XVI FISV CONGRESS

FISV
FEDERAZIONE ITALIANA
SCIENZE DELLA VITA

3R: Research, Resilience, Reprise

14 – 16 September 2022 Reggia di Portici, Naples

ABSTRACT BOOK

AAI ABCD ADRITELF AGI BITS SIB SIBE SIBBM SIBV SIBS SICA SIFarma SIFisio SIGA SIGU SIMAG SIMGBM SIPMET SIPAV SITOX

I am pleased to announce the ***XVI edition of the Congress of the Italian Federation of Life Sciences (FISV)****, which will take place from 14 to 16 September 2022 in Naples at the Università degli Studi di Napoli Federico II, Department of Agriculture, located in the historical Reggia di Portici.*

Our Federation is presently composed by twenty Scientific Societies (AAI, ABCD, ADRITELF, AGI, BITS, SIB, SIBBM, SIBE, SIBS, SIBV, SICA, SIF (The Italian Society of Pharmacology), *SIF* (The Italian Society of Physiology), *SIGA, SIGU, SIMAG, SIMGBM, SIP MeT, SIPaV, SITOX), representing different aspects of the Life Sciences. I would like to thank them all for having contributed to put together an outstanding program, aiming at fostering interdisciplinary exchange at the forefront of the life sciences. Internationally renowned scientists from Italy and abroad will present their most recent data; young Italian and foreign scientists will be welcomed to present their research in a highly stimulating and interactive scientific environment. We will also hold round tables on selected hot topics as well as talks dedicated to a broader audience to enhance the interaction between scientists and non-scientists.*

We are sure that you will enjoy very much this exciting Congress.

Gennaro Ciliberto
FISV President

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IRCCS Regina Elena National Cancer Institute

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AGI
Associazione Genetica Italiana

SIMGBM
Società Italiana di Microbiologia Generale e Biotecnologie Microbiche

SIB
Società Italiana di Biochimica e Biologia Molecolare - 1951 -

SIBE
Società Italiana di Biologia Evoluzionistica

SICA
Società Italiana di Chimica Agraria

SIF
SOCIETÀ ITALIANA DI FARMACOLOGIA

S.I.M.A.G.
Società Italiana di mutagenesi Ambientale e Genomica

abcd
Associazione di Biologia Cellulare e del Differenziamento

SIMGBM
Società Italiana di Microbiologia Generale e Biotecnologie Microbiche

A.D.R.I.T.E.L.F.

SIBS
Società Italiana di Biologia Sperimentale
1925

SIF
Società Italiana di Fisiologia
Fondata nel 1947

SIPaV
Società Italiana di Patologia Vegetale
Italian Phytopathological Society

SIP MET
Società Italiana di Patologia e Medicina Traslazionale

SITOX
Società Italiana di Tossicologia

AAI
Associazione Antropologica Italiana

SOCIETÀ ITALIANA DI BIOLOGIA VEGETALE

Società Italiana Genetica Agraria

BITS
Società di Bioinformatica Italiana

Società Italiana di Genetica Umana

preclinics ITALIA

Promega

EMBL
European Molecular Biology Laboratory

BMR Genomics

Nikon

SIC Società Italiana Chimici
Life Sciences

GILSON

deltek DX

IGATech

tebu-bio
Innovative Lab Services & Reagents

EuroClone
serving science through innovation

LGC Standards

ThermoFisher
SCIENTIFIC

AUROGENE
#weservescience

MERCK

SEQUENTIA
DECODE • DISCOVER • DISRUPT

ZEISS

SARSTEDT

VODEN

EdiSES

BIOSPA

FEBS

PeerJ
Life & Environment

Biosigma
a Dominique Dutscher Company

EMBO
excellence in life sciences

ZANICHELLI

14th SEPTEMBER

13.30 **Welcome**
Prof. Gennaro Ciliberto, FISV President

14.00-15.00 **The EMBO Keynote Lecture**
Riccardo Cortese
Chair:

Angela Nieto
(*Istituto de Neurociencias, CSIC-UMH, Alicante, Spain*)
Aberrant activation of developmental programs in adult disease

15.00-15.30 ***Coffee Break***

15.30-17.00 **Poster Vision 1**
P1. Environmental, Evolutionary and Developmental Biology
P2. Genetics, Epigenetics and Chromosome Biology
P4. Current Trends in Biotechology
P6. Health and Disease

17.00-17.15 **Takis presentation**

17.15-17.30 **Preclinics Italia Presentation**

17:30- 19:30 **PARALLEL SYMPOSIA**

1a. From plastics revolution to bioplastics evolution

Chairs: *Raffaele Porta & Paola Chiarugi*

Vincenzo Busico (*Naples*)
The plasticsrevolution: a critical review
Loredano Pollegioni *(Varese)*
Plasting-eating enzymes: a protein engineering studies
Loredana Mariniello *(Naples)*
Additives and Processing Aids for Bio-based Plastics from Renewable Sources.
Fabrizio Adani *(Milan)*
Bioplastic in the circular economy

1b. Diversity and evolution of genomes

Chairs: *Omar Rota Stabelli, Maria Ercolano, Luca Sineo*

Manuela Sironi (*Lecco*)
Evolutionary genomics of human coronaviruses
Donato Giovannelli (*Naples*)
Trace elements, proteins and biogeochemistry: a comparative genomicperspective.
Giorgio Bertorelle (*Ferrara*)
The mutation load in small populations
Stefania Vai (*Florence*)
Surfing on the Evolution of the Human Genome

19.30 ***AMA Choir in concert – La natura in musica***

20.00 ***Welcome Cocktail***

15th SEPTEMBER

09:00 11:00 PARALLEL SYMPOSIA

2a. Microbiome modulation for improving human and plant health

Chairs: *Franco Faoro, Loredana Baccicalupi*

Gabriele Berg (*Graz University of Technology, Austria*)
The plant microbiome explored: implications for plant as well as planetary health

Maria Rescigno *(Milano)*
The microbiota and the immune system in gut-liver-brain axis.

Duccio Cavalieri (*Florence*)
Microbiome a gut-brain axis

Silvia Perotto ***(****Turin)*
Synergistic and complementary interplay between roots and associated microorganisms to boot plant resilience.

2b. The COVID-19 pandemic/tsunami: a scientific revolution from mechanisms to drugs

Chairs*: Giulia Piaggio & Gianni Sava*

Mario Capasso *(Naples)*
Human genetic susceptibility to COVID-19: implications in prevention and treatment

Giuseppe Nocentini *(Perugia)*
Come la pandemia ha cambiato i paradigmi di ricerca e sviluppo di farmaci e vaccini

Mirko Cortese *(Naples)*
Molecular and structuraldeterminants of SARS-CoV-2-induced cellularremodeling

Marta Giovanetti *(Rome)*
Facing the coronavirus crisis: Genomic evolution of SARS-CoV-2"

11.00 -11.30 ***Coffee Break***

11.30-13.00 Tavola Rotonda

Chairs: *Michele Morgante & Gennaro Ciliberto*

Nuove opportunità per la ricerca Italiana.

Centro Agritech: Matteo Lorito

Centro RNA: Rosario Rizzuto

Centro Biodiversità: Danilo Porro

13.00-13.30 ***Lunch Break***

13.30-15.00 Poster Vision 2

P1*. Environmental, Evolutionary and Developmental Biology*

P3*. Genomics, Proteomics and System Biology*

P4*. Current trends in Biotechnology*

P5*. Food and Nutrition*

P6*. Health and Disease*

15.00 - 17.00 PARALLEL SYMPOSIA

3a. A Plant adaptation and resilience to climate change

Chairs: *Manuela Rigano & Francesco Loreto*

Josep Peñuelas *(Barcelona)*
Climate and nutrient imbalances as drivers of a recent global decline in the effects of CO2 and nitrogen fertilization on vegetation growth

Luisa Maria Sandalio *(Granada)*
Reactive oxygen species in the interface of environmental changes sensing and plant resilience

Anca Macovei *(Pavia)*
DNA Damage Response (DDR) and genome stability at the crossroad between plant development and stress resilience

Matthew Haworth *(Firenze)*
Stomatal control of photosynthesis in response to past and potential future climate change

3b Updates in Neuroscience: a comparative and phylogenetic glance

Chairs: Roberto Feuda & Giuditta Gambino

Emiliano Bruner *(Burgos)*
Fossils and neurosciences: synergies for evolutionary anthropology
Maria Ina Arnone (*Napoli*)
The journey of a nerve net towards a brain: a lesson from the sea urchin

Marina Boido *(Turin)*
Neuromuscular junctions: from development to aging and disease
Marco Rasile, (*Milan*)
Effects of maternal immune activation on the offspring's neurovascular unit

17.00-17.30 ***Coffee Break***

17.30-18.00 **Cornelius Gross**
(EMBL, Monterontondo)
The Future of Molecular Biology – The EMBL Perspective

18.00-20.00 ***Societies Time***

20.15 ***Social Dinner at Villa Signorini***

16th SEPTEMBER

08.45-09.45 **FEBS National Lecture**
Chairs: *Paola Chiarugi & Martino Bolognesi*
Carlo Catapano (*Bellinzona*)
Mitochondrial plasticity and stemness in human cancers

09.45 – 10.00 **Sequentia presentation**

10.00-10.30 ***Coffee break***

10.30-13.30 **MINI SYMPOSIA**

1. Environmental, Evolutionary and Developmental Biology

Chairs: *Michela Zottini – SIBV, Alessandro Achilli – AGI, Emiliano Trucchi – SIBE*

Maria Teresa Vizzari *(Ferrara)*
Robust demographic inference from low-coverage whole-genome data through Approximate Bayesian Computation
Sara Del Duca *(Sesto Fiorentino)*
Molecular mechanisms for the evolution of gene structure and organization: the histidine case
Anna Olivieri *(Pavia)*
Barn swallows: mitogenome relationships and phylogeography
Andrea Siverj *(San Michele All'Adige)*
Large-scale genome reconstructions from human gut metagenomes to study phage-host relationships
Sonia Renzi *(Sesto Fiorentino)*
The transition from a traditional to a Western lifestyle and its effect on the interrelation between diet, gut microbiome and health
Costanza Cannariato *(Florence)*
A molecular portrait of ancient Pompeians
Serena Capitanio *(Turin)*
Investigating the role of clathrin-mediated endocytosis in Myc-factors perception in arbuscular mycorrhizas
Edoardo Tosato *(Bologna)*
The likely role of Arabidopsis thaliana cytochromes b561 in intracellular ascorbate redox homeostasis and ROS-mediated signaling
Andrea Pagano *(Pavia)*

Genotoxic damage and nucleolar stress response in primed and overprimed Medicago truncatula seeds

Serena Farrotti *(Rome)*
The air is dry: an integrative quest to safeguard crops from a hotter and drier future

Riccardo Di Mambro *(Pisa)*
Auxin dynamics during developmental programmed cell death events in the Arabidopsis root

Simone Landi *(Naples)*
The circle of reductants to respond to low temperature: the role of the oxidative pentose pathwayOS-mediated signaling

2. Genetics, Epigenetics and Chromosome Biology

Chairs: *Domenico Carputo – SIGA, Gianni Cenci – AGI, Tiziana Bonaldi – SIBBM, Fulvio Chiacchiera – SIBBM*

Giovanni Messina *(Rome)*
Evolutionary conserved relocation of chromatin remodeling complexes to the mitotic apparatus: a new class of moonlighting proteins preventing genetic instability

Fulvio Chiacchiera *(Trento)*
ARID1A preserves tissue homeostasis by preventing genomic instability and chronic inflammation

Rodolfo Negri *(Rome)*
A truncated and catalytically inactive isoform of KDM5B histone demethylase accumulates in breast cancer cells and regulates H3K4 tri-methylation and gene expression

Stefano Zoroddu *(Sassari)*
Recovery of the correct balance of EZH2 and EZH1 proteins as a new strategy for the treatment of rhabdomyosarcoma

Liliana Tullo *(Rome)*
Assessing an unprecedented role for Heterochromatin Protein 1a (HP1a) at mitochondria

Romina Burla *(Rome)*
The nuclear envelope-associated ESCRT factor CHMP7 is needed for telomere integrity

Francesca Gorini *(Naples)*
8-oxodG accumulation within super-enhancers marks fragile CTCF-mediated chromatin loops

Tiziana Angrisano *(Naples)*
Retinoic Acid promotes a totipotent-like stem cell through Zscan4 epigenetic activation

Vittoria Brambilla *(Milan)*
Molecular control of flowering in rice

Sebastiano Giallongo *(Catania)*
Lactate rewires uveal melanoma metabolic profile promoting cellular quiescence

Armando Di Palo *(Caserta)*
The long non-coding RNA SPACA6-AS1, miR-125a and its mRNA targets establish a novel ceRNA regulatory network in hepatocarcinoma cells

Cinzia Calvio *(Pavia)*
The power of SwrA: from a minor protein to a game changer in B. subtilis biofilms

3. Genomics, Proteomics and Systems Biology

Chairs: *Maria Benedetta Mattei – SIBV, Alessio Mengoni – SIMGBM, Federico Giorgi – AGI, Gianni Cuda – SIBBM*

Martina Aulitto (Naples)
Genomic analysis reveals new insights into the biotechnological and industrial potential of Weizmannia coagulans

Mariarita Brancaccio *(Naples)*
Identification of key microRNAs in preeclampsia by bioinformatics analysis

Sara Bruschini *(Rome)*
Leveraging malignant pleural effusions from lung adenocarcinoma patients to investigate the immune landscape of advanced non-small cell lung cancer

Lisa Cangioli *(Sesto Fiorentino)*
Study of the regulatory cascade involved in the megaplasmids conjugation in the model plant symbiotic bacterium Sinorhizobium meliloti by the creation of a GFP-based reporter system

Michele Costanzo *(Naples)*
Proteomic study of the interactions of MMACHC protein

Guido Domingo *(Varese)*
Phosphoproteomic analysis reveals novel mechanisms by which cAMP affects cellular processes and response to heat in tobacco cells

Nicolaj Jeran *(Milan)*

Study of three putative Plastid Peptide Transporters mediating chloroplast-to-nucleus signalling in response to folding stress in Arabidopsis thaliana chloroplasts

Cristiana Lucia Rita Lipari *(Catania)*
Deletion of VDAC1 in human cells affects mitochondrial respiration

Michele Menotta *(Urbino)*
Multi-omics approaches for ataxia telangiectasia therapy development

Elena Perrin *(Sesto Fiorentino)*
A system biology approach to study microbial adaptation to global warming

Francesca Pirozzi *(Naples)*
A specific lipidomic signature defines serum lipid content of patients affected by glycogen storage disease Ia (GSDIa)

Anna Santin *(Naples)*
Integrative molecular analyses and first functional characterization of low-affinity nitrate transporters in diatoms, diNPFs

4. Current trends in Biotechnology

Chairs: *Rachele Isticato – SIMGBM,* *Matteo Ballottari – SIBV, Vittoria Brambilla (SIBBM SIGA), Gerardo Puopolo – SIPAV*

Ivan Baccelli *(Sesto Fiorentino)*
New fungicides inspired by natural molecules: plant disease protection by targeted modifications of the Trichoderma peptaibol Trichogin GA IV

Michele Perazzolli *(San Michele all'Adige)*
Taxonomic and functional analysis of the microbiota associated with cold-adapted plants

Sara Ragucci *(Caserta)*
Sodins, ribosome-inactivating proteins from Salsola soda L.

Michela Cecchin *(Verona)*
RNA interference-based techniques to increase sustainability and safety in the fruit and vegetable supply chain

Tania Vanzolini *(Urbino)*
Development of new biological drugs for the treatment of fungal infections

Nicola Zambrano *(Naples)*
Strategies for development of H. simplex-based oncolytic viruses in cancer immunotherapy

Massimo Zollo *(Naples)*
Long-chain polyphosphates impair SARS-CoV-2 infection and replication: prophylactic and therapeutic options against the 6th wave

Daniela Eletto *(Fisciano)*
Potential role of trefoil factor 1 as bioactive peptide in Helicobacter infection

Miriam Carbonaro *(Naples)*
More than just a cellulase: Cel9_Am a new multifunctional GH9 enzyme from Alicyclobacillus mali FL18

Riccardo D'Incà *(Rome)*
Solanum lycopersicum CRISPR/Cas9 mutants for a polyamine oxidase gene exhibit improved drought stress tolerance and water-use efficiency

Gabriella Fiorentino *(Naples)*
A hyperthermoactive-Cas9 editing tool reveals the role of an arsenite methyltransferase in the arsenic resistance system of Thermus thermophilus HB27 and provides a new sensitive arsenic bioreporter

Valeria Bontà *(Pavia)*
Applications of engineered Bacillus subtilis strain for agri-food waste valorization

5. Food, and Nutrition

Chairs: *Katia Petroni – AGI,* *Francesca Marcon – SIMAG, Stefania Iametti,* *Francesco Bonomi – SIB*

Stefany Castaldi *(Naples)*
Halotolerant PGP Bacillus amyloliquefaciens enhance the salinity stress tolerance of Lotus japonicus cv gifu

Maurizio Chiurazzi *(Naples)*
LjNRT2.3 plays a hierarchical role on the control of root nitrate uptake in L. japonicus

Nadia Lombardi *(Portici)*
Biological treatments of grapevines improve the phenolic composition and sensory properties of Aglianico wine

Roberta Marra *(Portici)*
Sustainable approaches to improve productive and qualitative traits of industrial tomato plants using biostimulants and mulch biofilms

Francesca Messina *(Pavia)*
Sustainable seed priming treatments to improve germination in lettuce

Angelo Santino *(Lecce)*
Biofortification strategies for the improvement of the nutritional value of tomato

Antonio Caporale *(Portici)*

Fostering food safety and quality in Neapolitan contexts of sustainable urban agriculture
Paola Antonia Corsetto *(Milan)*
Selective impact of polyunsaturated fatty acids on lipid composition and metabolism in breast cancer cells
Mattia Di Nunzio *(Milan)*
Modulation of proteolytic activity by dietary polyphenols is dependent on both substrate and enzymes
Katia Petroni *(Milan)*
Anthocyanins confer cardioprotection against Doxorubicin by modulating the AMPK-SIRT1-p53 pathway
Giuditta Heinzl *(Milan)*
Investigating the mechanisms of action of conglutin-γ in regulating D-glucose uptake: the role of intestinal barrier
Manuela Leri *(Florence)*
Olive polyphenols act as biomimetic molecules in hormone replacement therapy for Alzheimer's disease prevention in menopausal women
Giulia Magni *(Milan)*
Purple corn anthocyanins as a nutraceutical approach to control the progression of multiple sclerosis and associated trigeminal pain: role of the gut-brain axis
Laura Mosca *(Naples)*
"Greco" grape canes as a valuable source of bioactive compounds with antioxidant properties and anticancer activity on head and neck squamous cell carcinoma

6. Health and Disease

Chairs: *Marco Oggioni – SIMGBM, Paola Minghetti – ADRITELF, Giuseppe De Rosa – ADRITELF, Sheridan Lois Woo - SIPAV*

Andrea Bosso *(Naples)*
GVF27, a new human host defence peptide, shows anti-inflammatory and anti-biofilm activity against the Burkholderia cepacia complex (Bcc)
Lorenzo Ceccarelli *(Pisa)*
Human microglia derived Extracellular Vesicles: effects of inflammatory stimuli on production, content, and biological function
Costanza Cicchi *(Florence)*
Characterization of the enzymatic activity of Lscβ and Lscγ, two levansucrases from Pseudomonas syringae pv. actinidiae
Luigi Fattore *(Rome)*
Systemic delivery of miRNA-loaded nanoparticles blunts resistance to therapy in BRAF-mutant melanoma
Barbara Giordani *(Bologna)*
Nanocarriers for the delivery of Lactobacillus crispatus BC1 biosurfactant: alternative strategies to fight Candida biofilms
Sarah Giulietti *(Viterbo)*
Elucidation of the Arabidopsis NPK1-related protein kinases (ANPs) role in cell wall biosynthesis and signalling
Vittoria Nicolis di Robilant *(Rome)*
Inactivation of the nijmegen breakage syndrome protein (NBS1) abrogates SHH-driven cerebellar development/tumorigenesis through the primary cilium
Marco Rinaldo Oggioni *(Bologna)*
Ex vivo perfusion of the human spleen to study infection
Lara Russo *(Pisa)*
Biomimetic scaffolds for tendon/ligament regeneration
Chiara Siniscalchi *(Caserta)*
Human microRNAs binding to SARS-CoV-2 sequences: computational analysis and experimental validation
Mariavittoria Verrillo *(Portici)*
Antiflammatory activity and potential dermatological applications of characterized humic acids from a lignite and a green compost
Guendalina Zuccari *(Genoa)*
Fenretinide-loaded mesenchymal stem cells-derived extracellular vesicles as novel drug carriers against human neuroblastoma

13.30-14.30 ***Lunch Break***

14:30 16:00 **PARALLEL SYMPOSIA**

4a. Insights into insect biology to control pathogen vectors and agricultural pests

Chairs: *Guido Favia & Laura Rinaldi*

Guido Favia (*Camerino*)
Microbial symbiosis in insect vectors and insect pests
Paolo Gabrieli (*Milan*)
Digging into the biology of mosquito reproduction to design novel control tools
Francesco Pennacchio *(Naples)*
Insect multitrophic interactions for bioinspired plant protection

4b. Spatial profile of gene expression.

Chairs: *Lucia Altucci & Massimiliano Corsi Romanelli*

Raffaele Calogero (*Turin*)
Single cellomicsintegration with spatialtranscriptomics
Tiziana Bonaldi (*Milan*)
Unravelling non-genetic Inter- and Intratumor (epigenetic) Heterogeneity by spatial tissue proteomics
Francesca Ciccarelli *(London)*
Unravelling the dynamic interplay between genetics and immunity in driving cancer evolution

16.00 CONCLUSIONS AND PRIZES

Sommario

1.Environmental, Evolutionary and Developmental Biology

#1

Vincenzo Agostini • Pavia

The worldwide spread of Aedes albopictus: new insights from mitogenomes

V. Battaglia[1], Vincenzo Agostini[1], E. Moroni[1], G. Colombo[1], G. Lombardo[1], N.R. Migliore[1], P. Gabrieli[1,2], M. Garofalo[3], S.Gagliardi[3], L.M. Gomulski[1], L. Ferretti[1], O. Semino[1], A.R. Malacrida[1], G. Gasperi[1], A. Achilli[1], A. Torroni[1], A. Olivieri[1]*

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[2]Dept of Biosciences and Pediatric Clinical Research Center "Romeo ed Enrica Invernizzi", University of Milan, Milan, Italy
[3]Molecular Biology and Transcriptomic Unit, IRCCS Mondino Foundation, Pavia, Italy

The tiger mosquito (Aedes albopictus) is one of the most invasive species in the world and a competent vector for numerous arboviruses, thus the monitoring of its fast worldwide spread is crucial for global public health. The study of mitochondrial DNA represents a key tool for reconstructing phylogenetic and phylogeographic relationships within a species, especially when analyzed at the mitogenome level. Here the mitogenome variation of 76 tiger mosquitoes was investigated. This analysis significantly improved the global mtDNA phylogeny of Ae. albopictus, uncovering new branches and sub-branches within haplogroup A1. Our phylogeographic approach shows that the current distribution of tiger mosquito mitogenome variation has been strongly affected by clonal and sub-clonal founder events, sometimes involving wide geographic areas, even across continents, thus shedding light on the Asian sources of worldwide adventive populations. In particular, different starting points for the two major clades within A1 are suggested, with A1a spreading mainly along temperate areas from Japanese and Chinese sources, and A1b arising and mainly diffusing in tropical areas from a South Asian source.

#2

Francesca Alderotti • Sesto Fiorentino

To save water on not to save, that is the question: tree-ring stable isotopes explain the dieback of Quercus ilex in southern Tuscany (Italy)

Francesca Alderotti[1,2], P. Cherubini[3,4], L. Brilli[5], F. Bussotti[1], M. Centritto[2,6], F. Ferrini[1,2], A. Gori[1,2], M. Pollastrini[1], M. Saurer[3], C. Brunetti[2,1]

[1]University of Florence, Dept of Agriculture, Food, Environment and Forestry, Sesto Fiorentino, Florence, Italy
[2]National Research Council of Italy (CNR), Institute for Sustainable Plant Protection, Sesto Fiorentino, Florence, Italy
[3]WSL Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf, Switzerland
[4]Dept of Forest and Conservation Sciences, University of British Columbia, Vancouver BC, Canada
[5]CNR-IBE, National Research Council Institute for the BioEconomy, Florence, Italy
[6]Ente Nazionale Idrocarburi-CNR Joint Research Center "Water - Hypatia of Alexandria", Metaponto (MT), Italy

Quercus ilex L. dieback has been recently observed in Tuscany (Italy). Two stands with different levels of dieback, a declining (D), where 80% of the trees exhibited crown-defoliation and dead branches, and a non-declining (ND) (dieback affected 20% of the trees) were studied. We hypothesized that different damage levels are connected to different strategies to cope with drought. Dendrochronological and tree-ring δ13C analyses were applied to investigate the impact of two major drought events (2012 and 2017) on Q. ilex growth and water use efficiency (WUE). Overall, D showed a lower growth rate than ND. Furthermore, δ13C was significantly reduced in D than in ND from 2000 to 2020, highlighting a water-spending strategy for Q. ilex in D. Drought in 2017 significantly decreased growth in D, but not in ND. Drought in 2017 was stronger than in 2012, as shown by the increased WUE revealed by tree-ring δ13C. Our results suggest that the differences in Q. ilex dieback in the two stands could potentially be linked to the different water-use strategies adopted. A more conservative use of water may have helped Q. ilex to cope with drought in ND and to maintain a higher growth rate than D.

#3

Deborah Bastoni • Rome

Linking plate tectonic settings and microbial functions on a global scale

Deborah Bastoni[1], J. Buongiorno[2], S. Morrison[3], R. Hazen[3], A. Prabhu[4], A. Eleish[4], P. Barry[5], D. Bekaert[6], S. Zahirovic[7], S. Narkar[4], A. Cordone[1], D. Giovannelli[1]

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[3]Earth & Planets Laboratory, Carnegie Institution for Science, Washington, DC, USA
[4]Tetherless World Constellation, Rensselaer Polytechnic Institute, Troy, NY, USA
[5]Marine Chemistry and Geochemistry Department, Woods Hole Oceanographic Institution, Woods Hole, MA, USA
[6]Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA, USA
[7]EarthByte Group, School of Geosciences, The University of Sydney, NSW, Australia

Microbial metabolisms are responsible for many core redox reactions that control biogeochemical cycles of elements and nutrients, mainly due to enzymes that use transition metals as core catalytic cofactors. As Earth’s Biosphere and Geosphere have coevolved over time, metal concentrations at Earth’s surface have changed, influencing surface redox conditions leading to new and more abundant reductant-oxidant pairs, and likely influencing the evolution and distribution of the metabolic processes that play a central role in the complex geosphere-biosphere interactions of today's Earth. The delivery of biologically relevant metals is controlled, to a first order, by tectonic processes. We present here a geochemical and microbiological coupled analysis, looking for links between the delivery of transition metals to the surface through plate tectonics and microbial functional diversity. Our dataset reveals correlations between key microbial functions and differences in the delivery of the key metal cofactors to the surface of our planet.

#4

Agnese Bellabarba • Sesto Fiorentino

Exploration of salt tolerance of Sinorhizobium meliloti strains during the first steps of the symbiosis

Agnese Bellabarba[1], F. Decorosi[1], C. Fagorzi[2], F. Bracali[1], A. Bekki[3], F. Pini[4], A. Mengoni[2], C. Viti[1]

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Rhizobium inoculants are widely used in agriculture, providing one of the most cost-effective ways to boost legume performances. Nowadays, salinity is one of the largest constraints in agriculture for plant growth in stricken regions. The progressive salinization of soil strongly influences the symbiotic interaction between alfalfa and their associated rhizobia affecting the early stages of the symbiotic process. Hence, the need to select salt-tolerant rhizobia useful to increase alfalfa yield in saline conditions. An extensive screening of in vitro salt tolerance of Sinorhizobium meliloti strains, isolated from Algerian soils, was performed on increasing concentrations of NaCl. Also, the efficiency of the symbiotic pairs S. meliloti-Medicago sativa was evaluated at 0 mM and 100 mM NaCl in controlled conditions. The strains able to efficiently improve plant growth at 100 mM are different and specific for each cultivar, highlighting how specificity and (microbial) G x (host) G interaction in this symbiosis has to be considered in the selection of the rhizobia inoculants. Overall, the results obtained allowed us to identify 5 strains as candidate inoculants to test in saline soils.

#5

Manuel Benedetti • L'Aquila

Radical cation scavenging activity of berberine bridge enzyme-like oligosaccharide oxidases acting on short cell wall fragments

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OG-oxidases (OGOXs) and cellodextrin-oxidase (CELLOX) are plant berberine bridge enzyme-like oligosaccharide-oxidases (OSOXs) that oxidize, respectively, oligogalacturonides (OGs) and cellodextrins (CDs) by inactivating their elicitor nature and concomitantly releasing H2O2. Little is known about the role of oxidized oligosaccharides as generated by OSOX activity. By using an ABTS•+-reduction assay, we identified a novel reaction mechanism through which the activity of OSOXs on cell wall oligosaccharides scavenged the radical cation ABTS•+ with an efficiency dependent on the type and size-length of the oxidized oligosaccharide. In contrast to the oxidation of longer oligomers such as OGs (degree of polymerization from 10 to 15), the activity of OSOXs on short galacturonan- and cellulose-oligomers (degree of polymerization ≤ 4) successfully counteracted the radical cation-generating activity of a fungal laccase. These results suggest that OSOX enzymes can generate radical cation scavenging activity in the apoplast with an efficiency proportional to the extent of degradation of plant cell wall, with possible implications for redox homeostasis and plants stress defense.

#6

Alessandro Bevilacqua • Onna

Exploiting bovine serum albumin liquid--liquid phase separated protocells to unravel enzyme activity tuning dependent on the protocell’s size and composition

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Liquid--Liquid Phase Separation (LLPS) is a reversible cellular phenomenon whereby biomolecules separate into discrete liquid phases with different compositions. LLPS has been hypothesized to have several functions in cells, such as concentration of biomolecules, filtering, compartmentalization of solutes to activate or inactivate biological processes and enzymatic reactions. Enzymes’ activity is known to be affected by some of these mechanisms, especially when its solvation is modified by the LLPS along with substrate/product availability. However, how the enzyme activity could be influenced by this new phase’s environment is still not fully explained in literature. In this study, the Polyethylene Glycol--Bovine Serum Albumin (BSA) system is used to obtain liquid-liquid phase separated BSA protocells in vitro. By compartmentalizing β--Galactosidase inside BSA droplets, the enzyme kinetics is characterized inside the new liquid phase using different substrates. When compartmentalized, the enzyme shows varied kinetic parameters for different substrates, suggesting a role of the inner composition of the new liquid phase itself on modulating enzyme activity on specific substrates.

#7

Rajiv Boscolo Agostini • Ferrara

Reconstructing the global invasion routes of Halyomorpha halys through Approximate Bayesian Computation

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The brown marmorated stinkbug (Halyomorpha halys) is a polyphagous insect pest native to Asia, which has rapidly spread worldwide causing extensive damage to global agriculture. Investigating the genetic diversity among H. halys populations is essential to understand the patterns of colonization and invasion history of local populations. Recently genomic data (ddRAD) from multiple worldwide populations of H. halys have been published, and preliminary analyses suggested a complex pattern of invasion. In this study we analyze published ddRAD sequencing data of 389 individuals from 12 worldwide populations of H. halys, with the final aim of explicitly compare different demographic models in order to shed light on the dispersal process of the species. We identified 1577 high quality single nucleotide polymorphism (SNPs), that have been used to investigate the fine-scale population structure and the genetic diversity of H. halys. Our results show a peculiar genetic structure that suggests multiple invasions from China to Europe and America. This hypothesis will be explicitly tested simulating alternative demographic scenarios employing an Approximate Bayesian Computation framework.

#8

Giulia Caioni • L'Aquila

Long-term neurotoxicity induced by early-life exposure to propylparaben in zebrafish model

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Propylparaben is an effective bactericide and it is widely used as a preservative in personal care products, pharmaceuticals and foods. Due to its high solubility in water, it can easily disperse in the aquatic systems. It has been detected in environmental and biological matrices, suggesting the frequent human exposure to this chemical. The available data provide evidence of its endocrine disrupting activity. There is limited information about its role in neurodevelopment. This study aimed at investigating the effect of propylparaben on neurodevelopment in the zebrafish model. Zebrafish embryos were exposed for 96 hours post-fertilization to two concentrations of propylparaben (10 and 1000 μg/L) and different biological end-points were performed in adults to evaluate the long-term effects. Molecular analysis revealed alterations in the expression of some genes involved in neurodevelopment and the onset of neurological disorders. The proteomics profiles of brains showed the modulation of proteins, some of which are also involved in autistic disorders and autosomal recessive non-syndromic intellectual disability. All these changes are also accompanied by behavioral alterations.

#9

Ilaria Camponeschi • Rome

Smuorz e llaits: response of the yeast Kluyveromyces lactis to light

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In unicellular organisms like yeasts, which do not have specialized tissues for protection against environmental challenges, the presence of cellular mechanisms to respond and adapt to stress conditions is fundamental. In this work, we aimed to investigate the response to light exposure in Kluyveromyces lactis. Yeast lacks specialized light-sensing proteins; however, Saccharomyces cerevisiae has been reported to respond to light by increasing hydrogen peroxide level and triggering nuclear translocation of Msn2. This is a stress-sensitive transcription factor also present in K. lactis. To investigate light response in this yeast, we analyzed the different phenotypes generated by the deletion of the hypoxia responsive and lipid biosynthesis transcription factor KlMga2. Alterations in growth rate, mitochondrial functioning, ROS metabolism, and fatty acid biosynthesis provide evidence that light was a source of stress in K. lactis and that KlMga2 had a role in the light-stress response. The involvement of KlMsn2 in light stress was also explored.

#10

Costanza Cannariato • Florence

A molecular portrait of ancient Pompeians

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The ancient city of Pompeii is a UNESCO World Heritage site, retrieved after the eruption of Vesuvius caused its destruction in 79 CE.

Excavations of the ruins have unearthed many human remains belonging to people died during the eruption, whose DNA is often well preserved.

We analyzed 124 human samples from Pompeii using state-of-the-art ancient DNA techniques. The aim of the project is to describe the genetic structure of the population, and to infer the main processes that shaped it.

The molecular analyses allowed to determine the sex and kinship relations among individuals found in the same place. Preliminary Principal Component Analyses (PCA) of genomic data demonstrated that Pompeians overlap with present-day Central Eastern Mediterraneans, as well as with the previously reported Imperial samples from Rome and surrounding regions. However, genetic diversity in Pompeii appears higher than elsewhere in the Roman Empire, presumably because of the extensive commercial network of Pompeii, also evidenced by archaeological materials and historical records found in the city.

#11

Giorgia Capasso • Naples

Characterization of a glucose-6-phosphate dehydrogenase from Chlamydomonas reinhardtii

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The recombinant G6PDH from C. reinhardtii was kinetically characterised.
CrG6PDH reacted against P1-G6PDH and P2-G6PDH antisera, showing peculiar kinetic properties. Activity was 80% inactivated in vitro after 1h by 80% at 100 mM DTT. Different thioredoxins were tested for their effect on CrG6PDH: the measurement of the redox potential indicates CrTrx m as the physiological regulator of CrG6PDH.
Purified CrG6PDH showed hyperbolic kinetics versus G6P and NADP+ and a mixed inhibition by NADPH. The inhibition by glucosammine-6P versus G6P was competitive; glucosammine-6P versus NADP+ showed a non-competitive inhibition. CrG6PDH activity depends on Mg++; we argued that Mg++ is involved in the stabilization of CrG6PDH structure, facilitating NADP+ binding. A kinetic analysis suggests a sequential reaction mechanism with random first substrate.
Post-translational modification were investigated. The activity of CrG6PDH was modulated by PTMs, including S-nytrosilation, H2O2-dependent oxidative treatment and alkylation. Furthermore, cysteines can undergo various redox modifications: diamide (TMAD) 1mM caused an activity decrease after 1h by 80%; 0.2mM DTNB decreased by 75% after 30min.

#12

Serena Capitanio • Turin

Investigating the role of clathrin-mediated endocytosis in Myc-factors perception in arbuscular mycorrhizas

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Clathrin-mediated endocytosis (CME) is a major endocytic pathway in plants, driving the internalization of membrane-bound receptors.

Previous studies using tyrphostin A23, a CME inhibitor, demonstrated that CME is required for the regulation of symbiotic genes in response to Nod-factors in legume-rhizobium symbiosis (Wang et al., 2015). Growing evidence indicates that this symbiosis recruited part of the signaling pathway supporting arbuscular mycorrhiza (AM). We therefore decided to investigate whether CME is also involved in AM signaling.

To this aim, we treated M. truncatula roots with AM fungal signals in the absence or presence of CME inhibitors (tyrA23, Dynasore), analyzed the expression for early AM marker genes and monitored nuclear Ca2+ spiking (a hallmark of symbiotic signaling). Symbiotic gene regulation was strongly impacted by CME inhibition. Nevertheless, no significant reduction was observed in Ca2+ spiking, suggesting CME is required for gene regulation but not for upstream symbiotic signaling. We here discuss the new questions opened by such unexpected results, which contrast with our current model of symbiotic signaling in legumes.

#13

Benedetta Caraba • Rome

Calorie restriction suppresses premature aging in pro-apoptotic LSM4 yeast mutants.

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LSM4 is an essential gene involved in mRNA decapping and pre-mRNA splicing. We previously reported that the expression of a truncated form of the homologous gene KlLSM4, named KlLsm4Δ1, from the biotechnological yeast Kluyveromyces lactis can restore cell growth in both K. lactis and S. cerevisiae not expressing the endogenous protein. However, cells showed loss of viability during stationary phase and undergo to regulated cell death (RCD). Here we report that cells expressing Sclsm4Δ1, a truncated form of LSM4 also shows the same apoptotic markers and increased sensitivity to hydrogen peroxide and acetic acid, two inducers of RCD in yeast.

We found that the extension of chronological lifespan induced by caloric restriction in Sclsm4Δ1 is higher compared to the wild type, representing an efficient survival mechanism.

Moreover, data on autophagic process during growth and caloric restriction in wild type and Sclsm4Δ1 mutant will be presented.

#14

Federica Carducci • Ancona

Omics approaches as useful tool to provide an exhaustive view of marine species: the case of Chamelea gallina

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In the Mediterranean basin, the bivalve mollusc Chamelea gallina represents a precious fishing resource that in the last two decades, in the Adriatic Sea, experienced a continuous decline. Moreover, the shell hardness, a feature important for the survival of rejected undersized samples and quality of catch, varies in clams coming from sites having different environmental parameters. Therefore, we employed a multidisciplinary approach to investigate the functional and genetic status of this species. The population study, performed using ddRAD-Seq data, highlighted a considerable genetic variability, probably due to the high larval dispersal rate. This feature might play a compensatory role against factors threatening this species. Moreover, the functional status of C. gallina was investigated through RNA-Seq evidencing the ability of this species to modulate gametogenesis and shell mineralization processes in relation to abiotic factors such as salinity and food availability. Our results contributed to increase knowledge on the striped venus that might represent a valuable aid in the adoption of science-based management plans for this fishery resource.

#15

Federica Casagrande • Rome

Characterization of light-dependent regulation of flower opening in model plant Arabidopsis thaliana

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While the environmental and endogenous factors regulating flowering of flowering plants are well known, considerably less is known on how these factors regulate flower opening (or "anthesis"), despite it being a trait of high biotechnological and agronomic interest. Our and other laboratories have shown that anthesis is regulated by an intricate hormonal network. While auxin slows down flower opening, gibberellins
promote it. Both these hormones converge on the regulation of jasmonic acid: auxin inhibits its production through ARF6 and ARF8, while GA promote its synthesis. We set to investigate the role of light on the regulation of anthesis in the model plant Arabidopsis thaliana, through a genetic (using mutants of light signaling and hormones) and molecular (analysis of the transcriptional profile, followed from identification and functional confirmation of target genes and reconstruction of downstream pathways) analysis. Our
results suggest that a specific cross-talk between light and hormone signaling is required to finely regulate flower opening. The results of this research can be used in programs aiming at improving crop genomes, to increase their quality and productivity.

#16

Stefano Cazzaniga • Verona

Turning a green alga red: astaxanthin biosynthetic pathway in Chlamydomonas reinhardtii improves resistance to light stress and biomass productivity at high irradiances

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Microalgae are photosynthetic unicellular organisms which can be exploited to capture CO2 and produce biomass. Their yield is limited by the dichotomy between the need of continuous light incoming and the dangerous effects of excess light energy that generates reactive oxygen species damaging cells. Contrasting photoinhibiton is mandatory to improve microalgae productivity. Astaxanthin is one of the strongest antioxidants found in nature, but not accumulated in many species. Engineering astaxanthin biosynthetic pathway in Chlamydomonas reinhardtii, we obtained a strain (bkt5) that constrictively accumulate astaxanthin as main carotenoid noticeably changing its color from green to red. This strain was characterized by a far greater resistance to light stress and photoinhibition that led to improved photosynthetic efficiency and higher biomass productivity. When bkt5 and its parental strain were co-cultured in extreme high light, in a ”competitive test”, the bkt5 outcompete the control and, in a few days, more than 90% of the cells in the bioreactor were bkt5. Changing carotenoids composition could be a viable strategy to mitigate light stress in photosynthetic organisms.

#17

Costanza Ceccanti • Pisa

Responses of photosynthesis and antioxidant system to salinity stress in Beta vulgaris L. under different levels of nitrogen supplementation

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Salinity has become an important constrain especially in reason of Climatic Change. Beta vulgaris has been identified as salt-tolerant species likely thanks to its adaptive betacyanin accumulation. In the present study, effect of different levels of nitrogen (N0, deprived; N10, optimum and N40, supra-optimum) was investigated in B. vulgaris L. var. Bull's blood (red leaves; R) and B. vulgaris L. var. Robuschka (green leaves; G) seedlings under NaCl (150 mM) stress. Gas exchange, chlorophyll a fluorescence, glutathione and ascorbate contents, antioxidant activity and N and Na+ assimilation were evaluated. Interestingly, the G leaves responded rapidly, with a reduction of CO2 assimilation rate and PSII efficiency if compared with R leaves. However, both the species showed a decrease of antioxidant activity and glutathione content with salt stress whilst ascorbate increased in R salt-stressed plants under N40. The latter showed an interesting behaviour in Na+ assimilation in R leaves, suggesting that, after N addition, Na+ content is favourably regulated, which might be associated with mitigation of NaCl stress by N supplementation.

