3rd IBBR Memorial Workshop

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M A R I A C I A R A M E L L A

November 10-11, 2022

Area di Ricerca NA1 – Aula Magna
Via Pietro Castellino 111, Napoli

Istituto di Bioscienze e BioRisorse

Consiglio Nazionale delle Ricerche

Dipartimento di Scienze BioAgroalimentari

Maria Ciaramella (1958-2018)

On December 2, 2018, Maria Ciaramella passed away in Naples at the age of 60. She was born in Naples in 1958 and received her degree in Biology *magna cum laude* in 1980 discussing a thesis under the supervision of Prof. John. F. Pulitzer. From 1982 until 1992 she worked as research scientist in Naples at the Institute of Genetics ad Biophysics (IGB) of the National Research Council of Italy (CNR). In the Pulitzer's group, she worked on the genetic analysis of the interactions of virus-host during the infection of phage T4 on *Escherichia coli*, on the regulation of the transcription in *Saccharomyces cerevisiae*, and on the heterologous expression in yeast of enzymes from hyperthermophilic microorganisms.

From 1993, she moved into the Institute of Protein Biochemistry (IBP) of CNR in Naples directed by Prof. Mosè Rossi, where, settled as group leader, she focused her scientific interests on the adaptation of organisms to high temperatures, by analysing the molecular mechanisms of stabilization of DNA, proteins and enzymes in hyper-thermophilic Archaea. Promoted as senior scientist in 1999, Maria joined the new Institute of Biosciences and BioResources of CNR in 2014, where from 2015 she also served as Responsible of the Operative Unit in Naples.

The scientific community will remember Maria Ciaramella for her studies on the mechanisms of regulation of gene expression and on protein/enzymes involved in DNA transactions in mesophilic and (hyper)thermophilic organisms. In the last 20 years, her scientific activity has been focused on the study of genome structure and stability, by analysing the molecular mechanisms involved in DNA damage response and repair. In particular, her findings have clarified the biochemical role of DNA manipulating proteins and enzymes (DNA binding proteins, topoisomerases, helicases) in hyperthermophiles. Especially, the studies on the reverse gyrase topoisomerase provided essential information about the *in vivo* function of this thermophilic hallmark. More recently, she also made substantial contributions to elucidate for the first time the structure-function relationships governing activity and stability of a thermostable protein involved in DNA alkylation damage repair. The knowledge from these studies also led to the development of new *protein-tags*, expanding biotechnological applications on thermophilic model systems. On 18 September 2018, Maria Ciaramella delivered her last talk: "*Peculiar hyperthermophilic genome-protecting enzymes: new techniques, applications and perspectives*", as invited speaker at the 12th International Congress of Extremophiles in Ischia, Naples, Italy.

Throughout her career, she inspired and mentored many young students and scientists, and her approach to research was characterized by creativity, collaboration, and determination. On top of her professionalism, she also transpired much kindness and was a good friend to many people. Maria was an enlightened and enlightening woman for all those who worked with her, touching the lives of many people at the laboratory and in her field nationally and internationally.

The Organizers and the Scientific Committees welcome the scientific community to this third appointment, thanking all the young researchers who will contribute to the success of this event.

Anna, Pino and Roberto

The Institute of Biosciences and BioResources- CNR

The Institute of Biosciences and BioResources (IBBR) comes from the reorganization and optimization of research centres recently carried out by Italian National Council of Research (CNR). IBBR has been recently founded (Nov 2013) by merging the former IGV (Plant Genetics Institute) with part of the activities carried out at IGB (Institute of Genetics and Biophysics) and IBP (Institute of Protein Biochemistry). The headquarter is located in Bari (Apulia, Italy) and has 5 different divisions spread over national territory (Florence, Naples, Palermo, Perugia and Portici). Current Director is Dr. Giovanni Giuseppe Vendramin. Research activities of the IBBR aim at the enhancement of basic knowledge in biology. Its main scientific commitment is to investigate the molecular and genetic basis of agro-food productions focusing on the sustainable management of BioResources in the biomedical, biochemical and environmental fields. These topics are basics for the European agricultural policy, the National Research Plan, and the R&D framework programmes of the EU, in particular for the VIII framework programme "Horizon 2020". The IBBR acts accordingly to the needs of a strategic sector of the Italian research and economy such as agriculture, in collaboration with several University departments, experiment Institutes (e.g., MiPAF, ENEA) and local administrations. People at the IBBR has long experience in the field of germplasm collection and management, and in the study and appraisal of the genetic variability of herbaceous, fruit and forest species.

Beppe Vendramin
(Director)

Gianna Palmieri
(Head UOS Naples)

On behalf of the Institute of Biosciences and BioResources, and in particular of the UOS of Naples, we are pleased to welcome the scientific community to honour and remember our dear Maria Ciaramella, on the occasion of this third Meeting dedicated to her. We wish all participants a fruitful scientific discussion, which is an important opportunity especially for young researchers who have been selected as speakers or will participate with a poster at this event.

Gianna and Beppe

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Mariella **FERRANTE**	*Dip. di Ecologia Marina Integrata, Stazione Zoologica di Napoli "Anton Dohrn"*

PROGRAM

Thursday, November 10 – Session 1

*Session 1a (chair: Martina **AULITTO**)*

09:00 – 09:50 *Registration*

09:30 – 09:50 *Greetings and Opening*

09:50 – 10:10 L01: Ida **LETTIERO**
Istituto di Bioscienze e BioRisorse (IBBR - CNR)
Anticancer potential of natural compounds from *Glycyrrhiza glabra* leaves

10:10 – 10:30 L02: Marta **MALLARDO**
Dipartimento STABiF, Università della Campania "Luigi Vanvitelli"
Adiponectin reduces proliferation of B lymphocytes: focus on the expression of the main factors involved in angiogenesis

10:30 – 11:10 *coffee break and poster session*

*Session 1b (chair: Federica **SCOTTO DI CARLO**)*

11:10 – 11:30 L03: Maria **MAROTTA**
Istituto per l'Endocrinologia e l'Oncologia Sperimentale "G. Salvatore" (IEOS -CNR)
Novel therapeutic combinations that induce immunogenic cell death in anaplastic thyroid carcinoma

11:30 – 11:50 L04: Federica **SARACINO**
Istituto di Genetica e Biofisica "Adriano Buzzati Traverso" (IGB - CNR)
Exploring a drug repurposing approach to treat pancreatic ductal adenocarcinoma

11:50 – 12:10 L05: Fortunato **PALMA ESPOSITO**
Dipartimento di Biotecnologie Marine, Stazione Zoologica di Napoli "Anton Dohrn"
Combining OSMAC Approach and Untargeted Metabolomics for the Identification of New Glycolipids with Potent Antiviral Activity Produced by a Marine *Rhodococcus*

12:10 – 12:40 L06: Ludovica **D'AURIA**
Dipartimento di Biologia, Università degli Studi di Napoli "Federico II"
Role of p73 and p63 in skin inflammation

12:40 – 14:00 *light lunch and poster session*

Thursday, November 10 – Session 2

*Session 2a (chair: Rosanna **MATTOSSOVICH**)*

13:00 – 14:00 *Registration*

14:00 – 14:20 L07: Rita **RUSSO**
Istituto di Biostrutture e Bioimmagini (IBB - CNR)
Galectins: structure, functions and potential therapeutic inhibitors

14:20 – 14:40 L08: Maria **MARONE**
Istituto di Biochimica e Biologia Cellulare (IBBC - CNR)
A mesophilic phosphotriesterase-like lactonase shows high stability and proficiency as quorum quenching enzyme

14:40 – 15:00 L09: Giovanni **DI MAURO**
Istituto di Bioscienze e BioRisorse (IBBR - CNR)
First thermostable CLIP-tag by rational design applied to an archaeal O^6-alkyl-guanine-DNA-alkyl-transferase

15:00 – 15:40 *coffee break and poster session*

*Session 2b (chair: Alessia **AMETRANO**)*

15:40 – 16:00 L10: Giuseppina **TOMMASINI**
University of Zaragoza, Istituto di Scienze Applicate e Sistemi Intelligenti (ISASI - CNR)
Magnetic hyperthermia: triggering different biological effect tuning magnetic nanoparticles composition

16:00 – 16:20 L11: Angela **PENNACCHIO**
Istituto di Scienze dell'Alimentazione (ISA - CNR)
The recombinant Human Heparanase: fluorescent Assay design

16:20 – 16:40 L12: Chiara **FORENZA**
Istituto di Biochimica e Biologia Cellulare (IBBC - CNR)
Identification of factors active on human monocytes/macrophages from *N. meningitidis* and *N. gonorrhoeae*-derived OMVs and development of cross protection by innate immunity

Friday, November 11 – Session 3

*Session 3a (chair: Luciano **PIRONE**)*

09:00 – 09:50 *Registration*

09:50 – 10:10 L13: Sara **MAGLIACANE TROTTA**
Istituto di Genetica e Biofisica "Adriano Buzzati Traverso" (IGB - CNR)
Unraveling the role of tumor-derived exosomal Alu RNAs in colorectal cancer

10:10 – 10:30 L14: Sara **RAGUCCI**
Dipartimento STABiF, Università della Campania "Luigi Vanvitelli"
Sodins, ribosome-inactivating proteins from *Salsola soda* L.

10:30 – 11:10 *coffee break and poster session*

*Session 3b (chair: Carla Lucia **ESPOSITO**)*

11:10 – 11:30 L15: Laura **FIORETTO**
Istituto di Chimica Biomolecolare (ICB – CNR)
Immunomodulant activity of Sulfavants and its correlation with colloidal self-assembly

11:30 – 11:50 L16: Gianluca **SCERRA**
Dipartimento di Medicina Molecolare e Biotecnologie Mediche, UNINA
A signal peptide dictates the timing for PD-L1 expression control

11:50 – 12:10 L17: Emanuela **PALOMBA**
Istituto di Bioscienze e BioRisorse (IBBR - CNR)
Extracellular self-DNA inhibits microalgae growth: insights on self-inhibition in freshwater and marine environment

12:10 – 12:30 L18: Anna **SANTIN**
Dip. di Ecologia Marina Integrata, Stazione Zoologica di Napoli "Anton Dohrn"
Integrative molecular analyses and first functional characterization of low-affinity nitrate transporters in diatoms, diNPFs

12:40 – 14:00 *light lunch and poster session*

Friday, November 11 – Session 4

*Session 4a (chair: Mariella **FERRANTE**)*

13:00 – 14:00 *Registration*

14:00 – 14:20 L19: Ilaria **MOTTOLA**
Istituto di Biochimica e Biologia Cellulare (IBBC - CNR)
The microbial endopeptidase E40 efficiently detoxifies gluten proteins in complex food matrices: implications for oral treatment of gluten intolerance

14:20 – 14:40 L20: Deborah **GIORDANO**
Istituto di Scienze dell'Alimentazione (ISA - CNR)
Molecular simulations to investigate protein structure and function

14:40 – 15:00 L21: Lucia **VERRILLO**
Istituto di Genetica e Biofisica "Adriano Buzzati Traverso" (IGB - CNR)
Defective corticogenesis in Arx mouse model of developmental and epileptic encephalopathy caused by polyalanine elongations

15:00 – 15:40 *coffee break and poster session*

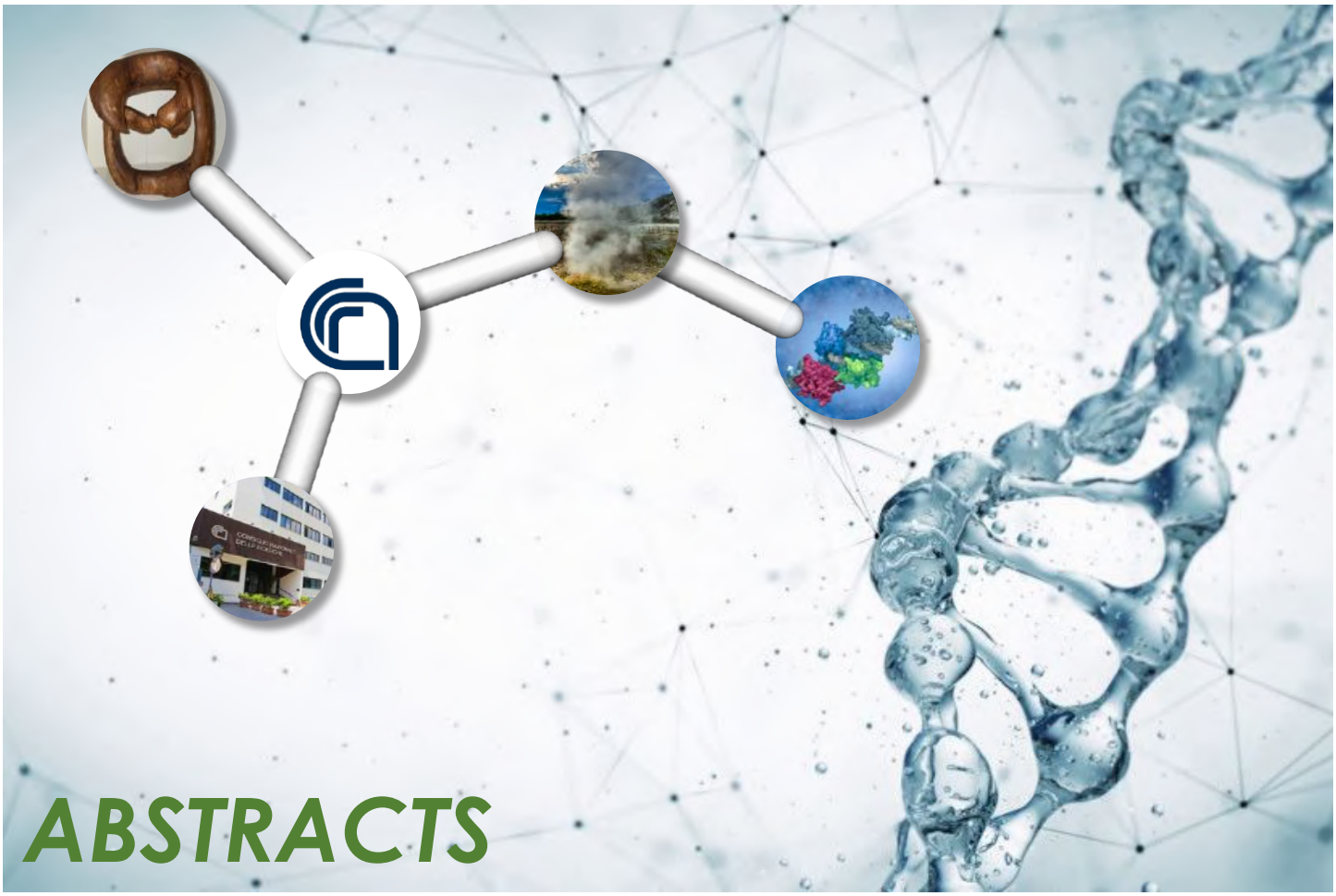
*Session 4b (chair: Valentina **MARCHESANO**)*

15:40 – 16:00 L22: Marcello **MERCOGLIANO**
Istituto di Chimica Biomolecolare (ICB – CNR)
Synthesis of BODIPY-based fluorescent analogue of the molecular adjuvant Sulfavant

16:00 – 16:20 L23: Giuliana **GIAMUNDO**
Dipartimento di Biologia, Università degli Studi di Napoli "Federico II"
Induction of autophagy promotes clearance of RHOP23H aggregates and protects from retinal degeneration

16:20 – 16:40 L24: Lucia **TREPPICCIONE**
Istituto di Scienze dell'Alimentazione (ISA - CNR)
The intestinal immune response to transamidated gliadin in a mouse model of gluten sensitivity

16:40 – *Closing and Poster Award*



ABSTRACTS

L01
Anticancer potential of natural compounds from *Glycyrrhiza glabra* leaves

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ABSTRACT

Background-Aim: Colorectal cancer (CRC) is considered the second most common cancer in women and the third in men worldwide. Patients with this type of cancer are treated with 5-fluorouracil-based chemotherapy, which involves several side effects for the patient. Recent studies are therefore focusing on using natural compounds with anti-cancer activity to counteract the growth of cancer cells and any side effects of chemotherapy treatment[3]. Particularly relevant is the use of Glycyrrhiza glabra (GGL)[1,2] which seems to show some effect on cell viability. The project aim is to characterize the use of GGL as a marker for therapy for CRC.

