2nd IBBR Memorial Workshop

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

October 25-26, 2021

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.

After the first Workshop held just before the Covid-19 epidemic in 2020 dedicated to our dear Maria, the Organizing and Scientific Committees welcome the scientific community to this second appointment, thanking all the young speakers who will contribute to the success of this event.

Anna, Pino and Roberto

The Institute of Biosciences and BioResources- CNR


IBBR Headquarter- Bari

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.


IBBR UOS Florence


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IBBR UOS Napoli


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 the second Meeting dedicated to her. We wish all participants, in presence and via streaming, a fruitful scientific discussion, which is an important opportunity especially for young researchers who have been selected as speakers at this event.

Gianna and Beppe

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PROGRAM

Monday, October 25 – Session 1

13:00 – 14:00 *registration*

14:00 – 14:20 *greetings and opening*

14:20 – 14:40 Federica SCOTTO DI CARLO
Institute of Genetics and Biophysics "A. Buzzati Traverso" (IGB-CNR), Napoli
The loss of Profilin 1 causes chromosome instability in osteosarcoma through impairment of mitotic fidelity.

14:40 – 15:00 Clorinda FUSCO
Istituto per l'Endocrinologia e l'Oncologia Sperimentale "G. Salvatore" (IEOS-CNR), Napoli
Role of a novel Foxp3Exon2+ Treg cell subset in the tumor microenvironment of breast cancer subjects.

15:00 – 15:20 Paola PUNZO
Institute of Biosciences and BioResources (IBBR-CNR), Portici (NA)
From an expression-based functional screening to the identification of a splicing regulator involved in stress tolerance.

15:20 – 15:50 *break*

15:50 – 16:10 Rosanna MATTOSSOVICH
Institute of Biosciences and BioResources (IBBR-CNR), Napoli
O^6-alkylguanine-DNA-Alkyltransferase (AGT): lights on the scene.

16:10 – 16:30 Salvatore FIORINIELLO
Institute of Genetics and Biophysics "A. Buzzati Traverso" (IGB-CNR), Napoli
Glycosphingolipid metabolic imbalance in Rett syndrome and development of a pharmacological treatment in Rett models.

16:30 – 16:50 Ermenegilda VITALE
Department of Biology, University of Naples Federico II, Napoli
Effect of ionizing radiation on photosynthesis and recovery strategies: implications for plant growth in Space.

16:50 – 17:10 Alessandra CAMARCA
Laboratory For Molecular Sensing, Institute of Food Sciences (ISA-CNR), Avellino
Characterization of NMN Deamidase Mutants as Possible Probes for an NMN Biosensor.

Also available in streaming *via* GoToMeeting at the following link:
https://global.gotomeeting.com/join/754044957

access code: 754-044-957

Tuesday, October 26 – Session 2

09:00 – 10:00 *registration*

10:00 – 10:20 Michele CILLO
Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Napoli
Interfering with NF-kb in anaplastic thyroid carcinoma.

10:20 – 10:40 Federica DE LISE
Institute of Biosciences and BioResources (IBBR-CNR), Napoli
From the discovery of hyperstable CAZymes to translational recoding: the archaeal α-L-fucosidase from *Saccharolobus solfataricus*.

10:40 – 11:00 Antonio VARRIALE
Laboratory For Molecular Sensing, URT-ISA at Department of Biology, University of Naples Federico II, 80126 Napoli
Protein-based biosensor as tool for "Farm to Fork" strategy.

11:00 – 11:30 *break*

11:30 – 11:50 Lorena GRATINO
Institute of Biosciences and BioResources (IBBR-CNR), Napoli
Sustainable plant-based production of SOD from *Deinococcus radiodurans* for food applications.

11:50 – 12:10 Jessica PAZZAGLIA
Department of Integrative Marine Ecology, Stazione Zoologica Anton Dohrn, Napoli
Living with global environmental changes: the case of *Posidonia oceanica*.

12:10 – 12:30 Donatella DELLE CAVE
Institute of Genetics and Biophysics "A. Buzzati Traverso" (IGB-CNR), Napoli
LAMC2 drives tumorigenicity and metastasis in pancreatic ductal adenocarcinoma.

12:30 – 12:50 Elisa CAPPETTA
Institute of Biosciences and BioResources (IBBR-CNR), Portici (NA)
Sustainable biorefineries: strategies for modifying metabolic fluxes in cardoon cells.

Also available in streaming *via* GoToMeeting at the following link:
https://global.gotomeeting.com/join/340338069

access code: 340-338-069

Tuesday, October 26 – Session 3

13:00 – 14:00 *registration*

14:00 – 14:20 Martina AULITTO
Department of Biology, University of Naples Federico II, Napoli
Pangenomic analysis of *Bacillus coagulans* MA-13: a promising thermophilic strain for production of bioactive molecules from agricultural wastes.

14:20 – 14:40 Marta MALLARDO
Dipartimento di Scienze e Tecnologie Ambientali, Biologiche, Farmaceutiche, Università della Campania "Luigi Vanvitelli", Caserta
Adiponectin protects human neuroblastoma cells against cytotoxicity induced by cerebrospinal fluid from patients affected by multiple sclerosis.

14:40 – 15:00 Iolanda SCOGNAMIGLIO
Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Napoli
Breast cancer-derived exosomes trigger stromal fibroblasts in tumor microenvironment via microRNA cargo.

15:00 – 15:30 *break*

15:30 – 15:50 Francesco DI MEO
Department of Medicine, Indiana University School of Medicine, Indianapolis, IN, USA
Mapping the high-risk Multiple Myeloma cell surface proteome identifies T-cell inhibitory receptors for immune targeting.

15:50 – 16:10 Alessia AMETRANO
Institute of Biochemistry and Cell Biology - National Research Council of Italy, Napoli
CRISPR-mediated editing of the murine immunoglobulin heavy chain gene locus for the generation of an "antarctized" monoclonal antibody.