#18

Ana Chero Osorio • Pavia

Mitochondrial DNA haplogroup X2a: an enigmatic presence among North American Indigenous people

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Many founding mitochondrial DNA (mtDNA) haplogroups of Asian/Beringian origin have been identified so far among Indigenous Americans. Most are defined as “pan-American” because of their presence throughout the double continent. They were probably brought by the first late Pleistocene/early Holocene settlers who spread from eastern Beringia along the Pacific coast since 18-15 thousand years ago. However, the Indigenous American haplogroup X2a harbors distinctive features, including a distribution restricted to northern North America, which has led to alternative entry scenarios. Here, we analyzed previous and novel X2a complete mitogenomes from 68 modern and ancient individuals. Two major branches, known as X2a1 and X2a2, were found to be differentiated into several sub-branches. Thanks to the availability of ancient mitogenomes, ages and distributions of branches were assessed with a higher degree of accuracy in order to provide new insights on how and when the enigmatic Indigenous haplogroup X2a reached and spread into North America.

#19

Pasquale Chiaiese • Portici, NA

Exposure to transition metals and metalloid to eggplant (Solanum melongena L.) affect ionome and specific metal transporters gene expression

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The uptake, distribution, and accumulation of mineral in the plants is regulated by a wide array of ions transporters. These carriers have low ions selectivity and plants can also absorb non-essential elements such as heavy metals. In this study we have evaluated the effects of As or Cd, or Cr; on ionomic variation and on specific gene expression transporters on six different cultivars of eggplant (S. melongena) growing in hydroponic culture. The treated plants shown visible damage symptoms and their intensity varied according to cultivar and the chemical specie and its concentration. ICP-MS analysis shown that roots are the main organs that accumulate the three ions and translocation index (TI) indicate that are not easily transported to the above ground organs. Moreover, ionome variation of other elements was detected. By a bioinformatic approach it was possible to identify transporter genes related to As, Cd and Cr. Based on TI we have identified the minus and plus cultivar for each elements assayed and these genotypes we have performed gene expression analysis. Those genes modified their expression according to treatment and /or genotype in an organ specific manner.

#20

Giulia Colombo • Pavia

New insights on past human movements by combining modern and ancient Y-chromosome data in a single phylogeny: the test case of haplogroups C and Q

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Advances in sequencing technologies are providing unprecedented numbers of modern and ancient human Y-chromosome sequences. However, their classification into haplogroups (hgs) is often based on the already known sequence variation, without further investigating for new phylogenetic relationships.

To highlight the importance of phylogenetic meta-analyses, we focused our attention on hgs C and Q. Both originated outside of Africa and are observed from Europe to America, but they are characterised by different phylogenetic features and demographic histories. To perform this study all the available Y-chromosome sequences ascribable to these two haplogroups were collected and a computational workflow was developed, based on the imputation of missing data for low-coverage and ancient individuals, to insert them into a robust reference phylogeny built with high-coverage sequences.

Here, we provide the most up-to-date phylogeny and nodes dating for hgs C and Q, with a focus on novel structures, and discuss, by considering this information and present geographic distributions, the potential migratory routes followed by the two haplogroups during their movements in Eurasia and the Americas.

#21

Ilaria Colzi • Florence

Do tomato plants like microplastics? Study of the impact on crop yield and physiology

Ilaria Colzi, M. Dainelli, A. Coppi, M. Mabily, S. Marchetti, C. Gonnelli

Microplastic (MNP) contamination in agricultural soils may represent a crucial issue in terms of yield and economic losses for crops. The food chain could also be affected in the case of uptake of plastic fragments by the plant. Therefore, monitoring the MNP impact on crops deserves urgent attention to ensure safe food production practices.

This work aimed at investigating the effects of MNP-polluted soil on crop biomass and performance, together with the fruit production and quality. The study was achieved on the model crop species Solanum lycopersicum cv. Micro-Tom which was grown in controlled conditions and exposed to soil artificially polluted with different types of MNPs (0.5% w/w) commonly found in agroecosystems, namely polyvinyl chloride (PVC) and polyethylene terephthalate (PET). The effects of MNPs on tomato was investigated through different physiological analyses (e.g. photosynthetic efficiency, pigment content, productivity, nutrient concentrations). The tested MNPs showed to interfere with the fruit production, by slowing down the ripening and reducing the number of tomatoes, even when the plant growth and photosynthetic efficiency were not significantly affected.

#22

Lorenzo Cotrozzi • Pisa

Hyperspectral assessment of physiological and morphological leaf traits related with drought in three varieties of Aucuba japonica with different leaf variegation

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Advancements in techniques to assess plant status are needed for nursery management. Hyperspectral data have great potential to rapidly and non-destructively monitor plant stress. However, this method has been exclusively developed on green leaf species, although plants with variegated foliage are a prerogative of nurserymen, because of their aesthetic peculiarity. This study examined the capability of reflectance spectroscopy (400-2400 nm) to characterize responses to water deprivation of three varieties of Aucuba japonica with no, mild and high leaf variegation. Partial least squares regression models were built to predict from spectra an array of leaf photosynthetic, water and morphological traits related with drought. Predictive models for photosynthetic traits were not accurate (R2: 0.10-0.58), whereas those for water and morphological ones showed an excellent prediction accuracy (R2: 0.65-0.94), this because only these latter models did not include the visible spectral region (400-700 nm) which is highly susceptible to leaf variegation. Variations of spectral indices and spectra-derived traits confirmed A. japonica as drought tolerant, especially the not variegated variety.

#23

Rossana Cuciniello • Naples

Oxidative stress defense impairment affects neuronal differentiation

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As we age, the cells of our brain lose the ability to respond to endogenous and environmental stress, determining the outbreak of neurodegenerative diseases caused by neuronal cell loss, mostly caused by oxidative stress. Moreover, in several neurodegenerative diseases, neurons show an impaired glucose uptake, long time before the onset of the disorder's symptoms.

A crucial cofactor involved in the scavenging of oxidative stress is NADPH, mainly generated in the Pentose Phosphate Pathway, that provide reducing energy to cells oxidizing glucose-6-phosphate.

We have previously demonstrated that a mouse Glucose 6 Phosphate Dehydrogenase-null embryonic stem cells (G6pd delta ES cells) are extremely sensitive to oxidative stress.

G6PD delta ES cells can differentiate in vitro into neuronal cells, but they fail to fasciculate. We have demonstrated that failing in fasciculation is a direct consequence of their oxidative stress defense impairment. To identify the deregulated genes involved in the mechanism of fasciculation, we have performed a Genechip transcriptome analysis with the aim of compare the expression of G6PD delta and WT neuronal cells.

#24

Rosanna Culurciello • Naples

Protective effects of recombinant human angiogenin in HaCaT cells: new insights on skin stress response mediated by RNases

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Human angiogenin (ANG) is involved in different pathophysiological processes, including tumorigenesis, neuroprotection, inflammation, regeneration of damaged tissues and stress cell response depending on its intracellular localization. Under physiological conditions ANG moves to the cell nucleus where enhances rRNA transcription; conversely, recent reports indicate that under stress conditions, ANG accumulates in cytoplasmic compartment modulating the production of tiRNAs, a class of small RNAs that contribute to translational inhibition and recruitment of Stress Granules (SGs). To date, there are still limited experimental evidence relating to the ANG role in the epidermis, the outermost layer of human skin, continually exposed to external stressors. This study shows that endogenous ANG can modify its subcellular localization on HaCaT cells depending on different cellular stresses. Furthermore, the use of recombinant ANG allowed to determine that this special enzyme is effectively able to counter at various levels the alterations of cellular homeostasis in HaCaT cells, opening a new vision on the possible functions that ANG can support also in human skin the stress response.

#25

Matteo Daghio • Florence

Poly (lactic acid) biodegradation study in simulated composting bioreactors

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Poly (lactic acid) (PLA) is a biobased polyester that finds many applications due to its favorable properties. However, PLA's safe disposal is an emerging problem because its demand for disposable packaging is constantly rising. Although the ability to degrade PLA is not widely spread in microorganisms, microbial degrading activity is still both an environmentally and economically preferable method. Consequently, more PLA-biodegradability tests in real composting condition, and PLA-degrading microorganisms' evaluation must be performed to face the waste problem.

In this project, simulated-composting-facility bioreactors were designed to evaluate the degradation of four types of PLA (IM002, 4032D, EXT404, IM502(M)) that differ in their chemical-physical and mechanical properties. The degree of degradation was determined by measuring the loss of mass of the samples. Rates of weight loss increased in the order IM002 (7%/month) < 4032D (25%/month) < EXT404 (42%/month) < IM502(M) (100%/month), with the decrease of the optical purity. Novel thermophilic-PLA-degrading microorganisms were isolated from the biomass attached to the samples and characterized through 16sRNA gene sequencing.

#26

Federica De Lise • Naples

Transcription regulation and translational recoding of the alpha-L-fucosidase from Saccharolobus solfataricus in vivo

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Decoding of genetic informationis a dynamic mechanism due to programmed deviation of ribosomes from standard translational rules, known asrecoding. Recodingplaysa role in the regulation of gene expression and it has been suggestedto have implication in the adaptation of life in extreme environments (Ling et al. 2015. NRM). In extremophilic Archaearecoding has been described for termination codon readthrough,which regulates the incorporation of the unusual amino acids selenocysteine and pyrrolysine, and for -1PRFwhich allows the expression of an α-L-fucosidase in the crenarchaeon S.solfataricus (De Lise et al. 2021. Front microbiol.; Cobucci-Ponzano et al. 2006. NAR.) . We report here on the analysis of the transcription of the fucA recoded gene and of its full-length mutant in different growth conditionsin vivo, suggesting thatthe increased level of fucA mRNA in certain growth conditions is related to translation efficiency by recoding (De Lise et al. 2021. Molecules) . In addition, the analysis of the expression of the interrupted fucA gene in the archaeon S. Acidocaldarius allowed, for the first time,the identification of the frameshifting site for this gene in vivo.

#27

Maria Concetta de Pinto • Bari

GUN1 involvement in the redox changes occurring during biogenic retrograde signaling

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Communication between plastids and nucleus is essential for chloroplast biogenesis. Retrograde signals communicate the functional and developmental state of chloroplasts to change nuclear gene expression, accordingly. GENOMES UNCOUPLED 1 (GUN1), a chloroplast-localized protein, and reactive oxygen species (ROS) are reported as key players of retrograde signaling.
To study the interaction between GUN1 and redox signaling occurring during chloroplast biogenesis, we investigated redox changes in Arabidopsis wild type and gun1 seedlings, grown under control conditions or in presence of lincomycin, which perturbs chloroplast development.
Under control conditions, gun1 mutants show low activity of superoxide dismutase and ascorbate peroxidase, and an increase in O2- levels and lipid peroxidation, indicating that GUN1 could optimize chloroplast biogenesis minimizing oxidative damage. Lincomycin treatment causes a decrease of ROS-scavenging systems and H2O2 accumulation only in wild type seedlings. These results suggest that perturbation of plastid protein homeostasis could be communicated to the nucleus by GUN1-dependent oxidation of cellular environment.

#28

Sara Del Duca • Sesto Fiorentino

Molecular mechanisms for the evolution of gene structure and organization: the histidine case

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One of the most studied metabolic pathways is histidine biosynthesis and analyses of the structure of his genes revealed that different molecular mechanisms played an important role in shaping this route. The aim of this work was to explore the molecular mechanisms that shaped metabolic pathways during evolution, using the histidine biosynthesis as a model and experimentally simulating these events in Escherichia coli. Through bioinformatic analyses, genome editing techniques and directed evolution experiments, we i) studied the structure and organization of histidine biosynthetic genes in bacterial phyla, highlighting a high variety of gene structures and organizations; ii) analyzed the compartmentalization of histidine biosynthetic enzymes in E. coli; iii) investigated the evolutionary molecular mechanisms of gene elongation, frameshift mutation and homologous recombination using the hisF gene as a model, simulating its possible early evolution; iv) explored the HisF involvement in different cellular processes in the bacterial world. Results obtained from the proposed analyses could represent a further step towards the understanding of metabolic pathways' evolution.

#29

Raffaele Dello Ioio • Rome

microRNA165 and 166 control plastic development of the Arabidopsis root meristem in response to salt stress

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In plants, developmental plasticity allows modulating organ growth in response to environmental challenges. In the root, plasticity can rely on changes in the activity of the meristem, an apical region of this organ where a set of self-renewing undifferentiated cells sustain growth. Here, we show that variations in the dosage of the Arabidopsis homeobox protein PHABULOSA (PHB) modulate root meristem activity, and that these variations in turn depend on the activity of miRNA165 and 166. Specifically, by means of a genetic and molecular analysis we show that the levels of miR165 and 166 respond to abiotic stresses such as high salt concentration, and that miR165 and 166-dependent PHB modulation is fundamental for root growth response to this stress. Our data provide direct evidence of how the miRNA-dependent regulation of transcription factors dosage regulates plastic development in plants.

#30

Giovanni Di Gregorio Barletta • Naples

CotG controls spore surface formation in response to the temperature of growth in Bacillus subtilis

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Bacterial spores of the Bacillus genus are isolated from a variety of different environments, including extreme habitats. Such wide distribution mainly reflects the spore resistance properties and adaptability. In fact, it is known that in some Bacillus species growth and sporulation in different conditions affect the structure and the resistance properties of the produced spore. In B. subtilis the temperature of growth and sporulation has been shown to influence the structure of the spore surface throughout the action of a sporulation-specific and heat-labile kinase CotH. Here we report that CotG, an abundant component of the B. subtilis spore surface and a substrate of the CotH kinase, assembles around the forming spore but also accumulates in the mother cell cytoplasm where it forms aggregates with at least two other coat components. Our data suggest that the thermo-regulator CotH contributes to the switch between the coat of 25°C and that of 42°C spores by controlling the phosphorylation levels of CotG that, in turn, regulates the assembly of at least two other coat components.

#31

Riccardo Di Mambro • Pisa

Auxin dynamics during developmental programmed cell death events in the Arabidopsis root

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Programmed Cell Death (PCD) represents a key developmental program to sculpt organs. In plants, characteristic PCD events are crucial for the correct development of the lateral root cap, the most external tissue of the root. The lateral root cap is responsible to interpret environmental stimuli into internal signals, thus governing root growth and architecture. The uppermost lateral root cap cells (ULRC) are characterized by a recurrent PCD, as a result of which ULRC are sloughed off the root into the soil, thus facilitating penetration. This cellular turnover ensures tissue proper development and functions. Several evidences provided a correlation between the activity of the phytohormone auxin and PCD events, including those occurring in the ULRC. Nonetheless, the molecular mechanism that triggers ULRC PCD is still not clear. Exploiting a novel live imaging analysis combined with cell type specific gene editing, we show that the auxin polar transport affects the timing of ULRC PCD occurrence by adjusting auxin levels. We thus suggest a ULRC-specific molecular circuit that confers positional cues to coordinate cellular turnover and whole organ development.

#32

Conrado Jr Duenas • Pavia

Variation in germination behavior of Italian rice varieties in response to drought and poly-γ-glutamic acid treatments

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Climate change, water stress, and increasing cost of farming inputs are the major challenges faced in agricultural food production. To meet the daunting task of the SDG's aim for ZERO HUNGER by 2030 amidst these obstacles, implementing versatile agricultural practices offers putative solutions in addressing the need to increase productivity in global food production. Seed priming is a method that regulates germination by tackling the metabolic activity within the initial phases of germination. In this study, five popular Italian rice varieties (Lomello, Carnaroli, Cerere, Unico, and Apollo) representing three genetical Italian rice clades were subjected to priming treatment using the non-toxic biopolymer poly- γ-glutamic acid (γ -PGA). The treated seeds were subsequently sown under mild drought stress soil condition and well water treatment as control. Preliminary data on selected germination parameters show variability in their response to buffer drought stress. Expression of DNA Damage Response (DDR) genes and the levels of Reactive Oxygen Species (ROS) are also being investigated to further understand the observed behavioral variability.

#33

Antonia Esposito • Sesto Fiorentino

Temporal evolution of bacterial communities associated to Phragmites australis exploited in phytodepuration of wastewater

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Phytodepuration is considered an efficient and cost-effective treatment of wastewater based on the use of plants and their associated microorganisms to remove and/or transform pollutants. This goal is achieved using constructed wetlands (CW), which are engineered systems designed to mimic the natural processes that occur in natural wetlands. Samples of P. australis, soils and permeates were collected from the CW of Calice (Tuscany, Italy) during five sampling campaigns spanning 22 months, with the first one performed before the activation of the CW. In a previous study, the bacterial culturable community associated to the roots of P. australis was investigated. The aim of the present work is the full characterization of the bacterial communities associated to the collected samples, through a next-generation sequencing-based investigation. Results obtained revealed a high conservation of the plant associated bacterial communities during time. On the contrary, permeates showed remarkable sampling-/temporal-related bacterial composition variations. These results suggest that the plant-microbiome interaction is stable despite environmental variations.

#34

Sergio Esposito • Naples

Magnesium di-cations as regulators of plastidic glucose-6P dehydrogenase activity in plants

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Native and cysteines-mutagenized plastidic G6PDH from Populus trichocarpa (PtP2-G6PDH) were overexpressed, purified, and identified by using both specific anti-P2-G6PDH antisera.

These preparations were utilized for the determination of kinetic properties and the determination of a possible reaction mechanism.
The results suggest the enzyme obeys to a sequential mechanism, with cofactor probably binding first. Interestingly, the properties of the double active-cysteines mutant, mimicking the reduced, low activity enzyme, imply that the reduction of thiols severely inhibits the access of coenzyme to the active site. At this regard, Mg++ plays a main role on enzymatic activities, suggesting a key role of Mg++ in determining the correct binding of NADP+ in the active site. Displacement experiments using Cu++ confirmed the specificity of Magnesium in the binding/release of the cofactor NADP+/NADPH during the reaction. These results may indicate the diel fluctuation of Mg++ in plastids as a modulating mechanism of oxidative pentose phosphate pathway in in plants.

#35

Serena Farrotti • Rome

The air is dry: an integrative quest to safeguard crops from a hotter and drier future

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Drought is the leading cause of a world-wide reduction in crop yields and poses a major threat to the global food supply. Low soil moisture and high atmospheric water demand (air drought; referred to as vapor pressure deficit, VPD) are the two main drivers of drought stress. However, while soil drought has been extensively studied, the effects of high VPD on crop growth and yield remain less explored. Thus, the aim of this research is to dissect and characterize the independent effect of high VPD on plant fitness and yield. To this goal, we have developed an integrated experimental system that allows us to identify the morphological, physiological and molecular traits underlying the response to air drought of seedlings of the model plant Arabidopsis thaliana. This research will thus help us to close the gaps in our understanding of the specific role of air drought as a key component of drought stress, and on the long term can contribute to the development of crops resistant to a drier and warmer future with increasing demand of water.

#36

Edgardo Filippone • Portici, NA

Ionome-transcriptome data integration from a subset of Solanum pennellii introgression lines reveals traits for tomato fruit mineral content

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The main aim of ionomics is to relate the content of inorganic elements in plant tissue to the gene expression. In this study we have evaluated the genome impact of wild tomato species Solanum pennellii on fruit mineral content in a subset of introgression lines (ILs) and their cultivated tomato cv M82 by ionome-transcriptomic data integration. The content of 20 inorganic elements obtained by ICP-MS analysis was assessed. Pairwise mineral relationships and ionomic perturbations, triggered by S. pennellii were studied by Pearson correlation. K, and Ca displayed a high variation content in the ILs fruits, while Mn and Se was less pronounced. A strong correlation has been found in Na and Mg, Mn and Co. In order to identify candidate genes involved in tomato fruit mineral accumulation we integrated the ionome data with to the expression fold change of 383 tomato genes identified as encoding elements transporters. The fold change genes expression was correlated to the concentration fold change of some target elements for food safety and for biofortification. Forty-one out genes were highly significantly correlated ($|r|>0.5$; $p<0.01$) with at least one of the 10 target elements.

#37

Anna Fiorillo • Rome

Role of Salt Tolerance-Related Protein (STRP) in salt stress responses in Arabidopsis thaliana

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Despite its self-explicative name, the role played by the Salt-Tolerance Related Protein (STRP) in salt stress remains largely unknown. STRP has been previously characterized as a LEA-like intrinsically disordered protein involved in plant responses to cold stress.

Our experiments show a strong increase of STRP levels in response to salt stress, due to a reduction of proteasome-mediated degradation of the protein. Physiological and biochemical responses to salt stress were studied by comparing wt plants with the strp mutant and STRP-overexpressing plants. We observed that several salt stress-related alterations are more marked in the strp mutant than in the wt plants, whereas they are reduced in the STRP-overexpressing plants. Moreover, strp mutant accumulates more H2O2 and is more susceptible to the oxidative damage induced by salt stress, compared to wt plants. Accordingly, H2O2 production and oxidative damage are reduced in STRP-overexpressing plants. Results obtained suggest a role for STRP in plant protection against salt stress, proposing this protein as a multitasking regulator acting at different levels in the response mechanisms to abiotic stress in Arabidopsis thaliana.

#38

Elide Formentin • Padua

ROS, cytokinin and high salinity affect primary root development in rice

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The root is a fundamental organ for plants as it is involved in nutrition, anchoring and perception of soil-born stresses. The root structure is the result of genetic and environmental factors, and it is dynamic, so that its development is modulated during the plant growth in response of environmental conditions. This ability is related to the activity of the root apical meristem (RAM) which has been shown in Arabidopsis be regulated by hormones and second messengers. In a translational work, we observed that primary root development and plasticity in rice (Oryza sativa, L.) were influenced by cytokinin, ROS and high salinity. We also found candidate genes involved in the regulation of root development that showed different expression profiles in salt-tolerant and salt-sensitive rice plants. Our present results show that similar mechanisms regulate RAM activity in rice and Arabidopsis laying the base for understanding rice root plasticity under high salinity conditions, a major threat to rice productivity worldwide.

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#39

Giobbe Forni • Bologna

Genome evolution in Enterobacteriaceae endosymbionts.

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Enterobacteriaceae include several endosymbiotic lineages which evolved independently and thus represent an extraordinary testing ground to understand the mechanisms underlying endosymbionts genome evolution. Yet, most attention has been focused on a few closely related species — specially endosymbiont of insects — due to the lack of a comprehensive phylogenetic framework. Enterobacteriaceae endosymbionts strongly reject the assumption of common models of sequence evolution (homogeneity, stationarity, and reversibility; HSR) being characterized by an accelerated evolutionary rate and a strong AT bias, compared to free-living species. Here, we leveraged the genomes of 210 Enterobacteriaceae species to infer a novel phylogenetic resolution of the clade, employing methods that can mitigate systematic biases derived from HSR rejection. Our novel phylogenetic resolution shows that endosymbiont genome reduction largely happened concurrently with the shift to their novel lifestyle and that gene transfers are extremely limited. Coherently with theoretical expectations, genes under relaxed selection in free-living species are most likely to be lost in endosymbionts.

#40

Silvana Francesca • Portici, NA

Flowering time and leaf morphology traits are key for heat tolerance in one tomato genotype

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The increase in heat waves is putting global food security at great risk. Crop improvement for tolerance to increased temperatures is therefore of vital importance. High temperatures can cause several physiological changes in plants including flower abscission and photosystems damages, which are related to a decrease of crop production. Previously, we selected a tomato genotype (E42) that demonstrated to have a stable yield under elevated temperatures. We analyzed plant functional traits, growth parameters and physiological responses of E42 to high temperatures in different environmental conditions. E42 was able to maintain a long flowering period, producing flowers even in the latest stages of development, and exhibited good pollen vitality under high temperatures. Moreover, E42 grown during hot periods presented smaller leaf area and major leaf thickness compared to control conditions. Since leaf thickness is related to strategies of resource acquisition and use, we hypothesize that thinning of the leaves of E42 might be regarded as an acclimation mechanism. Further investigation will allow us to confirm these initial outcomes and to better characterize the response of E42.

#41

Ilaria Fraudentali • Rome

Role of CuAOβ and RBOHD-derived hydrogen peroxide in wound-induced local and systemic signal propagation leading to stomatal closure in Arabidopsis thaliana

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In plants, propagation of abiotic stress-induced signaling involves production of hydrogen peroxide (H2O2) that mediates a wide range of functions among which stomatal closure. In guard cells, NADPH oxidases are the best-known indirect sources of apoplastic H2O2, though recent evidence has demonstrated that also polyamine catabolism contributes to H2O2 production in these cells. Here, the specific role of Arabidopsis NADPH oxidase isoform D (RBOHD) and copper-containing amine oxidase β (CuAOβ) in wound-induced stomatal closure in response to leaf or root wounding has been investigated both at injury and distal sites. Analysis of stomatal closure in cuaoβ mutants shows that CuAOβ-derived H2O2 mediates both local and distal responses, as revealed by the complete unresponsiveness of cuaoβ upon both leaf and root wounding. In contrast, analysis in rbohD mutants shows that RBOHD-derived H2O2 is involved only in wound-induced leaf-to-leaf signal propagation, as revealed by the rbohD partially unresponsiveness exclusively in distal leaves. These results suggest distinct roles of CuAOβ and RBOHD in wound-induced local and distal signal propagation pathways leading to stomatal closure.

#42

Isidora Gjata • Bari

Phytotoxicity of rare earth elements cerium and neodymium in onions and lentils

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Many advanced technological applications remarkably increased in the last decades and were associated with an intensive extraction of rare earth elements (REE) from their ores. Increasing amounts of REE-containing by-products are reaching the environmental systems at global levels. To date, few data in the literature concern the effect of REE application in terrestrial vascular plant species and at different physiological stages. This study evaluates the exposure of Lens culinaris Medik. seedlings and Allium cepa L. bulbs to cerium chloride (CeCl3) and neodymium chloride (NdCl3) at different concentrations for 72h. The results highlighted that both Ce and Nd induced alterations in the mitotic activity and the growth and biomarkers of stress (reactive oxygen species, malondialdehyde and antioxidant compounds). Lentils showed a higher sensitivity for lower concentrations (5, 25, 50 µM) than onions. Regarding Nd, a higher cell oxidative status appeared in the lentil seedlings compared to Ce. Lower concentrations of Ce positively affected the growth rate of lentils. This study aimed to improve the knowledge of ecotoxic responses to different REEs in terrestrial plants.

#43

Adriano Griffo • Pavia

Determination of reactive oxygen species (ROS) during seed priming and germination

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Hydropriming is a water-based technique widely used by seed technologists to improve stress tolerance and seed vigour in terms of germination potential. It allows seed rehydration to trigger the metabolic processes normally activated during pre-germinative stress. In this phase, Reactive Oxygen Species (ROS) accumulation is a critical molecular event of the pre-germinative metabolism that influences seed germination. ROS have been proposed as molecular messengers during seed germination and are currently recognized as important factors in seed dormancy. For instance, ROS can control seed dormancy and germination through the interaction with plant hormones. Moreover, ROS production and accumulation by embryos lead to dormancy alleviation. To understand the impact of ROS on germination, two assays, FOX-1 (Ferrous Oxidation in Xylenol orange method 1) and DCFH-DA (Dichloro-dihydro-fluorescein diacetate), were used to determine ROS and oxidative stress levels in seeds from different species subjected to hydropriming treatments. The data collected so far evidence correlations between several germination parameters and ROS levels.

#44

Libero Gurrieri • Bologna

Inhibitory loss of glutathionylation as an alternative regulatory mechanism for Arabidopsis β-amylase 1

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Proteins are fundamental components of the cellular machinery. Post-translational modifications (PTM) influence protein function and, among these, the importance of redox PTM is emerging more and more. Reversible disulfide bonds are recognized as enzyme regulators. Protein cysteines can also form disulfides with glutathione (GSH), a cysteine-containing tripeptide responsible for redox buffering in eukaryotes. Usually, glutathionylation prevents thiols overoxidation by reactive oxygen/nitrogen species.

In Arabidopsis thaliana plants, β-amylase 1 (BAM1) is the only one inhibited by reversible disulfide bond. This regulation is essential to tune starch degradation and maintain stomata opening, as well as sustaining the drought response. BAM1 sensitivity to other redox PTM was explored by testing the effect of H2O2 and GSH on enzyme activity. Interestingly, H2O2 reversibly inactivates BAM1 and together with GSH triggers glutathionylation. Glutathionylated BAM1 is still active but spontaneously releases GSH turning into an inhibited state likely due to disulfide bond formation.

This in vitro results support the role of BAM1 in stomata opening and as a stress-adapted enzyme.

#45

Sondos Hejazi • Naples

Bio-inspired materials manufactured with poly-γ-glutamic acid and chitosan

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The demand for developing natural biodegradable polymers is an emergence-driven force for bio-plastic industries due to the public concern of the impact of plastic materials on the environment. To this aim, promising novel bio-composite materials were manufactured by blending poly-γ-glutamic acid (γ-PGA) and chitosan (CH) under specific experimental conditions. γ-PGA is an anionic homopolyamide synthesized by Bacillus bacteria, whereas chitosan is a linear heteropolymer derived from the deacetylation of chitin, the second most abundant polysaccharide in nature. In particular, low Mw-CH (50-190 kDa) and low Mw (18 and 55 kDa) ultra-filtrate γ-PGA were blended at pH 3.5 using specific ratios (CH/γ-PGA: 1-5/9-5, w/w). The obtained materials appeared as moldable hydrogels when suspended in water, whereas they resulted extremely hard when dehydrated as well as completely insoluble in organic solvents. FTIR analyses indicated strong electrostatic and hydrogen interactions between the two biopolymers. On the other hand, the additional physicochemical properties of the new materials, still under study, suggest their potential exploitation in multiple industrial sectors.

#46

Maurizio Iovane • Naples

Heat treatment on microsporogenesis affects pollen functionality and timing of pollen ontogenesis

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Pollen functionality is essential for the successful reproduction of all plant species and therefore plays a key role in the crop species in producing seeds and /or fruits. In the current climate change scenario, extreme weather events such as heat waves, are becoming more frequent endangering crop productions.

Previous experiments showed that heat treatments on pollen negatively affect pollen germinability in crops. Differently, here we tested if high temperature (HT) occurring during microsporogenesis (MS) can affect pollen formation and its subsequent functionality in the stage of pollen (microgametophyte). Data on Solanum lycopersicum ‘Micro-Tom’ showed that HT during MS decreased pollen germination and heat-treated microspores developed into not germinable pollen. Further, we assessed that in the HT treatment, all the developmental stages resulted shifted forward compared to the control. We thus hypothesize that HT during MS cause a premature transition of microspores into mature pollen ready for dispersal.

Therefore, HT during the earliest stage of pollen development (MS), accelerates pollen senescence endangering pollen germination and consequently reproductive success.

#47

Nataliia Kozak • Pavia

Disentangling the genetic history of modern Ukrainians through mitogenomes

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The territory of Ukraine has been a crossroads of people and cultures since the Palaeolithic due to its strategic location on the northern shore of the Black Sea at the intersection between East Europe and the Pontic Caspian steppe. These features make the assessment of Ukrainians' genetic variation and history of particular value to fully reconstruct the genetic landscape of West Eurasia.

In this work we analysed 94 mitogenomes from modern Ukrainians collected in the territory of Donetsk Oblast. Thanks to detailed genealogical data, each individual was redistributed according to the birthplace of the last known terminal maternal ancestor (TMA). It is worth mentioning that one third of them were from western Russia. We detected extensive mitochondrial DNA variation in the entire dataset, testified by lineages typical of both eastern and western Europe. However, we also identified a differential distribution of specific mitochondrial haplogroups between individuals with a TMA from Ukraine and Russia, pointing to different ancestral roots from a female perspective. The results will be also discussed in comparison with those obtained from the Y chromosome on the same dataset.

#48

Simone Landi • Naples

The circle of reductants to respond to low temperature: the role of the oxidative pentose pathway

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Cold stress represents one of the most detrimental abiotic conditions, inducing a number of negative effects, from photosynthetic stress to oxidative damages. Unexpected low temperature events, often triggered by climate change, impact on biomass and yields in crops. A critical factor in plant response to abiotic stress is the supply of reductants, e.g. NADPH, requested by the antioxidant system. In plants, the oxidative pentose phosphate pathway, and particularly the first-phase of the cycle, provides a major portion of NADPH. In this work we aim to elucidate the possible roles of the main regulatory enzyme - glucose 6P dehydrogenase - in the tolerance to cold stress in higher plants.

Arabidopsis plants defective in chloroplastic isoform (P1-G6PDH) were exposed to cold stress, displaying an improved response, a modification of lipid metabolism and a modified chloroplast development. Our results suggest a prominent role of cytosolic and peroxisomal G6PDHs during cold stress and recovery, recognizing a central role of these enzyme in plant acclimation to adverse conditions. The functions of the G6PDH isoforms present in plant tissues under cold stress and acclimation are discussed.

#49

Cecilia Lasorella • Bari

Chloroplast-localized GUN1 contributes to the acquisition of basal thermotolerance in Arabidopsis

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Temperature rises are a critical threat to plant growth. Heat stress (HS) alters cellular homeostasis and inhibits photosynthesis, causing serious damage to components operating in different metabolic processes. Chloroplasts are crucial in heat stress response (HSR), signalling to the nucleus the environmental stress and adjusting metabolic and biosynthetic functions, accordingly. GENOMES UNCOUPLED 1 (GUN1), a chloroplast-localized protein, has been recognized as one of the main actors of chloroplast retrograde signalling. Here, we studied the HSR in fourteen days-old wild type and gun1 plants subjected to 3 hours of HS at 45°C, focusing on the role of reactive oxygen species (ROS), and ROS-scavenging systems in the signalling activating defence responses. The results show that gun1 mutants result more sensitive to HS than wild type plants. The analysis of heat shock proteins and redox systems indicates that the major differences occur in the phase of recovery from HS. The data suggest that GUN1 could be required to oxidize the cellular environment, participating to the acquisition of basal thermotolerance through the redox-dependent plastid-to nucleus communication.

#50

Riccardo Lorrai • Rome

Plant cell wall perturbations trigger hormonal changes impairing apical hook development

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The plant cell wall (CW), mostly composed of polysaccharides, provides mechanical support to plant cells, countering turgor pressure and controlling cell shape and size. Seedlings via differential epidermal cell elongation form a protective structure, the apical hook (AH) which prevent damage to the apical meristem. The role of the phytohormones auxin (IAA) gibberellins (GAs) and ethylene (ET) in this process is well characterized, but their interplay with the CW status is still elusive. Exposure to biotic or abiotic stresses alter CW structure leading to the activation of compensatory mechanisms, such as the accumulation of defence-related phytohormones, jasmonic acid (JA) and salicylic acid (SA), which, conversely to IAA, GAs and ET, antagonize AH development. Here we have investigated the interplay between CW alterations and the regulation of GA signalling in AH formation. CW alterations impaired AH development via the downregulation of GA biosynthetic genes and the upregulation catabolic ones. Alleviation of CW perturbation recovered both AH development and GA gene expression. Finally, we dissected the crosstalk between JA, GA and CW during AH development.

#51

Francesca Lucibelli • Naples

The Phalaenopsis PeDL2 gene is a possible new regulator of labellum development

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In many plant species, the YABBY transcription factor DL/CRC is involved in carpel development. The Phalaenopsis orchid has two DL/CRC genes, PeDL1/2, with conserved function in regulating gynoecium development. However, in silico differential expression analysis, confirmed by qPCR experiments, showed that the PeDL2 expression pattern resembles that of the class B MADS-box PeMADS3/4 and is opposite to PeMADS2/5. These MADS-box genes are key regulators of the orchid perianth formation. In the wild-type flower, PeDL2 is highly expressed in the lip, the modified inner median tepal that attracts pollinators. In contrast, it is expressed at similar levels within the perianth structures of peloric mutant. To characterize PeDL2, we performed the computational prediction of its structure and possible protein interactions, the intracellular localization of the full-length protein and individual domains through transient expression in Nicotiana benthamiana, and the screening of possible promoter interactors by in silico analysis. Finally, we set protoplast dsRNAi experiments to follow the expression level of PeDL2 genes after AP3/DEF-like genes silencing.

#52

Francesco Magnanimi • Rome

Mechanisms and molecular basis of the effect of natural oils on the development and resilience of plants aimed at the eco- sustainable production of vegetable species

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Natural substances represent an alternative to mitigate the excessive use of chemicals, giving contribution to eco-sustainable agriculture. Essential oils have been extensively applied in organic farming with effective controls against plant diseases due to both pathogens and pests. However, studies are needed to understand plant responses at the physiological and molecular levels in order to unravel fundamental mechanisms, optimize oil usage and preserve crop quality. Moreover, studies on the effects of oil on plant organ differentiation and development are still in their infancy. Within a context of circular bioeconomy, a research program has been developed (industrial doctorate and the project TOP CROP – Gruppi di Ricerca by Regione Lazio) to study the effects of lavender and jasmine oils on flowers of Brassicaceae using a translational biology approach with model (A. thaliana) species and local crops (Brassica rapa sylvestris, cime di rapa) through lab and open field experiments. Here, we report the project outline and preliminary results regarding the oil effects on flower opening.

#53

Pina Marotta • Naples

Insights into the molecular mechanisms of diatoms life cycle

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Oceans are populated by a myriad of unicellular eukaryotic and prokaryotic organisms composing the phytoplankton, whose knowledge is precious to understand ocean functioning and the evolutionary history of life on earth. Here, we focus on diatoms, microalgae at the base of aquatic food webs, characterized by high biodiversity and adaptive capacity. Their life cycle is characterized by a progressive cell size reduction; this would inevitably lead to death if they do not restore the initial cell size, generally through sexual reproduction.

Using Pseudo-nitzschia multistriata as a model system, we are exploring the factors controlling transitions between the different phases of diatom life cycle: mate perception, meiosis, sexual competence acquisition and sex determination. Transcriptomic and transgenesis experiments outlined the basis of sex determination and the physiological changes occurring in the early phase of mating; moreover, ongoing experiments are elucidating the chemical crosstalk regulating mate perception.

Comparisons with environmental metadata will help to establish sex markers at sea and the identified pheromones could be exploited in biotechnological applications.

#54

Carmine Merola • Teramo

Is methylparaben an endocrine disrupting-chemical inducing generic toxicity in zebrafish model?

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Methylparaben (MeP) is widely used as a preservative in personal care products, food commodities, and pharmaceuticals due to its antimicrobial properties. Its widespread use resulted in the contamination of the aquatic environment and raised concerns about the potential adverse effects on human health, especially in developing organisms. The present study aimed to evaluate the embryotoxicity of MeP in zebrafish's early-life stages focusing on the investigation of lethal and sublethal alterations. Zebrafish embryos exposed to MeP had several developmental defects. Zebrafish larvae treated with 30 mg/L of MeP showed behavioral changes including tremor behavior with head, pectoral fins, and spine tremors. To characterize this behavioral phenotype, zebrafish larvae exposed to MeP were compared with a pharmacological model of epilepsy, the pentylenetetrazole (PTZ), and the evaluation of genes commonly involved in the epileptic behavior, namely bdnf and c-fos, was performed. The results of the molecular analysis showed a similar trend of gene modulation in MeP-exposed zebrafish compared to PTZ-treated larvae, with an up-regulation of the gene expression for both genes.

#55

Michela Molinari • Rome

A correlation between sulfur and glutathione metabolism in Oryza Sativa under salt stress

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Rice (Oryza sativa) is a staple food for 50% of the world's population and is also one of the most salt-sensitive crops among cereals. Soil salinization is an increasing environmental challenge that reduces the yield of crops. Studies of intra-species variability have been significant for the identification of traits involved in salt stress tolerance. The involvement of antioxidant system is crucial in the response to abiotic stress. Since we previously reported different glutathione biosynthesis and levels of glutathionilated proteins between tolerant and sensitive Italian varieties (Cimini et al. Antioxidants 2022), we investigated the involvement of miR395 whose targets are ATP sulphurilase (ATPS) and S transporters'(SULTR) genes in salt stress resistance. Inorganic and organic sulfur concentration and distribution, with a focus on cysteine and methionine in roots and leaves, were also analysed. The obtained data support a strong relation between S metabolism and stress resilience in the rice salt tolerant variety.

#56

Francesco Montemagno • Naples

Shark microbiome in the Mediterranean sea: microbiome analysis demonstrates unique microbial communities

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Our knowledge of the role of the microbiome in fish health has been steadily increasing for commercial species, while there are still relatively few studies focusing on the shark microbiome, due to both the lower economic interest behind this group, and the greater difficulty in sampling these animals. Most studies on shark microbiome mainly focus on the microbial diversity present on shark teeth, or evaluating the presence of certain pathogens in healthy or diseased specimens. Only a handful of studies have the intent to evaluate the link between the host ecology, certain population-level dynamics or phylogeny on the shark microbiome. Our study investigates the microbiome diversity in different species of sharks in the Mediterranean sea, to get a better understanding of the microbiome of these animals, how it changes throughout different body parts and how much it is influenced and determined by the ecology and phylogeny of the host.

To our knowledge, this is the first study comparatively analysing the microbiome diversity of different shark species and anatomical location in the Mediterranean Sea.

#57

Maria Monti • Naples

Identification and functional characterization of Toxoneuron nigriceps ovarian proteins involved in the early suppression of host immune response

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Toxoneuron nigriceps is an endoparasitoid of the larval stages of Heliothis virescens. During oviposition, it injects into the host body the egg, the venom, the calyx fluid, containing a Polydnavirus and the Ovarian Proteins (OPs). OPs are involved in precocious host immune system alterations. To functionally characterize the OPs, we firstly incubated hemocytes with OPs in toto, detecting several alterations on host cells that can explain the high mortality of hemocyte and the loss of their encapsulation ability. Then, we evaluated the effect of the 28 HPLC fractions deriving from in toto OPs. Among them, 2 fractions caused a reduction in hemocyte viability and were tested to detect changes in hemocyte morphology and functionality. Analogously to results obtained with in toto OPs, the 2 fractions caused severe oxidative stress, actin cytoskeleton disruption and loss of functionality. By a transcriptome and proteomic approach we identified 8 proteins in the 2 fractions that might be involved in the observed host hemocyte changes. Our findings can contribute to better understand the ovarian components and their role in parasitic wasp strategy to escape the host immune responses.

#58

Elisabetta Moroni • Pavia

Analysis of ancient human genomes from a medieval site in Northern Italy

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History can be studied from different perspectives, including the one provided by archaeogenomics, the study of human past through ancient genomes. Up until now, only few publications have included individuals from medieval Southern Europe and even less from Italy. In particular, there is no genetic data from populations of medieval Lombardy. In this study, 20 ancient human bones recovered from the medieval church of San Biagio in Cittiglio (Varese, Italy) were processed to obtain low-coverage whole genomes through shotgun sequencing on Illumina platform. Sequenced reads underwent an extensive validation procedure and, eventually, 15 whole genomes were eligible for further investigation. This focused on sex determination, phylogenetic and phylogeographic analyses of mitogenomes and assessment of kinship at genome-wide level. The results contributed to reconstruct the biological profile of the individuals and the genetic past of Northern Italy.