Methods: Cck-8 assay, western blot

Results: Our GGL extract was obtained by exhaustive UAE (ultrasound assisted extraction) of licorice leaf tissues supplied by Consortium "Liquirizia di Calabria". Our preliminary data suggest significant effects of GGL treatment on the viability, proliferation, and death of cancer cells, in particular on HCT-116 (human colorectal carcinoma cell line). Several assays, at different time and different concentrations of GGL, showed a decrease in cell proliferation and increase of death induction. Furthermore, western blot analysis suggests the involvement of apoptosis intrinsic pathway by Caspase-9 activation. The same assays were carried out on non-tumor cells: HaCaT (Human immortalized keratinocytes). The preliminary results showed that this extract does not produce toxic effects on a healthy organism, favouring exclusively its potential anticancer use.

Conclusions: GGL is a good extract for selectively fighting cancer cells. Our promising data need further investigation related the molecular mechanism of GGL and the specific fraction responsible of its anticancer activity. Recent study [1] characterized the individual fractions of the extract (pinocembrin, glabranin, lycoflavanone, and prenylateddihydrostylbenes). *De facto*, the single molecules isolated from GGL compound will be analysed and deeply investigated as anticancer new drugs/molecules.

REFERENCES

1. Celano R, Docimo T, Piccinelli AL, *et al,* (2021). *Industrial Crops and Products*, vol 170.
2. Nourazarian S.M, Nourazarian A, Majidinia M, *et al,* (2015). *Asian Pac J Cancer Prev*, vol 16(18): 8563–8566.
3. Chen Y, Cheng C, Tan HY, *et al,* (2021). *Frontiers in Oncology*, vol 11: 629-632.

L02
Adiponectin reduces proliferation of B lymphocytes: focus on the expression of the main factors involved in angiogenesis

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ABSTRACT

Adiponectin is one of the main adipokines secreted by adipocytes. Different biological functions of adiponectin have been described ranging from anti-diabetic, anti-inflammatory to immune suppressive. A plethora of experimental evidence supports a suppressive role for adiponectin in several obesity-related cancer. On the contrary, literature data describe a reverse regulatory mechanism of adiponectin expression in non-Hodgkin lymphoma (NHL) patients, being adiponectin levels increased. In this study, we aimed to investigate the effects of adiponectin on NHL cells in terms of proliferation, and aggressiveness. Therefore, we selected an in vitro model of NHL, the JVM2 cells, and performed treatments with AdipoRon, an adiponectin agonist largely utilised. After 24h, 48h, and 72h treatment, we verified adiponectin effects in term of proliferation, VEGF, HIF1-a and CXCL1 expression. We found that AdipoRon reduces the proliferation rate of JVM2 cells, while rises VEGF secretions accompanied by increased expression of HIF1-a and CXCL1. Also, conditioned media from JVM2 exposed to AdipoRon stimulate angiogenesis and tube formation in HUVEC endothelial cells, suggesting that adiponectin possibly sustain angiogenesis of JVM2 cells. Our data indicate that adiponectin is able to reduce proliferation and to enhance angiogenic capacity of the JVM2 lymphoma cells; such evidence supports a functional role for adiponectin in the regulation of NHL establishment and progression. Further studies are needed to investigate additional molecular characteristics of cancer cells, such as apoptosis, invasiveness, and autophagy regulated in vitro by AdipoRon.

REFERENCES

1. Parida S, Siddharth S, Sharma D (2019). *Int J Mol Sci*, vol 20(10): 2519.
2. Tilija Pun N, Pil-Hoon Par. (2018). *Br J Pharmacol*, vol 175(7): 1066-1084.
3. Nigro E, Daniele A, Salzillo A, *et al.* (2021). *Int J Mol Sci*, vol 22(11): 5569.

L03
Novel therapeutic combinations that induce immunogenic cell death in anaplastic thyroid carcinoma

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ABSTRACT

Anaplastic thyroid carcinoma (ATC) is the most aggressive malignancy of the thyroid follicular epithelium, characterized by a very poor prognosis and overall survival, despite the use of multimodal therapies.

We searched for novel therapeutical targets in ATC and we identified the interleukin-8 (IL-8)/CXCR1/2 circuit as a promoter of ATC cell malignant features and tumor forming potential. These activities were inhibited by Reparixin, a CXCR1/2 inhibitor.

We also found that ATC cells express the immune checkpoint Programmed death-1 (PD-1) and its ligands PD-L1/2. We showed that the PD-1/PD-L1 autocrine circuit, by activating a SHP2/RAS/ERK signaling cascade, stimulates ATC cell proliferation, migration and xenograft growth; accordingly, PD-1 blockade by Nivolumab, or SHP2 inhibition by SHP022, reverted these effects.

We then asked whether the combination of IL-8- and PD-1-inhibition could induce immunogenic cell death (ICD) in ATC cells. ICD is a regulated apoptosis characterized by ER stress, release of inflammatory factors, and induction of adaptive immunity against dying cancer cells. To this aim, we selected human ATC cell lines, for *in vitro* experiments, and mouse ATC cell lines syngeneic with 129SV mice for *in vivo* experiments, and we tested different combinations of Reparixin, Nivolumab and SHP099. We found that SHP099/Nivolumab and SHP099/Reparixin significantly reduced human and mouse ATC cell viability and induced apoptosis in culture. Importantly, cell death was characterized by ICD hallmarks, including eIF2a phosphorylation, cell surface calreticulin (CRT) exposure, ANXA1 and CXCL10 protein increase, ATP and HMGB1 release. We will use syngeneic mice models of ATC to verify whether these drug combinations could induce *in vivo* ICD, tumor regression and adaptative immunity against ATC.

L04

Exploring a drug repurposing approach to treat pancreatic ductal adenocarcinoma

Federica SARACINO[1], Eduardo IBELLO[1,2], Donatella DELLE CAVE[3], Filomena AMOROSO[1,2], Silvia BUONAIUTO[3], Vincenza COLONNA[3], Enza LONARDO[3], Eduardo Jorge PATRIARCA[1], Gabriella MINCHIOTTI[1], Cristina D'ANIELLO[1].

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ABSTRACT

The pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive and the most prevalent malignancy of the pancreas characterized by a dense tumor-associated stroma consisting of extracellular proteins, including collagens, which generate a stiff extracellular matrix[1,2]. The crosstalk between tumor cells and the microenvironment promotes PDAC growth and dissemination[3,4]. Recent studies showed that collagen provides a source of amino acids to fuel tumor growth and that PDAC cells exploit the environment to support metastatic dissemination[2]. The identification of compounds able to reduce the fibrotic component and the acquisition of mesenchymal traits in PDAC cells is of great interest to prevent tumor progression.

Budesonide, a glucocorticoid used to treat asthma, has been recently identified as a potent inhibitor of collagen deposition and of the acquisition of mesenchymal traits in both pluripotent stem and breast cancer cells[5]. Our aim is to evaluate the potential repositioning of budesonide for the treatment of PDAC.

We have assessed the effects of budesonide on human pancreatic cancer cell lines and patient -derived primary cells, by combining molecular and functional analysis. Genome-wide analysis revealed that budesonide alters the expression of a large set of genes enriched in key biological processes related to tumor progression and dissemination, including cell migration/locomotion and collagen fibril organization. Accordingly, budesonide reduces collagen accumulation both *in vitro* and *in vivo*, cell invasion in 2D culture and 3D spheroids and the acquisition of mesenchymal/invasive traits, without affecting cell proliferation and apoptosis. Our findings point to a potential repurposing of this drug to treat PDAC progression.

REFERENCES

1. Cascinu *et al.* (2010). *Ann Oncol*, vol 21 (5): 55-58.
2. Olivares *et al.* (2017). *Nat Commun*, vol 8: 16031.
3. Cukierman, Bassi, *et al.* (2010). *Semin Cancer Biol*, vol 20 (3): 139-145.
4. Pickup, Mouw, *et al.* (2014). *EMBO Rep*, vol 15 (12): 1243-1253.
5. D'Aniello, *et al.* (2019). *Cancer Res*, vol 79 (13): 3235-3250.

L05

Combining OSMAC Approach and Untargeted Metabolomics for the Identification of New Glycolipids with Potent Antiviral Activity Produced by a Marine *Rhodococcus*

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ABSTRACT

Natural products of microbial origin have inspired most of the commercial pharmaceuticals, especially those from Actinobacteria. However, the redundancy of molecules in the discovery process represents a serious issue. The untargeted approach, One Strain Many Compounds (OSMAC), is one of the most promising strategies to induce the expression of silent genes, especially when combined with genome mining and advanced metabolomics analysis. In this work, the whole genome of the marine isolate *Rhodococcus* sp. I2R was sequenced and analyzed by antiSMASH for the identification of biosynthetic gene clusters. The strain was cultivated in 22 different growth media and the generated extracts were subjected to metabolomic analysis and functional screening. Notably, only a single growth condition induced the production of unique compounds, which were partially purified and structurally characterized by liquid chromatography high-resolution tandem mass spectrometry (LC-HRMS/MS). This strategy led to identifying a bioactive fraction containing >30 new glycolipids holding unusual functional groups. The active fraction showed a potent antiviral effect against enveloped viruses, such as herpes simplex virus and human coronaviruses, and high antiproliferative activity in PC3 prostate cancer cell line. The identified compounds belong to the biosurfactants class, amphiphilic molecules, which play a crucial role in the biotech and biomedical industry.

L06
Role of p73 and p63 in skin inflammation

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ABSTRACT

Cutaneous squamous cell carcinoma (cSCC) is one of the most frequent cancers in the human population. Mutations in the tumour suppressor TP53 are commonly found in damaged skin after extensive UV exposure leading to SCC formation[1]. TP63 and TP73, two transcription factors members of TP53 family, are often overexpressed in this type of cancer[2]. While the role of TP63 is widely studied in the etiogenesis of SCC, the role of TP73 in physiological and pathological conditions is still unclear.

In this study, we investigate the role of TP73 (p73) alone and in concert with TP63 (p63) in skin inflammation, a condition that precedes tumour formation. We used p73 knock-out (p73 -/-) and p73 knock-out; p63 heterozygote (p73-/-; p63 +/-) murine models obtained crossing p73 fl/fl and p73fl/fl; p63+/- mice with K14-Cre expressing mice to obtain gene deletion specifically in the skin at late stages of embryonic development. These mutant mouse models were treated with a pro-inflammatory tumour-promoting agent (12-O-tetradecanoylphorbol-13-acetate) to obtained acute skin inflammation treatments. H&E and immunofluorescence analyses on epidermis controls and mutants revealed a significant increase in epidermal thickness and showed a statistically significant decrease in cell proliferation in both mutants compared to wild type mice. RNA-seq analyses performed on wild-types and mutants mouse epidermis confirmed this preliminary results. Taken together these data suggest that mice lacking p73 exhibit decreased proliferation and increased epidermal thickness, possibly due to a general alteration of keratinocyte shape caused by cytoskeleton rearrangements. Skin hypertrophy suggests that TP73 may play a significant role during skin tumorigenesis.

REFERENCES

1. Madan A, Cebrian M, Lazer D, Pentland A (2010). UbiComp '10, Sep 26-Sep 29, 2010, Copenhagen, Denmark. Copyright 2010 ACM 978-1-60558-843-8/10/09.
2. Alonso-Rasgado T, Zhang Q-H, Jimenez-Cruz D, *et al.* (2018). *Med Biol Engin Comput*, vol 56: 1091-1105.

L07
Galectins: structure, functions and potential therapeutic inhibitors

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ABSTRACT

Galectins represent an ancient family of lectins binding specifically β-galactoside residues on glycoconjugates. The Carbohydrate Recognition Domain (CRD), used to bind ligands, shows a high degree of conservation among all the members of this protein family. In human there are several evidences of the involvement of galectins in carcinogenesis as well as other pathologic conditions, as chronic inflammation, heart failure and metabolic disorders[1]. For this reason, galectins have received a great deal of attention from researchers, paving the way for the discovery of compounds with an inhibitory effect. To date, numerous synthetic inhibitors have been described in the literature and, of these, the most promising class is represented by thiodigalactosyl derivatives. On the other hand, research is also moving within the plant kingdom to discover galectin inhibitors derived from natural sources. However, the limitation of almost all discovered inhibitors is the low selectivity among human galectins. Therefore, in order to identify more selective galectin inhibitors, our research group analysed three synthetic compounds, unsymmetrical selenoglycoside (Unsymm(Se)), galactosyl selenide (SeDG) and the benzylated derivative of the latter (BenEST), exploring a panel of different binding partners. Furthermore, the anti-tumour activity of BenEST has been studied in cellular models, finding a good inhibitory effect on angiogenesis and migration of tumour cells[2-3]. Similarly, we are taking the first steps in the field of naturally derived galectin inhibitors by selecting a plant matrix from which to extract bioactive molecules that act as potential new galectin inhibitors.

REFERENCES

1. Ludger J, Ralf J, Hakon L (2018). *J Cell Sci*, vol 131(9): jcs208884.
2. Pirone L, Nieto-Fabregat F, Di Gaetano S, *et al.* (2022). *Int J Mol Sci*, vol 23(15): 8273.
3. Di Gaetano S, Pirone L, Galdadas I, *et al.* (2022). *Int J Mol Sci*, vol 23(05): 2581.

L08

A mesophilic phosphotriesterase-like lactonase shows high stability and proficiency as quorum quenching enzyme

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ABSTRACT

The Phosphotriesterase-Like Lactonase (PLL) family was discovered in our laboratory in collaboration with another group[1]. The first characterized members were the hyperthermophilic crenearchaeon *Saccharolobus solfataricus* (Sso Pox) and *Saccharolobus acidocaldarius* (Sac Pox)[2,3] and successively others thermophilic and mesophilic members were added[4]. These enzymes were studied as phosphotriesterases at first and subsequently it was discovered that their primary activity was a lactonase activity and acyl-homoserine lactones (AHLs). The SsoPox structure also revealed an unexpected active site topology, and a unique hydrophobic channel that perfectly accommodates the lactone substrate[5].
In the frame of a project granted from the Italian Ministry of Research, we are exploiting the lactonase activity of different enzymes to counteract *Pseudomonas aeruginosa* infections. The human opportunistic pathogen *P. aeruginosa* (PAO1) orchestrates the expression of many genes in a cell density-dependent manner by using quorum sensing (QS). Two acyl-homoserine lactones (AHLs) are involved in QS circuits and contribute to the regulation of virulence factors production, biofilm formation, and sensitivity to antimicrobials. Disrupting QS, a strategy referred to as quorum quenching (QQ) can be achieved using exogenous AHL-degrading lactonases[6]. In the mesophilic bacterium *Rhodococcus erythropolis* a PLL enzyme has been identified and partially characterized[1]. It is 28% identical with *P. diminuta* PTE, and 40% identical with respect to the thermostable SsoPox. Here we report the cloning, expression and purification procedure to produce a His-tagged Ahla, namely his-Ahla, that has been further characterized as a lactonase enzyme. It is highly stable over time and also under oxidizing conditions. It has showed to be a proficient quorum quenching enzyme, able to hydrolase acyl-homoserine lactones, 3oxo-C12-HSL and C4-HSL, and to reduce more than 50% the biofilm formation of PAO1. The combination of enzymes with different abilities as quorum quenchers could represent a powerful tool to be employed against pseudomonas infections. Here we will also report a comparison of the different enzymes under study.

REFERENCES

1. Afriat L, Roodveldt C, Manco G, *et al* (2006). *Biochemistry*, vol 45: 13677-13686.
2. Merone L, Mandrich L, Rossi M, *et al.* (2005). *Extremophiles*, vol 9: 297-305.
3. Porzio E, Merone L, Mandrich L, *et al.* (2007). *Biochimie*, vol 8: 625-636.
4. Porzio E, Del Giudice I, Manco G (2016). *Grand Challenges in Biology and Biotechnology*, vol 1: Biotechonology of Extremophiles, 978-3-319-13520-5, 331325_1_En.
5. Elias M, Dupuy J, Merone L, *et al.* (2008). *J Mol Biol*, vol 379: 1017-1028.
6. Thi MTT, Wibowo D, Rehm BHA (2020). *Int J Mol Sci*, vol 21: 8671.

L09

First thermostable CLIP-*tag* by rational design applied to an archaeal O^6-alkyl-guanine-DNA-alkyl-transferase

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ABSTRACT

Self-labelling *protein tags* (SLPs) are resourceful tools that revolutionized sensor imaging, having the versatile ability of being genetically fused with any protein of interest and undergoing activation with alternative probes specifically designed for each variant (namely, SNAP-*tag*, CLIP-*tag* and *Halo-tag*)[1-3]. Commercially available SLPs are highly useful in studying molecular aspects of mesophilic organisms, while they fail in characterizing model organisms that thrive in harsh conditions. In Perugino's lab was first designed an engineered variant of the alkylguanine-DNA-alkyl-transferase (OGT) from the hyper-thermophilic archaeon *Saccharolobus solfataricus* (*Ss*OGT-*H*^{5}), with no DNA-binding activity, able to covalently react with O^6-benzyl-guanine (BG-), which was the first thermostable SNAP-*tag* to date[4,5]. Recently, by applying an integrated computational and structural approach, we further obtained from *Ss*OGT-*H*^{5} the first thermostable CLIP-*tag*, named *Ss*OGT-*MC*^{8}, reactive only orthogonal substrates O^2-benzyl-cytosine (BC-) derivatives[6].

