haematological malignancy remains incurable. Therefore, the identification of novel therapeutic targets is crucial. Approximately 50% of MM patients present aberrant activation of the MAPK pathway due to somatic mutations in proto-oncogenes such as *NRAS* and *KRAS*. Among *NRAS* mutations, the Q61K and Q61R mutations are the most frequent and are associated with poor prognosis and drug resistance. However, only a few studies have explored their molecular role in the disease. Moreover, despite pan-RAS inhibitors are under development, no approved therapies currently target NRAS mutations specifically.In this study, we investigated the potential of NRAS Q61K/R mutations as therapeutic targets in MM, with a focus on their functional role. We designed a panel of short interfering RNA (siRNAs) selectively targeting mutant or wild type *NRAS*. Notably, silencing of mutant *NRAS* alleles led to decreased ERK phosphorylation and downregulation of MAPK pathway, resulting in reduced cell viability and cell cycle arrest. These effects were not observed upon wild-type *NRAS* silencing. Additionally, silencing of NRAS Q61K significantly impaired cellular growth in zebrafish xenograft model. Beyond providing a better understanding of the biological role of mutated *NRAS* in MM, our findings provide a foundation for exploring how endogenous or environmentally induced mutations contribute to disease progression and therapy resistance.

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Minisymposium n. 1: Environmental mutagenesis and genomics: methods and models

P. 1.2.

Tradescantia andersoniana as model plants for the evaluation of mutagenic substances and air quality in situ biomonitoring

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Urban air, particularly in high traffic areas, is usually contaminated by different pollutants mainly from combustion engines. Several epidemiological studies revealed that the exposure to polluted air causes adverse health effects and risks to urban populations. Regarding environmental monitoring carried out through the use of plants, the most frequently used species is *T. clone* #4430.
The aim of the study is to compare two species of plants belonging to the genus, in particular, *Tradescantia clone* #4430, and *Tradescantia andersoniana* which has never been used before for these types of studies and presents a better adaptability to a wider temperature range.
In the first part of this work, to better understand whether the response of *T. andersoniana* was comparable to that of *T. clone* #4430, we performed micronucleus and Comet assays following exposure to known mutagenic substances: maleic hydrazide (MH) and ethyl methanesulfonate (EMS). *T. andersoniana* showed similar sensitivity to *T. clone* for both treatments in the MN assay. In addition, Comet assay showed an induced DNA damage in the two plants following treatment with MH.
In the second part of this work, we used these two species to conduct an in-situ biomonitoring study in the area of Borgo Val di Taro, Italy, to assess the air quality. Specifically, the overall objective of the study was to provide homogeneous and comparable data on the quality of the environment, and in particular on the presence of airborne genotoxic agents.

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Minisymposium n. 1: Environmental mutagenesis and genomics: methods and models

P. 1.3.

DNA damage reduction in human intestinal cell line Caco-2 by leaf extracts of Posidonia oceanica. A potential resource for human health

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Bioactive compounds derived from marine organisms may exhibit antibacterial, antiviral, anticancer, and anti-inflammatory properties, representing an interesting potential while treating various human diseases. The present research focused on studying the potential anti-genotoxic activity of leaf extracts from *P.oceanica* (POE) in e, the human intestinal epithelial adenocarcinoma cell line caco-2 chosen as an *in vitro* cell model. Cells were exposed to different concentrations of POE in ethanol, obtaining two final dilutions of 1:500 or 1:1000 for an exposure time of 24 hours. Caco-2 cells were also treated with two different doses of hydrogen peroxide (H_2O_2), 200mM and 400mM, to induce oxidative DNA damage following different treatment times: 10 minutes before the 24 hour treatment with POE (a); co-exposure with POE for 24 hours (b); and 10 minutes before the end of the treatment with POE (c). Cell viability was assessed by Trypan blue test, while primary DNA damage by Comet assay. Both dilutions of POE resulted to be not genotoxic for Caco-2. Both (b) and (c) protocols induced DNA damage amount to come back to the control level. Specifically, this was observed for the dilution of POE 1: 1500 and H_2O_2 concentration of 200 mM after 24 hours of co-exposure and for the dilution of POE 1:1000 and H_2O_2 200 mM added 10 minutes before the end of the treatment. Overall, these preliminary data suggest the anti-genotoxic potential of leaf exytracts from *P. oceanica* in human intestinal cells and invite further investigation into the antioxidant capabilities in the same experimental set-up.