#59

Yuri Luca Negroni • Padua

Genetically encoded biosensors in grapevine for studying abiotic stresses

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Plants are continuously exposed to abiotic stresses especially due to extreme weather events that are now more frequent than ever, as a consequence of global climate changes. Once the plant is exposed to stress, the intracellular homeostasis is altered and the release of different mobile signaling factors, such as ROS, calcium and hormones (SA and ABA), is triggered, In the last few years, the interest in these stress response mechanisms is increasing. Understanding the molecular players involved and their dynamics could help us find innovative solutions for aiding crop plants in their battle against harsh environmental conditions. Among crops, grapevine cultivation is an economically relevant sector for several Mediterranean Countries. Genetically encoded fluorescent protein sensors can be used to follow the kinetics of different intracellular signal in vivo upon exposure to various stimuli. Generation of grapevine lines harboring ABA, Ca2+ and H2O2 sensing probes, will allow us to better understand the dynamics of these signaling factors under the exposure of grapevine on abiotic stresses, deepening our understanding on how in crops can be enhance the plant resistance to stress.

#60

Stefano Nenciarini • Sesto Fiorentino

Yeast Extracellular vesicles as means of immunomodulation

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Extracellular vesicles (EVs) are lipid-bilayered particles released by cells for different functions. In fungi, functions of EVs include modulation of the host immune system. One possible hypothesis for this modulation is through the interaction between host immune cells and small RNAs transported by EVs. This study aims to assess variety in production levels and immunological effects of EVs released by yeast strains from different species and origin. Specifically, strains of S. cerevisiae and P. fermentans, isolated either from Tuscany grapes or fermented milk, have been screened for their ability to produce high levels of EVs in different growth conditions. Then, immunomodulation potential of isolated EVs have been studied through immune assays after internalization from human dendritic cells. Results showed a significative reduction of antigen presentation ability of dendritic cells treated with EVs from isolated from fermented milk strains. In conclusion, this study suggest that different yeast strains produce EVs with different effects, and that a possible mechanism of beneficial effects of fermented beverages is based on the release of EVs from their yeast strains.

#61

Anna Olivieri • Pavia

Barn swallows: mitogenome relationships and phylogeography

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The barn swallow (Hirundo rustica) is one of the most widely distributed bird species. Here we analysed the sequence variation of 411 complete mitogenomes, from the European H. r. rustica and other subspecies. We observed subspecies-specific haplogroups, which we employed together with estimated radiation times to postulate a model for the geographical and temporal worldwide spread of the species. The female barn swallow carrying the H. rustica ancestral mitogenome left Africa (or its vicinity) around 280 thousand years ago (kya), and her descendants expanded first into Eurasia and then, at least 51 kya, into the Americas, from where a recent (< 20 kya) back migration to Asia took place. The exception to the haplogroup subspecies specificity is represented by the sedentary Levantine H. r. transitiva that shares haplogroup A with the migratory European H. r. rustica and haplogroup B with the Egyptian H. r. savignii, thus providing evidence that admixture events occurred between different subspecies in specific geographic areas. Demographic analyses confirm this species' strong link with climate fluctuations and human activities.

#62

Andrea Pagano • Pavia

Genotoxic damage and nucleolar stress response in primed and overprimed Medicago truncatula seeds

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Desiccation tolerance is crucial for survival under seed priming protocols and variable field conditions. Nonetheless, the molecular and ultrastructural features underlying the maintenance and loss of desiccation tolerance are not fully elucidated. The present study explores the responses of Medicago truncatula seeds to two hydropriming conditions displaying contrastive effects in terms of desiccation tolerance. Priming and overpriming were carried out through a 24 h imbibition step followed by 6 h of desiccation (dry-back). Primed seeds did not display radicle protrusion before dry-back and were desiccation tolerant, whereas overprimed seeds irreversibly entered germination and developed aberrant phenotypes after dry-back.

The accumulation of reactive oxygen species and DNA damage, the expression profiles of antioxidant and DNA Damage Response genes, the accumulation of rRNA molecules, and the ultrastructural features of nucleus and nucleolus were assessed as hallmarks of the seed response to desiccation stress, highlighting a consistent response to overpriming during dry-back and providing novel insights on seed metabolism responding to seed priming and stress conditions.

#63

Anna Maria Pappalardo • Catania

Evidence for selection on mitochondrial DNA in the Mediterranean killifish Aphanius fasciatus Valenciennes, 1821

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Protein subunits involved in the oxidative phosphorylation pathway (OXPHOS), encoded by 13 mitochondrial genes and several nuclear genes, are subject to high functional constraint. However, variation in environmental factors leading to changes in metabolic requirements could potentially act selectively on OXPHOS genes. In this study, the Cytochrome Oxidase I (COI) mitochondrial gene was analyzed in 113 specimens of the killifish Aphanius fasciatus, from 6 Mediterranean (MED) localities. The species is endemic in MED and tolerates extreme conditions of temperature, salinity and oxygen concentration. The COI sequence analysis identified a non-synonymous transition (G to A) at position 64 resulting in amino acid change. All COI sequences of the Greek population had a Threonine (T) in position 22 of the amino acid sequence where an Alanine (A) was detected in the other populations. Both MEME and FUBAR selection tests showed episodic and diversifying positive selection at 9 and 111 codon position respectively. Mutations at such sites may experience transient positive selection, followed by purifying selection to maintain the change, and could play a key role in adaptative evolution.

#64

Chiara Pedalino • Rome

Plant as a whole: rapid long-distance signaling mediated by Ca2+-ATPases and glutamate receptor-like channels triggers wound-induced stomatal closure

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The Arabidopsis auto-inhibited Ca2+-ATPases 8 and 10 (ACA8/10) and the glutamate receptor-like channel 3.3 (GLR3.3), all three especially localized in guard cells, are involved in [Ca2+]cyt transient changes and cell-to-cell communication, respectively. In this study, the role of these proteins in wound-induced stomatal closure both at the injury and distal sites, in response to local-leaf wounding and distal-leaf/root wounding, was investigated by loss and gain of function genetic approaches. In wild-type (WT), distal and local wounding induced a rapid stomatal closure, while aca8/aca10 single and double mutants and ACA8 over-expressing lines were unresponsive possibly due to dysregulation of calcium homeostasis. Interestingly, glr3.3 mutants showed similar stomatal modulation as compared to WT upon local injury while showing a partial closure upon distal wounding. These results suggest that ACA8/10 may be necessary for the modulation of cytosolic calcium signature in guard cells in response to both local and distal wounding, in a mechanism coupled to GLR3.3-mediated long-distance signal propagation leading to stomatal closure.

#65

Elisa Pellegrini • Pisa

Early biochemical responses of resistant and susceptible clones of cypress infected with Seiridium cardinale

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Seiridium cardinale is a pathogenic fungus responsible for cypress canker disease, which has caused severe mortality worldwide in many species of Cupressaceae. To face this epidemic, a series of canker-resistant clones of Cupressus sempervirens have been selected so far. This study aims to elucidate the early biochemical responses to S. cardinale infection of canker-susceptible and resistant cypress clones. In inoculated susceptible cypress, a high production of ethylene and jasmonic acid (more than 4-fold higher than uninoculated ones) occurred at 3 and 4 days post inoculation (dpi) in the bark. However, a partial redox rearrangement of antioxidant compounds was recorded in the foliage. In inoculated resistant individuals, an increased production of ethylene was observed at 3 and 13 dpi in the bark (more than 2-fold higher). Conversely, a rise of salicylic acid levels (more than 4-fold) occurred in the foliage at 1 dpi, concomitantly with an accumulation of abscisic acid, that reached the maximum values at 9 dpi. The activation of phytohormones and signaling molecules may counteract oxidative burst and prevent lipid peroxidation by offering protection to bark tissues and foliage.

#66

Federica Pennisi • Verona

Investigation of the role of B-box MicroProteins in the tomato reproductive development

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Microproteins (miPs) are single-domain proteins that act as post-translational regulators of protein complexes. A central role of two Arabidopsis miPs, miP1a and miP1b, in the control of flowering time has recently been identified. These miP1s are members of the BBX family, a group of zinc-finger transcription factors and regulators. BBX proteins are characterised by a B-Box motif, responsible for protein-protein interaction. It has been shown that both miP1a and miP1b actively participate in the Arabidopsis flowering process by mediating the recruitment of CONSTANS (CO) into a repressor complex with TOPLESS (TPL). In Arabidopsis, the interaction of CO with miP1a/b and TPL causes a reduction in the expression of FLOWERING LOCUS T resulting in a delay in flowering. SlBBX16 and SlBBX17 are the tomato homologs of miP1b and miP1a, respectively. As there is no evidence in the literature for the presence in tomato of a flowering inhibitor complex similar to that in Arabidopsis, we checked the interactions between SlBBX16/17 with key flowering regulators by yeast-two-hybrid analysis. We also monitored the reproductive phenotypes of Arabidopsis and tomato plants overexpressing SlBBX16/17.

#67

Silvia Perretti • Ferrara

Understanding the process of skin lightening in Europeans: selection and migration

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Variation in skin pigmentation is one of the best-known products of human adaptation. Protection against photolysis of folate, and enhanced synthesis of vitamin D, account for, respectively, dark and light skin colours. The exact combination of selective and migration processes that lead to the spread of light pigmentations in Europe is still far from clarified.

Ancient DNA studies showed that only 10,000 years ago hunting-gathering populations living as high north as the British islands had dark skin, and the first genetic signals of lightening did not appear until the spread of Neolithic farmers, almost 3,000 years later.

Here we present an innovative approach based on Artificial Intelligence to infer skin pigmentation from DNA haplotypes. We compared multilocus data in modern populations and key ancient individuals, representing the main prehistoric groups migrating into Europe. Based on state-of-art bioarchaeological approaches (aDNA, 14C dates and cultural information on the samples), we reconstruct some of the main evolutionary events that shaped the current diversity of human skin pigmentation.

#68

Alex Pessina • Milan

A novel strategy for ammonia production using yeast

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Ammonia, with a worldwide production of 235 million tonnes is one of the main chemical commodities produced, extensively used as fertilisers with growing interest as an energy storage vector. The conventional Haber-Bosch synthesis requires about 2-3% of total world's energy commonly derived from fossil fuels, generating disastrous effects for the environment. To achieve a carbon-free society, sustainable routes for its production are needed.

Among the green approaches, some bacteria and yeasts have already been used to produce ammonia from biomass, respectively, by metabolic engineering and by displaying an amino-acid-catabolizing enzyme on the cell surface. In our laboratory, with a Saccharomyces cerevisiae strain, we investigated the natural ability of yeast to release ammonia, opening new process possibilities. We worked to validate the ammonia release and further increase this feature. Furthermore, the preliminary results showed very promising extracellular ammonia accumulation which could have a valuable biotechnological application. The final goal of this project will be to create a cell factory able to valorise different waste biomasses for a renewable and sustainable process

#69

Matteo Pivato • Verona

Dissecting the role of intracellular Ca2+ signalling in the responses to the environment in the green microalga Chlamydomonas reinhardtii

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Calcium (Ca2+)-dependent signalling plays a well-characterized role in the perception and response mechanisms to environmental stresses in plant cells. Although Ca2+ is known to be important in disparate physiological processes in the model green alga Chlamydomonas reinhardtii, many of these signal transduction pathways still need to be characterized, as well as the evolution of the involved Ca2+ signalling machinery. To investigate the role of intracellular Ca2+ signalling in the mechanisms of response to a range of environmental stressors (high light, nutrient availability, osmotic stress and temperature), we targeted a genetically encoded ratiometric Ca2+ indicator to different subcellular compartment of C. reinhardtii cells. Cytosolic, chloroplast and mitochondrial localization in stably transformed lines were confirmed by confocal microscopy. Through in vivo single-cell imaging we report compartment-specific [Ca2+] transients, characterized by stimulus-specific kinetic parameters. Our data point out a crucial role of Ca2+-dependent signalling at the level of specific subcellular compartments in the response mechanisms to several environmental stressors in C. reinhardtii.

#70

Miriam Porretti • Messina

Plasticisers content in women’s blood at different ages

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Phthalic acid esters (PAEs) are used as additives in the formulation of plastic polymers. Phthalates are regarded as endocrine disruptors and may have adverse effects on human health. Currently, instead of PAEs, non-phthalate plasticisers (NPPs) are used, including adipates (DEHA), sebacates and terephthalates (DEHT).

The presence of PAEs in the ecosystem is ubiquitous, including the marine environment, sediments, and foods, such as fish, meat, cheese, herbs, and spices. Inevitably they are ingested or absorbed by the human body. However, the bibliography on the presence of plasticisers in human blood is limited. In this work the plasticisers were evaluated in women’s blood at different ages, ranging between 20 and 60 years, from the same geographical area. Extraction of plasticiser residues was performed on 40 whole blood samples in hexane and simultaneously qualitative-quantitative analysis was performed by GC/MS-MS. Results show that residues of PAEs and NPPs were found in all samples analysed, assuming presumably an age-varying residue content. Further research will be devoted to investigating the effects of such molecules on blood cells.

#71

Adele Preziosi • Rome

2-hydroxyisobutyrate (2-HIBA), an unspecified mammalian metabolite, modulates ageing and fat deposition depending on the high glucose diet in C. elegans animal model

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2-hydroxyisobutyrate (2-HIBA) is a metabolite found in human urine. Recent studies have shown that 2-HIBA levels were increased in patients with obesity and hepatic steatosis, suggesting that it could potentially be involved in clinical conditions. We analyzed the effect of 2-HIBA on the model organism Caenorhabditis elegans, in standard and High-Glucose Diet (HGD) growth conditions, by targeted transcriptomic and metabolomic analyses, Coherent Anti-Stokes Raman Scattering and fluorescence microscopy. In standard conditions, 2-HIBA was able to extend the lifespan, delay ageing processes and stimulate the oxidative stress resistance in wild type nematodes through the activation of insulin/IGF-1 signaling (IIS) and p38 MAPK pathways with a reduction of ROS levels and an increase of lipid droplets accumulation. 2-HIBA pro-longevity effect on C. elegans grown on HGD appeared to be correlated to tryptophan degradation pathway mediated by 2,3-dioxygenase (TDO) activity. Tryptophan levels, higher in treated worms, may play a role in restoring the decreased viability observed in the untreated ones. The effect of 2-HIBA on HGD worms resulted in a reduction of the lipid droplets deposition.

#72

Sonia Renzi • Sesto Fiorentino

The transition from a traditional to a Western lifestyle and its effect on the interrelation between diet, gut microbiome and health

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Industrialization has historically led to rural-to-urban migration, alongside a change in nutrition from mostly plant-based foods to globalized hyper-caloric diets. Currently, several regions in the world, such as Burkina Faso, are experiencing a differential transition towards urbanization. All the demographic transitions significantly affect both human microbiome and physiology because of (i) a change in the exposure to microorganisms, (ii) a nutritional transition to a "western" diet. Among the multiple factors that influence human microbiome composition and activity, diet has been identified as one of the key players. The advances in omics technologies have improved our understanding of microbiome and metabolome, but the integration of these data and the understanding of the gut microbiome role in maintaining health as well as in the onset and progression of chronic diseases is still far to be achieved. The main goal of this study is the assessment of the complex interaction between diets and host metagenomic, through nutritional analysis and targeted and untargeted sequencing of the microbiome to unravel the cause-effect relation of diet-microbiome-health.

#73

Maria Manuela Rigano • Portici, NA

Modulation of AsA biosynthesis and pectin-related gene expression by genetic introgression to enhance resistance to Botrytis cinerea in Solanum lycopersicum

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Cell wall structure and cellular redox state both play a key role in plant resistance to pathogens. Tomato (S. lycopersicum L.), one of the most important vegetable crops, is susceptible to a wide range of pathogens.

The S. lycopersicum × S. pennellii introgression line IL12-4-SL with higher content of ascorbic acid (AsA), produced by the uronic acid pathway, have been exploited to provide information on the link among AsA, pectin metabolism and plant response to pathogens.

Transcriptomic analysis of genes involved in pectin-mediated AsA metabolism indicates increased expression of pectic genes such as UGLcAE involved in galacturonic acid biosynthesis, pectin methylesterases (PMEs) and polygalacturonases (PGs) and a concomitant increase in PME activity. Remarkably, a reduced susceptibility of the IL12-4-SL plants to B.cinerea was observed with respect to the tomato variety M82. HPAEC-Dionex analysis of cell wall monosaccharide composition consistently indicates greater cell wall integrity in IL12-4-SL plants compared to M82 after fungal infection. Defence gene expression profiling during infection is in progress to understand the molecular basis of disease resistance in IL plants.

#74

Teresa Rinaldi • Rome

Carbonatogenic bacteria on the ‘Motya Charioteer’ sculpture

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The marble statue ‘Motya Charioteer’, one of the greatest masterpieces of the archaic Greek sculpture, was discovered in 1979 in island of Motya, Sicily. In 2019 and in 2020, microbiological surveys were performed to assess the type of biodeterioration, i.e., the presence of bacteria which can metabolize calcium carbonate and contribute to the damage of the statue. Indeed, we selected bacterial strains able to dissolve calcium carbonate mainly from the damaged areas of the statue; among 31 strains showing a calcium carbonate metabolism (precipitation and/or dissolution), 24 were bacilli; although we cannot prove that the bacteria are responsible for the statue degradation, nevertheless their metabolism is fully competent to contribute to marble dissolution. In two damaged areas of the statue, we also found Staphylococcus haemolyticus, a common component of human skin flora; this strain demonstrated a fast calcium carbonate dissolution property, which had not previously been established for this species. Two Bacillus strains showed carbonatogenic features suitable for a bioconsolidation intervention on the sculpture.

#75

Antonio Massimiliano Romanelli • Fisciano,SA

Cytotoxicity of the emergent environmental pollutant 4-octylphenol in human cell lines

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The pollutant 4-octylphenol (4-OP) originates from the environmental degradation of alkyl phenol ethoxylates, compounds largely used in several industrial applications. Together with 4-nonylphenol (4-NP), 4-OP represents a health risk for animals and humans which can be exposed to it through contaminated food and water and through inhalation and dermal absorption. Here, we investigated 4-OP-induced cytotoxicity in three human cell lines (HepG2, Caco-2, MRC5 cells). By performing a MTT assay, we found a dose-dependent reduction of cell viability in all cell lines treated with 4-OP in the range 25-100 mM. Interestingly, treatments with 4-OP, together with 4-NP, seemed to produce synergic cytotoxic effects, in particular in MRC5 cells. In HepG2 cells, we also observed a significant reduction of cell cycle progression, an increased caspase-3 cleavage and
chromatin fragmentation and a stimulation of the ER-stress markers. Our preliminary findings highlight potential 4-OP-induced damaging effects on organs homeostasis and physiology, and indicate the importance to further investigate biological effects of the concomitant exposure to different alkyl phenols.

#76

Simone Samperna • Rome

Cyclopaldic acid induces PCD and autophagy in Arabidopsis thaliana

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Cyclopaldic acid is one of the main phytotoxic metabolites produced by fungal pathogens of the genus Seiridium, causal agents, among others, of the canker disease of plants of the Cupressaceae family. Previous studies showed that the metabolite is toxic to different plant species, thereby proving to be a non-specific phytotoxin. Despite the remarkable biological effects of the compound, information about its mode of action is still lacking. In this study, we investigated the effects of cyclopaldic acid in A.thaliana plants and protoplasts, in order to get informations about subcellular targets and mechanism of action. Results of biochemical assays showed that cyclopaldic acid induced leaf chlorosis, ion leakage, hydrogen peroxide production, inhibited root proton extrusion in vivo and plasma membrane H+-ATPase activity in vitro. Confocal microscopy analysis of protoplasts showed that cyclopaldic acid targeted the plasma membrane H+-ATPase, inducing depolarization of the transmembrane potential, mitochondria, disrupting the mitochondrial network and eliciting overproduction of ROS, and vacuole, determining tonoplast disgregation and induction of vacuole-mediated PCD and autophagy.

#77

Irene Sbrocca • Rome

Phenotypical and bio-chemical characterization of durum wheat (Triticum durum) genotypes under salt stress

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Durum wheat (Triticum durum) is the most widely grown crop in the Mediterranean basin, as its end-products are traditionally associated with the Mediterranean diet. Breeding is one of the most cost effective and environmentally safe ways to cope with the future challenges that durum wheat productivity will face due to the effects of climate change on environment, such as soil salinization. The identification of genetic resources and the study of genetic variability and their effects on plant physiology could contribute to the increase and stability of production in future adverse climatic conditions. On these bases, we performed a screening of 10 different durum wheat genotypes resulting from a breeding program between "Primadur", an elite cultivar (yellow) and "T1303", a pigmented cultivar (purple). We evaluate the salinity stress tolerance at two different concentrations (50 and 100 mM NaCl) during seed germination and seedling developmental phase. Based on germinability, we identified four salt-resistant genotypes, two of which also showing good growth performance at the seedling developmental phase. The anti-oxidative properties and polyphenols content were also investigated.

#78

Valentina Scafati • L'Aquila

Characterization of two 1,3-β-glucan acting enzymes from Penicillium sumatraense reveals new insights into 1,3-β-glucan metabolism of fungal saprotrophs

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1,3-β-glucan is a polysaccharide widely distributed in several phylogenetically distant organisms such as bacteria, fungi, plants, macro- and micro-algae. To elucidate the molecular mechanisms of 1,3-β-glucan metabolism in fungal saprotrophs, the putative exo-1,3-β-glucanase G9376 and the truncated glucan endo-1,3-β-glucosidase ΔG7048 from Penicillium sumatraense AQ67100 were heterologously expressed in Pichia pastoris and characterized. G9376 converted laminarin and 1,3-β-glucan oligomers into glucose by acting as an exo-glycosidase, whereas ΔG7048 displayed a 1,3-β-transglucanase/branching activity towards 1,3-β-glucan oligomers that made the branched products more recalcitrant to the hydrolysis acted by G9376. The 1.9 Å resolution crystal structure of the catalytic domain of G7048 shared the $(\beta/\alpha)8$ TIM-barrel fold characteristic of all GH17 family members and a V-shaped catalytic cleft containing the two conserved catalytic glutamic residues. The antagonizing activity between ΔG7048 and G9376 suggested how opportunistic fungi belonging to Penicillium genus may feed on substrates similar for composition and structure to their own cell wall without incurring in a self-damage.

#79

Anna Scortica • L'Aquila

Berberine bridge enzyme-like oligosaccharide oxidases act as enzymatic transducers between microbial glycoside hydrolases and plant peroxidases

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OGOX1 and CELLOX have been recently identified as members of berberine bridge enzyme-like (BBE-l) proteins from Arabidopsis thaliana. They act as oligosaccharide-oxidases (OSOXs) capable of oxidizing specific cell wall fragments [i.e., oligogalacturonides (OGs) for OGOX1 and cellodextrins (CDs) for CELLOX] with nature of damage-associated molecular patterns (DAMPs). The oxidation of cell wall DAMPs by OSOXs quenches their elicitor activity by concomitantly releasing H2O2, a molecule with multiple functions in the cell wall strengthening and signalling. Little is known about the role of oxidized oligosaccharides and the H2O2 released from their oxidation. By a multiple-enzyme assay approach, we provide an in-vitro demonstration that the combined action of a microbial glycosyl hydrolase (GH) and a specific plant OSOX converts the hydrolysis of a cell wall polysaccharide to a controlled production of H2O2. If a plant peroxidase (POD) is added to such reaction, the oxidizing potential of H2O2 can be directed towards reactions typical of plant defenses such as monolignol and IAA oxidation, pointing to OSOXs as enzymatic transducers between microbial GHs and plant PODs.

#80

Matteo Selci • Naples

Geobiological processes controlling methane cycling at the Central American convergent margin

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Convergent margins are gateways to Earth's interior where volatile species such as carbon, water, hydrogen, and sulfur are cycled between the surface and the interior of the planet. While carbon fluxes in volcanic-arc regions have been constrained, little information is available on the fate of carbon in fore-arc and back-arc regions. In these regions, carbon is released in different forms, with geobiological processes largely mediated by subsurface microbes that help determine the relative contributions of these carbon species. Among these, CH4 represents the most reduced possible form of carbon and contributes greatly to greenhouse effects and climate stability. Here we present coupled geochemical and microbiological data from 80 geothermal fields, spanning the Costa Rica and Panama convergent margin, and analyze the presence and diversity of CH4 cycling microorganisms coupling it to data on its origin using clumped isotope data. This work provides a snapshot of the geobiological processes controlling CH4 cycling in the shallow fore-arc of convergent margins, providing a deeper understanding of the role of the deep microbial biosphere in controlling CH4 cycling in these areas.

#81

Giulia Semenzato • Sesto Fiorentino

The phytobiome of the medicinal plant Origanum vulgare: exploring the link between essential oil composition and its associated endophytic communities

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One of the advances in addressing the global issue of antibiotic resistance is the discovery that microorganisms residing inside medicinal plants may contribute to the production of metabolites of pharmaceutical interest. This work aims to characterize the bacterial endophytic communities associated with Origanum vulgare L. spp. and to understand if the aroma profile of their essential oil (EO) might be influenced by these bacteria. EO was hydrodistilled and the cultivable bacterial communities were isolated from the same plant. The structure and the composition of the microbiomes were evaluated by Random Amplified Polymorphic DNA analysis and 16S rRNA gene sequencing, which revealed a high degree of biodiversity and a low degree of strains sharing between subspecies, and compartments of the same plant. Antagonism tests revealed endophytes' potential to synthesize antibiotics, including volatile ones, and analysis of genome sequences might shed light on the metabolic pathways involved in their biosynthesis. The set-up of an in vitro axenic model of the plants might prove endophytes' ability to modulate plant metabolome and their adaptation to specific anatomical parts of plants.

#82

Andrea Silverj • San Michele All'Adige

Large-scale genome reconstructions from human gut metagenomes to study phage-host relationships

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Bacteriophages are viruses that infect bacteria. They are extremely abundant in the human gut, yet their diversity, evolution and the ecological relationships with their bacterial hosts are still underexplored. To increase the number of phage genomes that can be phylogenetically modelled and to test coevolution with their bacterial hosts, we mapped all known viral reference genomes from RefSeq, together with a newly developed resource of putative viral genomes from highly purified viromes, against a large set of previously assembled contigs from more than 9,000 human metagenomic samples and 3,000 viromes, obtaining more than 180,000 putative viral genomes. To reconstruct the molecular phylogenies of the newly characterised phages, we devised a new strategy to detect and remove possible alignment artefacts. We then used host predictions generated by CRISPR spacer matches to link the retrieved phages to their hosts, building a catalogue of phage-host pairs that cooccurred in the same samples. Our methodology allowed us to reconstruct the phylogenies of both phages and their putative hosts from the same samples, helping to clarify phage-bacteria coevolution in human metagenomes.

#83

Mario Soccio • Foggia

Genome-wide analysis and expression profiling of glyoxalase I genes in durum wheat (Triticum durum Desf.) under hyperosmotic stress

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Glyoxalase I (GLYI), a ubiquitous enzyme responsible for the GSH-dependent detoxification of the glycating agent methylglyoxal (MG), has been demonstrated to play a crucial role in providing abiotic stress tolerance in various plant species.

In this study, the full GLYI gene family was identified in durum wheat (Triticum durum Desf.) by a comprehensive genome database analysis. Based on analyses of phylogenetic relationships and conserved binding sites, nine candidate genes (TdGLYI) were predicted to encode for functionally active TdGLYI enzymes, putatively localized in cytoplasm, plastids and mitochondria. Expression profile, performed by qRT-PCR in root and shoot tissues from 4-day-old seedlings exposed to salt and osmotic stresses, showed an up-regulation of most TdGLYI genes with different pattern depending on stress and tissue type. An increase (up to 40%) of GLYI activity and MG content was also observed in the same experimental conditions, accompanied by a decrease of GSH (up to -60%) and an increase of GSSG content (up to 7-fold).

Taken together, these results shed some light on the crucial role of GLYI in the early response of durum wheat seedlings to hyperosmotic stress.

#84

Francesca Sparla • Bologna

The role of sucrose phosphate synthase A2 in directing carbon skeletons along different pathways

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In plants, sucrose is the main transported photoassimilate and acts as an energy source, signalling molecule and source of carbon skeletons. One of the limiting steps in the biosynthesis of sucrose is the production of sucrose-6-phosphate catalyzed by sucrose phosphate synthases (SPSs). The Arabidopsis genome encodes four isoforms of SPSs, whose specific functions are not clear yet.

Although SPSA2 is not the most abundant isoform in leaves, in silico data suggest transcriptional activation of SPSA2 in response to osmotic stress and increased levels of this protein in response to cold stress has been demonstrated. In this work, the role of SPSA2 in response to drought was studied in Arabidopsis plants lacking this isoform. In seeds and seedlings, major phenotypic traits were not significantly different between wild-type and mutants. Conversely, adult plants clearly showed changes in the use of carbon skeletons. Our findings strongly suggest that in the absence of SPSA2, instead of being directed to sucrose biosynthesis, hexoses are preferentially consumed by the Oxidative Pentose Phosphate pathway which might be activated by the downregulation of the inhibitory isoform 4 of G6PDH.

#85

Edoardo Tosato • Bologna

The likely role of Arabidopsis thaliana cytochromes b561 in intracellular ascorbate redox homeostasis and ROS-mediated signaling

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As an antioxidant and substrate for numerous enzymes, ascorbate (ASC) is one of the most important metabolites in all organisms. In plants ASC is involved in developmental processes and in response to biotic and abiotic stresses. Cytochromes b561 (CYB561s) are ubiquitous proteins catalysing the bidirectional, single electron transmembrane transfer from ASC to monodehydroascorbate and their function in plants is still unclear.

To elucidate their physiological role in Arabidopsis, molecular phenotyping of single mutants, each lacking one of the four CYB561 isoforms encoded in the genome, was performed. The phenotypic traits observed in mutants depleted of isoform A, whose localization in the tonoplast was demonstrated by YFP-fused CYB561-A, suggested an alteration of intracellular ASC homeostasis and, possibly, of ROS-mediated signaling. Indeed, mutant plants showed an increase in ASC content and ASC peroxidase activity, with a concomitant decrease in ROS levels. This latter trait correlates with delayed onset of flowering, which was molecularly confirmed by the repression of FLOWERING LOCUS T. Preliminary results for the other 3 isoforms suggest overlapping functions between CYB561s.

#86

Anna Valenti • Naples

A proteomic approach to uncover Deformed Wing Virus-honey bee interactions

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Honey bee pollination is important for maintaining ecosystems, however the colony losses is a big problem still poorly understood. A specific causal agent has not been identified, but the ectoparasitic mite Varroa destructor, which feeds on honey bee haemolymph, and the vectored viral pathogens, in particular the Deformed Wing Virus (DWV), seem to play a key-role in the induction of this syndrome. DWV is an RNA virus, with a virion made of 3 subunits, VP1, VP2, and VP3, which are arranged into a capsid with icosahedral symmetry. VP1 is the most abundant protein and may play a role on virus introduction, however very little is known regarding the mechanisms of viral infection. The identification of host proteins interacting with the viral proteins may help to better understand the infection process. To date, no studies on interactions among DWV and bee proteins are available. We performed coimmunoprecipitation experiments coupled with mass spectrometry approaches to identify honey bee proteins associated with VP1. The data analysis showed that most of identified bee proteins are involved in fundamental biological processes which may have a potential role in the viral infection.

#87

Margherita Vanni • Florence

Genomic analyses of human skeletal remains from the prehistoric site of Corna Nibbia (Bione, Brescia)

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The Corna Nibbia shelter, in Valle Sabbia (BS), is characterised by a long human habitation that began around 3400 BC, at the start of the Copper Age, when the site was used as a collective necropolis. Palaeogenetic analyses have been carried out on 9 individuals found at the site, with the aim of providing new elements useful for studying the dynamics of exploitation of the necropolis and the characteristics of its population. The genetic material was extracted from the petrous portion of the temporal bone through a minimally invasive sampling strategy characterised by extreme care for the preservation of the finds. The genomic analyses allowed the molecular determination of the sex of the individuals, the verification of kinship relationships and the identification of phenotypic traits linked both to the somatic appearance and to factors related to metabolism or disease. Also, the genomic data will be used for population genetics studies by comparison with other ancient and modern data. This will allow to point out the level of genetic affinity with other populations either contemporary or distant in time and expand our knowledge of the European and Italian Copper Age.

#88

Maria Teresa Vizzari • Ferrara

Robust demographic inference from low-coverage whole-genome data through Approximate Bayesian Computation

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The reconstruction of past demographic processes relies on the pattern of genetic variation shown by the sampled populations and an accurate estimation of genotypes is crucial for a reliable inference of populations' dynamics. Approximate Bayesian Computation (ABC) is a robust and flexible approach to reconstruct past demographic events, which can be coupled with coalescent simulations to generate the expected level of variation under different evolutionary scenarios. Low sequencing depth drastically affects the ability to reliably call genotypes, thus making low-coverage data unsuitable for such an approach.

Here, we present a new ABC framework, based on the Random Forest algorithm, to infer past population processes using low-coverage data. We summarized the data using the full genomic distribution of the four mutually exclusive categories of segregating sites (FDSS). The FDSS is not calculated from known genotypes, but rather estimated using genotype likelihoods, so as to take into account the uncertainty of low-coverage data.

Our results showed that the use of genotype likelihoods integrated within the ABC framework provides a reliable inference of past population dynamics.

#89

Clarissa Zanotti • Rome

Analysis of the role and functionality of plant-derived exosome-like nanovesicles (PELNVs): a new research strategy and therapeutic application

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Plant-derived exosomes-like nanovesicles (PELNVs) are similar for characteristics to mammalian exosomes: they consist of a lipid membrane with a diameter of 50-150 nm and are composed by proteins, lipids, nucleic acids. They are involved in many physiological processes, indeed participating in plant cell crosstalk and in cross-kingdom communication. Plant exosomes perform multiple activities into the biological environment and carry biochemical cargo to the receiving cells, giving them new properties or functions. Moreover, for their natural source PELNVs are minimally cytotoxic and immunogenic and show good tissue-specific targeting. Plant exosomes preparations have been carried out from different natural sources, such as edible plants, sprouts or roots. Growing evidence indicates that PELNVs exert many therapeutic effects in the treatment of diseases, with anti-inflammatory, anti-carcinogenic, anti-oxidant activities. In this study, we investigate on composition, biochemical properties and activities of PELNVs to provide a new and alternative nanotherapeutic platform to deliver bioactive components in clinical applications, especially in the nutraceutical and cosmeceutical fields.

#90

Valentina Zaro • Florence

A genetic picture of Bologna (Italy) during the Etruscan period

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The high genetic variability of present-day Italians reflects a complex scenario of past population dynamics dating back not only to the Late Paleolithic and Neolithic but also Metal Ages. During the Early Iron Age, the Etruscan civilization developed from the Villanovan culture in the Etruria territories with local expansions into neighboring regions, including the Po Valley. Recent genomic studies on Etruscans show that individuals with a genetic profile associated with Central European populations were present in Etruria before the 7th century BCE.

To better investigate the relationship between Etruscans and Celtic-related populations, we applied next-generation sequencing technologies and in-solution capture to generate genome-wide data from 52 ancient individuals linked to the Etruscan culture and coming from several necropolis excavated in Bologna (Italy) and dated to the 8th- 5th century BCE.

In addition to the identification of genetic sex and kinship relations, the analysis of both uniparental markers and 1.2 million single nucleotide polymorphisms will allow to identify the potential impact of Celtic-related groups on the genetic pool of Bologna during the Etruscan period.

2. Genetics, Epigenetics and Chromosome Biology

#1

Roberta Amato • Rome

Telomeric replicative stress promotes ALT mechanism in ATRX and p53 depleted cells

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Cancer cells maintain their telomeres by telomerase or alternative lengthening of telomeres (ALT). ALT tumors are characterized by ATRX and p53 inactivating mutations and display specific hallmarks. Data from literature suggest that telomerase-positive cancer cells may activate ALT (e.g. as a resistance mechanism to anti-telomerase drugs). The modulation of the most reliable ALT markers was analyzed as a function of ATRX and p53 status, in two isogenic colon cancer cell lines (HCT116 and HCT116 p53-/-) transiently or permanently silenced for ATRX. Several papers suggest that telomeric chronic replicative stress, exacerbated by the absence of ATRX, may be one of the triggers of DNA damage and telomeric recombination; for this reason, a telomeric G-quadruplex-ligand (RHPS4), known to induce replicative stress and telomeric DNA damage, was used. Our results suggest that RHPS4 treatment induces the activation of a cell cycle checkpoint at the G2/M boundary partially governed by p53, uncovering aspects of p53 and ATRX functional interaction in the activation of the ALT mechanism. Experimental procedures and results will be shown in the poster.

#2

Susanna Ambrosio • Naples

Dissecting the relationship between DNA repair and autophagy to improve the efficacy of anticancer treatment

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It is becoming increasingly clear that, despite being a cytoplasmic process, autophagy plays a key role in maintaining genomic stability. Many studies provided evidence that autophagy may modulates DNA repair pathways, with major implication for therapy-induced responses and acquired resistance in cancer, although the exact mechanism behind this connection remains a matter of debate. To investigate how autophagy modulates DSB damage response, we have generated a MCF10A cell line expressing the fusion proteinconsisting of the AsiSI restriction enzyme and a modified hormone-binding domain from the estrogen receptor. Cell exposure to 4-hydroxytamoxifen results in nuclear accumulation of the AsiSI-ER protein and in the rapidinduction of ~150 sequence-specific DSBs across the genome. This cellular system enables us to investigate recruitment of DNA repair factors at specific DSBs by using ChIP-based approaches. Our goal is to investigate how autophagy regulates DNA repair proficiency and pathway choice throughout the cell cycle and themolecular events underlying these regulatory mechanisms.

#3

Tiziana Angrisano • Naples

Retinoic Acid promotes a totipotent-like stem cell through Zscan4 epigenetic activation

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In culture, embryonic stem cells (ESCs) are a heterogeneous population that includes several cell intermediates with different degrees of potency. In particular, the subpopulation marked by Zscan4 expression, resembling molecular and epigenetic two-cell stage preimplantation signature, results enhanced after RA-treatment. We detected the epigenetic mechanisms involved in Zscan4 activation RA-dependent. We identified the minimal Zscan4 promoter region responsive to epigenetic stimuli, such as AZA and TSA, using transgenic ESCs stably transfected with different deletions of the Zscan4 promoter regions and their specific mutation. Thus, we identified a minimal promoter region responsive to epigenetics stimuli, demonstrating that Zscan4 activation is related to the histone acetylation status and DNA demethylation. This Zscan4 promoter region, at -300 bp to TSS, contains binding motifs for the DUX and TBX transcription factors and three specific CpG sites responsible for Zscan4 RA-activation. The next step will be identifying the proteins that bind and regulate the Zscan4 activation RA-induced by using a dCAS9 system, followed by a Mass Spec analysis.

#4

Federica Barbato • Rome

DNA Damage induced by Ionizing radiation activates the innate immune response: study and characterization of the cGAS-STING pathway

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Ionizing radiation (IR) has been shown to modulate immune response processes. IR-mediated immune system modulation involves several players (such as stress sensors and cytokines) regulating immune and inflammatory response. Among them, the cyclic -GMP-AMP synthase (cGAS), a free cytosolic DNA sensor, is capable of recognizing DNA fragments that, after IR treatment, could be observed as micronuclei (MNi)1. Activation of cGAS causes GAMP dependent-STING activation and promotes phosphorylation and translocation into the nucleus of IRF3 and IRF7 transcription factors that induce type I interferon (IFN)2. In the present work the induction of cGAS-positive MNi was evaluated in immortalized human keratinocytes (HaCaT) exposed to X-rays (250 Kev; 0.5, 1 and 2Gy) and fixed 24-120h after treatment, to determine the dose-response and activation kinetic. In addition, type I IFN gene expression and ISG15 protein levels were analyzed. The relationship between DNA damage and innate immune system activation will provide useful information about the role of radiation exposure in immune response.

#5

Ludovica Bonanni • Rome

The role of BLM and FANCJ DNA-helicases in the response to G-quadruplex-stabilizing ligand RHPS4

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G-quadruplexes (G4s) are secondary DNA structures arising spontaneously in guanine-rich regions (e.g. telomeres).

G4s have a role in regulating important biological functions but they might represent an obstacle for DNA metabolism. To prevent G4-related genomic instability, their unwinding is mediated by helicases such as BLM and FANCJ.

In this study we focused on the role of BLM and FANCJ in response to a G4 stabilizer compound (RHPS4) able to induce telomeric replicative stress. To this end, we generated CRISPR/Cas9 U251MG cells knock-out for BLM and silenced for FANCJ, in order to evaluate single and combined contribution of the two helicases in response to treatment. Whereas BLM depletion did not affect cell proliferation, siFANCJ cells displayed a moderate cell growth delay and defects in telomeric replication upon RHPS4 treatment. In BLM/FANCJ depleted cells, proliferation was totally inhibited already in the absence of G4-ligand treatment and this evidence correlates with a significant increase in chromosomal aberrations. Further experiments are in progress to clarify whether G4-ligands may be an attractive therapeutic strategy to overcome chemo-resistance in cancer cells.

#6

Vittoria Brambilla • Milan

Molecular control of flowering in rice

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Plants regulate their development in response to environmental cues. Under vegetative growth, cells of the shoot apical meristems (SAM) differentiate into leaves indefinitely but, after reproductive reprogramming, these differentiate into inflorescences and determinate flowers. We study the flowering process in rice, a crop of tropical origins that flowers in response to short days. Under flowering-inducing conditions, two florigenic proteins are produced in leaves and then translocated to the SAM. These, namely Hd3a and RFT1, function as long-distance signals and as transcriptional activators taking part of transcriptional complexes at the SAM. We studied Hd3a and RFT1 targets and we found genes that not only control cell identity but also coordinate other developmental changes that are linked to flowering. Of these targets, we are currently molecularly characterizing PREMATURE INTERNODE ELONGATION 1 (PINE1) that is a zinc finger transcription factor that represses stem elongation under vegetative growth by modifying chromatin conformation at target genes sites and BROAD TILLERING 1 (BRT1) that is an F-BOX protein required to narrow tiller angle during flowering induction.

#7

Cinzia Calvio • Pavia

The power of SwrA: from a minor protein to a game changer in B. subtilis biofilms

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SwrA is a short Bacillus subtilis protein without any recognizable structural feature. Due to a frameshifting polynucleotide tract in the ORF, SwrA can be lost and regained with high frequency. The functional allele, present in wild isolates, is always lost during domestication. So far, SwrA was shown to be crucial only for swarming motility and the production of the polymer poly-γ-glutamate. In both these pathways, SwrA acts by modifying the activity of the transcriptional regulator DegU.