The presented construct is able to recognize and to covalently bind BC- substrates with a marked specificity, and showing a remarkable thermal stability that broadens the applicability of SLPs. The rational mutagenesis that, starting from *Ss*OGT, led to the production of *Ss*OGT-*MC*^{8}, was first evaluated by structural predictions to precisely design the chimeric construct, by mutating specific residues involved in protein stability and substrate recognition. The final construct was further validated by biochemical characterization and X-ray crystallography, allowing us to present here the first structural model of a CLIP-*tag* establishing the molecular determinants of its activity.

REFERENCES

1. Keppler A, *et al.* (2003). *Nat Biotechnol*, vol 21: 86–9.
2. Gautier A, *et al.* (2008). *Chem & Biol*, vol 15: 128–36.
3. Los GV, *et al.* (2008). *ACS Chemical Biology*, vol 3: 373–82.
4. Vettone A, *et al.* (2016). *Extremophiles*, vol 20: 1–13.
5. Visone V, *et al.* (2017). PLoS ONE, vol 12: e0185791.
6. Merlo C, *et al.* (2022). *Comp Struct Biotech J*, vol 20: 5275-5286.

L10
Magnetic hyperthermia: triggering different biological effect tuning magnetic nanoparticles composition

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ABSTRACT

Magnetic nanoparticles (MNPs) have gained growing attention due to their capability of responding to an external magnetic field allowing their remote manipulation. For this reason, MNPs have been widely used in the recent years to develop different biomedical approaches[1]. Most interestingly, MNPs are capable to produce heat under an alternating magnetic field (AMF), a phenomenon known as magnetic hyperthermia (MHT), acting as remote-switchable devices or restricting heat production to defined areas. A previous study showed how MHT in melanoma cells and *Hydra* polyps treated with MNPs induces the gene modulation of *hsp70*, suggesting a general mechanism to delivery sublethal thermal doses[2].

In this work, we present a new approach for tuning the composition of a set of manganese-iron oxide nanoparticle ($Mn_xFe_{3-x}O_4$) improving their magneto-thermal behavior, to trigger the activation of specific physiological processes. After investigating the heating performance using different combinations of alternating magnetic fields (AMFs), we studied *in vitro* and *in vivo* the impact of selected $Mn_xFe_{3-x}O_4$ nanoparticles with variable Mn^{2+} content using two biological models: pancreatic tumoral cells (MIA PaCa) and the freshwater invertebrate polyps *Hydra vulgaris*. In both systems, we first evaluated the nanotoxicology of the different MNPs and the internalization by fluorescence microscopy and ICP spectrometry. In order to understand if we could induce the activation of different physiological processes, we studied the biological effects of internalized MNPs after applying mild magnetic hyperthermia, both triggering apoptosis to promote cancer cells death and to enhance the regeneration process and reproduction rate in *Hydra* polyps.

REFERENCES

1. Kumar CSSR, Mohammad F (2011). *Adv. Drug Deliv. Rev*, vol 63(9): 789-808.
2. Moros M, Ambrosone A, Stepien G, *et al*. (2015). *Nanomedicine (Lond)*, vol 10(14): 2167-83.

L11
The recombinant Human Heparanase: fluorescent Assay design

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ABSTRACT

Human Heparanase (HPSE), an endo-β-D-glucuronidase, degrades heparan sulfate (HS), one of the extracellular matrix (ECM) components. It catalyzes the cleavage of the β (1,4)-glycosidic bond between glucuronic acid and glucosamine residue[1]. HS cleavage results in remodelling of the ECM as well as in regulating the release of many HS-linked molecules such as growth factors, cytokines and enzymes involved in inflammation, wound healing and tumour invasion[2]. HPSE is an enzyme implicated in a multiplicity of physiological and pathological processes. In recent years, different studies have shown that the enzyme is overexpressed in human tumours, it was identified to be a promising target for cancer diseases[3]. The heterodimer biologically active of the Human Heparanase is composed of two subunits, 8 and 50 kDa, which are noncovalently associated. Several studies have been performed to express in a bacterial system the enzyme in high yield and to define the substrate specificity due to the ECM heterogeneity. It would be convenient to elaborate a fast, easy and quantitative fluorescence assay to determine enzyme activity, synthesizing a new low-molecular-weight fluorogenic substrate with a unique β (1,4)-glycosidic bond, as site of cleavage, in order to have a measurable response.

REFERENCES

1. Hao Jin and Shaobo Zhou (2017). *Medicinal Chemistry*, vol 17, 541-548.
2. Pennacchio A, Capo A, Caira S, *et al.* (2018). *Biotechnol Appl Biochem*, vol 65(1): 89-98.
3. Edovitsky E, Elkin M, Zcharia E, *et al.* (2004). *J. Nat. Cancer Inst*, vol 96: 1219-1230.

L12

Identification of factors active on human monocytes/macrophages from *N. meningitidis* and *N. gonorrhoeae*-derived OMVs and development of cross protection by innate immunity

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ABSTRACT

Vaccination against *Neisseria meningitidis* (*Nm*) with 4CMenB vaccine, which includes outer membrane vesicle (OMV) from New Zealand strain, induces protection against *Neisseria gonorrhoeae* (*Ng*) likely due to vaccine-induced antibodies that cross-react with *Ng* OMVs proteins[1,2]. However, the role of the innate immune system in this cross-protection has not been explored. Preliminary results suggested that *Nm* OMVs knock-out in *Neisseria* adhesin A Regulator (NadR)-encoding gene impair human monocytes/macrophages cytokines secretion. In *Ng* is present a structural and functional homologous protein of NadR, the Fatty Acid Resistance Regulator (FarR). The aims of this project are: 1) characterize the molecular determinants in both *Ng* and *Nm* OMVs *FarR* and *NadR* knock-out involved in the impairment of cytokines secretion by human monocytes/macrophages; 2) study the role of the innate memory in the response of human monocytes/macrophages to priming/challenge with wt and KO *Nm* and *Ng* OMVs and 3) assess if this mechanism is an active player leading to the observed protection of the *Nm* OMV on gonococcus infection. Herein, preliminary results on the ability of OMVs wild type from both *Neisseria* species to induce innate memory in human monocytes/macrophages are presented. Innate memory is the ability of innate immune system to resist to reinfection and is mediated by metabolic and epigenetic reprogramming of innate immune cells[3]. Isolated monocytes from healthy donors have been primed and challenged with different combinations of OMVs from the two *Neisseria* species and inflammatory and anti-inflammatory cytokines in cell supernatants have been measured. Our preliminary data show a tolerance effect of OMVs challenge in primed compared to un-primed cells.

REFERENCES

1. Semchenko EA, Tan A, Borrow R, *et al* (2019). *Clin Infect Dis*, vol 13: 1101-1111.
2. Petousis-Harris H, Paynter J, Morgan J, *et al.* (2017). *Lancet*, vol 390: 1603-1610.
3. Netea MG, *et al.* (2016). *Science*, vol 352.

L13
Unraveling the role of tumor-derived exosomal Alu RNAs in colorectal cancer

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ABSTRACT

The Alu repeat elements are the most abundant short interspersed repeated elements (SINEs) in human genome. Recently, it has been showed that Alu RNAs accumulation is responsible for a human disease, such as a geographic atrophy, one of the main causes of human blindness. Interestingly, increased levels of Alu RNAs and reduced DICER1 expression have been observed in several cancers, although no correlation has yet been established. Furthermore, we recently demonstrated that Alu RNA accumulation induces epithelial-mesenchymal transition (EMT) and it is associated with colorectal cancer (CRC) progression[1].

Moreover, it has been demonstrated that tumors-derived exosomes are enriched for Alu sequences. Indeed, increasing evidence demonstrated that exosomes play an important role in intercellular communication and can control a wide range of pathway bolstering tumor development, metastasis and drug resistance.

In this scenario, we purified exosomes by ultracentrifugation from cell culture and we demonstrated that Alu transcripts are stored and transported by exosomes. Interestingly, we demonstrated that exosomal Alu RNAs can be transferred to CRC cells and promote invasion and growth in anchorage-independent manner by inducing NLRP3 inflammasome activation and EMT. Finally, we purified exosomes both in vitro from cell culture and in vivo from plasma of xenotransplanted mice with human CRC cells and, more interestingly, we found that Alu RNA abundance correlates with malignancy and tumor size, respectively.

Together these preliminary findings suggest that the abundance of tumor-derived exosomal Alu RNAs correlates with CRC progression and they could yield new approaches for tumor prognosis and therapy.

REFERENCES

1. Di Ruocco F, Basso V, Rivoire M, *et al.* (2018). *Oncogene*, vol 37: 627-37.

L14
Sodins, ribosome-inactivating proteins from *Salsola soda* L.

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ABSTRACT

Salsola soda L., known as "agretti" in Italy, is an annual, edible halophytic plant widespread in south Europe. Its tissues are rich in flavonoids with anti-inflammatory and antidiabetic potential. In the past, the plant was also used for the production of soda (sodium carbonate), from which the name 'soda'.

S. soda belongs to Caryophyllales order known as a source of ribosome inactivating proteins (RIPs). These enzymes (EC: 3.2.2.22) are rRNA N-glycosylases, which remove a specific adenine (A_{4324} in rat) of the Sarcin Ricin Loop (SRL) in the 28S rRNA, involved in the interaction of ribosome with elongation factors. This irreversible damage leads to the inhibition of translation and cell death by apoptosis pathway.

Here, in light of the possible biotechnological applications of RIPs in agriculture and medicine, we investigated their presence in leaves, roots and seeds of *S. soda*, found a higher amount of toxins in the latter. In particular, we: i) set-up the protein purification protocol; ii) evaluated N-glycosylase action by β-fragment release after incubation with rabbit ribosomes; and iii) verified the polynucleotide : adenosine glycosylase activity (PNAG) on salmon sperm DNA.

L15

Immunomodulant activity of Sulfavants and its correlation with colloidal self-assembly

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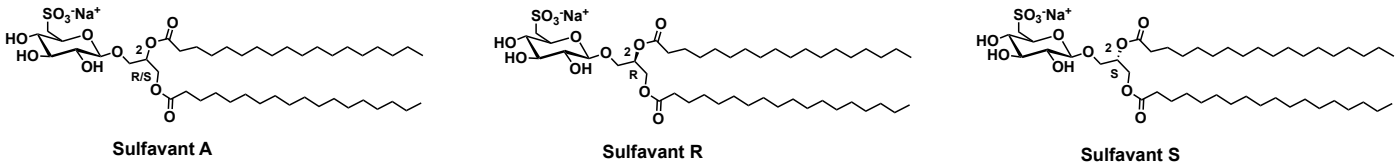
ABSTRACT

In the last decades, the improved understanding of how innate mechanisms influence the adaptive immunity system has prompted the research of new vaccine adjuvants by a rational design of new chemical entities.

In recent years we have been interested in the study and develop of new immunomodulatory substances from diatoms. This research led to isolation and characterization of α-sulfoquinovosides (α-SQDG)[1] as possible adjuvant candidates for vaccines. These substances showed a promising activity and their biological optimization by chemical synthesis led to the derivative 1,2-O-distearoyl-3-O-β-D-sulfoquinovosyl glycerol named Sulfavant A. The synthetic product induces maturation of human dendritic cells (DCs) with no expression of inflammatory cytokines and, in vivo, boost of an immune protective response.[1] Later on, two other synthetic analogues of Sulfavant A (named Sulfavant S and Sulfavant R) were synthesized, and they showed the capability to elicit DC maturation at a concentration 1000 times lower than that the progenitor molecule[2].

Investigation on the colloidal arrangement of Sulfavant A, R and S pointed out the formation of different kind of aggregates for the three molecules (Figure 1). The supramolecular interactions and stability of these aggregates affect the different immunomodulant behavior of these molecules[2].



REFERENCES

1. (a) Manzo E, Cutignano A, Pagano D, *et al.* (2017). *Sci. Rep,* vol 7: 6286. (b) Fontana A, Manzo E, Cutignano A, De Palma R, IT1417828 2015; PCT/IB2014/062098; (c) Manzo E, Fioretto L, Pagano D, *et al.* (2017). *Mar. Drugs*, vol 15: 288; (d) Gallo C, Manzo E, Barra G, *et al.* (2022). *CMLS*, vol 79(7): 369.
2. Manzo E, Gallo C, Fioretto L, *et al.* (2019). *ACS Omega*, vol 4: 7807.

L16
A signal peptide dictates the timing for PD-L1 expression control

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ABSTRACT

The majority of transmembrane proteins is equipped with an amino-terminal signal peptide (SP), a short protein region characterized by high frequency of hydrophobic amino acids: SPs recruit endoplasmic reticulum (ER)-translocation machinery immediately after their egress from ribosomal exit tunnel, and they are sufficient to drive protein translocation across the lipid bilayer. Programmed Death-Ligand 1 (PD-L1) protein is a single-pass transmembrane protein, mainly known for its central role in cancer immunotherapy, and its ER-translocation is SP-mediated. Many PD-L1 post-translational modifications have been described: it has been proposed that its phosphorylation occurs in the lumen of the ER by kinases such as AMPK and JAK1; Although such kinases lack a canonical SP for ER-translocation, their luminal localization has been proposed. Since much evidence suggested that a ribosomal nascent chain can be modified once arising from a ribosome, we hypothesized an alternative way for cytosolic enzymes to act as PD-L1 modifiers. Our evidence suggests PD-L1 ribosomal nascent chains acquire post-translational modifications in the cytosol, and they do so before ER-translocation completion. The temporal window may be created by PD-L1 SP, since it contains polar/charged amino acids: they may be responsible for an inefficient SP recognition by ER-translocation machinery, exposing PD-L1 ribosomal nascent chains to the activity of cytosolic enzymes, eventually dictating protein stability and function. These findings suggest that a less efficient kinetic of PD-L1 translocation into the ER could represent the key step for protein modifications and open interesting future perspectives for pharmacological applications based on PD-L1 SP targeting in cancer therapy.

REFERENCES

1. Cha JH, Hung MC, *et al.* (2018). *Mol Cell*, vol 71: 606-620 e607.
2. Chan LC, Hung MC, *et al.* (2019). *J Clin Invest*, vol 129: 3324-3338.
3. Robin MA, *et al.* (2002). *J Biol Chem*, vol 277: 40583-40593.

L17

Extracellular self-DNA inhibits microalgae growth: insights on self-inhibition in freshwater and marine environment

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ABSTRACT

The extracellular DNA (exDNA) is defined as the DNA outside the cell that can be actively or passively released[1]. It can be located in different natural habitats (e.g. soil, sediments, oceans and freshwater) as free molecule, complexed with inorganic and organic particles[2,3] or organized in extracellular structures[4-6].

The role of exDNA has been mainly investigated in soil, water column and in aquatic sediments, where nucleotides function as a nutrient[7], phosphate[8] and carbon[91] sources[8-10] or as building blocks for the synthesis of new molecules[11]. Recently, the exDNA has been shown to have an inhibitory effect on the growth of conspecific (self-exDNA), while the same was not evident for heterologous (nonself-) exDNA[12]. The inhibitory effect of self-exDNA was proposed as a universal phenomenon, although evidence is mainly reported for terrestrial species[13].

In this research, we show the inhibitory effect of self-exDNA on the growth of the microalgae *Chlamydomonas reinhardtii* and *Nannochloropsis gaditana*, a freshwater and a marine species, respectively. This study revealed the formation of peculiar heteromorphic structures embedded in an extracellular matrix after the treatment with self-exDNA. The differential effect of self and nonself-exDNA on both microalgae, accompanied by the inhibitory growth effect of self-exDNA are the first pieces of evidence provided about this phenomenon for species leaving in aquatic environments[14].

