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In silico evaluation of rare protein variants in a multiplex multiple sclerosis family

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Multiple sclerosis (MS) shows complex heritability; common GWAS variants explain only part of risk, suggesting a contribution from rare, higher-impact alleles. We performed whole-exome sequencing (WES) in a multiplex MS family and applied a standardized pipeline (coverage ~60×), obtaining ~20–25k variants per individual. Serial filters (quality, functional consequence, rarity, and segregation) reduced the set to 47 shared rare variants. Phenotype-aware prioritization using ACMG criteria and ensemble predictors (e.g., REVEL, CADD) highlighted three co-segregating missense candidates classified as VUS.

To probe functional plausibility, we mapped the substitutions on available 3D structures/AlphaFold models and performed structure-based analyses: residue–residue contact networks (RING), secondary structure (DSSP), and solvent accessibility (NACCESS), complemented by stability predictions (DynaMut2, DUET, INPS-MD). Two variants showed convergent signals across tools: loss/rewiring of aromatic and van der Waals contacts, location at interfaces or near a putative pocket, and predicted destabilization ($\Delta\Delta G > 0$ by at least two methods), consistent with potential functional impact. The third change lies in a likely disordered/low-confidence region, where modeling is inherently uncertain. Known common MS risk alleles did not explain the segregation pattern in this pedigree.

Our results illustrate an integrated strategy—WES, segregation, ACMG-guided prioritization, and protein-level *in silico* evaluation—to refine candidate lists in familial MS. While classification remains VUS pending functional evidence, the convergent structural signatures for two variants nominate them for targeted assays (e.g., expression/stability, ligand binding, pathway readouts) and replication in independent multiplex families. This work strengthens the hypothesis that rare coding variants can contribute to familial MS risk and delineates a practical path from genome to mechanism [1].

Reference

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The education group of the Italian Society of Biochemistry and Molecular Biology (SIB-GD): objectives and current initiatives

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The Education Group of the Italian Society of Biochemistry and Molecular Biology (SIB-GD) is a translational group committed to advancing and sharing innovations in biochemistry education. We maintain close contact with the FEBS Education Committee, cooperating to develop a European network of biochemistry teachers and education specialists who promote the translation of educational research into biochemistry teaching practice, as a strategy to improve students' competencies and active participation. In 2024, we took part in organizing the inaugural FEBS Education and Training Conference, which featured plenary talks, workshops, meet-the-expert sessions, and networking activities focused on innovative approaches to molecular life sciences education.

Alongside its educational initiatives, SIB-GD is actively engaged in training students and young researchers in new, hands-on, research-related biochemistry methods. Recently, We have also organized online courses and training sessions in collaboration with the ELIXIR life sciences infrastructure, focusing on omics data analysis and the use of online software for bioinformatics and biostatistics.

Building on these experiences, SIB-GD, in collaboration with SIB Young, is launching an initiative called "Bench Talks". This is a new series of online seminars to discuss practical laboratory applications, each focusing on a specific technique or experimental approach to examine technical and methodological issues. The primary goal is to foster dialogue among young researchers, enabling them to exchange experimental experiences and discuss emerging techniques and protocols for diverse applications. In each seminar, a young speaker will be invited to present the state of the art on selected methodological topics. During the talk, participants will be encouraged to ask questions and share experiences via chat. In the second part, moderators will facilitate the discussion by addressing participants' comments and questions to foster the exchange of experiences and insights on troubleshooting and best practices in laboratory techniques.

Dysregulated ceramide metabolism mediates the pro- oncogenic and chemoresistant phenotype induced by GATA-1_S in myeloid leukemia

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GATA-1 is a key transcription factor in hematopoiesis, existing in two isoforms: a full-length form (GATA-1_{FL}) and a short variant (GATA-1_S). While a balanced ratio between GATA-1_{FL} and GATA-1_S ensures normal hematopoietic regulation, aberrant expression of GATA-1_S exerts a pro-leukemic effect [1]. Recently, lipidomic analysis on myeloid leukemia K562 cells overexpressing GATA-1 isoforms identified significant alterations mainly associated with ceramides and fatty acid profiles with reduced PUFA content. These findings allowed us to unveil a ferroptosis-resistance mechanism contributing to the maintenance of survival pathways in myeloid leukemia cells. Furthermore, we found that the two GATA-1 isoforms differentially regulate ceramide metabolism by modulating the expression of key enzymes involved in the synthesis of specific ceramide and sphingolipid species that act as signaling mediators. Compared to their GATA-1_{FL} counterpart, GATA-1_S cells showed reduced expression of ceramide synthases (CERS1 and CERS2) and increased levels of ceramidases and sphingosine-related enzymes (CERK1, SPHK1, ASAH1, ACER2). These enzymes, primarily through the overproduction of sphingosine-1-phosphate (S1P), promote pro-proliferative and anti-apoptotic signaling. Notably, this is accordance with the pro-leukemic role of GATA-1_S. The evidence that S1P mainly contributes to the regulation of proliferative and apoptotic pathways prompted us to investigate the effects of modulating the expression levels of SPHK1 in these cells. SPHK1 silencing restored sensitivity to cisplatin-induced apoptosis, showing a mechanism to counteract the drug-resistance conferred by GATA-1_S [2]. These results suggest that GATA-1_S is able to shift cells toward an anti-apoptotic, pro-survival and chemoresistance phenotype by rewiring fatty acid and ceramide metabolism and indicate new therapeutic targets to overcome drug resistance in myeloid leukemia.

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Interconnected Roles of PARP12 in Estrogen Signaling, DNA Damage Response, and Immune Modulation in ER+ Breast Cancer

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ADP-ribosylation (ADPr) is a post-translational modification with diverse roles in cellular homeostasis, including DNA repair, transcription, apoptosis, and immune responses. Catalyzed by PARP enzymes, ADPr has emerged as a critical modulator of oncogenic signaling. PARP12, a mono-ADP-ribosyltransferase initially linked to antiviral defense, is increasingly implicated in tumorigenesis and drug resistance in estrogen receptor-positive (ER^+) breast cancer. In cell models, PARP12 promotes AKT activation and cell survival, while its inhibition triggers apoptosis and enhances DNA damage responses. This study investigates PARP12's multifaceted role in ER^+ breast cancer. Using RNA sequencing of PARP12-depleted MCF7 cells, we examined its influence on estrogen receptor α (ERα) signaling, immune-related pathways—particularly interferon signaling—and the DNA damage response beyond canonical PARP1/2 functions. An integrated experimental approach combined RNA-seq, live-cell imaging, western blotting, immunofluorescence, proximity ligation assay, and co-immunoprecipitation to elucidate PARP12 functions. PARP12 knockdown reduced proliferation and viability, impaired ERα activity, and altered estrogen-responsive gene expression and ERα nuclear localization. Immune signaling was activated, with increased STAT1 phosphorylation and upregulation of interferon-stimulated genes. Under genotoxic stress, PARP12 localized to DNA damage foci independently of PARP1/2 and exhibited dose-dependent auto-ADP-ribosylation, indicating adaptive recruitment to damaged chromatin. These results position PARP12 as a central regulator linking ERα signaling, immune activation, and genome maintenance. Its upregulation in hormone therapy–resistant breast cancer suggests potential as a therapeutic target, either to modulate estrogen signaling or enhance DNA damage responses. Future studies will dissect PARP12's regulation of STAT1, ERα phosphorylation, and MARylation-mediated genome stability to explore combination strategies for improved endocrine therapy efficacy. We can conclude that PARP12 acts as a multifunctional hub in ER^+ breast cancer, integrating hormone signaling, immune pathways, and DNA repair, highlighting its relevance in tumor homeostasis and as a promising target for therapy.

Role of the Unfolded Protein Response (UPR) in the anticancer effects of novel cathepsin L inhibitors

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Cathepsin L (CTSL) is a lysosomal cysteine protease that plays a critical role in cancer cell invasion, metastasis, and therapeutic resistance. Its overexpression correlates with aggressive phenotypes and poor prognosis, highlighting CTSL as a promising therapeutic target in melanoma and other malignancies [1]. However, the relationship between cathepsin inhibition and compensatory endoplasmic reticulum (ER) stress responses, particularly the unfolded protein response (UPR), remains poorly defined. In cancer, the UPR represents a double-edged mechanism: while it facilitates tumor adaptation to stress, its sustained activation can ultimately trigger various forms of cell death, including apoptosis [2,3].

Given the emerging role of CTSL inhibition as an anticancer strategy through disruption of lysosomal homeostasis, we tested a small library of synthetic compounds specifically designed to target CTSL. After preliminary FRET-based enzymatic assays demonstrating potent cathepsin L inhibition in the low nanomolar range, compounds were further evaluated in an in-house panel of tumor cell lines overexpressing CTSL. Through this screening, A375 melanoma cells were identified as particularly responsive. The most promising candidate was compound 82, which exhibited the strongest effects, inhibiting migration, proliferation and increasing reactive oxygen species (ROS) production. Apoptosis induction was confirmed by flow cytometry. Thioflavin T staining revealed intracellular accumulation of misfolded proteins under fluorescence microscopy, indicating endoplasmic reticulum (ER) stress and activation of the compensatory unfolded protein response (UPR). Western blot analysis further confirmed UPR activation, particularly through the IRE1 branch and its downstream effector NLRP3. These preliminary findings suggest that UPR activation may underlie the observed antitumor effects, highlighting the crucial role of this pathway in mediating stress-induced cell death following cathepsin inhibition. This study therefore lays the groundwork for further investigations into the mechanistic importance of UPR activation in cathepsin-targeted cancer therapy.

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Novel para-phenylenediamine- based derivatives as receptor tyrosine kinase-like orphan receptor 1 (ror1) inhibitors: an *in vitro* preliminary characterization

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ROR1 (Receptor tyrosine kinase like Orphan Receptor 1), reported as an orphan receptor for a long time, has been recently identified as target of Wnt signaling, which is an ancient and highly conserved family of proteins playing a pivotal role in embryonic development and adult tissue homeostasis [1]. The increased attraction in the pseudo-kinase functionality, because of its pro-survival mechanisms in multiple cancer cell lines can be further explained by its peculiar expressional behavior: ROR1 is an embryonic protein, missing in most tissues during adult life, while it results highly expressed in several tumor types, including chronic lymphocytic leukemia (CLL), mantel cell lymphoma (MCL), large B-cell lymphoma (DLBCL). ROR1 kinase is an underexplored promising target for the development of novel anticancer drugs, being strongly expressed in several cancer cell lines, but poorly in non-tumor cells [2]. This property, together with the scarce number of molecules effective against ROR1, led us to design and develop a research program aimed to the discovery of new chemical entities able to inhibit ROR1 thus interfering with its pro-tumoral activity [3]. Step-by-step in silico studies guided the design and synthesis of para-phenylenediamine-based compounds. SPR and CETSA analyses, coordinated with cytotoxicity assays carried out on JeKo-1 (mantle cell lymphoma) and SH-SY5Y (neuroblastoma cell) cell lines overexpressing ROR1, demonstrated the strong affinity and the anticancer potential of the synthesized molecules, respectively, further confirming its mechanism of action. Moreover, pharmacokinetic assessment revealed a good stability profile for these molecules, paving the way for additional SAR studies and for the development of new ROR1 inhibitors. These findings contribute to the biochemical understanding of ROR1 signaling in tumor progression and highlight the potential of this target for therapeutic intervention in oncology.

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A comprehensive *in vitro* characterization of a new class of anticancer compounds developed as selective haspin inhibitors

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Haspin is a serine/threonine protein kinase that plays a key role in cell division by phosphorylating histone H3 at threonine 3 (H3T3), a crucial step for proper chromosome alignment and segregation during mitosis [1]. Because Haspin is often upregulated in cancer cells, the development of selective inhibitors of this enzyme is considered a promising strategy for anticancer therapy [2]. In this project, we focused on the design, synthesis, and biochemical evaluation of a new series of indole-based molecules developed as selective Haspin inhibitors. A virtual library of approximately 14 million compounds was generated through in-silico modifications of a lead compound to screen potential Haspin inhibitors. Selected derivatives were tested for kinase inhibition with a focus on Haspin via FRET-based assays. Cell viability was evaluated using an in-house cellular panel (Raji, HeLa, A375, MiaPaca2, MCF7) known for Haspin expression. Hypodiploid nuclei and cell cycle disruptions were assessed by flow cytometry, and histone extraction by western blotting confirmed inhibition of Haspin-specific histone H3Thr3 phosphorylation. Confocal microscopy further revealed abnormal mitotic spindle formations post-treatment, suggesting that Haspin inhibition triggers biochemical pathways leading to mitotic catastrophe. Comprehensive cell-based testing identified compounds 47 and 60 as promising hits that synergistically enhanced paclitaxel's antitumor effects, particularly doubling its efficacy in 3D HeLa cell models. LC-MS further confirmed the chemical and metabolic stability of compounds 47 and 60, underscoring their potential as drug candidates. Overall, this study provides a thorough characterization of novel antitumor compounds targeting Haspin, clarifying structural features essential for kinase inhibition. Considering the scarcity of selective Haspin modulators and their potential in impacting tumor growth, these findings form a solid starting point for future drug development.

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Using chemical proteomic approach to investigate the biological space of HFR, a novel promising antitumor molecule, in cervical cancer cell line

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Proteomics is one of the most effective approaches for drug target discovery and for assessing the biochemical impact of plant compound for the treatment of human diseases. Since one of the main issues limiting the actual use of natural compounds as therapeutic agent, or even as led compounds for drug design, is the lack of information concerning their mode of action, we started a study focused on a novel putative antitumoral compound, 2-hydroxy-2', 6'dimethoxy-austrobailignan 10 (HFR), isolated from the plant *Hypoestes forskaolii* (Vahl) R.Br. (Acanthaceae). First, cytotoxic effect of HFR was evaluated on several human cancer cell lines, from lung to cervical cancer, including neuroblastoma and melanoma. Interestingly, the compound was shown to exert a specific cytostatic effect on HeLa cell line (IC_{50} = 7 μM). To study the biological effect of HFR, we analyze the proteome of HeLa cells incubated for different times with HFR or DMSO, as vehicle. A comparison between the obtained result showed that HFR had a significant impact on tubulin expression, thus suggesting a potential effect of this compound on dynamics of microtubules, important components of the eukaryotic cell cytoskeleton, as well as on the mitotic spindle and intracellular trafficking. Based on these results, we performed target identification using a mass spectrometry-based chemical proteomic approach, Drug Affinity Responsive Target Stability Assay (DARTS). Different proteins emerged as putative intracellular interactors of the molecule. However, by correlating the DARTS results with those achieved by global proteome analyses, the Ras-related GTP-binding protein family was identified as a potential target of HFR. Therefore, we hypothesize that the antiproliferative and cytostatic effects of HFR relays on its ability to inhibit the Ras protein, thus influencing the overall rate of GTP hydrolysis and consequently the microtubule dynamics.

β-Carotene suppresses stem-like properties associated with uL3- mediated chemoresistance in colorectal cancer *in vitro* and in CAM model

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Background and aims. Increasing evidence highlights the pivotal role of cancer stem cells (CSCs) in the progression, metastasis, chemoresistance, and recurrence of colorectal cancer (CRC). Recent studies suggest that dysregulation of ribosomal proteins may impact CSC properties [1]. In our previous studies, we identified an inverse correlation between the expression of the ribosomal protein uL3 and chemoresistance: tumors with low uL3 levels displayed a more aggressive phenotype, characterized by enhanced vascularization and metastatic potential. Moreover, we found that β-carotene exerts anti-tumor effects in CRC cells with low uL3 expression [2,3]. The present study aims to investigate the impact of uL3 dysregulation on the stem-like properties of CRC cells and to explore the potential of β-carotene in modulating CRC stem cells (CCSCs), to overcome uL3-associated chemoresistance. **Methods.** The expression of several CSCs markers was evaluated in 2D and 3D cultures of HCT $116^{p53-/-}$ expressing low or high levels of uL3 by qPCR and western blotting. The chicken chorioallantoic membrane (CAM) model was employed to study the impact of β-carotene on tumor growth, vascularization, and metastatic potential of HCT $116^{p53-/-}$ in conditions of both uL3overexpression and downregulation. **Results.** Our data revealed that uL3 downregulation was associated with a significant increase in the expression of stem cell markers such as CD133, ALDH1, and SOX2 in CRC cells, promoting a stem-like phenotype in CRC cells. Treatment with β-carotene effectively reduced the expression of these markers, indicating a suppression of CCSCs. In the CAM model, tumors with low uL3 showed enhanced growth, vascularization, and metastatic spread, all of which were mitigated by β-carotene administration. **Conclusion.** These findings highlight the potential of β-carotene as an adjuvant strategy to target CSCs, reduce tumor aggressiveness, and improve therapeutic outcomes in CRC patients with dysregulated uL3 expression.

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Combining proteomics and bioinformatics to identify breast cancer biomarkers using telomeric g-quadruplex

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The identification of reliable biomarkers is crucial for improving breast cancer detection, prognosis and treatment. Among the many molecular targets being explored, human telomeric DNA - consisting of tandem repeats of the G-rich sequence TTAGGG, which can fold into G-quadruplex (G4) structures - emerged as key player in cancer biology. Here, the sequence tel_{46} - mimicking the multimeric G4 structures in telomeric overhang - covalently anchored to a Controlled Pore Glass (CPG) solid support, has been chosen as a novel biomarker discovery tool. We have found 93 proteins from MCF7 nuclear extracts interacting with tel_{46} using affinity purification mass spectrometry (AP-MS). These proteins are related to DNA replication, repair and genome stability pathways, known to be altered in cancer. Integrating AP-MS data with quantitative proteomics, and comparing MCF7 to non-tumorigenic MCF10a cells, we have found 27 tel_{46} interactors among upregulated proteins. Functional analysis indicated an enrichment in pathways related to genome stability and repair, while downregulated proteins were linked to essential cellular processes, as expected in cancer. Thanks to additional bioinformatics assessments using the public cancer proteomics database, 19 proteins have been confirmed. Transcriptomic and clinical data-driven bioinformatics analysis identified MSH6, MSH2, ESRP1 and WDHD1 as the most promising candidate biomarkers for breast cancer. Validation analyses confirmed cancer specificity and direct binding of the candidates. AP-MS in non-tumorigenic MCF10A detected none of the four proteins, while recombinant pulldown assays showed specific retention of WDHD1, ESRP1, and MSH2 on tel_{46}-derivatized CPG (vs naked CPG), demonstrating direct interaction. Moreover, targeted MRM LC–MS/MS corroborated their overexpression in MCF7 relative to MCF10a, reinforcing their biomarker potential. This research [1] supports the application of tel_{46}-functionalized CPG in capturing cancer-associated proteins and highlights the relevance of telomeric G4-binding proteins as potential biomarkers for breast cancer detection and treatment, establishing a basis for further studies.

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New insights on prevalence and interpretation of microsatellite instability detection in bladder cancer

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Microsatellite instability (MSI) is considered an important step in carcinogenesis and a alteration of genetic pathway in many cancers. In colorectal cancer, several studies have shown that increase to microsatellite instability is associated to alterations in DNA repair complexes or to MLH1 promoter methylation. The MSI is also considered a biomarker for therapeutic treatments, in particular in colon rectal cancer. Until now, the role of MSI in bladder cancer (BC) has not been clearly defined and evaluation of MSI in BC has not yet been well documented in particular in the Italian population. Therefore, this study analyzed the association between MSI status and BC in Italian patients.

Materials and Methods: This study included Italian patients who underwent transurethral resection for bladder cancer between 2019 to date. For MSI analysis a panel of five quasi-monomorphic markers was used. The prevalence rates of microsatellite stable (MSS), MSI-low (MSI-L), and MSI-high (MSI-H) were determined thus, we examined the association of MSI status (MSS versus MSI-L/H) with clinicopathological features of analysed samples.

Results: MSI was observed in 46.6% of cases, including MSI high (H) (33.3%) and MSI low (L) (13.3%) status. Furthermore, the most unstable and stable markers in our study were NR-21 and BAT-26, respectively. MSI-H/MSI were observed more frequently in G3 tumors ($p = 0.028$ and $p = 0.019$, respectively).

Conclusion: The present study showed MSI status more frequently in carcinoma G3 grade, which may be considered a prognostic and therapeutical factor in BC. However, larger and more comprehensive studies are needed to confirm this statement. Use of dinonucleotide markers could improve MSI analysis in BC Italian patients.

In silico and nanotechnology- based approaches to overcome chemoresistance in NSCLC via miRNA modulation

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Background: Non-small cell lung cancer (NSCLC) is one of the leading causes of cancer-related mortality worldwide. Despite therapeutic advances, over 30% of patients do not respond to first-line treatments, mainly due to cancer stem cells (CSCs) [1]. These rare, drug-resistant cells drive relapse and progression. Non-coding RNAs (ncRNAs) are emerging as crucial regulators of CSC traits, including stemness, self-renewal, and metabolic adaptation [2,3].

Aim: To identify ncRNAs involved in CSC metabolic regulation and exploit them for therapeutic purposes using self-assembling nanoparticles (SANPs) as delivery systems.

Methods: Primary NSCLC cell lines were cultured to obtain CSCs at different de-differentiation stages. Stem-like traits were assessed through morphology and marker expression, followed by miRNA profiling. Five differentially expressed miRNAs were used for target prediction with four tools (mirDB, TargetScanHuman 8.0, DIANA microT-CDS, mirDIP), selecting only common targets. Differentially expressed genes (DEGs) were identified from GEO datasets (GSE44076, GSE44861, GSE113513) with GEO2R (adjusted $p \leq 0.05$, $|logFC| \geq 1$). Overlapping genes between predicted targets and DEGs were analyzed, followed by functional enrichment (DIANA Tools mirPath v3.0). Candidate miRNAs were validated with TCGA data using OncomiR and mirTV. SANPs were also developed and optimized for ncRNA delivery, showing low cytotoxicity and efficient uptake.

Results: MiRNA profiling revealed 5 differentially expressed miRNAs across CSC stages. A total of 775 common target genes and 528 DEGs were identified, with 45 genes overlapping and regulated by at least 2 miRNAs. Enrichment analyses highlighted the Neurotrophin (hsa04722) and ErbB (hsa04012) signaling pathways, along with lung cancer–specific pathways. Gene ontology confirmed roles in Fc-epsilon receptor (GO:0038095) and neurotrophin TRK receptor (GO:0048011) signaling. TCGA data validated dysregulated expression of 2 miRNAs across NSCLC subtypes, supporting their clinical relevance.

Conclusions: This study highlights the potential of targeting ncRNA-regulated metabolism in CSCs using SANPs to overcome chemoresistance, aiming to develop innovative therapies to enhance treatment efficacy and improve survival in NSCLC patients.