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Minisymposium n. 1: Environmental mutagenesis and genomics: methods and models

P. 1.4.

Exploring *NRAS* and *KRAS* mutations in bone marrow aspirates, peripheral blood mononuclear cells, and cell free-DNA in Multiple Myeloma

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Multiple Myeloma (MM) is a hematologic cancer characterized by the proliferation of monoclonal plasma cells (PCs) in the bone marrow, often preceded by premalignant conditions such as Monoclonal Gammopathy of Undetermined Significance (MGUS) and Smoldering Myeloma (SMM). Diagnosis currently relies on invasive bone marrow aspiration. Theoretically, liquid biopsies could represent a promising tool for detecting and, eventually, monitoring MM through the analysis of circulating MM cells (CMMCs) and circulating cell-free DNA (cfDNA) in blood samples.

Given the high prevalence of RAS gene mutations in MM—specifically *NRAS* (17–24%) and *KRAS* (22–27%)—this study focused on three of the most frequent mutations: *NRAS Q61R*, *NRAS Q61K*, and *KRAS Q61H*. We analyzed genomic DNA (gDNA) from bone marrow aspirates (BMAs), peripheral blood mononuclear cells (PBMCs), and cfDNA from plasma using digital PCR (dPCR). A total of 48 BMA samples from newly diagnosed MM or SMM patients were processed for gDNA extraction together with their PBMCs counterpart. Moreover, for 13 patients, cfDNA extracted from plasma was also analyzed.

Despite targeting highly recurrent mutations, only a few BMA samples tested positive, and in those cases, the corresponding mutations were rarely detected in PBMCs-derived gDNA. Notably, no mutations were identified in cfDNA samples.

These findings suggest that, although liquid biopsies hold potential, their application for detecting MM remains challenging due to insufficient sensitivity compared to traditional bone marrow analysis.

S.I.M.A.G.
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Annual Meeting 2025

Minisymposium n. 2: Chemical & Physical Mutagenesis-Implications for Public Health

P. 2.1.

Genotoxicity assessment of some industrially processed meat products in the human Caco-2 cell line using the alkaline Comet assay

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Industrially processed meat products are widely consumed around the world, particularly because of their availability in different lfavors and their low costc ompared to raw meats. Previous studies showed that these products have a positive correlation with the incidence of cancer. Nevertheless, the genotoxic risk associated with these products has not yet been assessed. Therefore, the aim of this study is to evaluate the potential genotoxic effect of eight industrially processed meat products made of beef and chicken source and sold on the market. Possible genotoxic compounds were extracted from the meat samples by maceration using a polar solvent (methanol) and a non-polar solvent (n-hexane). The alkaline version of the Comet assay was used to evaluate DNA damage stemming from meat extracts exposure in human Caco-2 cells. To avoid false positive results, cytotoxic threshold was firstly established using the Trypan blue exclusion assay. Data showed that three of hexane extracts tested resulted to be statistically significantly genotoxic in comparison with controls, whereas two different meat extracts among the eight dissolved in methanol resulted to possess a statistically significant genotoxic effect. Only three extracts were found to be not genotoxic both after methanol and n-hexane extraction. Our findings imply that today there are still products, the production procedures of which should be refined to reduce the potential risk of genotoxicity to consumers. Consequently, more investigations should be performed with an accurate analytical approach in order to be able to suggest new recommendations on the permissibile daily intake for those meat products.