REFERENCES

1. Ceccherini MT, *et al.* (2009). *Int J Gen Mol Microbiol*, vol 96: 653–657.
2. Thakur BK, *et al.* (2014). *Cell Res*, vol 24: 766-769.
3. Malkin EZ, Bratman SV (2004). *Cell Death Dis*, vol 11: 584.
4. Brinkmann V, *et al.* (2009). *Appl. Soil Ecol*, vol 42: 176-181.
5. Ascher J, *et al.* (2009) *Appl. Soil Ecol*, vol 42: 176-181.
6. Hawes MC, *et al.* (2011). *Plant Sci*, vol 180: 741-745.
7. Levy-Booth DJ, *et al.* (2007). *Soil Biol. Biochem*, vol 39: 2977-2991.
8. Dell'Anno A, Danovaro R (2005). *Science* (80-.). 309, 2179 LP – 2179 (2005).
9. Lennon JT (2007). *Appl. Environ. Microbiol*, vol 73: 2799–2805.
10. Dell'Anno A, Corinaldesi C (2004). *Appl. Environ. Microbiol*, vol 70: 4384–4386.
11. Morrissey EM, *et al.* (2017). *ISME J*, vol 11: 1890-1899.
12. Mazzoleni S, *et al.* (2915). *New Phytol*, vol 205: 1195-1210.
13. Mazzoleni S, *et al.* (2015). *New Phytol*, 206, 127–132.
14. Palomba E, *et al.* (2022). *Plants* (Basel, Switzerland), vol 11.

L18

Integrative molecular analyses and first functional characterization of low-affinity nitrate transporters in diatoms, diNPFs

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ABSTRACT

Diatoms constitute one of the most important phytoplankton groups in the ocean, thanks to their adaptative capacity to face environmental variations, among which nutrient availability. To cope with them, diatoms own sophisticated mechanisms to sense and transport nutrients from the external environment and to re-allocate them inside the cell, still largely unknown[1,2]. In this study, we provide the first characterization of the Nitrate/Peptide Transporter Family (NPFs) in diatoms. NPFs are well characterized in many organisms where they recognize a remarkably broad range of diverse natural substrates, ranging from di- and tri-peptides in bacteria, fungi and mammals to nitrate and phytohormones in plants, but for diatoms their exploration is still in its infancy. Using a multilevel approach which integrated -omics, phylogenetic, structural and expression analyses, we revealed an evolutionary divergence into two distinct groups, with a different predicted sub-cellular localization suggesting functional diversification[3]. We generated overexpressing strains and CRISPR/Cas9 loss of function mutants in the model species *Phaeodactylum tricornutum*[4]. Preliminary characterization of the mutants suggests that the two different PtNPFs could be respectively required for intracellular nitrogen re-allocation and for regulation of internal pH homeostasis, providing clues to the mechanisms in which they are involved. These results once again confirm the variety of processes involving this conserved family of transporters and lay the foundation for future investigations into their role in other organisms.

REFERENCES

1. Smith SR and Allen AE (2022). *The Molecular Life of Diatoms*, pp. 529–566.
2. Rogato A, Amato A, Ludicone D, *et al.* (2015). *Marine Genomics*, vol 24: 95-108.
3. Santin A, Caputi L, Longo A, *et al.* (2021). *Open Biology*, vol 11(4): 200395.
4. Russo MT*, Santin A*, Rogato A, Ferrante MI (2022). *Marine Genomics - Methods in Molecular Biology*, vol 2498: 327-336.

L19

The microbial endopeptidase E40 efficiently detoxifies gluten proteins in complex food matrices: implications for oral treatment of gluten intolerance

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ABSTRACT

Gluten proteins are the causative agent of celiac disease (CeD), a life-long food intolerance characterized by a chronic enteropathy. Gluten degrading enzymes, which are commonly referred to as "glutenases", represent attractive candidates for the development of a pharmacological treatment of CeD[1]. Endoprotease-40 (E40), a novel glutenase secreted by the actinomycete *Actinoallomurus A8* and recombinantly produced in *S. lividans TK24*, was shown to be active at acidic condition (optimum pH 5), resistant to pepsin and trypsin degradation and able to destroy immunotoxicity of the most active gluten peptides, as demonstrated in functional assay with celiac intestinal T cells[2]. In this study, we functionally assessed the capabilities of E40 to detoxify residual gluten immunogenic peptides in gastrointestinal digesta of food matrices made of soft and durum wheat. The INFOGEST model of simulated human gastrointestinal digestion was applied for the quantitative assessment of residual gluten in liquid (beer) and solid (bread and pasta) foods after the addition of E40 at increasing enzyme:wheat proteins ratios. Proteomic and immunological techniques (R5-ELISA and celiac-intestinal T cell assay) were used to identify residual gluten-derived immunogenic peptides in gastric and gastrointestinal digesta. During the gastric phase (2 h incubation time), the addition of E40 demonstrated an extensive (≥ 95%) dose-dependent detoxification of whole gluten in real food matrices. Overall, the residual gluten content was found at, or even below, the 20 ppm (gluten-free threshold) for beer, bread and pasta. Furthermore, unlike in untreated gastrointestinal digesta, none of the immunodominant α-gliadin peptides survived in E40-treated digesta. Traces of ω- and γ-gliadin immunogenic peptides were still detected in E40-treated digesta, but unable to stimulate celiac-intestinal T cells. In conclusion, E40 is a promising candidate for the oral enzymatic therapy for CeD[3].

REFERENCES

1. Gianfrani C, *et al* (2021). *In Advances in Celiac Disease. Improving Paediatric and Adult Care. Springer, ISBN: 978-3-030-82401-3 171-191.*
2. Cavaletti L, *et al.* (2019). *Sci Rep*, vol 9: 13147.
3. Mamone G, *et al.* (2022). *Front Nutr*, vol 9: 974771.

L20
Molecular simulations to investigate protein structure and function

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ABSTRACT

Recent developments of bioinformatics tools for the study of protein structure culminated with the publication by AlphaFold of more than 200millions of 3D structures of proteins, claimed as 'The entire protein universe'[1]. This result offers a powerful resource for the investigation of protein structure and function relationships.

Molecular simulations have now the opportunity to exploit this resource for potential applications to any known protein, looking at stability, molecular interactions and other protein features object of interest. Moreover, the *in-silico* analysis of protein-ligand interaction by bioinformatics and molecular docking simulation tools is a great help in understanding the possible health effects on humans of small molecules. These methods can be used to understand the action of compounds from plants and from food, with two complementary study approaches. It is possible to search for molecules capable of interacting with a given protein, or in a reverse procedure one can search for the protein targets of a given molecule (reverse docking approach).

The research of potential protein targets could also be performed by high-throughput screening by similarity on databases with millions of sequence, and the characterization of the peculiar structural features of the selected targets could be performed by further bioinformatics and computational tools.

In recent years, we have carried out several studies with these approaches, as in work aimed to investigate the molecular dynamics behaviors of two enzymes of industrial interest, i.e., microbial transglutaminases by *S. mobaraensis* and *K. albida*[2], and in another one focused on the search for the potential plant receptors of volatile organic compounds[3]. In this presentation, some examples of our bioinformatics and molecular simulations applications to protein structure and molecular interactions will be presented.

REFERENCES

1. Callaway E (2022). *Nature*, vol 608(7921): 15-16.
2. Giordano D, Langini C, Caflisch A, *et al.* (2022). *Comput Struct Biotechnol J*, vol 20: 3924-3934.
3. Giordano D, Facchiano A, D'Auria S, Loreto F (2021). *Elife, vol* 23: 10:e66741.

L21

Defective corticogenesis in Arx mouse model of developmental and epileptic encephalopathy caused by polyalanine elongations

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ABSTRACT

Developmental and Epileptic Encephalopathy (DEE) is a pediatric epilepsy characterized by abundant epileptiform activity resistant to traditional anti-epileptic therapies. A severe form of DEE affecting male children is caused by expanded runs of GCG repeats in the X-chromosome gene Aristaless-related homeobox (*ARX*), which encodes a homeotic transcription factor with a key role in mammalian corticogenesis (1-3). To ascertain cellular diversity and disease mechanisms associated to polyalanine elongations in *ARX*, we conducted single cell RNA sequencing (scRNA-seq) in the epileptogenic neocortex of the *Arx* polyalanine mouse (*Arx*$^{(GCG)7/Y}$) (4). This is a DEE model that spontaneously develops severe tonic-clonic seizures in a phenotype that recapitulates the chronic epilepsy detected in *ARX* male patients (3). We detailed cell-specific gene expression patterns, unveiling how transcriptional changes in distinct cell subpopulations are associated with a lower proportion of neuronal precursors (NPs) and higher proportion of immature neurons (INs), suggesting a defective cell composition of the *Arx* polyalanine neocortex. *in vivo* BrdU pulse-chase and immunofluorescence studies revealed that neurogenesis and cortical organization are both altered in the mutant embryos. Enrichment analysis in NPs identified consistently altered pathways implicated in cell cycle, chromatin remodelling and RNA metabolism; whereas functions involved in neuronal structure and synapse organization were widely damaged in INs. Finally, immunocytochemistry and morphometric analysis highlighted a defective neurite arborization and hypoconnectivity in the *Arx*$^{(GCG)7/Y}$ cortical neurons. These results provide new insights into the cell types and neuronal functions perturbed in the *Arx* polyalanine neocortex disclosing a defective brain development that could potentially underlie the DEE pathogenesis.

REFERENCES

1. Poeta L, Fusco F, Drongitis D, *et al.* (2013). *Am. J. Hum. Genet,* vol 92: 114-125.
2. Poeta L, Padula A, …, Verrillo L, *et al.* (2019). *Hum. Mol. Genet,* vol 28: 4089-4102.
3. Drongitis D, Caterino M, Verrillo L, *et al.* (2022). *Hum. Mol. Genet,* vol 31: 1884-1908.
4. Verrillo L, Mangano E, Drongitis D, *et al.* (2020). *J. Neurosci. Methods*, vol 1: 347.

L22
Synthesis of BODIPY-based fluorescent analogue of the molecular adjuvant Sulfavant

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ABSTRACT

The sea represents a huge source of biologically active compounds characterized by an amazing structural variety and a great therapeutic potential[1]. During the ongoing exploration of the immunomodulatory marine natural products, recently our activity has been focused on the identification of novel chemical candidates able to stimulate dendritic cells (DCs), a specific type of antigen-presenting cells (APCs) that operate as master regulator for the adaptive immune response. Inspired by natural immunomodulant a-6'-sulfoquinovosyldiacylglycerols (a-SQDGs), synthetic sulfolipids named Sulfavants have been characterized as a novel class of molecular adjuvants able to trigger differentiation and maturation of human dendritic cells (hDCs)[2]. Crucial steps for the pharmacological development of this class of adjuvants are the elucidation of the biological mechanism and sub-cellular localization. For this purpose, in this communication, a feasible synthesis of a fluorescent analogue of the Sulfavants bearing the 4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene moiety (Me_4-BODIPY) is provided (**Figure 1**). The new synthetized molecule, has been used for live cell imaging experiments, opening the way for more in-depth studies on the interactions with cellular targets and on the biological behaviour of this class of compounds.

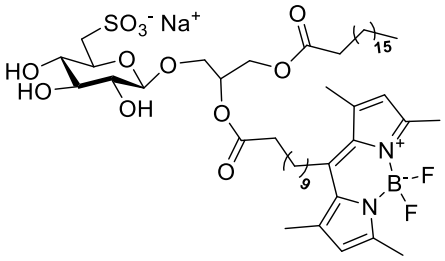

Figure 1: Fluorescent analogue of Sulfavant A.

REFERENCES

1. Thomford NE, Senthebane DA, Rowe A, *et al.* (2018). *Int J Mol Sci*, vol 19(6): 1578.
2. (a) Manzo E, Cutignano A, Pagano D, *et al.* (2017). *Sci Rep (Nature)*, vol 7: 1-10. (b) Gallo C., Manzo E, *et al.* (2022). *CMLS*, vol 79 (7), 369.

L23

Induction of autophagy promotes clearance of RHO^{P23H} aggregates and protects from retinal degeneration

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ABSTRACT

Autophagy is a critical metabolic process that acts as a major self-digestion and recycling pathway contributing to maintain cellular homeostasis. An emerging field of research supports the therapeutic modulation of autophagy for treating human neurodegenerative disorders, in which toxic aggregates are accumulated in neurons. Our previous study[1] identified Ezrin protein as an inhibitor of autophagy and lysosomal functions in the retina; thus, in turn, identifying it as a potential pharmacological target for increasing retinal cell clearance to treat inherited retinal dystrophies in which misfolded proteins have accumulated. This study aimed to verify the therapeutic inhibition of Ezrin to induce clearance of toxic aggregates in a mouse model for a dominant form of retinitis pigmentosa (i.e., RHO$^{P23H/+}$). We found that daily inhibition of Ezrin significantly decreased the accumulation of misfolded RHO^{P23H} aggregates. Remarkably, induction of autophagy, by a drug-mediated pulsatile inhibition of Ezrin, promoted the lysosomal clearance of disease-linked RHO^{P23H} aggregates. This was accompanied with a reduction of endoplasmic reticulum (ER)-stress, robust decrease of photoreceptors' cell death, amelioration in both retinal morphology and function culminating in a better preservation of vision. Our study opens new perspectives for a pulsatile pharmacological induction of autophagy as a mutation-independent therapy paving the way toward a more effective therapeutic strategy to treat these devastating retinal disorders due to an accumulation of intracellular toxic aggregates.

REFERENCES

1. Naso F, Intartaglia D, Falanga D, *et al.* (2020). *EMBO J*, vol 39: e102468.

L24
The intestinal immune response to transamidated gliadin in a mouse model of gluten sensitivity

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ABSTRACT

A life-long gluten free diet (GFD) is the only available therapy for people with Coeliac Disease (CD). However, the GFD is associated with important health, social and economic problems, influencing patient compliance. Therefore, research is now focused on identifying alternative strategies to the traditional GFD. Different studies have been performed in order to find a way to modify wheat gluten so that gluten epitopes are not recognised by the immune system. We have shown the ability of enzymatically transamidated gliadin to block the inflammatory phenotype induced by the native antigen in intestinal T cells isolated from CD patients[1]. The detoxification protocol of wheat flour produced transamidated water-soluble gliadins (spf) that lose their toxicity without influencing the main technological properties[2]. Very recently, we analysed the potential of spf to down-regulate the inflammatory response to wheat gliadin in the small intestine of HLA-DQ8 transgenic mice, a known model of CD[3]. We found that spf reduced the interferon-γ production of intestinal gliadin-specific T cells. This property was associated with the inability of transamidated gliadin to stimulate the pro-inflammatory pattern of innate immunity *in vitro*. Furthermore, spf appeared to modulate adaptive immunity by generating gliadin-specific T cells with a different immune phenotype. These data underscore the potential of transamidated gliadin to modulate the immunity triggered by native gliadin in the small intestine of sensitive mice.

REFERENCES

1. Gianfrani C, Siciliano RA, Facchiano AM, *et al.* (2007). *Gastroenterology*, vol 133: 780–789.
2. Mazzeo MF, Bonavita R, Maurano F, *et al.* (2013). *Biochi. Biophys Acta*, vol 183: 5166–5174.
3. Treppiccione L, Maurano F, Rossi S, *et al.* (2022). *Food Funct,* vol 13: 8941-8950.

P01
Analysis of the inhibitory effects of Self DNA in *Caenorhabditis elegans*

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ABSTRACT

All organisms, from bacteria to mammals, sense and respond to foreign nucleic acids to fight infections in order to survive and preserve genome integrity in all types of invaded cells.
As part of the immune response, specific molecular pathways are activated to sense both foreign DNA and damaged or aberrantly localised self-DNA inside the cell[1].
To avoid unwanted responses to regular physiological processes, recognition of endogenous nucleic acids is actively regulated by the cells and alteration of such mechanisms is associated with various diseases[2].
We investigated the effects of dietary delivered self-DNA in the nematode *Caenorhabditis elegans*. The hermaphrodite worms were fed on *Escherichia coli* genomic libraries: a *C. elegans* library (self) and a legume (*Medicago truncatula*) library (non-self). The presence of self-DNA in the food significantly decreases egg deposition, induced high embryo death, and negatively affected larval development. DNA damage and activation of p53/CEP-1-dependent apoptosis occur in gonadal germ cells[3].
The genetic tractability of *C. elegans* will help to identify the basic molecular pathways involved in such mechanisms.
The observed phenomenon suggests possible applications for the biocontrol of parasitic nematodes by appropriate delivery of their self-DNA in their growing environment.

REFERENCES

1. Hu MM, Shu HB (2020). *Annu Rev Immunol*, vol 38: 79-98.
2. Kawasaki T, Kawai T (2019). *Int Rev Cell Mol*, vol 344: 1-30.
3. Germoglio M, Adamo A, et al. (2022). *Biology*, vol 11(2): 262- 280.

P02
Investigation on the new immunomodulatory pathway triggered by Sulfavant A in the innate immune system cells

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ABSTRACT

In the last years we reported a novel immunomodulatory sulfolipid named Sulfavant A[1] able to prime maturation of dendritic cells (DCs) mediated by Triggering Receptor Expressed on Myeloid cells-2 (TREM2) and inducing a new homeostasis-inducing phenotype of DCs that we are naming HomeDCs (homeostatic dendritic cells)[2]. TREM2 is a transmembrane receptor expressed on macrophages, DCs, osteoclasts, and microglia. Previous evidence pointed out that TREM2 pathway could involve the transcriptional factor NFAT and exerts an anti-inflammatory activity by crosstalk with other biochemical pathways (e.g. Toll Like Receptor pathway). Anyway, the intracellular signal transduction machinery of this receptor remains to be fully elucidated.