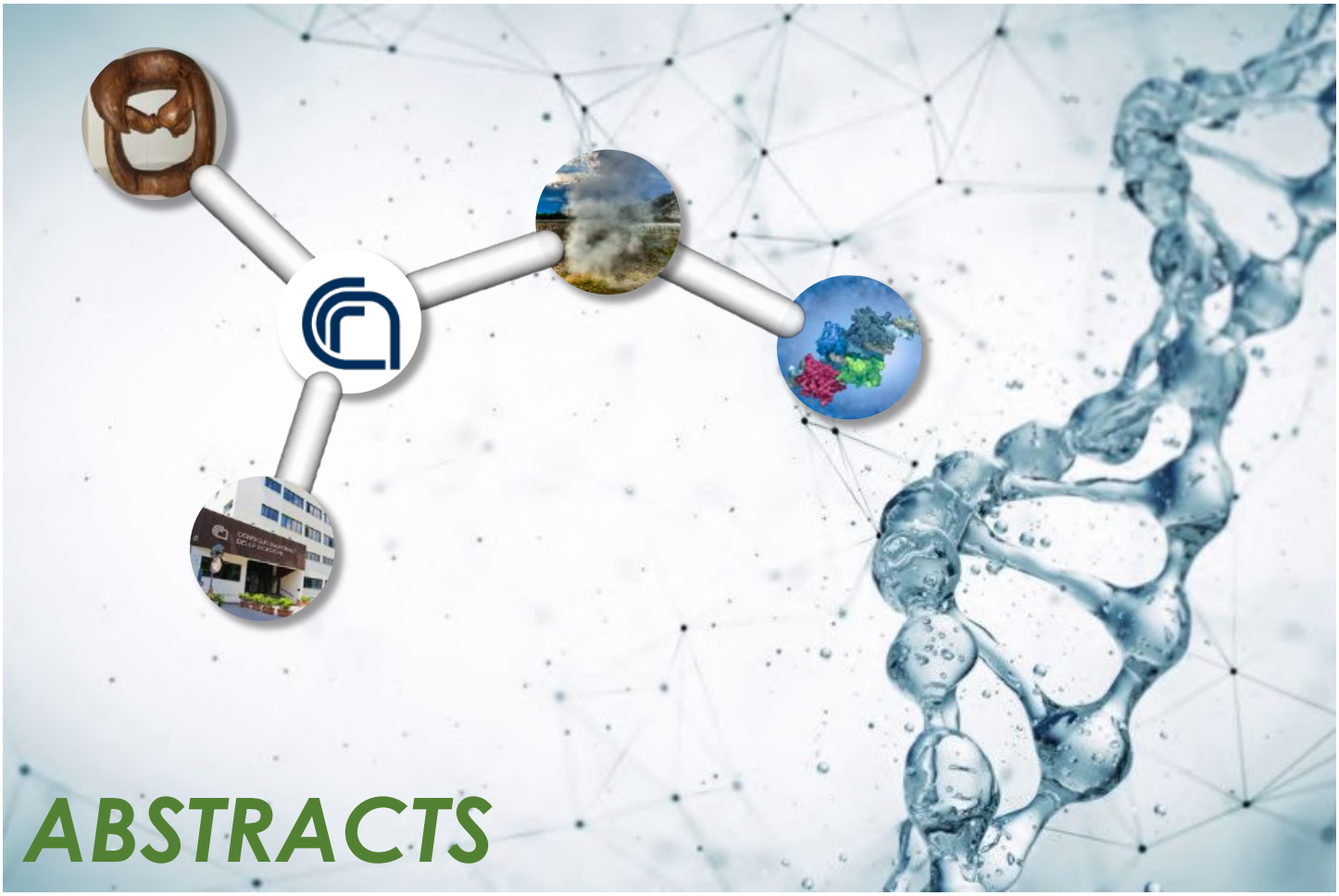
16:10 – 16:30 Carola MURANO
Stazione Zoologica Anton Dohrn, Napoli
Unravelling the effects of microplastic-associate biofilm in the uptake and immunological response in the sea urchin *Paracentrotus lividus*.

16:30 – 16:50 Daniela D'ARCO
CEINGE-Biotecnologie Avanzate Scarl, Napoli
Next Generation Sequencing (NGS) for the diagnosis of autosomal dominant/recessive polycystic kidney disease.

16:50 – 17:10 *closing remarks*

Also available in streaming *via* GoToMeeting at the following link:
https://global.gotomeeting.com/join/340338069

access code: 340-338-069



ABSTRACTS

The loss of Profilin 1 causes chromosome instability in osteosarcoma through impairment of mitotic fidelity.

Federica Scotto di Carlo, Fernando Gianfrancesco.

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ABSTRACT. Osteosarcoma (OS) is an aggressive tumour of mesenchymal origin, characterised by a great genetic heterogeneity due to high levels of chromosome instability. It may arise as degeneration of Paget's disease of bone (OS/PDB), showing a 5-year survival rate almost nil. We recently identified a germline loss-of-function mutation in the *PFN1* gene, encoding the Profilin 1, as responsible for familial OS/PDB, and disclosed loss of heterozygosity at somatic level in 54% of sporadic OS/PDB patients. Furthermore, we detected the loss of the *PFN1* locus (17p13) also in 30% of primary OS (unrelated to PDB).

Because chromosomal instability could be related to mitotic alterations, we investigated the role of Profilin 1 during cell division through confocal analysis. Notably, the immunofluorescence analysis highlighted a wide and constant distribution of Profilin 1 during all stages of mitosis, with a peculiar enrichment in the spindle midzone at anaphase, suggesting an essential role for this protein in regulating chromosome segregation and/or cleavage furrow formation. In agreement with our hypothesis, CRISPR-based *PFN1*-knock-out (KO) resulted in multiple mitotic defects in all cell types analysed (hTERT-RPE1, MC3T3, HCT116), including failures in mitotic rounding, delayed anaphase onset, and mitotic slippage at cytokinesis. Fluorescence time lapse imaging pointed out a remarkably higher frequency of chromosome mis-segregation events as a consequence of aberrant mitoses in *PFN1*-KO RPE1 cells stably expressing H2B-mCherry/EGFP-Tubulin. These events included chromosome mis-alignment on metaphase plates, tripolar metaphase spindles, formation of anaphase bridges between sister chromatids, and lagging chromosomes at telophase. Of interest, such mitotic errors resulted in p53 activation as well as phosphorylation of the H2AX histone variant (γH2AX), which accumulates in the presence of DNA double strand breaks. Accordingly, low-pass whole genome sequencing (WGS) analysis on *PFN1*-KO cells showed extensive chromosomal alterations (both structural and numerical) in all eight clones analysed, revealing the loss of *PFN1* as a cause of chromosome instability. In fact, WGS performed on 4 *PFN1*-mutated OS/PDB tumours and their healthy counterparts revealed high levels of copy number alterations associated with massive rearrangements, including phenomena of chromothripsis.

In conclusion, we describe the loss of function of *PFN1* as a novel mutational event underlying mitotic defects and chromosomal instability, explaining the chaotic genomes of osteosarcomas.

Role of a novel Foxp3Exon2+ Treg cell subset in the tumor microenvironment of breast cancer subjects.

Clorinda Fusco[1], Francesca Di Rella[2], Alessandra Colamatteo[3], Vincenzo Gigantino[4], Teresa Micillo[1], Francesca Collina[4], Antonio Porcellini[5], Francesco Perna[6], Antonello Accurso[7], Gerardo Botti[4], Veronica De Rosa[1].