Once phosphorylated by DegS kinase, DegU acts either positively or negatively on several promoters. The presence of SwrA dramatically alters its activity. In all phenotypes previously ascribed to the DegS/U, SwrA was found to completely subvert the function of the two-component system.

Through a bioinformatic approach, a sequence motif was identified in SwrA and other proteins. Single amino acid mutations were introduced in the SwrA motif; mutants are neither impaired in swarming motility nor in poly-γ-glutamate production, however each of them produces incredibly distinct biofilms phenotypes, suggesting, for this orphan protein, a major role in complex colony architecture.

#8

Fabio Caradonna • Palermo

Clonal evolution in genomically unstable rat astrocyte cell lines: cytogenetic, epigenetic and proteomic study

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Astrocytes represent a study model for CNS cancers. We previously gained astrocytic cell lines genomically increasingly unstable. To better understand astrocyte transformation, we subcloned the last-obtained most unstable clone (C6) to obtain the clone 7 (C7), with intriguing characteristics useful for understanding the chain of genomic instability events. C7 showed a novel round cell-morphology and detached by shake-off. Both clones showed an iso(8q) chromosome, and C7 showed even a double dose of this aberration (15% of metaphases), suggesting that it confers an ever-greater advantage. CpG islands methylation of DNMT1 and CYP19 rat 8q genes was different for some sites in C6, C7, with respect to the primary cells. Surprisingly, proteomic and western blotting analyses, comparatively performed on primary and C6 cells, suggested that the presence of 8q supernumerary genes does not result in their widespread increased expression.

In conclusion, genomically unstable cells could gain their selective advantage by duplication or triplication of 8q genes but then an epigenetic compensatory regulation of their expression could limit it.

#9

Pietro Salvatore Carollo • Palermo

Inhibition of FTSJ1, a tryptophan tRNA-specific 2’-O-methyltransferase as possible mechanism to readthrough premature termination codons (UGAs) of the CFTR mRNA

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Cystic Fibrosis (CF) is an autosomal recessive genetic disease caused by mutations in the CFTR gene, coding for the CFTR chloride channel. About 10 % of the mutations affecting the CFTR gene are "stop" mutations, which generate a Premature Termination Codon (PTC), thus resulting in the synthesis of a truncated CFTR protein. A way to bypass PTC relies on ribosome readthrough, that is the capacity of the ribosome to skip a PTC, thus generating a full-length protein. “TRIDs” are molecules exerting ribosome readthrough and for some of them the mechanism of action is still under debate. By in silico analysis as well as in vitro studies, we investigate a possible mechanism of action (MOA) by which our recently synthesized TRIDs, namely NV848, NV914 and NV930, could exert their readthrough activity. Our results suggest a likely inhibition of FTSJ1, a tryptophan tRNA-specific 2’-O-methyltransferase. In addition, we report that our TRIDs do not exert readthrough on natural termination codons.

#10

Fulvio Chiacchiera • Trento

ARID1A preserves tissue homeostasis by preventing genomic instability and chronic inflammation

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ARID1A is widely considered a tumour suppressor gene but whether this role is mainly due to its transcriptional-related activities or to its ability to preserve genome integrity is still a matter of intense debate. We show that while ARID1A is largely dispensable for maintaining enhancer activity, its presence is required for preserving genome integrity in vivo. ARID1A loss leads to the accumulation of DNA damage, activation of the interferon pathway, and chronic inflammation, severely affecting tissue homeostasis and favouring tumour formation. Our data strongly suggest that the oncogenic effects of ARID1A mutations should be ascribed primarily to compromised transcription-independent mechanisms. Possible therapeutic and prognostic implications of our data will be discussed.

#11

Marco Coluccia • Rome

Understanding the functional homology of CamA and VirK proteins in Shigella flexneri

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Shigella flexneri is an enterobacterium responsible for shigellosis, whose virulence phenotype is due to the presence of a virulence plasmid (pINV). camA (ybjX) is a gene of unknown function localized on the chromosome, and it shares homology with virK, located on the virulence plasmid. VirK protein is known to be involved in the post-transcriptional regulation of IcsA, required for intercellular spreading. Analysis of the predicted structures of CamA and VirK indicates that they share homology leading to the hypothesis that they might have a similar function in virulence. Since no data are available on the function of CamA in Shigella, we identified the camA promoter and analysed the positive role of PhoPQ in its regulation. We experimentally assessed that CamA is localized in the outer membrane. By isolating mutants lacking camA, virK or both genes we observed that the lack of either gene significantly reduces the resistance of S. flexneri to polymyxin B and that the double mutant is much more sensitive than the single mutants. We are currently defining the complementary role of the two proteins and the evolutionary significance of their presence in the Shigella genome.

#12

Fabio Coppedè • Pisa

Gene-environment interactions in autism spectrum disorders (ASD): artificial intelligence reveals sex-specific connections among maternal risk factors and the methylation levels of autism genes as well as the main contributors of symptoms severity in ASD children

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We used conventional statistics and artificial neural networks (ANNs) to unravel connections among BDNF, OXTR, HTR1A, RELN, EN2, BCL2, and MECP2 gene methylation levels, sex, maternal risk factors and symptoms severity (ADOS-2 score) in a cohort of 58 children with autism spectrum disorders (ASD). Sex differences were observed in blood DNA methylation levels of the studied genes, and ANNs revealed sex-specific connections among maternal risk factors and gene methylation. Furthermore, ANNs selected a set of variables allowing discriminating between high and low-moderate ADOS-2 scores with 86.8% overall accuracy. Particularly, high gestational weight gain, lack of folic acid supplements, advanced maternal age, pre-term birth, low birthweight, and living in rural context were the best predictors of high ADOS-2 score. Moreover, the analysis of saliva DNA samples revealed that Mir-28 methylation levels could represent a biomarker of disease severity in ASD children. In conclusion, ANNs revealed links among ASD maternal risk factors, symptoms severity and gene methylation levels, as well as sex differences in gene methylation levels that warrant further investigation in ASD.

#13

Ylenia Cortolezzis • Udine

Transcription regulation of Kirsten Ras (KRAS) gene: role of guanine-rich motifs in promoter and 5' UTR

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Genomic instability plays a key role in supporting carcinogenesis. Non-canonical DNA and RNA structures affect genome stability and gene expression. Among them, DNA and RNA G-quadruplex (G4s) are constituted of stacked guanine tetrads held by Hoogsteen hydrogen bonds and stabilized by monovalent cations. G4s regulate telomeres stability and the transcription of oncogenes, like c-MYC, NRAS and KRAS.

By applying promotor swapping techniques, we found that the 5’UTR of NRAS and KRAS contain a guanine-rich region overlapping G4 motifs that leads to mRNA instability. The chemical stabilization of these G4s increases NRAS and KRAS mRNA levels, while their G-to-A mutations increase the instability. RNApolII processivity is not affected by G4 formation in the 5’UTR of KRAS, while it is impaired in correspondence of the 32R G4, demonstrating a dynamic and locus-specific effect of the G4 stabilizers.

As RNA G4s may affect the pairing with anti-sense RNAs, we plan to use a biotin RIP-seq approach to identify all ncRNAs binding the 5’UTR region of KRAS, while a CRISPR-Cas13 approach will be applied to cleave the identified ncRNAs and prove their causal role in regulating KRAS mRNA stability.

#14

Flora Cozzolino • Naples

Lysines Acetylome and Methylome Profiling of H3 and H4 Histones in Trichostatin A–Treated Stem Cells

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Trichostatin A affects chromatin state through its potent histone deacetylase inhibitory activity. Interfering with the removal of acetyl groups from lysine residues in histones is one of many epigenetic regulatory processes that control gene expression. Histone deacetylase inhibition drives cells toward the differentiation stage, favoring the activation of specific genes. We investigated the effects of TSA on H3 and H4 lysine acetylome and methylome profiling in mice ES14, treated with TSA by using a new, untargeted approach, consisting of trypsin-limited proteolysis experiments coupled with mass spectrometry. The method was firstly set up on standard core histones to probe the optimized conditions in terms of enzyme: substrate ratio and time and, then, applied on ES14 cells. The proposed strategy was found in its simplicity to be extremely effective in achieving the identification and relative quantification of some of the most significant epigenetic modifications, such as acetylation and lysine methylation. Therefore, we believe that it can be used with equal success in wider studies concerning the characterization of all epigenetic modifications.

#15

Elena De Marino • Naples

Arf at the crossroad between cancer and differentiation

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p14/p19ARF is one of the most important tumor suppressor being its direct contribution to cancer largely demonstrated. Intriguingly, several more recent evidence underline the emergent role of ARF in the development, differentiation, and control of stem cell self-renewal, placing ARF at the crossroad between cancer and differentiation. In our lab, by using an established in vitro protocol, we demonstrated that Arf intracellular levels increase during pancreatic differentiation, suggesting its involvement in this process.
We address the role of Arf during the pancreatic differentiation process by generating genetically engineered ESCs in order to induce Arf conditional knockout. This will allow us to analyze the role of Arf in pancreatic progenitor cells homeostasis either in differentation and/or cancer susceptibility.

#16

Antonella Delicato • Naples

Involvement of Y-box binding protein 1 (YB-1) in DNA damage and repair mechanisms systems

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The Y Box 1 binding protein (YB-1) belonging to the Cold Shock Domain family of proteins, is highly conserved and is an important component of messenger ribonucleoprotein (mRNP) particles in various organisms and cells. Cold Shock proteins are multitasking proteins that bind other polypeptides and nucleic acids thus participating to a variety of cellular functions. Biological activities of YB-1 range from the regulation of transcription, splicing and translation, to the orchestration of exosomal RNA content.

Data from litterature indicates that YB-1 has high affinity towards damaged DNA. However the involvement of YB-1 in the cellular response to genotoxic stress and its ability to act as a modulatory factor in DNA damage recognition in response to oxidative stress or UV rays is still underinvestigated. Our preliminary data suggest that YB-1 appears to work to prevent and eventually repair genotoxic damage by promoting cell survival pathways. This aspect support the emerging evidence showing that YB-1 can allow cancer cells to evade conventional anticancer therapies and avoid cell death.

#17

Maddalena Di Nardo • Pisa

SMC1A downregulation as therapeutic target for colon cancer

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

This work was supported by an AIRC grant to A.M.

#18

Armando Di Palo • Caserta

The long non-coding RNA SPACA6-AS1, miR-125a and its mRNA targets establish a novel ceRNA regulatory network in hepatocarcinoma cells

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Non-coding RNAs play a crucial role in the hepatocellular carcinoma (HCC). In this study, we discovered a novel competing endogenous RNA regulatory network involving the long non-coding RNA SPACA6-AS1 (SP-AS), two oncosuppressive miRNAs, miR-125a and let-7e, and their mRNA targets. Firstly, the binding of the miRNAs to SP-AS was validated. Then, the boosting of either the miRNAs or SP-AS levels demonstrated their reciprocal inhibition. In addition, over-expression of SP-AS reduced the silencing activity of miR-125a and let-7e toward their key oncogenic targets, i.e. Lin28b, MMP11, SIRT7, Zbtb7a, Cyclin D1, CDC25B, HMGA2, that resulted upregulated. These expression data, together with cell proliferation assays, demonstrated that SP-AS could counteract the antiproliferative action of the two miRNAs. Finally, the analysis of 374 HCC samples in comparison to 50 normal liver tissues showed an upregulation of SP-AS and a reverse expression of miR-125a, not observed for let-7e; consistently, miR-125a oncogenic targets were upregulated. Intriguingly, additional regulatory loops involving miR-125a and the oncogenes Zbtb7a and Lin-28b have been identified as potential contributors of HCC.

#19

Camilla Fagorzi • Sesto Fiorentino

Unravelling the bacterial epigenome: the role of DNA methylation in gene transfer and gene expression regulation

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In prokaryotes DNA methylation plays roles during cell cycle and gene transfer with few data only on its influence in transcriptional control and in the population structuring. Bacterial genomes are often very diverse within the same species and characterized by a large fraction of horizontally transferred genes. May this genomic variability relate to genome-wide methylation patterns and have epigenetic impact on transcriptional control? By exploring the methylation patterns 21 Sinorhizobium meliloti strains, nitrogen-fixing plant symbionts with open pangenome structure, we aimed at understanding the phylogenetic signature and functional relevance of epigenomic modifications. The genomes were analyzed using PacBio SMRT sequencing to identify genome-wide methylations. A pipeline has been developed to parse the methylated positions within the genome and detect methylated-enriched regions. Our results showed the presence of a core and of a strain-specific set of DNA methylation patterns, and differential abundance with respect to gene position and genomic locations, suggesting that they may either act as barrier to gene transfer and have a role on gene expression regulation.

#20

Margherita Ferretti • Civitavecchia

Searching for NF-Y-dependent signalling pathways in the progression from colorectal primary tumor to liver metastases

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More than 50% of patients with colorectal cancer (CRC) develops liver metastases in their lifetime. Identifying molecular signatures associated with the epithelial-mesenchymal transition (EMT) leading to metastatic CRC could detect new specific therapeutic targets. NF-Y is composed of three subunits and one of these has two splicing isoforms, NF-YADex3 linked to an epithelial phenotype and NF-YA+ex3 to a mesenchymal one.

Preliminary RNA-seq data between primary CRCs and synchronous metastases detected the presence of deregulated genes between primary tumours versus metastases, containing NF-Y binding sites in their promoter. Based on this, we hypothesize that NF-Y plays a major role in the EMT.

Our aims are: to identify, among the NF-Y dependent signatures deregulated in colorectal primary tumours versus liver metastases, the most interesting ones in terms of EMT and/or therapeutic targets; to study the functional role of the NF-Y dependent genes in the CRC EMT; to investigate the role of the two splicing isoforms of NF-YA in the EMT of CRC.

By identifying potential metastatic mediators, our data will open new roads for therapeutic benefits.

#21

Sebastiano Giallongo • Catania

Lactate rewires uveal melanoma metabolic profile promoting cellular quiescence

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Despite being the most common intraocular cancer, uveal melanoma (UM) lacks proper strategies hampering its progression. Since several reports described the role of lactate receptor HCAR1 in cancer progression, we evaluated in vitro the impact of lactate on UM. Lactate increased the expression of Gpr81/HCAR1 and MCT1 transporter upon 24h treatment. Consequently, UM cells decreased proliferation and migration, overexpressing OXPHOS markers SIRT1, ATP-synthase, CoxII, CoxIV, and PGC1a. Interestingly, quiescence-related genes p53, p21, CytC, Foxo3, EZH2 expression was also increased, together with the percentage of G0 cells. Consistently, cellomics analysis also depicted more relaxed chromatin following lactate treatment as a result of an increase in H3K18 lactylation. These results were further confirmed in a clinical setting showing that in less aggressive epithelioid rather than choroid UM biopsies, lactate transporter MCT4 is overexpressed. These data were mirrored by GEO dataset analysis unveiling an increased MCT4 expression in choroid biopsies from UM patients. In conclusion, targeting lactate metabolism may represent a valuable strategy to counteract UM progression.

#22

Alessandro Giampietro • Rome

Depletion of the genome integrity-associated factor Ft1 induces DNA damage and cardiac dysfunction in mice

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Cardiac disease is associated with inflammation, DNA damage and senescence. However, more studies are needed to clarify mechanistic paths and to identify druggable factors. We previously described a factor linked to genome integrity named Ft1. Here we investigated whether Ft1 reduction causes cardiac disease and to what extent this is driven by cell- and age-specific events. We generated Ft1 knockout (Ft1ko) and cardiac targeted (sm22ko) animals. We profiled these mice macroscopically, histologically and at transcriptome level. Ft1 constitutive depletion reduced mouse survival, body and heart weight. In sm22ko mice, we observed same defects, but only after 20weeks. Cardiac histological alterations were robust at 1week and less prominent at 21weeks in both Ft1ko and sm22ko animals. The same early age-linked profile was observed for DNA damage and DNA damage-associated markers IL-6, p21. RNAseq mirrored histological and DNA damage sub-phenotypes. In Ft1ko mice we observed upregulation of heart disease-associated genes including Myl7, Mybphl, Myl4. Our data add information on the implication of Ft1 in disease and on the acute and chronic molecular alterations linked to cardiac disease

#23

Teresa Giannattasio • Rome

The RNA-binding protein FUS/TLS interacts with SPO11 and provides a link with PRDM9-dependent recombination hotspots

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Ewsr1-/- mice are sterile and display defects in synapsis of the XY chromosomes. Recent findings demonstrates that EWSR1 interacts with PRDM9, a methyl transferase that by introducing H3K4mer3 marks, guide meiotic recombination. EWSR1 belong to the family of FET proteins, that include TAF15 and FUS/TLS. Previous studies have shown that Fus-/- male mice are sterile and display defects of synapsis between the homologous chromosomes, suggesting a defect in meiotic recombination. We investigated the potential function of FUS/TLS at the site of DSBs formation by SPO11, onto autosomes and the pseudo autosomal region (PAR), the site of genetic recombination between XY chromosomes. We demonstrate that FUS interact with PRDM9 and SPO11 and localizes at autosomal hotspots identified by PRDM9. Moreover, FUS/TLS localizes in hotspots of the PAR. These results indicate a role of FUS/TLS in meiotic recombination and the alterations of its function are expected to represent a treat for proper and correct meiosis progression.

#24

Francesca Gorini • Milan

8-oxodG accumulation within super-enhancers marks fragile CTCF-mediated chromatin loops

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8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) is a major product of the DNA oxidization process. It has been proposed to have an epigenetic role in gene regulation and has been associated with genome instability. However, the 8-oxodG epigenetic role at a genome level and the mechanisms controlling genomic 8-oxodG accumulation/maintenance have not yet been fully characterized.

We identified and characterized a set of enhancers accumulating 8-oxodG in human epithelial cells. These oxidized enhancers are mainly super-enhancers and are associated with bidirectional-transcribed enhancer-RNAs and DNA Damage Response activation. Using ChIA-PET and HiC data, we also identified specific CTCF-mediated chromatin loops in which the oxidized enhancers and promoters are physically associated. Oxidized enhancers, and their associated chromatin loops, accumulate endogenous DSBs which are in turn repaired by NHEJ pathway through a transcription-dependent mechanism. Our work provides novel mechanistic insights on the intrinsic fragility of chromatin loops containing oxidized enhancers-promoters pairs and suggests that 8-oxodG accumulation in these latter occurs in a transcription-dependent manner.

#25

Valentino Maria Guastaferro • Portici, NA

Root adhesion and cell-to cell fusion are HAM-7-dependent in Fusarium oxysporum

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The fungal cell wall is a dynamic structure with protective and cell signaling functions. The cell-wall-anchored protein HAM-7 is highly conserved in filamentous ascomycetes where forms a sensor complex at the cell wall/plasma membrane interface for the activation of the MAK-1 cell wall integrity mitogen-activated protein kinase (MAPK) pathway. Absence of this GPI-anchored cell wall protein in N. crassa leads to severe defects in cell-to-cell fusion and sexual development. BlastP searches using the N. crassa HAM-7 protein as a bait identified a single 233-amino-acid long orthologue (64.19% identity) in the Fusarium oxysporum f. sp. lycopersici (Fol) genome. Here, we genetically dissected the contribution of Fol ham-7 gene in the regulation of stress response, vegetative hyphal fusion (VHF), hyphal agglutination, plant root adhesion and virulence. Similarly to N. crassa, Fol ham-7Δ mutants are severely impaired in VHF, but not in vegetative growth under stress conditions (i.e. cell wall, hyperosmotic and heat stress). Importantly, ham-7Δ mutants despite being unable to undergo hyphal agglutination and plant root adhesion they showed only minor defects in plant virulence.

#26

Mattia La Torre • Rome

The nuclear envelope-associated ESCRT factor CHMP7 is needed for telomere integrity

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Members of the endosomal sorting complex required for transport (ESCRT) are involved in sealing processes including nuclear envelope repair. This process is needed to re-establish envelope integrity post-mitotically. If an association between nuclear envelope and telomeres has been described less is known about molecular factors and mechanisms. Given the role of ESCRTs at nuclear envelope, we analyzed ESCRT members impact on telomere metabolism. We focused on CHMP7, ESCRT member specifically involved in nuclear envelope integrity. We observed that CHMP7 RNAi depletion triggers DNA damage response and telomere aberrations. CHMP7-RNAi phenotype was not linked to replication defects since aphidicolin co-treatment did not change it. As a second step, we monitored the effect of IST1 depletion ESCRT member involved in abscission and contributing to nuclear envelope sealing. IST1-RNAi cells had nuclear alterations but not telomere aberrations. These data show that CHMP7 is needed for telomere integrity. Given CHMP7 role at the nuclear envelope, it can be hypothesized that telomere aberrations arise from CHMP7 depleted cells inability to fully assemble the nuclear envelope post-mitotically

#27

Jessica Marinaccio • Rome

TERT role in response to oxidative stress: a putative player into mitochondria

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Telomerase Reverse Transcriptase (TERT), the catalytic subunit of telomerase, aside from the role in telomere lengthening, has non-canonical functions such as gene regulation and protection against apoptosis. We transfected normal primary fibroblasts HFFF2 with TERT protein, obtaining TERT overexpressing cells (HF-TERT). We investigated the response to oxidative stress (OS) induced by H2O2 treatment: HF-TERT showed a lower level of reactive oxygen species than HFFF2 and increased expression of some specific factors involved in the cellular antioxidant defense.

A mitochondrial targeting signal within TERT sequence allows the protein translocation into mitochondria: we showed the presence of TERT inside mitochondria in HF-TERT, and its increase after H2O2 treatment. To correlate the response to OS and TERT role into mitochondria, we have also evaluated some mitochondrial markers. The basal mitochondria quantity appeared to be reduced in HF-TERT and showed an additional reduction after OS; nevertheless, the functional mitochondrial membrane potential was conserved. Our results suggest for TERT a protective function against OS and a role in preserving mitochondria functionality.

#28

Giovanni Messina • Rome

Evolutionary conserved relocation of chromatin remodeling complexes to the mitotic apparatus: a new class of moonlighting proteins preventing genetic instability

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ATP-dependent chromatin remodeling complexes are multi-protein machines highly conserved across eukaryotic genomes. In particular, SRCAP and p400/Tip60 chromatin remodeling complexes in humans and the related Drosophila Tip60 complex belong to the evolutionary conserved INO80 family, whose main function is exchanging canonical with the variant H2A histone to regulate transcription and DNA repair.

Here we combined cell biology, genetics and biochemical approaches to study the subcellular distribution of a number of subunits belonging to the SRCAP and p400/Tip60 complexes and assess their involvement during cell division in HeLa cells. Interestingly, they accumulate at different sites of the mitotic apparatus and their depletion yielding aberrant outcomes of mitosis and cytokinesis. Importantly, this behavior is conserved in D. melanogaster, despite the evolutionary divergence (~780 million years).

Overall, our results disclose the existence of a massive and evolutionarily conserved phenomenon, whereby subunits of SRCAP and p400/Tip60 complexes relocate from the interphase chromatin to mitotic apparatus playing moonlighting functions required for proper cell division.

#29

Concetta Montanino • Caserta

Genetics and Epigenetics in Palmoplantar keratoderma: a case report

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Background: Palmoplantar keratoderma (PPK) is a group of skin diseases, inherited or acquired, characterized by thickening of the skin on the palms of the hands and soles of the feet very painful for patients. The most common mutations characterizing inherited PPK forms are KRT9, KRT1, AQP5, SERPINB 7.
Methods: Herein, we reported a case of a family affected by Vörner-Unna-Thost disease (autosomal dominant KRT1 mutation). Patients are submitted to a genetic analysis to evaluate telomerase activity and the epigenetic involvement of miR-21 in disease.
Results: PPK patients showed high expression levels of hTERT and hTR, the genes encoding for the telomeric subunits and high expression level of miR-21.
Conclusions: All members of family have multiple hyperkeratotic papules on the palmoplantar surface, show KRT1 mutation and express high hTERT, hTR and miR-21 levels. High telomerase activity interfere with apoptosis and an over-expression of miR-21 promotes a major activation of PI3K/AKT/mTORC1 pathway which determines cells growth, proliferation and survival. Both these functions are responsible of thickening of the skin characterizing disease.

#30

Rodolfo Negri • Rome

A truncated and catalytically inactive isoform of KDM5B histone demethylase accumulates in breast cancer cells and regulates H3K4 tri-methylation and gene expression

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KDM5B histone demethylase acts on H3K4me2/me3, playing a relevant role in transcriptional regulation, DNA repair and chemoresistance. It is over-expressed in many cancers and shows an ambivalent role in oncogenesis, which depends on the specific contest. This ambivalence could be explained by the expression of protein isoforms with different functional roles in different cancer cell lines. We show that one of these isoforms, KDM5B-NTT, accumulates in breast cancer cell lines, due to a remarkable protein stability relative to the canonical PLU-1 isoform, which shows a much faster turnover. This isoform is the truncated and catalytically inactive product of a mRNA with a transcription start site downstream of the PLU-1 isoform and the consequent usage of an alternative ATG for translation initiation. It also differs from the PLU-1 isoform for the inclusion of an additional exon, previously attributed to other putative isoforms. Over-expression of this isoform in MCF7 cells leads to an increase in bulk H3K4 methylation and induces de-repression of a gene cluster, including the tumor suppressor Cav1 and several genes involved in the interferon alpha and gamma response.

#31

Paola Pagano • Pavia

Phenotypic characterization of Arabidopsis thaliana tdp1α and tdp1β mutants subjected to genotoxic stress

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TDP1 (tyrosyl-DNA phosphodiesterase 1) hydrolyses the phosphodiester bond between a tyrosine residue and the 3’-phosphate of DNA in the DNA-Top1 (topoisomerase I) complex. Due to its function, it is involved in different DNA repair pathways. A small TDP1 gene subfamily, composed by TDP1α and TDP1β, have been identified in plants. While TDP1α has been demonstrated to be involved in the maintenance of genomic stability, the TDP1β gene has still unknown functions.
To investigate the role of plant TDP1 gene family in DNA damage response, an ad hoc system was developed, using the tdp1α and tdp1β mutants of A. thaliana treated with several genotoxic agents (hydroxyurea, camptothecin, cisplatin, curcumin, aphidicolin, azacytidine, NSC120686) for 20 days. An image segmentation-based (ImageJ) method was used to analyse the morphology of mutants and wild-type accession and to provide data regarding (i) germination parameters (ii) measurement of radicle and aerial part length (iii) number of leaves and (iv) percentage of abnormal phenotype. Statistical analysis on phenotype data highlight interaction between genotype and treatments.

#32

Miriam Pagin • Milan

Brain gene regulation in 3D: long-range promoter-enhancer functional interaction networks in mouse brain-derived neural stem cells, and their relevance for neurodevelopmental disease

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Vertebrate genomes contain thousands of non-coding cis-regulatory DNA elements with epigenetic and functional characteristics of transcriptional "enhancers". We showed that the transcription factor SOX2 is critical for the 3D chromatin organization in Neural Stem Cells (NSC) cultured from the mouse brain, by controlling a network of long-range interactions physically associating enhancers to gene promoters. SOX2 is essential for NSC maintenance and brain development and its mutation in humans causes Neurodevelopmental Disease (NDD), with significant brain defects (hippocampal dysplasia, learning disabilities, epilepsy, motor control problems, eye and vision defects). The genome-wide long-range interactions map provides many putative enhancers conserved in humans, and connected to genes responsible for NDD when mutated, such as Olig1 and Olig2, involved in Down Syndrome. An open question is which enhancers are relevant for the regulation of the connected gene promoter. We find that targeted silencing of specific putative Olig1 and Olig2 enhancers, using a CRISPR/dCas9 system, causes decreased expression of these connected gene. This may further represent a future therapy hypothesis

#33

Maria Pallotta • Pisa

Cohesin mutations and Wnt agonism: a new therapeutical approach for cancer

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Cohesin mutations are common in several cancers and it has been showed that cells carrying cohesin mutations are vulnerable to drug treatments. Previously, we found that cohesin-deficient cells were sensitivity to LY2090314 drug, a GSK3 inhibitor that acts as an agonist of the Wnt pathway. This observation is very innovative and deserves further investigations. To this aim, the NCI-60 a cancer cell line panel was first analysed by exome sequencing for searching cohesin mutations. We identified at least one cohesin mutation in nine cell lines. These cells were then treated with LY2090314. It is well-known that the accumulation of β catenin promotes the transcription of many oncogenes such as c-Myc. Here, we found that treatment caused a strong reduction of cell growth and β catenin stabilization. Unexpectedly the combination of LY2090314 treatment and cohesin deficiency led to the reduction of c-Myc expression. These results indicate that β catenin stabilization upon cohesin deficiency likely contributes to sensitivity of Wnt target genes, suggesting that Wnt agonism could be a new therapeutical approach for cohesin-mutant cancers.

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#34

Giuseppe Saccone • Naples

A single principle for male sex determination in major agricultural pests (Tephritidae): Maleness-on-the-Y (MoY), its evolutionary conservation and its usage for future genetic control

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The dipteran Tephritidae family includes dozens of major agricultural pest species, such as the mediterranean fruitfly Ceratitis capitata, the olive fly Bactrocera oleae, and the oriental fruit fly Bactrocera dorsalis, which it has been recently intercepted in Campania region. The climate change and the intensification of trading exchanges accelerated the invasion of invasive pest insects in different areas of the planet. Genetics can open the road for novel biocontrol strategies which are species-specific and alternative to pesticides.

The identification of the Y-linked Maleness-on-the-Y (MoY) gene of medfly, led to discover that MoY is a master gene for male sex determination and it is widely conserved in other related species. Present challenges are 1) understanding MoY molecular mechanism of action in repressing the downstream female determining transformer gene, influencing its sex-specific splicing, and 2) MoY biotech applications to harness innovative genetic control strategies for Tephritidae. Sterile Insect technique and synthetic biology approaches to the control of alien invasive harmful insect species will be also presented and discussed.

#35

Maria Virginia Santopietro • Rome

Beyond chromatin remodeling: evolutionary conserved roles of Drosophila melanogaster Tip60 complex in cell division

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The Drosophila Tip60 chromatin remodeling complex (dTip60) and its related SRCAP and p400/Tip60 complexes in mammals belong to the evolutionary conserved INO80 family, whose main function is promoting the exchange of canonical H2A with the H2A variant histone in different eukaryotic species. We found that four subunits of the dTip60 complex, DOM-A, MRG15, Tip60 and YETI, are recruited to the mitotic apparatus and midbody in S2 cells and their depletion affects cell division. A variety of mitotic defects were observed arising as consequence of the failure of spindle and/or midbody function, consistently with the localization of the analysed subunits. This phenomenon is not only restricted to mitosis but also to meiosis, thus affecting fertility. Taken together, our results highlight the existence of an evolutionarily conserved phenomenon where chromatin remodelers relocate from the nucleus to the mitotic/meiotic apparatus playing moonlighting functions required for proper cell division progression.

#36

Valentina Sassano • Padua

Deregulation of dNTP pools in cells derived from patients affected by Friedreich Ataxia

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Friedreich ataxia (FRDA) is an incurable autosomal recessive disease. At the cell level, the defect is caused by deficiency of the mitochondrial protein frataxin, encoded by a nuclear gene (FXN). The most frequent mutation is the expansion of an intronic GAA-repeat, causing transcriptional inhibition of the gene.

Frataxin function is linked to iron homeostasis, and to Iron-Sulfur clusters biogenesis and maintenance, so that FRDA cells may display mitochondrial disfunction and increased oxidative stress. Genomic instability is also reported.

We measured dNTP pools, mtDNA copy number, mitochondrial mass, in cycling and quiescent primary fibroblasts from three FRDA patients and four healthy subjects. The general trend is the reduction of both cytosolic and mitochondrial dNTP pool sizes in FRDA quiescent cells compared to controls. The relative abundance of the four precursors is also modified, but deviations are line-specific. mtDNA and mitochondrial mass quantifications are in progress, with the goal to verify a possible link among availability of DNA precursors, frataxin deficiency and mitochondrial dysfunction.

VS is a fellowship from Associazione "OGNI GIORNO" per Emma Onlus

#37

Andrea Stoccoro • Pisa

Artificial neural networks revealed several associations between the exposure to various prenatal environmental stressors and DNA methylation levels evaluated in placenta and in mothers and neonates buccal cells

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Prenatal exposure to environmental stressors influences the susceptibility of the new-born to certain chronic diseases by modulating epigenetic mechanisms. The aim of this study was to explore the connections between adverse environmental exposures during gestation and DNA methylation by applying artificial neural networks (ANNs). Information on the mother's lifestyle during gestation, placenta heavy metals and dioxins concentrations, and DNA methylation levels of placenta, maternal and neonatal buccal cells were obtained from 28 mother-infant couples.

ANNs analysis revealed several associations among mothers' lifestyle, heavy metals and dioxins placenta concentrations and DNA methylation levels of several genes, some of which involved in neurodevelopment, including BDNF and NR3C1, and in DNA repair, including MGMT, in placenta, mothers and neonatal buccal cells.

Current results show that methylation levels of genes important for embryonic development are sensitive to various environmental stressors during pregnancy and are modulated in placenta, potentially affecting fetal development, and in buccal cells, potentially providing peripheral biomarkers of environmental exposure.

#38

Luca Tadini • Milan

Perturbation of protein homeostasis brings plastids at the crossroad between repairment and dismantling

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The chloroplast proteome is a dynamic mosaic of plastid- and nuclear-encoded proteins. Plastid protein homeostasis is maintained through de novo protein synthesis and proteolysis, together with intracellular communication mechanisms. However, the maintenance of fully functional chloroplasts is costly and, under stress conditions, the degradation of damaged chloroplasts is functional to the maintenance of a healthy population of photosynthesising organelles. Here, we have addressed the chloroplast quality-control regulatory pathway by modulating the expression of two nuclear genes encoding the plastid ribosomal proteins PRPS1 and PRPL4 in Arabidopsis leaves. By transcriptomics, proteomics and transmission electron microscopy analyses, we show that the increased expression of PRPS1 gene leads to chloroplast degradation and early flowering, as an escape strategy from environmental challenges. On the contrary, the overaccumulation of PRPL4 protein is mitigated by the increased accumulation of plastid chaperons and other components of the unfolded protein response (cpUPR) regulatory mechanism.

#39

Rita Trirocco • Rome

Fatty acids abolish Shigella virulence inhibiting its master regulator VirF

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Shigella is a facultative intracellular pathogen that causes human bacillary dysentery, also known as shigellosis, an acute infection of the large intestine. The major regulator of the virulence phenotype is VirF, a DNA-binding protein belonging to the AraC family of transcriptional regulators. We made a reliable structural prediction of the VirF protein and observed that the VirF protein has a "jelly roll" pattern, a protein fold containing an enclosed binding pocket. We have chosen a set of molecules that are good candidates for interaction with VirF's “jelly roll” module. The results obtained show that fatty acids (FAs) are potential ligands that project deeper into the buried fissure of VirF. This binding hinders the functionality of this activator by inducing a structural remodeling of these and leading to the "closed" conformation, unable to activate the downstream genes. Given its cardinal position in the Shigella virulence system, the identification of these molecules and the characterization of their interaction with VirF, can provide a basis for the development of antivirulent compounds as therapeutic agents in the treatment of shigellosis.

#40

Liliana Tullo • Rome

Assessing an unprecedented role for Heterochromatin Protein 1a (HP1a) at mitochondria

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The Drosophila Heterochromatin Protein 1a (HP1a) is conserved non-histone protein with essential roles in heterochromatin maintenance and gene expression. We recently found that HP1a is also present on mitochondria, mostly associated with the outer mitochondrial membrane and with Drosophila VDAC1. Cellular fractionation from HeLa cells and from mouse liver and heart samples showed that also the mammal ortholog Hp1a localizes at mitochondria, further suggesting that HP1a is a mitochondrial resident protein and plays evolutionarily conserved roles in this organelle. Interestingly, loss of HP1a in Drosophila and in Hela cells associates with larger mitochondria number and increased mitochondrial mass without affecting mitochondrial biogenesis. This result prompted us to hypothesize a role for HP1a in the regulation of mitochondrial quality control and mitochondrial degradation. In support of this hypothesis, we recently found that a specific depletion of HP1a in Drosophila larval and adult muscles associates with impaired locomotor activity and altered mitochondrial ultrastructure, revealing an unexpected role of HP1a for mitochondrial activity at multiple levels during development.

#41

Stefano Zoroddu • Sassari

Recovery of the correct balance of EZH2 and EZH1 proteins as a new strategy for the treatment of rhabdomyosarcoma

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Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma in children. Metastasis, resistance or relapse are the main cause of death. It is characterized by the loss of myoblastic differentiation caused by genetic and epigenetic factors. We focused our attention on histone hypermethylation. Enhancer of Zeste Homolog 2 (EZH2) and Enhancer of Zeste Homolog 1 (EZH1) are the catalytic subunits of the Polycomb Repressive Complex 2 (PRC2). EZH2 is able to trimethylate lysine 27 of histone H3 (H3K27me3), which is highly expressed in RMS, whereas the role of EZH1 is not yet completely well known. We evaluated the effects of EZH2 downregulation in RMS knock-down lines. The results showed that downregulating EZH2 leads to an increase in myogenic regulatory factors (MRFs), resulting in partial restoration of the muscle phenotype. Furthermore, Co-IP experiments showed that there is a competition between EZH2 and EZH1 as catalytic subunits of PRC2; when EZH2 is depleted, EZH1-associated PRC2 levels increase, indicating a possible rescue and thus, preventing full myogenic differentiation. In conclusion, restoring EZH2 and EZH1 levels could be a good strategy for the treatment of RMS.

3.Genomics, Proteomics and Systems Biology

#1

Martina Aulitto • Naples

Genomic analysis reveals new insights into the biotechnological and industrial potential of Weizmannia coagulans

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In the last decades, several lactic-acid producer strains of W. coagulans have been used for the production of LA from lignocellulosic biomasses and so far, the genomes of 47 strains have been sequenced1,2,3,4. To expand our understanding of the intra-strains genomic diversity of W. coagulans and to provide new insights into its genetic potential in biotechnological applications, a comparative analysis study was performed. 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 W. coagulans Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) immune system along with CRISPR-associated (Cas) genes, was also investigated. Overall, this study expands our understanding of the strain's genomic diversity of W. coagulans to fully exploit its potential in biotechnological applications5.

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#2

Andrea Becchimanzi • Portici, NA

Analysis of the salivary gland transcriptome of the honey bee mite Varroa destructor

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Varroa destructor is a parasitic mite of honey bees and a major driver of honey bee colony losses. By feeding on the host’s body fluids, this obligate ectoparasite has a strong impact on honey bee physiology, causing the reduction of weight and longevity, and the spread of viral pathogens. Despite its importance, many aspects of Varroa lifestyle are still obscure. In particular, during feeding activity on the host, the mite injects salivary secretions only partly identified so far. Here we analyzed the salivary gland transcriptome of V. destructor and identified 15 transcripts encoding putatively secreted proteins. Using databases such as InterPro, UniProt and NCBI, we functionally annotated this secretome, which includes proteins belonging to cysteine protease, serine protease, endochitinase and phospholipase families. The identification and functional characterization of these proteins will offer new knowledge on the molecular basis of Varroa-bee interactions, on which to develop innovative and eco-sustainable mite control strategies.

#3

Mariarita Brancaccio • Naples

Identification of key microRNAs in preeclampsia by bioinformatics analysis

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Preeclampsia is a leading cause of perinatal maternal-foetal mortality and morbidity. The aim of this study is to identify the key microRNAs in preeclampsia and uncover their potential functions. We downloaded the miRNA expression profile of GSE93020 for trophoblast cells, GSE119799 for plasma and of GSE177049 for placenta. Each dataset consisted of 5 patients (PE) and 5 controls (N). In placenta, we selected the miRNAs on the basis of Fold Change. We found 15 upregulated miRNAs (Fold Change ≥ 2) and 20 downregulated miRNAs (Fold Change ≤ 0.5). Among these, we identified the significant ones (p-value < 0.05). In addition, the significant miRNAs in the placenta, we looked for them in the plasma and found that 12 miRNA were in common, of which 6 were upregulated and 6 downregulated. The deep bioinformatics analysis conducted during our study will allow us on the one hand to verify the targets of each identified miRNA; on the other hand, to use them both as new biomarkers and as therapeutic targets for the development of ad personam treatments.

#4

Sara Bruschini • Rome

Leveraging malignant pleural effusions from lung adenocarcinoma patients to investigate the immune landscape of advanced non-small cell lung cancer

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Immunotherapy with ICIs has turned out as much astounding as unsatisfactory, calling for new therapeutic targets for non-small cell lung cancer (NSCLC). A deeper characterization of the tumour immune microenvironment (TIME) is expected to shed light on the mechanisms of cancer immune evasion, providing opportunities for the development of better therapeutic strategies. We exploited malignant pleural effusions (MPEs) from lung adenocarcinoma (LUAD) patients as a model system to investigate TIME in advanced NSCLC.

Mononuclear cells from MPEs and peripheral blood, cell free pleural fluid and plasma were collected from 24 LUAD patients. Transcriptomic analysis of immune cells and cytokinome analysis of soluble factors in the pleural fluid depicted MPEs as a metastatic niche in which all the components required for an effective antitumor response are present but conscripted in a pro-tumoral and immune suppressive mode. Focusing on macrophages we identified an MPEs signature associated with worse clinical outcome in LUAD patients.

Our study reports a comprehensive characterization of LUAD MPEs and opens new perspectives for the rational design of new therapies for metastatic NSCLC.

#5

Martina Buonanno • Naples

Insights into the Winged Helix Domain of P150, a subunit of the Chromatin Assembly Factor-1

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In eukaryotes DNA is packaged into chromatin. The basic unit of chromatin is the nucleosome, characterized by DNA wrapped around an octamer of core histone proteins. The transition between the wrapping and unwrapping state of nucleosomes is mediated by histone chaperones, proteins that bind histones with high affinity. Among these, the human Chromatin assembly factor 1 (CAF-1) consists of three subunits: P150, P60 and P48. P150 is the largest one and it is considered the main character of the complex. In addition, P150 overexpression has been correlated with several tumours. However, information about P150 was mainly deduced from studies of the yeast homologue Cac-1.
Here we reported the identification of a winged helix domain (WHD) which covers residues from 721 to 860 of the full-length protein (P150721-860) and it is able to bind DNA. P150721-860 was produced in E. coli and biochemically characterized. The interaction of this region with DNA was in vitro investigated and an acid portion of the protein which modulates this binding was also identified. Results provided information about the DNA binding ability of this subunit and the relative regulatory mechanism.