In this project we started to investigate the effects on human monocyte-derived DCs treated with Sulfavant A in combination with an inflammatory stimulus (e.g. LPS) and evaluate the production of cytokines as immune mediators after the biochemical inhibition of NFAT activation.

To go deeply in the investigation of the immune regulatory signaling triggered by Sulfavant A, we then set up the characterization of the effect of this molecule on macrophages as a more representative cell model exposing TREM2 receptor.

REFERENCES

1. Manzo E, Cutignano A, Pagano D, *et al.* (2017). *Sci Rep*, vol 7: 6286.
2. Gallo C, Manzo E, Barra G, *et al.* (2022). *Cell Mol Life Sci*, vol 79(7): 369.

P03
Investigation of cytotoxic cembranoids from the Mediterranean gorgonian *Leptogorgia sarmentosa*

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ABSTRACT

Natural products (NPs) have always represented an enormous source of molecules with relevant potential in the pharmacological field. In the frame of the research project ADViSE[1], it was developed a platform of drug discovery of anticancer and immunomodulant Marine Natural Products (MNPs); between the most active organisms, the Mediterranean gorgonian species "*Leptogorgia sarmentosa*" was selected for its cytotoxicity. Fractions obtained by SPE methods[2] are tested in vitro bioassays both on immune cells and selected tumor cells, showing a great potential cytotoxic activity. To validate the biological response and identify the active components, the marine crude extract is purified on a silica gel column followed by HPLC. The isolated compounds were analyzed through NMR spectroscopy and Mass spectrometry; chemical investigation had led the identification of different classes of cembranoids. Previous studies have already showed the presence of these compounds in other gorgonian species, however only sterols[3] have been reported in literature from the species "*Leptogorgia sarmentosa*" until now. The study of isolation and characterization of these members of the cembranoids family will be presented.

REFERENCES

1. "Antitumor Drugs and Vaccines from the Sea (ADViSE)" project funded by POR Campania FESR 2014-2020 "Technology Platform for Therapeutic Strategies against Cancer".
2. Cutignano A, Nuzzo G, Ianora A, *et al.* (2015). *Mar. Drugs*, vol 13.: 5736-5749.
3. Cimino G, De Rosa S, De Stefano S, *et al.* (1984). *Experientia*, vol 40: 246-248.

P04

Modulatory effects of cholinergic signalling on insect immunity: functional study in *Drosophila melanogaster*

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ABSTRACT

The cholinergic system, traditionally associated with synaptic neurotransmission in animals, is not restricted to neurons, but is also found in non-neuronal tissues, where it plays important roles in the maintenance of physiological homeostasis. In humans, acetylcholine is involved in the regulation of immune functions, but information about its immunomodulatory potential in invertebrates is still limited. The finding that neonicotinoids, neurotoxic insecticides targeting insect nicotinic acetylcholine receptors (nAChRs), impair the immune response in *Drosophila melanogaster* and *Apis mellifera* provided a first hint that acetylcholine signaling may control insect immune pathways. To investigate this issue, we used a Gal4-UAS expression system to alter nAChR function in Drosophila immune tissues and tested if and how these manipulations affected fly survival to pathogen infection. The obtained results support the occurrence of acetylcholine-based immune regulation in *Drosophila*, whose impact on the fly ability to fight pathogen infections may vary depending on the specific infectious agent.

P05

An innovative biosensor for monitoring the presence of volatile organic compounds (VOCs) in food

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ABSTRACT

The demand for a wide choice of food that is safe, palatable, and of consistent quality is increasing every day. Consumers will not accept *off-flavors* that are atypical odors resulting from internal deterioration or contamination by a substance alien to the food. Odor response depends on the *volatile organic compounds* (VOCs), a broad group of chemicals that evaporate easily at room temperature. Therefore, their detection can provide information about food quality. The combination of gas chromatography and mass spectrometry (GC-MS) is the most powerful tool available for VOC detection, but it is laborious, costly, and unfit for everyone's use. Intending to develop a new, faster, and easy-to-use tool, we have designed a *non-Faradaic* impedimetric biosensor for monitoring VOCs involved in food spoilage, using the *pig odorant binding protein* (pOBP) as a molecular recognition element. *1-octen-3-ol*, *trans-2-hexen-1-ol*, and *hexanal* were selected as target VOCs. The affinity of the pOBP for these compounds was evaluated both by direct docking simulations experiments and competitive Fluorescence Resonance Energy Transfer (FRET) assays by using *1-aminoanthracene* (1-AMA) as a fluorescence probe. The impedimetric measurements were conducted both in a liquid solution and in the air. The impedance changes registered at 0.1 Hz allowed us to detect the analytes in the range of 0.1–10 µM (LOD of 0.1 µM in solution). We observed a similar behavior when the biosensor was exposed to air contaminated with the target odors. The collected data will be used to train neural networks, which will help us to discriminate the presence of different odorant compounds.

REFERENCES

1. Capo A, *et al.* (2022). *Int J Mol Sci,* vol 23: 4039.
2. Vincent F, *et al.* (2000). *J Mol Biol*, vol 300(1): 127-39.
3. Lakowicz JR (1999). Principles of fluorescence spectroscopy. 2nd ed. Kluwer Academic/Plenum: New York.
4. Randviira EP, Banks CE (2013). *Anal Methods,* vol 5: 1098-1115.

P06
Role of GADD45β following TNF-R triggering and its therapeutic implications

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ABSTRACT

GADD45β is an NF-κB-induced pro-survival factor capable of blocking TNFα-elicited JNK activity and cell death (CD) in p65 deficient cells[1]. Its high expression has been correlated with poor clinical outcomes in most human cancers, consolidating the general clinical significance of the GADD45β-mediated oncogenic mechanism in malignant disease[2]. A recently developed D-tripeptide (DTP3) inhibitor counteracts the apoptosis-inhibiting activity of the GADD45β / MKK7 interaction and exhibits a potent selective ability of cancer cells to induce JNK-dependent apoptosis of multiple myeloma without apparent toxicity for normal cells[3]. Although GADD45β is able to suppress caspase 8 activity, the molecular events that mediate this inhibition are still unclear. Here we investigate the role of GADD45β in the regulation of apoptotic and necroptotic cell death complexes. Necroptosis is a programmed cell death pathway that is activated by the necrosome complex, which includes protein kinases that interact with receptors such as RIPK1 and RIPK3. We examined the effect of GADD45β expression on the formation of these complexes on stimulation in an established cell system in which caspase activation and the onset of necroptosis are clearly followed[4]. Mechanically, we found a direct interaction between GADD45β and RIPK3, surprisingly this interaction affects both the execution of MLKL-dependent cell death and RIPK3-dependent NF-κB activation. The use of a RIPK3 kinase mutant (D160N) and a deleted RIPK3 mutant from the RHIM region (ΔRHIM) showed a GADD45β-RIPK3 interaction RHIM independent and a suppression of NF-κB RIPK3 independent kinase. Delineating the mechanism of apoptotic or necroptotic CD cancer is noteworthy for cancer therapy as the necroptotic program involves the coordination of cell death with extended cytokine synthesis.

REFERENCES

1. De Smaele E, Zazzeroni F, *et al.* (2001). *Nature*, vol 414: 308-313.
2. Verzella D, Bennett J, *et al.* (2018). *Cancer Research*, vol 78: 1275-1292.
3. Tornatore L, Sandomenico A, *et al.* (2014). *Cancer Cell*, vol 26: 495-508.
4. Pescatore A, Casale C, *et al.* (2021). *Methods in Molecular Biology*, vol. 2366.

P07
Hydrogenotrophic metabolisms in the subsurface: ecological and biotechnological applications.

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ABSTRACT

Hydrogen is a fundamental electron donor in diverse microbial metabolisms and it is considered the energetic currency exchanged within microbial communities in anaerobic environments. Hydrogen is also considered one of the keys to a greener energetic society. Excess energy produced from renewable sources can be converted into hydrogen for storage and subsequent use. An important technology which is under development involves subsurface storage of hydrogen (known as underground hydrogen storage, UHS) in a stable terrestrial reservoir for later use. If viable, UHS developments could enable this imminent energy transition.

However, when hydrogen is artificially stored in the subsurface, the extensive microbial ecosystem can interact with it, potentially reducing the production capacity of the storage site or converting the H to H_2S, FeS and other metabolic byproducts that can compromise the viability of storage. In order to safely store hydrogen underground it is thus necessary to investigate the diversity and distribution of natural hydrogenotrophic metabolisms in the subsurface. Given the high cost of drilling boreholes and/or accessing deep mines, one solution to characterize microbial communities in the subsurface is to use deeply-sourced seeps and hot springs, which carry fluids and gasses from depth to the surface and generate hot spots of microbial diversity.

The key enzymes controlling hydrogen utilization in microorganisms are called Hydrogenases, and are found in all domains of life, catalysing the oxidation of molecular hydrogen to protons and electrons. These diverse enzymes use tangled organometal complexes built around a binuclear Ni-Fe, Fe-Fe or Fe center, with bound CO and CN(-) groups, as well as multiple FeS centers. This work uses metagenomic approaches to investigate the diversity of Hydrogenases in samples from different types of deeply-sourced seeps. Our results will help to establish the baseline of hydrogenotrophic metabolisms in the subsurface, aiding in the development of subterranean hydrogen storage technology.

P08

Use of halotolerant plant growth-promoting bacteria as a safety strategy to counter the global increase in land salinity

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ABSTRACT

Halotolerant (HT) bacteria are a group of microorganisms able to thrive in environments with relatively high salt concentrations. Recently, HT microorganisms with plant growth-promoting (PGP) characteristics have been proposed to increase plant tolerance in salty soil[1]. To this aim, we evaluated the PGP properties at increasing NaCl concentrations of HT-Bacillus strains, previously shown to have beneficial effects under physiological conditions[2,3]. Most of the isolated showed indole acetic acid and ammonia production and were able to solubilize phosphate and suppress the proliferation of the phytopathogenic fungus *Macrophomina phaseolina* 2013-1 at high salt concentrations. One of the selected strains, *Bacillus amyloliquefaciens* RHF6, which retained its beneficial properties up to 400mM NaCl in vitro, was tested on the legume model plant *Lotus japonicus cv Gifu* under salt stress. The inoculation with RHF6 significantly improved the survival of plants under high salinity conditions compared with non-inoculated plants. Furthermore, the ability of RHF6 to induce a plant antioxidant response, secrete the osmoprotectant proline and reduce ethylene level via the enzymatic ACC deaminase activity indicated this strain as a potentially helpful PGPB for the treatment of degraded soils.

Furthermore, current studies on RHF6 in consortium with two other HT-Bacillus have confirmed the PGP proprieties also in the *Lactuca sativa L.* plant stressed by salinity. These results sustain that using HT-PGPB individually or in a consortium is a valid fertilization strategy in the agro-industrial field.

REFERENCES

1. Ullah A, *et al.* (2021). *Front Sustain Food Syst*, vol 5: 618092.
2. Castaldi S, *et al.* (2021). *Int J Mol Sci*, vol 24: 22-3324.
3. Castaldi S, Petrillo C, *et al.* (2021). *Front Microbiol*, vol 13: 12-715678.

P09
Applications of bioinformatics methodologies in the study of lipoxygenases from diatoms

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ABSTRACT

Diatoms are organisms living in any aquatic ecosystems and, being at the base of the trophic chain, play a very important ecological role. In diatoms, as in many plants, animals, cyanobacteria, and some fungi, a wide variety of secondary metabolites are produced, including those called oxylipins, as a consequence of abiotic or biotic stress. These molecules, derived from the oxidation of poly-unsaturated fatty acids (PUFA), influence not only the growth of phytoplankton, as the same diatoms, but also the growth of numerous organisms that make up zooplankton.

This work was born from the hypothesis that, as on a world scale, also along the Gulf of Naples coasts, diatoms are the main producers of oxylipins and that this production has a strong impact on marine flora and fauna.

In this project we focused on the research of Lipoxygenase (Lox) in diatoms using bioinformatics tools and database analysis, starting from the only protein sequences annotated as Lox in two species of diatoms, i.e, *Pseudo-Nitzschia Arenysensis* and *Fragilariopsis Cylindrus*.

Firstly, the sequence differences between the two enzymes have been investigated, in particular by studying the cofactor interaction sites typical of Lox (three His, one Asn and one Ile, coordination residues of Fe^{2+}). This has allowed focusing the attention on a probable insertion between two coordination residues, found on the sequence of Lox by *F. Cylindrus*.

Moreover, by querying different database resources, it was also possible to identify transcripts attributable to Lox in other species of diatoms, too. Subsequently, using the identified sequences, a phylogenetic analysis was developed which highlighted the presence of two groups of Lox diverging from each other.

Finally, the 3D structure of Lox enzymes from *P. arenysensis* and *F. Cylindrus* were modelled, evaluated, and compared. This structural level of the study has revealed that, despite their very low percentage of sequence similarity, the coordination sites of Fe^{2+} is not affected by variation, preserving the correct orientation of the five coordination residues necessary for the enzyme correct function.

P10

Unravelling the clinical significance and therapeutic management of unknown GANAB (glucosidase II subunit a) variations in polycystic kidney disease

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ABSTRACT

Polycystic kidney disease (PKD) is due to mutations in several distinct genes; among these, GANAB, if mutated, causes liver and kidney polycystic disease. GANAB encodes the a-subunit of Glucosidase II (GIIa) localized in the endoplasmic reticulum in which triggers quality-control assessment of glycoprotein folding, including Polycystin1 and 2 (PC1) (PC2), the two proteins mainly involved in kidney proper functioning and then in PKD. We identified a novel GANAB variant, c.2087G>A (p.R696Q), in an Italian patient. This variant is located in GIIa active site and then predicted to affect enzyme activity and/or folding of the protein. In this study, we aim: - to confirm the pathogenicity of the new variant; - to understand the molecular mechanisms; - to identify pharmacological inhibitors able of recovering GIIa function. Our plan is to express both wild-type and c.2087G>A cDNA in a mammalian plasmid, to test enzymatic activity and thermal stabilization of mutant cDNA compared to the wild type. Successively, we will investigate the impact of the c.2087G>A on PC1 and PC2 maturation, localization and functioning, using WB, glycosylation analysis, immunoprecipitation and confocal microscopy. Furthermore, we plan to perform the above-described tests in presence of sub-inhibitory concentrations of three potent GIIa inhibitors, to test their capacity of recovering GIIa function. The identification of both molecular pathogenic nature of disease as well as of novel pharmacological drugs may have important implications in the clinical practice leading to a more effective and specific therapeutic approach based on specific genotype in GANAB mutated patient.

REFERENCES

1. Waldrop E, Al-Obaide MAI, Vasylyeva TL. (2019). *Front Genet*, vol 10: 44.
2. Delbarba E, Econimo L, Dordoni C, *et al.* (2022). *J Nephrol*, vol 35(2): 645-652.
3. Porath B, Gainullin VG, Cornec-Le Gall E, *et al.* (2016). *Am J Hum Genet*, vol 98(6): 1193-1207.

P11

Hydra vulgaris and *Nematostella vectensis* as model organisms for *in vivo* study of new synthetic drugs

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ABSTRACT

Alzheimer's (AD) and Parkinson's disease (PD) are among the most studied neurodegenerative illnesses. The conventional treatments offer relatively little symptomatic benefit, consequently the research and development of more powerful and effective pharmacological tools are of daily interest. Arundic acid is a synthetic derivative of valproic acid, both are potential drugs for the treatment of AD and PD[1]. Some studies have shown that astrocytes are the primary target of these two agents[2,3]; however, the mechanism by which they carry out their glioprotective action as well as their toxicological profile is not fully defined. In this work, valproic and arundic acids have been tested in two animal models: *Hydra vulgaris* and *Nematostella vectensis*. These small polyps are considered excellent model organisms for both in vivo and in vitro studies, in fact they are equipped with a simple anatomical structure and a simple nervous system allowing functional studies, from toxicological to behavioral studies. With the aim to identify the arundic and valproic acids toxicological profiles and the mechanism of action, we investigated the impact of these compounds on morpho-functional response and gene expression. The data here reported are the starting point to devising new pharmaceutical forms (i.e. nanovehiculation) that improve administration, compliance and reduce side effects[4].