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Minisymposium n. 2: Chemical & Physical Mutagenesis-Implications for Public Health

P. 2.2.

Monitoring Air Pollution Effects on Children living near cement factories (MAPEC_Gubbio study): cytogenetic effect preliminary results

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Introduction: Air pollution presents a significant threat to human health due to exposure to individual contaminants and their mixtures, which may exert synergistic effects on various cellular targets. Fine particulate matter (PM), classified as Group 1 carcinogens by the IARC, is particularly concerning. Epidemiological studies have consistently demonstrated a strong association between PM exposure and the incidence/mortality of various diseases, including cancer, cardiovascular diseases, and diabetes. Therefore, detecting early adverse effects from air pollutant exposure is crucial, especially in children, who are more vulnerable in the short and long term.
As atmospheric pollutants are also related to industrial activities such as cement production, this molecular epidemiology study sought to evaluate the relationship between air pollutant concentrations and cytogenetic alterations in school-aged children from Gubbio (Umbria, Italy), where two cement factories are situated.
Methods: Children aged 6–8 from Gubbio (exposed group) and Città di Castello (control group) were recruited. Two exfoliated buccal mucosa cell samples were collected from each child (during autumn/winter and spring/summer), and the frequencies of cytogenetic alterations (micronuclei and nuclear buds) were measured. Simultaneously, air pollution data (*e.g.* NO_2, PM_{10}, $PM_{2.5}$) were obtained from the Regional Agency for Environmental Protection (ARPA Umbria) monitoring stations.
Results: Despite lower pollutant levels in Gubbio compared to Città di Castello, children from Gubbio exhibited consistently higher frequencies of cytogenetic damage.
Conclusion: The findings suggest the presence of an unidentified, unmonitored environmental genotoxic factor in Gubbio. Biomonitoring revealed health risks undetected by routine environmental monitoring systems.

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Minisymposium n. 2: Chemical & Physical Mutagenesis-Implications for Public Health

P. 2.3.

Pro-mutagen and pro-oxidant activity of digest cured meat on HT29 cell line

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Westernization of the diet is a major risk factor in the development of chronic diseases, primarily through chronic low-grade inflammation, which can lead to metabolic disorders, cardiovascular diseases, and cancer. The Western Diet (WD) is characterized by heavy consumption of processed foods, sugar and fats and low consumption of fruits and vegetables. This dietary pattern leads to the coexistence of excessive energy intake and deficiencies in essential vitamins and minerals. Among the typical foods of WD, consumption of processed meat is increasing. Although these products have been classified as carcinogenic to humans by the International Agency for Research on Cancer (IARC), the cellular and molecular mechanisms underlying their role in cancer development remain under debate. Furthermore, humans are continuously exposed to environmental compounds that may have detrimental effects on health. In this context, excessive consumption of such foods, at the expense of protective food groups, may increase the exposure burden, thereby reducing the organism's ability to counteract tumorigenesis. In this study, commercial cured meat products were selected and subjected to in vitro gastrointestinal digestion using the standardized INFOGEST protocol. The digested samples were tested on HT29 human colon cancer cells, both in the presence and absence of EMS (a pro-mutagen) and menadione (a pro-oxidant). The COMET assay was employed to evaluate DNA damage. The results indicate that digested cured meats may enhance the effects of mutagenic and oxidative agents. These findings support the hypothesis that an unbalanced diet can amplify exposure to harmful compounds, particularly when protective foods are under-consumed.