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The cam assay: an *in vivo* model to evaluate the biocompatibility and the efficacy of novel therapeutic compounds

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The chorioallantoic membrane (CAM) assay offers a valuable *in vivo* alternative to traditional animal experimentation, bridging the gap between *in vitro* and *in vivo* studies. The CAM is an extraembryonic membrane formed by the fusion of the chorion and allantois. Its dense vascular network, and inherent immunodeficiency make it an ideal platform for supporting the proliferation of transplanted tumor cells. This enables the rapid development of cell line-derived xenografts within a few days. The CAM xenograft model is widely used to study tumorigenesis, metastasis drug resistance [1].

Moreover, the CAM model has emerged as a highly useful platform for rapid screening of novel therapeutic agents and drug-delivery systems ensuring both efficacy and safety prior to clinical application [2].

Recently, our research group has established the experimental procedure to develop a CAM xenograft model of colorectal cancer (CRC). Specifically, we inoculated a chemoresistant CRC cell line stably silenced for uL3, namely uL3ΔHCT 116$^{p53-/-}$, onto the CAM [3].

In this study, we investigated the cytotoxic activity of two G-quadruplex binders, pyridostatin (PDS) and RHPS4, alone or combined with 5-fluorouracil in overcoming uL3-mediated chemoresistance. Our results revealed that CAM xenografts exposed to 5-fluorouracil, combined with PDS or RHPS4, exhibited a significant reduction in tumour weight and volume, validating the proposed therapeutic strategy *in vivo*.

In addition, the safety profile of two cell-penetrating peptides was investigated using *in ovo* toxicity test on chicken embryos, underscoring their potential for nanomaterial functionalization and drug delivery applications.

In conclusion, our results highlighted the versatility and utility of the CAM assay as a powerful *in vivo* platform for evaluating the efficacy and safety of novel therapeutic compounds. The CAM model allows rapid, cost-effective, and ethically favourable testing of anti-cancer agents and delivery systems, providing essential preclinical data to implement subsequent mammalian studies.

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Enhanced safety profile of novel gh625 derivatives for efficient membrane transport and drug delivery: *in vitro* and *in vivo* studies in chicken embryo model

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The assessment of safety profile for drug delivery systems is essential for advancing innovative treatment approaches, guaranteeing their effectiveness and safety before clinical application. Biocompatibility is typically tested through *in vitro* experiments to examine cell responses and potential toxic effects. Following this, *in vivo* studies are conducted to examine systemic effects and overall biocompatibility. Over recent decades, the chicken embryo model has gained increasing recognition as a valuable *in vivo* preclinical model, aligning with the 3R principle (reduction, refinement, and replacement), thus providing an ethical and effective alternative to traditional mammalian testing [1]. Cell-penetrating peptides, such as gH625, are extensively employed to facilitate the transport of nanomaterials into cells [2]. Four novel gH625 derivatives (gH-w10, gH-l7, gH-y13, and gH-combi) were developed by replacing L-amino acids at cleavage sites with D-enantiomers to enhance protease resistance. This study aims to evaluate their cell-penetrating ability and safety both *in vitro* and *in vivo*. Our findings demonstrated that the gH-combi peptide achieved the highest cellular uptake, followed by gH-y13, comparable to native gH625. In contrast, gH-l7 and gH-w10 showed reduced penetration capability compared to gH625. Notably, none of the peptides significantly affected cell viability in either normal or cancer cells, suggesting a favorable safety profile. The in ovo toxicity test revealed that both of gH625 and gH-combi were non-toxic, with no observable adverse effects on embryo development. Overall, these findings demonstrate that the gH-combi derivatives exhibit enhanced cell-penetrating ability and a high safety profile, supporting its potential for nanomaterial functionalization and drug delivery applications.

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Dissection of the role of differentially expressed microRNAs in head and neck cancers, their impact on cell proliferation genes and analysis of their therapeutic potential

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Head and neck squamous cell carcinomas (HNSCC) are characterized by rapid progression and poor survival. The multiprotein Dimerization partner, RB-like, E2F, And Multi-vulval class B (DREAM) complex maintains cells in quiescence by recruiting RB-like and E2F co-repressors to the MuvB core. CDK activation displaces these repressors, allowing BMYB and/or FOXM1 to bind and activate proliferation genes. MicroRNAs (miRNAs) affect most HNSCC tumorigenic processes and are both diagnostic biomarkers and potential therapeutic targets. We aim to identify new miRNAs that regulate the DREAM and BMYB/FOXM1-MuvB complexes in HNSCCs, characterize their effects on G_0-G1/S/M checkpoints, apoptosis, and proliferation, and assess in 2D/3D and xenograft models the potential synergy between miRNA targeting strategies along with CDK inhibitors (e.g., abemaciclib) to counteract laryngeal cancer progression. Differentially expressed miRNAs in laryngeal carcinomas and adjacent normal tissues were identified using TaqMan Array Card Type A. In silico analyses were performed to select miRNAs predicted to target DREAM/BMYB/FOXM1-MuvB complex components, whose expression was validated by quantitative real-time PCR (qRT-PCR) on HNSCC cell lines. Simultaneously, the effects of abemaciclib were assessed by WST-8, clonogenic assays, FACS analysis and western blotting.miR106a-5p; miR20a-5p;miR17-5p;miR204-5p and miR186-5p were found differentially upregulated/downregulated in laryngeal cancer patients' specimens and predicted to target DREAM members. QRT-PCR analysis confirmed miRNA expression levels in different HNSCC cell lines. In parallel, the CDK4/6 inhibitor, abemaciclib proved able to reduce cancer cell viability and colony forming ability. Preliminary FACS analyses showed cell accumulation in G1 or G2 cell cycle phases upon 48 and 72 h treatment with abemaciclib at the IC25 and IC50, respectively. Consistently, an increase in the expression of the cell cycle inhibitor p21 was observed, likely independent by p53. This was paralleled by a decrease in AKT activation. These results support the study of cell cycle regulation mechanisms in HNSCC, evaluating the potential role of targeting miRNAs that affect the DREAM function in synergy with the new clinically approved CDK4/6 inhibitors.

From cell cycle control to cancer: functional characterization of *CDKN1B* variants in MEN4 and breast cancer

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p27^{Kip1}, encoded by *CDKN1B*, is a key cell cycle gatekeeper. As a structurally unfolded protein, p27^{Kip1} is addressed to different post-translational modifications that govern its stability, localization, and interactions. Several *CDKN1B* somatic and germline mutations have been reported in multiple endocrine neoplasia type 4 (MEN4) and human cancers, underscoring its role as a bona fide tumor suppressor. In this study, we characterized the functional impact of some *CDKN1B* missense mutations (p.P117S and p.P133T) and nonsense mutations (p.Q163* and p.E171*). Expression vectors encoding wild-type and mutant p27^{Kip1} forms were transfected in ER^{+} MCF-7 and triple-negative MDA-MB-231 breast cancer cell lines for functional analysis on cell motility, cell cycle distribution, phosphorylations, degradation and interactions. All mutants abrogated the antitumor activity of p27^{Kip1}, resulting in increased cell growth and motility. Moreover, p27 mutants exhibited altered phosphorylation patterns, specifically the missense variant p27-P133T showed increase Ser10 phosphorylation, correlated to the increased cell motility. The removal of p27-WT is prevalently through the proteasome (for which the Thr187 is the major phosphodegron), as well as for the mutant forms P117S, P133T, and Q163* (showing faster degradation kinetics than the WT). Conversely, p27-E171* is degraded through the lysosome-dependent system, suggesting a region of the protein as crucial for the lysosome-dependent degradation. Finally, all the mutants showed an altered interaction with cyclin/cyclin-dependent kinase complexes and/or with proteins indirectly controlling this binding (particularly Pin1), providing a mechanistic basis for the hyperproliferative phenotype. Our findings reveal shared molecular signatures among cancer-associated *CDKN1B* variants, highlighting a convergent mechanistic basis for their functional impairment. Notably, the alterations in the binding with CDK4 and cyclin D1 might also be associated to mechanisms of resistance to drugs targeting the Cyclin Ds-dependent kinases in breast cancer.

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Synergistic Antitumor Effects of S-Adenosylmethionine and Cabazitaxel in mCRPC Cell Lines

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S-Adenosylmethionine (AdoMet), a naturally occurring sulfur-containing nucleoside and the principal biological methyl donor, is well known for its antiproliferative and proapoptotic properties. AdoMet's linkage with cancer has been widely explored, studying both its implication in tumor establishment and progression and as a potential therapeutic agent [1, 2].

Prostate cancer represents the second most frequently diagnosed malignancy in men. Metastatic castration-resistant prostate cancer (mCRPC) is an advanced stage of the disease that no longer responds to standard androgen-deprivation therapy, leading to a very poor clinical outcome. Thus, there is an urgent need for new therapeutic strategies that offer greater efficacy and fewer adverse effects.

In this work, we investigated the antitumor potential of AdoMet in combination with Cabazitaxel (CBZ), a second-generation semisynthetic taxane, in DU 145 and PC-3 mCRPC cell lines. Our data revealed that the combined treatment synergistically increased ROS generation and weakened the cancer cells' antioxidant defenses, with the reduction of glutathione, GPX4, and catalase after 72 hours of exposure to 400 μM AdoMet together with 0.7 nM CBZ in DU 145 cells and 1.5 nM CBZ in PC-3 cells. Moreover, elevated ROS levels resulted in DNA damage, as shown by confocal microscopy and by the increased γH2AX/H2AX ratio detected *via* Western blotting. This damage subsequently triggered apoptosis after 72 hours, as confirmed by cleavage of full-length PARP-1 and FACS analysis. We also evaluated the effects of combined treatment on mitotic spindle assembly, our results indicate that AdoMet induces significant morphological alterations consistent with mitotic catastrophe in mCRPC cells, particularly when combined with CBZ.

Overall, these results indicate that AdoMet enhances the sensitivity of mCRPC cells to the antitumor activity of CBZ, supporting its potential use in the development of innovative and cost-effective therapeutic approaches to improve outcomes for prostate cancer patients.

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ZNF224 connects AKT and TGF-β Signalling to Promote Melanoma Plasticity and Aggressiveness

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Melanoma is one of the most aggressive and plastic forms of cancer, whose progression relies on the interplay of multiple oncogenic signalling pathways [1]. Among these, the AKT and TGF-β cascades play pivotal roles in sustaining tumour growth, survival, and cellular plasticity [2,3]. Although these pathways are often co-activated in melanoma, the molecular mechanisms underlying their functional integration remain poorly defined.

Our studies identified ZNF224, a Krüppel-like zinc finger transcription factor, as a key mediator of melanoma aggressiveness through its involvement in both AKT and TGF-β signalling networks. We demonstrated that ZNF224 promotes uncontrolled proliferation and resistance to apoptosis by acting on the AKT/p21/p53 axis. Specifically, ZNF224 transcriptionally activates p21 in a p53-dependent manner and enhances its cytoplasmic retention, thereby reinforcing AKT-driven oncogenic signalling and preventing apoptosis (10.1111/febs.70114).

In parallel, ZNF224 amplifies the pro-oncogenic functions of TGF-β by positively modulating the expression of TGF-β itself and its receptors TGF-βRI and TGF-βRII, thus establishing a positive feedback loop that sustains pathway activation. This persistent signalling promotes epithelial–mesenchymal transition (EMT) and the acquisition of stem-like traits, as indicated by the upregulation of N-cadherin, Slug, Snail, and Vimentin [4].

Among these targets, vimentin emerges as a crucial convergence point between the AKT and TGF-β cascades. Beyond its role as a mesenchymal marker, vimentin acts as an effector of cytoskeletal remodelling and tumour invasiveness. Its dynamic organization is regulated by phosphorylation events reflecting pathway crosstalk: AKT phosphorylates vimentin at Ser39, enhancing motility and invasiveness [5], while PLK1, a kinase activated downstream of TGF-β, also phosphorylates vimentin, promoting metastatic dissemination and immune evasion [6].

Overall, these results suggest that ZNF224 functions as a potent mediator of interconnected signalling cascades that synergistically sustain melanoma plasticity and aggressiveness.

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Redox signals triggered by Formyl-peptide Receptor 2 regulate LAT1/SLC7A5-mediated amino acids uptake

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Cancer cells undergo metabolic reprogramming to sustain the elevated biosynthetic and energetic demand required for rapid proliferation. While non-essential amino acids (AAs) can be synthesized de novo, essential AAs must be acquired from the extracellular environment through specific transporters. Among these, the L-type amino acid transporter 1 (LAT1/*SLC7A5*) is overexpressed in various malignancies and mediates the uptake of branched-chain and bulky AAs. Its expression is transcriptionally regulated by the transcription factor c-Myc and post-transcriptionally by miR-126. LAT1/*SLC7A5* forms a heterodimer with CD98/*SLC3A2*, ensuring proper membrane localization and stability [1]. Formyl peptide receptor 2 (FPR2) is a G protein-coupled receptor involved in the activation of downstream kinases and in the NOX-dependent generation of reactive oxygen species (ROS) [2,3]. Metabolomic analysis of lung anaplastic carcinoma CaLu-6 cells revealed that stimulation of FPR2 with its cognate agonist WKYMVm induces intracellular accumulation of leucine, phenylalanine, tryptophan, and tyrosine. Based on these findings, we investigated the role of FPR2 in modulating LAT1/*SLC7A5* expression. FPR2 activation in CaLu-6 and ductal carcinoma HCC-1937 cells upregulates LAT1/*SLC7A5* in a NOX-dependent manner and promotes a time-dependent translocation of CD98 to the plasma membrane. Moreover, FPR2 regulates LAT1/*SLC7A5* expression at both transcriptional and post-transcriptional levels by enhancing c-Myc activation and reducing miR-126 levels. Given the role of leucine in activating mTORC1 and thereby promoting phosphorylation of its downstream targets S6K and 4E-BP1 [4], we analysed mTORC1 activation and found that FPR2 stimulation promotes phosphorylation of S6K and 4E-BP1 through a NOX-dependent mechanism. Overall, our findings demonstrate that FPR2 stimulation upregulates LAT1/*SLC7A5* expression, enhances c-Myc activation, reduces miR-126 levels, promotes CD98 translocation to the plasma membrane, and triggers redox-dependent activation of the mTORC1/S6K/4E-BP1 pathway. These data reveal a novel regulatory mechanism of the LAT1/mTORC1 pathway, identifying a potential therapeutic target for cancer treatment.

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Nanoemulsified Polyphenol Formulation Enhances Cellular Uptake and Protects Cells Against Oxidative Stress

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Nutritional strategies are increasingly recognized as complementary tools for promoting health. Among them, dietary polyphenols have gained attention for their ability to counteract oxidative stress (OS) and are therefore considered potential agents for the prevention of OS-related diseases [1]. In male reproductive system, OS significantly contributes to sperm dysfunction, affecting sperm concentration, motility, and DNA integrity [2]. However, the therapeutic potential of polyphenols is limited by poor oral bioavailability due to gastrointestinal degradation and first-pass metabolism [3].

To address these limitations, we developed oil-in-water nanoemulsions by high-energy emulsification with optimized surfactant/oil ratios and evaluated the effect of different polyphenols in modulating OS in different cellular models. Quercetin loaded-nanoemulsions were prepared, and their biocompatibility and cytotoxicity were evaluated in human spermatozoa. In parallel, the formulations were tested in the differentiated intestinal cell line HT-29, a representative cellular model the physiology of the intestinal epithelium. Cell viability was assessed using the Crystal Violet assay, while cellular uptake of quercetin was quantified by HPLC–UV/DAD.

Quercetin loaded-nanoemulsions were non-cytotoxic at concentrations of 0.5, 1, and 2 μM. Under OS induced by *tert*-butyl hydroperoxide, they provide significant protection, yielding lower intracellular peroxide levels compared to both the control and the equivalent doses of free quercetin. Furthermore, HPLC–UV/DAD confirmed intracellular accumulation of quercetin following exposure to the nanoemulsion, whereas the same doses delivered in DMSO remained below the detection threshold. In addition, preliminary results showed that human sperm exposed to quercetin exhibit an improvement in post-thaw quality.

Overall, these findings support nanoemulsified quercetin as a promising strategy to mitigate OS in different cellular models.

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Digested cow and buffalo milk inhibit colorectal cancer growth

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Background: Colorectal cancer (CRC) is the third most common neoplasm worldwide and the fourth most common cancer by mortality rate (900,000 cases per year) [1]. Key risk factors include obesity, physical inactivity, smoking and dietary choices. Epidemiological studies have attributed a third of deaths from neoplasms to unhealthy eating habits suggesting dairy products as a protective dietary factor against CRC [2,3].

Aim: The purpose of the present research is to evaluate the effects of cow and buffalo milk, whole and after to an in vitro enzymatic digestion, on CRC models to demonstrate its potential protective activity against CRC progression.

Materials and Methods: Cow and buffalo milk were processed to simulate physiological gastrointestinal digestion (GID) and all milk fractions were subjected to aqueous soluble phase extraction (WSE). After WSE treatment, cell viability in HT-29 and LoVo cancer cell lines was assessed using a colorimetric assay (WST-8). Subsequently, a clonogenic assay was performed to evaluate tumour potential and cell cycle progression was evaluated by flow cytometry. Mass spectrometry was performed to identify peptides or metabolites that characterised WSE of whole and digested milk fractions.

Results: Results showed that buffalo milk had stronger cytotoxic effects than cow milk, and the cell growth inhibition was even higher after treatment with the GID fractions. Additionally, the GID fractions from buffalo milk caused a significantly decrease in S/G2/M phase cells and a reduction in cell proliferation. Mass spectrometry results showed that four out of all peptides contained in the WSE whole and digested milk fractions are characterised by anticancer activity. These peptides, known for their anticancer effects, could justify in vitro results observed after treatment with WSE fractions.

Conclusions: Our results demonstrate that treatment with WSE derived from GID fractions could represent a potential approach to successfully prevent CRC progression.

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SGLT2 inhibition induces metabolic dysfunction and triggers ER stress- dependent cell death in colorectal cancer cells

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Colorectal cancer (CRC), the third most common neoplasia worldwide, is characterized by strong metabolic plasticity which plays a key role in its initiation and progression [1,2]. The sodium-glucose cotransporter 2 (SGLT2), overexpressed in several cancer models, modulates cell metabolism and promotes cancer onset and development [3]. In this context, SGLT2 inhibitors (iSGLT2) with anti-hyperglycemic, nephroprotective, and cardioprotective properties, have recently gained attention for their emerging anticancer properties [4,5,6]. The present study aimed to investigate the possible effects of iSGLT2, canagliflozin, on CRC cell metabolism and death mechanisms. Results showed that SGLT2 expression was higher in CRC cells compared to normal colonocytes CCD 841 CoN ($p < 0.01$). Treatment with iSGLT2 (50 μM for 72 h) reduced CRC cell viability ($p < 0.001$) and triggered metabolic dysfunction, characterized by impaired oxidative phosphorylation, glycolytic flux and ATP production ($p < 0.001$). These effects were associated with enhanced ROS generation, endoplasmic reticulum (ER) stress and activation of the autophagic flux, leading to cell death ($p < 0.001$). Result on the molecular mechanism underlying the antitumor activity iSGLT2 showed an increases SIRT3 protein levels following treatment with iSGLT2. Moreover, transient SIRT3 silencing attenuated the cytotoxic effects of iSGLT2, suggesting the role of the SGLT2/SIRT3 axis in mediating CRC cell death.

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Innovative lipophilic ruthenium (III) complex as an antiproliferative agent triggering ferroptosis cascade in triple-negative breast cancer

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In recent years studies have shown the hard-to-treat triple-negative breast cancer (TNBC) phenotypes as more sensitive to ferroptosis than other types of BC, thereby providing the opportunity to explore new and effective targets for cancer therapy. Ferroptosis is featured by uncontrolled lipid peroxidation and extensive membrane injury caused by unbalanced redox homeostasis and iron-dependent oxidative stress [1]. However, recent evidence demonstrates attractive crosstalk between different programmed cell death pathways, e.g., ferroptosis and apoptosis, encompassing the Bcl-2 protein family [2]. In this context, our research team has showcased that Ru(III)-based complexes can serve as pro-apoptotic multitarget antiproliferative agents capable of effectively inhibiting proliferation, migration and invasion of BC cells [3]. Besides apoptosis, we here demonstrate that ferroptosis is implicated in the mechanism of action of a novel lipophilic Ru(III) complex named PalmiPyRu, which has proven to be effective against the TNBC [4]. Following treatments in vitro, we show significant effects on iron homeostasis via changes in cellular content of Iron Regulatory Proteins, transferrin receptor, ferritin, and ferroportin, leading to labile iron pool enlargement and consequent iron-dependent oxidative stress. Thus, PalmiPyRu biological effects in BC models are very similar to those induced by a condition of iron overload. Moreover, targeted experiments show ROS production with oxidative damage, coupled to a consistent decrease in cellular antioxidant potential. GSH levels are significantly decreased after treatment with PalmiPyRu, as well as the expression of glutathione peroxidases (GPXs).

Distinctive cytomorphological hallmarks highlight that TNBC phenotypes are directed towards a ferroptotic death pathway. These findings prove the potential of PalmiPyRu as a candidate anticancer drug in effectively inhibiting cancer cell proliferation through multimodal molecular mechanisms.

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miR-223: A Key Player in Metabolic Reprogramming and Drug Resistance in Laryngeal Squamous Cell Carcinoma

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Background: Laryngeal squamous cell carcinoma (LSCC) represents one-third of all head and neck malignancies [1]. Delayed diagnosis and limited treatment options highlight the need for early non-invasive biomarkers and novel therapeutic targets, including promising noncoding RNAs [2,3].

Aim: Based on preliminary data identifying a 3-miRNA signature in LSCC [4], we focused on miR-223 to investigate its biological and functional roles *in vitro*. Specifically, we explored its molecular targets involved in tumorigenesis, lipid metabolism, ferroptosis, and resistance to conventional treatments.

Methods: LSCC HEp-2 and HNO-210 cell lines overexpressing or downregulating miR-223 were tested by functional assays and Nanolive Analysis. Dose-response curves were performed to assess cisplatin resistance. RT-qPCR and Western blot analyses were used to investigate the molecular bases underlying miR-223 pro-tumorigenic role. Bioinformatics tools were queried to select miR-223 putative targets and modulated pathways.

Results: miR-223 overexpression enhanced cell proliferation, clonogenicity, migration and invasion. Nanolive imaging revealed a marked increase in both number and size of lipid droplets in HEp-2 cells overexpressing miR-223. This accumulation correlated with the upregulation of lipid metabolism genes (FABP5, EC1, EHHDAH) in HNO-210 and ferroptosis-related genes (SLC7A11, GSTT1) in HEp-2. HEp-2 cells overexpressing miR-223 showed reduced sensitivity to cisplatin compared to negative control. *In vitro* data and bioinformatics revealed miR-223 involvement in cancer-related pathways, including EMT. EMT array profiling identified several pro-metastatic genes modulated by miR-223. Western blot analysis of HEp-2 cells overexpressing miR-223 revealed downregulation of tumor suppressors p53 and PTEN, alongside upregulation of oncoproteins eIF4E and Cyclin D1. RT-qPCR further confirmed EMT activation and cell cycle progression in both cell lines.