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PURPOSE. CD4+CD25+Foxp3+ regulatory T cells (Tregs) are a subset of T cells involved in the control of immune tolerance and in tumor escape through the induction of immunosuppression. Several studies have found that a large infiltration of Tregs in the tumor microenvironment (TME) is associated with poor clinical outcomes in several human cancers, such as estrogen and progesterone receptor positive, human epidermal growth factor 2 negative (ER+PR+HER2-) breast cancer (BC). We performed a phenotypical and functional characterization of a novel tumor-infiltrating Tregs subpopulation, expressing the Foxp3 Exon 2 (Foxp3E2) splicing variant, having strongest suppressive function.

METHODS. We enrolled a cohort of 30 BC subjects at diagnosis and 30 healthy controls, matched for age and sex. We performed multiparametric analysis, by flow cytometry, on freshly isolated Peripheral Blood Mononuclear Cells (PBMCs) and tumor inflammatory infiltrate cells (TILs) to measure the expression of Treg cell markers associated with proliferation, migration and suppressive capacity. Moreover, we evaluated the ability of Tregs from BC subjects to suppress *in vitro* the proliferation of conventional T cells (Tconvs) labeled with the division-tracking dye CFSE. Finally, we evaluated Foxp3 expression in *in vitro* induced-Tregs (iTregs) generated from Tconv cells after 24 and 36 hours of stimulation with anti-CD3/CD28, by Western Blot.

RESULTS. We observed that Foxp3E2+ Tregs, both in the tumor infiltrate and in peripheral blood of BC subjects, have higher expression of Treg cell markers and increased suppressive capacity, and this correlates with tumor cell growth and progression.

CONCLUSIONS. Our data unveil a novel strategy to study the relationship between peripheral blood and intratumoral Foxp3E2 Tregs, a novel target for the immunotherapy of BC.

From an expression-based functional screening to the identification of a splicing regulator involved in stress tolerance.

Paola Punzo[1,2], Alessandra Ruggiero[1], Antonello Costa[1], Giorgia Batelli[1], Stefania Grillo[1].

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ABSTRACT. Drought and salinity cause osmotic stress in plants, reducing growth and development. The identification of genes involved in stress tolerance mechanisms is a key goal in plant biology and breeding. Previously, several differentially regulated genes were identified in potato (*Solanum tuberosum*) culture cells adapted to high concentrations of polyethylene glycol (PEG). Here, we functionally analysed the role of fifty of these genes through a reverse genetics approach using their orthologues in *Arabidopsis thaliana*. The large-scale phenotype screening of homozygous *knockout* lines for each gene allowed the identification of the splicing factor *DNA-DAMAGE REPAIR/TOLERATION PROTEIN111 (DRT111)*, whose function in stress response was so far unexplored. We observed that the Arabidopsis *DRT111* interacts with Splicing Factor1 (SF1), involved in early spliceosome assembly and 3'splicing site recognition. Altered expression of *DRT111* affects the sensitivity to ABA concerning stomatal movements and seed germination. Accordingly, *DRT111* is highly expressed in seeds and stomata and is induced by long-term treatments of PEG and abscisic acid (ABA). Transcriptome analysis of *drt111* mutants dry seed showed the role of *DRT111* in the regulation of expression and splicing of genes involved in light signalling, ABA and osmotic stress responses. Moreover, *ABSCISIC ACID INSENSITIVE3 (ABI3)* alternative transcripts quantifications and double- and triple-mutant germination analysis show that *DRT111* controls splicing of *ABI3* and acts upstream of the splicing factor *SUPPRESSOR OF ABI3 (SUA)*. Moreover, the expression of *SOMNUS*, a negative regulator of light-dependent seed germination acting on ABA biosynthetic genes, is induced in *drt111* mutants. Together the results reveal the role of *DRT111* in the integration of ABA and light pathways during seed germination.

O^6-alkylguanine-DNA-Alkyltransferase (AGT): lights on the scene.

Rosanna Mattossovich[1], Nada Farag[2], Rosa Merlo[1], Ëukasz Nierzwicki[3], Giulia Palermo[3], Alessandro Porchetta[2], Francesco Ricci[2], Giuseppe Perugino[1].

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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[1]. AGTs are enzymes which mainly remove alkyl adducts from the O^6-position of guanines from DNA, by a peculiar irreversible reaction. 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) has a crucial clinical importance, because it contrasts the effect of chemotherapy's based on alkylating agents, making tumor cells resistant[2]. For these reasons, the development of hMGMT inactivators/inhibitors to be used in combination with this kind of chemotherapy is constantly evolving. As a consequence, 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[3].

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 O^6-methyl-guanine (O^6-MeG) nucleobases, as well as a FRET fluorophores optical pair[3]. 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[4]. I have characterized the folding mechanism induced by the enzymatic repair activity through fluorescent experiments and then I demonstrated that the folding-upon-repair DNA nanoswitches are universal AGTs' substrates, successfully applying to several enzymes, including the hMGMT, the bacterial *E. coli* AdaC, and the archaea *Saccharolobus solfataricus* AGT[5-7]. 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].

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Glycosphingolipid metabolic imbalance in Rett syndrome and development of a pharmacological treatment in Rett models.

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ABSTRACT. Rett syndrome (RTT, OMIM 312750) is a severe neurodevelopmental disease, classified as an autism spectrum disorder (ASD), and considered one of the leading causes of intellectual disability in girls. RTT is caused by mutations in the X-linked *Methyl-CpG-binding Protein 2 (MECP2)* gene, encoding a master epigenetic modulator of transcription with a key role in the nervous system functioning. Although RTT is reversible in principle, no effective therapies are available so far. The identification of new molecular pathways altered in RTT is instrumental to develop *ad hoc* therapeutic strategies focused to cure or ameliorate RTT symptomatology.

Altered content of glycosphingolipids (GSLs), which represent functional lipids enriched in brain, were preliminarily observed in RTT patients. In addition, mutations in ST3GAL5 gene, encoding a key enzyme of the GSL biosynthetic pathway, were found in patients with a RTT-like phenotype. However, neither in-depth approaches to understand the impact of GSL derangements in RTT pathogenesis, nor appropriate therapeutic treatments, have been attempted so far. We found altered distribution and levels of several GSLs in brain of the *Mecp2*$^{-/y}$ RTT mouse model that parallels a deregulation of several genes controlling GSL metabolism (glycogenes). Perturbations of GSL metabolism were further confirmed in murine embryonic stem cell-derived neurons carrying two common RTT mutations, and in iPSC-derived neurons from RTT patients. Moreover, we highlighted that the expression of *Autism susceptibility candidate 2 (AUTS2)* gene, which is mutated in ASDs and encodes an epigenetic factor with a key role in GSL metabolism in neurons, is up-regulated in murine and human RTT models. We demonstrated that in mouse brain MeCP2 directly controls *AUTS2* expression, and both MeCP2 and AUTS2 bind the promoter of *St3gal5*, with a putative regulatory function. Our findings highlighted a novel role of MeCP2 in the control of GSL metabolism in brain, possibly through the modulation of glycogenes and *Auts2* expression, and allow to hypothesize that a perturbation of this process contribute to RTT pathogenesis. In this frame, a therapeutic approach capable to target the MeCP2-AUTS2-GSL axis in RTT might alleviate the disease manifestations. In a pilot study, we showed that a treatment of *Mecp2*$^{-/y}$ mice with Fingolimod, a sphingosine receptor modulating drug known to modulate GLS metabolism, extended the average lifespan of mutant mice and ameliorate the RTT phenotype. The capability of Fingolimod to rescue GSL metabolic alterations in RTT models is currently under investigation.