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Minisymposium n. 4: Gene/environment interaction in complex diseases

P. 4.1.

MiRNAs analysis reveals potential diagnostic biomarkers for pleural mesothelioma

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Pleural mesothelioma (PM) is a malignancy arising following asbestos exposure. Despite many countries banning the extraction and use of asbestos decades ago, the number of mesothelioma cases is constantly increasing due to the presence of asbestos in the environment and its intensive use during the past years. Although people at risk undergo health surveillance programs, the lack of accurate and minimally invasive diagnostic biomarkers prevents a prompt diagnosis. Here, we evaluated the total miRNA concentration (miRNome) in the plasma of 33 PM patients and 34 subjects affected by benign respiratory diseases (BRD) with a history of asbestos exposure recruited at the University Hospital of Cisanello (Pisa; discovery cohort). Our analyses led to the identification of 166 miRNAs differentially represented among the two groups. Of these miRNAs, 5 resulted in a high diagnostic accuracy (AUC > 0.90) following a technical validation through RT-qPCR and the receiving operating characteristic curve analysis. The same miRNAs were also analysed through RT-qPCR in the serum of 25 PM and 21 BRD patients recruited at the Department of Chest Diseases, Eskisehir (Turkey, validation cohort). In this case, however, no significant difference was observed between the two groups. This lack of replication in the validation cohort may depend on several factors, including the demographic characteristics of the population and the different matrix employed for the analysis. In conclusion, these results suggest that the identified miRNAs may have a diagnostic significance, but further studies are warranted to investigate the lack of significance in the validation cohort.

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P.4.2.

Investigating Lymphoid Clonal Haematopoiesis as a Predisposing Factor in the Evolution of Multiple Myeloma

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Clonal haematopoiesis (CH) arises from somatic genomic alterations that drive clonal expansion of blood cells. Traditionally, clonal haematopoiesis of indeterminate potential (CHIP) has been linked to myeloid malignancies, while mosaic chromosomal alterations are associated with lymphoid cancers. However, recent findings challenge this distinction, identifying gene mutations that increase lymphoid malignancy risk and leading to the concept of lymphoid clonal haematopoiesis (L-CH). Unlike myeloid clonal haematopoiesis (M-CH), L-CH may originate from haematopoietic stem cells or more differentiated progenitors.
This study hypothesizes that stem or progenitor cells accumulate functional mutations over time, increasing susceptibility to multiple myeloma (MM). Whole Exome Sequencing was performed on nine bone marrow aspirate samples and their matched peripheral blood (PB) from seven MM patients at diagnosis. We applied a mutation consequence filter and considered only mutations with variant allele frequencies of at least 1% in the PB. A total of 54 mutations were identified within 235 L-CH-associated genes. Several L-CH genes were found to be mutated across all samples. In contrast, no mutations were identified among the 54 M-CH genes. The remaining genome (>19,000 genes) exhibited a total of 900 mutations. Preliminary findings lend support to the hypothesis that L-CH contributes to MM development and may originate from a common progenitor cell.
These preliminary results support the hypothesis that L-CH contributes to MM development and may arise from a shared progenitor cell. Studying L-CH could provide biomarkers for personalized medicine, especially for individuals with monoclonal gammopathies at high risk of progressing to MM.

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P.4.3.

Very-long-term COVID-19: clinical and molecular profiling of patients in different phases of the pandemic – An ongoing study

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Long COVID is a clinical syndrome affecting many individuals who continue to experience symptoms three months after SARS-CoV-2 infection, frequently persistent respiratory symptoms. While the impact of long COVID is well recognized, data on the very long-term (VLT) effects - beyond 12–24 months - remain scarce, particularly regarding potential chronic respiratory alterations.

This ongoing study investigates the clinical, genetic, and immunological characteristics of individuals who had acute COVID-19, focusing on persistent respiratory impairment (PRI) in the VLT. It aims to identify biomarkers and understand the mechanisms underlying PRI by comparing patients with long-term damage to those fully recovered.

The study cohort includes 190 patients discharged from Pisa University Hospital between March 2020 and March 2021, who completed a respiratory follow-up 12 months post-discharge. These individuals are now undergoing a second evaluation after at least 55 months. This VLT assessment includes clinical data collection, medical examination, spirometry, plethysmography, DLCO, and thoracic ultrasound.