Conclusions: Our data highlight miR-223 as a key regulator of cellular metabolism and drug resistance in LSCC. Both omics and molecular approaches will be employed to better understand the identified mechanisms. In parallel, we plan to establish cisplatin-resistant cell lines for a more detailed characterization of the interaction between miR-223 and key targets involved in drug resistance.

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Untargeteddiversity-orientedsynthesis(undos)forthediscoveryof novel antitumor agents: integrating ivs, bioinformatics and omics for targets deconvolution

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Nowadays cancer remains one of the main causes of death suggesting a still urgent need of novel pharmacological opportunities, despite the wide variety of available therapeutic treatments [1]. Diversity-Oriented Synthesis (DOS) represents a helpful tool in the identification of new antitumor agents, providing new chemical tools and novel potential drugs for cancer research [2]. In this work we describe an Untargeted Diversity-Oriented Synthesis (UnDOS) addressed to the identification of new antitumoral agents [3]. Starting from an *L*-amino acid as common reagent we designed and synthetized a set of twenty small molecules, which were preliminary screened for their anticancer activity using a cell viability assay on an in-house panel of immortalized cell lines. Among the tested derivatives, two compounds were selected for their micromolar activity and selectivity for further biochemical investigations on A-375 melanoma cells. To identify the binding partners and clarify the mechanism underlying the observed effects, we employed a helpful tool combining inverse virtual screening, bioinformatics and multi-omics analyses. The putative targets predicted by the integration of these approaches were filtered considering those with higher mRNA level in A-375 cells than in healthy HaCat cells. Then, the most promising targets suggested by the integration of all the obtained results, were corroborated carrying out binding, functional, and cellular assays, confirming a measurable interaction with several of them and identifying Transient Receptor Potential channels and Bromodomain-containing protein 4 as the most involved. Moreover, these data were supported in-cell assays that confirmed the pro-apoptotic effect of our compounds and their ability to induce protein misfolding and oxidative stress. Therefore, besides the identification of new potential hits, the novelty of this study regards the setting up of a platform integrating the principal approaches currently used for the target deconvolution, presenting a valuable tool for the identification of the biological counterpart of an untargeted compound set.

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Synergistic therapeutic strategy: 5-FU combined with G-Quadruplex ligands overcomes uL3-mediated chemoresistance *in vitro* and in preclinical CAM model

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Chemoresistance remains a major obstacle in the treatment of colorectal cancer (CRC), significantly reducing the efficacy of chemotherapy. In this context, gene expression heterogeneity plays a crucial role in determining cancer cell adaptability and survival under therapeutic pressure. Our previous data revealed that ribosomal protein uL3 is positively correlated with both chemoresistance and poor prognosis in CRC patients [1]. This study investigates an innovative therapeutic approach combining 5-fluorouracil (5-FU), the gold standard of first-line treatment for CRC, with two well-characterized G-quadruplex (G4) ligands—pyridostatin (PDS) and RHPS4—emerging as promising candidates for cancer therapy [2], aiming to counteract uL3-mediated chemoresistance. We found that resistant p53-deficient and uL3-silenced CRC cells exhibited vulnerability to the cytotoxic effects of both G4 ligands. The combination of 5-FU with PDS or RHPS4 demonstrated a synergistic effect, selectively targeting tumor cells. This strategy enabled a more than tenfold reduction in the required 5-FU dose, enhancing therapeutic efficacy while minimizing adverse effects. The effectiveness of this combination was further validated in vivo using uL3-silenced CRC cell-derived xenografts in the chick chorioallantoic membrane (CAM) model. Overall, our findings reveal a promising combination therapy to overcome chemoresistance in CRCs characterized by dysfunctional p53 and decreased uL3 expression levels.

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Dimethyl fumarate combined with cisplatin at sub-cytotoxic doses sensitizes cervical cancer towards ferroptosis and apoptosis through GSH restriction and p53 (re)activation

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Cervical cancer remains one of the leading causes of cancer-related mortality among women worldwide. Persistent human papillomavirus (HPV) infection is the primary cause of cervical cancer progression. HPV-mediated transformation relies on viral proteins that by interacting with proteins of the host cells (e.g. p53) dysregulate pathways essential to prevent tumor progression. Chemotherapy in cervical cancer is mainly based on cisplatin, but this drug has limited efficacy; therefore, alternative treatment options are needed. Ferroptosis represents a novel form of cell death. In cervical epithelium, ferroptosis occurs in the early neoplastic stages of HPV infection but shifts to evasion in carcinoma. We demonstrated that Dimethyl fumarate (DMF), an FDA-approved anti-inflammatory drug, induces ferroptosis in cervical cancer cells and inhibits growth in spheroid models. Since combined therapy has the potential to enhance cancer cell death and overcome resistance development, we co-treated cells with sub-cytotoxic dose of DMF and cisplatin. The results indicated that cell viability was decreased compared to either drug alone. Under DMF/cisplatin combination, cervical cancer cells underwent to glutathione depletion and p53 (re)activation, provoking both ferroptosis and apoptosis. We found a lowering of the x-CT system/glutathione mediated by p53, hinting a shift towards cell death. Our results suggest that administration of DMF plus cisplatin by targeting the dependency of cervical cancer cells on glutathione and (re)activating p53, can represent a promising option for anti-cancer therapy.

Growth-Dependent CDKL5 Phosphorylation in *Pseudoalteromonas haloplanktis* TAC125

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CDKL5, a member of the cyclin-dependent kinase family, is abundantly expressed in the central nervous system and participates in neuronal migration and synaptic function. Dysregulated CDKL5 expression or activity underlies the severe, currently incurable, CDKL5 deficiency syndrome (CDD). Because the phosphorylation status of CDKL5 is closely related to its functions and regulatory circuits, defining its activity and control mechanisms is urgently needed. The Antarctic marine bacterium *Pseudoalteromonas haloplanktis* TAC125 (PhTAC125) has emerged as a promising tool for the recombinant production of complex proteins, thanks to its atypical physiology and enabling molecular toolkit. Notably, PhTAC125 has been reported as the only prokaryotic host capable of producing full-length human CDKL5. Using five distinct culture conditions, we investigated how environmental inputs modulate CDKL5 phosphorylation and the implications for cell signalling, solubility, stability, and overall quality of the recombinant product. Mass spectrometry delineated the phosphorylation landscape and revealed site-specific phosphorylation for each condition, highlighting limited activity of the full-length protein. We then expressed only the catalytic domain to test whether the highly unstructured C-terminus compromises stability and activity. We also catalogued co-purifying proteins that could interfere with CDKL5 isolation, providing insight into purification outcomes. Preliminary data indicate that the catalytic domain alone improves stability and activity, and that specific contaminants measurably affect purification. Establishing optimal growth conditions should therefore provide useful information on CDKL5 phosphorylation levels and activity, guiding production strategies and ultimately facilitating the development of targeted drugs aimed at alleviating and/or improving neurodevelopmental disorders related to CDKL5 dysregulation.

A stable GH31 α-Glucosidase as a powerful model to investigate mutations causing glycogen storage disease type II

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GH31 glycosidases represent a large and diverse enzyme family found across all domains of life, yet fewer than 1% have been biochemically characterized. Among them, the human lysosomal acid α-glucosidase (GAA) is of particular interest, as its deficiency causes Pompe disease—a rare lysosomal disorder leading to glycogen accumulation, muscle degeneration, and premature death. Structural and functional studies of GAA mutants are limited by their instability and loss of enzymatic activity, hindering expression and purification.

Here, we present MalA, a GH31 α-glucosidase from a hyperthermophilic archaeon, as a stable and tractable homolog of GAA. Mala is highly expressible, easy to purify, and structurally well characterized, making it a valuable model for biochemical analyses. To validate this system, we generated the R400H mutant in MalA, corresponding to the pathogenic R600H variant in human GAA. The mutant showed a dramatic 1,200-fold decrease in catalytic efficiency and an 8 °C reduction in thermal stability, closely mirroring the effects observed in GAA.

Our results demonstrate that MalA provides a robust platform to investigate the molecular impact of disease-related mutations and to support the development of pharmacological chaperones aimed at restoring GAA function in Pompe disease.

Reference

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Mediterranean Herbs derived compounds: Bridging Antioxidant Power and Lipid Regulation

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Obesity remains a major global health issue, affecting both adults and children and contributing to chronic diseases such as metabolic syndrome, cardiovascular disorders, type 2 diabetes, and non-alcoholic fatty liver disease (NAFLD) [1]. NAFLD has become the most prevalent chronic liver condition in developed countries, strongly associated with poor dietary habits and sedentary lifestyles. As public health awareness increases, functional foods, defined as foods providing health benefits beyond basic nutrition, have gained growing attention for their potential in preventing and managing obesity-related disorders [2]. These foods often contain bioactive compounds such as polyphenols, flavonoids, and terpenoids, naturally occurring in herbs, fruits, and vegetables. Mediterranean herbs like basil, thyme, and oregano are particularly rich in health-promoting molecules including eugenol, carvacrol, and thymol, while lycopene, abundant in tomatoes, is well known for its beneficial properties [3]. This study investigates the biological activities of eugenol, carvacrol, thymol, and lycopene, individually and in combination, both in vitro and in cell-based models. The antioxidant potential of these compounds was first evaluated and subsequently used as a basis to assess their efficacy in counteracting lipid accumulation in a hepatic steatosis model using HepG2 cells. All molecules demonstrated full biocompatibility across a wide concentration range and were shown to modulate NAFLD induction in hepatocytes by influencing distinct molecular pathways. Overall, these findings highlight the potential of integrating bioactive compounds from Mediterranean herbs and tomatoes into functional foods, such as bioactive-enriched sauces, as part of nutritional strategies aimed at preventing obesity-related metabolic disorders and promoting liver health.

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Microalgae as a sustainable solution for modern agriculture

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Agricultural intensification driven by global population growth led to severe environmental consequences. Since their first synthesis in the 1930s, chemical pesticides have become a cornerstone of modern agriculture, contributing to increased crop yields and global food security. However, their excessive and improper use has raised serious concerns regarding human health and environmental safety [1]. In line with the European Union "Farm to Fork" strategy, there is growing interest in sustainable alternatives to synthetic agrochemicals. Microalgae and cyanobacteria are photosynthetic microorganisms characterized by a remarkable metabolic diversity, representing a potential reservoir of compounds endowed with biological activity against phytopathogens [2], making them a green, natural, sustainable alternative to synthetic pesticides [3]. In this study, bioactive molecules were extracted and characterized from different microalgae strains. Some of them were endowed with antimicrobial activity on selected phytopathogens associated with plants. The most promising strains were cultivated on Dairy Wastewater and Olive Mill Wastewater, in order to reduce freshwater footprint while facilitating nutrient recycling and effluent treatment. Highlighting the potential of microalgae bioactive compounds as sustainable biocontrol tools, allowing the integration of circular economy principles with eco-friendly agricultural innovation.

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From Gas to Growth: Using Carbonic Anhydrase for CO2 Bio- Fixation in Microalgae

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The continuous rise in atmospheric CO_2 represents one of the most pressing global challenges, demanding innovative and sustainable mitigation strategies [1]. Here, a biotechnological system that enhances CO_2 capture through the synergistic use of human Carbonic Anhydrase II (hCAII) increasing microalgal biomass production is reported. hCAII is an efficient metalloenzyme that catalyzes the reversible hydration of CO_2 into bicarbonate, increasing the availability of inorganic carbon for photosynthetic microorganisms [2]. The enzyme is entrapped in silica beads synthesized from tetraethoxysilane (TEOS), a robust and biocompatible material that provides a reusable matrix with high mechanical and chemical stability [3]. This configuration allows repeated use of the biocatalyst while maintaining enzymatic activity, offering a sustainable and cost-effective approach to CO_2 conversion. Once introduced into the culture medium, the TEOS–hCAII system increases bicarbonate availability, stimulating microalgal growth and improving biomass yield. The produced biomass can then be valorized in a cascade biorefinery process to obtain high-value compounds, such as pigments, proteins, and antioxidants, contributing to a circular bioeconomy. Although Carbonic Anhydrases have been extensively studied for CO_2 capture, their integration with TEOS-based supports in microalgal systems remains largely unexplored. This study demonstrates a promising route for combining enzymatic catalysis, material science, and microalgal biotechnology toward sustainable carbon management and renewable resource generation.

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Circular Bioconversion of Agri- Food Residues into Bacterial Cellulose and Natural Indicators for Smart Packaging Applications

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Within the framework of circular economy and sustainable innovation, the transformation of agro-food residues (AFR) through microbial processes represents a promising approach to reduce waste and generate value-added biomaterials [1]. This study investigates the bioconversion of agri-food residues into bacterial cellulose (BC) using Kombucha, integrating natural anthocyanin pigments to generate functional, pH-responsive materials [2].

Several AFR by-products were evaluated as alternative carbon and nitrogen sources for Kombucha fermentation. Additionally, anthocyanin-rich natural extracts were incorporated and characterized for their antioxidant activity and pH-dependent chromatic response. The resulting biocellulose–anthocyanin films exhibited reversible color transitions correlated with pH variations, demonstrating their potential as visual spoilage sensors for food packaging applications. This sustainable approach, which does not require chemical pretreatment, valorizes low-value agri-food residues by converting them into functional, biodegradable materials. In this way, the study provides a concrete example of how AFR can be transformed within a circular economy framework, supporting the development of smart coatings for sustainable food packaging and enhancing quality control throughout the food supply chain.

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A circular bioeconomy approach using a biotechnological integrated platform to valorise different fractions from buffalo second cheese whey for cosmeceutical, nutraceutical and food applications

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The disposal of by-products from dairy industries is often economically demanding due to the treatments that are required to reduce the environmental impact. This project is part of a circular economy perspective focused on the conversion of a potential "waste" difficult to manage into a resource to be exploited, and it embodies the concept of the three "R": Reduce, Recycle and Reuse. Specifically, the aim of this study regards the setup of an efficient lab-scale platform for the conversion of locally produced liquid waste materials, specifically whey and second cheese whey (SCW), into added-value products. Special focus was paid to the implementation of a downstream process that converts waste generated from food processing industries into added value products with potential applications. Initially, membrane processes were performed to fractionate bioactive components starting from buffalo milk whey. Owing to innovative downstream and biotechnological processes, we demonstrated up to the 20L scale that SCW fractions can be used as substrates in probiotic fermentations, in the nutraceutical sector and also to obtain biodegradable films with or without enzymatic crosslinking. In addition, specific fractions retentate 100 and 10 kDa, proved a protective effect in dehydration tests, 4-fold respect to a negative control, and a reparative effect in oxidative stress, 3.5-fold respect to a negative control [1]. Also wound healing experiments on human keratinocytes to gain further potential as cosmeceutical ingredients, obtaining complete closure of the wound after 20 hours compared to 48 hours in the control [2]. Moreover postbiotics obtained from the treatment of fermentation supernatants were tested against three different pathogens, *Enterococcus faecalis*, *Salmonella enterica subsp. enterica serovar Typhimurium* and *enteroinvasive Escherichia coli*, gave a reduction on viability by 6–7 log on average, proving an antimicrobial activity.

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Unveiling the potential of *Pseudococcomyxa simplex*: a stepwise extraction for cosmetic applications

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The cosmetic industry is one of the most profitable worldwide, with revenues projected to reach 129 billion USD by 2028. Skincare dominates this market and is expected to grow to 221.38 billion USD by 2030 (CAGR 4.79%). Increasing consumer preference for natural cosmetics reflects rising environmental and health awareness from synthetic compounds, such as allergies or hyperactivity [1]. Thus, companies are transitioning to natural, safe, effective, and sustainable products [2]. Microalgae are gaining attention as they are green factories of bioactive metabolites, with unique biological activities [3]. However, their use at an industrial scale is still a challenge because of high costs related to upstream and downstream processes [4]. Here, a biorefinery approach is proposed, starting from the biomass of the green microalga *Pseudococcomyxa simplex* for the extraction of two classes of molecules with potential use in the cosmetic industry. Carotenoids were extracted first by an ultrasound-assisted extraction, and then, from the residual biomass, lipids were obtained by conventional extraction. The chemical characterization of the ethanol extract indicated lutein, a biosynthetic derivative of α-carotene, as the most abundant carotenoid. The extract was found to be fully biocompatible on a cell-based model, active as antioxidant and with an *in vitro* anti-aging property. In particular, the lutein-enriched fraction was able to activate Nrf2 pathway, which plays a key role also in aging process. Finally, lipids were isolated from the residual biomass, and the isolated fatty acids fraction was composed of palmitic and stearic acids. These molecules, fully biocompatible, can find application as emulsifiers and softener agents in cosmetic formulations. Thus, an untapped microalgal species can represent a biotechnological source for cosmeceutical formulations.

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Isovitexin, a flavone glucoside present in *Passiflora edulis* Sims isolated and characterized using an innovative, environmentally sustainable extraction method

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Passiflora edulis Sims is a tropical climbing plant also known as "passion fruit", widespread in South America belonging to the *Passifloraceae* family, renowned for its beneficial properties on human health and used in local traditional medicine for the production of remedies against numerous pathologies. Currently, several food and pharmaceutical formulations of *P. edulis* Sims are available on the market [1]. The aim of this study was to produce and characterize hydroalcoholic extracts of *P. edulis* Sims leaves by comparing solid-liquid extraction techniques of different technological complexity: maceration or infusion, the most used traditional extraction method due to its simple application, Ultrasound Assisted Extraction (UAE), a highly technological and more recent technique based on the use of low frequency ultrasounds for extraction improvement and Rapid Solid-Liquid Dynamic Extraction (RSLDE), an innovative technique based on a principle of pressure and depression [2]. The extracts were analyzed using the Folin-Ciocâlteu and DPPH (2,2-diphenyl-1-picryl-hydrazyl) assays to determine total polyphenolic content and radical scavenging power, respectively. Furthermore, the extraction kinetics of the extracts obtained at different ethanol concentrations was evaluated by optimizing the parameters of a kinetic exponential function. The comparison between the different techniques showed how technological advances have allowed for a drastic reduction in extraction times while maintaining unchanged yields. The extracts obtained under the optimal conditions determined for each technique were purified using various chromatographic techniques, obtaining the main secondary metabolite present in the *P. edulis* Sims extracts. This compound of particular pharmaceutical interest was identified as the flavone glucoside isovitexin [3] using mass spectrometry, polarimetry and various NMR techniques and quantified in the various extracts by HPLC-UV, determining a high yield of isovitexin for each of the extracts. In particular, the comparison highlighted the possibility of obtaining this valuable bioactive compound through an ecologically sustainable green extraction method, in line with the current circular economy model.

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Biological activity of microbial melanin: insights into amyloid complex formation and UV protection in human keratinocytes

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Melanin is a natural polymeric pigment known for its remarkable biological properties, including antioxidant and antimicrobial activities, UV-visible light protection, redox behavior, and radical-scavenging capabilities, which make it promising for various biomedical applications [1]. However, despite these known functions, the biological properties of microbial melanin remain poorly understood [2]. In this study, we have performed a functional characterization of microbial melanin biotechnologically produced and purified from *Streptomyces nashvillensis* DSM 40314. Our results reveal that this pigment exhibits previously unexplored biological activities, including specific interactions with amyloid fibrils. In mammalians, functional amyloid fibrils are crucial in melanogenesis but their relationship with the intrinsic properties of microbial melanin still remains unexplored. To address this, we examined the activity of microbial melanin and melanin/amyloid complexes using a human keratinocyte (HaCaT) cell model. Specifically, incorporating microbial melanin into an amyloid scaffold significantly enhanced the protection of keratinocytes from UV-induced damage. Overall, this work provides essential preliminary insights into the functional and biological characterization of biotechnologically produced melanin and add new information regarding the potential role of amyloid in melanogenesis.

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Metal-ion driven biotechnological production of melanin

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Melanin is a poorly studied pigment with excellent chemical and physical properties, making it suitable for various industrial and biomedical applications. Nowadays, melanin production mainly follows two routes. The first is chemical synthesis, which is characterized by low yields and environmental unfriendly processes. The second is extraction from animal tissues, mainly from cuttlefishes, which ensures higher yields but raises ethical concerns. A more sustainable and cost-effective alternative to meet the demand of melanin manufacturing is to set up an innovative biotechnological production process. Between microorganisms, Streptomycetes have recently gained more and more attention as they are able to produce melanin during their secondary metabolism phase as extracellular product. In particular, in this work, *Streptomyces nashvillensis* DSM40314 melanin production was enhanced by setting up a supplementation strategy to the growth medium with metal ions. In shake flask experiments the addition of different concentrations (1.0, 1.5, and 2.0 g/L) of $CuSO_4$ or/and $Fe_2(SO_4)_3$ was tested singularly or, at the same time. Both copper and iron salt increased melanin synthesis when supplied singularly and they gave a synergistic effect up to a maximum of 4.0 ± 0.1 g/L melanin production when combined together. In fermentation batch experiments, in more controlled stirring and air flow conditions, this approach allowed to further boosting the pigment production up to 4.9 ± 0.1 g/L.

Citrus lignocellulosic waste biomasses as substrates for enhancing microbial melanin production

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Melanins are polymeric pigments found in the animal, plant and microbial kingdoms, having a role in defense mechanisms under stressful conditions, serving as protective agents against intensive UV-visible light radiation, and acting as scavengers against free radicals and reactive oxygen species. Nowadays they are mainly obtained from animal sources with long, expensive and not sustainable extraction procedures. Since some fungi and bacteria species can produce melanins as pigments, in the last years the possibility to develop biotechnological processes to produce melanins has gained more attention. The goal of this study was to set up a sustainable biotechnological process of melanin production by a new *Streptomyces nigra* strain, isolated from Messina soil (Sicily, Italy), by using lignocellulosic wastes as growth substrates. *Citrus sinensis*, *Citrus bergamia* and *Citrus limon* peel powders, wastes of food and cosmetic industries, were supplied in shake flasks at different concentrations (1.0 g/L or 2.5 g/L or 5.0 g/L) to a semi-defined growth medium, containing rice starch, soya peptone and malt extract, to support and enhance both bacterial growth and melanin synthesis. Addition of *Citrus sinensis* (1.0 g/L) drove to a 7.8 ± 0.1 g/L melanin production, 3.25-fold higher than the 3.3 ± 0.1 g/L pigment concentration obtained with *Citrus bergamia (*5.0 g/L) supplementation. *Citrus limon* addition*,* instead, at any concentration, did not enhance bacterial growth nor melanin production, compared to the control medium. The tyrosinase activity, the first enzyme involved in the melanin biosynthetic pathway, was also checked in the different conditions to correlate it with the pigment production.