REFERENCES

1. Johannessen Landmark C. (2008). *CNS Drugs*, (https://doi.org/10.2165/00023210-200822010-00003)
2. Chen PS, Peng GS, Li G, Yang S, *et al.* (2006). *Mol Psychiatry*, (https://doi.org/10.1038/sj.mp.4001893)
3. Asano T, Mori T, Shimoda T, *et al.* (2005). *Curr Drug Targets CNS Neurol Disord*, vol 4: 127-142.
4. Ambrosone A, Matteis L, Serrano-Sevilla I, *et al.* (2020). *ACS Biomater Sci Eng*, https://doi.org/10.1021/acsbiomaterials.9b01820

P12
Development of Peptides/Peptidomimetics for Targeted Diagnosis, Therapy and Drug Delivery

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ABSTRACT

Active targeting based on the ligand-target affinity is a common strategy to enhance the uptake of biosystems into specific tissues, cells and/or their microenvironment.
Peptides/peptidomimetics are widely employed as targeting ligands for their several advantages including the low immunogenicity, the ease of synthesis and chemical modification, and the production costs as well as the capability to recognize selectively specific diseases markers.
Over the last decades, we mainly focused on rational design, chemical synthesis, in vitro and in vivo characterization of peptides/peptidomimetics[1-7] for:

i) targeting of integrin receptors sub-types for theranostic application in cancer field;
ii) drug delivery of nanoparticles and nanoheterostructures by integrin selective targeting;
iii) mimicking NGF activity retaining some neurotrophic activities;
iv) targeting of FSH receptor for tumour and diagnosis therapy.

REFERENCES

1. Acconcia C, Paladino A, della Valle M, *et al.* (2022). *Int J Mol Sci*, vol 23: 11039.
2. Comegna D, Zannetti A, Del Gatto A, et al. (2017). *J Med Chem*, vol 60: 9874-9884.
3. Hill BS, Sarnella A, Capasso D, *et al.* (2019). *Cancers*, vol 11: 139.
4. Bolzati, C, Salvarese, N, *et al.* (2018). *J Med Chem*, vol 61: 9596.
5. Capasso D, Del Gatto A, Comegna D, *et al.* (2020). *Molecules*, vol 25: 4298.
6. Siciliano G, Corricelli M, Iacobazzi RM, *et al.* (2020). *Chemistry- A European Journal*, vol 26, 11048.
7. Comegna D, Del Gatto A, Saviano M, Zaccaro L (2019). *Organic Lett*, vol 21: 4454.

P13

Insights into the mechanisms of alternative macrophage polarization to circumvent cancer immunotherapy resistance

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ABSTRACT

Tumor-associated-macrophages (TAMs) share many characteristics with alternatively activated macrophages, also called M2. TAMs exert tissue repair and tumor-growth promotion activities and are responsible for tumor-tolerance and resistance to immunotherapy (IT). FKBP51 immunophilin is abundantly expressed by immune cells. The splicing isoform of the *FKBP5* gene, FKBP51s, is generated by alternative splicing and was found to be exploited by cancer cells to suppress undesired immunity and highly expressed in circulating monocytes of cancer patients resistant to IT. Aim of this study is to identify new potential therapeutic targets to reprogram TAMs. Macrophage polarization was assessed by qPCR, immunoblot, and flow cytometry both in a THP-1 cell line and in primary monocytes to obtain classically activated M1-macrophages, alternatively polarized M2-macrophages, and the deactivated M1D-macrophages. The Boyden chamber method was used to address invasive properties. Small interfering RNAs were transfected to specifically target gene expression. Alternative macrophage polarization showed typical features of M2-macrophages, such as STAT1 activities downregulation in favour of STAT3/6, and a shift towards arginase1 metabolism and scavenger receptor expression. Interestingly, FKBP5s levels strongly increased in M1D and M2-macrophages, thus suggesting the activation of the *FKBP5* alternative splicing in TAMs. The Boyden-chamber assay showed that M2-macrophages exert an increased ability in migrating/invading the extracellular matrix. Finally, the specific silencing of FKBP51s expression restored STAT1 activity to the detriment of active STAT3/6 levels. Overall, these findings suggest that during alternative macrophage polarization the *FKBP5* gene undergoes alternative splicing and that targeting FKBP51s may be a means to reprogram macrophages and restore IT-response.

P14
Screening of a tomato collection under recurrent drought stress

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ABSTRACT

Tomato is an annual herbaceous plant, native to Central America, South America and the southern part of North America. Because of its high thermal requirements, being very sensitive to frost, it predisposes to cultivation characterized by warm-temperate climates, finding its growing season in the summer period. What is of greatest concern is the water scarcity that has been occurring in recent years, especially during the summer season, which is characterized by long periods of drought that would affect the cultivation of the tomato plant, as it requires a high amount of water, accounting for 90-95 % of the tomato. Here, we performed a recurrent stress trial on 15 tomato genotypes using the HTP plant phenotyping platform at ALSIA. Drought stress was applied through a 70% reduction in irrigation water in two stress cycles followed by recovery stages. To evaluate the stress, we analyse the proline content; after the first drought cycle stressed plants showed 5-6 times higher amounts than that of the controls in all tested genotypes, while after the recovery and the second stress cycle the proline levels were comparable in the two conditions. On selected genotypes we analyse the expression of genes implicated in biosynthesis (*P5CS*) and catabolism (*P5CDH*) of proline. The gene expression results confirmed biochemical analysis, in particular after the second stress cycle the two genes showed the same expression. Changes in gene expression of stress related genes are in progress and will contribute to the identification of differential responses among genotypes.

P15

Insights into the interaction between the p60 subunit of chromatin assembly factor-1 and the histone chaperone anti silencing factor-1

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ABSTRACT

The proper assembly of newly synthesized and repaired DNA into chromatin is fundamental for maintaining genomic stability. Nucleosomes, which represent the repeating unit of chromatin, are made of four histones (H2A, H2B, H3 and H4) wrapped around with DNA[1]. The transport and deposition of H3/H4 histone dimers in human cells involve four different histone chaperones: Chromatin Assembly factor 1 (CAF-1), Histone cell cycle regulator (HIRA) and two paralogs Anti-Silencing Factors (ASF-1a and ASF-1b)[2].

It has been demonstrated that ASF-1 works in synergism with CAF-1 during nucleosome deposition and the interaction between the two chaperones is mediated by one of the three subunits of CAF-1, namely p60[2].

In details the association with ASF-1 occurs via the C-terminal domain of p60, involving two specific sequences known as "HIRA B-domain like motifs"[2].

Here we studied the interaction between different peptides of different length which encompass the B-domain like motifs of p60, and the N-terminal domain of ASF-1.

We confirmed the in vitro binding of the molecules and determined the dissociation constant (Kd) of the interaction by means of several techniques, such as Enzyme Linked Immunosorbent Assay (ELISA) and Microscale Thermoforesis (MST). Our data showed a Kd in the micromolar range, thus confirming the hypothesis of a weak binding between ASF-1 and CAF-1, which likely favors a transient contact and a rapid histone transfer between the two chaperones. Currently, the X-ray crystal structure of ASF-1 in complex with both the B-domain like motifs of p60 is underway, in order to elucidate the structural and molecular determinants responsible of the interaction.

Together, our data provide new information on the biological and structural principles that guide the assembly and activity of fundamental multi-protein chaperone complexes, leading to innovative prospects for the understanding of their physiological function and their usage as diagnostic, prognostic and eventually therapeutic targets.

REFERENCES

1. Kornberg RD (1977). *Annu Rev Biochem*, vol 46: 931-54.
2. Tang Y, Poustovoitov MV, Zhao K, *et al.* (2006). *Nat Struct Mol Biol*, vol 13(10): 921-9.
3. Mello JA, Silljé HH, Roche DM, *et al.* (2002). *EMBO Rep*, vol 3(4): 329-34.

P16
Comprehensive structural analyses of protein families by AlphaFold predictions: the case of KCTDs

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ABSTRACT

The occurrence of an intimate relationship between the structure and the function of proteins is the paradigmatic assumption of structural biology. Indeed, a full understanding of protein activities cannot be achieved without a profound knowledge of their structural and dynamic properties. The development and the release of effective predictive methods, based on machine learning techniques such as those implemented in the AlphaFold algorithm[1], is opening new and previously unforeseen scenarios in structural biology. Particularly appealing is the perspective that, in the AlphaFold era, comprehensive approaches aimed at unraveling structural similarities/dissimilarities among all members of a protein family have become feasible at a global level.

We are currently applying this approach to the KCTD (proteins containing a (K)Potassium Channel Tetramerization Domain) family[2], a class of proteins involved in an uncountable number of key physio-pathological pathways[3-7]. Over the years, the lack of structural data has negatively impacted the understanding of the activities of these proteins at atomic level. By exploiting AlphaFold predicted three-dimensional structures of single polypeptide chains of KCTDs, we have recently unraveled previously undetected molecular relationships among the C-terminal-domains of the members of the family[8]. More recently, by exploiting recent functionalities in AF predicting tools, we report an exhaustive prediction of the structure of KCTD functional oligomers[9] (**Figure 1**). Collectively these findings shed a new and global light into the structural properties of these proteins paving the way for structure to function analyses.

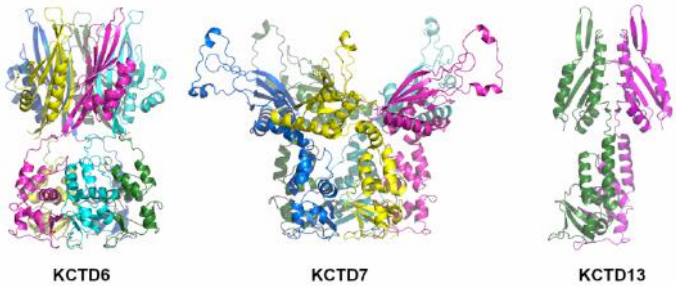

Figure 1. Important KCTDs with previously uncharacterized 3D structures.

REFERENCES

1. Jumper J, Evans R, Pritzel A, *et al.* (2021). *Nature*, vol 596: 583-589.
2. Liu Z, Xiang Y, Sun G (2013). *Cell Biosci*., vol 3: 45.
3. Pirone L, Smaldone G, Spinelli R, *et al.* (2019). *Biochim. Biophys. Acta Mol. Cell Biol. Lipids*, vol 1864: 158514.
4. Brockmann M, Blomen VA, Nieuwenhuis J, *et al.* (2017). *Nature*, vol 546: 307-311.
5. Escamilla CO, Filonova I, Walker AK, *et al.* (2017). *Nature*, vol 551: 227-231.
6. Canettieri G, Di Marcotullio L, Greco A, *et al.* (2010). *Nat. Cell. Biol.*, vol 12: 132-142.
7. Schwenk J, Metz M, Zolles G, *et al.* (2010). *Nature*, vol 465: 231-235.
8. Esposito L, Balasco N, Smaldone G, *et al.* (2021). *Biomolecules*, vol 11: 1862.
9. Esposito L, Balasco N, Vitagliano L (2022). *submitted* – see also the website http://alphafold.ibb.cnr.it

P17

Common and rare variants in TMEM175 gene concur to the pathogenesis of Parkinson's disease in Italian patients

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ABSTRACT

Parkinson's disease (PD) represents the most common neurodegenerative movement disorder. We recently identified 16 novel genes associated with PD[1].

In this study, we focused the attention on the common and rare variants identified in the lysosomal K+ channel *TMEM175*. Our findings demonstrated that *TMEM175* is highly expressed in dopaminergic neurons of the substantia nigra pars compacta and in microglia of the cerebral cortex in the human brain[2].

Clinical, genetic, and molecular analysis, performed on 400 patients and 300 healthy controls allowed to identify 4 variants associated with PD, including two novel variants, located in the Kozak consensus sequence and in the TM3II domain, respectively. We also disclosed 14 novel highly penetrant mutations. Importantly, our findings also suggested that detrimental mutations in the *TMEM175* gene may be sufficient to cause the disease in about 6% of Italian patients, and the presence of polygenic mutations correlated with an earlier disease onset[2].

In vitro functional analyses of the mutant channels revealed a loss of the K+ conductance and a reduced binding affinity for AKT protein. Moreover, we observed an impaired autophagic-lysosomal flux and the activation of UPR markers in patient-derived fibroblasts[2].

Our results, highlight the importance of studying common and rare variants of PD genes to obtain a comprehensive genetic contribution of the disease[2].

In this frame, to further investigate the impact of multiple mutations on PD pathogenesis, in dopaminergic background, we generated hiPS cells of PD patients and healthy subjects carrying the most promising combination of mutations. The analysis is ongoing.

REFERENCES

1. Gialluisi A, *et al.* (2021). *Mol Neurodegener*, vol 16(1): 35.
2. Fortunato G, Palomba NP, *et al.* (2022). *Molecular Neurobiology*, submitted

P18

24 weeks of training programs improve cardiometabolic health, body composition and adiponectin values in young obese male

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ABSTRACT

Obesity and related diseases represent a public health problem. The main factors predisposing to obesity are unhealthy lifestyle including the lack of physical activity (PA). A central role in studies on the pathogenesis of obesity is represented by adipose tissue as an endocrine organ secreting adipokines involved in numerous metabolic processes. Of particular importance is adiponectin, an adipokine involved in the regulation of insulin resistance and anti-inflammatory processes. The aim of the present study was to determine the effects of 24 weeks of two different training programs, POL and THR modified by Veronique Billat the first with a moderate volume intensity, the second with higher intensity, on body composition, physical capacities and adiponectin values in serum and saliva from male adult obese subjects at T0 (before starting) and at T1 (at the end of the program).

Although the results obtained did not show significant differences between the two training programs, we observed an increase in serum and salivary adiponectin concentration at T1 compared to T0 in all obese subjects. Such increase correlated to an improvement in anthropometric (Fat Free Mass, Fat Mass. BMI, BM) and cardiometabolic parameters (VO2max, HRmax). Our results suggest that 24 weeks of training program, independently from intensity and volume, are effective in ameliorating body composition and fitness performance. This improvement is strictly related to an increase expression in both serum and salivary adiponectin. Further studies are needed to deepen the relationship between the characteristics of training programs and the outcomes in terms of body composition and adipose tissue endocrine activity.

REFERENCES

1. Clark JE, (2016). *Biol Sport*, vol 33: 309-333.
2. Molinari, *et al.* (2020). *J Environ Res Public Health*, vol 17(24): 9250.
3. Nigro E, Scudiero O, Monaco ML, *et al.* (2014). *Biomed Res Int*, vol 2014: 658913.

P19
Antioxidant, heavy metal chelating and antihypertensive activities in V range legume products

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ABSTRACT

The legume consumption is constantly growing because this food represents a rich and sustainable source of plant proteins and other compounds, which are considered effective in the prevention of some chronic diseases, including those age-related. Therefore, there is a growing demand of details about their functional properties. With the aim to characterize the functional properties of some legume foodstuffs, the flours from industrial cooking processes of eight legumes were investigated. The compounds contained in the flour samples were extracted using two different protocols and the antioxidant, metal chelation and ACE-inhibition activity were analysed using specific spectrophotometric assays. Experimental results were interpreted using as a reference also compounds which are prescribed as drugs in medicine, namely EDTA and captopril. Lentils and borlotti bean displayed much higher antioxidant and metal chelation activity. Conversely, lentils were the less rich in ACE-inhibition activity, whereas beans, in particular borlotti, had the highest amount of this activity. In conclusion, the analysed legume flours contain good and variable amounts of functional activities. These data could contribute to formulate personalized diets to address the needs of different subjects and could attract the food industry interested in the development of food products with known amounts of health-promoting activities.

P20
Physiological responses of contrasting Tunisian durum wheat genotypes to drought stress

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ABSTRACT

Tunisia is highly vulnerable country to climate change. This means that the increased aridity and rainfall dependency will severely threaten agriculture in Tunisia, especially wheat yields and eventually food security[1]. Therefore, screening wheat genotypes that produce high and stable yield with less water becomes crucial under actual dry conditions. In this study, we aim to compare the dynamics of elastic and osmotic properties of leaf tissues of four Tunisian wheat cultivars.

We observed that in drought-susceptible genotype (Mahmoudi AG3), drought stress (DS) induces reduction of apoplasmic water content, lower osmotic adjustment with high level of lipid peroxidation, leading to great yield loss. On the contrary, in drought-tolerant genotypes (D117 and Syndiouk), physiological activities were maintained through improved fraction of apoplasmic water content (AWC), which induces osmotic adjustment due to the accumulation of osmotically active compounds (soluble sugars and proline). The redistribution of water outside the cell walls allows plant to increase the concentration of solutes, resulting in greater tolerance to water deficit [2]. Moreover, DS remarkably alter the cell-wall elasticity by increasing the modulus of elasticity (εmax)[3]. The osmotic and elastic adjustment allow greater membrane stability in tolerant genotypes with lower level of lipid peroxidation compared to susceptible ones.