Overall, our findings might open new perspectives for the set-up of novel therapeutic protocols for RTT patients.

Effect of ionizing radiation on photosynthesis and recovery strategies: implications for plant growth in Space.

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ABSTRACT. Long-duration space missions depend on the realization of Bioregenerative Life Support Systems (BLSSs), which may be considered a 'miniature ecosystem' where humans, plants, and microbes support each other. In BLSSs, plants have fundamental ecological functions: through photosynthesis, the regenerate air, remove CO_2 and produce O_2 and biomass. However, Space is a harsh environment for living organisms, including plants, for its different temperature and pressure conditions, microgravity and high doses of ionizing radiations (IR), which may prevent plant survival. Generally, the effects exerted by IR on plants ranging from detrimental at very high doses to stimulatory at low levels. Besides dose, radiation quality (low or high Linear Energy Transfer - LET), time of exposure, and other plant characteristics (species, developmental stage, intrinsic physiological and genetic traits) differently influence plants' responses.

This overview summarizes the effects of low and high-LET IR on the photosynthetic process in dwarf bean and tomato plants and analyses the safety strategies engaged by photosynthetic machinery to face radiation-induced injuries.

Our results showed that high doses of low-LET IR, namely 50 and 100 Gy X-rays, negatively affected photosynthetic performance in both tomato and bean, reducing total chlorophyll content, photochemical efficiency, carbon assimilation and Rubisco activity. However, these levels also induced photoprotection mechanisms, such as a rise of antioxidants, wall cell phenolic compounds and PARP activity which improved plant radioresistance. This property depends on the plant phenological phase, being young plants more vulnerable to radiation-induced damage than adults.

The exposure of tomato seeds to high-LET IR (25 Gy Ca ions) stimulated photosynthesis and PSII photochemistry in sprouted plants, upregulating the levels of D1 protein and photosynthetic pigments. As a result, these plants also produced fruits with higher contents of ascorbic acid, carotenoids, and anthocyanins than control. However, a different fashion was found in dwarf bean plants germinated by irradiated seeds with 10 Gy C ions. IR reduced photosynthetic activity and light-harvesting pigment content but triggered an overproduction of free radicals acting as a signal for activating plant defence mechanisms promoting antioxidant synthesis and PARP activity.

Our results showed that high doses of low-LET IR compromise the photosynthetic apparatus functionality and act as a signal to induce protective responses. Conversely, low doses of high-LET IR, comparable to levels reached on BLSSs, may be perceived by some plants as a stimulus to produce antioxidants and secondary metabolites to protect photosynthetic apparatus and the whole cell from oxidative stress.

Characterization of NMN Deamidase Mutants as Possible Probes for an NMN Biosensor.

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ABSTRACT. Biosensors are powerful tools for detecting analytes of different nature and monitoring changes in their concentration, not only *in vitro*, in biological samples, but also *in vivo*, at cellular and extracellular levels[1]. Recently, several biosensing strategies have been explored, for example, for the detection of cellular metabolites, providing valid methodologies alternative to HPLC/MS-MS and fluorometric assays[2,3].

In this context, we are interested in the development of a biosensor for Nicotinamide Mononucleotide (NMN), a key intermediate in the nicotinamide adenine dinucleotide (NAD^+) biosynthesis, whose supplementation has demonstrated beneficial effects on several diseases.

The first, crucial step, for designing a biosensor is the identification of an appropriate molecular recognition element (MRE), working as a probe to capture the analyte of interest, and inactivated enzymes are among the biomolecules of choice as MREs for small-molecule detection. Indeed, it is of fundamental importance that the MRE is able to bind the substrate specifically, with a relatively high affinity (with regards to the physiological concentrations to be measured), but not to transform it into a product[4].

Here, our recent results on the characterization of two NMN deamidase (PncC) inactive mutants, as MREs for an NMN-specific biosensor, will be discussed. In particular, results of thermal stability assays and steady-state fluorescence spectroscopy measurements performed to address the binding of NMN and related metabolites (NaMN, Na, Nam, NR, NAD, NADP, and NaAD) to the PncC mutated variants, will be presented[5].

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Interfering with NF-*k*b in anaplastic thyroid carcinoma.

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ABSTRACT. Most human cancers are characterized by alterations of the NF-kB pathway[1,2]. Despite NF-kB represents an ideal candidate for targeted therapy, to date no pharmacological inhibitors have been approved for clinical practice, due to its pleiotropic roles and to the adverse effects resulting from its global suppression[3,4]. For these reasons, in the last two decades, efforts have been made to identify and target downstream effectors of the NF-kB pro-survival activity.
GADD45β, a member of the Growth Arrest and DNA Damage family proteins, was identified as an anti-apoptotic gene transcriptionally regulated by NF-kB. The GADD45β pro-survival activity relies on its ability to bind and inhibit MKK7, thus blocking prolonged pro-apoptotic JNK signaling[5-7]. In this context, Anaplastic Thyroid Carcinoma (ATC) is characterized by a strong activation of NF-kB and consequently also GADD45β expression is increased both in primary samples as well as in validated cell lines[8]. It was recently developed a D-tripeptide, named DTP3, which can physically displace the interaction between GADD45β and MKK7 thus preventing the suppression of the JNK pathway[9]. The DTP3 was shown to exhibit a potent and cancer-cell selective ability to induce JNK-dependent apoptosis of Multiple Myeloma cell lines and primary cells from patients, both *in vitro* and *in vivo*, without any toxicity[10].
On this basis, we are currently evaluating the feasibility of DTP3 treatment also for ATC. Our cellular model is composed of three ATC cell lines: BHT-101, CAL-62 and 8505c, which display a differential expression of GADD45β. The cell entry of FITC-tagged DTP3 and a control scrambled peptide (SCR) was confirmed by Flow Cytometry and Confocal Microscopy. We will discuss the effects of DTP3 treatment in terms of proliferation, apoptosis, alterations of the cell cycle and preliminary biochemistry, with particular regards for the high GADD45β expressing line CAL-62, which also showed the highest sensitivity towards the DTP3.