Decoding copper adaptation mechanisms in marine bacteria through multi-omics and network analysis

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This study, conducted in collaboration with the Stazione Zoologica Anton Dohrn (Naples, Italy), investigates adaptative molecular mechanisms of marine bacteria isolated from metal-contaminated sediments in the Gulf of Naples and Bagnoli. [1][2] All isolates were screened by determining the maximum tolerance concentration (MTC) to As, Cd, Co, Cu, Zn, and Pb.[2][3] Among them, *Pseudohalocynthiibacter aestuariivivens* P96 was selected for in-depth investigation under copper exposure. Copper was chosen for its dual significance as both an essential enzymatic cofactor and a strategic raw material recognized under the European Union's *Critical Raw Materials Act* [4], making it relevant for both bioremediation and bioleaching applications.[5] Genomic and proteomic analyses were performed to elucidate the mechanisms underlying bacterial adaptation to copper. *MRGs* screening revealed determinants related to copper homeostasis, including multicopper oxidases, efflux systems, and transports then confirmed by differential proteomic analysis. [6] Due to the phylogenetic distance of this isolate from reference organisms, conventional annotation and pathway reconstruction tools proved largely ineffective; to overcome this limitation, a correlation-based approach integrating omics data was applied for enabling the reconstruction of putative pathways involved in copper response, further supported by literature validation. [7]

This integrative omics strategy provides new insights into bacterial adaptation to copper stress and demonstrates the effectiveness of correlation-based network protein analysis for functional inference in non-model organisms, identifying promising candidates for in situ bioremediation and bioleaching in metal-contaminated environments

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Chemical and functional characterization of free and bound polyphenols from ancient wheat flour Risciola (*Triticum aestivum* L.)

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In recent years, ancient grains have attracted growing interest from the scientific community, due to evidence suggesting a superior nutritional and functional profile compared to modern cereals, primarily attributed to their higher concentration of bioactive compounds [1]. In this study, a locally cultivated ancient wheat flour, Risciola, was analyzed at different degrees of sifting and characterized for its nutritional and functional properties. The antioxidant and antiproliferative activities of its phenolic compounds were evaluated in vitro using the human colorectal adenocarcinoma cell line HT-29.

Free phenolic compounds were extracted with ethanol/water, while bound phenolics were recovered through sequential alkaline and acid hydrolysis followed by solvent extraction. Total phenol content was determined using the Folin-Ciocalteu method, and antioxidant activity was assessed spectrophotometrically by evaluating radical scavenging capacity against DPPH and ABTS radicals. Following preliminary characterization, the bound polyphenolic extract (EP-bound) from whole wheat Risciola flour was selected for biological assays. Cell viability was assessed using the Crystal Violet assay after treatment with increasing concentrations of the extract. Intracellular reactive oxygen species (ROS) production was quantified using the DCFH-DA probe, while cytotoxicity was evaluated by measuring lactate dehydrogenase (LDH) release. Apoptotic marker activation was further analyzed via SDS-PAGE and immunoblotting.

Risciola whole wheat flour exhibited a high concentration of bound polyphenols and significantly greater antioxidant activity compared to refined flours. The EP-bound extract demonstrated cytotoxic effects on HT-29 cancer cells, accompanied by reduced ROS levels and indications of potential adaptive cellular responses. At low concentrations, a hormetic effect was observed, suggesting dose-dependent biological benefits.

These findings provide a promising foundation for future studies aimed at the chemical characterization of EP-bound and the identification of specific compounds responsible for the observed bioactivities.

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Citrus waste valorization for the sustainable production of the plant probiotic *K. pseudosacchari* TL13 and of its exopolysaccharide with potential biotechnological applications

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Recently the study of plant growth promoting bacteria (PGPB) is gaining considerable attention due to the increasing need for sustainable agricultural practices that reduce reliance on chemical fertilizers and pesticides. *Kosakonia pseudosacchari* TL13 is a recently isolated PGPB with potential applications in biotechnology due to its ability to metabolize and grow on different substrates that make it attractive in a biorefinery perspective. As other rhizobacteria the strain was found to produce a high molecular weight exopolysaccharide (EPS) probably involved in modulating plant growth and resistance to abiotic stresses.

In this study, the use of *K. pseudosacchari* TL13 was evaluated to produce low-cost inoculant biomass and metabolites, by combining medium optimization through DoE (design of experiments) analysis with fermentation on renewable waste substrates. A small scale plackett-Burmann screening approach with 5 factors and 13 runs was initially used in a defined medium to identify components that addressed metabolism towards biomass and/or EPS production. Growth of *K. pseudosacchari* TL13 was next evaluated in controlled 0.5 L bioreactors using citrus waste as substrate. Supplementation of a nitrogen source increased by two-fold the production of viable cells that reached a final titer of about $2.7\pm0.04\times10^{10}$CFU/mL to the detriment of polysaccharide production. This was also confirmed by the analysis of the rheological behaviour of the broths at the time zero and at the end of the process.

Overall citrus waste demonstrated a cheap and nutritionally valid substrate not only to support sustainable production of the plant probiotic *K. pseudosacchari* TL13 but also to obtain a value-added biopolymer with potential biotechnological applications.

KNR50: a new, promising, cryptic antimicrobial peptide from bovine casein αS2

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The rapid increase in drug-resistant infections represents a serious challenge for antimicrobial therapies thus focusing the attention on the necessity to continuously develop new and powerful drugs. In this context a class of very interesting molecules are antimicrobial peptides (AMPs).

There is currently thriving research on new potential AMPs and on innovative methods to identify cryptic AMPs hidden in larger proteins. At this regard, we previously developed an in silico tool [1,2] allowing to identify potential cryptic AMPs in AMP-releasing proteins and to predict their antimicrobial potency. Recently, by using this approach, we identified a novel cryptic AMP corresponding to the last 50 amino acid residues of bovine alpha S2 casein, we named KNR50 [3].

KNR50 was produced in Escherichia coli, purified and characterized. It emerged that KNR50 possess a strong antimicrobial activity on both planktonic and sessile cells. Intriguingly, by studying the bacterial killing kinetics, it was observed that KNR50 determines a significant reduction of the CFUs in less than three hours, likely through a lytic mechanism of action.

Has been highlighted that KNR50 also has moderate antibiofilm activity on three different stages of biofilm formation. Furthermore, we found that KNR50 is also a promising anti-viral agent thanks to its membranotropic activity and able to act in different phases of innate immunity as anti-inflammatory cytokine release, attenuation of ROS production and wound healing. All collected data, strongly, suggest that KNR50 is a powerful host defense peptide ideally suitable for various application fields including the formulation of new potential therapeutical agents.

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Green Biosynthesis of Silver Nanoparticles from Microbial Secretomes and Agri-Food Waste: A Circular Bioeconomy Approach

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Silver nanoparticles (AgNPs) are attracting increasing interest due to their unique physicochemical properties and broad applications in medicine, catalysis, and antimicrobial technologies [1]. In this study, AgNPs were biosynthesized using the secretome of *Geobacillus stearothermophilus* GF16, an extremophilic bacterium isolated from the hydrothermal volcanic area of Pisciarelli (Italy) [2].

The microbial secretome, rich in extracellular enzymes and bioactive metabolites, was employed for nanoparticle synthesis. To further enhance the functional properties of the AgNPs, the secretome was combined with phytochemical-rich extracts derived from agri-food by-products, specifically tea and grape residues. This dual approach aimed to exploit the synergistic potential of microbial and plant-derived components within a sustainable, low-impact synthesis framework.

The AgNPs produced under both conditions were extensively characterized through spectroscopic, biochemical, and biological analyses. The incorporation of agri-food waste extracts resulted in nanoparticles with significantly enhanced antioxidant and anti-inflammatory properties and improved hemocompatibility. Additionally, these AgNPs exhibited promising catalytic activity in the degradation of environmental pollutants.

Overall, the results support the use of microbial secretomes in combination with agro-industrial waste as an effective and eco-friendly strategy for the synthesis of high-value nanomaterials. The proposed method aligns with the principles of the circular bioeconomy, offering innovative opportunities for waste valorization and sustainable nanobiotechnology. This approach holds strong potential for scalable applications in biomedical devices, environmental remediation, and green industrial processes.

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Novel medicated wound dressing based on self-esterified Hyaluronan and the human antimicrobial peptide PAP A3 to promote wound healing and prevent/reduce in situ infections

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The research aimed to obtain a novel wound dressing combining recently developed self-esterified hyaluronic acid (HA) matrices with the human antimicrobial peptide PAP A3 to improve management of infected skin wounds. Self-esterified HA matrices proved an innovative delivery system capable of gradually releasing only native HA of defined molecular weight without releasing exogenous substances. PAP A3, a human cationic peptide released during pepsinogen activation in the stomach, was selected for its potent activity against Gram-positive and Gram-negative bacteria commonly found in infected wounds as well as for its endogenous origin [1]. A pharmaceutical-grade 230kDa (Mw/Mn=2.0) HA sample was used. Four self-esterified HA matrices with nominal esterification degrees of 5, 10, 30, and 100% (XHA5,XHA10,XHA30 and XHA100) were prepared [2]. Swollen in an aqueous peptide solution to load PAP A3, then lyophilized. Peptide release from the matrices, under physiological conditions, was monitored by UV spectrophotometry. Data indicated that PAPA3 release is inversely correlated with the degree of HA-esterification: PAP-A3 was completely released (100 wt%) within 7 days from the XHA5 and XHA10 matrices, with a faster release rate observed from XHA5 while no significant release was detected from the XHA30 and XHA100 matrices. The bioavailability of released PAP A3 was assessed by antimicrobial activity using a microdilution assay against *Pseudomonas aeruginosa* PAO1.

Results were in line with release kinetics, MIC values between 0.8 and 2.5 μM (for the XHA5 and XHA10), indicating that peptide-HA interactions do not compromise antimicrobial efficacy. No antimicrobial activity was detected for the other matrices and for the control.

Overall, data demonstrated that the developed XHA- PAP-3 system shows high potential as a novel device that, unlike typical approaches where peptides are covalently conjugated to HA, often compromising their natural activity, is able to preserve and deliver both HA and PAP-3 in their endogenous bioactive forms.

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Active Chitosan Films Incorporating *Callistemon citrinus* Extract

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Background

The growing environmental impact of synthetic plastic waste has accelerated efforts to develop biodegradable solutions for food packaging. Among these, chitosan, a natural polymer valued for its film-forming capacity, antimicrobial effects and antioxidant activity, stands out as a promising candidate. Incorporating plant-based extracts like *Callistemon citrinus* (C.E.) into chitosan films may further enhance their functional properties, particularly in extending food shelf life and improving preservation performance [1].

Objectives

This research aimed to formulate and evaluate chitosan-based bioplastics enriched with C.E., focusing on their film-forming behaviour, antimicrobial and antioxidant activities, oxygen permeability, water and moisture content, and lipid oxidation control during cheddar cheese storage.

Methods

Bioplastic films containing varying levels of C.E. were tested for colour, opacity, oxygen permeability, and antimicrobial efficacy against S. aureus, L. monocytogenes, P. aeruginosa, and E. coli. Antioxidant capacity was assessed and cheddar cheese samples wrapped in the films were monitored over 28 days for weight variation, moisture retention, lipid oxidation (K_{232}, K_{270}), and consumer acceptance [2].

Results

Film opacity increased proportionally with higher concentrations of C.E. The incorporation of 5% C.E. significantly improved the film's resistance to oxygen permeation and selective antimicrobial activity was observed against S. aureus and L. monocytogenes. Packaged cheese experienced higher weight loss (11.63%) compared to LDPE (2.90%) but retained satisfactory moisture (76.62%) and exhibited reduced lipid oxidation (K232 = 2.90). Consumer feedback was positive, with 73% expressing interest in purchasing cheese wrapped in C.E.-based films.

Conclusions

The addition of *Callistemon citrinus* extract enhances the antioxidant and antimicrobial properties of chitosan bioplastics, supporting their potential as eco-friendly food packaging materials. Further refinement is needed to improve moisture retention and ensure broader applicability.

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Unlocking the Human Proteome: Encrypted Antimicrobial Peptides from MMP19 as Next-Generation Therapeutics

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The human proteome represents a largely untapped reservoir of encrypted bioactive peptides with significant therapeutic potential [1,2]. We identified and characterized three antimicrobial encrypted peptides [3] derived from human matrix metallopeptidase 19 (MMP19), corresponding to residues 1–19, 1–33, and 247–279. These peptides were recombinantly expressed in bacterial cells and found to exhibit potent broad-spectrum antimicrobial activity against a range of Gram-positive and Gram-negative bacteria, including clinically isolated and multidrug-resistant strains. Mechanistic investigations revealed that their antimicrobial effects are mediated by membrane depolarization and permeabilization, leading to rapid bacterial killing. The peptides also demonstrated strong antibiofilm activity, both by inhibiting biofilm formation and by disrupting established biofilms. Notably, the peptides displayed selective antiviral activity against enveloped viruses. Furthermore, peptides were found to be neither hemolytic nor toxic toward mammalian cells. They were also found to possess immunomodulatory properties. Combination therapy assays revealed synergistic or additive effects when the peptides were used alongside conventional antibiotics. Importantly, prolonged bacterial exposure to peptides did not result in the emergence of resistance. A D-amino acid analogue of the lead peptide retained full antimicrobial activity, demonstrated excellent cytocompatibility, and showed therapeutic efficacy in a murine skin infection model. Collectively, these findings highlight human MMP19 as a novel source of multifunctional encrypted peptides and establish secreted human proteins as a promising reservoir for the development of next-generation antimicrobial and antiviral therapeutics.

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Escherichia coli Outer membrane vesicles (OMVs) surface functionalization with a variable heavy chain nanobody directed against PDL1: ClyA-PDL1 chimeric protein recombinant expression optimization

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Most bacteria produce Outer Membrane Vesicles (OMVs) through controlled membrane bulging mechanisms. These small proteo-liposomes, averaging 100 nm in size, perform multiple biological functions in vivo and can be readily engineered through surface modification or cargo loading to achieve site-specific targeting. Such properties make OMVs attractive candidates for developing drug delivery systems. In this context, antibody-mediated targeting represents a particularly promising strategy.

This study describes the surface functionalization of Escherichia coli (E. coli) OMVs with a camelid nanobody variable heavy chain domain (VHH) consisting of a single heavy-chain variable domain. VHH fragments are also referred to as single-domain antibodies (sdAbs), and they are generally smaller and more stable than conventional antibodies. We designed a chimeric protein composed of the membrane protein cytolysin A (ClyA), serving as a membrane scaffold, fused at the C-terminus to a VHH nanobody directed against the PD-L1 ligand. A C-terminal His-tag was added for protein detection in cells and vesicles.

The chimeric construct, termed ClyA-PDL1, was expressed in three E. coli strains, BL21(DE3), TUNER, and ROSETTA-GAMI (Merck), under varying recombinant expression conditions. Following expression, cells were harvested and OMVs purified from the culture supernatants. The presence of the chimeric protein in both cells and OMVs under the different conditions was analyzed by SDS-PAGE and Western blotting.

Results demonstrated successful expression of ClyA-PDL1 in all tested strains, albeit with variable efficiency. Notably, OMV-associated levels of ClyA-PDL1 differed depending on the bacterial strain and expression conditions. These findings indicate that expression optimization is crucial for maximizing nanobody incorporation into OMVs. Future experiments will focus on confirming the correct surface exposure of ClyA-PDL1 on OMVs and assessing their binding efficiency toward the PD-L1 ligand to establish a functional, targeted nanocarrier platform.

Sustainable biotechnological production of melanin by the novel streptomyces nigra mt6 isolate

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Melanins are natural pigments widely used in the food, cosmetic, and textile industries, typically obtained through unsustainable extraction from cuttlefish. Biotechnological production offers a sustainable and scalable alternative, yet melanin biosynthesis by Streptomyces species remains underexplored. This study investigates the potential of a novel isolate, Streptomyces nigra MT6, for melanin production using bergamot peel, an agro-industrial waste, as a carbon source.

The strain produced a diffusible brown pigment, and genome analysis confirmed genes related to melanin biosynthesis. A semi-defined medium devoid of animal-derived nitrogen sources was optimized using a Design of Experiment (DOE) approach to assess the effects of glucose, $CuSO_4$, $FeSO_4$, and L-tyrosine on melanin yield. Production ranged from 1.5–2 g/L, with copper sulfate and L-tyrosine significantly enhancing melanin synthesis.

Scaling up in 0.5 L bioreactor batch cultures resulted in a six-fold increase, reaching 3.0 ± 0.3 g/L after 48 h in the optimized medium. Substituting pure glucose with hydrolyzed bergamot peel extract yielded comparable results (3.1 ± 0.4 g/L), demonstrating an eco-friendly and cost-effective approach to melanin biosynthesis.

This work highlights the potential of waste-derived substrates for sustainable pigment production and contributes to advancing green biotechnological processes.

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Measurement of Hepcidin-25 in human serum: development of an intrinsic Surface Plasmon Resonance - based biosensor for a femtomolar detection strategy

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Hepcidin-25 is a 2.8 kDa hepatic peptide regulating body iron homeostasis. The determination of serum Hep-25 concentration might be useful for diagnosing and monitoring iron-related diseases since its blood levels are altered in hemochromatosis and some forms of anemia. It has also gained interest in sports medicine as an indirect marker of doping in conditions of erythropoietin administration [1]. So far, mass spectrometry and competitive ELISA are the most common methods used for Hep-25 dosage in serum and urine. Of interest, only a few facilities perform such assays, and, despite significant efforts to overcome technical limitations, the development of a quantitative assay that is user-friendly, robust, and with a low limit of detection (LoD) is still challenging. Furthermore, the lack of harmonic data on synthetic Hep-25 handling for standard solutions made it difficult to standardize interlaboratory results [2]. In this work, we first demonstrated that nickel(II) sulfate was effective in solubilizing Hep-25 peptide, obtaining a stable calibrator for quantitative assays. Secondly, we exploit surface plasmon resonance (SPR) for developing a plastic optical fiber (POF) biosensor [3]. To be noted, the presented SPR-POF biosensor shows the lowest LoD so far reported (48 fM), and, most importantly, we proved its capability to dose Hep-25 in human serum.

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Tailoring calcium-alginate hydrogels: a systematic study of molecular weight and concentration effects

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Alginate is a natural biopolymer typically obtained from seaweed, widely used in biomedical applications due to its biocompatibility and ability to form hydrogels through ionic gelation with divalent cations. Although the biochemical properties of alginate-based hydrogels are expected to correlate with alginate molecular-weight (Mw) and concentration (c), these relationships have not been thoroughly investigated, and in most cases the alginate used for hydrogel production are not adequately characterized. Therefore, commercially available alginate with low, medium, and high viscosities were hydrodynamically characterized by size exclusion chromatography–triple detector array (SEC-TDA). The alginates were dissolved at various concentrations and crosslinked with a 5% calcium- ions solution. To investigate the correlations, the resulting hydrogels were analyzed in terms of rheological properties, swelling, stability, and porosity. Additionally, the response of human-dermal-fibroblasts (HDFs) to the matrices was evaluated. Characterization revealed Mw of 120,250, and 400 kDa for the starting alginates. Hydrogels with storage moduli(G') ranging from 4 to 369 kPa were obtained at tested concentrations. A novel mathematical correlations between G' and alginate Mw and concentration were established. Stability in Phosphate-buffered saline was sound and found to increase with both Mw and concentration. HDFs remained viable and proliferated within the hydrogels in 10 days experiments, collagen I expression was reduced as in a quiescent state similar to in vivo, homogeneous spatial distribution of cells was observed regardless of Mw and c. Overall, these results provide key insights into the relationship between alginate Mw/c and the biophysical properties of hydrogels, and their effects on HDFs, offering a reference for optimizing hydrogel performance.

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Mitigation of Acrylamide and Hydroxymethylfurfural in Fried Potatoes Using Pectin–Chitosan Nanocomposite Coatings Enriched with Olive Leaf Extract

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The formation of acrylamide (ACR) and 5-hydroxymethylfurfural (HMF) during frying poses significant public health concerns due to their potential carcinogenic and genotoxic effects. This study investigated the efficacy of pectin (PEC)-based edible coatings enriched with olive leaf extract (OLE) and chitosan nanoparticles (CH-NPs) in mitigating ACR and HMF formation in French fries prepared by deep fat frying (190 °C, 9 min) and hot air frying (200 °C, 18 min). Physicochemical characterization of coating solutions revealed that OLE and CH-NPs substantially enhanced antioxidant activity (78.9%) without markedly affecting zeta potential or particle size distribution. Quantitative HPLC-UV analysis showed that air frying produced significantly higher ACR and HMF levels than deep frying. However, the combined PEC + OLE + CH-NPs coating achieved the greatest mitigation, reducing ACR by 53% in deep frying and 58% in air frying, and decreasing HMF formation by 65% and 39.5%, respectively. Moreover, this coating lowered oil uptake by 55% while retaining higher moisture content (up to 61.9%) and lighter color parameters (L* = 77.0). Sensory evaluation indicated that deep-fried coated fries were more acceptable to panelists than air-fried samples, suggesting that edible coatings can improve both safety and quality. These findings demonstrate the synergistic potential of combining natural antioxidants with nano-biopolymer reinforcements to develop healthier frying strategies. The study highlights that although air frying reduces fat content, it may increase heat-induced toxicants, emphasizing the importance of consumer awareness and technological optimization in domestic frying practices.

Hybrid Gelatin Methacryloyl and Glycosaminoglycan Bioinks Enable 3D-Printed Scaffolds for Tissue Engineering and *In Vitro* Models

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Three-dimensional (3D) scaffolds have emerged as powerful tools in tissue engineering, enabling the development of biologically relevant models that better mimic native tissue architecture and function. In particular, 3D skin models offer clear advantages over traditional two-dimensional cultures, providing a more physiologically accurate environment for studying skin biology and testing therapeutics.

This work aims to develop and characterize functional scaffolds based on natural biopolymers, including gelatin methacryloyl (Gel-MA) and glycosaminoglycans (GAGs), such as hyaluronic acid (HA), and two types of chondroitin: a sulfated form from shark cartilage (CS) and an unsulfated, biofermentative variant (BC), for in vitro 3D skin models.

Cylindrical porous scaffolds with controlled geometry were fabricated via extrusion-based printing using 15% w/v Gel-MA, with or without 2% w/v GAGs (HA/BC or HA/CS), and characterized for microstructure, gel fraction, swelling, GAG release (by capillary electrophoresis), and mechanical properties.

Subsequently, human dermal fibroblasts were seeded onto the scaffolds and cultured under static and dynamic conditions using indirect perfusion bioreactors. Cell viability, adhesion, and proliferation were assessed, along with the expression of skin-specific markers at gene and protein levels through immunohistochemistry, immunofluorescence, and western blotting. Scaffold morphology and tissue organization were further analyzed by scanning electron microscopy.