These results will help future studies dealing with drought adaptive mechanism to predict wheat tolerance in dryland ecosystems. Based on those features, the genotypes D117 and SYN can be used as a breeding material to develop drought resistant wheat genotypes.

REFERENCES

1. Ayed S, Mlouhi S, Bouhaouel I (2021). Plants, vol 10(11): 2379.
2. Silva H, Valenzuela C, Garrido M, *et al.* (2021). *Irrig Sci, vol* 39: 789-801.
3. Al-Yasi H, Attia H, Alamer K, *et al.* (2020). *Plant Physiol Biochem*, vol 150: 133-139.

P21
Selective targeting of DNA Methylation through nucleic acid aptamers for non-small cell lung cancer therapy

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ABSTRACT

Lung cancer is the leading cause of cancer death worldwide, and about 80% of all lung cancers are non-small cell lung cancer (NSCLC). NSCLC is often associated with resistance to conventional therapy relapses and poor prognosis[1]. Thus, the development of innovative and effective therapeutics has become a fundamental challenge. Among new therapeutic targets, DNA methylation is a key epigenetic mechanism regulating gene expression. Several studies have established an important link between aberrant DNA methylation and different cancers including NSCLC, indicating that its inhibition can restore normal gene function and revert malignant cell transformation[2]. However, medical intervention has been limited to toxic, unspecific demethylating drugs[3]. To overcome these limits, we addressed DNA methylation targeting by exploring nucleic acid aptamers, promising ligands with high affinity and specificity[4]. We developed nuclease-resistant RNA aptamers (named aptaDiRs) able to bind and neutralize the major epigenetic player DNA methyltransferase (DNMT) 1, showing the potential to reprogram aberrant DNA methylation in cancer[5]. Here we aimed to demonstrate the therapeutic efficacy of these aptamers for NSCLC selective therapy. To this end, we have characterized the functional effects of one of the best aptaDiRs on different NSCLC cells, demonstrating its ability to impair cell viability and migration. The analysis of the effects on DNA methylation and gene expression are currently ongoing. Collectively, our results provides a first study of efficacy of a new RNA-based epigenetic therapy for NSCLC treatment.

REFERENCES

1. Economopoulou P, Mountzios G (2018). *Ann Transl Med*, vol 6(8): 138.
2. Feng S, De Carvalho DD (2021). *FEBS J*.
3. Yin Y, Morgunova E, Jolma A, *et al.* (2017). *Science*, vol 356(6337).
4. Rotoli D, Santana-Viera L, Ibba ML, *et al.* (2020). *Int J Mol Sci*, vol 21(17): 6075.
5. Esposito CL, Autiero I, Sandomenico A, *et al. Under review in Nat Comm*

P22
New photodynamic therapy approaches tested in *Hydra vulgaris*

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ABSTRACT

In the last years, photodynamic therapy (PDT) has been widely used for clinical treatment of several types of cancer (brain, lungs, etc.) since its minimal invasiveness. In PDT, a compound with photosensitizing properties (photosensitizer, PS) is accumulated in cells and activated by light in the presence of oxygen. The activated PS generates reactive oxygen species (ROS) that are cytotoxic and can cause the death of neoplastic cells. Recent studies reported the use of proteins (human serum albumin, HSA)[1] or phages (M13 bacteriophage)[2,3] as innovative carriers for several photosensitizers (rose bengal, chlorin e6, oligothiophene ECB04), showing the possibility to induce cell death by apoptosis in cancer cells. To overcome the intrinsic limitations of *in vitro* models (lack of a behavioral and animal physiology response) and mammalian models (raising ethical concerns) here we propose the freshwater polyp *Hydra vulgaris* as an innovative *in vivo* model to investigate the biocompatibility and the bioactivity of advanced formulation for photodynamic therapy. The transparency, softness and body simplicity together with the lack of ethical issues make *Hydra* a suitable model for this type of studies, allowing us to perform functional comparative analyses. By challenging the polyps with HSA and phages functionalized with ECB04, we tested the efficacy of the bioconjugates using morpho-functional and molecular approaches, and identified possible molecular pathways activated by PTD.

REFERENCES

1. Cantelli A, *et al.* (2021). *JACS*, vol 1: 925-935.
2. Ulfo L, *et al.* (2022). *Nanoscale*, vol 14(3): 632-641.
3. Bortot B, *et al.* (2021). *Free Radic Biol Med*, vol 179: 242-251.

P23

Plant cell culture as an alternative method to boost the release of secondary metabolites by Mediterranean species

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ABSTRACT

Plant cell cultures (PCCs) have recently gained a renewed popularity as advantageous platforms to increase the production of secondary metabolites (SMs) that are of commercial interest as natural drugs for humans and plants[1]. PCCs offer several benefits such as: *i*) no seasonal dependence; *ii*) cell cultivation under controlled conditions preventing an excessive use of water, fertilizers and pesticides; *iii*) high rate of cell growth[2]. The aim of our research is to produce novel improved PCCs accumulating high-value secondary metabolites. Given this, we developed protocols for efficient callus cultures that were obtained from plant's organs (leaf, stem, fruit) of Mediterranean species, such as *Malus pumila*, *Cistus creticus*, and *Solanum lycopersicum*. Furthermore, calli were subjected to a qualitative analysis by performing high performance liquid chromatography (HPLC). SMs are well-known to have anti-inflammatory, anti-microbial, anti-cancer, and antihypertensive properties[3]. Therefore, future work will concentrate on in-depth study of polyphenols biosynthetic pathway within the developed Mediterranean plant cell cultures to optimize the production of bioactive compounds whose amount is comparable or higher than reference material.

REFERENCES

1. Gubser G, Vollenweider S, Eibl D, *et al.* (2021). *Eng Life Sci*, vol 21: 87-98.
2. Moscatiello R, Baldan B, Navazio L (2012). *Plant Mineral Nutrients. Methods in molecular biology*, vol 953.
3. Chiocchio I, Mandrone M, Tomasi P, *et al.* (2021). *Molecules*, vol 26(2): 495

P24

Interaction between *Fusarium oxysporum* and *Candida albicans* during Dual-Species Biofilm Formation and a Novel Treatment Strategy

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ABSTRACT

A single or multispecies biofilm may form during an infection. Molds, such as Fusarium spp. and yeasts, such as Candida spp., can caused non-dermatophyte infections. Treatment of these infections are particularly difficult due to the capability of the two species to form a mixed biofilm [1,2]. Approximately 20% of human fusariosis is caused by *Fusarium oxysporum* [3], while *Candida albicans* is responsible for superficial mucosal and dermal infections and for disseminated bloodstream infections with a mortality rate above 40% [4]. Importantly, *Fusarium* species are resistant to most common commercial antifungals [5]. The increased incidence of mixed infections and of resistance to traditional antifungal required the develop of novel antimicrobial molecules are request. The aim of this study was to investigate the interactions between *C. albicans* and *F. oxysporum* dual-species biofilm, considering variable formation conditions. Further, the ability of the WMR peptide, a modified version of myxinidin, to eradicate the mixed biofilm when used alone or in combination with fluconazole (FLC) was tested, and the efficacy of the combination of WMR and FLC at low doses was assessed, as well as its effect on the expression of some biofilm-related adhesin and hyphal regulatory genes. Finally, in order to confirm our findings *in vivo* and explore the synergistic effect of the two drugs, we utilized the *Galleria mellonella* infection model. We concluded that *C. albicans* negatively affects *F. oxysporum* growth in mixed biofilms. Combinatorial treatment by WMR and FLC significantly reduced the biomass and viability of both species in mature mixed biofilms, and these effects coincided with the reduced expression of biofilm-related genes in both fungi. Our results were confirmed *in vivo* since the synergistic antifungal activity of WMR and FLC increased the survival of infected larvae and reduced tissue invasion. These findings highlight the importance of drug combinations as an alternative treatment for *C. albicans* and *F. oxysporum* mixed biofilms.

REFERENCES

1. Butassi E, Svetaz L, Carpinella MC, *et al.* (2021). *Antibiotics*, vol 10: 1053.
2. Oliveira LT, Medina-Alarcón KP, Singulani JdL, *et al.* (2020). *Front Microbiol*, vol 11: 551256.
3. Nucci M, Anaissie E (2019). *Clin Microbiol Rev*, vol 20: 695-704.
4. Bajpai VK, Khan I, Shukla S, *et al.* (2019). *Biotechnol Bioprocess Eng*, vol 24: 436-444.
5. Sav H, Rafati H, Öz Y, *et al.* (2018). *J Fungi*, vol 4: 16.

P25

Monitoring the DNA repair activity of the *O*⁶-alkylguanine-DNA-alkyl-transferases (AGT) and DNA nanoswitches: "light on the scene"

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ABSTRACT

Cellular DNA is subjected to covalent modifications by intracellular chemical compounds and coming from the external environment. Alkylating agents are reactive molecules transferring chemical groups to nucleobases causing alterations in their functions. AGTs are enzymes which remove alkyl-adducts from the *O*⁶-position of guanines from DNA, by a peculiar irreversible reaction[1]. Although their evolutive function represents the major factor in contrasting the effects of alkylating agents on DNA, on the other side the human representative of this class of enzymes (hMGMT) contrasts the effect of chemotherapy's based on alkylating agents, making tumor cells resistant. For these reasons, the development of hMGMT inactivators/inhibitors to be used in combination with this kind of chemotherapy is constantly evolving. In fact, rapid and reliable tests are needed for the measure of the methyltransferase activity. To this aim, DNA nanotechnology offers the possibility to create DNA nanodevices to monitor DNA repair activity. In this work, I present a new class of DNA-based substrates that, upon enzymatic DNA repair by AGTs, could undergo a conformational switch, followed by a change in a fluorescent signal. Such folding-upon-repair DNA single strand oligonucleotides, called DNA-nanoswitches, are synthetic DNA sequences containing as O6-methyl-guanine nucleobases, as well as a FRET fluorophores optical pair. These molecules are canonical DNA duplex, but they are rationally designed so that only upon enzymatic repair by demethylation of the *O*⁶-MeG nucleobases they can form stable intramolecular Hoogsteen interactions and fold into a DNA triplex structure, which is optically different from the initial DNA duplex form[2]. I have characterized the folding mechanism induced by the enzymatic repair activity and then I demonstrated that the folding-upon-repair DNA nanoswitches are universal AGTs' substrates. These innovative substrates will allow the high-throughput screening of alkylated DNA containing biological samples, as well as the selection of novel potential hMGMT inhibitors for cancer studies[3].

REFERENCES

1. Perugino G, *et al.* (2012). *J Biol Chem*, vol 10: 1074.
2. Idili A, *et al.* (2014). *J Am Chem Soc*, vol 10: 1021.
3. Farag N, *et al.* (2021). *Angew Chem Int Ed*, vol 60: 7283-9.

P26

Proteomic approach and identification of VP1 interactors to uncovering deformed wing virus-honey bee relationship

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ABSTRACT

Honey bee pollination is important for maintaining ecosystems and the production of many crops. Large-scale colony losses are a big problem of global importance, still poorly understood. The honey bee colony loss is a multifactorial syndrome induce by interacting stress factors, which reduce the immune competence and allow an uncontrolled proliferation of parasites and pathogens. A central role in this syndrome is played by the symbiotic association between the viral pathogen Deformed Wing Virus (DWV) and its ectoparasitic mite vector Varroa destructor, the two major stress agents that severely impact honey bee immunity and health[1-2]. The DWV is a positive-strand RNA virus. The DWV virion is constructed by VP1, VP2, and VP3 subunits which are arranged into a capsid with pseudo-T3 icosahedral symmetry. VP1 is the most abundant protein and may play a key role on virus introduction, driving the RNA into the cytosol, however very little is known regarding the mechanisms of viral infection and the strategies used from the host to counteract the virus [3]. The identification of host proteins interacting with the viral proteins may help to better understand the infection process and the pathogenesis of viruses. To date, no study of protein interactions between DWV and honeybee proteins has been performed yet. In this study, we performed coimmunoprecipitation experiments coupled to mass spectrometry approaches to analyse the composition of honey bee proteins associated to VP1 viral protein. The data analysis showed that most of identified honey bee proteins are involved in fundamental biological processes suggesting a potential role in the viral infection.

REFERENCES

1. Anderson DL, Trueman JWH (2002). *Exp Appl Acarol*, vol 24: 165-189.
2. Lanzi G, *et al.* (2006). *J Virology*, p. 4998–5009.
3. Wu, *et al.* (2021). *Frontiers in Microbiology*.

P27
Involvement of miR-137 in the regulation of MSH2 gene

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ABSTRACT

The Lynch syndrome (LS) is associated to sequence variants in Mismatch Repair (MMR) genes. The MMR genes dysregulation of play a fundamental role in the pathogenesis of LS. In the previous study, we identified a sequence variant, the c*226A>G in 3'UTR of MSH2 gene, that was not predicted to be a pathogenic variant, in some LS patients. This variant determined an increase of MSH2 gene expression likely resulting in a non¬functional MMR complex. This overexpression effect was likely related to a loss of MSH2 gene regulation causing by sequence variants that prevented binding with regulatory factors, such as also the microRNAs. For this regard, the aim of this study was investigated if this variants c*226A>G located in the 3'UTR region of MSH2 gene disrupts a putative binding site for a regulatory factors or miRNAs. Bioinformatic analysis identified a putative binding site for hsa¬miR137 in MSH2 3'UTR region which could be altered own by the presence of the c*226A>G variant. Luciferase assays allowed to verify that in the 3' UTR region of MSH2 gene is located effectively the seed ¬region of miR¬137. Relative luciferase activity was significantly reduced in the presence of pre¬miR¬137 for MSH2 3'UTR wild type construct, but not for 3'UTR three mutants constructs. Moreover, the functional relevance of miR¬137 in regulating the expression of MSH2 was established by overexpressing miR¬137 in human cell lines SW480. To confirm what we were observing we conducted the same experiment in another human HT29 cell line. Our results indicate that both MSH2 mRNA and protein levels were regulated by Mir 137 transfection and anti Mir-137 in both cell lines. In conclusion, our data showed that MSH2 gene is target gene of miR¬137 and that this miR¬137 down-regulates MSH2 expression. These findings will open new possibilities in detection, diagnosis and treatment for LS.

P28
Enhancement of CRISPR/Cas12a trans-cleavage activity using hairpin DNA reporters

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ABSTRACT

In the recent years, the use of CRISPR technology have been boosted by the discovery of the *trans-cleavage* nuclease activity of CRISPR type V (Cas12a) system, especially in the field of diagnostic applications[1]. Cas12 protein is an RNA-guided component of the bacterial adaptive immune system which can integrate single- and double-stranded DNA recognition, signal transduction and amplification in one system. Its *trans-cleavage* activity is activated upon base-paring of a nucleic acid target to a complementary crRNA guide and can lead to a specific increase of fluorescence in case of FRET-based single-stranded DNA reporter. However, although this technology has demonstrated vast potential in diagnostic and biosensing applications, the detection of the target is limited in absence of a pre-amplification step. Moreover, the enhancement of *trans-cleavage* activity often results in increased costs of chimeric DNA/RNA guide[2].
In response to the above considerations, here we report the rational design of single-stranded harpin DNA reporter having a fluorophore and a quencher at the two ends of self-complementary portions. In this work, we demonstrate that the Cas12a protein presents an improved affinity (K_m) for this harpin DNA reporter, resulting in a faster rate of *trans-cleavage* activity and enhancing the sensitivity and the specificity of CRISPR/Cas12a technology. The single-stranded harpin DNA reporter not only represents a step to better understand the nuclease activity of Cas12a but also offers the opportunity to expand the use of CRISPR/Cas12a in high-throughput applications[3].

REFERENCES

1. Tang Y, Gao L, Feng W, *et al.* (2021). *Chem Soc Rev*, vol 50: 11844-11869.
2. Nalefski E.A, Patel N, Leung PJY, *et al.* (2021). *Iscience*, vol 24: 102996.
3. Rossetti M, Merlo R, Bagheri N, *et al.* (2022). *Nuc Ac Res*, vol 50: 8377-8391.