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From the discovery of hyperstable CAZymes to translational recoding: the archaeal α-L-fucosidase from *Saccharolobus solfataricus*.

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ABSTRACT. Genetic decoding is not frozen but flexible, due to programmed deviation of the ribosomes from standard translational rules, globally named recoding[1]. Recoding, which includes stop codon readthrough, programmed ±1 frameshifting, and ribosome bypassing, has been found in all domains of life and reported in Archaea for stop codon readthrough, that regulate the incorporation of 21st and 22nd amino acids selenocysteine and pyrrolysine, and for -1 programmed frameshifting (-1 PRF) which allows the expression of a fully functional α-L-fucosidase in the crenarchaeon *Saccharolobus solfataricus*[2-5].

Although this phenomenon is important for the regulation of protein expression, the physiology and the adaptation of organisms, little is still known about the genes whose expression could be regulated by recoding in Archaea. Here we report on the serendipitous identification of the first archaeal glycoside hydrolase expressed by programmed -1 frameshifting, and on the transcription analysis of this recoded gene and of its full-length mutant in different growth conditions, *in vivo*[6]. In addition, the discovery of new potential recoded archaeal genes in metagenomes from extreme environments, suggests that recoding is a universal mechanism of regulation of gene expression and that interrupted genes could be functional in vivo and might provide an evolutionary advantage in extreme environments.

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Protein-based biosensor as tool for "Farm to Fork" strategy.

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ABSTRACT. During the last years, emerging biosensor technologies are developed for the detection of analytes of interest for agri-food, environment, security, and health. Consequently, biosensors have acquired increasing importance in a wide range of applications.

Typically, a biosensor device results composed of at least of three principal elements[1]: (1) the molecular recognition elements (MREs) that recognize the molecular target and, consequently, upon the binding of the target molecule, it generates a detectable signal; (2) a "transducer" that is able to convert the generated signal; (3) an amplifier, that is able to quantify and transfer the signal to the operator.

The central core of the biosensor is the biological element (enzyme, binding protein, nucleic acid sequence, antibody, microorganism, part of a tissue, cell, etc.) that defines its specificity and selectivity. In this context, proteins[2], even isolated from extremophiles[3], organisms possess special structural and functional features (such as high specificity and selectivity towards a target substrate), and they provide numerous advantages if used as MREs.

Here, I will present the latest developments in protein-based biosensors to detect contaminates (toxins[4], antibiotics[5], hormones[6], and food allergens) in the agri-food field to improve the effectiveness and efficiency of food safety and food security chain, objectives of the EU program "Farm to Fork". These approaches allow to monitor the quality chains from food farming, production, process, packaging, transportation to all the consuming ways.

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Sustainable plant-based production of SOD from *Deinococcus radiodurans* for food applications.

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ABSTRACT. Oxidative stress generates harmful compounds that are responsible for the gradual reduction of sensory and nutritional quality of highly perishable foods such as meat and fish, thus affecting consumer acceptance. Therefore, the control and minimization of this process in fresh products is of great interest to the food industry. In view of this, some technologies have been developed and to prevent the deleterious effects of oxidation and to increase the shelf-life of these products, such as addition of antioxidants. However, great consideration is paid to the utilization of natural antioxidants due to the harmful side effects imparted by synthetic counterparts.

In our study, superoxide dismutase (SOD) deriving from the polyextremophilic bacterium *Deinococcus radiodurans* (SOD_{Dr}) was expressed in *Solanum lycopersicum* (tomato) cell cultures. The plant extracts enriched in extremoenzyme were used for the treatment of tuna fillets, to evaluate their potential use as natural additives, taking advantage of the extraordinary features of SOD_{Dr} in terms of high stability and UV resistance. The obtained results demonstrated that the tuna fillets treated with the plant extracts enriched in SOD_{Dr} preserved their colour and brightness after 8 days of treatment respect to those treated with the untransformed-cell extracts. This study suggests that the recombinant plant extracts could be advantageously used in formulations for the food preservation industry to prevent the deleterious effects of oxidation and preserve the sensory properties of fresh products, extending their shelf life.

Living with global environmental changes: the case of *Posidonia oceanica*.

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ABSTRACT. Coastal marine environments are particularly affected by rapid and extreme environmental changes with dramatic consequences for the entire ecosystem. The intensification of anomalous events of seawater warming and the co-occurrence of different anthropogenic stressors such as the increase of nutrients inputs are threatening coastal marine habitats, including seagrasses, which form extensive underwater meadows. Seagrasses form a unique group of marine plants that have colonized heterogeneous environments along coastlines supporting diverse and productive ecosystems. Among seagrasses, *Posidonia oceanica* (L.) Delile (1813) is an endemic species of the Mediterranean Sea, ranks amongst the slowest- growing and longest-lived plants on earth. However, sea warming and the rapid occurrence of different anthropogenic pressures result in cumulative impacts that are forcing native populations to respond quickly. In this context of rapid environmental changes, studying their responses to different environmental stresses is fundamental for exploring their resilience capacity. Here, we explored the degree of phenotypic responses of *P. oceanica* plants growing in environments with different nutrients conditions (oligotrophic plants, Ol; eutrophic plants, Eu) to single and multiple stresses. For this purpose, collected shoots were exposed to temperature and nutrient enrichment in a multi-factorial experiment performed in a mesocosm system. Plants' performances were assessed using an omic approach exploring the physiological status at the leaf level and transcriptional profiles in leaves and shoot apical meristems (SAMs) in both Ol and Eu plants. Since epigenetic mechanisms, including DNA methylation, consist of regulatory machinery involved in gene regulation under environmental stresses, we also explored dynamics of DNA methylation changes in both Ol and Eu plants during the experiments analysing global DNA methylation levels and the expression of key genes involved in DNA methylation. Results revealed a different degree of responses among plants. Nutrients induced the greatest effect in the leaf in both Ol and Eu plants, with the regulation of different nutrient-balancing strategies. The transcriptomic analysis also differed between Ol and Eu plants and confirmed different vulnerabilities to nutrients at the leaf level. Ol plants were more vulnerable to nutrients stress in respect to Eu plants where the treatment with temperature increases induced the largest transcriptomic regulation. Leaf and SAMs tissues displayed different transcriptional profiles with the last activated a larger number of biological processes under warming conditions. Global DNA methylation levels and the expression dynamics of selected genes were influenced by both plants' origin and the duration of the imposed stresses. These findings suggest that different life histories of *P. oceanica* plants are crucial to understanding the future persistence of this species under rapid environmental changes. Local environmental conditions modify plant responses to environmental stresses through the appearance of different phenotypes affecting transcriptional regulation. Moreover, DNA methylation levels appeared to be a dynamic process that could potentially regulate phenotypic responses to environmental changes depending on local environmental conditions experienced by plants in their home environments. These considerations are crucial for better understand the resilience capacity of marine plants to future local and global environmental changes and to support management strategies.