The results demonstrated that the presence of GAGs did not significantly affect the biophysical and mechanics of the scaffolds, but interestingly enhanced cell viability and biological activity.

Notably, despite the shear stress induced under dynamic conditions, the scaffolds maintained proper biocompatibility and supported cell viability, highlighting their robustness do develop appropriate full thickness skin models for in vitro studies Specifically valuable for topical and injective class III medical devices.

Development of nanoparticles encapsulating miRNA-603 and miRNA- 221 to overcome chemoresistance in glioblastoma

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Glioblastoma (GBM) is a highly aggressive brain neoplasm with a poor prognosis. Temozolomide (TMZ), an alkylating agent used as a standard treatment, shows limited efficacy in many patients due to chemoresistance, mainly caused by the DNA repair enzyme O6-methylguanine-DNA methyltransferase (MGMT), which repairs the damage induced by TMZ.

MGMT expression is regulated by epigenetic mechanisms, including microRNAs. In particular, miR-603 and miR-221 have been shown to suppress MGMT expression, suggesting a possible therapeutic approach to overcome chemoresistance.

A self-assembling nanoparticle system, PLCaP-NP, encapsulating miR-603 and miR-221, was developed and characterized by DLS analysis. The in vitro cytotoxic effects of PLCaP-NP and miRNA release efficiency were evaluated by MTT assay and real-time PCR in GBM cell lines (U-87 MG, T98G and LN-229), respectively. The potential synergistic effect between TMZ and encapsulated miRNAs was studied by both viability and clonogenic assays. Also, the migratory and invasive capacity of the cells was evaluated after treatment.

The previously optimized miRNA-encapsulating PLCaP-NPs showed good stability and no hemolytic activity on our biological model. MTT assay and real-time PCR analysis allowed to select PLCaP-NPs with the lowest cytotoxicity and excellent miRNA internalization efficiency, respectively. Subsequently, drug combination studies using TMZ and PLCaP-NP-encapsulating miRNAs were performed on GBM cells: miRNA co-delivery via SANPs in combination with TMZ treatment strongly reduced cell viability and tumorigenic potential compared to TMZ or single miRNAs alone.

This strategy offers a rational basis for the development of new therapies based on the targeted and effective delivery of miRNAs, which play a crucial role in tumor suppression. This opens up new perspectives for the personalized treatment of cancer.

Inhalable nanoparticles delivering antimicrobials to enhance the local treatment of lung infections in cystic fibrosis

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Cystic fibrosis (CF) is a genetic disease where chronic lung infections, mainly caused by biofilm-forming, antibiotic-resistant bacteria, are the main cause of morbidity and mortality [1]. Prolonged antibiotic use worsens resistance, limiting treatment options thus highlighting the urgent need for new antimicrobials and innovative delivery strategies to overcome resistance and effectively treat biofilm-associated infections.

In this context, the development of alternative therapeutics and advanced inhalable formulations is particularly interesting to improve treatment outcomes in CF patients. Unfortunately, overcoming lung barriers and achieving good biodistribution inside the lungs is difficult, particularly in the case of cationic antimicrobial peptides and peptidomimetics. These antimicrobials offer promising antibacterial, antibiofilm, and anti-inflammatory properties with low resistance risk [2-3] but their relatively high molecular weight and the abundance of cationic and hydrophobic residues often reduce the diffusibility promoting their adhesion to mucus and thus a poor biodistribution.

Inhalable polymeric nanoparticles (NPs) based on poly(lactide-co-glycolide) (PLGA) blended with poloxamers P188 or P407 widely used in the treatment of lung infection [4] were developed to deliver antibiotics, antimicrobial peptides and peptidomimetics, alone or in combination. Tobramycin, colistin, and the antimicrobial peptoid P13#1 [5] were chosen as representatives of the three classes of antimicrobials. Corresponding nanoparticles exhibited favourable properties for inhalation (size, ~ 200 nm; ζ-potential, ~ -25.0 mV), high encapsulation efficiency, and sustained drug release particularly for tobramycin.

Antibacterial assays against *Pseudomonas aeruginosa* PAO1 confirmed preserved activity post-encapsulation. In biofilm eradication, colistin-loaded NPs performed slightly better than tobramycin NPs even if complete eradication was obtained in both cases. These findings strongly support nanoparticles-based inhalable delivery as a promising strategy for lung infections.

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Proteomic characterization of outer membrane vesicles isolated from *K. Pneumoniae* resistant strains exposed to ciprofloxacin and tigecycline

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Klebsiella pneumoniae (*K. pneumoniae*) is globally recognized as one of the five major pathogens responsible for nearly half of all deaths caused by drug-resistant bacterial infections. Its various strains exhibit multiple levels of resistance, particularly to third-generation cephalosporins and carbapenems. Recent studies highlight the crucial role of outer membrane vesicles (OMVs) secreted by pathogens in modulating virulence and antibiotic resistance.

This study presents the proteomic profiling of three multi-resistant *K. pneumoniae* clinical isolates and their OMVs to investigate vesicle proteome variations under antibiotic pressure. Antimicrobial activity assays revealed that the isolates were resistant to ciprofloxacin and tigecycline. Both clinical isolates and an ATCC reference strain were cultured in the presence or absence of these antibiotics at half their minimum inhibitory concentration (1/2 MIC). OMVs were isolated, and proteomic analyses were performed on both cell lysates and vesicles.

Clinical isolates exhibited a general overexpression of homeostasis and stress response proteins in both OMVs and cell lysates. Under antibiotic exposure, OMVs showed an increased number of differentially expressed proteins compared to the control strain. In the presence of ciprofloxacin, proteins enriched in all clinical strains were mainly transport proteins and efflux pumps, whereas tigecycline exposure enhanced carboxylic acid metabolic processes. OMVs under ciprofloxacin pressure contained fluoroquinolone resistance proteins, Qnr peptides, and DNA topoisomerases, while porins and multidrug efflux pumps were

consistently upregulated and Type VI secretion systems were downregulated. Under tigecycline pressure, TetR proteins, involved in efflux pump transcriptional regulation, were increased.

Overall, our findings indicate that antimicrobial pressure induces a reduction in virulence mechanisms through OMV-mediated remodeling of transport and membrane proteins, suggesting a general reorganization of the OMV proteome in resistant *K. pneumoniae* strains. However, each drug seems to activate a specific resistance strategy in clinical isolates.

Hydrogel-Based Bio-Adhesives as an Alternative to Animal Glues in the Conservation of Cultural Heritage

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This project explores the development of an innovative bio-adhesive based on hydrogel systems, aimed at replacing traditional animal glues historically used in "colle pasta" [1] formulations for the conservation and restoration of cultural heritage. The objective is not only to replicate the adhesive performance of conventional glues but to surpass them by leveraging the unique properties of hydrogels—such as high biocompatibility, tunable swelling behavior, mechanical stability, and the ability to tailor chemical and rheological characteristics [2]. By integrating natural components traditionally found in conservation practices (e.g., starches, honey, molasses, turpentine) with modern hydrogel technology, the resulting bio-glue retains the beneficial features of historical recipes while overcoming key limitations such as microbial degradation, thermal sensitivity, and preservation complexity. A crucial ethical dimension of this research lies in the elimination of animal-derived ingredients, replacing them with renewable molecules as represented by chitosan (CH) and poly-γ-glutamic acid (γ-PGA). CH is a polysaccharide made of β-(1 → 4)-D-glucosamine and N-acetyl-D-glucosamine units and derived from the deacetylation of chitin, whereas γ-PGA is a polymer in which glutamic acid units are linked by peptide bonds between α-amino and γ-carboxylic acid groups. It is produced by strains of *Bacillus* species, with interesting attributes, like water-solubility, biodegradability, and non-toxicity. The hydrogel were produced taking advantage of a polyelectrolyte approach by blending the polycation CH and polyanion γ-PGA, and they were evaluated for their chemical-physical and adhesive properties and detachment resistance on canvas specimens. The results showed that hydrogel-modified formulations based on CH and γ-PGA exhibit a viscoelastic behavior typical of structured and stable materials maintaining the main functions of the "colle pasta", with improved adhesive performance.

The presented approach offers a significant step in the design of smart materials for heritage science, balancing technological innovation with cultural and environmental responsibility.

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Structural and Biochemical Characterization of Bacterial Metal-Binding peptides as Biorecognition Elements for Heavy Metal Biosensors

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Accelerated human activities (industrialization, mining, and agriculture) have globally contaminated the environment with toxic heavy metals. To address the critical need for rapid detection methods, this study explores using metal-binding domains derived from bacterial metal-resistance proteins as highly specific biorecognition elements for a sensitive and selective biosensor for detection of these contaminants. Three metal-binding proteins from thermophilic and mesophilic bacteria were selected: *Tt*ArsX (a membrane ATPase responsible for cadmium and arsenic ion efflux [1]) and *Tt*ArsM (an arsenite methyltransferase [2]) from *Thermus thermophilus* HB27; MerA (a mercuric reductase) from *Pseudomonas aeruginosa*. *In silico* structural analyses of the full-length *Tt*ArsX and *Tt*ArsM proteins were performed to identify conserved metal-binding domains (MBDs). Based on these analyses, the coding regions corresponding to the 64-residue soluble MBDs of *Tt*ArsX and the 80-residue of metal binding domain of *Tt*ArsM were cloned into pET30(a)+ expression vectors for recombinant production in *E. coli*. Based on the structural and biochemical characterization reported by Ledwidge et al. [3], the N-terminal 69-residue domain of *P. aeruginosa* MerA, responsible for mercury binding, was cloned into the pET-30a(+) vector for expression in *E. coli*. All three domains were successfully expressed and purified, and the biochemical characterization was accomplished to assess structural stability and metal-binding specificity.

The results highlight that the three protein domains are promising candidates for the development of real-time and portable biosensors for cadmium, arsenic and mercury detection in environmental samples.

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Exploring the Antioxidant and Regenerative Potential of *Triticum vulgare* Extracts in 2D and 3D Skin Models

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Plant extracts and microbially derived molecules, particularly oligo- and polysaccharides, are well recognized for their contribution to tissue repair processes. The aqueous extract of *Triticum vulgare* (TVE), obtained from whole germinated plants, has long been incorporated into wound care formulations registered for topical use in Europe. This extract is characterized by a complex mixture predominantly composed of oligo- and polysaccharides, accompanied by a wide range of bioactive phytochemicals present at low concentrations, many of which have been reported to exert functional roles in wound healing [1, 2, 3]. In this study, we investigated the potential antioxidant properties and protective effects of TVE against dryness, factors crucial for dermal regeneration, using both 2D and 3D in vitro models. TVE significantly enhanced HaCaT keratinocyte migration in time-lapse scratch assays, outperforming low concentrations of hyaluronic acid (HA), used as a positive control for its well-established role in tissue regeneration [4]. While no significant differences in migration were observed in scratched HDF monolayers, TVE markedly stimulated HDF proliferation and upregulated the expression of extracellular matrix proteins, including collagen I, collagen III, and elastin. Moreover, TVE promoted the expression and biosynthesis of the antimicrobial peptide human β-defensin-2 (HBD-2) to a greater extent than HA. Under stress conditions, TVE effectively preserved cell viability in both HaCaT and HDF cultures, demonstrating notable antioxidant and protective activities. Results obtained from 3D skin models, including those exposed to lipopolysaccharide (LPS), were consistent with the 2D data, further confirming the regenerative efficacy of TVE in a tissue-like context. Collectively, these findings highlight the regenerative potential of TVE, showing efficacy comparable to HA across multiple parameters relevant to wound healing.

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Exploring new classes of fungal biosurfactant proteins

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Proteins are intrinsically amphipathic molecules often acting as high molecular weight biosurfactants (BS). Among fungal BS, hydrophobins (HPBs) are the best known: small, cysteine-rich proteins produced by most filamentous fungi. Their biological role is crucial in fungal development, as they self-assemble at hydrophilic–hydrophobic interfaces into stable, amphipathic layers that reduce surface tension, enabling the growth of aerial hyphae. HPBs have been extensively characterized for their surfactant and self-assembling properties, making them promising biomolecules for various biotechnological applications. However, recent findings from our research group indicate that other classes of fungal proteins may exhibit comparable or even superior surface activity, suggesting that the range of fungal BS proteins extends well beyond hydrophobins.

In collaboration with the *Mycotheca of the University of Turin*, which provides fungal strains isolated from diverse environments, we have identified new surface-active proteins. Fungi isolated from hostile or highly anthropized environments are a promising source of novel BS proteins, as their adaptive strategies often include the secretion of molecules that facilitate interaction with hydrophobic substrates. One example is given by two *Fusarium solani* strains collected at a plastic dumpsite. These fungi secrete an uncharacterized protein -containing the conserved CFEM domain- that displayed remarkable emulsifying and stabilizing abilities [1]. Similar examples have been reported, such as a BS protein secreted by *Penicillium chrysogenum* from marine environments, whic showed hydrophobin-like properties despite not belonging to that family [2].

These findings highlight the existence of a broader diversity of fungal BS proteins, opening new perspectives for the discovery of naturally derived, eco-friendly surfactants with potential applications in biotechnology, materials science, and sustainable industry.

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A PLGA-Capsaicin-delivery strategy to improve encapsulation eBiciency and proapoptotic eBects in HepG2 cells

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Over time, Capsaicin (Cap), a lipophilic alkaloid derived from *Capsicum annuum*, has shown anticancer effects on more than 40 cell lines including pancreas, colon, prostate, liver, bladder, lung cell types [1]. However, the clinical use of Cap is restricted due to its low bioavailability, short half-life, and side effects as mouth and stomach irritations and burning sensation. To overcome these limitations, we realized a Cap delivery system based on Poly(lactic co -glycolic acid) (PLGA) nanoparticles (NPs). We synthesized the Cap-PLGA-NPs by the single emulsion solvent evaporation method. All parameters were optimized in order to obtain NPs with the best characteristics. Indeed, our preparation showed a very high encapsulation efficiency (EE) (96%) and a smaller size compared to those reported in the literature [2]. By confocal fluorescence microscopy, we revealed a rapid distribution of NPs in the cell cytoplasm of HepG2 cells, a model of hepatocellular carcinoma. MTT assay highlighted that both PLGA-Cap and free Cap influenced in similar way the viability of HepG2 cells. Furthermore, by Hoechst labeling, we observed that both treatments induced an increase in the number of dysmorphic nuclei, indicative of apoptosis. Western blot analyses after treatments with free Cap or PLGA-Cap showed a modulation of the Bcl-2/Bax ratio, thus confirming the induction of apoptosis. Moreover, PLGA-Cap induced a greater increase of caspase-3 level expression and of its activity, measured with a fluorimetric assay, compared to free Cap. ROS accumulation, detected with DCFDA staining, was also more augmented in PLGA-Cap than in free Cap samples, suggesting that apoptosis could depend on an efficient generation of oxidative stress. In conclusion, the observed improvement in EE and in proapoptotic activity of our PLGA-Cap-NPs preparation render it a promising candidate for the development of nanocarrier-based anticancer strategies.

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A Novel Thermophilic 4-α-Glucanotransferase from Extreme Environments: Biochemical Characterization and Insights into Resistant Starch Production

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Thermostable carbohydrate-active enzymes from extremophilic microorganisms represents a valuable resource for biotechnological applications [1]. Among the starch-active enzymes in the Carbohydrate Active enZyme (CAZy) database, glycosyl hydrolase family 77 (GH77) displays monospecific amyloglucosidase activity, catalysing both the cleavage and transfer of α-1,4-linked glucan chains. GH77 includes more than 22,000 sequences, with only a few thermophilic representatives characterized [2].

In this study, a 4-α-glucanotransferase (4αGT), *Par*GT, from the thermophilic archaeon *Pyrobaculum arsenaticum* was identified in the metagenomic dataset of the Pisciarelli solfataric hot spring (85 °C, pH 5.5) in Naples, Italy [3]. *Par*GT was biochemically characterised and showed optimal disproportionation activity at 100 °C and pH 5.5, with a specific activity of approximately 1,300 U/mg on maltotriose. In addition to activity on soluble glucans, producing maltooligosaccharides of varying degrees of polymerization, *Par*GT was able to act on native high-amylose starch (HAS) granules, which possess compact and thermally stable structural architecture.

Under annealing-like conditions, *Par*GT enzymatic treatment promoted surface-level reorganization of HAS, resulting in elongation of α-1,4-linked glucan chains, an increase in molecular order, and reduced susceptibility to enzymatic digestion. These structural modifications resulted in functional effects with the increase in resistant starch content, as confirmed by *in vitro* digestibility assays. Overall, the thermostability, catalytic efficiency, and capacity to remodel native starch structures indicated that *Par*GT is a promising biocatalyst for the tailored production of starch-based materials with enhanced nutritional and functional properties.

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Spent coBee grounds upcycling to prebiotic oligosaccharides: An integrated pretreatment and enzymatic hydrolysis approach

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Spent coffee grounds (SCG), a lignocellulosic waste byproduct of coffee production, represent a rich source of carbohydrates, mainly galactomannan, arabinogalactan type II, and cellulose. To minimize waste and integrate SCG valorization into a circular bioeconomy, this study explores the use of mild pretreatment methods followed by an enzymatic hydrolysis strategy to efficiently convert SCG polysaccharides into high-value oligo- and monosaccharides. Thermostable glycoside hydrolases (GH) [1], selected for their activity and stability at elevated temperatures and broad pH ranges, were combined into a tailored enzymatic cocktail targeting the recalcitrant polysaccharides in pretreated SCG. Mild delignification using microwave-assisted pretreatment enhanced substrate accessibility while minimizing hemicellulose loss. Using this approach, 52% of SCG polysaccharides were converted to oligo- and monosaccharides. Notably, the application of a thermostable endo β-mannanase enabled the production of 62.3 mg of mannooligosaccharides from 500 mg SCG. *In vitro* experiments demonstrated that these mannooligosaccharides significantly promoted the growth and biofilm formation of probiotic bacteria, underscoring their potential as novel prebiotics derived from waste lignocellulosic biomass. This strategy yields multifunctional saccharides suitable for food, biofuel, and materials applications while aligning with sustainable processing principles by reducing chemical input and energy demand. Collectively, the results establish a framework for efficient SCG valorization via mild pretreatment and thermostable enzymes, offering an effective and eco-friendly pathway for biowaste upcycling [2].

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Neuroprotective Extracellular Vesicles from Salvia Hairy Roots: A multi-omic study in Parkinson's disease cellular model

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Extracellular vesicles (EVs) have become highly promising instruments in the realm of nanomedicine [1]. The isolation of mammalian-derived EVs entails intricate procedures, and their therapeutic utilization introduces numerous safety and regulatory considerations. Recently, plants have emerged as unconventional reservoirs of therapeutically significant EVs. In this context, we have identified hairy roots (HRs) from medicinal plants as innovative biotechnological platforms for manufacturing EVs with the goal of improving human health [2].

In this study, we present a comprehensive exploration of the purification, omics profiling, and bioactivity of EVs derived from HRs of medicinal plants, specifically *S. sclarea* and *S. dominica* [3]. A detailed biophysical analysis of *S. sclarea* HR EVs has been conducted, along with the characterization of their proteome, revealing a distinctive molecular signature. Metabolomic analyses of HR EVs from both *S. sclarea* and *S. dominica* unveiled a conserved cargo of secondary metabolites, predominantly triterpenoids renowned for their antioxidant properties.

Utilizing an in vitro model of Parkinson's disease involving SH-SY5Y cells treated with 6-hydroxydopamine (6-OHDA), we demonstrated the safety, cellular entry, and potent anti-apoptotic effects of HR EVs. Cell metabolomics showcased that EVs maintain metabolic homeostasis and alleviate cellular oxidative stress when co-administered with 6-OHDA. Mechanistically, HR EVs impede 6-OHDA autoxidation and significantly reduce the accumulation of its oxidative byproducts, mitigating the toxicity induced by 6-OHDA.

In summary, our results offer compelling evidence that EVs derived from the hairy roots of Salvia species represent promising non-mammalian alternatives for innovative therapies in neurological disorders.

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Identification and Characterization of a Thioredoxin–Metallothionein Chimeric Protein from *Runella aurantiaca*

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Metallothioneins (MTs) are a family of small (6–7 kDa), cysteine-rich, metal-binding proteins involved in metal homeostasis, as well as in the detoxification and remediation of heavy metal contamination in polluted environments [1,2]. In this study, a naturally occurring chimeric protein composed of thioredoxin (Trx) and metallothionein (MT) domains was identified through bioinformatic analysis (accession number RDB06694.1) from the Gram-negative bacterium *Runella aurantiaca* [3]. The MT domain contains cysteine residues arranged in distinct metal-binding motifs and shares high sequence identity with known bacterial MTs. A synthetic gene encoding this putative chimeric protein was designed, synthesized, and heterologously expressed in *Escherichia coli*. The recombinant protein, referred to as Trx-MT, was purified and characterized to assess both its Trx reductase activity and heavy metal-binding capabilities. Trx activity was evaluated using the insulin disulfide reduction assay, while metal-binding properties were analyzed using UV spectroscopy, circular dichroism (CD), and isothermal titration calorimetry (ITC). *In vivo* metal-binding activity was assessed by growing the recombinant *E. coli* strain BL21(DE3)RIL/pET28-TrxMT in the presence of various metal ions. The results indicate that this chimeric protein is a promising candidate for heavy metal sequestration and may be suitable for bioremediation applications.

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Coffee silverskin green extracts protect uva-stressed keratinocytes via PERK–Nrf2 activation and ferroptosis inhibition *in vitro* and *in vivo*

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Coffee silverskin (CSS) extracts obtained via eco-friendly microwave-assisted extraction are rich in bioactive compounds, including phenols and melanoidins, which contribute to their antioxidant and functional properties [1]. However, the protective mechanism is not fully understood. In this study, we investigated the cytoprotective and antioxidant effects of two green CSS extracts, a phenol-rich fraction and a melanoidin-rich fraction, using UVA-stressed HaCaT cells and *in vivo* zebrafish larvae. Our results highlight the crucial role of the unfolded protein response (UPR), a compensatory mechanism activated upon endoplasmic reticulum (ER) stress triggered by various detrimental stimuli, including UVA irradiation [2], in mediating the protective effects of these extracts, at least in part.

CSS extracts significantly attenuated reactive oxygen species (ROS) and reactive nitrogen species (RNS) production, as well as the accumulation of misfolded proteins, which underlies maladaptive ER stress, while confocal analysis showed enhanced nuclear accumulation of Nrf2, indicating activation of the antioxidant pathway following CSS extracts treatment. Notably, pharmacological inhibition of PERK, a central mediator of ER stress signaling, abolished these protective effects, highlighting the role of controlled ER stress in mediating Nrf2-dependent antioxidant responses. Moreover, the extracts inhibited tyrosinase activity and melanin synthesis, and reduced ferroptosis markers both *in vitro* and *in vivo*. Collectively, these findings demonstrate that CSS extracts exert multifunctional protective effects against UVA-induced skin damage, supporting their potential as sustainable dermaceutical ingredients.