#6

Luisa Canè • Naples

Characterization of human TSLP isoforms

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Thymic stromal lymphopoietin (TSLP) is a pleiotropic cytokine highly expressed by epithelial cells and several cells of the immune system. TSLP exerts its biological roles by binding to the heterodimeric receptor TSLP-IL-7Ra present on a plethora of immune system cells and plays a central role in several inflammatory disorders and cancers. In particular, overexpression of TSLP is involved in orchestrating allergic inflammation in asthma and other atopic diseases. There are two isoforms of TSLP in humans: a long form of 159 amino acids (lfTSLP), which is up-regulated in inflammatory conditions, and a short form (sfTSLP), comprising the last 96 amino acids at the C-terminus of lfTSLP. There is compelling evidence that overexpression of TSLP may represent a biomarker of human airway inflammation. We are investigating the existence of both TSLP isoforms in unstimulated and stimulated human peripheral blood monocytes and lung-resident macrophages, by using targeted (MRM) and untargeted proteomics approaches. We are also evaluating whether lfTSLP can be cleaved by tryptase and chymase, proteases secreted by activated human mast cells, by limited proteolysis and MALDI-MS.

#7

Lisa Cangioli • Sesto Fiorentino

Study of the regulatory cascade involved in the megaplasmids conjugation in the model plant symbiotic bacterium Sinorhizobium meliloti by the creation of a GFP-based reporter system

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Sinorhizobium meliloti is a model plant symbiotic bacterium with a genome composed by a chromosome and the megaplasmids pSymA and pSymB, responsible of symbiotic nitrogen fixation and growth in the rhizosphere. The conjugal transfer of them in S. meliloti is highly regulated and repressed through the rct system composed by 7 key genes. However, the environmental signals which trigger the rct regulatory cascade are still unknown. Here, we aim to decipher the signals of rct activation. rct genes promoters were cloned upstream to green fluorescent protein (GFP) gene in the pOT2 plasmid in E. coli, transferred to S. meliloti and we tested them in induction conditions in liquid cultures of M9 minimal medium supplemented with luteolin, sucrose, root exudate and plant macerate. Results showed that higher levels of GFP fluorescence were obtained using root exudate and plant macerate, suggesting that conjugal transfer activation occurs during the interaction with the host plant. However, further studies are necessary to investigate the chemical nature of the inducing molecules and the interplay between rtc and the quorum sensing system on the conjugational transfer of megaplasmids.

#8

Ludovica Celli • Pavia

Dissecting the involvement of GC-AG introns in alternative splicing regulation

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Alternative splicing (AS) is the mechanism that cells use to generate different mature transcripts from the same pre-mRNA, through the differential usage of splice sites. We recently demonstrated an involvement of introns with the non-canonical GC donor site in gene expression regulation, and their correlation with organismal complexity (Abou Alezz et al. 2020). Further studies allowed us to i) demonstrate a role of GC-AG introns in alternative 5’ splicing and ii) to interpret the molecular mechanism beneath their usage. Moreover, GC-AG containing genes resulted enriched in DNA damage repair functions. In particular, genes belonging to the Fanconi Anemia (FA) pathway occured as the most prevalent. This led us to focus our studies on the intron 41 of the FANCA gene, which contains a GC at the 5'splice site (5'ss) and a downstream GT 5'ss, differentially used.

These results show that GC-AG represent a new regulatory motif with important functional implications, and their accumulation in higher organisms seems to be due to their capacity to enhance alternative splicing (AS).

#9

Himanshi Choudhary • Udine

Long-term NRF2 depletion in KRAS-mutated pancreatic cancer cells leads to the activation of metabolic short-circuits sustaining cancer cells fitness and overcoming KRAS addiction

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More than 90% of patients with PDAC carry KRAS-activating mutations. Despite its KRAS dependence, the inhibition of MAPK and KRAS has proven to have limited efficacy. Therefore, we explored an alternative strategy focused on the inhibition of the KRAS-NRF2 axis. By CRISPR-Cas9 we knockout NRF2 in mutant KRAS PDAC cells. A global decrease in the malignancy and invasive properties of NRF2-/- cells switches to a compensatory AKT1-MYC pro-survival pathway leading to resistance to conventional therapies. Long-term deletion of NRF2 induces a metabolic reprogramming of Panc-1 cells, which become unresponsive to glucose deprivation but rely on arginine catabolism. Rescue experiments demonstrate the direct role of NRF2 in controlling arginine catabolism. NRF2-depleted cells become dependent on iNOS for NO generation and maintenance of invasive properties. They also show a dependence on CKB for ATP generation. Our study demonstrates a critical role of NRF2 in controlling cellular metabolism; highlighting the existence of metabolic short-circuits that allow cancer cells to adapt to non-permissive metabolic conditions, providing new targets for therapeutic approaches.

#10

Michele Costanzo • Naples

Proteomic study of the interactions of MMACHC protein

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MMACHC protein is a cytosolic chaperone known to participate in the biosynthetic pathway of adenosylcobalamin, which serves as cofactor of the mitochondrial methylmalonyl-CoA mutase enzyme for the production of Krebs cycle intermediates. Actually, mutations in the MMACHC gene concur to the onset of the methylmalonic acidemia type cblC, a rare inborn error of the propionate metabolism.

Despite the recognition of its role in the biosynthesis of adenosylcobalamin, the exact functions of MMACHC are not completely elucidated yet. To this aim, the interactome of MMACHC was investigated using an affinity purification-mass spectrometry (AP-MS) strategy in HepG2 cells. The results of the AP-MS interactomics study showed the identification of several putative MMACHC-interacting partners clearly connected with propionate metabolism. Furthermore, several mitochondrial proteins were identified as MMACHC interactors, comprising proteins related to mitochondria biogenesis and function, as well as microtubule-regulating proteins. These findings can help to extend the knowledge about the physiological functional role of MMACHC, possibly providing therapeutic targets for cblC patients.

#11

Nunzio D'Agostino • Portici, NA

Towards the analysis of the transcriptome dynamics to understand the molecular basis of the complex interaction process between the parasitic weed Phelipanche ramosa and tomato.

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Phelipanche ramosa (broomrape) severely affects tomato (Solanum lycopersicum L.) cultivation. One of the most effective approaches to control this pest is to develop resistant varieties. Some lines of evidence indicate that the wild species Solanum pennellii is resistant. Therefore, some S. lycopersicum x S.pennellii introgression lines (ILs) may be tolerant to parasitic weed infestations. We are testing the ability of ILs to stimulate P. ramosa seed germination and induce the production of haustoria and the development of tubercles. The purpose of this screening is to identify the most resistant and susceptible ILs to be subjected to dual RNA-Seq. The analysis of transcriptome dynamics is essential to reveal the molecular mechanisms that mediate host/parasite crosstalk. Tomato root samples and samples of P. ramosa at different stages of infection (pre-attachment, haustorial differentiation, tubercle development) will be collected, the total RNA extracted and then sequenced. The analysis of the tomato mRNAs and non-coding RNAs could lead to expanding the panel of candidate genes useful as targets in future mutagenesis programs to obtain tomato varieties resistant to broomrapes.

#12

Claudia De Vitis • Rome

Multiomic analysis reveals ALDOC and ENO2 as master regulators of cancer stem cells metabolism

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Cancer Stem Cells (CSCs) are responsible for cancer initiation, metastasis and chemoresistance and due to their plasticity adapt to different environmental nutrient conditions, concentration and ratio of given metabolites. A used model to study CSCs enriched cultures is to grow cancer cells as 3D spheroids. To identify functional features specific of CSCs analysed 2D vs 3D cultures by transcriptomics, proteomics and metabolomics and started to integrate the data obtained. Multivariate analysis showed a significant shift from the 2D typical oxidative metabolism to a glycolytic/gluconeogenesis metabolism in 3D cultures. Multiomics approaches highlighted the upregulation of the glycolytic enzymes ALDOC and ENO2, at both gene and protein levels, in 3D vs 2D cells. Comparative analysis match with the increased intracellular ratio L-lactic acid/Glucose monophosphate 3D vs 2D cells, thus representing the hubs in CSCs expansion. Silencing of ALDOC and ENO2 decreases 3D propagations, cell viability and the levels of lactate, thus restrong 2D levels of this metabolite. Multiomics approaches are tools for the direct profiling of cell metabolism to uncover phenotypic changes peculiar to CSCs.

#13

Guido Domingo • Varese

Phosphoproteomic analysis reveals novel mechanisms by which cAMP affects cellular processes and response to heat in tobacco cells

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Even though, in plants, cyclic adenosine monophosphate (cAMP) is involved in the signaling pathways of various biological processes, including (a)biotic stress responses, the exact role of this messenger remains elusive, as well as the existence of kinases that respond to its concentration changes. To elucidate the phosphorylation changes involved in cAMP signaling we analyzed the phosphoproteome of tobacco Bright Yellow-2 cells in response to cAMP depletion or heat stress condition.

Our data indicate that the cAMP-dependent phosphoregulations involve kinases such as MAPKs, RLKs and CDPKs. At phosphorylation level, cAMP mainly regulates RNA splicing, cell organization, autophagy, protein trafficking and folding. Our results showed a significant overlap between cAMP- and temperature-responsive phosphosites, however, proteins involved in processes like autophagy are phosphoregulated in the opposite way by cAMP depletion and heat stress. Finally, a two-way ANOVA analysis allowed us to distinguish phosphoproteomic changes that represent the specific impact of cAMP depletion on heat stress response.

#14

Sara Donzelli • Rome
no preference.

PI3KCA-mutant breast cancer metastatic patient-derived organoids envisage Alpelisib treatment for multiple secondary lesions

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Breast cancer is a powerful example of intra and interpatient heterogeneity, and the BC treatment scenario is still characterized by ample grey areas. This is especially true for the metastatic disease (mBC). Organoid cultures represent a suitable model for studying the histological complexity and genetic heterogeneity of parental tumors, with credit for assessing treatment strategies at a single-therapeutic challenge posed by the mBC. Here, we report a proof-of-concept test case coupling the setting-up of organoid cultures from metastatic BC specimens to fast drug sensitivity testing with molecular-lesion driven treatment. Based on the relevant impact of PIK3CA gene mutations on breast cancer progression and resistance to therapy, herein we assessed the efficacy of Alpelisib (BYL719; Novartis Pharma AG, Basel, Switzerland), a specific PIK3-a inhibitor, on organoid cultures derived from multisite mBC carrying specific PIK3CA gene mutations.

#15

Francesca Guarino • Catania

Regulation of VDAC1 gene expression in HeLa cells mediated by HIF-1α

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Voltage Dependent Anion-selective Channel 1 (VDAC1), the most abundant protein of mitochondrial outer membrane, is the regulator of ions and metabolites exchange between cytosol and mitochondrion. VDAC1 is involved in cellular metabolism, apoptosis and mitochondrial bioenergetics balance in physio-pathological conditions. We are exploring the transcriptional regulation of VDAC1 in HeLa cells in adaptive response to stress impairing mitochondrial function. Here, we investigated the role of transcription factor HIF-1α on VDAC1 gene expression regulation in hypoxic condition. VDAC1 transcript levels and promoter activity were increased upon exposure to CoCl2 inducing hypoxia. We investigated the role of HIF-1α as regulator of VDAC1 transcription. The functional activity of HIF-1α on VDAC1 promoter in hypoxia was confirmed by binding sites mutagenesis, and HIF-1α transcription factor overexpression experiments. We also revealed that HeLa cell viability is compromised upon treatment with CoCl2. In conclusion the results might let to characterize the molecular mechanism of VDAC1 involvement in cell survival or apoptosis of HeLa cells in hypoxia a typical feature of various tumours.

#16

Nicolaj Jeran • Milan

Study of three putative Plastid Peptide Transporters mediating chloroplast-to-nucleus signalling in response to folding stress in Arabidopsis thaliana chloroplasts

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Chloroplasts are DNA-containing organelles originated from endosymbiosis with cyanobacteria ancestors. The physical separation of nuclear and plastid genomes drove the evolution of signalling pathways that ensure coordinated nuclear and plastid gene expression, throughout development and in response to the environment. Albeit the signalling molecules behind the chloroplast-to-nucleus communication are still not fully understood, pieces of evidence suggest that peptides derived from proteolysis could act as signalling molecules. Studies conducted in nematode and yeast have revealed that mitochondrial ABC transporters mediate the extrusion of peptides from mitochondria to activate the mtUPR in response to heat. In this context, we identified three putative Plastid-located Peptide Transporters (PPTs) in the Arabidopsis genome. Lack of PPT proteins abolishes the peptide extrusion from chloroplasts upon heat-shock, altering the regulation of CLPB3 plastid unfoldase and increasing heat-sensitivity. The extruded peptides were analysed via mass spectrometry. In addition, PPTs are capable of complementing heat-sensitive phenotype in yeast, corroborating their function as peptide extruders.

#17

Cristiana Lucia Rita Lipari • Catania

Deletion of VDAC1 in human cells affects mitochondrial respiration

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Mitochondria play a crucial role in cell signaling events, inter-organellar communication, aging, cell proliferation, diseases and cell death. Voltage Dependent Anion Channel isoform 1 (VDAC1) is the most expressed pore-forming protein of the outer mitochondrial membrane (OMM). It allows passive diffusion of ions, ATP/ADP, NAD+/NADH and metabolites feeding Krebs'cycle and electron transport chain between the cytosol and the mitochondrion. In this work we investigated the effects of VDAC1 knock out on HAP1 cells by High-Resolution Respirometry (HRR) tests. Our findings indicate a significant reduction of oxygen consumption in the main respiratory states, but an increase of the specific contribution of complex I to the maximal capacity. This correlates to a decrease of the respiratory reserve capacity, consisting in the total production of extra ATP that can be produced by oxidative phosphorylation in case of energy request. In conclusion, our results indicate once again the importance of VDAC1 as a key regulator of mitochondria functionality.

#18

Alessia Medugno • Naples

Involvement of miRNA 137 in hereditary gastrointestinal cancer predispostion trought MSH2 gene regulation

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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 regards, the aim of this study was investigate 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. Our results indicate that both MSH2 mRNA and protein levels were regulated by transfection of miR-137 and anti-miR-137 in SW480 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.

#19

Michele Menotta • Urbino

Multi-omics approaches for ataxia telangiectasia therapy development

A. Ricci, F. Biancucci, G. Morganti, M. Magnani, Michele Menotta

Ataxia Telangiectasia (A-T) is a very rare severe neurodegenerative disease, caused by mutations in the Ataxia Telangiectasia Mutated (ATM) gene that codes for the protein of the same name, a member PIKK family. Currently no cure is available but positive effects of neurologic features in A-T patients have been achieved by dexamethasone (dex) administration through autologous erythrocytes (EryDex) leading the researchers to explore the molecular mechanisms of dex action. During the studies, new ATM variants originated from alternative splicing of ATM messenger were discovered. Some of the new ATM variants, alongside an in silico designed one, were studied as potential biological drug for gene delivery or gene therapy. To this intent, metabolomic, proteomic and phospho-proteomic investigations were performed by HRMS. Hundreds of compounds and proteins were found as modulated by ATM variants, thus recovering some of the molecular functions usually impaired in A-T. The primary characterized metabolites were involved in red-ox homeostasis and mitochondrial functions, while the main found master proteins were implicated in vesicles dynamics, ER-phagosome pathways and insulin signaling.

#20

Angela Messina • Catania

An emerging role as a biomarker for VDAC1P8 pseudogene in acute myeloid leukemia

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Voltage dependent anion selective channels (VDACs) are the most abundant mitochondrial outer membrane proteins encoded in mammals by three genes, VDAC1, 2 and 3, expressed in most tissues. As 'mitochondrial gatekeepers', VDACs control organelle and cell metabolism and are involved in many diseases. Despite the considerable importance of these mitochondrial proteins, little is known about the mechanisms controlling their expression, both in physiological and in disease contexts.

In this work, we investigated the expression in human normal and pathological tissues of the numerous processed pseudogenes of VDAC genes. Using high-throughput tools and querying many genomic and transcriptomic databases, we highlighted that some VDAC pseudogenes are specifically transcribed in different tissues and probably regulate the parental gene. Specifically, from our in-silico comparative analysis between the VDAC1 gene and its pseudogene VDAC1P8 and experimental data produced in an acute myeloid leukemia (AML) cell model, it appears that the VDAC1P8 pseudogene plays a role in AML, probably through downregulation of VDAC1. This is the first report on the significance of human VDAC pseudogenes.

#21

Elena Perrin • Sesto Fiorentino

A system biology approach to study microbial adaptation to global warming

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Global warming affects ocean ecosystems in several ways, including alteration of temperature, acidification, oxygen content and nutrient inputs. Since microbes play a dominant role in global biogeochemical cycles that are crucial for planet's sustainability, their response to climate changes affect the overall ecosystem functioning. The Antarctic strain Pseudoalteromonas haloplanktis TAC125 (PhTAC125) is one of the model organisms of cold-adapted bacteria, and its ability to growth in a wide range of temperature makes it also an ideal model for studying the effects of climate change. The main objective of this work is the systems-level understanding of the adaptation process of this model bacterium to environmental fluctuations imposed by the on-going global climate change. We combined experimental evolution, -omics data and mathematical metabolic modelling to provide an outlook on the adaptation of PhTAC125 to global warming, evaluating the impact of projected changes in some key environmental chemico-physical parameters and describing the molecular, evolutionary and ecological strategies that PhTAC125 may adopt to face such conditions.

#22

Francesca Pirozzi • Naples

A specific lipidomic signature defines serum lipid content of patients affected by glycogen storage disease Ia (GSDIa)

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Glycogen storage disease type Ia (GSDIa) is an inherited disorder of carbohydrate metabolism caused by deficiency of the glucose-6-phosphatase-α (G6Pase-α) involved both in gluconeogenesis and glycogenolysis. GSDIa is characterized by the accumulation of glycogen and fat in kidneys and liver resulting in renal disease and hepatomegaly (1). The clinical presentation of GSDIa patients includes fasting hypoglycaemia, elevated lactate, metabolic acidosis, hypercholesterolemia, hypertriglyceridemia and hyperuricemia. Despite dietary treatment, GSDIA patients may develop long-term complications, including liver adenomas, chronic kidney disease and metabolic syndrome, including insulin-resistance. In order to better investigate the exact pathophysiology of GSDIa we performed a serum lipidomic study for an in-depth characterization of GSDIa hyperlipidemia. Extensive lipidomics profiling of GSDIa patients showed broader lipid metabolism involvement possibly affecting various cellular functions (e.g., cell proliferation, insulin response) and highlighting currently unknown pathomechanisms. Specific lipid species may provide potential novel biomarkers for GSDIa.

(1) Rossi et al. JIMD 2018

#23

Rosita Russo • Caserta

Unravelling the CD3+CD56+ T cell proteome by Tandem Mass Tag-Based High-Resolution LC-MS/MS

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Effective immune responses require the coordinate actions of immune cell subsets belonging to both innate and adaptive immunity. Peripheral blood contains a lymphocytes population characterized by the co-expression of the typical surface molecule of T-cells (CD3) and NK cells (CD56), referred as NKT or CD3+CD56+ T-cells. Besides their known cytotoxic activity, the ability to modulate proliferation and effector function of CD8 T cells was recently reported for NKT1. Also, the frequency and function of CD3+CD56+ T-cells were reduced in autoimmune condition, such as type 1 diabetes1. Despite these significant progresses, there is still limited information on the molecular signature of NKT cells.

Here, the molecular profile of CD3+CD56+ T-cells in comparison with CD4+, CD8+ and NK cells in healthy individuals was explored by nanoLC-ESI-MS/MS. Proteins related to cytotoxic functions and calcium homeostasis were differentially modulated in NKT cells compared with other immune subsets. This study offers new insights on molecular determinants regulating NKT cells functions sustaining an effective immune response under physiological conditions.

1Terrazzano et al., Nat Metab 2, 142-152 (2020)

#24

Veronica Russo • Caserta

MucR is a new prokaryotic histone-like protein.

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MucR from Brucella abortus belongs to Ros/MucR zinc-finger protein family comprising members that bind DNA and control the expression of genes important for the successful interaction of α-proteobacteria with their eukaryotic hosts (Baglivo I. et al., PNAS 2009).
MucR binds AT-rich DNA sequences, containing T-A steps, present in the promoter of target genes.
The N-terminal region of MucR contains a conserved hydrophobic region involved in the oligomerization process that allows forming higher-order oligomers. The modality adopted to bind DNA, the ability of this protein to oligomerize, the heat stability and the presence of many basic regions support the hypothesis that MucR is a histone-like protein that regulates gene expression by structuring the Brucella genome (Pirone L. et al., Sci Rep 2018; Russo V. et al., Biomolecules 2020).
Performing the expression of the N-terminal region and the C-terminal DNA-binding domain, we have obtained new structural insights confirming that MucR is the histone-like protein never found before in Brucella and in many other α-proteobacteria.
These findings will help to fill the gap in our knowledge about genomic DNA compaction in bacteria.

#25

Anna Santin • Naples

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

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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. In this study, we provide the first characterization of the Nitrate/Peptide Transporter Family (NPFs) in diatoms. 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. We generated overexpressing strains and CRISPR/Cas9 loss of function mutants in the model species Phaeodactylum tricornutum. 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 and foundations for future investigations of their role in diatoms.

#26

Giovanni Scala • Naples

MultiOmics Network Embedding for SubType Analysis

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Biological systems are complex entities whose behavior emerges from an enormous number of reactions taking place within and among different internal molecular districts. The dissection and the modeling of the entities and the interactions constituting these interactions are essential in biological processes behind normal and pathological conditions as well as the perturbations induced by the exposure to external molecules like drugs. The recent explosion of omics data fueled the creation of diverse systems biology models. The majority of these are focused on the representation of interactions taking place in single molecular districts and have been successfully used to perform sample stratification, especially in cancer disease. One step forward in this direction is the creation of multi-omics models capturing the dynamics taking place within and between omics layers.

Here we present a novel approach for the identification of relevant multi omics relationships between biological samples.

This approach has been applied in the identification of different cancer subtypes using multiomics data from the The Cancer Genome Atlas and the Clinical Proteomic Tumor Analysis Consortium datasets.

#27

Shraddha Shridhar Gaonkar • Pavia

Profiling of LEA proteins in Medicago truncatula dry seed proteome

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High quality seeds are crucial for agriculture. Seed priming is a pre-sowing technique in which seeds undergo controlled imbibition followed by dry-back. This treatment activates the pre-germinative metabolism, preparing the seeds for faster germination and more efficient stress response. Desiccation tolerance is the ability of the seed to resist water loss during maturation and under drought stress, and it is crucial also for seed priming. Seed maturation phase in orthodox seeds are characterized by accumulation of late embryogenesis abundant (LEA) proteins which are thermostable proteins facilitating re-folding of denatured proteins during desiccation. Despite their importance, the role of LEA proteins is not deeply explored in post-maturation events and seed priming. The present work identified and classified the LEA proteins in the dry seed proteome of model legume Medicago truncatula and characterised their putative functions by exploring different databases and bioinformatic tools. The results of this research line can be used to refine our understanding of seed metabolism responding to dehydration stress and seed priming.

#28

Mariangela Valletta • Caserta

Profiling of lactic acid bacteria in milk kefir by high-resolution tandem mass spectrometry

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The consumers' attention towards functional foods containing probiotics is growing for their positive effects on human health. Kefir is a fermented dairy beverage produced by fermentation of milk by bacteria and yeasts present in kefir grains. Given the emerging market of kefir, there is an increasing demand of new methodologies to certify product claims such as values of colony-forming units/g and bacterial taxa. MALDI-TOF MS proved to be attractive for bacteria identification in clinical diagnostics and agri-food applications. Recently, LC-MS/MS approaches were also applied to the identification of proteotypic peptides from lactic acid bacteria (LAB) in fermented food matrices1.

Here, we developed a sensitive methodology based on nano-LC-ESI-MS/MS for LAB profiling in commercial milk kefir products at genus, species and sub-species level. The proposed workflow allows the authentication of kefir labeling claims declaring probiotic starters. An overview of LAB composition was also obtained for kefir without advisory labeling, highlighting the great potential of LC-MS/MS as a sensitive tool to address fermented foods authenticity.

1 Russo et al., Food Chem 285:111-118 (2019)

#29

Federico Vita • Bari

Molecular responses to phosphate fertilization in durum wheat plants under different fertilization regimes

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Phosphorus nutrition is one of the major factors limiting crop plants' growth and production, which affects many processes since it is directly involved in photosynthesis, cell division, seed formation, root development and formation.

Durum wheat (Triticum durum Desf.) is an important cereal crop whose cultivation extends beyond the Mediterranean basin to Canada. A wide range of projects worldwide has been focusing on understanding the role of phosphate in the uptake, assimilation, and utilization to improve the efficiency of phosphate recovery. Whilst the physical processes of phosphate remobilization have been studied in detail, the genetic control of these processes and their contribution to agronomic productivity are less well understood.

In this view, a proteomic approach was employed to detect differences in leaf proteome using a durum wheat cultivar (Anco Marzio) subjected to different fertilization regimes (organic and conventional). Results were then coupled with data from pigments, phosphate, and qPCR analyses on selected genes involved in phosphate uptake and remobilization, to get a proper picture of the metabolic response of durum wheat to phosphate fertilizers.

4. Current trends in Biotechnology

#1

Alberto Alfano • Naples

Biotech processes towards waste valorization in nutraceutical and cosmeceutical applications

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Waste reduction and reuse is a crucial target of current research efforts. The present study focused on the implementation of an integrated biotechnological process based on the use of enzymes and/or ultrafiltration membranes to convert waste generated from local agro-industrial processes into added value products with potential nutraceutical and cosmeceutical applications. In particular, either liquid or solid waste that contain potential substrates for the production of value-added chemicals (e.g., succinic acid, lactic acid), antimicrobial peptides and probiotic biomass. Probiotic biomass obtained from processing waste is a very discussed topic in recent years. In fact, besides targeting gastrointestinal disorders, food supplements based on probiotics may improve immunomodulation and protection of the host against infections caused by bacterial pathogens. Interestingly, also inactivated microbial (probiotic) cells and their metabolic products, indicated as parabiotics and postbiotics, respectively, are crucial and act as mediators in the modulation of the host's immune function.

#2

Ivan Baccelli • Sesto Fiorentino

New fungicides inspired by natural molecules: plant disease protection by targeted modifications of the Trichoderma peptaibol Trichogin GA IV

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Peptaibols are non-ribosomal linear peptides naturally produced by fungi. These metabolites possess the ability to act on lipid membranes and cause the formation of pores, and have been long studied as antibiotics and anticancer drugs in human medicine. In this work, we modified by chemical synthesis the 11-residue lipopeptaibol Trichogin GA IV, a natural product of Trichoderma longibrachiatum, to develop new eco-friendly molecules to be used in plant protection. Several Trichogin analogs were rationally designed to enhance their water solubility, helix stability and biological activity. The obtained analogs are active to a varying degree against different fungal plant pathogens in vitro and on their host plants (tomato, rice, barley). Peptide hydrophilicity, rather than helix stability, appears as the major determinant of their fungicidal activity, which is consequent to conidia membrane damage and autophagic cell death, as supported by ultrastructural images and transcriptomic data. In treated plants, reactive oxygen species (ROS) and gene expression analyses suggest that the peptides protect from fungal diseases with a limited impact on plant defenses. Work funded by PRIN2017.

#3

Carmen Bianco • Naples

Broad-spectrum activity of endophytes isolated from African rice

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We demonstrated that endophytic bacteria isolated from the African rice Oryza glaberrima, a variety resistant to environmental stresses and able of growing almost in the absence of external inputs, colonizeed rice plants grown in temperate areas (Oryza sativa) stimulating their nitrogen-fixing capacity and improving plant resistance to salt stress. In the present study, two varieties of durum wheat (Cappelli and Primadur) were inoculated with the endophyte Klebsiella pasteurii BDA134-6, a nitrogen-fixer synthesizing and releasing indole-3-acetic acid (IAA), the most common plant auxin. Under controlled laboratory conditions, BDA134-6 was able to colonize the internal tissues of both varieties. When the non-inoculated plants were used as control, BDA134-6-inoculated plants subjected to strong water stress (simulated with PEG) showed a good level of N-fixing activity and a significant increase in the activity of enzymes (CAT, GR and SOD) involved in the detoxification of ROS. Phenotyping experiments are currently underway to evaluate the response of inoculated plants to water deprivation. This strategy might promote the adaptation of plants to ongoing climate changes.

#4

Valeria Bontà • Pavia

Applications of engineered Bacillus subtilis strain for agri-food waste valorization

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Bacillus subtilis is an important industrial workhorse equipped with wide range of hydrolytic enzymes necessary to thrive on decaying biomass, acquired during its evolution as soil bacterium. We evaluated the biotechnological application of B. subtilis strains in which the production of enzymes for the deconstruction of lignocellulosic biomasses had been optimized. One of the engineered strains was able to increase the nutritional values of several dairy cow forages, thereby showing a promising application as feed additive suitable to enhance animal productivity without impacting on natural resources. Another optimized strain could facilitate the recovery of nutraceutical compounds from vegetable waste when applied as pre-treatment before the extraction procedure. The recovery of bioactives correlated with the amount of cellulolytic enzymes applied, demonstrating that the bacterial assisted pretreatment represents an efficient and sustainable approach to promote the recovery of valuable substances from the vegetable matrix.

#5

Miriam Carbonaro • Naples

More than just a cellulase: Cel9_Am a new multifunctional GH9 enzyme from Alicyclobacillus mali FL18

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Cellulolytic and hemicellulolytic enzymes are biocatalysts widely used in many industrial processes; in the bio-based era they are considered flagships for their role in the conversion of recalcitrant biomasses and for this reason, many microorganisms are characterized as sources of carbohydrate-active enzymes1. In this context, a recently discovered thermo-acidophilic microorganism, Alicyclobacillus mali FL182 is a wide reservoir of putative glycosyl-hydrolase enzymes. In our work, a novel enzyme belonging to glycoside hydrolase family 9 (GH9) from A. mali FL18 (Cel9_Am) was investigated. The gene has been expressed in E. coli and the recombinant protein purified and characterized. Enzyme assays showed hydrolysing activity on a broad range of β-glycosidic linkages of cellulose (crystalline and not), lichenan, mannan and xylan; this is not a general feature within GH9 members. This work gives insights into the multifunctional GH9 enzyme family, through the characterization of an enzyme with potentialities for industrial processing.

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#6

Simona Carfagna • Naples

Microalgae cross-fertilization: short-term effects of Galdieria phlegrea extract on growth, photosynthesis and enzyme activity of Chlorella sorokiniana cells

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Galdieria spp. are polyextremophile microalgae known for their important antioxidant properties in different biological systems. Nowadays, the beneficial and bio-stimulant effect of microalgal extracts is widely tested on crops. Here, for the first time, potential positive effects of aqueous extracts from Galdieria were tested on a second microalgal culture systems. Chlorella sorokiniana cultures were supplemented with Galdieria phlegrea extracts and the short-term effects of extract addition on growth and biochemical and physiological parameters were monitored and compared to those of non-supplemented. In Chlorella cultures, cells growth was improved by Galdieria extracts supplementation and photosynthetic pigments contents appreciably increased. The pigments increase was associated with higher photosynthesis and lower non-radiative dissipation of light as indicated by chlorophyll fluorescence parameters. Reduced activities of antioxidant enzymes and increased total antioxidant capacity suggested that these cultures were under a low oxidative status. In conclusion, a positive effect of the addition of G. phlegrea extracts on growth and physiology of C. sorokiniana was demonstrated.

#7

Martina Cascone • Naples

Hydrogenotrophic metabolisms in the subsurface: ecological and biotechnological applications

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Hydrogen is a fundamental microbial electron donor considered the energetic currency exchanged within microbial communities in anaerobic environments. A currently under development technology involves subsurface storage of hydrogen (UHS) in a stable terrestrial reservoir for later use. If viable, UHS development could enable the urgent energy transition.

When hydrogen is artificially stored underground, the in situ microbial community interacts with it, converting the H2 to metabolic byproducts, potentially compromising the storage viability. It is thus necessary to investigate the diversity and the distribution of hydrogenotrophic metabolisms in the subsurface. Given the difficulty of drilling boreholes and/or accessing deep mines, we suggest using deeply-sourced seeps, notoriously hot spots of microbial diversity.

Hydrogenases are the key enzymes controlling hydrogen utilization and oxidation, which are found in all domains of life. This work uses metagenomic approaches to investigate the hydrogenases diversity in samples from deeply-sourced seeps in order to establish the baseline of hydrogenotrophic metabolisms in the subsurface and aiding in the development of UHS technology.

#8

Michela Cecchin • Verona

RNA interference-based techniques to increase sustainability and safety in the fruit and vegetable supply chain

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RNA interference (RNAi) is an innovative and sustainable way to improve the quality of plant production and reduce the use of pesticides. There are two main strategies for RNAi-based plant disease control: Host-Induced Gene Silencing (HIGS) and Spray-Induced Gene Silencing (SIGS). HIGS can be applied in fruit trees to produce a rootstock that confers pathogen resistance to non-GM scion through the transfer of dsRNA molecules. We employed grafted non-GM grapevine plants on HIGS rootstock expressing dsRNA against Botrytis cinerea and we are evaluating the presence of siRNA in the leaves to verify its effects on the scion. Apoplastic vesicles emerged in HIGS plants as responsible for plant-pathogen communication. We are studying these vesicles in grafted plants of Nicotiana benthamiana to evaluate the presence of siRNA from the HIGS rootstock. SIGS represents a new “green” alternative to chemical pesticides, but high costs of dsRNA have limited its commercial application. For this reason, we propose the green algae, Chlamydomonas reinhardtii, as a biofactory of dsRNA. Overall, this work aims to propose new insights into the fight against pathogens through RNA interference techniques.

#9

Roberta Cirincione • Palermo

Application of polybutylene succinate electrospun microfibrillar artificial scaffold for promoting bone regeneration: in-vivo study on rabbit model

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Polybutylene succinate (PBS) has high potential as a scaffold in bone tissue engineering. This study aimed to evaluate in vivo bone regeneration promotion and osteointegration ability of microfibrillar PBS-based scaffolds implanted in cranial bone defects experimentally created in a rabbit model. Two circular defects (8 mm) were formed on the left and right calvarial bones of 9 rabbits, followed by the implantation of the scaffold in only one defect (treated). The contralateral one had spontaneous repair (control). Bone formation was evaluated by computed tomography (CT) imaging and histological analysis at 4 (Group 1), 12 (Group 2), and 24 (Group 3) weeks post-implantation. At CT imaging, treated defects showed a larger area of new bone formation compared with the controls (10%, 30%, and 60% vs 8%, 25%, and 54% at 4, 12, and 24 weeks, respectively). Histologically, scaffolds induced significantly better bone repair as demonstrated by an intense periosteal expansion osteogenesis, the presence of numerous osteoblasts and isolated hematopoietic niches. This study showed that PBS scaffolds represent a promising solution for enhancing the amount of bone regeneration in bone defects.

#10

Corentin Clavé • Portici, NA

Plant-mediated effects of the entomopathogenic fungus Beauveria bassiana on Spodoptera littoralis

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Beneficial microorganisms constitute a significant and promising alternative to agrochemicals thanks to their capacity to promote plant growth and their defence barriers against different biotic stress agents. Beauveria bassiana is one of the most important entomopathogenic fungi and can colonize a wide variety of plant species. Experimental evidence has already shown that it can contribute to limit growth and survival of plant pests and pathogens. Here we further contribute to this research topic by studying the effect on Spodoptera littoralis (Lepidoptera, Noctuidae) of tomato plants colonized by B. bassiana. Unexpectedly, endophytic colonization of plants has no effect survival of larvae feeding on them, which also showed a weight increase. These larvae proved to be more sensitive to B. bassiana infection, given their reduced immune competence. These results shed new light on the complex network of plant multitrophic interactions which underlie the evolution of entomopathogenic lifestyle in soil fungi.

#11

Sara Colanero • Milan

Engineering water use in tomatoes by generating novel allelic variation for ABA signaling genes via Crispr-Cas9.

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The enormous demand for water by world agriculture increases rapidly. Tomato is a major crop plant, and it is becoming very important to identify genotypes with superior productivity with reduced use of resources. CRISPR-Cas9- based approaches provide us with the tools for targeted mutagenesis approaches (knockout and gene editing) of genes that modulate pathways related to abiotic stress responses. Abscisic acid (ABA) is the key signal molecule that mediates the acclimation of plants to water deficit, therefore increasing ABA sensitivity may represent an obvious strategy for improving water use efficiency (WUE). The goal of our research is to generate a wide allelic variability on different components of ABA signaling.

The target genes modified are (i) the putative ortholog of the tomato AtMYB60 gene, which functions as a positive regulator of stomatal opening, and (ii) a family of genes encoding type 2C phosphatase proteins (PP2C), which are negative regulators of ABA signaling. We have provided a large pool of alleles from which different combinations can be mined that confer a trade-off between water deficit tolerance and growth performance.

#12

Patrizia Contursi • Naples

Production of bioactive molecules from agri-food and lignocellulose biomasses using a novel strain of Weizmannia coagulans

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Industrial processes based on lignocellulose degradation exploit hydrolytic enzymatic cocktails for biomass treatment. A thermophilic W. coagulans strain (MA-13) has been recently isolated (1,2) and shown to be able to produce lactic acid (LA) from lignocellulose biomass. W. coagulans MA-13 turned out to be exceptionally resistant to toxic compounds derived from the thermo-acidic treatment of lignocellulose, thus pointing to this microorganism as a promising candidate for the production of LA (3) .

One of the most challenging food consumption issues is how to ameliorate the digestibility of foods containing some not-digestible galactosides. The intra- and extra-cellular glycosyl hydrolase (GH) repertoire of MA-13 was biochemically analyzed and a full biochemical characterization of a β-galactosidase, revealed that the enzyme was able to produce GOS, thus proving the ability of B. coagulans MA-13 to eco-friendly produce prebiotics from dairy food waste (4).

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#13

Iolanda Corrado • Naples

Exploitation of inulin rich biomass for bioplastics production

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Polyhydroxyalknaoates, PHAs, are microbial polyesters introduced as alternative to traditional plastics. Despite the high potential, PHAs production is hampered by high production costs thus the research is currently concerned with use of cheap and renewable feedstocks to use as starting materials. In this work inulin, a plant-derived polysaccharide, was exploited as potential renewable carbon source for PHA production. A protocol for aqueous inulin extraction from Cynara cardunculus roots was set up and the extract was characterized. To be used as carbon source by microorganisms, inulin needs to be converted into fermentable sugars (mainly fructose and glucose) by inulinases. In this work, a mixture of inulinases was produced by Peniciullium lanosocoeruleum and characterized for hydrolytic performances. Moreover, a Simultaneous Saccharification and Fermentation (SSF) process was investigated as strategy for the conversion of inulin into PHA by Cupriavidus necator, a well-known PHA producer. A maximum of 3.2 g L-1 of PHB accumulation, corresponding to 82% polymer content, was achieved.

#14

Salvatore Cubisino • Catania

Determination of electrophysiological properties of envelope (E) protein from SARS-CoV-2

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The envelope (E) protein of SARS-CoV-2 is the smallest structural protein produced by the virus. It is an integral membrane protein composed by 75 amino acids distributed in three distinct domain: the hydrophilic N-terminus, the hydrophobic transmembrane domain (TMD) and the long C-terminus predicted as a single hydrophilic domain. In particular, it was supposed that TMD is involved in oligomerization of E polypeptides leading to the formation of an ionic channel, as showed by Liao et al. for the homologous E protein of SARS-CoV (BBRC, 2004). We cloned SARS-CoV-2 E gene into the pET21a vector and optimized an experimental protocol for protein expression and purification in E.coli. Preliminary results reported here provide a comprehensive electrophysiological characterization of the channel that could be useful for the identification of metabolic pathways that can be activated by the permeation of ions from the channel to the cytosol. Alongside, we performed molecular dynamics simulations finalized to understand how this channel works: preliminary results show a correlation with electrophysiology measures.

#15

Sabrina Cuomo • Naples

Holocellulose from Poseidonia oceanica egagropili sea balls: a waste source valorized as enhancer of cellulase biotechnological production by Streptomyces roseochromogenes

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Posidonia oceanica egagropili accumulate on the Mediterranean Sea coasts and could be valorized, as such or by deriving holocellulose, in biotechnological processes as substrate to obtain fermentable sugars. In this work the cellulase production by Streptomyces roseochromogenes ATCC13400 was investigated for the first time, and the enzyme production was prompt by addition of the holocellulose, isolated from egagropili. In shake flasks with 2.5 g·L−1 of holocellulose addition, the cellulases activity increased of 4.3-folds compared to the control, up to 268 U·L−1 in 72 h, with a productivity of 3.72 U·L−1 ·h−1. In 2 L batch experiments the production reached 347 U·L−1 in only 45 h, with a doubled productivity of 7.7 U·L−1 ·h−1. The hydrolytic activity was due by three different cellulases having molecular weights between 115 and 47 kDa. The enzyme pool showed a maximum of activity at 60 °C and at pH 5.0. The extracellularly produced cellulases were recovered from the broth supernatant by two sequential ultrafiltration and diafilfration steps on 10 and 3 kDa membranes. These cellulases proved able to degrade different β-linked carbohydrate substrates.

#16

Gennaro D'Ambrosio • Portici, NA

Phycoremediation of persistent pollutants: Polycyclic Aromatic Hydrocarbons (PHAs) and microplastic debris (MPs)

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Since the industrial revolution, human pressure by releasing many pollutants on environment has notably increased. Among them, PAHs and MPs have gathered significant environmental concerns for their toxicity and several approaches have been applied to remove them. Here, we report the identification of microalgae able to grown in presence of benzoanthracene B[a]A or polyethylene terephthalate (PET). By growth inhibition assays (GI) were identified 4 microalgae specie with able to grow at 43.8 mM of B[a]A. These algae were able to degrade 90% B[a] after only 21 days of growth. While inhibition growth assay, performed on 37 microalgae accession, has identified 6 species able to grow in plastic enriched liquid medium. Oxidoreduction by laccase has been reported in literature as a step to biodegraded these two pollutants. Therefore, we have performed an in-silico analysis of laccase gene in green alga model Chlamydomonas reinhardtii and we found several nucleotide sequences including one with a secretion leader sequence. Finally, the laccase activities of cell-free supernatants and intracellular form identified microalgae culture growing with xenobiotics will be reported.

#17

Sergio D'Ambrosio • Naples

Agri-food waste biomasses for the production of Lactic Acid from Lactobacillus rhamnosus IMC501

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Green economy aims at reducing environmental risks and ecological scarcities, toward a more sustainable development without degrading environment. Potential use of waste biomasses as carbon sources for biotechnological processes is widely established. In particular, in the present work, different waste product biomasses (eg. whey from diary factories, strawberry leafs and orange waste) were used as substrate for the growth of Lactobacillus Rhamnosus IMC 501 probiotic strain for the production of high amount of lactic acid. This homofermentative strain is a natural producer of lactic acid that is a valuable product with numerous established industrial applications such as food additive and it is a building block for the synthesis of polylactide (PLA) a biodegradable polyester. Tests were performed booth in presence or not of ammonia pre-treatment and finally the biomasses were hydrolyzed with the Cellic CTec2 (Novozyme) enzyme mix. Bottle growth experiments were performed in 100 mL bottles with hydrolyzed biomasses to evaluate capability of L. rhamnosus to produce lactic acid on these waste biomasses.