LAMC2 drives tumorigenicity and metastasis in pancreatic ductal adenocarcinoma.

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ABSTRACT. Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of cancer-related mortality with limited therapeutic options. Cancer stem cells (CSCs) are key player in PDAC chemoresistance, tumor initiation and metastatic spreading, but the mechanism through which they acquire metastatic traits is not well understood. Hence, targeting the CSC niche and their plasticity could be a complementary therapeutic strategy against cancer. Laminin subunit-γ-2 (LAMC2) is an epithelial basement membrane protein, which controls cell motility and adhesion and is widely expressed in the majority of human tumors. However, its role in PDAC remains largely unknown. Here, we propose LAMC2 as a key driver of PDAC stemness and tumorigenicity.

In several patient cohorts we observed that high levels of LAMC2 significantly correlated with shorter overall survival. In addition, the tissue microarray analysis on PDAC sections revealed prognostic significance of LAMC2 expression in tumor with high grade of aggressiveness (i.e., G2 and G3). To determine the role of LAMC2 in sustaining tumorigenicity, we knocked down it in patient-derived xenografts (PDX) cells using lentiviral shRNA constructs. The silencing of LAMC2 resulted in decreased self-renewal, invasiveness and gemcitabine resistance both *in vitro* and *in vivo*. To track the LAMC2 tumor cell population in an intact environment we engineered primary PDAC cells that carry EGFP cassette knocked in the LAMC2 locus through the CRISPR-Cas9 technique. *In vivo* experiments revealed increased tumorigenicity of the LAMC2High-EGFPHigh cells respect to LAMC2Low-EGFPLow cells, while RNA-seq data obtained from cells extracted from tumors confirmed a gene program similar to that of highly metastatic stem cells and that they initiate and propagate both the primary tumor and the metastasis to recipient mice very efficiently compared to their counterpart. In addition, Gene Set Enrichment Analysis (GSEA) indicates that LAMC2 is enriched in the squamous molecular subtype of pancreatic cancer, which is the one associated with the worse prognosis.

In conclusion, we identified a highly metastatic subpopulation of cancer stem cells, characterized by high levels of LAMC2. Strategies aimed at targeting the LAMC2 population may be effective in reducing tumor aggressiveness in combination with conventional therapy.

Sustainable biorefineries: strategies for modifying metabolic fluxes in cardoon cells.

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ABSTRACT. The biomass demand to fuel a growing global bio-based economy is expected to heavily increase over the next decades, and projections indicate that dedicated biomass crops will satisfy a large portion of it. In this context, cultivated cardoon (*Cynara cardunculus* L.), a multi-year food crop requiring low agronomical inputs and producing high biomass even in limiting growth conditions, has raised attention for cultivation in marginal lands, where it does not compete with food and feed production. Indeed, cardoon is considered one of the most promising biomass crops in Mediterranean areas and has attracted large investments as a sustainable source of biomaterials for bioplastic production. However, as a crop-derived commodity, cardoon biomass suffers from seasonal availability and variable quality and quantity. In this regard, cardoon cell cultures could be a valuable alternative to field cultivation, providing large-scale production of fatty acids and polyphenols to be used as bioplastics and biofuel precursors, as well as biochemicals and valuable compounds, thus exemplifying the concept of biorefinery. To explore the potential of cardoon cell cultures as sustainable biorefineries, we designed strategies to direct metabolic fluxes towards the accumulation of the molecules of interest. Increased oleic acid content was addressed through metabolic engineering of fatty acid biosynthesis. Cardoon leaves were selected as the most suitable tissue for obtaining calli cultures. Specific fatty acid biosynthesis genes, SAD (stearic acid desaturase) and FAD2.2 (fatty acid desaturase), were selected and used to transform leaf-derived calli through *A. tumefaciens*. SAD-overexpressing and FAD-silenced transgenic calli lines were molecularly and metabolically characterized to verify the metabolic flux towards higher accumulation of oleic acid, confirming increased unsaturated fatty acids content. Abiotic stress tolerance mechanisms are known to be associated with enhanced biosynthesis of biologically active compounds. Moreover, the accumulation of biomolecules of interest could be achieved by manipulating either cell growth and/or molecular fluxes. The DNA-binding with one finger (*Dof*) family is a class of plant-specific TFs involved in several biological processes, including response to abiotic stresses and plant development. In order to identify possible targets for genetic manipulation, we performed the first genome-wide identification of *Dof* TFs in cardoon, resulting in 39 family members. Bioinformatic analyses allowed selection of seven candidates and among these, *CcDof18* and *CcDof20* resulted highly induced in salt- or in cold-stressed cardoon calli, respectively. Their functional exploration is ongoing in order to confirm their possible role(s) in the regulation of the response to abiotic stresses, and their suitability for biotechnological approaches to improve the yield of compounds for green chemistry. Scale-up of suspension cell cultures of the transgenic lines at a pilot-scale and the use of industrial and/or agricultural waste/by-products as components of nutritive substrates could contribute to the set-up of a sustainable, innovative biotechnological approach to provide valuable supplies for biocosmetics and green chemistry, according to the principles of Circular Economy.

Pangenomic analysis of *Bacillus coagulans* MA-13: a promising thermophilic strain for production of bioactive molecules from agricultural wastes.

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ABSTRACT. Lignocellulosic biomasses represent an alternative to the finite nature of fossil fuels as promising and suitable energy source. Recently, a novel thermophilic *B. coagulans* strain, designed as MA-13, has been isolated from canned beans manufacturing and shown to be able to produce lactic acid from lignocellulose biomass[1,2]. Interestingly, it was demonstrated that pre-exposure of *B. coagulans* MA-13 to hydrolysate supports physiological adaptation to the fermentation medium, resulting in a considerable reduction of lactic acid production costs[3].

Furthermore, *B. coagulans* strains are generally recognized as safe (GRAS). One of the most challenging food consumption issues is how to ameliorate the digestibility of foods containing not-digestible galactosides, associated with intestinal gas and discomfort. A full biochemical characterization of the β-galactosidase (named *Bc*GalB) from of *B. coagulans* MA-13, revealed that the enzyme is able to produce GOS in homo- and hetero-condensation reactions from artificial and natural substrates, thus proving the ability of *B. coagulans* MA-13 to produce prebiotics from dairy food waste[4].