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Temporin-A Targets the PilSR Regulatory System to Inhibit Motility and Virulence in *Pseudomonas aeruginosa*

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Antimicrobial peptides (AMPs) have emerged as promising alternatives to combat multidrug-resistant (MDR) pathogens such as *Pseudomonas aeruginosa (P. aeruginosa),* a Gram-negative opportunistic bacterium with intrinsic resistance mechanisms and a remarkable ability to form biofilm. These characteristics complicate the treatment of both acute and chronic infections, especially in immunocompromised individuals or patients with cystic fibrosis [1]. The global health threat of antimicrobial resistance (AMR) has emphasized the urgent need for innovative therapeutic strategies, and AMPs are seen as potential enablers due to their broad spectrum of activity and multiple mechanisms of action [2]. Temporin-A (TA), an α-helical, cationic AMP with 13 residues derived from the skin of *Rana temporaria*, has shown promising activity against Gram-positive bacteria [3]. Although TA does not exhibit bactericidal activity against *P. aeruginosa* under standard conditions, recent studies have demonstrated its ability to interact with and perturb bacterial membranes, suggesting potential intracellular effects. To identify a potential intracellular target, a functional proteomics approach identified PilR, the response regulator of the PilS-PilR two-component system (TCS)—a conserved signaling mechanism that helps bacteria adapt to environmental changes through the interplay between a sensor kinase and a response regulator. PilR specifically regulates motility, surface attachment and biofilm formation mediated by type IV pili and also influences virulence by controlling the expression of type III and type VI secretion systems [4]. *In silico* molecular docking simulations predicted a strong interaction between TA and PilR, supporting the hypothesis that TA could interfere with PilR-mediated signaling. Ongoing *in vitro* fluorescence-based assays using recombinant PilR protein aim to validate this interaction at the molecular level. *In vivo* motility assays demonstrated that TA significantly reduces both swimming and swarming motility in *P. aeruginosa*, indicating the alteration of flagellar and pili. These processes are crucial for bacterial colonization and the establishment of persistent infections. Together, these findings suggest a novel mechanism of action for TA through interference with a key regulatory system in *P. aeruginosa*, providing a potential foundation for the development of anti-virulence therapies.

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Proteomic profiling highlights mechanisms of functional activation in primary equine fibroblasts treated with advanced-platelet rich fibrin plus (A-PRF+)

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Wounds are frequent in horses and often cause complications such as infection, delayed healing, and scarring. Consequently, developing affordable and effective treatments are increasingly important in veterinary research. In this context, Advanced-platelet-rich fibrin plus (A-PRF+) shows regenerative potential like humans, but its molecular mechanisms at the cellular level remain poorly understood. Previous evidence has reported high levels of growth factors and pro-inflammatory cytokines in A-PRF [1], its ability to stimulate fibroblast activity and wound healing in vitro [2], and its superior biological performance compared to other platelet concentrates [3].

To this aim, the study employed a label-free quantitative (LFQ) proteomic approach to characterize the secretome of Advanced Platelet-Rich Fibrin Plus (A-PRF+) and to investigate the molecular mechanisms underlying its regenerative activity in primary equine fibroblasts [4].

Comprehensive proteomic analysis identified more than 2000 proteins across A-PRF+ samples, defining the specific molecular signature induced by A-PRF+ treatment.

At the cellular level, treatment with A-PRF+ enhanced cell proliferation, migration, and cell cycle progression, effects that were demonstrated to be at least partially dependent on reactive oxygen species (ROS). Specifically, increased ROS production and stimulated organelle metabolism were observed, alongside upregulation of genes and proteins associated with cell proliferation and wound regeneration, indicating a coordinated and active cellular response. Furthermore, fibroblasts exposed to A-PRF+ exhibited clear proteomic reprogramming, with strong upregulation of proteins related to energy metabolism, cytoskeletal dynamics, protein synthesis, and extracellular matrix stability aimed at sustaining fibroblast proliferation, migration, and remodeling. These findings provide insights into the molecular profile and functional responses of equine fibroblasts exposed to A-PRF+ , contributing to our understanding of its biochemical effects, supporting further exploration of this product in regenerative medicine applications.

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Investigating Phox2B aggregation: a crucial process in the onset of congenital central hypoventilation syndrome

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Expansion of the polyAlanine (polyAla) region in the Phox2B protein is associated with Congenital Central Hypoventilation Syndrome (CCHS, MIM 209880), a rare neonatal disease characterized by abnormal ventilatory response to hypoxia and hypercapnia, owing to failure of autonomic respiratory control[1].

Increasing evidence indicates that protein aggregation plays a significant role in this pathology. In a previous study, we determined the domain architecture of Phox2B, suggesting that it comprises a globular homeodomain, named HD, and a more flexible C-terminal region, that includes the polyAla tract [2,3]. In this work, we further investigate the biophysical properties of the two isolated domains, the full-length Phox2B protein, its parologue Phox2A and several polyAla expansion variants.

Using dynamic light scattering, circular dichroism, and spectrofluorimetric analyses, we assessed the aggregation tendencies of these proteins to pinpoint regions that may act as triggers in the aggregation process. Our findings will be extensively discussed alongside structural characterization results obtained through NMR spectroscopy.

Collectively, our results support a model in which the thermodynamic stability of Phox2B is primarily determined by the HD domain, while expansion of the polyAla tract significantly enhances the protein's aggregation propensity.

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Insights on the role of metformin in counteracting the age-induced endothelial damage and cardiotoxicity

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Advanced Glycation End Products (AGEs) are a heterogeneous group of compounds that originate from the glycation reaction, a non-enzymatic and non-selective process that originates from the reaction between reducing sugar and free amino groups of proteins, nucleic acids, and lipids. AGEs are implicated in aging and diseases such as diabetes, along with related vascular complications [1,2] In diabetic conditions, the hyperglycemic state markedly increases plasma AGE levels, and their accumulation contributes to endothelial dysfunction mainly through oxidative stress, inflammatory responses and apoptosis. Therefore, preserving endothelial function is a major therapeutic goal in the prevention of diabetic vasculopathies. Metformin (MET), a widely prescribed antidiabetic agent, in addition to its hypoglycemic effect, possesses antioxidant, anti-inflammatory and anti-glycation properties that may contribute to counteracting the deleterious vascular effects of AGEs in diabetes [3]. We report that MET prevents AGE-induced cytotoxicity by suppressing oxidative stress in both endothelial cells and cardiomyocytes. Protection from oxidative stress by MET is associated with activation of AMPK/NRf2 signaling pathways and inhibition of cellular apoptosis via deactivation of ERK/p38 MAPK. These findings broaden our understanding of the mechanism by which MET modulates AGE-induced apoptosis under oxidative stress conditions, with important implications for the potential use of MET for the treatment of diabetic vascular complications.

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Exploring antimicrobial and immunomodulatory activities of native and denatured versions of human angiogenin

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Ribonucleases (RNases) are a Superfamily of proteins with enzymatic activity capable of degrading RNA [1]. Among them, RNase 5, also known as angiogenin (ANG), is a 14-kDa single-chain cationic protein with three disulfide bonds, primarily studied for its roles in stress response and tRNA cleavage [2]. However, according to the literature, limited information is available regarding the potential antimicrobial and immunomodulatory properties of ANG. Given that many antimicrobial agents exploit their net positive charge to disrupt microbial membranes, and considering the intrinsic cationic nature of ANG, a denatured form of the protein was developed for further investigation. Denaturation was achieved using cystamine, which forms disulfide bridges with cysteine residues, stabilizing the protein in a denatured state. This variant, termed ANGdm, together with wild-type ANG (ANGwt) and the catalytic mutant H13A, was recombinantly produced in Escherichia coli, purified, and characterized. Preliminary analyses on prokaryotic cells revealed that ANGdm shows significant antimicrobial activity against the tested strains, markedly higher than ANGwt and H13A, which were active only at the highest concentrations. This suggests that the denatured form possesses increased cationicity, enhancing its interaction with bacterial membranes. The immunomodulatory activities of the proteins were evaluated in murine macrophages (RAW 264.7). Unlike the bacterial assays, both ANGwt and ANGdm exhibited dose-dependent immunomodulatory effects, possibly through different mechanisms. ANGdm appeared capable of binding LPS from *Pseudomonas aeruginosa* O1 with a low dissociation constant, suggesting a scavenger-like role, whereas ANGwt, which doesn't bind LPS, is known to enter in some cell types. These findings indicate that different conformational states of the same protein can drastically influence its biological functions, providing new insights into ANG biology and expanding the potential of RNases as multifunctional biomolecules.

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The Reactivity of Cysteines of 3CLpro affects the oligomerization state of the Sars- CoV-2 protease

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SARS-CoV-2 encodes a 3C-like protease (3CLpro) that is essential for viral replication. This cysteine protease cleaves viral polyproteins to release functional non-structural proteins, making it a prime target for antiviral drug development. We investigated the inhibitory effects of halicin, a known c-Jun N-terminal kinase inhibitor, on 3CLpro. Mass spectrometry and crystallographic analysis revealed that halicin covalently binds to several cysteine residues in 3CLpro. As expected, Cys145, the catalytic residue, was found to be the most targeted residue by halicin. Secondly, Cys44 was found to be modified, suggesting a potential inhibitory role of this residue. A mutant protease (Cys44Ala) was generated to further understand the function of Cys44. In silico and enzymatic assays showed that the mutation significantly reduced the stability and activity of 3CLpro, indicating the importance of Cys44 in maintaining the active conformation of the protease. Differential scanning fluorimetry assays confirmed this evidence, showing a reduced thermal stability of the mutant compared to the wild-type protease. Our results highlight the potential of halicin as a multi-target inhibitor of 3CLpro and underline the importance of Cys44 in the function of the protease. These findings contribute to the development of effective antiviral therapies against COVID-19 by targeting critical residues in 3CLpro.

Structural and Dynamic Insights into GALT–Pharmacochaperone Complexes: Implications for Classical Galactosemia Treatment

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Classical galactosemia is a hereditary metabolic condition caused by mutations in the galactose-1-phosphate uridylyltransferase (GALT) gene, with the p.Q188R substitution representing one of the most prevalent and severe variants. A promising therapeutic approach involves the use of pharmacological chaperones, small molecules that can stabilize misfolded enzymes and help them regain their functional conformation.

In this work, we applied computational techniques to explore how potential pharmacochaperones could enhance the stability of the GALT enzyme. Compounds previously proposed through target-based screening and molecular docking were further analyzed using 600-nanosecond molecular dynamics (MD) simulations. The systems were prepared with CHARMM-GUI for force field setup, and the simulations were executed with GROMACS on the Leonardo high-performance computing (HPC) platform at CINECA.

Both the wild-type and p.Q188R mutant forms of GALT were simulated in complex with their physiological substrates—galactose-1-phosphate (G1P) and 5,6-dihydrouridine-5-monophosphate (H2U)—alongside the pharmacochaperone candidates. The resulting trajectories were analyzed to characterize conformational dynamics, structural stability, and protein–ligand interaction patterns.

This computational pipeline enabled a detailed comparison between the wild-type and mutant enzymes, offering molecular-level insights into their dynamic behavior and the potential stabilizing effects of the tested pharmacochaperones.

Interaction network of zinc finger proteins from eukaryotes and prokaryotes

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Zinc finger proteins (ZFPs) are characterized by the presence of a zinc finger (ZF) domain folding around a zinc ion tetrahedrally coordinated by two histidine and two cysteine residues. Most of ZFPs function as transcription factors mediating protein–nucleic acid and protein–protein interactions. The human ZBTB2, a BTB/POZ zinc-finger transcription factor, is implicated in proliferation, cancer, and DNA methylation, and was recently identified as a new NuRD complex partner. ZFPs are also found in prokaryotes, where they contribute to DNA binding and genome structuring. Members of the Ros/MucR protein family are formed by an N-terminal domain responsible for oligomerization and a C-terminal zinc finger domain able to bind DNA. This study aims to explore the molecular interactions and functional roles of two human zinc finger proteins, ZBTB2 and ZNF639, in chromatin remodeling and gene expression regulation. Additionally, it investigates the interactions of the prokaryotic zinc finger protein MucR from *S. meliloti*, focusing on its role in gene regulation and symbiosis in α-proteobacteria. Using immunoprecipitation and high-resolution mass spectrometry, we identified the interacting partners of these proteins. The ZBTB2 interactome includes ZNF639 and chromatin remodelers, notably the NuRD complex. Some proteins, such as CTCF, a key chromatin organizer, have been identified as a ZBTB2 interactor but are absent in the ZNF639 interactome, suggesting distinct roles for the two proteins, which are reported as couple working together in molecular functions. In *S. meliloti*, MucR mainly interacts with proteins related to energy metabolism and molecule transport rather than genome structuring, showing a wide role of this protein thus not only involved in gene expression regulation. Overall, these findings highlight the evolutionary functional versatility of zinc finger proteins and their crucial role in several molecular processes.

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Characterization of the celiac cellular phenotype in patient- derived intestinal fibroblasts

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Celiac disease (CD) is an inflammatory intestinal disease caused by the ingestion of gluten-containing cereals by genetically predisposed individuals. Constitutive differences between cells from CD patients and control subjects, including levels of protein phosphorylation, alterations of vesicular trafficking and Ca^{2+} homeostasis, regulation of type 2 transglutaminase (TG2), have been reported in skin-derived fibroblasts from CD and control subjects, a suitable model to study differences that are independent from gluten exposure [1]. In the present work we investigated constitutive differences between CD and control cells in intestinal-derived fibroblasts from atrophic CD and control subjects. To identify biological pathway responsible of the phenotypical variations in CD and control subject cells, we analysed proteins differentially expressed by using a proteome approach. We identified 117 proteins highly expressed in CD subjects and 160 less expressed in CD subjects compared to control ones. By Gene Ontologies analysis we found proteins involved in cell adhesion, vesicular transport and extracellular vesiculation. In particular, among all identified proteins we found that TG2, a protein with a key role in the disease, [2] were expressed at lower level in CD respect to control subject cells. Also, TG2 activity was lower in CD subjects compared to control both at the basal level and upon stimulation with Thapsigargin, a calcium release-inducing drug. In conclusion, our findings highlight the presence of a peculiar CD cellular phenotype involving the differential expression of several proteins and also comprising differential TG2 expression and activity at the level of CD intestinal fibroblasts.

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Proteomics mapping of the molecular signature of surgically treated epileptic lesions for the development of a comprehensive diagnostic refinement protocol

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Epilepsy, one of the most prevalent chronic neurological diseases, affects 1% of the global population [1]. While anti-seizure medications are effective for approximately 70% of patients, 30% remain resistant to therapy. For those with drug-resistant epilepsy (DRE), surgery is a safe and effective treatment. DRE surgical specimens exhibit a broad spectrum of structural brain lesions, including focal cortical dysplasia (FCDs; types I, II, and III), hippocampal sclerosis, low-grade developmental tumours, and other less common alterations [2]. This study aims to compare protein expression profiles in lesional, perilesional, and control tissues using a shotgun proteomic approach to identify biomarkers for epileptic lesions. The initial phases of the project focused on optimizing protein extraction methods from FFPE (formalin-fixed paraffin-embedded) to maximise the yield of extraction procedure and of sample clean-up. After the application of each extraction protocol, the optimisation procedure was monitored according to the peptide quantification and the numbers of proteins identified. Moving forward, a label-free differential proteomics approach has been employed for the analysis of samples representative of different epileptic brain lesions to univocally identify potential protein targets whose abundance is altered in correspondence with the lesions. The non lesional surrounding region of each sample has been excised and analysed as control.

Up to now, 8 lesions from FCDIIa and 6 relative perilesions have been analysed, as well as 14 FCDIIb lesions and 12 perilesions; fold changes of up and down regulated proteins have been measured among all biological replicates for each subset of samples to characterize the molecular signatures associated with each histopathological entity. The results obtained from differential proteomic approach will be compared with those obtained using MALDI-imaging strategy to establish a comprehensive molecular profile for each FCD subtype.

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Central Precocious Puberty and MKRN3 mutations: new insights on the protein RING finger structure, ubiquitination, and localization

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MKRN3 (Makorin Ring Finger Protein 3) is a maternally imprinted intronless gene in the Prader-Willi syndrome locus. The encoded protein controls puberty timing, working as a brake of gonadotropin-releasing hormone secretion [1]. *MKRN3* loss-of-function mutations are the primary cause of central precocious puberty (CPP) [2]. MKRN3 protein is an E3 ubiquitin ligase with three C3H1 zinc fingers, a Makorin-type Cys-His zinc finger, and a C3HC4 RING finger. The correct folding of these domains is required for full protein activation. Numerous CPP-related *MKRN3* mutations cluster prominently in the RING [3]. This leads to the hypothesis that the RING acts as the hub of MKRN3-dependent control of pubertal onset. This study explores the effects of some *MKRN3* mutations identified in CPP patients. We focused on missense mutations falling inside the RING domain. Exome sequencing revealed *MKRN3* mutations (two of which inside the protein RING domain) in five females with familial CPP. We employed transient overexpression of WT and mutant MKRN3 plasmids to investigate the debated electrophoretic pattern of MKRN3, the ubiquitination of each immunoreactive signal, and protein subcellular localization. To find out if RING mutations could result in MKRN3 inactivation for RING motif improper folding, treatments with zinc chelators and antagonists were employed. By comparing the electrophoretic patterns of mutant and wild-type MKRN3, we found that alterations in the RING sequences (p.Arg328Cys and p.Gln352Arg) significantly impacted the band ratios, leading to a notable increase in the intensity of the higher signal. Additionally, the presence of either Zinc antagonists or the exposure to EDTA during MKRN3 expression indicated that the highest band corresponds to a partially folded MKRN3 (lacking Zn in the RING). Our study represents an in-depth analysis of MKRN3 forms and suggests new effects of MKRN3 RING mutations in determining the Holo→Apo MKRN3 transition with possible roles on MKRN3 inactivation.

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Proteomic insights into brain vulnerability in DiGeorge syndrome

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DiGeorge syndrome, or 22q11.2 Deletion Syndrome (22q11.2DS), is the most frequent chromosomal microdeletion disorder, caused by the loss of about 3 Mb on chromosome 22q11.2. This region contains roughly 90 genes essential for embryonic development and neurogenesis, and its deletion is strongly associated with neurodevelopmental and psychiatric disorders. Among the affected genes, the T-box transcription factor *Tbx1* plays a central role in cardiovascular, craniofacial, and brain development [1].

This study investigated brain proteomic alterations in a mouse model of 22q11.2DS carrying a *Tbx1* loss-of-function mutation (*Tbx1*$^{+/-}$). Whole-brain tissues from wild-type and *Tbx1*$^{+/-}$ mice were analyzed at 2, 4, and 7 months using high-resolution mass spectrometry to identify age- and genotype-dependent molecular changes. Proteomic profiling revealed a clear temporal progression of alterations. Early stages showed disruption of synaptic and axonal proteins, followed by intermediate changes in neurotransmission and energy metabolism. In older mice, pronounced metabolic dysfunction and signs of neurodegeneration emerged. These results indicate that *Tbx1* haploinsufficiency initiates a progressive molecular cascade leading from early structural vulnerability to later functional decline. Overall, this work underscores the value of brain proteomics in identifying dynamic molecular signatures and defining critical time windows for potential therapeutic interventions in DiGeorge syndrome.

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Mono-ADP-ribosylation as a Host Defense Strategy: Implications for Viral Infections and Immune Signaling

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The innate antiviral response serves as the first line of defense against viral infections, initiated by pattern recognition receptors (PRRs) that detect viral components like double-stranded RNA. This triggers the production of type I interferons (IFNs), leading to the expression of interferon-stimulated genes (ISGs). Among these, enzymes of the PARP family have emerged as important regulators of antiviral immunity, possibly through the transfer of single ADP-ribose units to target proteins or nucleic acids.

To investigate the role of mono-ADP-ribosylation (MARylation) in antiviral defense, we stimulated or infected human cell lines (A549 and THP-1) using synthetic PRR agonists or SARS-CoV-2 and analyzed ADP-ribosylation profiles. We observed that Poly(I:C) treatment and SARS-CoV-2 infection both enhance global ADP-ribosylation profile. Mass spectrometry analysis of ADP-ribosylated proteins from Poly(I:C)- and IFN-treated A549 cells revealed an enrichment of antiviral proteins, RNA-binding factors, and IFN signaling components.

Key modified proteins included STAT1, ISG15, PARP12, and PARP14. STAT1, a central IFN signaling transcription factor, was found to be ADP-ribosylated alongside ISGylation and phosphorylation, suggesting coordinated regulation by multiple post-translational modifications. PARP12 and PARP14, known for restricting viral replication, were also identified as active MARylating enzymes. Additionally, we validated ADP-ribosylation of STAT1 and the SARS-CoV-2 nucleocapsid protein in SARS-CoV2 infected A549 cells.

These findings highlight a novel interplay between ADP-ribosylation and other antiviral mechanisms, particularly ISGylation, suggesting that MARylation fine-tunes antiviral responses by modulating protein function, stability, and localization. This study expands the known repertoire of ADP-ribosylated substrates and underscores the role of MARylation in host-virus interactions, establishing PARP enzymes as master regulators of innate immune defense and promising targets for pharmacological intervention.

Sex dimorphism in pulmonary fibrosis correlates with impaired expression of Formyl Peptide Receptors-NOXs axis

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Formyl Peptide Receptors (FPRs) belong to a subfamily of the classical chemoattractant G protein-coupled receptors (GPCRs) functionally expressed in various tissues and cell types. FPRs recognize a plethora of ligands and activate either inflammatory or anti-inflammatory responses, thus promoting cell adhesion, migration, and NADPH oxidase (NOX)-dependent superoxide production [1]. FPRs can mediate pattern recognition of damage-associated molecular patterns (DAMPs), whose massive accumulation contributes to trigger chronic inflammation responsible for the development of several pathologies, including idiopathic pulmonary fibrosis (IPF) [2]. IPF is a chronic, progressive, interstitial lung disorder characterized by a survival of 3-5 years after diagnosis and a well-established male predominance [3]. FPRs and NOXs have been recognized as key targets for understanding sex differences in the pathogenesis of inflammatory processes [4, 5]. We evaluated the expression levels of different members of the FPR family and of NOX isoforms in a mouse model of PF induced by 4-week bleomycin injection. Our results showed a significant FPR2 and FPR3 enhanced expression in bleomycin-treated male mice compared to female counterparts, as well as sex differences in the expression levels of NOX4 enzyme. In FPR2-stimulated lung cancer cells, we previously demonstrated NOX-dependent Nrf2 activation, and consistently, in bleomycin-treated female mice, we observed a higher level of Nrf2 and the dysregulation of Nrf2 target genes involved in oxidant defense. Overall, these results identify novel molecular signatures mediating sex dimorphism in IPF that could be relevant for the discovery of new potential targets for IPF therapy.