#18

Caterina D'Angelo • Naples

Characterization of a new anti-adhesive molecule from Psychrobacter sp. TAE2020

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Biofilm is a major issue in medical settings and Staphylococcus epidermidis is one of the pathogens involved1 in biofilm-related infections. Polar marine bacteria represent potential candidates for the discovery of new anti-biofilm compounds2-3. Moreover, our attention was focused on the ability of the Antarctic bacterium, Psychrobacter sp. TAE20204, to produce molecules able to impair the attachment of staphylococci strains to the surfaces. The anti-adhesive and surfactant properties of TAE2020 cell-free supernatant were evaluated and we demonstrated that the cell-free supernatant displays anti-adhesive, emulsifying, and surfactant properties. The anti-adhesive and the emulsification activities were associated with a new high-mass complex of proteins and polysaccharides, that we named Catasan. An enriched sample allowed the identification of Omp38, a 40kDa outer membrane protein participating to the Catasan complex. TAE2020 Omp38 shares 46% of sequence identity with AlnA, a protein of the Alasan complex, a bioemulsifier produced by Acinetobacter radioresistens KA535.

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#19

Riccardo D'Incà • Rome

Solanum lycopersicum CRISPR/Cas9 mutants for a polyamine oxidase gene exhibit improved drought stress tolerance and water-use efficiency

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The polyamines putrescine, spermidine, spermine (Spm) and thermospermine (T-Spm) are involved in important plant developmental and defence processes. In Arabidopsis, five distinct polyamine oxidases (AtPAO1 to AtPAO5) are involved in polyamine catabolism. AtPAO5 preferentially oxidizes Spm and T-Spm and contributes to the control of plant development, xylem differentiation and tolerance to abiotic stresses. Three AtPAO5 homologs (SlPAO2, SlPAO3, SlPAO4) were identified in Solanum lycopersicum and SlPAO3 knockout mutants (slpao3) were obtained by CRISPR/Cas9-mediated genome editing. Compared to wild-type plants, slpao3 mutants exhibit altered T-Spm levels, growth parameters and expression levels of auxin-related genes, along with increased tolerance to drought stress. Furthermore, slpao3 mutants differ from wild-type plants in the number, size and lignification level of the xylem elements, the transpiration rate, the water transport capacity and the vulnerability to xylem embolism. Altogether, our data suggest that slpao3 mutants are characterized by improved water-use efficiency highlighting that polyamine metabolism can be exploited to transfer stress tolerance traits in crops.

#20

Giovanna De Leva • Portici, NA

Impact of Deformed Wing Virus infection on honey bee gut microbiota

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Honey bee health decline represents a problem of global importance for the remarkable impact of these pollinators on the environment and human economy. The reduced bee survival is the final result of a multifactorial syndrome triggered by several stress factors, that may synergistically interact, inducing a reduction of bee immunocompetence. A common element to all collapsing colonies is the high loads of parasites and associated pathogens, such as Deformed Wing Virus (DVW). DWV is an endemic immunosuppressive virus that generates asymptomatic covert infections, kept in check by the bees' immune system when not exposed to stress agents which weaken antiviral barriers. Here we focus on the role that DWV viral infections can have on the modulation of honey bee microbiota. We compared microbiota composition of bees with low and high DWV levels, pointing out the occurrence of a gut dysbiosis in highly infected bees, which are characterized by a reduced level of Lactobacillus species. The comprehension of DWV effect on microbiota will likely allow to define blends of probiotic microorganisms which may help to rescue the decay of honey bee immune competence.

#21

Maria Giovanna De Luca • Portici, NA

I funghi benefici del genere Trichoderma sono efficaci agenti di controllo delle larve di falena notturna

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I funghi benefici appartenenti al genere Trichoderma hanno effetti ben noti sulla biologia vegetale, come la promozione della crescita, l'efficienza dell'assorbimento dei nutrienti, il tasso di germinazione dei semi e la difesa delle piante contro agenti di stress sia biotici che abiotici, compresi gli insetti fitofagi. Qui abbiamo ottenuto piante di pomodoro colonizzate, rivestendo i semi con spore del ceppo T22 di Trichoderma afroharzianum (pianta T22), e abbiamo valutato gli effetti mediati dalle piante di T22 sulla falena notturna Spodoptera littoralis. La colonizzazione delle piante ha aumentato la mortalità e ridotto il peso larvale. Inoltre, le larve nutrite con piante T22 hanno mostrato un fenotipo immunosoppresso che è più suscettibile a dosi subletali di un biopesticida contenente l'entomopatogeno Bacillus thuringiensis . I risultati ottenuti hanno mostrato un effetto vegetale di T. afroharzianum sulle larve di S. littoralis che potrebbe impedire alla riduzione dell'uso di pesticidi in agricoltura sopprimendo la parassita o aumentando la sua suscettibilità agli agenti di biocontrollo.

#22

Angelo De Paolis • Lecce

Biotechnological production of natural agrochemicals by cell cultures of Dittrichia viscosa

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Plant cell and tissue cultures can be used as biofactories of valuable natural compounds for various applications, from the production of food additives to pharmaceuticals, cosmetic ingredients or bio-pesticides. Dittrichia viscosa (L.) Greuter, a plant species widespread in the Mediterranean regions, represents a valuable source of natural bioactive compounds with potential applications in pest management, including insects, diseases, and weeds. With the aim of producing natural agrochemicals, callus cultures of D. viscosa were established by optimizing in vitro growth conditions. Fast-growing cell suspensions were obtained from friable calluses and maintained at controlled temperature and light conditions. At the stationary phase, D. viscosa cells and spent medium were separately collected, lyophilised and extracted using organic solvents. Phytotoxic and moderate fungicide activities of the obtained extracts were observed by using suitable bioassays. The identification and quantification of the bioactive compounds are in progress. From these preliminary results, D. viscosa cell cultures appear to be a promising biotechnological system for the production of natural agrochemicals.

#23

Benedetta Di Chiara Stanca • Lecce

Chemical, structural and biological characterization of CGF and analysis of its osteogenic potential

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Concentrated Growth Factors (CGF) represent new autologous blood-derived biomaterial, attracting growing interest in the field of regenerative medicine. In this study, the chemical, structural and biological characterization of CGF was achieved. CGF molecular characterization was performed by GC/MS to quantify small metabolites and by ELISA to measure growth factors release; structural characterization was carried out by SEM analysis and immunohistochemistry; CGF has been cultured and its primary cells were isolated for the identification of surface markers by flow cytometry, Western blot and real-time PCR. The osteogenic differentiation of CGF primary cells was evaluated through matrix mineralization by alizarin red staining and mRNA quantification of specific markers by real-time PCR. We found that CGF has a complex inner structure capable of influencing the release of growth factors, metabolites and cells. These cells, which could regulate the production and release of the CGF growth factors, show stem features and are able to differentiate into osteoblasts. These data highlight interesting new perspectives for the use of CGF in regenerative medicine.

#24

Daniela Eletto • Fisciano (Salerno)

Potential role of trefoil factor 1 as bioactive peptide in Helicobacter infection

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Trefoil factor 1 (TFF1) is a small peptide mainly expressed by foveolar cells of gastric mucosa with the aim of maintaining mucosa homeostasis and limiting Helicobacter infection. Since its depletion in mice causes the spontaneous appearance of antro-pyloric adenomas and ultimately carcinomas, TFF1 is considered a key factor for the stomach and fluctuations of its expression may predispose to gastric lesions following Helicobacter infection. We, therefore, aimed to investigate the potential role of TFF1 as a bioactive peptide. To this aim, we expressed TFF1 as dimeric recombinant protein and evaluated its effect on Helicobacter pylori at the morphological and functional levels. Our data show that TFF1 favors bacterial aggregation slowing down migration of Helicobacter across the mucus layer and ameliorating the whole infection. To corroborate the protective role of TFF1, we also investigated how it is modulated during the infection and found that it is initially stimulated but gradually reduced when the infection becomes chronic. Understanding the molecular mechanisms leading to TFF1 silencing during chronic infection will make considerable progress in future clinical applications.

#25

Carmen Ercolano • Naples

Functional metagenomics approach for the identification of new (hyper)thermophilic carbohydrate-active enzymes of biotechnological interest

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Dependence on fossil fuels has increased the atmospheric concentration of CO2, contributing to the global warming. With the diminishing supply of oil, this problem prompted to search for alternative energy sources, as biofuels [1]. Lignocellulosic biomasses are a promising reservoir, involving a closed circle of carbon due to the CO2 conversion in organic compounds. However, the bottleneck of the bioprocess is the hydrolysis step. This problem justifies the search for new cocktails of enzymes to degrade completely lignocellulosic material [2]. In this study, functional metagenomics approach is applied to discovery and characterize new enzymes that can help the complete conversion of lignocellulose in monosaccharides fermentable in second-generation biorefineries. Starting from the extraction of metagenomic DNAs of samples from geothermal sites, the objective will be carried out screening fosmidic expression libraries for exo- and endo-(hemi)cellulase activities. Preliminary analyses showed that 9 samples from geothermal sites in Argentina could be a good starting point for the functional screening.

References

[1] DOI: 10.1080/09593331003710236.
[2] DOI: 10.1111/febs.15080.

#26

Michela Famiglietti • Naples

Mechanical, thermal, and barrier properties of amylose-based bioplastics

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Looking for new sources to produce energy and materials and valorizing by-products, that otherwise would get wasted, are the aims of the circular economy. In this work, amylose, obtained by RNA interference technique from barley plants, and proteins, derived from argan oil cake, were exploited to produce novel blended bioplastics. Amylose provides added-value functionalities to the normal starch reducing or eliminating the need for subsequent chemical modification or blending with synthetic polymers.

Argania spinosa is a plant typically widespread in arid regions of Northern Africa, which plays a fundamental ecological role. Recently argan oilcakes have been attracting attention as a waste to be recovered to obtain high-added value products for different applications.

This work aimed to investigate the possibility to produce novel hydrocolloid bioplastics, improving the performance of amylose-based films and argan proteins-based films in terms of mechanical, barrier, and thermal properties. The effect of the enzyme microbial transglutaminase as reticulating agent for the argan protein component blended with amylose was also studied.

#27

Arianna Filippelli • Siena

Profiling of potential pharmacological activity of sweet chestnut wood distillate as antioxidant and anti-inflammatory agent

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Wood distillate (WD) is a bio-based product, extracted from residual sweet chestnut biomass employed in agriculture as bio-fertilizer. The WD is produced through a controlled pyrolysis process without any environmental risk. Polyphenols, phenols, and tannins are stably conserved into WD composition. We previously profiled the safety of exposure to WD in vitro, using human cell models involved in transcutaneous absorption and distribution into the blood stream. Based on the composition of WD and the safety profile of its low concentrations, our study aims to assess the anti-inflammatory and antioxidant activity of this bio-based product. Keratinocytes, mucosal model, dermal fibroblasts, and endothelial cells were exposed to low concentration of WD, with or without a condition of cytokines-induced inflammation. The modulation of the inflammatory markers was assessed by western blot and immunofluorescence assay and endothelial hyperpermeability, main pathological signals of inflammatory condition, was strenghten by the analysis of cell-cell adhesion proteins. In conclusion, WD shows a promising anti-inflammatory and antioxidant activity that may suggest novel application fields.

#28

Gabriella Fiorentino • Naples

A hyperthermoactive-Cas9 editing tool reveals the role of an arsenite methyltransferase in the arsenic resistance system of Thermus thermophilus HB27 and provides a new sensitive arsenic bioreporter

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Progress in genome editing techniques has provided new tools to engineer microbial genomes. Hyperthermophiles, microorganisms able to thrive in extreme environments, are attractive hosts in industrial platforms; however, their use is limited by the low number of suitable tools for their genetic manipulation. Among thermophilic bacteria, Thermus thermophilus is a good candidate as chassis for metabolic engineering due to its high biomass yields, expression of a very efficient natural competence system and metabolic versatility. Here we describe the set-up of a highly efficient hyperthermoactive-Cas9 editing tool for T. thermophilus active up to 65°C1. Based on our previous knowledge of the arsenic resistance molecular mechanisms, the tool has been used to define the role of a thermoactive arsenite methyltransferase and to develop a highly sensitive whole-cell biosensor for arsenic monitoring. This work is among the first examples of the application of the CRISPR-Cas9 technology to hyperthermophiles and could represent a novel starting point to engineer hyperthermophiles for biotechnological applications in environmental and industrial fields.

1 Gallo G et al:10.1128/mBio.02813-21

#29

Rosa Gaglione • Naples

Novel bioactive peptides identified in a protein from human plasma to counteract bacterial infections

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Several proteins with functions not necessarily related to host defence act as a source of host defence peptides (HDPs). This is also the case of proteins from human plasma, such as human Apolipoprotein B, from which three novel HDPs have been identified by using a bioinformatic tool. Peptides were found to be effective as antimicrobial, antibiofilm and anti-fungal agents. Moreover, they showed excellent cytotoxicity profiles.

ApoB-derived peptides have been also used to functionalize the surface of medical devices by employing the polymer polydimethylsiloxane. The obtained polymer showed antimicrobial properties, stability upon storage at 4°C and the ability to prevent biofilm attachment.

To ensure translatability of these molecules, we synthesized a retro-inverso version of the shorter identified peptide that appeared less susceptible to serum proteases while retaining its anti-infective properties and biocompatible profile. Interestingly, it was also able to attenuate infection in a murine model and was successfully employed to develop an effective hydrogel-based system.

Obtained results open interesting perspectives to the applicability these molecules in biotechnological field.

#30

Laura Giannotti • Lecce

Osteogenic differentiation of CGF cells can be induced by silicon scaffold

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The application of scaffolding materials together with stem cell technologies plays a key role in tissue regeneration. Therefore, in this study we used CGF (concentrated growth factors) that represents an autologous and biocompatible blood derived product with scaffolds made of hydroxyapatite and silicon (HA-Si). The aim was to evaluate osteogenic differentiation of CGF primary cells induced by HA-Si scaffolds. Initially, we determined by MTT assay the cellular viability of primary cells cultivated on HA-Si scaffolds and we performed a structural characterization of this complex carried out by SEM analysis. Moreover, we evaluated the matrix mineralization of CGF primary cells cultivated on HA-Si scaffolds through alizarin red staining and mRNA quantification of osteogenic differentiation markers by real-time PCR. We found that HA-Si scaffolds are not cytotoxic for CGF primary cells and allow them to grow and proliferate. Furthermore, they are able to induce the osteogenic differentiation of these cells. In conclusion, our results suggest that HA-Si scaffolds can be used as a biomaterial support for CGF application in the field of tissue regeneration.

#31

Laura Gioia • Portici, NA

Reduction of copper-based fungicides use for controlling phytopathogens by Trichoderma application

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Frequent applications of Copper (Cu)-based agricultural products determined the accumulation of Cu in soil and water sources. The development of alternative products for reducing Cu release in the agroecosystems is needed. The research was aimed at testing the efficacy of Trichoderma sp. in combination with reduced doses of copper-based fungicide (Cupravit Bioadvance®, Bayer) for controlling Botrytis cinerea and Alternaria alternata on tomato. In order to select Trichoderma Cu tolerant strains, the growth of 4 isolates was monitored on PDA enriched with growing concentration of fungicide. T. afroharzianum T22 and T. asperellum T25 were able to growth on medium contained up to 90 mg/L of Cu (metal), resulting the best candidates for in vivo biocontrol assays. A consistent diseases severity reduction was observed on plant treated with 1/2 and 1/4 dose of Cupravit in combination with both Trichoderma strains. T25 + 0,45 g/L $CuSO_4$ was found more effective than full dose fungicide in reducing Alternaria diseases symptoms. The data suggested that Cu tolerant Trichoderma strains can be used with Cu-based fungicides for the control of phytopathogens, reducing Cu release in the environment

#32

Moira Giovannoni • L'Aquila

Valorization of lignocellulosic wastes for the production of fungal enzymes of industrial relevance

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By using an algal trap, we captured a filamentous fungus that was identified by whole genome sequencing as a novel Penicillium sumatraense isolate (AQ67100). This fungus assimilated Chlorella vulgaris by secreting an enzymatic arsenal mainly composed of proteases, glycosidases and 1,3-β-glucanases. The treatment of C. vulgaris with the fungal filtrate improved the release of chlorophylls and lipids from the algal cells by 42.6% and 48.9%, respectively, highlighting the potential of saprotrophs in the bioprocessing of microalgae for the production of biofuel and bioactive compounds. As a further step towards sustainability and smart waste recycling, we evaluated the possibility of using lignocellulosic wastes to stimulate the production of enzymes from P. sumatraense AQ67100. The fungus grew in a wheat straw-supplemented medium by producing huge amounts of different commercially relevant enzymes such as β-glucosidases (12240 ± 752 U.kg-1 wheat straw), xylanases (54406 ± 891 U.kg-1 wheat straw) and proteases (1,1*107 ± 3,4*106 U-tame.kg-1 wheat straw), demonstrating the potential valorization of agricultural waste to produce high value-added products as industrial enzymes.

#33

Rachele Ingrisano • Bologna

Microalgae: from photosynthetic scavengers to green biofactories

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The rapid population growth, with its demand for water, food and energy, is also responsible for the increase in waste production. Domestic and industrial drains are rich in inorganic and organic substances and require pre-treatment before returning to the water supply. Microalgae are promising candidates for wastewater remediation. With their adaptable metabolism, microalgae can grow both by exploiting the energy of sunlight and in mixotrophy. Through mixotrophic growth, microalgae can absorb compounds from wastewater, recycling them to produce biomass. The algal biomass, in fact, is emerging as a valuable resource in several economic and industrial sectors.

In this context, our research is focused on the biomass production of five Chlorella spp. strains grown in a photobioreactor with and without organic compounds. A general increase in biomass yield was observed in algae grown in mixotrophy, with a similar partitioning of the newly formed biomass into carbohydrates and proteins. Only C. vulgaris showed significant starch enrichment, which could be a sustainable source for bioethanol production. Future analyses will focus on determining lipid and pigment content.

#34

Carmen Laezza • Portici, NA

The use of plant cell culture as an alternative method for the production of dihydrochalcones and flavonoid

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In the last years, there has a been an extensive adoption of plant cell culture (PPC) as a renewed approach to increase the production of secondary metabolites (SM) which have a consolidate role as natural drugs for plants and humans. The aim of our research is to develop novel improved PPCs accumulating high level of dihydrochalcones and flavonoids. The attention will be focused on catechins and epicatechins particularly known to have anti-inflammatory, anti-microbial, anti-cancer, and antihypertensive properties and on dihydrochalcones like phloridzin well-known to reduce blood glucose levels. Given this, we are developing protocols for callus and suspension cultures, starting from different plant's organs (leaf, stem, fruit) of Mediterranean species such as, Malus pumila, Cistus creticus, Solanum Lycopersicum, and Vitis vinifera. Subsequentially, mature callus and suspension, elicited or not, will be used to extract and analyze the content of SMs. The final goal of this project is the in-depth study of polyphenols biosynthetic pathway within Mediterranean plant species to deliver bioactive compounds in quantitative amounts that are comparable or higher than reference material.

#35

Giulia Magoga • Milan

COins: a reference database of COI sequences for insect species molecular identification

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Due to the high level of specialization required for the morphological identification, the species richness and ubiquity, insects are one of the groups for which DNA-based identification is most frequently adopted. Nowadays, insects biodiversity surveys using DNA metabarcoding are increasingly common, but species level identifications are not easily obtained with such approach. A DNA metabarcoding reference database specifically curated for insect identification can improve this result. Here we present COins, a database of COI-5P sequences of insects that includes over 532,000 sequences of more than 106,000 species. Through a combination of automated and manually curated steps, we developed this database starting from all COI sequences of insects available in BOLD, focusing on sequences that comply to several standards, including species-level identification. COins was then validated on previously published data (54 bulk samples obtained from Malaise traps) and its efficiency compared with other public reference databases. In our case study, COins allowed an increase of up to 30% of species identifications. COins represents a valuable resource for insects molecular identification.

#36

Emanuela Maresca • Naples

Alicyclobacillus mali FL18, a promising microorganism for degradation of agri-food wastes: from synthetic substrates to a novel consortium with Weizmannia coagulans MA – 13 on agri – food wastes

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Extremophiles have been considered as good candidates in lignocellulosic biomass degradation, a resource for green applications. In this research, the degradative properties of two thermophiles, Weizmannia coagulans MA – 13 and Alicyclobacillus mali FL18 were investigated., W. coagulans MA – 13, isolated from bean processing waste, was able to metabolize synthetic substrates at high temperature and showed promising results for industrial production of lactic acid from lignocellulose biomass 1,2. The aim of this work has been to investigate the degradative properties of A. mali FL18, a new arsenic tolerant and thermoacidophilic strain, isolated from Pisciarelli, firstly on synthetic substrates and then on agri – food wastes 3. To enhance the degradation and valorization of agri-food wastes, a second goal was to set up thermophilic consortium with A. mali FL18 and W. coagulans MA – 13.

Aulitto M. et al. (2017)
Aulitto M. et al. (2019)
Aulitto M. et al. (2021)

#37

Federico Martinelli • Sesto Fiorentino

Development of a protocol for CRISPR/Cas9-edited for legumes highly recalcitrant to in vitro regeneration

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Chickpea and lentils are very difficult legume crops to be genome-edited using CRISPR/CAS9 due to the low in vitro regeneration rates. Only one report of CRISPR/Cas9 has been documented in chickpea while no articles are present for lentil. Our project aims at: 1) improving the regeneration protocols for these two important grain legumes, 2) obtain DNA-free edited plants using a viral strategy, 3) improve some key qualitative/agronomic aspects such as drought tolerance, reduced branching, increased amounts of bionutrients. Benzilaminopurine (2 mg/L) and Thidiazuron (2 mg/L) in combination with different concentrations of α-naphthaleneacetic acid (0.1, 0.3, 0.6 mg/L) were tested. The fine-tuning of this latter molecule seems to play a critical role in the development of regenerants. Combining qRT-PCR of 21 genes analyzed in both legumes, the following gene targets have been selected: CBF2/DREB1c (drought tolerance), LAP1 and FAR1 (branching), ANS and DFR (anthocyanin biosynthesis). Results and approaches of viral infections will be shown to develop DNA-free editing of these legumes.

#38

Rosanna Mattossovich • Naples

New DNA-Nanoswitches for Monitoring the Activity of DNA Repair Enzymes

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Cellular DNA is subjected to covalent modifications by alkylating agents. O6-alkyl-guanine-DNA-alkyl-transferases (AGTs), remove alkyl adducts from DNA by a irreversible reaction. The human methyltransferase (hMGMT), protects cancer cells from the cytotoxic effect of alkylating agents-based chemotherapy: this has a crucial clinical importance, leading to a constant development of new hMGMT inactivators/inhibitors to be used in combination with chemotherapy. We present a new class of AGTs' fluorescent substrates (DNA-nanoswitches) that, upon enzymatic repair, could undergo a conformational change mechanism, leading to a change in FRET signal. DNA-nanoswitches are synthetic DNA sequences containing O6-methyl-guanine (O6-MeG) nucleobases and labelled with a fluorophore/quencher pair. These nanoswitches are rationally designed so that only upon enzymatic demethylation of O6-MeG they can form stable intramolecular Hoogsteen interactions and fold into an optically active triplex DNA structure. We demonstrated that DNA nanoswitches are suitable and specific substrates for AGTs allowing an easy and specific AGTs' high-throughput assay for the screening of novel potential AGTs inhibitors.

#39

Seyedeh Fatemeh Mirpoor • Naples

Manufacture of polyhydroxyalkanoate coated pectin-based bioplastics

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Biodegradable active packaging, i.e. materials able to promote food preservation while avoiding plastic waste accumulation is expected to play a key role in the manufacture of new generation materials. Renewable films composed of citrus pectins (CP) by-products functionalized with bioactive molecules derived from spent coffee grounds (SCGs), the waste obtained after coffee processing, were developed with potential applications in active packaging. CP-based films were prepared at acidic pH by means of casting and then, coated with a layer of polyhydroxyalkanoate (PHA), a family of biodegradable polyesters produced by various bacteria, which are gaining increasing interest as green-alternatives to petroleum-based plastics. The effect of the PHA coating on the hydrophobicity, mechanical properties and transparency of the functionalized CP-based materials was investigated and the interaction between the two biomaterials was investigated through FT-IR spectroscopy. Moreover, the functionalized films were evaluated for antioxidant and antimicrobial activity. The PHA coating turns out to improve the stiffens, opacity and permeability to the gas of the films.

#40

Eleonora Montuori • Messina

Microalgal compounds with immunomodulatory activities

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Modulation of the immune system is defined as any change in the immune response that can result in the induction, expression, amplification or inhibition of any phase of the immune response for therapeutic purposes. Recently, there has been a growing interest from the scientific community in the search for natural compounds capable of modulating the immune response as these compounds have potential applications in the fields of immunopharmacology and oncotherapy. Microalgae have attracted a growing interest because they can be cultivated in large quantities in an eco-sustainable way. My PhD thesis in applied biology and experimental medicine focuses on the screening of microalgae and their compounds on immune cells by analyzing the release of immunomodulatory mediators. The pipeline includes microalgal growth, chemical extractions, biological screenings on human cells, identify and characterize the active extracts, fractions or compounds and define the cellular mediators. In addition, it also includes transcriptome analysis of different microalgae in order to identify metabolic pathways responsible of the synthesis of immunomodulatory compounds for future genome editing approaches.

#41

Ohiemi Benjamin Ocheja • Bari

Development of yeast-based biosensor for precision agriculture

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Increasing daily demand for food and new challenges related to agriculture worldwide requires the development of precision agriculture (PA) methods. PA relies on multiple sensing layers for monitoring of chemicals and pollutants. Biosensors are considered as alternatives to conventional sensors in PA. Recently, there is a growing interest in biosensors based on yeast cells due to their low cost, stability and higher tolerance to harsh environment. We assayed the response of the budding yeast Saccharomyces cerevisiae to copper as a biorecognition element (BRE) with the final goal to design and develop a prototype yeast-based biosensor to be used in PA applications. Different strains of S. cerevisiae compared for growth and metabolic parameters showed concentration dependent sensitivity to copper. Yeast immobilization protocols by using natural matrix were optimized to improve cell stability and to assess detection and transduction mechanisms in the BRE.The obtained results lay the basis to develop the BRE of the biosensor. Analyte quantification via electronic devices and signal readout will be objective of our future work for development of prototype.

#42

Francesca Paragliola • Naples

Characterization and engineering of thermophilic α-glucosidase MalA from the hyperthermophilic crenarchaeon Saccharolobus solfataricus

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So far, in CAZy database enzymes active on substrates with α-GlcNAc are classified into GH89 family, including only mesophiles, both of bacterial and eukaryotic origin [1]. Thus, the identification of novel (hyper)thermophilic biocatalysts for the hydrolysis and chemo-enzymatic synthesis of oligosaccharides containing α-GlcNAc in extreme conditions, is of particular biotechnological interest. In 2019, α-GlcNAcase activity was identified in S. solfataricus [2], through the cooperative action of de-N-acetylase and GH31 α-glucosidase MalA. We engineered the latter enzyme to obtain a novel α-GlcNAcase. Docking studies with α-GlcNAc suggested that the residue R400 with its steric hindrance could prevent the entry of the α-GlcNAc substrates into the active site. So, the substitution with less bulky residues could favour the accommodation of the functional group in the C2 position. Preliminary results with R400 mutants confirmed its involvement in enzymatic catalysis and stability. We report here the screening on different substrates showing for the first time in GH31 family a new activity on galactopyranoside substrates.

References

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[2] 10.1128/AEM.01879-18

#43

Michele Perazzolli • San Michele All'Adige

Taxonomic and functional analysis of the microbiota associated with cold-adapted plants

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Plant-associated microorganisms can contribute to plant survival under stress conditions, but scarce information is available on the taxonomic structure and functional roles of bacteria associated with cold-adapted plants. The aim of this study was to identify bacterial communities associated with Antarctic pearlwort and alpine plants, in order to assess the contribution of psychrotolerant bacteria to plant growth at low temperatures. Bacterial communities associated with Antarctic pearlwort differed according to the collection site and growth conditions. In particular, global warming simulated by open-top chambers (OTCs) severely affected the taxonomic structure of bacterial communities, indicating possible impacts of climate changes on the microbiota of Antarctic plants. Moreover, alpine plants hosted complex communities of bacterial endophytes that differed according to the plant species and tissue type. Psychrotolerant bacterial endophytes were able to colonize tomato seedlings and promote plant growth at low temperatures, indicating potential biotechnological applications in plant bio-fertilization and cold protection.

#44

Federico Perozeni • Verona

Microalgae for the future: use of Chlamydomonas reinhardtii to produce high value products for a growing population

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Ensuring food, energy and raw material for a growing population in one of the bigger challenges of our century. Algae play a central role, as alternative to plants. Wastewaters and flue gas can be used to ensure nutrients and CO2 for carbon fixation; moreover, water can be reused after biomass harvesting generating a "green circular economy". Unfortunately, algae domestication is necessary to enhance biomass production and break down cultivation costs. One of the most promising strategies is the use of algae as green factories to produce high value products. Here we report the metabolic engineering of Chlamydomonas reinhardtii to produce 3 different products each one with specific characteristics and applications. First, Astaxanthin, a ketocarotenoid used for its antioxidant power in Alzheimer, Parkinson and several types of cancer treatments or as food supplements. Second, geraniol, a monoterpene used in food industry with the potential to change algae taste to be more appreciate to consumers. And third, Zeolin, recombinant protein obtained by merging γ-zein (Z. mays) and phaseolin (P. vulgaris) to obtain a food with a balanced amino acid content.

#45

Simone Pitton • Milan

Effective and eco-friendly delivery of a Bacillus thuringiensis-based insecticide in Aedes albopictus larvae

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Climate change and trade globalisation is causing the expansion of mosquitoes areals to non-endemic regions, together with the pathogens transmitted by their bite. To control this spread, we focused on the production of an innovative biorational floating raft containing a bioinsecticide and an attractant (i.e., Bacillus thuringiensis var. israelensis, Bti, and the yeast Saccharomyces cerevisiae) to control mosquito larvae.

We performed bioassays with a Bti-based commercial bioinsecticide to measure susceptibility to this product in larvae of different colonies of the Asian tiger mosquito (Aedes albopictus). Then, we tested the toxicity of chitosan-based hydrogel rafts containing the bioinsecticide alone or with yeasts in laboratory and semi-field conditions. The “MosChito” rafts resulted attractive for the larvae that eroded and ingested the hydrogel, and proved to be very effective in killing larvae over a month period demonstrating that the hydrogel preserved the bioinsecticide activity.

Our results showed that “MosChito” rafts may represent an original, eco-based and user-friendly floating solution for mosquito larval control in residential or urban environments.

#46

Luigia Principio • Portici, NA

Use of CRISPR/Cas9 to study ascorbic acid metabolism in a S. pennellii introgression subline

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In recent years, Solanum pennellii introgression lines (IL) have been exploited to improve several quality traits in tomato. In previous studies, one subline (R182) of chromosome 7, which carries only a small region of wild genome in the cultivated genetic background (M82), showed a higher ascorbic acid content compared to the cultivated line. This work aimed to study two genes, with the use of CRISPR/Cas9 technology, coding for a Nucleobase Ascorbate Transporter (NAT) and for a Major Facilitator Superfamily Protein (MFSP), which could play an indirect role in the biosynthesis and accumulation of AsA and in controlling other qualitative key traits. The genotyping of T1 edited plants confirmed the heritability of the mutated alleles. In both the cases of T1 NAT- and T1 MFSP-edited R182 lines, five different mutated alleles were identified. Among the T1 MFSP-edited R182 lines, two showed the insertion of one base and three resulted in a deletion of 4 bp, 7 bp and 81 bp, respectively As for the T1 MFSP-edited M82 lines, two mutated alleles were detected having a 3 bp or 21 bp deletion, rescpectively. Biochemical and physiological analyses on all the genotyped lines are under way.

#47

Yuri Prozzillo • Rome

Adaptation of dTAG system in Drosophila melanogaster

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Direct targeted protein degradation (TPD) allows an acute and re-versible knockdown of protein of interest (POI) without affecting its transcript. Recently, a degradation TAG (dTAG) system was developed to achieve proteasome-mediated proteolysis of POI exploiting the pow-er of degradation signal peptide sequence (FKBP12F36V). Indeed, the dTAG system developed by Nabet et al. is based on the heterobi-functional activity of dTAG-13 degrader, a molecule that link the FKBP12F36V-fused POI to the recognition unit of E3-ubiquitin ligase complex to trigger protein degradation. The dTAG system have been well established in vitro and in vivo in several species, unless in Drosophila. Bioinformatics analysis has revealed a high score of identity in the dTAG-13 binding domain of E3-ubiquitin ligase complex. Here, we report the feasibility of the dTAG system in Drosophila melanogaster S2 cell line and propose a pipeline for its in vivo application in this model organism.

#48

Sara Ragucci • Caserta

Sodins, ribosome-inactivating proteins from Salsola soda L.

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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 (A4324 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.

#49

Elia Russo • Portici, NA

Host-parasitoid developmental interactions are modulated by venom components of Aphidius ervi (Hymenoptera, Braconidae)

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Aphidius ervi regulates physiology and reproduction of the pea aphid, to enhance its nutritional suitability for the developing offspring. Here we investigated the functional role of the main components of A. ervi venom, Ae-γ-glutamyl transpeptidases (Ae-γ-GTs), using RNA interference. The suppression of Ae-γ-GTs was obtained through microinjection of dsRNA solution in A. ervi female pupae, and their relative expression was checked by qRT-PCR. Parasitism by knocked down females induced a significant increase of aphid size and bacterial load of the primary aphid’s symbiont Buchnera aphidicola. Similarly, both A. ervi larvae and teratocytes showed a significant increase in size. The silencing of Ae-γ-GTs significantly reduced the host castration which resulted similar to that induced by wild type parasitoid females. Parasitoid adults were of larger size, but this trait was associated with a reduced survival and fecundity, suggesting a trade-off of the body size increase. These results shed light on the role of venom in the intricate network of interactions among the parasitoid, the host aphid and its symbiont, which finely orchestrate the development of parasitoid’s offspring.

#50

Flora Salzano • Naples

Eco-friendly extraction of antioxidants from agri-food residues

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Agri-food residues represent a sustainable source of phenolic compounds with antioxidant, anti-inflammatory, and anticancer capacities [1,2]. Bio-based economy aims to take advantage of these raw materials wastes through novel eco-friendly processes of extraction that could bypass harsh chemical treatments [3]. To set up bio-based methods to extract phenolic/antioxidant compounds, different agri-food wastes underwent heat treatment combined with an enzyme-assisted extraction (EAE) with multi-enzymatic complex ViscozymeL and Cellulase. Our results showed increased phenolic content and antioxidant capacity in coffee, sunflower, and citrus agri-food residues. The characterization of novel enzymatic activities (cellulases and hemicellulases) is underway, to produce a new enzymatic cocktail to improve the release of antioxidants from agri-food residues using the EAE method.

1. doi: 10.1007/s13197-014-1295-9
2. doi: 10.1016/j.fbp.2019.03.009
3. doi: 10.1007/978-3-030-39137-9

#51

Emily Schifano • Rome

Development of functionalized masks: improvement of personal protective equipment using nanomaterials

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The microbiological contamination of surfaces in the healthcare sector remains an issue of great concern for public health. Pathogens are able to persist in such environments and spread through healthcare personnel and patients, or simply through contact with contaminated surfaces and equipment. The objective of this work is to achieve the creation of personal protective equipment with antimicrobial activities, through functionalization with appropriate nanostructured materials, with the aim of increasing the degree of protection of healthcare personnel who working in high-risk biological environments. To this aim, samples of surgical masks were coated with a mixture of polymer (POL) and Graphene Nanoplatelets (GNPs) and subsequently contaminated with different types of pathogenic bacteria or fungi. Treated samples showed antibacterial effects compared to untreated ones: indeed, in most of the bacteria tested, a consistent cell mortality can be observed already after two hours of treatment. Concerning the antifungal activity Candida albicans strain, instead, excellent results were obtained already after the first hour of treatment.

#52

Angelica Severino • Naples

Pseudoalteromonas haloplanktis TAC125 as host for the recombinant production of the hCDKL5-1 catalytic domain

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The Antarctic strain Pseudoalteromonas haloplanktis TAC125 is considered a model organism of cold-adapted bacteria because of its peculiar cellular properties1. Hence, it was implemented as an alternative expression system for recombinant production of difficult proteins, some of which are of human origin2. Its unique cellular physiochemical conditions and protein folding processes combined with protein production at low temperatures ensure the mitigation of insoluble aggregates3. These exceptional qualities enabled the production of a soluble and active form of the human protein CDKL5-1, whose mutations are accountable for the CDKL5 Deficiency Disorder. This protein is a S/T kinase consisting of an N-terminal catalytic domain and an intrinsically disordered C-terminal domain4, which is susceptible to degradation complicating the downstream analysis. Thus, to study functional mutations of hCDKL5-1 we focused on the recombinant production of the catalytic domain (ΔC). Here we report the overexpression and purification of the recombinant protein H6-SUMO-CDKL5ΔC by comparing E. coli BL21(DE3) and PhTAC125.

1. Parrilli et al 2021
2. Parrilli, Tutino 2017
3. Dragosits 2011
4. Olson 2019

#53

Alessia Staropoli • Portici, NA

Metabolomics for the selection of beneficial microorganisms and/or their metabolites for new bioformulates

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Metabolomics is an important tool to guide the selection of active substances of new bioformulates based on beneficial microbes and/or their natural products, and to evaluate treatments performance. A metabolomic approach was used to investigate the compatibility of different beneficial microbial strains and to test the selected combinations on different crops. The application of microbial products resulted in significant beneficial effects on treated crops, in terms of pathogen control and improvement of nutritional value. Statistical analysis of plant extracts revealed a modulation of metabolic profiles: it was possible to identify several metabolites, whose relative abundance was increased in treated samples compared untreated control. Moreover, the chelating properties of harzianic acid (a Trichoderma harzianum tetramic acid derivative) were studied with the aim to enhance soil quality. The solutions consisting of harzianic acid and bivalent metal cations (biologically relevant or toxic), highlighted the formation of neutral or charged complexes depending on pH of the solution.

#54

Maria Carmen Valoroso • Portici, NA

Characterization of immune genes and responses to entomopathogens in Aedes albopictus larvae

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Due to climate change and globalization, mosquitoes are expanding in non-endemic regions, causing an increasing number of tropical diseases. The Asian tiger mosquito Aedes albopictus is a vector of several arboviruses, spread worldwide and adapted to domestic and peridomestic environments. In this context, mosquito control efforts are needed to limit mosquito and arbovirus diffusion.

Studies on the larvae of lepidopteran pests revealed that the RNAi-mediated immune suppression increases their susceptibility to microbial bioinsecticides. An in-depth study of immune genes and their responses in mosquito larvae is pivotal to extending this approach to integrated vector control since larvae immunity is still neglected. To identify potential RNAi targets in mosquito larvae, we identified the full-length mRNA of several putative immune genes in Ae. albopictus larvae and characterized their expression after exposure to entomopathogens. The results obtained suggested the involvement of these transcripts in humoral and cellular immune responses in Ae. albopictus larvae and further work will be performed to functionally characterize their precise role.

#55

Tania Vanzolini • Urbino

Development of new biological drugs for the treatment of fungal infections

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Fungal infections are rising great concern, especially for the spread of new pathogenic species intrinsically multidrug-resistant. Given the limited drug arsenal and their low efficiency, innovative approaches are urgently needed. The employment of monoclonal antibodies (mAb) is a novel therapeutic strategy. 2G8 is a murine mAb that selectively recognizes β-1,3 glucans, vital components of the fungal cell wall. It resulted efficient in controlling fungal infections, but its murine nature represents an immunogenicity risk. We derived and characterized in vitro two humanized mAbs, a full-length mAb, Dia-T51, and a scFv, scFv-3T.

Their efficiency was tested on C. auris and C. glabrata (including resistant strains) both alone and in combination with commercially available antifungal drugs. They demonstrated additivity with echinocandins and synergy with amphotericin B moreover, Dia-T51 is effective in inhibiting the fungal growth and adhesion also alone and, since it has a full-length format, it enhances the phagocytosis. These promising results suggest that Dia-T51 and scFv-3T could be new drug candidates for the treatment of fungal infections and particularly, candidiasis.

#56

Gennaro Volpe • Naples

RNAi-mediated silencing of an immune gene in Spodoptera littoralis (Lepidoptera, Noctuidae) alters its embryonic development

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The Sl102 gene encodes a protein involved in the cellular immune response of Spodoptera littoralis. Knockdown of Sl102 gene in S. littoralis larvae increases the susceptibility to pathogens, providing the basis for the development of new insect pest control strategies. Here we explore the phenotypic effects of Sl102 gene silencing on embryonic development by soaking S. littoralis eggs in a dsRNA solution. The experimental results showed that gene silencing is associated with a drastic reduction in egg hatching and a very high mortality rate of the few hatched larvae. Structural and ultrastructural analyses showed morphological alterations in treated embryos indicating that the Sl102 gene, in addition to its immune function, has an important role in the regulation of embryonic development so far unrecognized, which is worth of further research efforts. This study contributes a novel member to the growing list of insect immune genes participating in other biological processes and offers the opportunity to exploit the pleiotropy between development and immunity for the setup of effective pest insect control strategies.

#57

Ehtisham Wahid • Bari

A Matlab-based algorithm for image quantification of yeast cell growth and survival on Agar plates

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Spot assay is a rapid microbiology test routinely used to assess the growth and/or the survival of bacteria or yeast cells in different stress experimental contexts. It essentially provides a qualitative growth measurement, but quantification methods have been developed although with several limitations such as human error, variable threshold values and reproducibility. We have set up a stand-alone MATLAB-based algorithm, which is an improvement of a previous quantitative protocol based on ImageJ. The algorithm detects and quantifies subtle differences in yeast growth by measuring the density of cells within a single spot of defined size in an image. The results obtained from the quantification of each analyzed spots were comparable to previous studies. Parameters such as size of the spot and threshold values of pixels are pre-set for an experiment unlike ImageJ-based analysis. This reduces human errors and enhances reproducibility of results. The algorithm can be also adapted for high throughput colorimetric analysis. This protocol will be useful for biological and biotechnological applications requiring a reliable quantitative assessment of stress sensitivity in yeast

#58

Nicola Zambrano • Naples

Strategies for development of H. simplex-based oncolytic viruses in cancer immunotherapy

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Further than inducing tumor cytolysis and mediating innate mechanisms, oncolytic viruses drive adaptive antitumor immune responses thus, acting as an endovaccine; so, the possibility to manipulate viral genomes by introduction of cargoes for local immunomodulation of the tumor microenvironment adds a growing spectrum of possibilities for cancer immunotherapy.