Finally, to expand our understanding of the intra-strains genomic diversity of *B. coagulans* and to provide new insights on its genetic potential in biotechnological applications, a pangenomic study is under way. A full repertoire of CAZymes, secretion systems and resistance mechanisms to environmental challenges was analysed to shed light on the genetic potential of this specie in biotechnological applications. Moreover, the B. coagulans Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) immune system along with CRISPR associated (Cas) genes, was also investigated. Overall, this pangenomic analysis expands our understanding of the inter-strains genomic diversity of *B. coagulans* in order to fully exploit its potential in biotechnological applications.

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Adiponectin protects human neuroblastoma cells against cytotoxicity induced by cerebrospinal fluid from patients affected by multiple sclerosis.

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ABSTRACT. Adiponectin (Acrp30) is an adipokine linking energy metabolism and immune system. Recently, we showed that Acrp30 is detectable in cerebrospinal fluid (CSF) and its increase is related to severity and prognosis of Multiple Sclerosis (MS), an autoimmune demyelinating disease of the human central nervous system.

The aim of this study was to investigate the effects of Acrp30 on SHSY5Y cells, *in vitro* model of neuroblastoma, exposed to CSF from MS patients.

To meet this purpose, we first verified the expression of Acrp30 receptors (AdipoRs and T-cadherin) and then SH-SY5Y cells were treated with CSF and/or Acrp30 to evaluate cell viability, oxidative stress, and the expression levels of some inflammatory mediators involved in the pathophysiology of MS (IL-6, IL-10, TNF-alpha, INF-γ) by MTT, nitrite assay and Real time PCR.

Our results demonstrated that Acrp30 moderately reduces CSF cytotoxic activity on SH-SY5Y cells. The CSF toxic effects are partially mediated by oxidative stress, as demonstrated by induction of nitric oxide; again, Acrp30 is able to reduce release of nitric oxide induced by CSF exposure. Finally, we found that CSF treatment induces the expression of INF-γ on SH-SY5Y cells and that Acrp30 partially reverts this effect.

Taken together, our data demonstrated that Acrp30 protects SH-SY5Y cells against MS CSF-induced cytotoxicity modulating the oxidative stress and the expression of INF-γ, a major mediator involved in inflammatory response in MS.

Breast cancer-derived exosomes trigger stromal fibroblasts in tumor microenvironment via microRNA cargo.

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INTRODUCTION. Triple negative breast cancer (TNBC) is the most aggressive breast cancer subtype, because of its high metastasis potential. TNBC clinical prognosis and patient treatment are undermined by the recruitment of a strong and unique tumor microenvironment (TME), that is mainly composed of activated fibroblasts (Cancer-Associated Fibroblasts-CAFs) able to endorse tumor hallmarks. Increasing evidence demonstrated that exosomes can mediate the cross-talk between cancer cells and TME. Accordingly, we examined the contribution of TNBC-derived exosomes and their microRNAs (miRNAs) cargo in the activation of normal fibroblasts (NFs) towards CAFs.

METHODS. Exosomes were obtained from TNBC cell line (MDA-MB-231) and incubated with primary cultures of normal fibroblasts (NFs) to study their activation. Thus, *in vitro* transwell migration and contraction assays, together with western blot and RT-PCR analysis were performed on NFs upon TNBC-derived exosome treatment to demonstrate the functional and molecular conversion of NFs to CAFs. Moreover, an organotypic co-culture model was set up with exosome-activated fibroblasts and normal epithelial breast cells (MCF10A) to study the impact of fibroblast activation on breast cells invasion ability in an *in vivo*-like environment. Finally, a small-RNA sequencing was performed on NFs treated with TNBC-derived exosomes to investigate the involvement of exosomal miRNA cargo in the transformation of NFs into CAFs.

RESULTS. We examined the contribution of TNBC-derived exosomes with their miRNA cargo in the transformation of normal fibroblasts (NFs) into CAFs. Firstly, we demonstrated that NFs treated with TNBC-derived exosomes exhibited increased collagen contraction and cell migration abilities, widely reported as the principal hallmarks of activated fibroblasts within the TME. Furthermore, we observed that those exosome-activated fibroblasts promoted the invasion potential of normal breast epithelial cells as assessed by the organotypic co-culture model. Therefore, we investigated the role of exosomes cargo in mediating the activation of NFs to CAFs, by performing a small RNA-sequencing on NFs treated with TNBC-derived exosomes compared to control. We found several miRNAs up-regulated in NFs after exosome treatment. In particular, we observed that the synergistic action of miRNAs-185-5p, -652-5p, and -1246 was able to strongly activate fibroblasts. Indeed, the combination of these three miRNAs boosted fibroblast migration and contraction abilities, thus promoting a specific CAF subspecialization towards a pro-migratory functional state.

CONCLUSIONS. All together these data highlighted the role of breast cancer cells in the re-education of TME, thus contributing to tumor evolution.

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Mapping the high-risk Multiple Myeloma cell surface proteome identifies T-cell inhibitory receptors for immune targeting.

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Background. Multiple myeloma (MM) is an incurable malignancy of mature plasma cells. Despite major advances in the therapeutic armamentarium of MM, only 50.7% of the patients survive more than 5 years after diagnosis, with significantly lower rates (21%) for high-risk patients.

Chimeric Antigen Receptor (CAR) T-cell therapy targeting BCMA (B-cell maturation antigen) shows high response rates in relapsed/refractory patients. However, most patients have disease remission that lasts less than 18 months, prompting the search for additional and synergistic therapeutic approaches.

Methods. We unbiasedly mapped the cell surface proteome of MM by integrating Mass-Spectrometry (MS) and RNA-seq analyses from 7 MM cell lines and 904 primary MM patient samples bearing high-risk cytogenetics. To identify cell surface proteins, we ran a pool of 4,761 proteins and 16,000+ transcripts through five repositories. An integrated scoring database was developed by scoring each ID based on the number of databases it was identified in, with 0 denoting the molecule was not found in any and 5 denoting the protein was found in all five.

Results. We identified 402 proteins with a surface score equal or higher than 3 and commonly detected in MM cell lines and patient samples by transcriptomics and proteomics. We prioritized the 326 candidates that were more highly expressed in patients. Based on functional enrichment analyses, we found that these proteins formed three main protein networks with immune mechanisms representing the largest cluster followed by transporters and adhesion proteins. Based on a previously developed pipeline (Perna F *et al.*, Cancer Cell 2017) we further selected 67 candidates minimally expressed in normal tissues. Validation in primary patient samples by western blot and flow-cytometric analyses enabled the identification of 10 top proteins most highly and frequently expressed. As importantly, we defined candidate protein abundance in highly-purified normal hematopoietic stem cells and activated T-cells, narrowing down the list to 6 potential immunotherapeutic targets.