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The amino acid sequence of hortensin 4: type 1 ribosome inactivating protein from *Atriplex hortensis* L. (mountain spinach) seeds

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Ribosome-inactivating proteins (RIPs) are specific N-β-glycosylases (EC 3.2.2.22) that remove a conserved adenine residue from the large rRNA within the Sarcin-Ricin Loop (SRL) [1]. This region plays a key role in mRNA-protein translation [2]. Specifically, the enzymatic action of RIPs on the SRL damages ribosomes, blocking protein synthesis, and inducing apoptosis [3]. Several RIPs have been isolated from edible plants of the Amaranthaceae family, such as quinoin from *Chenopodium quinoa* Willd. and sodins from *Salsola soda* L. In this framework, I purified four novel type 1 RIPs, named hortensins 1, 2, 4, and 5, from the seeds of the Amaranthaceae *Atriplex hortensis* L. [4]. These enzymes are endowed with both N-β-glycosylase activity, as determined by Endo's assay and polynucleotide:adenosine glycosylase activity [4].

After that, I elucidated the primary structure of hortensin 4, main type 1 RIP isolated from the seeds of *Atriplex hortensis* L. [4]. The complete sequencing was achieved by mass spectrometry coupled with Edman degradation using as a reference the sequence deduced from *A. patents* cDNA (AC: ABJ90432.1). It consists of 254 residues, without cysteines, and has a N-Acetyl-hexosamine chain at position Asn231. Experimental evidence and 3D model revealed that hortensin 4 has a protein fold typical of RIPs and the amino acid residues involved in the catalytic mechanism are conserved.

The structural characterization of hortensin 4 is crucial for future structural/functional studies. Therefore, further investigation will aim to compare its potential biological activities with those of other type 1 RIPs.

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Unravelling the TR3-56 cell proteome in healthy and T1D pediatric patients by tandem mass tag-based high resolution mass spectrometry

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Type 1 diabetes (T1D) is an autoimmune disease characterized by the destruction of pancreatic β cells by autoreactive T lymphocytes, with $CD8^+$ T cells playing a central role in this process. Although the pathogenic mechanisms underlying T1D onset have been extensively investigated, the complex interplay between the various immune cell populations has not been completely understood. Recently, TR3-56 cells have been proposed as a novel regulatory immune population involved in T1D pathogenesis. Published data revealed that while in healthy subjects TR3-56 cells suppress the function of T cell receptor (TCR)-activated CD8+ T lymphocytes, in recent-onset T1D children TR3-56 cells are defective in number and suppressive capability [1]. Despite the biological role of these cells has been explored, studies addressing the comprehensive molecular asset under physiological and pathological conditions of this novel regulatory T cell subset are still missing. Using an advanced quantitative proteomic approach based on Tandem Mass Tag (TMT) isobaric labelling and nano-liquid chromatography coupled with high-resolution mass spectrometry (nanoLC MS/MS), we investigated the proteomic signature of TR3-56 cells in both healthy and recent-onset T1D children. Our findings reveal that proteins related to cytotoxic functions and activation pathways are

differentially modulated in recent-onset T1D children TR3-56 cells compared to healthy individuals. Specifically, TR3-56 cells in pediatric T1D patients exhibit a molecular program more characteristic of cytotoxic effector cells, than that of immunoregulatory subsets. Understanding the mechanisms driving this phenotypic alteration also during disease progression may provide new insights into T1D pathogenesis.

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Decoding Galectins Selectivity through a Multidisciplinary Approach

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Galectins are β-galactoside-binding proteins involved in a wide range of biological processes, and their dysregulation has been linked to several diseases [1]. The development of selective inhibitors capable of blocking galectin activity therefore represents a promising therapeutic strategy [2,3], although research in this field is still at an early stage. To address this challenge, we adopted a multidisciplinary approach combining the design of a screening platform based on X-ray crystallography for identifying novel drug candidates followed by the validation of the compounds by performing ITC experiments and cell-based assay. Galectins (-1, -3, -7, and the R144S mutant of Gal-3) were first expressed, purified, and characterized using biophysical techniques, confirming that all proteins were properly folded and exhibited well-defined oligomeric states. The proteins were then crystallized, and soaking experiments were performed with sugar-based compounds, synthesized by our team. The resulting structures revealed key molecular interactions underlying ligand recognition and selectivity. ITC experiments confirmed the binding and selectivity of the ligands, complemented the crystallographic data. Finally, the most promising compounds were tested in cellular assays to evaluate cytotoxicity and therapeutic potential in melanoma cell lines.

Together, these results demonstrate the effectiveness of our integrated screening strategy for the discovery of novel selective galectin inhibitors, paving the way for the development of innovative therapeutic approaches targeting galectin-mediated pathologies.

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SHP-1 modulates apoptosis and senescence pathways in retinal cells: implications for age-related macular degeneration

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Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss in the elderly and is primarily driven by dysfunction and degeneration of retinal pigment epithelial (RPE) cells. Central to AMD pathogenesis are apoptosis and cellular senescence, which contribute to chronic inflammation within the retinal microenvironment [1]. However, the upstream regulators responses remain insufficiently characterized. Shp is a protein tyrosine phosphatase known as a negative regulator of signaling pathways governing cell growth, differentiation, and survival [2]. Recent findings suggest that Shp1 may be involved in cellular senescence, although its mechanisms of action remain poorly understood [3]. In this study, we investigated the role of Shp1 in modulating apoptosis and senescence in RPE cells under AMD-relevant stress conditions. Shp1 knockout (Shp1-KO) ARPE-19 cells were generated via CRISPR-Cas9. Apoptosis was induced by genotoxic stress and quantified through Annexin V staining and Western blot analysis of PARP1 and caspase-3 activation. Senescence was triggered by exposure to H_2O_2 and evaluated by γH2AX foci formation, cell cycle profiling, and Western Blot analysis.

Additionally, pharmacological inhibition of Shp1 was employed to corroborate its functional role. Shp1-KO cells exhibited significantly reduced apoptosis following stress, as demonstrated by decreased caspase-3 cleavage and attenuated Akt/p53 phosphorylation. Annexin V assays confirmed lower apoptotic rates compared to wild-type controls. Under H_2O_2 treatment, control RPE cells displayed dynamic Shp1 phosphorylation, while senescent cells showed impaired Shp1 activity. Notably, Shp1 inhibition reduces DNA damage accumulation and different cell cycle progression. Overall, our findings identify Shp1 as a pivotal regulator of apoptosis and senescence in RPE cells, highlighting its potential as a therapeutic target to preserve retinal integrity in AMD.

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Revealing novel roles of Spike from SARS-CoV-2: from receptor binding to metabolic rewiring

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, has rapidly spread worldwide since 2019, largely due to the emergence of numerous variants with mutations in the viral Spike (S) glycoprotein. Spike is responsible for mediating viral entry by binding specific receptors on host cell membranes. Although the angiotensin-converting enzyme 2 (ACE2) is the canonical receptor, increasing evidence indicates that SARS-CoV-2 can infect cells expressing low ACE2 levels, suggesting the presence of alternative host targets.

To explore this hypothesis, affinity purification–mass spectrometry (AP-MS) analyses were performed using the recombinant S1 subunit of Spike in non-pulmonary human cell lines, including kidney (HK-2), normal colon (NCM460D), and colorectal adenocarcinoma (Caco-2) cells. Shotgun proteomics identified several S1-interacting membrane proteins implicated in adhesion, endocytosis, and signaling pathways, indicating ACE2-independent entry mechanisms[1]. The three interactomes revealed the presence of lactate dehydrogenase B (LDHB) as potential Spike interactor. Although we did not identify any direct interaction between S and LDHB, we confirmed their physical proximity in cells by co-immunoprecipitation and immunofluorescence colocalization. In addition, functional studies demonstrated that S1 inhibits LDHB catalytic activity by competing for NAD^+ binding, resulting in lactate accumulation and a metabolic shift toward anaerobic glycolysis. Limited proteolysis-mass spectrometry (LiP-MS) and structural mapping identified Trp436 within the S receptor-binding domain as a key residue mediating this interaction[2]. Collectively, these findings shed light on a dual mechanism exploited by SARS-CoV-2: beyond receptor recognition, Spike S1 can reprogram host cell metabolism through LDHB inhibition, unveiling a new layer of viral strategy to subvert cellular homeostasis and sustain infection.

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Isolation of novel type-1 ribosome-inactivating proteins from saBron bulbs (*Crocus sativus* L.)

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Ribosome-inactivating proteins (RIPs) are rRNA N-glycosylases (EC 3.2.2.22) mainly found in higher plants. These enzymes irreversibly inactivate ribosomes by depurating a specific adenine in the α-Sarcin-Ricin Loop (SRL) of large rRNA, blocking protein synthesis and inducing apoptosis [1]. RIPs are structurally classified into two main groups based on the presence or absence of quaternary structure: single-chain type-1 RIPs (~30-kDa; pI ≥ 9.0), and two-chain type-2 RIPs (~60-kDa; neutral pI). The latter are more toxic for the presence of a lectin-binding B-chain, which facilitates the cellular entry of active A-chain (homologous to type-1 RIPs) [2]. Despite their physiological role in plants remains unclear, RIPs exhibit several properties, including antiviral, antifungal and anticancer activities [2-4]. In this context, during my first PhD year, I found novel type-1 RIPs in *Crocus sativus* L. bulbs, commonly known as saffron. This plant holds significant economic value, not only because it is the world's most expensive spice, but also for its medicinal and nutraceutical applications [5]. However, saffron cultivation is challenging due to its susceptibility to fungal disease [6]. Therefore, modulating the expression of RIPs in saffron could enhance crop resistance to pests and diseases. These findings will be the starting point of my PhD project.

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Engineered adhesive–antimicrobial proteins for the functionalization of biodegradable packaging materials

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Active packaging represents an emerging approach to improve food safety, extend shelf life, and reduce environmental impact without relying on synthetic preservatives. Within this framework, the MATE4MEAT project aims to develop biodegradable packaging systems that integrate antimicrobial active biomolecules into sustainable polymeric structures. Biobased microbial polyesters (polyhydroxyalkanoates, PHAs), with their universal biodegradability, have been selected as renewable alternatives to petrochemical plastics [1]. Among the proposed active molecules, engineered adhesive proteins have been designed to combine antimicrobial and surface-anchoring functionalities. These chimeric proteins merge an amphiphilic adhesive domain (HPB) [2] with antimicrobial peptides (AMPs), yielding bioengineered molecules capable of forming stable and active coatings directly at the food–polymer interface. The combination of the adhesive properties with the biological functionality allows the formation of active protein layers capable of inhibiting bacterial growth directly at the food–polymer interface.

Encouraging results were obtained with selected chimeric constructs, which exhibited both adhesive behavior toward biopolymer surfaces and antimicrobial activity against target meat spoilage bacteria. These strains are recognized as typical red meat contaminants responsible for the alteration of its organoleptic properties.

These findings highlight the potential of adhesive–antimicrobial chimeras as a versatile biotechnological approach for designing sustainable packaging materials capable of preventing food contamination and extending product shelf life. Future analyses will focus on testing additional chimeric variants, including potential combinations of multiple constructs, with the goal of maximizing both surface anchoring and antibacterial efficiency.

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MucR2 and MucR3 from *Sinorhizobium meliloti*: two new atypical members of Ros/MucR protein family

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Although zinc-finger proteins were thought to be present only in eukaryotes, a single prokaryotic Cys_2His_2 ZF domain was also identified in prokaryotes in the C-terminal domain (CTD) of Ros from *Agrobacterium tumefaciens* (1). Ros homologs, such as MucR and Ml5, have been identified mainly in α-proteobacteria, giving rise to the Ros/MucR protein family. Ros/MucR proteins regulate the expression of genes involved in infection of and symbiosis with eukaryotic hosts. These proteins bind DNA by the ZF, form higher-order oligomers by the N-terminal domain (NTD) and are thought to be H-NS-like proteins [1].

We identified by mass spectrometry two new Ros/MucR family members, MucR2 and MucR3 from *Sinorhizobium meliloti*. MucR2 differs from MucR1 and MucR3 in that it possesses a prolonged random coil N-terminus. MucR2 and MucR3 NTDs show a substitution of a hydrophobic residue, key for oligomerization of MucR proteins, by a Thr. By LS analysis we demonstrate that MucR2 is a monomeric protein unable to oligomerize because of the simultaneous presence of the prolonged N-terminus, Arg9 and Thr64. MucR3 forms oligomers only at high concentrations, while MucR3-L9R mutant cannot oligomerize even reaching high concentrations. Neither MucR2 nor MucR3 can bridge DNA. The transcriptome analysis under free living conditions of *WT*, *ΔmucR1*, *ΔmucR2* and *ΔmucR3 S. meliloti* reveals that MucR2 and MucR3 act mostly as activators of gene transcription, rather than repressors as expected for MucR homologs. Knockout mutants of *S. meliloti* show that only the absence of *mucR1* gene strongly affects symbiosis with *Medicago Sativa* plant. Our results lead us to define MucR2 and MucR3 as atypical members of Ros/MucR family as they are not H-NS-like proteins. Transcriptome analyses of *S. meliloti mucR* mutants in symbiosis with *Medicago Sativa* plants will help to further elucidate the biological roles of MucR2 and MucR3.

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Cipollotto nocerino extract protects against NAFLD lipotoxicity activating sirtuin 1

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Nutraceuticals derived from Mediterranean agri-food by-products are a sustainable source of bioactive compounds with potential benefits in metabolic disorders [1]. The Cipollotto Nocerino (CN), a spring onion variety of Southern Italy, is rich in antioxidant and bioactive molecules [2], yet its effects on non-alcoholic fatty liver disease (NAFLD) remain unexplored. This study evaluated the hepatoprotective activity of a CN leaves extract in HepG2 cells exposed to oleic and palmitic acid (OA/PA) to induce steatosis, in both two- and three-dimensional culture systems. CN markedly improved cell viability, attenuated lipid droplet accumulation and reduced steatosis markers, restored redox balance preserving mitochondrial membrane potential, alleviated endoplasmic reticulum (ER) stress. Real-time PCR analysis of inflammatory markers, together with confocal imaging of NF-κB localization, confirmed the anti-inflammatory action of the extract, which can be further associated with a reduction in ER stress.

Sirtuin 1 (SIRT1) expression and activity were enhanced by CN in the presence of OA/PA, and the pharmacological modulation of this enzyme confirmed its partial involvement in protective effects.

These findings demonstrate that CN counteracts free fatty acid-induced lipotoxicity through an integrated mechanism targeting lipid metabolism, oxidative and ER stress, mitochondrial function, and inflammation, with SIRT1 activation acting as a central, though not exclusive, mediator supporting its potential as a promising, sustainable nutraceutical for the treatment of NAFLD.

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Biochemical mechanisms underlying the cytoprotective effects of *Vaccinium myrtillus* in intestinal cells under oxidative stress

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Blueberry (*Vaccinium myrtillus L.*) represents a valuable source of polyphenolic compounds with recognized antioxidant, anti-inflammatory, and cytoprotective activities [1]. This study aimed to elucidate the biochemical and molecular mechanisms underlying the protective action of a standardized blueberry extract (BLUBE) on IEC-6 intestinal epithelial cells exposed to oxidative stress induced by H_2O_2 [2].

BLUBE chemical profiling by UHPLC-PDA-ESI-Orbitrap-MS/MS revealed a complex mixture of phenolic acids (chlorogenic acid derivatives), flavonoids (quercetin glycosides), and anthocyanins (delphinidin and peonidin hexosides), consistent with their known redox activity [3]. In vitro assays (DPPH, ABTS, FRAP) confirmed the high antioxidant capacity of BLUBE, while cell-based experiments demonstrated significant cytoprotection against H_2O_2 induced damage. The extract effectively reduced intracellular ROS and RNS, limited hypodiploid nuclei, preserved actin cytoskeletal organization, and promoted wound healing and clonogenic recovery, thus enhancing epithelial regeneration.

Metabolomic profiling revealed that BLUBE reprogrammed cellular metabolism by modulating pathways involved in oxidative stress response. Sphingolipid and glycerophospholipid metabolism were stabilized, supporting membrane integrity and signaling. Moreover, taurine/hypotaurine and cysteine/methionine metabolism were significantly upregulated, enhancing sulfur-based antioxidant defenses and glutathione biosynthesis [4]. The partial restoration of sphingosine-1-phosphate, taurine, and glutamate levels indicate the activation of protective metabolic circuits that sustain redox balance and cell survival under oxidative stress conditions.

Overall, these findings provide a comprehensive biochemical interpretation of *Vaccinium myrtillus* cytoprotective effects, demonstrating its ability to modulate redox-related metabolic pathways and maintain intestinal epithelial homeostasis. The integration of metabolomics with classical biochemical assays highlights the potential of nutraceuticals in preventing oxidative stress–associated intestinal disorders.

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Endothelial protection by nanocarrier-loaded natural compounds in an *in vitro* model of the blood–brain barrier

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Vascular dysfunction and chronic inflammation, both of which worsen with aging,are hallmark features of neurodegenerative diseases. A promising therapeutic strategy to counteract these processes involves targeting the molecular mechanisms underlying endothelial dysfunction. In this study, we aimed to identify bioactive compounds capable of protecting against blood-brain barrier (BBB) impairment by modulating specific endothelial targets. We evaluated the protective effects of selected bioactive molecules (quercetin, curcumin, resveratrol, luteolin, and ergothioneine) using b.END3 cells as an in vitro BBB model. All tested compounds enhanced the expression of tight junction proteins, suggesting an improvement in barrier integrity. Furthermore, a significant increase in intracellular accumulation of Rhodamine 123 was detected, indicating inhibition of P-glycoprotein activity, a major efflux transporter at the BBB. All compounds, except resveratrol, significantly reduced LPS-induced TNFα levels, demonstrating their anti-inflammatory properties. Among them, only curcumin showed a marked antioxidant effect, decreasing reactive oxygen species (ROS) accumulation under oxidative stress conditions. The bioactive molecules were successfully encapsulated in liposomes, retaining their biological activity in the BBB cell model. Based on the overall findings, curcumin and quercetin, which exhibited the most promising individual effects, were selected for further evaluation in combination. The co-encapsulation of curcumin and quercetin in liposomes led to improved endothelial integrity and reduced oxidative stress. These results contribute to the development of an optimized liposomal formulation of bioactive compounds aimed at enhancing bioavailability and providing synergistic endothelial protection against early mechanisms leading to BBB dysfunction and central nervous system disorders.

Adaptability of Kombucha Microbial Consortia in Dairy and Plant-Based Milk

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Kombucha is a fermented tea beverage produced by a symbiotic culture of bacteria and yeast (SCOBY) that offers a versatile platform for functional beverage production. The microbial consortium is a flexible and robust community that can adapt to diverse substrates, including waste-derived agrifood and plant-based media, providing a sustainable approach for functional fermented beverages [1]. The quality of the final product depends on fermentation parameters, choice of raw materials, and the SCOBY starter culture. In this study, a preadaptation trial was conducted to evaluate whole milk and four plant-based milks (soy, almond, rice, oat) as fermentation substrates, considering pH decrease (1–2 units), °Brix reduction (≥2 units), and bacterial cellulose production. Based on these criteria, whole milk and rice-based milk were selected as the best substrates and used to produce kombucha beverages using two SCOBY variants (Italian and Polish). Microbiological analyses of the final beverages after 3 days of fermentation showed stable fermentation across all substrates, with microbial counts increasing from ~0–2 log CFU/mL at Day 0 to ~3–10 log CFU/mL at Day 3, depending on the matrix. These results demonstrate that both dairy and plant-based substrates support efficient kombucha fermentation and highlight the adaptability of the microbial consortium, helping to improve the knowledge on new potential and innovative fermented beverages using alternative raw materials.

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Effects of acute hypobaric hypoxia on human immune system

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Although it is known that acute hypobaric hypoxia (AHH) affects the function and activity of immune cells through the hypoxia-inducible factor 1 alfa (HIF-1), there is a knowledge gap in how AHH impacts the immune system, alters the expression of key cellular regulators, and shifts immune cell population homeostasis. We investigated the immune response in healthy volunteers exposed to a simulated altitude of 3800 m in the climate chamber terraXcube at EURAC Research Center (Bolzano). Saliva and blood samples were collected before and during AHH (1, 6 and 24 hours). We isolated peripheral blood mononuclear cells to assess the expression profile of genes encoding HIF-1, B and T cell subpopulation markers, and cytokines by qPCR. We observed an increase in the expression of PAX5 and PRDM1 within 1 hour of hypoxia, along with HIF-1α, suggestive of a shift in B-cell status and plasma cell differentiation early in the response to AHH. High expression of CD27 and CD38 genes indicated an expansion of the regulatory B cells (Bregs), which are essential in maintaining homeostasis of adaptive immune response. Likewise, high early expression of FOXP3 highlights that the regulatory T cells (Tregs), crucial for immune tolerance, are predominant at that early stage in response to acute AHH. Accordingly, purified monocytes exhibited subject-specific expression patterns of IL-1β, TNF-α, and TGF-β, suggesting that AHH elicits a heterogeneous inflammatory response that can influence the downstream immune adaptation. We did not detect appreciable changes in humoral and endocrine responses. In conclusion, the present study describes for the first time alterations in the expression of B and T cells gene markers under AHH, highlighting a possible key role of Bregs and Tregs in the response to AHH. These results may contribute to identify key molecular nodes involved in the immune response to hypoxia for new therapies for hypoxic diseases.

Aliophen-XP Antiproliferative Effects via ROS-Mediated Mechanism in Chronic B-Cell Leukaemia Cells

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A formulation named Aliophen®, derived from barley malts and hops, was developed through a patented process (PCT/IB2018/056283) and has previously shown promising effects in disease prevention models [1]. To enhance its efficacy, a new formulation, Aliophen-XP (patent submitted), was subsequently developed by removing interfering components and increasing the concentration of bioactive molecules.

This study aimed to evaluate the biological effects of Aliophen®, with particular focus on Aliophen-XP, on the chronic B-cell leukaemia cell line HG3, emphasizing its antiproliferative potential.

Polyphenols were extracted using Sep-Pak C-18 columns and quantified by the Folin-Ciocalteu assay. Cell viability was assessed using CyQuant assay, reactive oxygen species (ROS) levels were analysed with DCFH-DA staining, and flow cytometry was employed to evaluate cell cycle progression and apoptosis.

Aliophen-XP contained approximatively 13-fold more polyphenols than the original Aliophen® formulation. Both formulations exhibited dose-dependent cytotoxicity; however, Aliophen-XP showed a markedly stronger effect. Cell cycle analysis revealed G2/M phase arrest, followed by an increase in apoptotic cell death in Aliophen-XP treated cells. Intracellular ROS level rapidly increased upon treatment with Aliophen-XP; suggesting that its pro-oxidant activity mediated cell death induction. Indeed, the antiproliferative effect of Aliophen-XP was completely abolished in the presence of N-acetylcysteine, a ROS scavenger.