H. simplex virus HSV-1 provides several advantages for oncovirotherapy, including the possibility to manipulate its genome for selective cancer-redirected tropism and local release of modulators, priming adaptive antitumor responses and abscopal effects. We developed a series of HSV-1 vectors re-directed to selected tumor antigens, such as Mesothelin, and releasing immunostimulatory cytokines or enzymes, such as adenosine deaminase, to clear the tumor microenvironment from immunosuppressive agents. Local inactivation of immune checkpoints was also exploited, via the release of the related scFvs within the tumor microenvironment. Suitable cellular systems were developed, for optimized production of recombinant HSV-1 oncolytic viruses, amenable to preclinical evaluation and clinical development.

#59

Massimo Zollo • Naples

Long-chain polyphosphates impair SARS-CoV-2 infection and replication: prophylactic and therapeutic options against the 6th wave.

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Inorganic polyphosphates (polyPs) are linear polymers composed of repeated phosphate units linked together by multiple high-energy phosphoanhydride bonds. Here, we investigated the antiviral activities of long-chain polyPs against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. In molecular docking analyses, ELISA and limited proteolysis assays, confirm interaction between polyPs with angiotensin-converting enzyme 2 (ACE2) and the viral RNA-dependent RNA polymerase (RdRp) to enhance their proteasomal mediated degradation. PolyP120 impairs replication of SARS-CoV-2 variants, including the newly identified Omicron 2 in primary human nasal epithelial cells. A multiOmic approach (including gene-expression, metabolomics and proteomics) indicated the polyPs impairing the inflammatory and metabolic pathways correlated to the patients severe Covid19 disease. A rapid method of synthesis and delivered via a nebulized formula show an antiviral activity in vivo and in a K18-ACE-2 transgenic murine model. These results open the window for future applications in both prophylactic and therapeutic options against Covid19 pandemia.

#60

Monica Zoppè • Milan

Handling proteins. Tangible models in soft rubber allow composition of complex structures.

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Proteins can be represented in many ways, but they are still hard to conceptualize. It is possible to 3D print them, as proposed in the NIH 3Dprint initiative [1], but hard plastic (PLA) requires printing each object and the print process is rather cumbersome. Therefore we prepared plastic molds, which can be used multiple times to cast protein models with a soft material.

We 3D designed and printed two-part molds and used them to make subunits that can be assembled into complex [2]: F-actin, Hemoglobin, and Histone octamer.

The models are made of white rubber and can be marked by the users to indicate sites of interest such as contact surfaces, binding spots, or electrically active parts.

The models have been presented to high school teachers, who reported their utility in the explanation of biochemical concepts and also strong interest by the students. All models are in the same scale of ten million times [3] and can be compared directly.

Since the fundamental information is the PDB structure, the process can be applied to other molecules. The models will be available at the conference for participants to handle them and experience this new dimension of protein representation.

#61

Michela Zottini • Padua

Grafting as a new strategy for endophyte transferring in grapevine

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Grapevine is a perennial woody plant of global economic importance. In the field, grapevine is colonized by endophytes, which are bacteria or fungi that reside in plant tissues and promote plant growth. Grafting is a common practice for grapevine propagation and trait improvement, so we want to verify whether it can be used to mediate the transfer of endophytes in plants of interest to improve their growth. In this contest, grapevine plants populated by specific endophytes are used as rootstocks in the grafting procedure of grapevine plants lacking that endophytic population. To demonstrate the efficiency of endophyte transfer, GFP-labelled endophytic bacteria are inoculated in endophyte-free plants that are used as rootstock for endophyte-free grapevine plants. At different times after grafting, stems and leaves of the plant to which the bacteria should have been transferred are analysed by confocal microscopy.

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5. Food And Nutrition

#1

Cristina Angeloni • Ravenna

Anti-inflammatory and antioxidant activities of essential oil from Cannabis sativa in activated microglial cells

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Neurodegenerative diseases are characterized by a multifactorial etiology involving common triggering causes like oxidative stress and inflammation. Excessive microglial activation produces a large number of cytokines and ROS causing progressive neuronal loss. Essential oils (EO) obtained from hemp inflorescences displayed different biological activities. This study aimed to investigate the anti-inflammatory and antioxidant properties of an EO obtained from hemp inflorescences in BV-2 microglial cells. Cells were treated with different concentrations of EO for 2 h and then activated with LPS for 24 h. Nitric oxide production was significantly reduced by EO treatment with respect to LPS. The treatment with EO decreased the expression of inflammatory mediators such as IL-1β, IL-6, TNF-a, COX-2, iNOS, and NLRP3 and increased the expression of anti-inflammatory mediators such as IL-4 and Arg1 with respect to LPS. EO showed antioxidant activity increasing antioxidant enzymes such as NQO1, peroxiredoxin, GSH peroxidase and GSH reductase. These results suggest that EO could be used as protective agent in neurodegenerative diseases due to its antioxidant and anti-inflammatory properties.

#2

Attilio Anzano • Portici, NA

Metabolomic analysis and antimicrobial activity of Moringa oleifera seeds, leaves and oil

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Introduction: Moringa (Moringa oleifera) is one of the traditional food crops widespread in Asiatic, African and South American regions. It is very important for the ability to grow in harsh conditions, for the high nutritional value, and for its cardioprotective, anti-inflammatory, antioxidant and antimicrobial properties.1 In this work, we investigated the metabolome of moringa polar and non-polar extract of leaves and seeds, and of moringa oil, using proton Nuclear Magnetic Resonance spectroscopy (1H-NMR) and Gas Chromatography-Mass Spectrometry (GC-MS). We also evaluated their antimicrobial activity against four bacterial strains.
Results: The metabolomic profiling provided a wide set of metabolites that were identified and quantified. Moreover, non-polar extracts from seeds showed an antimicrobial activity against Staphylococcus aureus and Staphylococcus epidermidis, that was associated to the content of specific fatty acids.
Conclusion: Our results remarked the importance of the metabolomic approach for the identification of bioactive molecules by recognizing the molecules responsible for the antimicrobial properties of M. oleifera seeds extract.

#3

Benedetta Bottiglione • Bari

Red and blue light-emitting diode differently affect physiological parameters in lentil seedlings

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In the last decades, light-emitting diode (LED) proved to be a sustainable tool for plant growth and development. This study aims to evaluate the effects of light intensity and spectra composition on physiological parameters in lentil (Lens culinaris Medik.) seedlings grown in hydroponics. Two monochromatic LED lights (red and blue) and three light intensity treatments (100, 300, and 500 µmol m-2 s-1) were used, under 24 h of continuous light and with dark as a control. The effects of LED lighting on 3- and 5-day-old lentil seedlings were investigated by analyzing biometric (fresh and dry matter, root and aerial part length) and biochemical (ascorbate, phenols, pigments, redox status) parameters. Interactions between light quality and intensity were highlighted. In particular, red light promoted elongation growth and blue light enhanced the bioactive compounds of lentil seedlings, although the promotion effects varied with light intensities. Further research is required to deeper understand the interaction between LED lighting and plants and establish the optimal guideline for improving seedlings' quality and productivity, in a sustainable agricultural approach.

#4

Maria Cammareri • Portici, NA

Biotechnological approaches to increase triterpene production in sweet pepper

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Sweet pepper (Capsicum annuum) is an important source of vitamins and several other bioactive metabolites, which mainly include carotenoids, flavonols and phenolic acids but also small amounts of triterpenes. These latter compounds show a variety of pharmacological properties including antiviral, antitumoral, anti-inflammatory, as well as immunoregulatory activity. To pave the way for biotechnological approaches aimed at increasing the triterpene content in pepper, we investigated the expression patterns of the genes coding for squalene, b-amyrin and lupeol synthases in fruit of six sweet pepper cultivars/ecotypes. Specifically, we employed two yield-enhancement strategies: elicitation and metabolic engineering. In vitro elicitation was applied using two concentrations of salicylic acid and two elicitation times. The results will be discussed.Moreover, in order to test a metabolic engineering strategy, the CaLUP gene, which encodes for lupeol synthase, was cloned into the binary vector PG0029 under the control of the CaMV35S promoter and cotyledonary explants of cv. Quadrato were transformed with the A. tumefaciens strain GV3101. The analysis of regenerants is in progress.

#5

Antonio Caporale • Portici, NA

Fostering food safety and quality in Neapolitan contexts of sustainable urban agriculture

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Urban farming can be threatened by several issues, such as soil degradation/contamination, loss of ecosystem services and food safety. UrbanSoilGreening project (financed by University of Naples Federico II, Programma per il Finanziamento della Ricerca di Ateneo 2020 - Linea A) aims to assess soil fertility and crop safety/quality in many urban vegetable gardens of Naples, to foster a sustainable use of soil and resources and enhance food safety and quality.
Soil characterisation evidenced sandy-loam texture, neutral-to-sub-alkaline pHs and overall good fertility and nutrient bioavailability. As well, a low-to-moderate occurrence/bioavailability of potentially toxic elements and organic compounds were found. After soil characterisation, crop species were planted and managed by site-specific sustainable cultivation practices, such as organic farming and synergistic techniques, with an active involvement of local citizens and associations.
Ongoing chemical and microbiological analyses are evaluating food quality (content of minerals and bioactive compounds such as polyphenols, carotenoids and vitamin E) and safety (possible presence of contaminants and pathogenic microorganisms).

#6

Stefany Castaldi • Naples

Halotolerant PGP Bacillus amyloliquefaciens enhance the salinity stress tolerance of Lotus japonicus cv gifu.

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Over the past decades, an increase in abiotic stresses has negatively impacted agricultural lands. Among them, salinity stress is a major environmental challenge threatening agriculture and the economy worldwide. Furthermore, current remediation methods by physical and chemical processes are time-consuming and very expensive, causing an ecological imbalance in nature. In this contest, Plant Growth-Promoting rhizobacteria are receiving increasing attention since it represents a cheaper and eco-friendly approach. These beneficial microorganisms may promote plant growth and enhance plant stress tolerance through several specialized mechanisms when applied directly to the degraded soil. In this work, we have evaluated the plant-growth-promoting properties of seven halotolerant Bacillus strains by studying the whole genome and with biological experiments. The results showed that only one strain, RHF6 retained the PGP performance up to 400mM NaCl. Furthermore, the inoculation of the RHF6 strain in the legume plant Lotus japonicus under saline conditions confirmed the ability to mitigate abiotic stress damage in plants, and to secret the proline involved in osmolarity cellular plants.

#7

Benedetta Cerasuolo • Sesto Fiorentino

Dietary shift from traditional rural Tanzanian to globalized diet significantly affects the gut mycobiome.

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The Western world has long since undergone a demographic transition from rural to urban context with a consequent change in lifestyle and eating habits. This transition significantly affects the composition of the gut microbiome and its impact on human health. This event led to a change in disease exposure, with particular emphasis on non-communicable inflammatory and autoimmune diseases, such as inflammatory bowel diseases. Nowadays African populations are gradually changing their dietary habits replacing foods rich in fiber, typical of local rural areas, with those rich in simple sugars and fats, commonly consumed in the Western diet. In order to evaluate the impact of the dietary transition on the gut mycobiota, the stool from healthy male individuals between 20-40 years residing in a rural and urban area have been sampled before and after 2 weeks in which they have switched their eating habits. For the analysis of the gut mycobiome the ITS1 region was amplified and sequenced with an Illumina MiSeq platform yielding to significant changes in the fungal species pattern and abundance.

#8

Sofia Chioccioli • Florence

Faecal microbiome as determinant of the effect of diet on colorectal-cancer risk: comparison of red meat based versus pesco-vegetarian diets in rodent models

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Colorectal cancer (CRC) is strongly affected by diet. To understand the contribution of gut microbiome in red/processed meat-mediated CRC risk, Pirc rats, a genetic model of CRC developing spontaneous tumors due to an Apc mutation, were fed a red meat-based, red meat-based supplemented with tocopherol or pesco-vegetarian diets respectively associated with high, medium or low CRC risk. We found a diverse microbial community among the three diets where Roseburia, showing the strongest inverse relation to colon tumors, was the most abundant in pesco-vegetarian diet. In addition, faecal microbiota transplant from Pirc rats fed the three different diets into germ–free rats in which colon carcinogenesis was induced with Azoxymethane, demonstrated that microbiome with specific metabolomics profile were able to transmit cancer risk: WT rats transplanted with the meat-based diet faeces, had a significantly higher number of preneoplastic lesions related with specific microbiome profile and relative metabolites. Our results thus demonstrate how the diet is able to modulate the gut microbiota composition and how the microbiome can influence the colorectal tumors development.

#9

Maurizio Chiurazzi • Naples

LjNRT2.3 plays a hierarchical role on the control of root nitrate uptake in L. japonicus

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Nitrate is often a limiting resource for supporting plant growth. The high-affinity Nitrate Transporters (NRT2) represent a small family of proton-coupled transporters. We present here the preliminary functional characterization of the L. japonicus gene LjNRT2.3. The transcriptional regulatory profile indicates a strong induction of LjNRT2.3 in roots of plants grown in N-starvation conditions, which is quickly inhibited by addition of KNO3. The spatial profile of expression in transgenic hairy roots, confined to epidermal cell layer and root hairs suggests a role on nitrate uptake. In particular, these patterns indicate a crucial role for predisposition of Lotus roots to nitrate acquisition under N starvation conditions. This is confirmed by the phenotypic characterization of two independent Ljnrt2.3 knock out mutants. The Ljnrt2.3 mutants display a reduction of nitrate content after transfer from N starvation to low nitrate concentration conditions. Nitrate-mediated signalling pathways are also involved in the control of root development as well as formation of N2-fixing nodules in legume plants and we are now analyzing the possible role played by LjNRT2.3 in these processes.

#10

Alessandra Cona • Rome

Plant-based biorepellents against large white butterfly caterpillars in Brassica oleracea

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Biological pesticides and repellents represent an eco-friendly, highly biodegradable and reduced-risk alternative to synthetic products suitable to promote a sustainable agriculture. Plant-based products derived from waste/loss of agri-food supply chain represent zero-cost resources harmless for human and environment health in a circular economy based-model for a sustainable development. The main focus of this project is to develop new natural repellents from agri-food waste for the sustainable control of the phytophagous Pieris brassicae in the Brassica oleracea horticultural crop. Natural extract effects on plant agronomic and physiological traits were also evaluated in target and model plants (Brassica oleracea and Arabidopsis thaliana). In particular, the deterrence feeding activities of extracts from waste of agro-food supply chain as sources of alkaloids were tested by no-choice feeding test. Tested natural extracts showed a strong anti-feeding activity within 48 h from the onset of treatments, without showing any evident toxicity on caterpillars and treated target and model plants.

#11

Paola Antonia Corsetto • Milan

Selective impact of polyunsaturated fatty acids on lipid composition and metabolism in breast cancer cells

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Fatty acids are closely involved in lipid synthesis and metabolism in tumor, especially in breast cancer. Their cell amount and composition are dependent on dietary supply and tumor microenvironment. Polyunsaturated fatty acids (PUFA), in particular docosahexaenoic acid (DHA), play beneficial roles in breast cancer prevention and therapy. The present study investigated the impact of DHA incorporation in triple-negative breast cancer (TNBC) and Luminal A human breast adenocarcinoma cells on lipid metabolism, lipid peroxidation and energy pathways.

After DHA exposure, the TNBC cells showed a selective sensitivity compared to the Luminal A cells correlating with a distinct lipid phenotype. This specific response is strictly closed to the peculiar lipid profile and metabolism that characterizes each cell subset. Moreover, DHA treatment determines a significant alteration of the MUFA and PUFA contents of the endoplasmic reticulum in TNBC cells, with consequent changes in resident enzyme activity.

This study sustains the crucial role of lipid metabolism as an innovative hallmark to discriminate breast cancer subclasses and to develop personalized nutritional and pharmacological strategies.

#12

Patrizia D'Aquila • Rende, CS

Effects on bacterial growth and Quorum Sensing of Essential Oils from aromatic plants

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A fundamental role has been identified for essential oils (EOs) extracted from aromatic plants for their nutritional, antimicrobial, and antioxidant properties, and as food preservatives.
In the present study, EOs from plants used routinely to impart aroma and taste to food, were evaluated for their antibacterial activity against Chromobacterium violaceum by measuring the minimum inhibitory concentration. We found that EOs exert different activity, with Origanum vulgare and Foeniculum vulgare showing the greatest inhibitory effect on bacterial growth.
The effects of these oils to interfere in the Quorum Sensing (QS) were also studied. Sub-lethal concentrations of EOs were found to reduce the amount of violacein, a violet pigment produced by C. violaceum as a result of QS activity.
The results obtained in this study contribute to a further characterization of the antimicrobial properties of EOs which seem to be mediated by the interference in the QS system and open new scenarios in the evaluation of the role of EOs in different fields, spanning from the microbial to the nutritional and clinical, which can lead to innovative food preparations.

#13

Ida De Chiara • Caserta

In vitro evaluation of probiotic properties of newly LAB strains isolated from natural whey cultures

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The growing market of probiotic food products stimulates scientific research towards the continuous search for new probiotic strains. We investigate the probiotic properties of strains previously obtained from natural whey cultures. In this contest, screening for acid and bile salt tolerance, antibiotic resistance, hydrophobicity and autoaggregation ability, production of antimicrobial and neuroactive substances, was performed on new isolates. All the strains maintained high survival rate in presence of 0,3% pepsin at pH 3.0 and in presence of 0.3% bile salts at pH 8.0. The same isolates did not show any antibiotic resistance towards ampicillin, tetracycline, penicillin, and vancomycin, but some of them showed resistance to gentamycin. In hydrophobicity and autoaggregation assays strain specific differences were observed; hydrophobicity values varied from 2 to 79%, whereas autoaggregation ability ranged from 36% to 52%. Isolated strains were also screened for the presence of gadB gene (GABA production) and for production of antimicrobial substances. Five best performing strains will be further investigated in vivo for their potential applications in the food industry.

#14

Marta Del Bianco • Rome

Microgreen production for space applications and earth benefit

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The feasibility of human exploration of Space and the realization of long-term settlements on the Moon and Mars will rely on our ability to produce fresh food away from Earth. Before we reach full self-sufficiency, food production will be aimed at integrating the astronaut’s diet with nutraceutical compounds that could help protect humans from the effects of the space environment.
Microgreens are a new class of leafy vegetables appreciated for their flavour and, most essentially, for their nutraceutical value. Microgreens are small and rich in desirable compounds, have low energy and growth needs, and a fast production cycle. For these reasons, the Italian Space Agency is fostering multiple projects for the study of possible space applications of microgreen production.
Advances in food production technologies for Space exploration could have an important impact back on Earth. Both in Space and on Earth, there is a need to find new sustainable ways to use the limited available resources and guarantee food security, and microgreens cultivation could help to give answers to those needs.

#15

Mattia Di Nunzio • Milan

Modulation of proteolytic activity by dietary polyphenols is dependent on both substrate and enzymes.

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Phenols/polyphenols (PPs) have a role in the regulation of metabolism and a beneficial impact on some chronic diseases. However, PPs are often referred to as anti-nutritional factors, being reportedly able to inhibit proteases. Reports on this topic are often conflicting - mostly because of different experimental conditions - stressing the need to evaluate the effect of PPs through standardized approaches. Here, we evaluated the effect of several PPs (at physiological concentrations) on pepsin and trypsin activity using albumin, lysozyme, gluten, and hemoglobin as substrates. Results show that PPs may affect proteolytic activity in even opposite ways, depending on both the protein substrate and the enzyme. Although further analysis is required to define the intervening interactions in the case of specific substrate/enzyme/PPs combinations, these results underscore the possibility of considering PPs as “digestion promoting agents” in the formulation of functional foods.

#16

Francesca Ferranti • Rome

The SOLE project: a plant greenhouse demonstrator for fresh food production in space

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Future human long-term space exploration demands for fresh food production during missions, independently on periodic supplies from Earth.

The possibility to grow plants in space positively impacts both astronaut diet and psychological wellbeing. It has been estimated that a facility of a few cubic meters is sufficient to supply the diet of 4 to 6 crew members with key vitamins and fresh bioactive substances. Soilless cultivation is the best suited for production of high quality food in space, where resources are limiting factors.

In this context, the project SOLE aimed to realize a hydroponic greenhouse based on LED lighting systems for growing plants, specifically microgreens, for space applications. The best performing light recipe, in terms of duration, intensity and spectral quality, was identified, based on plant response analyzed by nondestructive morphometric and hyperspectral systems. Data on plant growth and bioactive metabolites accumulation were monitored, in real time and even remotely, and elaborated.

The dimension of the SOLE demonstrator can be easily adapted for space applications as growing plant onboard the International Space Station or in mini- /micro-satellites.

#17

Venera Ferrito • Catania

A molecular strategy for species recognition: the Sparidae family as a case report

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Recent investigations have shown that the illegal practice of species substitution (sps) and mislabeling is the most common fraud detected in the fish trade worldwide. In this study, the COIBar–RFLP strategy (Cytochrome Oxidase I Barcode–Restriction Fragment Length Polymorphism) is applied to unveil cases of sps in the Sparidae family purchased in Sicilian fish markets. 11 species were morphologically and molecularly identified and used as reference samples (rs) after sequencing and BLAST validation, while 15 samples sold whole or as fillets were molecularly identified by DNA barcoding. For restriction digestion, the in silico analysis selected the MspI enzyme as the most suitable for sparid species discrimination. The digestion pattern obtained, allowed to discriminate all detected species unveiling 3 cases of sps: Pagellus erythrynus in place of Dentex dentex; Pagrus pagrus for P. erythrinus; Spondyliosoma cantharus for Diplodus sargus. These outcomes indicate the necessity to define molecular strategies for fighting fraud along the seafood supply chain and suggest COIBar–RFLP as a molecular tool to assess seafood authenticity.

#18

Giuditta Heinzl • Milan

Investigating the mechanisms of action of conglutin-γ in regulating D-glucose uptake: the role of intestinal barrier

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Conglutin-γ (Cg) is the major glycoprotein from the edible seed L. albus, long studied for its postprandial glycaemic regulating action. Several cell model systems (liver, pancreatic, myoblast, musculoskeletal) have been used to investigate in vitro mechanisms of action, all attributable to insulin-mimetic actions. However, it still lacks clear information on what could happen at the meeting point between the protein and the organism: the intestinal barrier.

We compared an in vitro system involving Caco2 and IPEC-J2 cells with an ex vivo system using pig jejunal segments to study Cg transport from the apical to the basolateral compartment, its effects on the D-glucose uptake and glucose transporters protein expression. Finally, we studied its potential in modulating glucose metabolism by assessing the possible inhibition of α-amylase and α-glucosidase. RP-HPLC analyses showed that Cg could be found in the basolateral side in the in vitro system but not in the pig intestines, where it causes tissue damage with albumin release. Cg dispensing was able to promote a decrease in glucose uptake in both cells and jejunum independently from the expression of the SGLT1 and GLUT2 transporters.

#19

Silvana Hrelia • Rimini

The impact of Lactiplantibacillus Plantarum fermentation on the antioxidant and immunomodulatory properties of Vaccinium Floribundum berries

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Fermentation with lactic acid bacteria influences the bioavailability of bioactive molecules, improving the activity of natural compounds. We evaluated the effects of Lactiplantibacillus Plantarum fermentation on antioxidant and immunomodulatory properties of Vaccinium floribundum (VF) berries in human umbilical vein endothelial cells (HUVECs) and macrophages (RAW264.7). The polyphenol profile determined by HPLC-MS/MS analysis showed an increase in quercetin aglycone levels after fermentation. Spectrophotometric (SP) assays ensured the safety of berries solutions on HUVECs and RAW264.7 viability, with a reduction in H2O2-induced cell death in HUVECs pre-treated for 24h with fermented berries solution (10 µg/mL). The effects of berries fermentation, measured by a CL bioassay, RT-qPCR, and SP techniques, showed: i) an improvement in antioxidant capacity in HUVECs, as indicated by a decrease in intracellular H2O2 production and in H2O2-induced HO-1 gene expression and an increase in Glutathione Reductase activity; ii) modulation of immunity, as indicated by increased proliferation and levels of iNOS in RAW 264. Our data show that LP fermentation enhances VF berries properties.

#20

Nicola Landi • Caserta

Nutritional values and amino acid content of 'Curniciello' beans grown in 'Piana di Monte Verna' district, Italy

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'Curniciello' beans are known as a typical legume of Southern Italy and are consumed as a traditional food. The seed is small in size, with a brown colour reminiscent of a monk's habit and a small white eye. The objective of the present investigation was to determine the nutritional values and amino acid profile of 'Curniciello' seeds (Phaseolus vulgaris L.) grown in 'Piana di Monte Verna' (Caserta) because there are no nutritional data available on them.

The seeds contain high raw proteins levels (23.40 g/100 g) and are rich in essential amino acids (7.1 g/100 g). Moreover, different unsaturated fatty acids contribute to the quality of lipids content (1.87 g/100 g). Indeed, the essential PUFA α-linolenic (1.17 g/100 g) and linoleic (0.49 g/100 g) acids are among the most abundant. In addition, trypsin and chymotrypsin inhibitors considered as antinutritional factors decrease after boiling procedure.

Our data confirm that 'Curniciello' beans are a functional food with beneficial qualities and can be used to promote their consumption, since the consumers' interest in choosing a healthy diet, such as the Mediterranean diet.

#21

Manuela Leri • Florence

Olive polyphenols act as biomimetic molecules in hormone replacement therapy for Alzheimer's disease prevention in menopausal women

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Epidemiological data indicate that the higher prevalence of Alzheimer's disease (AD) in women than in men is in part linked to estrogen decrease during menopause. Nowadays, the traditional hormone therapy has controversial activity in prevention of AD onset. In this contest, natural polyphenols (phytoestrogens) have considerable interest considering their beneficial effects on health, in particular, polyphenols of extra virgin olive oil (EVOO). EVOO is enriched in oleuropein aglycone (OleA) and its main metabolite, hydroxytyrosol (HT). Their ability to counteract the amyloid aggregation path and toxic effects of different proteins involved in amyloidosis have been widely demonstrated. Here, the Estrogen Receptor beta (ERβ) activation and localization, intracellular pathways regulation, including the IGF1-, AMPA- and NMDA receptors involvement have been investigated. Moreover, the cellular metabolic alteration induced by polyphenols treatment has been explored through ATR-FT-IR analysis. The results of these studies add in-depth knowledge on the effects of olive polyphenols on the AD-menopause relation and on the impact of the metabolic effects induced by diet in AD prevention

#22

Nadia Lombardi • Portici, NA

Biological treatments of grapevines improve the phenolic composition and sensory properties of Aglianico wine

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Beneficial microbes and their bioactive metabolites (BAMs) represent promising alternatives to chemicals for crop protection in sustainable agricultural production. Fungi of the genus Trichoderma, as well as their BAMs, are known to produce beneficial effects in vineyards in terms of pathogens control and yield improvement of harvested table and wine grapes. In this study, treatments of vine plants (Vitis vinifera cv. Aglianico) with Trichoderma afroharzianum strain T22 and the fungal metabolite 6-pentyl-α-pyrone were carried out, and their effects on chemical composition and sensory properties of grapes and the resulting wines were evaluated. Both treatments increased anthocyanin content and color of grapes and wine compared to control. Interestingly, grape skins showed a lower content of low molecular weight flavanols, thus contributing in reducing bitterness in red wines such as Aglianico. Sensory analyses of the corresponding wines revealed a more complex odor profile, with higher intensity of floral, tobacco and black pepper odors. These are due to volatile terpenoids, whose biosynthesis could have been stimulated in vine by the biological treatments.

#23

Giulia Magni • Milan

Purple corn anthocyanins as a nutraceutical approach to control the progression of multiple sclerosis and associated trigeminal pain: role of the gut-brain axis

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The aim of this work was to investigate whether an anthocyanin (ACN)-enriched purple corn dietary supplement can exert beneficial effects on trigeminal (TG) pain associated to multiple sclerosis (MS) and on the onset and progression of the disease.

Experimental autoimmune encephalomyelitis (EAE) was induced in Dark Agouti rats as described [Magni et al., Brain Behav Immun 89:268-280, 2020]. Eleven days before EAE induction rats were assigned to drink water, yellow corn (containing all flavonoids except for ACNs) as control, or purple corn extracts. From day post-immunization 1 to 21 rats were weighed daily, the development of EAE was evaluated by a scale of ascending paralysis and spontaneous TG pain was measured by von Frey test. Fecal samples were collected at significant time points for the analysis of microbiome composition and ACN metabolites. Results show that, thanks to gut ACN metabolism, purple corn positively influences the progression of EAE motor symptoms and protects from associated TG pain through multiple mechanisms. Our findings suggest a possible application of purple corn as adjuvant approach to MS and associated symptoms to reduce drug dosage and side effects.

#24

Roberta Marra • Portici, NA

Sustainable approaches to improve productive and qualitative traits of industrial tomato plants using biostimulants and mulch biofilms

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In agriculture the use of living beneficial microbes or natural products represents a sustainable alternative to chemical pesticides and fertilizers for the improvement of crop production or the control of phytopathogens. In this work the effects of two commercial products based on the fungal antagonist Trichoderma sp. and a seaweed extract from Ascophyllum nodosum were tested in combination with the use of biodegradable plastic mulch films on industrial tomato plants. Up to 30% yield increase was observed in treated plants compared to controls. Single and combined applications of biostumlants affected also qualitative parameters, such as fruit texture and colours, and concentration of total polyphenols, carotenoids and lycopene. Metabolomic analysis revealed that plant metabolic profiles varied according to the presence of biofilm as well as to the application of biostimulants. An increase in the concentration of some alkaloids and flavonoids in tomato berries and leaves was observed following the application of the Trichoderma-based product. These results confirm the efficacy of combining the application of mulch biofilms with biostimulants for industrial tomato productions.

#25

Francesca Messina • Pavia

Sustainable seed priming treatments to improve germination in lettuce

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High quality seeds are fundamental to ensure successful production for sustainable agriculture especially considering the effects of climate change. Seed Priming (SP) is an efficient option to boost crop performance in fragile ecosystems. Its benefits have been widely reported and include faster and synchronous germination, seedling uniformity, and optimised harvesting even under a wide range of temperatures. However, the success of SP protocols is strongly related to the genotype, seed lot, seed vigour, year of harvest and environmental conditions. Fast and uniform germination is important to produce fresh-cut lettuce that are harvested when the seedlings reach the four- to eight-leaf stage of growth. With this project, we plan to develop sustainable SPs in lettuce (Lactuca sativa L.) cultivars, whose seeds display thermodormancy under high temperatures. Lettuce seed could enter a state of dormancy and consequently fail to germinate at temperatures above 25°C. Several interventions have been investigated to circumvent thermodormancy but seed priming is probably one of the most effective ways to overcome thermodormancy.

#26

Laura Mosca • Naples

“Greco” grape canes as a valuable source of bioactive compounds with antioxidant properties and anticancer activity on head and neck squamous cell carcinoma

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Grape canes represent a poorly valorized waste despite being produced in large amounts. In the present work, we characterized the antioxidant capacity of grape cane extracts of “Greco di Tufo” a typical cultivar of the Campania region. We demonstrated, for the first time, the anticancer potential of the polyphenol component of the extract in head and neck squamous cell carcinoma (HNSCC) and we investigated the underlying mechanism. Aqueous extracts were prepared at different pH and extraction times and the total phenolic and reducing sugar content, as well as the antioxidant power, were estimated. We provided evidence that a polyphenol-enriched fraction prepared from the extract obtained at pH 7 and 60 min exerted antitumor effects toward oral Cal-33 and laryngeal JHU-SCC-011 HNSCC cell lines by inducing cell-cycle arrest via cyclin downregulation and p21 upregulation and by triggering apoptosis through caspase cascade activation, PARP-1 cleavage, and increase of Bax/Bcl-2 ratio. Altogether, these findings highlighted “Greco” grape canes as a valuable source of bioactive polyphenols that may represent good candidates for the design of innovative adjuvant therapies in HNSCC.

#27

Cristina Nali • Pisa

Physiological changes induced by a pulse of ozone improve grapevine defence against Botrytis cinerea

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Grapevine (Vitis vinifera L.) is a major fruit crop worldwide, but it is susceptible to many pathogens, such as the fungus Botrytis cinerea (Bc). Although ozone (O3) is known as a phytotoxic gas pollutant, exposure to an adequate dose of O3 can represent an eco-friendly tool for plant protection against pathogens. This study investigated whether a pulse of O3 (100 ppb for 3 h) influences the responses of V. vinifera to Bc. Ozone per se increased ethylene (Et) at 6 and 12 h from the end of treatment (FET; +68% than controls kept under filtered air and not inoculated), whereas it was decreased at 24 and 48 h FET (-64%). Only a raise of salicylic acid (SA) was observed at 48 h FET, so O3 did not mimic a systemic acquired resistance. Bc infection per se (24 h from inoculation) stimulated the production of Et (+35%), indicating that induced systemic resistance occurred, and the concomitant accumulation of hydrogen peroxide confirms that Et is a self-amplifying system. In plants experiencing O3 exposure and subsequently Bc infection (i.e. O3 + Bc treatment), a reduction of SA occurred at 12 h FET (-67%), and this is explainable as an O3-induced improved defense against Bcrather than a signalling regulation, considering the overexpression of the pathogenesis-related genes PR1 and PR6.

#28

Veronica Panichi • Bologna

Oleuropein and hydroxytyrosol in cartilage protection: molecular mechanisms

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The impact of age-related diseases is increasing due to lifespan elongation. Osteoarthritis (OA), the first cause of disability in the elderly, is characterized by low-grade inflammation and ECM remodeling leading to articular cartilage degeneration and joint functional failure. Despite the latest research advances, the mechanisms of pathogenesis are still poorly understood and no resolutive therapy is available. Dietary nutraceuticals with known anti-inflammatory properties could be an innovative therapeutic approach. In our study, we investigated the effects of hydroxytyrosol (HT) and oleuropein (OE), two olive-derived polyphenols in in vitro cellular models of OA. Pre-treatment with either HT or OE exerted significant effects in preventing LPS-induced inflammation and decreasing oxidative stress. Gene expression studies also indicated that either HT or OE reduced the activation of signaling pathways linked with the onset and progression of OA (NOTCH-1, NF-κB). Taken together, our data show that these compounds exert anti-inflammatory and chondroprotective effects and could represent a promising therapeutic "support" strategy in OA management.

#29

Stefania Peddio • Monserrato

Protein alpha amylase inhibitors from Sardinian common beans cultivars: biological activity and phylogenetic analysis

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Obesity and diabetes are increasing health concerns. In this perspective, Phaseolus vulgaris α-amylase inhibitor (α-AI) gained commercial interest. This protein inhibits mammalian α-amylase activity, reducing carbohydrates absorption [1].
Here, 10 Italian P. vulgaris cultivars (mainly from Sardinian Agricultural Agency) were screened for α-amylase and α-glucosidase inhibiting activity and antinutritional compounds.
Besides, the partial nucleotide sequence of α-AI gene was identified with the degenerate hybrid oligonucleotide primer (CODEHOP) strategy.
Only 2 cultivars had no α-AI activity, whereas the 3 highest values were present in cv Fazadu Nieddu Pattada, Nieddone Ploaghe, and Bianco Flumini (respectively 70.2±3.7, 71.2±5.1, 75.2±2.3 IAU/mL).
The CODEHOP strategy identified a fragment of the gene of interest of 235 nucleotides. Preliminary results showed genetic variability, possibly linked to the functional α-AI differences.
This study can be the basis for increase the knowledge about this class of proteins and developing commercial preparations from Italian common beans cultivars.

[1] Peddio S. et al. Phytotherapy Research (2022) doi:10.1002/ptr.7480

#30

Pasquale Perrone • Naples

Olive oil phenols prevent mercury and calcium induced phosphatidylserine exposure and morphological changes in human erythrocytes

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Mercury (Hg) is a metal not essential for nutrition, but diet represents the most important routes of chronic exposure to Hg in humans. Despite, some foods contain bioactive compounds able to reduce its harmful effects. The data indicate that structurally related olive oil phenols prevent alterations induced in intact human erythrocytes (RBC) exposed to HgCl2 (5–40 µM) or Ca2+ ionophore (5 µM), measured as phosphatidylserine exposure, ROS generation, GSH depletion and microvesicles formation. The protective effect is observed in a range of 1–30 µM, hydroxytyrosol being the most effective; its metabolite homovanillic alcohol still retains the activity of its dietary precursor. Significant protection is also exerted by tyrosol, in spite of its weak scavenging activity, indicating that additional mechanisms are involved. When RBC alterations are mediated by an increase in intracellular calcium, protection is observed at higher concentrations, indicating that these phenols mainly act on Ca2+-independent mechanisms, identified as protection of GSH depletion. Our findings strengthen the nutritional relevance of olive oil bioactive compounds in its claimed health-promoting effects.

#31

Katia Petroni • Milan

Anthocyanins confer cardioprotection against Doxorubicin by modulating the AMPK-SIRT1-p53 pathway

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Doxorubicin (Doxo) is one of the most effective chemotherapeutic drugs, but its clinical use is severely limited by its side effects, leading to cardiomyopathy and heart failure. An anthocyanin (ACN)-rich diet from purple corn (RED diet), which mainly contains cyanidin 3-glucoside (C3G) and its acetylated derivatives, was proved to be effective in reducing Doxo-induced cardiotoxicity in mice. Aiming at unveiling the molecular mechanisms involved in ACN protection, we decided to consider the AMPK-SIRT1-p53 pathway that recently gained interest for its cardioprotective role. HL-1 murine cardiomyocytes were treated with Doxo in presence or absence of purple corn extract (RED) and activation level of AMPK, SIRT1, p53 and its apoptosis-related target genes were evaluated. Our results showed that RED decreased Doxo-induced AMPK activation and increased p53 acetylation, which resulted in decreased levels of cleaved-Caspase 3, Puma and p21 transcripts and apoptosis. The RED-induced p53 acetylation and its cardioprotective role is currently under validation in mouse primary cardiomyocytes. In conclusion, RED may prevent cardiomyocytes apoptosis through AMPK and SIRT1 modulation.

#32

Valentina Ramundi • Rome

Wild edible plants chemical profile and their antimicrobial properties

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This study aims to explore wild edible plants (WEPs) as a potential source of novel antibacterial agents targeting Methicillin Resistant Staphylococcus aureus (MRSA). An ethnobotanical study on WEPs of the Mediterranean area was carried out and, some plant species not completely investigated were selected. Antimicrobial and antioxidant activity of those WEPs was tested and the main phenolic compounds in the phytoextracts showing the highest antimicrobial activity were determined, notably species belonging to Glechoma, Silene and Sonchus genera. All the phytoextracts have been tested for biofilm production, starting with S. aureus wild type strain ATCC 25923. Our results showed a consistent reduction of the mature biofilm production in S. aureus. Spectrophotometric analysis with Folin-Ciocalteu reagent was performed to assess the content of phenolic compounds. Antioxidant activity was evaluated by ABTS, FRAP and DPPH assays. A method coupling ultra-performance liquid chromatography with photodiode-array detector and electrospray ionization mass spectrometry with an ion trap analyzer was developed for the identification and quantitation of the main phenolic compounds in the extracts

#33

Samuele Risoli • Pisa

Biocontrol of Fusarium Head Blight in wheat (Triticum spp.): A meta-analysis

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Fusarium Head Blight (FHB) is a major fungal disease affecting wheat (Triticum spp.) worldwide, being responsible for huge yield losses and mycotoxin contamination of kernels. The use of biocontrol agents (BCAs) coping with plant pathogens by direct and/or indirect mechanisms of action is an appealing eco-friendly strategy for FHB management, but a better understanding of their functioning and effectiveness was necessary. This meta-analysis, including 41 pathometric, biometric, physiochemical, genetic and mycotoxin parameters reported in 51 studies published worldwide in the last 20 years, shows overwhelming evidence of the overall BCA effects on FHB infection in wheat, and highlights the differences between the use of BCAs and agrochemicals in FHB control. In brief, this study confirms the beneficial effects of BCAs in terms of FHB control, wheat production and mycotoxin contamination. On the other hand, it highlights that agrochemicals - despite the collective awareness of their environmental impacts, as well as of the future further restrictions on their use - are still more effective than the BCAs at the moment available in reducing FHB pressure and mycotoxin contamination.

#34

Giuseppe Sabbioni • Ferrara

Properties of Arabidopsis thaliana P5C synthetase 1 enable stress-induced proline accumulation

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Water stress tolerance is a major goal for plant genetic improvement, and a need to secure crop productivity threatened by ongoing climate changes. Under drought or excess salt, most plants accumulate proline inside the cell, which is believed to help counteracting the adverse effects of low water potential. This increase mainly relies upon transcriptional induction of d1-pyrroline-5-carboxylate synthetase (P5CS), the enzyme that catalyzes the first two steps in proline biosynthesis from glutamate. In several plant species, two P5CS isogenes have been identified that showed non-overlapping roles. In Arabidopsis thaliana, AtP5CS1 appears as the major contributor to stress-induced proline accumulation, whereas AtP5CS2 plays a pivotal role in embryo development and growth. Contrary to the large amount of information available on the regulation of P5CS gene expression, very little is known about the biochemical features of P5CS isoenzymes, and the occurrence of post-translational regulatory mechanisms. Here we report a preliminary characterization of AtP5CS1, showing some peculiar properties that seem functional to proline accumulation under hyperosmotic stress.

#35

Angelo Santino • Lecce

Biofortification strategies for the improvement of the nutritional value of tomato

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Tomato is one of the most widely grown and consumed horticultural crop in the world and an ideal candidate for the improvement of its nutritional value, since it naturally accumulates only low levels of polyphenols, mainly in the fruit skin. To enhance the nutritional value of tomato, metabolic engineering has been shown to be as an effective strategy to increase the content of polyphenols or even to induce the synthesis of novel compounds in fruit. An example of such approach is represented by the “Bronze” tomato line, which is enriched in several classes of polyphenols (flavonoids, anthocyanins, stilbenes) and which has been used in experimental diets to unravel the possible beneficial effects in mice models of gut chronic inflammation (inflammatory bowel disease, IBD).
Another example of nutritional improvement is represented by a new tomato line bio-fortified for Vitamin D content, recently obtained by CRISPR/Cas9 genome editing. These evidences suggest that the application of consolidated or new plant biotechnologies can be considered useful tools to speed up the process to obtain novel plant varieties with an improved nutritional value.