Additionally, peripheral blood is analysed for primary DNA damage, oxidative stress, DNA methylation, chromosomal aberrations, pro-inflammatory cytokines, Epstein-Barr virus, telomere length, and expression of DNA damage response genes.

Data collection is ongoing. Preliminary findings, including patient characteristics and enrolment response rates, will be presented. The final results may help estimate the incidence of VLT PRI in COVID-19 patients and identify predictive factors and molecular biomarkers. These insights could guide future preventive strategies and clarify why some patients develop chronic respiratory issues while others recover ful

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Minisymposium n. 5: Genotoxicity and emerging pollutants

P.5.1.

Evaluation of sub-lethal effects of oral exposure to different chemicals in *Osmia bicornis* through a multi-biomarker approach

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Pollinators are experiencing alarming declines, with chemical pollution recognized as a key driver. While ecotoxicological studies often focus on insecticides, pollinators in agricultural environments are also exposed to mixtures of other compounds such as fungicides, herbicides, heavy metals and veterinary drugs. The combined ecotoxicological effects of these pollutants remain poorly understood, despite their potential to induce biochemical and cellular alterations in pollinators. This study is part of the PRIN 2022 project "An interdisciplinary approach to unravel the effects of combined exposure to chemical pollutants on insect pollinators (POLYPOLL)". The present study aims to test the sub-lethal effects of oral exposure to different chemicals in *Osmia bicornis*, using a multi-biomarker approach that includes biochemical and cellular endpoints. Females were exposed to a heavy metal (copper) and a commercial fungicide (boscalid), alone and in combination. Four additional groups were treated with an herbicide (glyphosate) and a veterinary drug (ivermectin), alone and combined. From each insect, haemolymph, gut and head were collected for biomarker analysis. Immune system function, detoxification and metabolic responses, neurotoxicity and genotoxicity biomarkers were evaluated. An induction of detoxification pathways was observed after exposure to copper and its mixture with boscalid. Ivermectin, alone and with glyphosate, induced metabolic alterations. No changes in immune system markers were observed across treatments. The analysis of all biomarker responses proved to be a useful and integrated tool for better understanding the sub-lethal effects of contaminants on the health status of these insects.

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Minisymposium n. 5: Genotoxicity and emerging pollutants

P.5.2.

A multi-biomarker approach to evaluate the effectiveness of agricultural mitigation measures on wild pollinators

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Pollinator insects offer fundamental ecosystem services for the environment, agriculture and economy. However, wild pollinators have experienced a significant decline in abundance and diversity, mainly due to human activities. The EU launched the European Green Deal to counter this trend, including specific measures to safeguard wild pollinators and biodiversity. Nevertheless, the real benefits of these strategies on pollinator health remain unclear, making pollinators' health status monitoring crucial to evaluate their efficacy. This study is part of the PRIN-PNRR 2022 project "Assessing the effectiveness of mitigation measures on pollinator decline: an integrated multi-biomarker approach" (ÆM-POLLY). It aims to monitor and compare the health status of wild pollinators sampled in different sites (natural and agroecosystems with or without mitigation measures), using a multi-biomarker approach (to highlight changes in physiological responses) and to assess mitigation measures effectiveness. We selected two crop types: vineyards in Tuscany and orchards in Emilia-Romagna, characterised by presence (agro-ecological) or absence (conventional) of mitigation measures and adjacent natural areas. During late spring and summer, we sampled the most abundant pollinators: *Apis mellifera*, *Bombus terrestris* and hoverflies. On the specimens, we applied a set of biomarkers to evaluate immune responses, detoxification and metabolic processes, energy mobilization, neurotoxicity and genotoxicity effects. Honeybees from conventional Tuscany vineyards showed higher DNA damage than those from agro-ecological and natural sites. Immune alterations were more pronounced in specimens from Emilia-Romagna orchards compared to Tuscany vineyards. Honeybees from agroecological sites showed better health than those from conventional or natural areas, confirming the positive impact of mitigation measures on pollinator well-being.