In conclusion, Aliophen-XP demonstrates enhanced biological activity, exerting a potent antiproliferative effect through in HG3 cells through ROS-mediated mechanisms. These findings warrant further investigation into its mechanisms of action and potential applications in disease prevention or supportive therapies.

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Effect of polyphenolic extracts from of mediterranean plants on enzymes involved in the oxidative stress, and neurodegeneration useful as adjuvants in cancer treatment

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In this investigation, we report the ability of polyphenolic extracts from Mediterranean plants to affect the activity of key enzymes involved in the inflammation and neurodegeneration. The polyphenol extracts used in this report were obtained from either Mediterranean forage crops, and from flesh and peels of Mediterranean fruits, such as apple and lemon. The effect of the polyphenol extracts was tested on catalase, xanthine oxidase, acetyl- cholinesterase, butyryl-cholinesterase, monoamine oxidase A, and monoamine oxidase B, determining the concentration of the extract required to get 50% inhibition. In addition, the kinetic analysis of the enzyme activity inhibition, allowed the identification of the inhibition mechanism and the determination of the inhibition constant. Furthermore, the cytotoxic effect exerted by the polyphenolic extracts was tested on human cancer cell lines. In particular, the effect on cell viability was checked on the gastric cancer cell lines MKN-28 and AGS and the neuroblastoma cell line SH-SY5Y. The results obtained suggest a putative use of some of the tested polyphenol extract as adjuvants in the anticancer treatments.

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Biochar Dust: How this biomass influences human cell lines

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Biochar is a carbon-based biosolid produced by pyrolysis of waste and biomass. Given its physical-chemical properties, it is largely employed to ameliorate soil quality and retain contaminants from polluted waters. To date, only a few studies have explored the effects of biochar on living organisms and the potential risks also for human health, as a consequence of biochar environmental diffusion. Here, we investigated the biological effects of three biochars obtained from different raw biomasses (pruning chippings, almond shells, and stabilized organic fraction) on human keratinocyte and lung cell lines. We found that 24 h of treatment with biochar, even at low doses (1-10 μg/mL), caused a reduced cell viability, a cytoskeleton reorganization, and an increased apoptosis. Moreover, biochar seemed to perturb homeostatic physiologic responses, such as autophagy and antioxidant activity. Different properties of the three biochars, mainly particle dimension, composition, and roughness, could be responsible for differences in the intensity of biological responses we registered. We also noticed that each cell line displayed its own intrinsic sensitivity to each type of biochar. On the whole, our findings highlight that the large-scale employment and the consequent diffusion of biochar in the environment might have detrimental consequences for living organisms, including humans.

Effect of annurca apple polyphenols on mercury-induced oxidative injury in human erythrocytes

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Mercury (Hg) exposure is a significant environmental risk factor, linked to oxidative stress and cardiovascular disease. Human red blood cells (RBC) are highly susceptible to oxidative damage due to their oxygen exposure and limited repair capacity, making them sensitive indicators of systemic redox imbalance [1]. This study investigated the protective effects of polyphenolic extracts from Annurca apple flesh and peel, from both ripe and unripe fruit, on $HgCl_2$-exposed human RBC. Key markers of oxidative stress were assessed, including ROS generation, GSH content, lipid peroxidation (TBARS), MetHb formation, SH group levels, microvesicle (MV) release, and RBC morphology. Peel extracts, especially from ripe fruit, exhibited the strongest antioxidant and cytoprotective effects, effectively counteracting Hg-induced oxidative damage [2]. These extracts restored redox balance, preserved GSH levels, reduced ROS and TBARS accumulation, prevented MetHb formation, and minimized MV release and morphological changes [3]. Our findings suggest multifactorial protective mechanisms and highlight the potential of Annurca apple extracts as nutraceutical agents against heavy metal-induced oxidative stress, with implications for health promotion and by-product valorization.

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Effects of Vitamin-Based Nutraceuticals on Corneal Nerve Fibers in a Diabetic Chick Embryo Model

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Background and aims. The chorioallantoic membrane (CAM) of the chicken embryo is an experimental model of great interest in biomedical research [1]. Beyond its classical use for studying angiogenesis, the CAM assay provides a promising platform for investigating ocular pathologies characterized by an altered vascular and neural balance, such as diabetic retinopathy [2]. The present study aims to explore the effects of vitamin-based nutraceuticals on corneal nerve fiber preservation and inflammatory regulation under diabetic conditions. **Methods.** Fertilized chicken eggs were incubated under controlled conditions, and diabetes was induced at embryonic day 12 (EDD12) by administering streptozotocin (1.2 mg/egg) into the amniotic fluid. At EDD17, embryos received a vitamin-based nutraceutical treatment, and samples were collected at EDD18 for molecular analysis. Glucose plasma levels were measured to confirm the diabetic phenotype, and RT-qPCR was employed to evaluate the expression of angiogenic and inflammatory markers in embryonic corneal tissues. **Results.** Our data revealed that diabetic embryos exhibited a marked upregulation of VEGF-A and MMP-9, indicating enhanced angiogenic and extracellular matrix remodeling activity, along with increased IL-1β expression, suggesting inflammation. The treatment with the vitamin-based nutraceutical normalized VEGF-A, MMP-9, and IL-1β mRNA levels to those observed in control embryos. Additionally, the nutraceutical promoted the expression of substance P, a neuroprotective and trophic peptide associated with corneal nerve regeneration, while pigment epithelium-derived factor (PEDF) remained unaltered. These molecular changes correlated with observed improvements in corneal sensitivity and epithelial repair. **Conclusion.** This work establishes a diabetic model in chick embryos via streptozotocin administration and demonstrates that vitamin-based nutraceutical supplementation mitigates diabetes-induced corneal damage. The findings highlight the potential of the CAM model for preclinical screening of nutraceutical therapies targeting diabetic ocular neuropathy and angiopathy.

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Combination of *lactococcus lactis* and essential oils from mediterranean herbs to counteract non-alcoholic fatty liver disease: an *in vitro* study

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Nowadays, non-alcoholic fatty liver disease (NAFLD) is a very common disease in the world but functional foods have been identified as key agents in liver disease prevention and treatment [1]. Furthermore, recent *in vitro* and *in vivo* studies demonstrated that probiotic strains may be helpful in counteracting gut-liver axis upheaval coupled to gut microbiome alterations [2].

Here, we explored the biological properties of Lactococcus lactis I7 (Ll-I7) alone and mixed to mediterranean natural extracts (*e.g.* basil, thymus, oregano) in NAFLD *in vitro* model.

Probiotic was obtained by Ll-I7 fermentation, centrifugation and freeze-drying [3]. Postbiotic fraction was derived by heat-treatment of broth and freeze-drying. NAFLD *in vitro* model was based on human liver cancer cells (HepG2) insulted with fatty acid (FA) [4]. HepG2 were incubated with Ll-I7 based mixtures alone and coupled to essential oils at concentrations of 1-5-10 mg/mL for 24h and 48h to test cell viability. Furthermore, intracellular lipid droplets were evaluated by Oil Red O-staining and metabolic pathways related to fatty acids metabolism affection was evaluated by quantitative RT-PCR and western blotting.

A reduction of lipid droplets was recorded in all treated samples, particularly with both strain and herbs. These results were confirmed by specific biomarkers expression analyses; Peroxisome Proliferator Activated Receptor (PPAR-γ), sterol regulatory element-binding transcription factor (SREBP)-1c and fatty acid synthetase (FAS).

The results showed that proposed formulations can contribute to the inhibition of *de novo* lipogenesis and the prevention of triglyceride accumulation providing data for potential anti fatty liver diseases formulations.

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Autore	**Tematica**
Abate Marianna	BDT 19, BIOTEC 27
Abate Teresa	BDT 22
Acquistapace Isabella	MEMPRO 23
Adabbo Eva	NUTRA 6
Al-Asmar Asmaa	BIOTEC 25
Alfano Alberto	BIOTEC 7, BIOTEC 30, NUTRA 11
Alfieri Mariaevelina	BIOTEC 37
Ali Waqar	MEMPRO 12, BDT14
Aliberti Anna	BIOTEC 34
Aliberti Michela	BDT 4
Altieri Antonella	MEMPRO 11
Alvarez-Rivera Gerardo	BIOTEC 8
Amante Chiara	BIOTEC 37
Ambrosone Alfredo	BIOTEC 37
Ametrano Alessia	NUTRA 5
Ammendola Rosario	BDT 17, MEMPRO 15
Amodio Giuseppina	BDT 3
Annaz Hassan	BIOTEC 8
Annunziata Marco	BIOTEC 23
Apicella Gennaro	BDT 10
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Arcadio Francesco	BIOTEC 23
Arciello Angela	BDT 8, BIOTEC16, BIOTEC20
Arcone Rosaria	NUTRA 7
Arpino Roberta	BDT 15
Aulitto Martina	BIOTEC 22
Ausiello Raffaella	BIOTEC 6, NUTRA 4
Avitabile Marika	BIOTEC 19
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Basilicata Giovanna Manuela	NUTRA 2
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Bertamino Alessia	BDT 3, BDT 4, BDT 5, BDT 23, MEMPRO1, MEMPRO 3
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Bonora Simone	ABC 1
Borriello Adriana	MEMPRO 12, BIOTEC 23, BDT 14
Bosso Andrea	BIOTEC 28, BIOTEC 16, MEMPRO 6
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Bruzzaniti Sara	MEMPRO 17
Cabedo Mas Luis	MEMPRO 22
Cadoni Francesca	BDT 15
Cafaro Valeria	BIOTEC 16, BIOTEC 18, BIOTEC 20, BIOTEC 28, MEMPRO 6
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Calabrese Laura	BIOTEC 26
Calvanese Marzia	BIOTEC 1
Campani Virginia	BIOTEC 27
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Campiglia Pietro	BIOTEC 37, BDT 3, BDT 4, BDT 5, BDT 23, MEMPRO1, MEMPRO 3, NUTRA 1, NUTRA 2
Candia Umberto	BDT 16
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Capace Daria	BDT 10
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Costanzo Noemi	BIOTEC 15
Costanzo Paola	BDT 16
Cozzolino Flora	BDT 8, BIOTEC 1, BIOTEC 13, MEMPRO 7
Crescente Giuseppina	BIOTEC 14, BDT 18
Cristiano Maria	BIOTEC 16, BIOTEC 28, MEMPRO 6
Crocetto Felice	BDT 9
Culurciello Rosanna	BIOTEC 16, BIOTEC 20, BIOTEC 28, MEMPRO 6
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Cusano Andrea	BIOTEC 34
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D'Auria Giovanni	BDT 19
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Dando Ilaria	BDT 10

Danisi Camilla	BDT 7, BDT11, BDT12, BDT24 NUTRA 10
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De la Fuente-Nunez Cesar	BIOTEC 20
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Longo Giuseppe	BDT 22
Lubrano-Lobianco Alessia	MEMPRO 11
Luce Amalia	BDT 19, BDT 22
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Maffia Michele	NUTRA 5
Maglione Barbara	BIOTEC 32
Malacrida Sandro	NUTRA 5
Malatesta Francesco	ABC2
Malune Paolo	MEMPRO 7

Mancini Paolo Francesco	NUTRA 3
Manfra Michele	NUTRA 1, 2
Marabotti Anna	ABC1, ABC 2, MEMPRO 8
Marchetti Gianluca	BIOTEC 30
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Maresca Emanuela	BIOTEC 6
Marini Federico	NUTRA 2
Mariniello Loredana	BIOTEC 10, BIOTEC 11, BIOTEC 12, BIOTEC 19, BIOTEC 25
Marmo Elvira	BIOTEC 21, BIOTEC 29
Marotta Angela	BIOTEC 30
Marrone Stefano	BDT 17, MEMPRO 15
Maruccio Lucianna	MEMPRO 15
Masi Marco	BIOTEC 9
Masullo Mariorosario	NUTRA 7
Maurelli Maria Anna	NUTRA 3
Mautone Francesco	NUTRA 3
Melone Viola	BDT 2
Mensitieri Francesca	BIOTEC 20, BIOTEC 21, BIOTEC 29, BIOTEC 37, ABC 2
Mentino Maria Rosaria	BDT 8
Minopoli Giuseppina	BDT 25
Miranda Maria Rosaria	BIOTEC 37, BDT 3,BDT 4, BDT 23, MEMPRO 1, MEMPRO 3, NUTRA 1, NUTRA 2
Mirpoor Fatemeh Seyedeh	BIOTEC 19
Misso Gabriella	BDT 22
Moccia Stefania	BDT 18
Moltedo Ornella	BIOTEC 37, BDT 3
Monaco Vittoria	MEMPRO 20
Montefusco Antonio	BIOTEC 34, MEMPRO 10, NUTRA 8
Montesarchio Daniela	BDT 8, BDT 21
Monti M. S.	BIOTEC 5
Monti Maria	BIOTEC 1, BIOTEC 4, BIOTEC 5,BIOTEC 8, BIOTEC 13 BDT 8, MEMPRO 7,MEMPRO 11,MEMPRO 20
Monti Maria Maurilia	BIOTEC 4
Moracci Marco	BIOTEC 2, BIOTEC 35,BIOTEC 36
Morasso Stefano	MEMPRO 7
Morelli Marco	BIOTEC 30
Morone Chiara Maria	BDT 9
Mosca Laura	BDT 15
Motta Gaetano	BDT 22
Motta Giovanni	BDT 22
Mozzillo Enza	MEMPRO 17
Mulè Chiara	BIOTEC 34
Musella Federica	BIOTEC 17, BIOTEC 31
Musella Simona	BDT 4, BDT 23,
Musumeci Domenica	BDT 8
Napolitano Valeria	BDT 4, BDT 5, BDT 23

Nardini Marco	MEMPRO 23
Nasso Rosarita	NUTRA 7
Naviglio Daniele	BIOTEC 9
Naviglio Silvio	MEMPRO 5
Neffe-Skocińska Katarzyna	NUTRA4
Nolano Antonio	BDT 9
Notomista Eugenio	BIOTEC 13, BIOTEC 18, BIOTEC 20, BIOTEC 28
Novi Sara	NUTRA 2
Oliva Rosario	MEMPRO 6
Oliviero Carlo	BIOTEC 32
Ostacolo Carmine	BDT 4, BDT 5
Ottolenghi Sara	NUTRA 5
Palmero Paola	BIOTEC 24
Palumbo Domenico	BDT 2
Palumbo Ida	BIOTEC 16,BIOTEC 28, MEMPRO 6
Palumbo Stefania	MEMPRO 12
Paolella Gaetana	BIOTEC 34, MEMPRO 10, NUTRA 8
Paragliola Pia Maria Francesca	BIOTEC 2
Parecha Kumar Darshan	BIOTEC 7
Parente Daniela	MEMPRO 12, BDT 14
Parisi Valentina	BDT 6
Paroni Rita	NUTRA 5
Parrilli Ermenegilda	BIOTEC 1
Pascarella Stefano	MEMPRO 20
Pecoraro Annalisa	BDT 7, BDT 11, BDT 12, BDT 24, NUTRA 10
Pecoraro Michela	BDT 6
Pedone Emilia	MEMPRO 4, MEMPRO 18, MEMPRO 23,BIOTEC 38
Pedone Vincenzo Paolo	MEMPRO 9, MEMPRO 23
Peluso Riccardo	BIOTEC 12
Penna Y.M.	NUTRA 6
Pentimalli Francesca	BDT 13
Pepe Giacomo	BIOTEC 37, BDT 4, BDT 5, NUTRA 1, NUTRA 2, MEMPRO 1, MEMPRO 3
Pérez Gámez José	MEMPRO 22
Perlingieri Caterina	BIOTEC 30
Perrella Shana	BIOTEC 10, MEMPRO 5
Perrone Pasquale	NUTRA 9
Persico Carolina	BDT 24
Petrone Zeudi	BDT 7, BDT 11, BDT 12, BDT 24, NUTRA 10
Piacentini Emma	BIOTEC 15
Piccolo Erika	BIOTEC 20
Piccolo Marialuisa	BDT 21
Piemonte Erica	MEMPRO 17
Pirone Luciano	BIOTEC 38, MEMPRO 4, MEMPRO 18, MEMPRO 23
Piscitelli Alessandra	BIOTEC 33, MEMPRO 22

Pitocchi Rossana	BIOTEC 33, MEMPRO 22
Pizzo Elio	BIOTEC 16,BIOTEC 20, BIOTEC28 MEMPRO 6
Platella Chiara	BDT 8
Porcelli Marina	BDT 15
Porrino Francesco	BIOTEC 30
Porru Manuela	BIOTEC 27
Prete Ludovica	BIOTEC 9
Punziano Carolina	BDT 25
Quaranta Miriana	MEMPRO 20
Ragucci Sara	MEMPRO 16, MEMPRO 21
Randazzo Antonio	BDT 24
Recine Maria	BIOTEC 14
Remondelli Paolo	BDT 3
Restaino Francesca Odile	BIOTEC 10,BIOTEC 11, BIOTEC 12, BIOTEC 19
Riccardi Claudia	BDT 21
Ricchi Paolo	BIOTEC 23
Ricciardiello Filippo	BDT 22
Righelli Dario	BIOTEC 13
Rispo Francesca	BIOTEC 18, BIOTEC 24
Rizzo Emanuela	MEMPRO 22
Romanelli Massimiliano Antonio	BIOTEC 34, MEMPRO 10, NUTRA 8
Rossi Antonietta	MEMPRO 15
Rossi Mariagrazia	ABC 1
Rubino Maria Federico	NUTRA 5
Rullo Rosario	NUTRA 7
Ruocco Michelina	BIOTEC 4
Ruoppolo Margherita	MEMPRO 13
Russo Annapina	BDT 7, BDT 11, BDT 12, BDT 24, NUTRA 10
Russo Gian Luigi	BIOTEC 14, BDT 18, NUTRA 3, NUTRA 6
Russo Giulia	BDT 7, BDT 11, BDT 12, BDT 24, NUTRA 10
Russo Luigi	MEMPRO4
Russo Margherita	BIOTEC 27, BDT
Russo Maria	NUTRA, BDT 18
Russo Roberta	NUTRA 5
Russo Rosita	MEMPRO 23, MEMPRO 9, MEMPRO 17
Sabbah Mohammed	BIOTEC 25
Sacchi Mariapia	MEMPRO 19
Sacco Oriana	BIOTEC 35, BIOTEC 36
Salvati Alessandro	BIOTEC 9
Salviati Emanuela	NUTRA 2
Samaja Michele	NUTRA 5
Sanchez-Safont Estefania	MEMPRO 22
Santamaria Rita	BDT 21
Santoro Valentina	BIOTEC 37
Sapio Luigi	MEMPRO 5

Sarnelli Sara	BIOTEC 23
Saviano Michele	MEMPRO 18
Scafuri Bernardina	MEMPRO 8
Scafuro Giuseppe	BDT 22
Scala Carmina Maria	BDT 5
Scattoni Luisa Maria	ABC 1
Schibeci Martina	BIOTEC 20
Schiraldi Chiara	BIOTEC 3, BIOTEC 7 BIOTEC 15, BIOTEC 18, BIOTEC 22, BIOTEC 24, BIOTEC 26, BIOTEC 30, BIOTEC 32, NUTRA 11
Scotto Sara d'Apollonia	BIOTEC 4
Serafim Seuanes Luísa	BIOTEC 36
Serpieri Ludovica	BDT 2, MEMPRO 14
Sessa Raffaele	BDT 1
Severino Angelica	BIOTEC 1
Sgambati Domenico	MEMPRO 9, MEMPRO 23
Shabbir S. Bin	BIOTEC 22
Shaikh-Ibrahim Ali	BIOTEC 35, BIOTEC 36
Shehi Haidi	MEMPRO 23
Silvestri Ilaria	MEMPRO 18
Sirangelo Ivana	BIOTEC 10, MEMPRO 5
Sirangelo Ivana	MEMPRO 5, BIOTEC 10
Smaldone Gerardina	BDT 4, BDT 5, BDT 23
Solimeno Ilaria	BIOTEC 30
Sommariva Arianna	BDT 16
Sommella Maria Eduardo	BDT 23, MEMPRO 3
Spagnuolo Carmela	NUTRA 3, NUTRA 6
Stampone Emanuela	BIOTEC 23, BDT 14, MEMPRO 12
Stellavato Antonietta	BIOTEC 3
Stiuso Paola	BDT 19
Storici Paola	MEMPRO 7
Strapazzon Giacomo	NUTRA 5
Strazzulli Andrea	BIOTEC 2
Svensson Birte	BIOTEC 35
Tabolacci Claudio	ABC 1
Tammaro Chiara	BDT 22
Tammaro Lidia	MEMPRO 2
Tancredi Francesco	BIOTEC 11
Tarallo Roberta	BDT 2
Tarricone F.	NUTRA 6
Tecce Felice Mario	BDT 4, BDT 5, NUTRA 1, NUTRA 2
Tedesco I.	NUTRA 6
Tedesco Pietro	BIOTEC 13
Terracciano Daniela	BDT 9
Tian Yu	BIOTEC 35
Tornesello Lina Maria	BDT 25

Torres D. T. Marcelo	BIOTEC 20
Tosti Elisabetta	BDT 18
Tramontano Enzo	MEMPRO 7
Tringali Giovanni U.	MEMPRO 11
Trombetti Silvia	BDT 1, BDT 9
Trovato Maria	MEMPRO 14
Tutino Maria Luisa	BIOTEC 1
Ungaro Francesca	BIOTEC 28
Varasi Ilenia	MEMPRO 14
Varone Alessia	MEMPRO 19
Vassallo Valentina	BIOTEC 3, BIOTEC 26, NUTRA 11
Ventorino Valeria	BIOTEC 15
Vestuto Vincenzo	BIOTEC 37, BDT 3, BDT 4, BDT 5, BDT 23, MEMPRO 1, MEMPRO 3, NUTRA 1, NUTRA 2
Vicenti Ilaria	MEMPRO 14
Vietri Mariapia	BDT 3, BIOTEC 37
Viscusi Gianluca	NUTRA 8
Vitale Del Vecchio	BDT 14
Vitale Laura	BIOTEC 13
Vitiello Annamaria	BIOTEC 38
Vivenzio M. V.	BIOTEC 5
Volpe Grazia Maria	BIOTEC 14
Wang Yu	BIOTEC 35
Zannella Carla	BIOTEC 16, BIOTEC 20
Zappavigna Silvia	BIOTEC 27, BDT 10, BDT 13, BDT 19
Zarrelli Armando	BIOTEC 8
Zeni Luigi	BIOTEC 23
Zielińska Dorota	NUTRA 4
Zollo Massimo	MEMPRO 20
Zullo Alberto	NUTRA 3