In order to define the function of this group of promising cell surface targets, we used a CRISPR/Cas9 inducible system in KMS11 cells. We found that knock-out of *CCR1*, *LRRC8D* and *SEMA4A* individually reduces the cell growth in time manner by ~60, 50 and 50 respectively, 120 hours after seeding and leads to an >80% inhibition of their migration capacity in vitro. Co-culture assays with KO MM cells and healthy donor T-cells showed increased number of T-cells, that arrives at ~50% with CCR1 KO, and 20% with LRRC8D and SEMA4A KO, enhanced killing of MM cells compared to controls, with a reduction of MM cells about 40%.

Conclusions. This work suggests that targeting CCR1, LRRC8D and SEMA4A might promote T-cell mediated immune responses, representing a novel therapeutic approach in MM.

CRISPR-mediated editing of the murine immunoglobulin heavy chain gene locus for the generation of an "antarctized" monoclonal antibody.

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ABSTRACT. Immunoglobulin M (IgM) is the major circulating Ig isotype in teleost fish. Unique features of the IgM molecule have been uncovered in Antarctic fish species, e.g., an extraordinary long hinge region, crucial for the flexibility of the Ig molecule[1]. This structural peculiarity can be viewed as a result of adaptive evolution to enhance the functionality under very cold environmental conditions. This finding prompted the idea to modify a murine monoclonal antibody (mAb) by replacing its hinge with that from Antarctic Ig by using the CRISPR-Cas9 system[2]. Over the past few years, given its simplicity and flexibility, the CRISPR-Cas9 system has been successfully applied also in the field of immunology to edit mouse and human Ig genes[3-4]. A stepwise approach was chosen for targeted genome editing of a hybridoma cell line secreting IgG mAbs. The first step was the creation of a targeted DNA double-stranded break at the hybridoma Ig heavy chain constant region gene locus to be modified. Homology-directed repair was then used to insert the Antarctic hinge sequence through recombination of the target locus with a DNA donor template. The correct sequence insertion was assessed by using a fluorescent protein as selection marker. The "antarctized" mAb was successfully produced by the engineered hybridoma cell line. A preliminary biochemical and functional characterization in comparison to the wild type counterpart was performed. Overall, the results of this work project could be a promising starting point for engineering mAbs with an innovative and versatile technology.

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Unravelling the effects of microplastic-associate biofilm in the uptake and immunological response in the sea urchin *Paracentrotus lividus*.

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ABSTRACT. Thanks to their peculiar surface properties, microplastics (< 5 mm, MPs) are colonized by a microbial biofilm, representing a new habitat with a distinct biological community, known as "plastisphere". Such colonization contributes to the continuous transformation of MPs in the marine environment. Although the microbial biofilm growing on MPs has been the object of several investigations, little is known about how this might affect MPs interaction and response in marine organisms. To this aim, adult sea urchins *Paracentrotus lividus* were exposed to either virgin or biofilm-covered polystyrene microbeads (micro-PS, 45µm) and the effect of microbial colonization on the uptake, biodistribution and immune response investigated. Bacteria were predominant in micro-PS biofilm as evidenced by Scanning Electron Microscopy and 16S rRNA sequencing. Colonized micro-PS were higher internalized in sea urchins compared to virgin ones, suggesting a role of the plastisphere in the micro-PS uptake. However, both colonized and virgin micro-PS showed the same distribution pattern inside sea urchin body and mainly retained by the digestive system. On the other hand, colonized micro-PS induced a significant increase in catalase and total antioxidant activities compared to virgin ones in digestive system of sea urchins. Furthermore, colonized micro-PS caused a significant decrease in the number of coelomocytes. Moreover, a general time-dependent increase of reactive oxygen species and decrease in nitrogen ones was observed upon exposure to both colonized and virgin micro-PS. Overall findings suggest that micro-PS colonization influences both uptake and toxicological responses of the Mediterranean Sea urchin *P. lividus*.

Next Generation Sequencing (NGS) for the diagnosis of autosomal dominant/recessive polycystic kidney disease.

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ABSTRACT. Autosomal dominant polycystic kidney disease (ADPKD) is an autosomal dominant disorder with an onset of 1:1000. The genetic alterations responsible for ADPKD are within the PKD1 and PKD2 genes. The pathogenic variants in PKD1 cause a previous onset and a most severe phenotype of the disease than those in PKD2. Autosomal recessive polycystic kidney (ARPKD) is a fibrocystic hepatorenal disease characterized by an early end stage renal disease and high morbidity and mortality caused by mutations in PKHD1 gene. PKD3 is an autosomal dominant PKD caused by mutations in GANAB gene which encodes the subunit alpha's glucosidase II and characterized by the presence of renal and liver cysts.

Families might benefit from the genetic diagnosis improving the clinical management approaching to emerging therapeutic options. Obtaining a genetic diagnosis of ADPKD is complex because of historical segmental intrachromosomal duplication of three-quarters of the PKD1 gene, which produce six pseudogenes with a 99% sequence homology with PKD1.

The aim of our study was to perform the genetic analysis of PKD patients through a Next Generation Sequencing (NGS) and a Sanger sequencing approach. We established and validated a NGS panel of 20 genes known for PKD and analysed a cohort of 60 patients from the nephrology and gastroenterology department of the University of Naples "Federico II". An additional aim of this study was to perform a genotype-phenotype correlation to contribute to predict the prognosis.

Our results revealed mutations in PKD1, PKD2, PKHD1 and GANAB genes in 63%, 17%, 5% and 2% of affected patients respectively, with a detection rate of 87%.

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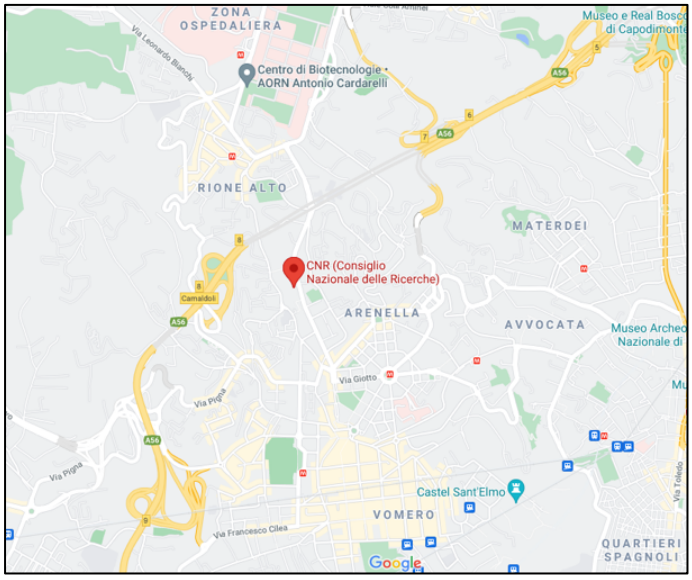

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