P29
Generation of a new AIF peptide targeting Human Cyclophilin A to inhibit AIF-mediated cell death

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ABSTRACT

The interaction between apoptosis inducing factor (AIF) and cyclophilin A (CypA) has a crucial role in neuronal cell death following apoptotic stimuli[1]. Blocking this interaction through a synthetic fragment mimicking the amino acid region of AIF, covering residues 370-394 (AIF (370-394)), induces neuroprotection in neuronal cell models of glutamate-induced neurotoxicity and prevents brain injury in neonatal mice after hypoxia-ischemia[1,2]. Here, we report the generation of a shorter and cyclic variant of the AIF (370-394) and its biochemical and structural characterization by NMR in the free and CypA-bound state.[3] The new peptide (AIF(381-389)), despite its small size, less than half of the precursor, shows an *in vitro* affinity comparable to that of AIF(370-394). NMR-based 3D models of isolated AIF(381-389) and in complex with CypA demonstrate that cyclization significantly limits the conformational space explored by the compound, resulting in the formation of intramolecular hydrophobic interactions and a broad network of hydrogen bonds involving the atoms of the backbone which significantly stabilize a β-hairpin structure very similar to that adopted by the amino acid sequence in the protein, unlike the linear peptide which is very flexible. Furthermore, the novel peptide exhibits significantly greater resistance to degradation in human serum and an improved ability to prevent cell death in a cell model of glutamate-induced neurotoxicity compared to the parent peptide AIF (370-394)[3].

The obtained structural data are very useful for drug development programs based on structure-activity-relationship studies and on computational approaches to improve peptide activity and to predict new highly effective, selective, stable inhibitors.

REFERENCES

1. Doti N, Reuther C, Scognamiglio PL, *et al.* (2014). *Cell Death Dis*, 5,e993.
2. Zhu C, Wang X, Deinum J, *et al.* (2007). *Exp Med*, vol 204(8): 1741-1748.
3. Russo L, Mascanzoni F, Farina B, *et al.* (2021). *J Med Chem*, vol 64(15): 11445-11459.

P30
Gelatinous zooplankton and their parasites (Trematoda, Digenea) from the Gulf of Trieste

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ABSTRACT

More and more evidence show that jellyfish are not just background characters in the oceans, they can interfere in the trophic webs and alter ecosystem structures[1]. Gelatinous zooplankton parasites, in particular digeneans, are one underinvestigated aspect of these organisms[2].

In this study, 58 individuals of *Rhizostoma pulmo* and 324 *Mnemiopsis leidyi* from the Gulf of Trieste have been dissected and parasites counted. For both species high prevalence (P) and intensity (I) were recorded (100% P, 214.8 I and 91% P, 8.24 I, respectively). All digeneans resulted to belong to the same genus, *Clavogalea*, confirmed by both morphological and genetic analyses (*M. leidyi* molecular results are preliminary).

Clavogalea sp. is present year-round in all sites, from Grado Lagoon to the bays of Trieste and Piran, showing remarkable tolerance of this species to environmental and seasonal variations (water temperature fluctuates between about 9°C and 25°C). High P recorded in both species (>90%) hints that these organisms are true intermediate hosts for digeneans, a key step in their life cycle. Plus, high P and I suggest fish predation on these species in the Gulf, since trophic transfer is fundamental for parasites to complete their cycles. Last, since jellyfish tissues do not last long in fish stomachs[3,4], parasitological studies and data may act as a useful tool to integrate traditional analysis (gut content and stable isotopes) in addressing trophic dynamics between fish and gelatinous zooplankton, a key but still obscure aspect in fish and jellyfish ecology[5].

REFERENCES

1. Graham W M, Gelcich S, Robinson KL, et al. (2014). *Frontiers in Ecology and the Environment*, vol 12(9): 515-523.
2. Marcogliese DJ (1995). *Reviews in Fish Biology and Fisheries,* vol 5: 336-371.
3. Hays GC, Doyle TK, Houghton JD (2018). *Trends in ecology & evolution*, vol 33(11): 874-884.
4. Lamb PD, Hunter E, Pinnegar JK, *et al.* (2017). *Royal Society open science*, vol 4(11): 171421.
5. Arai MN (2005). *Journal of the Marine Biological Association of the United Kingdom*, vol 85(3): 523-536.

P31
Study of the potential biological activity of substances extracted from agri-food industry waste

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ABSTRACT

The agri-food industries produce a large amount of waste along the entire production chain, which represents a huge problem both from an economic and environmental point of view. For this reason, there is an increasing interest to reduce the impact of their production, by seeking novel ways to exploit them, transforming waste in resource. One approach to the recycle waste is to enhance its value by considering the presence of some bioactive compounds. Large interest is focused on molecules from food sources that exert biological activity by reducing the risk of developing numerous chronical diseases (e.g. diabetes, cancer, cardiovascular diseases, neurodegenerative diseases), and that therefore could play a fundamental protective action on human health.

The aim of this project was to define protein targets for a group of polyphenols derived from processing waste from agri-food industries, to hypothesize molecular mechanisms by which these molecules carry out their biological activity within the cells, and suggest their recovery for its reuse.

Polyphenols are of great interest for their known anti-oxidant, anti-inflammatory, cardioprotective, antitumor, antidiabetic, antiobesity, antimicrobial activities. We performed a large-scale search for targets of selected polyphenols, among all proteins expressed in various human tissues. We applied an integrated analysis based on bioinformatics tools for the screening of proteins for both the potential interaction with the selected molecules and the involvement in functional pathways of interest. The results showed a significant involvement of the selected protein targets in cancer, diabetes and neurodegenerative diseases. Computational validation of the potential protein-ligand interactions was performed by molecular docking approach, obtaining the characterization of the binding sites and related binding energies. The best interactions, in terms of binding energy values and selectivity, have been analyzed in detail in order to define the binding pockets and compare them to known inhibitory sites. The results suggested that some polyphenols from agri-food waste may bind the selected protein targets and potentially inhibit their biological function, thus playing a positive effect on human health.

P32
Genome wide Analysis of *Dof* genes and their response to salt stress in wild and cultivated potatoes

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ABSTRACT

The plant DNA-binding with one finger (Dof) gene family is a class of plant-specific transcription factors involved in several biological processes, including response to biotic and abiotic stresses. Genome-wide identification of this family has been reported in the cultivated potato *Solanum tuberosum*, but clues about the roles of several *dof* genes are still lacking. Here, we performed a comprehensive bioinformatics analysis to identify the entire Dof family in potato and in its wild relative *S. commersonii*, which displays a much higher adaptation to environmental constrains compared to the cultivated species. This approach is a valuable way to highlight evolutive divergences between the two species and identify useful genes/alleles for breeding pourposes. By using the protein sequences of *Arabidopsis thaliana* as baits, a total of 44 and 37 *dof* candidate genes were fetched in *S. tuberosum* and *S. commersonii*, respectively. Phylogenetic analysis of potato Dof proteins revealed that our candidates can be classified in four clades (I, II, III, IV), in line with other reported analyses. We focused on clade I since homologs members in *Arabidopsis*, reported as cycling Dof factors (CDFs), are reported to have a crucial role in plant response to abiotic stresses. Interestingly, we found six CDF factors in *S. tuberosum* and only five CDFs in *S. commersonii*. To ascertain their involvement in stress response, saline stress (80 mM NaCl) for short (48 h) and prolonged (7 days) time was imposed on *in vitro* growing *S. tuberosum* and *S. commersonii* plantlets. Molecular analyses on stressed and non-stressed materials were performed. Expression analysis of selected *St*Dof genes on root and aerial tissues, using qRT-PCR, revealed different tissue specific gene expression induction between *S. tuberosum* and *S. commersonii*. Parallelly, biochemical changes measured by total phenols (TP), proline and MDA (malonyldialdeyde) upon saline treatment showed a diverse stress perception for both potato species. Overall, this study provides a better understanding of the involvement of CDF genes in response to salt and enables the discrimination of potential candidates for further functional studies.

P33

The long non-coding RNA *COMETT* as new potential target in $BRAF_{V600E}$-mutated papillary and anaplastic thyroid carcinomas

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ABSTRACT

Long non -coding RNAs (lncRNAs) deregulation has been reported in multiple tumor types[1], including thyroid cancer, and their targeting has relevant therapeutic potential[2-3]. We recently identified *COMETT* as oncogenic lncRNA highly expressed in papillary thyroid carcinomas (PTCs) and not in normal tissues[4]. Recently, we explored how its knockdown (KD) by Antisense LNA-GapmeRs impairs the tumorigenic potential of $BRAF_{V600E}$-mutated papillary and anaplastic thyroid (ATC) cells, highlighting the oncogenic role also in the latter tumor type. We investigated its silencing as adjuvant to standard therapy based on B-raf inhibition (i.e., FDA-approved vemurafenib). Interestingly, *COMETT* KD – other than markedly reducing viability and colony forming ability of tumor cells – enhances their responsiveness to vemurafenib, even at low drug doses. To understand how *COMETT* acts in tumor cells we 1) performed transcriptome analysis upon COMETT-KD (to identify the network of altered genes), and 2) combined *COMETT* RNA pull-down to Mass Spectrometry (MS; to identify its binding partners). Preliminary RNA-Seq analysis indicates that *COMETT* KD strongly represses EGFR, WNT and JAK-STAT signaling pathways, whereas MS data reveal the binding of *COMETT* to splicing factors and multiple ribosomal proteins, suggesting a role in translation and splicing. Lastly, using genome engineering (CRISPR/dCas9 system) we generated PTC clones stably over-expressing COMETT, which will be used to validate its oncogenic properties as well as for mechanistic studies. Overall, our results support the oncogene-like properties *COMETT* in BRAF-mutated ATC and PTC, indicating its silencing as potential adjuvant approach in the therapy, especially to overcome drug resistance in BRAF-mutated tumors.

REFERENCES

1. Aprile M, Katopodi V, Leucci E, Costa V (2020). *Cancers*, vol 12(11): 3220.
2. Winkle M, El-Daly SM, Fabbri M, Calin GA (2021). *Nature Rev. Drug Discovery*, vol 20(8): 629-651.
3. Arun G, Diermeier SD, Spector DL (2018). *Trends in Molecular Medicine*, vol 24(3): 257-277.
4. Esposito R, Esposito D, Pallante P, *et al.* (2019). *Cancer Res*, vol 79(9): 2124-2135.

P34
Structure-function correlation of PHOX2B in the Congenital Central Hypoventilation Syndrome (CCHS)

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ABSTRACT

Congenital Central Hypoventilation Syndrome is a rare neonatal disease characterized by abnormal ventilatory response to hypoxia and hypercapnia, owing to failure of autonomic respiratory control. Frameshift mutations and polyalanine triplet expansions have been detected in the coding region of the transcription factor PHOX2B gene, responsible for the proper development and function of the autonomic nervous system. Increasingly long polyalanine tracts lead to an increased tendency for protein oligomerization and toxic effects. The transcriptional dysregulation and protein misfolding might be key mechanisms of CCHS pathogenesis[1]. PHOX2B consists of 314 amino acids and it is composed by a 97-residue N-terminal domain, a DNA-binding motif (HD) and two poly-Ala stretches of 9 and 20 residues, within the C-terminal domain. We carried out a biochemical comparison of PHOX2B containing the correct C-terminal (20 Ala) stretch with one of the most frequent variant with a poly-Ala expansion (27 Ala), demonstrating that surprisingly the longer poly-Ala stretch of the pathological PHOX2B variant could act as a nucleus for fibrils formation[2]. Here we present a biophysical characterization of the PHOX2A paralogue, hypothesizing its possible role in the stabilization of pathological forms of PHOX2B. Finally, progress in the resolution of NMR structures is outlined. The resolution of the structure of the wild-type and mutated PHOX2B forms will help to elucidate the molecular mechanisms involved and will allow a rational design of therapeutic drugs.

REFERENCES

1. Di Lascio S, *et al.* (2021). *Front Neurosci*, vol 14: 615-666.
2. Pirone L, *et al.* (2019). *FEBS J*, vol 286(13): 2505-2521.

P35
Stabilization of anthocyanin production in potato cell cultures through knock-out editing of StISAC

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ABSTRACT

Anthocyanins are natural pigments with a multidisciplinary scientific interest due to their extensive range of colours and their relevant role against several non-communicable human diseases[1]. The New Breeding Techniques (NBTs) can effectively be employed to increase the accumulation of these important compounds in horticultural crops[2]. In the current work, we were able to increase the total level of anthocyanins in potato cells by using gene editing. As starting material, we used the potato variety "Blue Star", which naturally accumulates anthocyanins both in the tubers and in the leaves. Through CRISPR/Cas9 targeted mutagenesis, we knocked-out StISAC, a target gene encoding for a negative R3-MYB regulator of anthocyanin biosynthesis. The induced modifications led to a frameshift mutation and a premature stop codon, which was predicted to truncate the encoded protein after about the first 20 amino acids. The edited cells (stisac) doubled the total phenolic compounds and anthocyanin content compared to wt cells, as well as their antioxidant activity. What is more, the production of these important pigments was stabilized over time. Up to the present, gene editing has never been used in potato or in cell cultures to change anthocyanin content. Our results provided evidence that precise gene mutagenesis represents an efficient and effective strategy to increase the level of anthocyanins and total antioxidants in plants.

REFERENCES

1. Speer H, D'Cuhna NM, Alexopoulos NI, *et al.* (2020). *Antioxidants*, vol 9(5): 366
2. Marone D, Mastrangelo AM, Borrelli GM, *et al.* (2022). *Plant Phys Biochem*, vol 172: 48-55.

P36
FKBP51 Affects TNF-Related Apoptosis Inducing Ligand Response in Melanoma

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ABSTRACT

Melanoma is one of the most immunogenic tumors and has the highest potential to elicit specific adaptive antitumor immune responses. Immune cells induce apoptosis of cancer cells either by soluble factors or by triggering cell-death pathways. Melanoma cells exploit multiple mechanisms to escape immune system tumoricidal control. FKBP51 is a relevant pro-oncogenic factor of melanoma cells supporting NF-κB-mediated resistance and cancer stemness/invasion epigenetic programs. Herein, we show that FKBP51-silencing increases TNF-related apoptosis-inducing ligand (TRAIL)-R2 (DR5) expression and sensitizes melanoma cells to TRAIL-induced apoptosis. Consistent with the general increase in histone deacetylases, as by the proteomic profile, the immune precipitation assay showed decreased acetyl-Yin Yang 1 (YY1) after FKBP51 depletion, suggesting an impaired repressor activity of this transcription factor. ChIP assay supported this hypothesis. Compared with non-silenced cells, a reduced acetyl-YY1 was found on the DR5 promoter, resulting in increased DR5 transcript levels. Using Crispr/Cas9 knockout (KO) melanoma cells, we confirmed the negative regulation of DR5 by FKBP51. We also show that KO cells displayed reduced levels of acetyl- EP300 responsible for YY1 acetylation, along with reduced acetyl-YY1. Reconstituting FKBP51 levels contrasted the effects of KO on DR5, acetyl-YY1, and acetyl-EP300 levels. In conclusion, our finding shows that FKBP51 reduces DR5 expression at the transcriptional level by promoting YY1 repressor activity. Our study supports the conclusion that targeting FKBP51 increases the expression level of DR5 and sensitivity to TRAIL-induced cell death, which can improve the tumoricidal action of immune cells.

P37

Molecular networking combined with culture media variation reveal the metabolic potential of *Pantoea eucrina* isolated from a sponge living in a volcanic vents system

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ABSTRACT

Ischia is an island located in the gulf of Naples (South Italy), and its environment represents a singular marine habitat because of the volcanic origin of the island. Here, the presence of underwater CO_2 emissions, caused by the effect of secondary volcanism, creates a unique area of naturally lowered pH[1].

Different sponges were collected in this acidified area and subjected to microbial isolation. Among the isolates, the Gram-negative strain *Pantoea eucrina*, isolated from the sponge *Chondrosia reniformis*, was selected for further studies. Its metabolome was explored by a combined approach involving culture media variation[2] and Molecular Networking[3].

This approach yielded a very complex molecular network, allowing the annotation of several metabolites, among them two biosurfactants clusters: lipoamino acids and surfactins. Noteworthy is the production of unusual new surfactin derivatives, which is reported here for the first time from *Pantoea* genus. Their production was only triggered by using inorganic nitrogen as sole nitrogen source, while it was totally abolished in the other conditions.

Major metabolites belonging to N-lipoamino acids were characterized through HRMS/MS, 1D and 2D NMR spectroscopy, some of them were identified for the first time as natural products.

Antimicrobial activity assessment of pure metabolites towards a panel of human pathogens indicated a relevant activity; in particular, the leucine containing N-lipoamino acids revealed to be very effective towards *Staphylococcus aureus*, *Staphylococcus epidermidis* along with a clinical isolate of the emerging food pathogen *Listeria monocytogenes*.

REFERENCES

1. Foo Shawna A, Byrne M, Ricevuto E, Gambi MC (2018). *Oceanography and Marine Biology*, 237-310.
2. Romano S, Jackson SA, Patry S, Dobson ADW (2018). *Marine drugs*, vol 16(7): 244.
3. Wang M, *et al.* (2016). *Nature Biotech*, vol 34(8): 828-837.

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