#36

Daniel Savatin • Viterbo

Re-Waste: a green, sustainable and circular strategy for crop resilience

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In the EU, an estimated 20% of the total food produced is lost or wasted. Food waste is rich in highly valuable biomolecules, such as oligosaccharins, able to cause changes in crop important physiological processes. The purpose of the current study is to exploit waste by-product to develop a sustainable strategy for crop plant growth and resilience to (a)biotic stresses. We, thus, conducted agronomic trials on two model species for agriculture (wheat and tomato) by using agro-food waste, that was compacted and dehydrated, and the solid residue (SR) obtained was directly added to growth substrate. Different morpho-physiological parameters were positively influenced by the addition of low doses of both SRs hinting that they may be effective in regulating plant physiology. These SRs represent a potential reservoir of bioactive products, including oligosaccharins, such as chitooligosaccharides and oligogalacturonides, both potential alternative to traditional agrochemicals. We propose here a novel, safe and sustainable strategy based on a model that promotes circular economy by recycling wet-organic waste, replacing chemical fertilizers, reducing pollution and high management costs.

#37

Marta Toccaceli • Milan

Anti-inflammatory effect of upland potato varieties in THP-1 macrophages

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Chronic inflammation represents a risk factor of various diseases. Since anthocyanins (ACNs) were found to counteract many inflammation-related diseases, including obesity-related inflammation, and consumption of carotenoids has been inversely correlated to the incidence of many chronic diseases, we evaluated the possible anti-inflammatory properties of pigmented upland potato varieties enriched in different combinations of phytonutrients as possible source of beneficial bioactives. Three commercial upland potato varieties namely Bleuet (purple skin and flesh), Desiree (red skin and yellow flesh) and Kennebec (yellow skin and white flesh) were checked as ACN-rich, carotenoid-rich and chlorogenic acid-rich respectively. THP-1 macrophages were treated with extracts from the three cultivars and challenged with LPS. The dose-dependent effects on gene expression and protein levels of TNF-α, IL-1β and IL-6 were evaluated. While Desiree and Bleuet acted as anti-inflammatory already at lower doses, Kennebec was effective only at higher doses. These potato varieties may exert a significant anti-inflammatory activity, representing possible functional foods against chronic inflammation.

#38

Giorgia Maria Varalda • Rome

Unexpected genotoxic activity of two non-volatile nitrosamines, N-nitroso-thiazolidine-4-carboxylic acid (NTCA) and N-nitroso-2-methyl-thiazolidine-4-carboxylic acid (NMTCA)

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Nitrosamines (NA) are organic compounds to which humans may be exposed through water, food, tobacco, cosmetics, pharmaceuticals. Most NA are genotoxic and form DNA adducts by the electrophilic diazonium ion after metabolic activation by liver enzymes. Recently a gap-of-knowledge was identified in the assessment of the genotoxic hazard of non-volatile nitrosamines (NVNA), used as food additives at significantly higher levels than volatile NA. The identification of a toxic hazard for NVNA could raise a potential safety concern for human health.
Herein the genotoxic potential of NTCA and NMTCA, NVNA not previously assessed, was evaluated by Ames and in vitro micronucleus (MN) tests. Negative results were obtained in the Ames test, while statistically significant increases of MN were observed. NTCA: 65.5±28.99 and 17.5±1.5 MN‰, NMTCA: 28±1.41 and 21±14.1 MN‰ in the presence and absence of metabolic activation, respectively (p<0.001).
The positive findings were unexpected based on the lack of structural alerts for genotoxicity, as assessed by in silico method (QSAR analysis). NTCA and NMTCA genotoxic mode of action need to be further clarified for a proper risk assessment.

#39

Alessandra Villani • Bari

Changes in antioxidant metabolism and growth rate of wild rocket Diplotaxis tenuifolia (L.) DC as affected by different nutrient supply levels and growing systems

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Wild rocket is gaining popularity as a ready-to-eat salad, and the cultivation of this plant is in further expansion, both in greenhouse and open field conditions. However, a relatively few investigated aspects relate to the impact of cultivation system and nutrient solution composition on the nutritional quality and redox state of antioxidant components in this species. Therefore, a study was conducted to examine the effect of high- and low-input fertilization program on the physiological properties, antioxidant components, quality, and mineral content of wild rocket leaves grown in soil and soilless. Soil–bound cultivation led to higher fresh weight, leaf number and area, ascorbate, and chloride. Conversely, in soilless cultivation higher content of sulphates and polyphenols, and enhanced activity of some antioxidant enzymes activities, such as dehydroascorbate reductase, and monodehydroascorbate reductase were detected. This study provides useful new information for soil and soilless fertilization management for wild rocket cultivation and for further research aimed at enhancing the yield, quality, and bioactive value of this popular fresh-cut product.

#40

Alessandro Vitale • Milan

Marked increase of globulin-1S in seeds of the italian purple maize (Zea mays) Moradyn variety

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Seed storage proteins (SSPs) are the main protein source for human and animal nutrition. However, their profile has evolved to ensure germination and is sub-optimal for our nutritional needs. Prolamins, the main class of cereal SSPs, are poor in lysine and tryptophan. Moreover, some prolamins are allergenic. Maize SSPs are mainly constituted by the endosperm-located zein prolamins, that accumulate in endoplasmic reticulum-derived protein bodies (PBs). However, maize embryo accumulates significant amounts of globulins, which are usually the major SSPs in dicotyledons and accumulate in storage vacuoles. Searching for maize varieties with lower content of allergenic prolamins and higher nutritional value, we have found that Moradyn purple maize has a lower content of 27kD and 50kD gamma-zeins. In a PB fraction from Moradyn seeds, we found unusually high amounts of globulin-1S, which is known as a maize embryo vacuolar SP and contains almost 4% lysine. Rebalancing of protein content is known to increase embryo SPs in maize lines with lower amounts of zeins, but the co-purification of globulin-1S with PBs calls for further investigation on its subcellular destiny in Moradyn seeds.

6. Health and Disease

#1

Saverio Alberti • Messina

Giant recursive cell membrane platforms drive calcium, phosphatidylinositol and kinase signaling

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High temporal and spatial resolution techniques have provided insight into nanoscopic, millisecond-lasting membrane signaling platforms. However, multiple signaling events, including growth factor receptor activation, Ca2+ signaling, lipid and protein kinase stimulation, operate over much broader space and time ranges. To reconcile such dimensional scales, we investigated cell signaling mechanisms for induction of cell growth, by confocal/ super-resolution/ electron microscopy, using kinases, CD9, Trop-1, Trop-2 as benchmarks. Signaling capacity was acquired through corecruitment of activated growth inducers into micrometer-sized cell membrane areas, that recursively assembled over hundreds of seconds (‘docks’). Docks were induced by growth factors and were shown to drive cell growth. Our findings shift current nanodomain-centered signaling models to orders of magnitude-larger dimensional scales. Over decades, many studies have recognized large areas of recruitment of individual signaling components in various cell membrane compartments. Our findings indicate that these compartments share distinct signaling platform traits, suggesting common, fundamental organizing principles.

#2

Mariaevelina Alfieri • Naples

Identification of uPAR variants acting as ceRNAs in leukaemia cells

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The urokinase receptor (uPAR) concentrates proteolytic activities on cell surface, is an adhesion receptor for vitronectin and activates intracellular signals promoting cell adhesion, migration, proliferation and survival. Increased uPAR expression is associated with poor prognosis in several malignancies. In leukaemia cells, the uPAR-3′UTR upregulates the expression of pro-tumor factors by recruiting microRNAs, thus acting as a competitive endogenous RNA (ceRNA). Three uPAR transcript variants containing 3′UTR were identified and characterized in KG1 and U937 leukaemia cells expressing low and high uPAR levels, respectively. These variants lack exon 5 (uPAR-Δ5) or exon 6 (uPAR-Δ6) or part of exon 6 and exon 7 (uPAR-Δ6/7). uPAR-Δ5 and uPAR-Δ6 transcript levels were higher in U937 cells compared to KG1 cells and in AML blasts compared to CD34+hematopoietic cells from healthy donors. Overexpression of the uPAR-Δ5-3’UTR induced upregulation of some pro-tumor factors and increased cell adhesion, migration and proliferation. Identification of miRNAs bound by the uPAR-Δ5-3′UTR variant is actually in progress.

#3

Maria Laura Amenta • Naples

Functional analysis of acetylcholine signaling in Drosophila melanogaster immune response to bacterial pathogens

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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.

#4

Francesca Ascenzi • Rome

Lung Adenocarcinoma cells cultured as 3D spheroids and enriched for Cancer Stem Cells undergo a phenotypic switch associated with resistance to ferroptotic cell death

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Scientific literature supports the evidence that Cancer Stem Cells (CSCs) retain inside low Reactive Oxygen Species levels and are therefore less susceptible to cell death, including ferroptosis, a type of cell death dependent on iron-driven lipid peroxidation. An established collection of LUAD primary cell lines derived from malignant pleural effusions of patients was used to obtain 3D spheroids enriched for stem-like properties. We observed that the ferroptosis inducer RSL3 triggers lipid peroxidation and cell death in a panel of LUAD cell lines; interestingly however, when grown in 3D condition, all cell lines exhibit resistance to RSL3 and protection against ferroptotic cell death. Molecular analyses show that this phenomenon correlates with an increased expression of antioxidant genes and high levels of proteins involved in iron storage and export, indicating protection against oxidative stress and low availability of iron for the initiation of ferroptosis. Our results show that CSCs-enriched cultures undergo phenotypic changes that make them resistant to ferroptosis. Further studies are needed to investigate in greater detail the molecular mechanism underlying the process.

#5

Francesca Romana Auciello • Rome

Metabolism and tumour plasticity

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Pancreatic ductal adenocarcinoma (PDAC) is an aggressive cancer that is projected to be the second biggest contributor to cancer deaths by 2030. PDAC tumours are characterized by an abundant stromal reaction, and a particularly hostile tumour microenvironment. The tumour-resident pancreatic stellate cells (PSCs) are the major contributors to the PDAC fibrotic reaction. Here we showed that lipids, released by PSCs, promote PDAC cell growth and migration. We found that PSCs release lysophospholipids, used by PDAC cells for membrane synthesis and growth. In addition, PSCs produced lysophosphatidic acid (LPA), by releasing both the LPA-generating enzyme autotaxin and its precursor lysophosphatidylcholine (LPC). LPA signalling activated the Akt pathway, and enhanced growth and migration. This was also observed in vivo, where autotaxin is abundantly expressed in PDAC tumours, and its inhibition reduced the rate of tumour development. This work highlighted an unanticipated role for PSCs in producing the oncogenic LPA signalling lipid and demonstrated how PDAC tumour cells co-opt the release of wound healing mediators by neighbouring PSCs to promote their own proliferation and migration.

#6

Daniela Bellincampi • Rome

Olive mill waste water valorization: bioactive molecules-enriched fractions recovered by tangential-flow membrane filtration (TFMF) to elicit plant defence responses

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In modern olive oil extraction process (Multi-phase Leopard Decanter), in addition to olive oil and a kernel dehydrated fraction, a hydrated pulp named "Patè" is produced. After centrifugation, the vegetative water (VW) can be recovered from Patè as supernatant. VW is rich of bioactive molecules such as oligosaccharides and phenols effective in plant protection against pathogens. VW have been fractionated by TFMF. NMR spectroscopy analysis indicated the presence of bioactive phenols such as tyrosol, hydroxytyrosol and oleuropein with a known antimicrobial activity. The profile of TFMF fractions obtained by HPAEC-PAD indicated the presence of pectin-derived oligogalacturonides (OGs) well known elicitors of plant defence responses as the early intracellular increase of Ca2+ levels. A dose dependent elicitation of Ca2+ was induced by OGs enriched TFMF fractions. Remarkably, pre-treatment with specific OGs-enriched TFMF fractions reduces plant disease symptoms against microbial pathogens. The possibility that phenols cooperate with OGs in inducing plant immunity is not excluded. This finding highlights the potential of this waste as bio-stimulant of plant defense responses.

#7

Dina Bellizzi • Rende, CS

Blood circulating bacterial DNA in COVID-19 patients

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Coronavirus disease (COVID)-19 is a heterogeneous condition caused by SARS-COV-2 infection. Clinically, it is generally characterized by interstitial pneumonia that can lead to impaired gas-exchange, acute respiratory failure, and death. The pathogenesis is complex, and a variable combination of factors has been described in critically ill patients.

This study aimed to establish a link between Blood circulating Bacterial DNA (BB-DNA), which has been associated with a few pathological conditions, and clinical severity in COVID-19 patients. BB-DNA levels were determined, by quantitative real-time PCRs targeting the 16S rRNA gene, in 149 patients with COVID-19 (age range 65-99 years). Cox regression analysis reveals an association between BB-DNA and in-hospital mortality. Furthermore, a negative correlation with IFN alpha, and a positive association with the neutrophil count parameter, which increases in deceased patients, were observed.

The association of BB-DNA levels to immune-inflammatory parameters, previously correlated to non-survival, suggests their potential role as a biomarker of unfavourable outcomes of the disease.

#8

Valentina Bigini • Viterbo

Increasing durum wheat resistance to Fusarium graminearum by using cell wall-derived oligogalacturonides

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Plant diseases cause substantial crop losses worldwide and compromise food safety because of the presence of toxins associated to fungal contamination. Thus, sustainable yield increase, diminishing usage of chemicals, enhancing crop resilience to biotic stress represent the main, concomitant, targets to be pursued in agriculture in the shortest period. Among the current approaches to crop protection, the use of elicitors, able to activate the natural defense mechanisms of the plant, is a strategy gaining increasing attention. Numerous studies indicate that local application of plant cell wall (CW)-derived elicitors, such as oligogalacturonides (OGs) derived from partial degradation of pectin, induce systemic resistance against pathogens in different plant species. The aim of this study was to establish the efficacy of OGs in protecting durum wheat, characterized by an extreme susceptibility to fusariosis caused by Fusarium graminearum. Furthermore, the study of durum wheat plants with potentially altered endogenous OG levels, i.e. OG-Machine lines, is facilitating the elucidation of molecular mechanisms regulating plant defense activation upon sensing danger signals in cereals.

#9

Andrea Bosso • Naples

GVF27, a new human host defence peptide, shows anti-inflammatory and anti-biofilm activity against the Burkholderia cepacia complex (Bcc)

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Strains belonging to the so-called Burkholderia cepacia complex (Bcc) are clinically relevant since they display a variety of potential virulence factors, such as a unique LPS conferring antimicrobial resistance, and the ability to form biofilm which may protect them from conventional antibiotics. Host defense peptides (HDPs) are one of the most promising alternatives to antibiotics since they could be used to treat bacterial infections, especially those caused by multidrug-resistant pathogens. We previously identified in silico a cryptic HDP named GVF27, and here we report its promising anti-biofilm and immunomodulatory activities on two members of the Bcc: Burkholderia multivorans and Burkholderia cenocepacia. GVF27 shows anti-biofilm effects at different stages of biofilm formation towards both Bcc strains, the intriguing ability to work in combination with ciprofloxacin, and a high affinity for LPSs isolated from B. cenocepacia that allows the peptide to mitigate the release of proinflammatory cytokines in LPS-treated human cells. The case of GVF27 confirms that the potentialities of the human proteome as a source of new peptide therapeutic agents are still largely unexploited.

#10

Chiara Brignola • Naples

MDM2/4 inhibitors as novel therapeutic strategy to enhance 5-FU induced nucleolar stress and apoptosis in colon cancer cells

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Recent evidence suggest that the p53-dependent nuclear stress induced by some chemotherapeutics, such as 5-Fluorouracil (5-FU), might not be enough to produce cell cycle arrest or apoptosis, given the hyperactivation of MDM2/4, main inhibitors of the p53 activity. It has been recently discovered that Pep3, a 12-mer peptide, is able to disrupt the MDM2/4 complex stabilizing p53. In this study, we developed a library of Pep3 derivatives with sequence lengths ranging from 5- to 8-mer. Of note, the newly truncated peptides showed lower IC50 values than Pep3. We incorporated the most active peptides (VLP-13 and VLP-24), alone or in association with 5-FU, in biodegradable nanoparticles, and we analyzed their cytotoxicity against p53 proficient colon cancer cells. We found that the cytotoxic activity of VLP-24 was higher than VLP-13 both alone and in association with 5-FU. The combined treatment 5-FU/VLP-24 caused a significant unbalance of ribosomal RNA precursor levels associated to a strong increase of p53 expression levels triggering apoptosis. In conclusion, our data reveal VLP-24 as a promising molecule able to enhance 5-FU mediated activation of p53-dependent nuclear stress.

#11

Francesca Bruno • Catania

Human gene polymorphisms and the resistance to SARS-CoV-2 infection

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During the first wave of COVID-19 infection in Italy, the number of cases and the mortality rates were among the highest compared to the rest of Europe and the world. We have observed several cases of virus infection resistance in subjects living in close contact with infected subjects, thus, to explain the predisposition to SARS-CoV-2 infection and to disease progression we must consider the allelic variants of specific human genes, directly or indirectly related to the life cycle of the virus. We analyzed three human genetic polymorphisms of the TMPRSS2 and CCR5 genes in a sample population from Sicily to investigate the possible correlation with the resistance to viral infection and/or to COVID-19 disease progression. Our results did not show any correlation of rs35074065, rs12329760, and rs333 polymorphisms with SARS-CoV-2 infection or with disease severity. Further studies should be done to identify the major human determinants of SARS-CoV-2 viral resistance.

#12

Roberto Campagna • Ancona

Role of paraoxonase-2 in triple-negative breast cancer: effect of shRNA-mediated gene silencing on cell proliferation and chemosensitivity

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Triple-negative breast cancer (TNBC) accounts for 20% of all breast cancer (BC) cases and displays a high aggressiveness and mortality rate. Since TNBC is not sensitive to endocrine or targeted therapy, there is an urgent need to identify novel molecular targets to improve the outcome of TNBC patients. In this work, we focused on the enzyme paraoxonase-2 (PON2), which exerts an antioxidant role and whose upregulation has been reported in several tumors. PON2 expression has been evaluated in formalin-fixed and paraffin-embedded tissue specimens of different BC subtypes and statistical analyses have been performed to correlate enzyme levels with tumor prognostic parameters. Results showed that BC samples displayed a significantly higher PON2 expression compared with that detected in controls. Moreover, PON2 knockdown in TNBC cell lines led to a decrease of cell proliferation in vitro and enhanced sensitivity to cisplatin, 5-fluorouracil and doxorubicin, used alone or in combination. Data reported in this study suggest that PON2 may represent an interesting biomarker for TNBC, thus highlighting its promising role as a diagnostic determinant and therapeutic target.

#13

Nicoletta Campolattano • Caserta

Characterization of the MSMEG-3762/63 efflux pump in Mycobacterium smegmatis

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Drug-resistant tuberculosis (TB) is one of the most difficult challenges facing global TB control. Here we explore the role of efflux pumps in drug tolerance using the model organism M. smegmatis. Previous studies describe the function of the TetR-like MSMEG-3765 repressor that regulates the MSMEG_3762/63/65 operon and the Rv1687c/86c/85c in Mtb. The first two genes of the two operons encode for an ABC transporter. To characterize the efflux system, the deletion mutant M. smegmatis (ΔMSMEG_3763) was generated, and comparative analyses with the wt, using different antimicrobials, suggest the ability of the MSEMG-3762/63 pump to bind and extrude rifampicin and ciprofloxacin. The putative role of these antibiotics as ligands of the MSMEG-3765 regulator, functional to a de-repression mechanism for the expression of the MESMEG_3762 operon, was investigated. Moreover, the role of the efflux pump in membrane potential was evaluated by cytofluorimetric analyses. Since the MSMEG_3762 operon is up-regulated in acid-nitrosative stress, a condition mimicking the macrophage environment, its expression in M. smegmatis infected macrophages was also studied.

#14

Barbara Carrese • Naples

Doxorubicin loaded albumin modified nanoparticles for targeted chemo-photothermal therapy in HS578T breast cancer cells

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Nanotheranostic is an emerging strategy based on the use of nanoparticles (NPs) as carriers for contrast agents and drugs, to perform targeted diagnosis and therapy in tumors.
In this study, MelaSil_Ag NPs-HSA, previously tested for the photoacoustic imaging, were loaded with DOX to perform photo- and chemo- therapy. Results showed that NPs@DOX are more effective on HS578T breast cancer cells, compared to free drug under the same experimental conditions, allowing the usage of lower time exposure and drug concentration. This effect is probably associated to targeted delivery and to overcoming of multidrug resistance.
Successively, the cytotoxic effect of the DOX-loaded NPs after photothermal laser irradiation at 808 nm was investigated. The synergistic action of temperature rise and drug toxicity, produces a relatively higher cytotoxicity compared to dark conditions, after 6h of treatment at the lowest concentration.
All together, these results demonstrate that photothermal therapy increases NPs@DOX therapeutic efficacy. In conclusion, the use of DOX loaded NPs, thanks to their chemo- and photothermal properties, allows the reduction of time exposure and DOX effective concentration.

#15

Francesca Carriero • Pavia

Photodynamic therapy using berberine photosensitizing to induce apoptosis in human astrocytoma established cells

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Glioblastoma (GBM) is the most common and aggressive malignant brain tumor in adults. Despite many standard therapeutic approaches including surgical removal, radiation, and chemotherapy, GBM remains a poor prognosis neoplasm with a high rate of recurrence. Therefore, in clinical oncology GBM treatments became a challenging task.

Photodynamic therapy (PDT) is a promising anticancer strategy involving light and a photosensitizing (PS) designed to elicit cell death through the generation of reactive oxygen species (ROS). In this work we have exploited the cytotoxicity of the plant alkaloid berberine (BBR) and investigate the potentially use as photosensitizing agent. An in vitro specific BBR-PDT scheme was developed in human established astrocytoma cell lines to study a possible cell death. BBR-PDT scheme preferentially induce the activation of intrinsic apoptotic pathway with an alteration in mitochondrial membrane potential, massive ROS accumulation and the subsequent activation of caspase pathway.

Overall, our results demonstrated that BBR is an efficient PS agent and its association with PDT could be a novel strategy for high malignant gliomas.

#16

Luca Cavinato • Rome

Trikafta therapy improves cystic fibrosis monocytes antimicrobial activity against Pseudomonas aeruginosa

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Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene is the cause of Cystic Fibrosis (CF). CFTR mutations may impact protein synthesis, folding, stability, trafficking, turnover or activity. Mutation-specific small molecules, named modulators, have been developed to restore the activity of mutated CFTR. Based on its activity on CF epithelial cells, (Trikafta) has been recently approved to treat CF patients with at least one F508del mutations. It is well accepted that CF phagocytes are characterized by reduced phagocytic and bactericidal activity against lung pathogens, including Pseudomonas aeruginosa. Thus, to analyze the possible effect of Trikafta on CF phagocytes, cells isolated from peripheral blood prior and after Trikafta therapy have been investigated. We observed an improvement of the phagocytic and microbicidal activity of CF monocytes after therapy. Moreover, ROS production and NOX2 activation were lowered by Trikafta, suggesting that the reduced level of ROS production may have enhanced the microbicidal activity of CF phagocytes. Collectively, these results suggest that Trikafta is also effective in CF phagocytes.

#17

Lorenzo Ceccarelli • Pisa

Human microglia derived Extracellular Vesicles: effects of inflammatory stimuli on production, content, and biological function

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Microglial cells are a major component of the immune system in the brain. Their secretome also contains Extracellular Vesicles (EVs). EVs can be divided into two major populations: small (SEV) and large EVs, carrying different cargo which reflects the status of parental cells and can lead to beneficial and detrimental effects in many physio-pathological states.
Herein, a workflow for extracting and characterizing EVs from two human microglia cell lines (HMC3 and C20) was reported. Their biochemical and biophysical properties were analyzed by Western Blot, TEM, DLS, and CONAN assay. C20-derived SEVs, on the contrary to HMC3-derived, were demonstrated to interfere with the proliferation of glioblastoma cells, depending on their different levels of miRNAs cargo. C20- (IL1-β, and LPS) inflammatory stimulation produced an increased number of SEVs with a smaller mean diameter, and each stimulus differently modified the miRNA cargo. Our data help to fill a gap in the knowledge of human microglia EVs, shedding light on the effects exerted by different inflammatory stimuli.
TEM=Transmission Electron Microscopy, DLS=Dynamic Light Scattering, CONAN=Colorimetric Nanoplasmonic Assay.

#18

Ylenia Cerrato • Rome

Cytochrome c as a marker of oxidative stress in follicular thyroid cancer

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Thyroid cancers (TC) account for 4% of human cancers and are represented for about 12% by the follicular variant (FTC). To date, there is still no certain diagnostic method for distinguishing between benign and malignant forms and for staging classification of TC. Our group showed that Raman spectroscopy investigation of FTC human tissues provides reliable cancer diagnosis highlighting cytochrome c accumulation with an accuracy of ∼ 90%1. Here, we demonstrated by transcriptional and protein analyses a significant reduction in mitochondrial metabolism and an increased glycolytic rate in the tumor lobe compared to the matched healthy lobe of each FTC patient. Besides, an increase of the antioxidant response (e.g., HO-1, NQO1, SOD1) was reported in FTC lobes. The increase of cytochrome c levels in FTC supports increased levels of oxidative stress, cytochrome c acting as an antioxidant molecule scavenging superoxide radical. Data here obtained, in addition to defining in vivo alterations in energy metabolism in FTC tumors, allowed the identification of potential diagnostic markers and therapeutic targets.

1Sbroscia et al. Thyroid cancer diagnosis by Raman spectroscopy. Sci.Rep. 2020;10:13342.

#19

Armando Cevenini • Naples

Compromission of endolysosomal functions in cellular models of methylmalonic acidemia

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Methylmalonic acidemia (MMA) is an autosomal recessive inherited metabolic disorder due to deficient activity of methylmalonyl-CoA mutase (MUT). In presence of its cofactor adenosylcobalamin (AdoCbl), MUT converts methylmalonyl-CoA into succinyl-CoA, a Krebs cycle substrate. MMA patients are diagnosed with defects in the AdoCbl synthesis or mutations in the MUT gene, showing a poor outcome due to multi-organ damage. Despite the mitochondrial defects, the exact pathomechanisms of cell stress in MMA are poorly understood and the therapeutic options remain unsatisfactory. A proteome analysis conducted on a MUT-knockout cellular model of MMA has revealed a dysregulation in autophagy- and lysosomal-related pathways, besides metabolic alterations. Here, we further explore the molecular insights related to such pathways in fibroblasts from MMA patients using western blot and confocal microscopy analyses to demonstrate the compromission of the endolysosomal compartment in MUT-deficient cells. Lysosomal defects might be linked with persistence of dysfunctional mitochondria and might give hints to promote mitophagy and mitochondrial turnover as a mean to limit organ damage in MMA.

#20

Roberta Chiavetta • Palermo

Applying RNA editing technology to correct the W1282X stop mutation in the CFTR mRNA in human bronchial cells

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Premature termination codons (PTCs), caused by stop mutations, are the cause of CFTR impairment for approximately 10% of worldwide cystic fibrosis patients. Contrarily to DNA editing, RNA editing is safer to correct mutations and an in vitro/in vivo strategy alternative to pharmacological drugs. We used the REPAIRv2 system (RNA Editing for Programmable A to I Replacement, version 2) to edit the W1282X (c.3846 G>A) nonsense mutation in the CFTR mRNA. REPAIRv2 is based on an inactive CAS13 subunit fused with ADAR2DD (deamination domain) that once targeted to the mutant codon by a specific guide RNA, deaminates the adenosine into inosine that is then read as guanosine by the ribosome.

Our data show that the CFTR protein appears to be recovered by the REPAIRv2 system in the CFF-16HBEge CFTR W1282X cells harboring the W1282X non-sense mutation. REPAIRv2 is successfully delivered in 33% of the transfected cells, some of which show CFTR localization at the plasma membrane by immunofluorescence assays following 48 hours of RNA editing.

These preliminary, encouraging data pave the way for future gene therapy applications of sequence-specific RNA editing in cystic fibrosis.

#21

Costanza Cicchi • Florence

Characterization of the enzymatic activity of Lscβ and Lscγ, two levansucrases from Pseudomonas syringae pv. actinidiae

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P. syringae pv. actinidiae biovar 3 (Psa3) is the causal agent of the bacterial canker of kiwifruit, a disease that has destroyed many orchards in Italy.
Psa3 produces levan, a fructose exopolysaccharide that may be involved in pathogenesis and that is synthetized by levansucrases (Lscs) that hydrolyse the glycosidic bond of sucrose and use fructose for polymerization. Two genes encoding functional Lscs isoforms (Lscβ and Lscγ) have been identified in Psa3 genome. Lscβ and Lscγ have a different enzymatic activity: while Lscβ hydrolyse sucrose at pH 7, Lscγ mainly polymerizes levan at pH 5 (1).
This work is aimed to identify some synthetic molecules which may inhibit Lscs activities. Enzymatic assays were set up with the active compounds and the kinetic parameters (IC50, Ki) were measured. The data obtained with some inhibitors suggest a competitive inhibition. A selective inhibitor for levan polymerization was found. The results suggest that Lscs have a complex active site that may catalyse differently the two reactions.
Next steps will be structural analysis and in vivo experiments to study the impact of Lscs inhibition on Psa3 pathogenicity.

1) Luti et al, 2021, Int J Biol Macromol

#22

Ernesto Comite • Portici, NA

Application of microbial inoculants on food crops in Kenya and evaluation of rhizosphere microbiota

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Cultivation in small farms of Kenya are based on manual agronomic practices and manure soil applications, while water is a limiting factor because it’s related to the seasonality. Interactions with microorganisms may improve plant nutrient assimilation in conditions of abiotic stress. Two food crops (green gram and sorghum) were seed-treated with Trichoderma afroharzianum strain T22 and T. asperellum. This study aimed to characterize the rhizosphere microbiota of plants treated with Trichoderma after short or long rain seasons in Kaliku-Kenya. Bacterial and fungal diversity were assessed in rhizosphere soil by Illumina amplicon sequencing. Actinobacteria was the most abundant bacterial phylum in both crop groups, followed by Firmicutes and Proteobacteria. The abundance of these phyla and Chloroflexi reduced during the long-rain season, whereas Acidobacteria, Planctomyces and Gemmatimonadetes increased. Ascomycota was the most abundant fungal phylum, with Dothideomycetes and Sordariomycetes classes that showed a seasonal pattern in green gram rhizosphere, with a negative correlation. Finally, these variations of rhizosphere microbiota were due to treatment and seasonality.

#23

Federica Corrao • Palermo

LPS Responsive Beige-Like Anchor (LRBA) protein rescue by treatment with Translational Readthrough Inducing Drugs (TRIDs) in human fibroblasts harboring the c.5047 C>T (R1683*) nonsense mutation

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Nonsense mutations affect about 11% of the inherited diseases. They are due to single-nucleotide substitutions in gene codifying sequences, leading to the creation of a premature termination codons (PTC) in the mRNA, and causing the production of truncated and non-functional proteins. The suppression therapy, by readthrough of the stop codon, is one of the proposed approaches to restore protein expression. In this work the ability of three new optimized TRIDs (NV848, NV914, NV930) to rescue the expression of the LRBA protein in LRBA (c.5047 C>T, R1683X) primary human fibroblasts has been evaluated. Since it is known that the readthrough either basal or induced by TRIDs allows the insertion of Trp, Arg, or Cys in the place of a premature stop codon UGA, the possible aminoacidic substitutions were analyzed by structure assembly simulations to determine the influence on the protein structure. Finally, by NGS analysis we evaluated the correct mRNA sequence after TRIDs treatment to understand a possible interference of the molecules with the fidelity of the transcription process.

#24

Alessia Cuccurullo • Pontecagnano

Resistance against broomrapes (Orobanche and Phelipanche spp.) in tomato: phenotypic comparison of CRISPR/Cas9 tomato mutants targeting genes responsible for the biosynthesis of strigolactones

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Broomrapes are root holoparasites belonging to the genera Orobanche and Phelipanche spp. They are responsible of severe field infestations especially in Mediterranean climates. Phelipanche aegyptiaca and P. ramosa represent a serious threat for tomato cultivation, determining heavy production losses. Furthermore, conventional management practices for their control are mostly inadequate. CRISPR/Cas9 knock-out tomato lines for the four main genes involved in strigolactones (SLs) biosynthesis – D27, CCD7, CCD8 and MAX1 – was recently produced. SLs are carotenoid-derived phytohormones, acting as the main germination stimulus inducing broomrapes seeds germination, besides having fundamental roles in plant development. For these edited genotypes, a phenotypic comparison programme is underway, in which morphological, reproductive, productive and qualitative traits were considered. Moreover, biochemical analysis of root exudates was performed together with in vitro pathogenicity assay, to determine SL content and susceptibility to P. agegyptiaca and P. ramosa. Additionally, the interaction with AMF Glomus intraradices was also assessed to characterize the response to mycorrhization.

#25

Antonino Cucinotta • Rome

Blocking the hedgehog-dependent tumor growth by a new selective endoplasmic reticulum aminopeptidase 1 inhibitor

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Aberrant Hh signaling occurs in a wide range of human cancers, such as medulloblastoma (MB), the most common brain malignancy in childhood. Recently, we identified Endoplasmic Reticulum Aminopeptidase 1 (ERAP1), a key player of the immune response, as a previously unknown positive regulator of the Hh pathway and promising therapeutic target for Hh-driven tumors. However, the lack of availability for highly specific chemical inhibitors for ERAP1 has constrained the progress in this area.

To identify novel selective and effective ERAP1 inhibitors, we performed a docking-based virtual screening of a library of natural compounds against crystallographic structure of the ERAP1 catalytic domain and among eleven selected molecules, we identified compound N1 as a potent ERAP1 inhibitor. We demonstrated that this compound, blocking ERAP1 activity: i) significantly reduces stability of GLI1, thus counteracting Hh signaling; ii) impairs self-renewal ability and clonogenicity of tumor-derived MB stem-like cells; iii) suppresses MB growth in vitro and in vivo. Our finding strongly indicates N1 as a good candidate for further preclinical studies in the treatment of Hh-dependent tumors.

#26

Eleonora Da Pozzo • Pisa

Intermittent hypoxia promotes cellular priming and exaggerated inflammatory response of human microglia in vitro.

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Obstructive sleep apnoea syndrome (OSAS) is a sleep-disordered breathing characterized by cycles of blood oxygen partial pressure oscillations, leading to tissue damage due to recurrent intermittent hypoxia (IH) episodes. OSAS-derived IH could lead to cognitive impairment through not fully cleared mechanisms, thus we developed a new in vitro model to mimic IH and to study cellular effects on human microglia. First, a validation of in vitro model was performed by measuring HIF-1a, ROS, and mitochondrial activity in response to different IH protocols. The microglia inflammatory state was tested by evaluating mRNA levels of NF-kB and IL-6. Results show that, after IH treatments followed by a normoxic period, a real inflammation did not occur in microglia§; however, microglia resulted in a state characterized by increased NF-kB expression and markers related to a primed phenotype. Therefore, microglia were stimulated with IL-1β after IH; the results showed an exaggerated inflammatory response with increased IL-6, suggesting the role of primed microglia in OSAS-driven neuroinflammation.

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#27

Marco Dainelli • Florence

Under fungal attack on a microplastic-contaminated soil: a case study on Arabidopsis thaliana

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Plants must adapt to several stressful conditions, among which the accumulation of plastic pollutants in the environment represents one of the most threatening. Microplastics (MPs, 100nm - 5mm) and nanoplastics (NPs, <100nm), mainly generated via fragmentation from larger plastic debris, have been reported to negatively impact on plant life.

This work tested if two widespread plastic polymers, polyethylene terephthalate (PET) and polyvinyl chloride (PVC), are able to modify plant-pathogen interaction. Seeds of Arabidopsis thaliana were germinated in soils with different concentrations of PET/PVC MPs (0.2 and 0.5 % w/w, particles size < 330 µm) and grown for three weeks. At the end of the treatment, leaves were exposed for 48h either to cerato-platanin (CP), a PAMP protein from Ceratocystis platani, or to Botrytis cinerea conidia.

Even if plant growth and photosynthesis were not significantly affected by MPs, 0.2% PET/PVC treated plants showed a higher phytoalexin production after CP exposure. Plants treated with 0.2% PVC exhibited a better resistance to B. cinerea, as demonstrated by a lower development of leaf lesions. Hence, MPs are demonstrated to interfere with pathogen attack.

#28

Valeria De Pasquale • Naples

Molecular mechanisms triggered by heparan sulfate proteoglycans in Mucopolysaccharidosis lysosomal diseases

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Mucopolysaccharidoses (MPSs) are lysosomal storage diseases due to deficiency in glycosaminoglycan catabolizing enzymes. MPS patients show central nervous system involvement, neurodegeneration, degenerative joint disease and reduced bone growth. Current therapies exhibit limited efficacy, thus other therapies are under development for the cure of the neurological phenotype of the MPSs. In this scenario we have developed an innovative therapeutic approach, which allowed us to unravel a novel pathogenetic role of heparan sulfate proteoglycans (HSPGs) in the pathogenesis of MPSs. A competitive binding molecule of HSPGs is able to reduce the accumulated product and lysosomal defects in MPSs cellular model systems and MPS IIIB mouse model. We demonstrated that accumulation of HSPGs on the cell membrane of the MPS cells caused inactivation of growth factors signaling and consequent lysosomal exocytosis. Our findings, besides unravelling a novel pathogenetic role of the HS in the MPS diseases, indicate that targeting of the extracellular accumulated HS could be a promising therapeutic approach for MPS treatment, especially for the subtypes characterized by progressive neurodegeneration.

#29

Tonia De Simone • Verona

New miR-34a target genes identification by applying the CRISPR / Cas9 system for the generation of human miR-34a knock-out cell lines

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MicroRNAs (miRNAs) are short single-stranded non-coding RNAs mainly active in the regulation of gene expression by binding to complementary sequences located often in the 3' untranslated region (3'-UTR) of target mRNA. MiR-34a acts as a tumor suppressor transcriptionally activated by P53 and it has been associated with tumorigenesis, cancer progression, invasion, and metastasis. Although multiple miR-34a targets have been identified, several putative miR-34a target genes still need to be validated. The present study aims to identify new miR-34a targets potentially involved in tumorigenesis. For this purpose, we applied the CRISPR/CAS9 system to develop two miR-34a knockouts (KO) cell lines (HeLa and HEK293T). RNA-sequencing and enrichment analyses identified a set of genes that are deregulated in absence of miR-34a, including MAP1B, ONECUT2, and KMT2D. ONECUT2 and KMT2D were significantly up-regulated in both KO cell lines, compared to controls, indicating they could be new miR-34a target genes. Future analyses with 3’-UTR reporter assay and miR-34a over-expression will functionally validate its binding to predicted sites in the ONECUT2 and KMT2D transcripts.

#30

Samuele Di Cristofano • Rome

CD38 restrains the activity of cGAMP released by multiple myeloma cells through enzymatic breakdown.

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The second messenger 2’3’-cGAMP (or simply cGAMP) is a central mediator of the innate immunity. Multiple myeloma is a hematological malignancy arising from the proliferation of cancerous plasma cells in the bone marrow. The progression of the disease is associated with the establishment of a highly immunosuppressive tumor microenvironment. A promising frontier of immunotherapy is represented by the cGAS-STING pathway, in which the STING activator cGAMP is released in the microenvironment by the tumor, inducing cytokine-producing cells to orchestrate a strong antitumor response. However, the extracellular hydrolase ENPP1 can degrade cGAMP, thereby restraining its antitumor activity. To date, no other mammalian enzymes are known that can inactivate cGAMP. Here we provide in silico evidence (subsequently confirmed in vitro), that cGAMP is a novel substrate of the receptor/ectoenzyme CD38 and it could be degraded by CD38 through direct hydrolysis. We used molecular docking and multi-copy μs-long MD simulations to model and quantitatively analyze all interactions between cGAMP and CD38, in order to provide a mechanistic hypothesis for the enzymatic activity.

#31

Francesca Di Modugno Damiani • Rome

Tumoral and stromal hMENA isoforms influence tertiary lymphoid structure localization and predict response to immunocheckpoint blockade in lung cancer patients

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The tumor immune microenvironment includes cell types whose interaction affects cancer progression, clinical outcome and therapy response. Here, we highlighted the role of hMENA isoforms as crucial in communication among tumor cells, CAF, T and B cells. We demonstrated that in NSCLC cells hMENA11a increases the expression of the Tertiary Lymphoid Structure (TLS) regulator LTβR, reduces fibronectin (FN1) and favors CXCL13 production by tissue resident T cells. Conversely in CAF, hMENA/hMENAΔv6 favor FN1 production, inhibit LTβR-related NF-kB pathway and CXCL13 secretion. Consistently, NSCLC tumors with hMENA11ahigh expression, paucity of hMENA+ CAFs and stromal FN1low have TLS within the tumor core (TLS-IT). Notably, TLS-IT associate with memory B cells in tumor tissues and are predictive of longer survival in N0 NSCLC patients. In ICB treated patients, hMENA isoform pattern, FN1 and LTβR expression may discriminate responding or not-responding NSCLC patients.
Our findings reveal a novel role of the actin regulator hMENA alternative splicing as promising biomarker of clinical outcome in N0 NSCLC patient and likely response to ICB.

#32

Matteo Digregorio • Pisa

Zebrafish knockout model to enlighten neurodevelopment defects and social impairments caused by setd5 loss of function

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Loss of function (LoF) mutations of the methyltransferase SETD5 in humans are strongly related to the onset of Intellectual Disability (ID) and autistic spectrum disorders (ASD). We generated a zebrafish setd5 CRISPR-mutant model to characterize the molecular, histological and behavioral role of this epigenetic regulator. Mutants show microcephalic features and a significant reduction of telencephalon and optic tectum size. These defects are coupled with an alteration of mRNA levels of genes encoding for synaptic proteins and cell cycle regulators. Interestingly, the telencephalic ventral-ventricular zone displays a significant reduction of PHH3 positive cells, suggesting a deregulation of the proliferation mechanisms in this adult stem niche. Moreover, setd5 LoF leads to behavior and memory defects. Notably, we found that setd5 maternal contribution greatly impacts the future development of the individuals, as heterozygous fish carrying mutant maternal transcripts present the worst outcome.

The setd5 mutants are promising models to deepen our understanding of the neural defects underlying the clinical and behavioral effects associated to SETD5 haploinsufficiency.

#33

Alfio Distefano • Catania

Electronic Nicotine Delivery Systems (ENDS) vs Cigarettes: ENDS have demonstrated lower toxicity - "The Replica Study Experience"

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Replica Project Group is an international research group which independently replicated three relevant studies on the cytotoxicity and inflammation induced by cigarette smoke and ENDS aerosol. Our primary objective was to establish the reliability of the results obtained by these studies. In order to evaluate the cytotoxicity of smoke and aerosol, we exposed human bronchial epithelial cells (H292) to cigarette smoke and ENDS, aerosol at the air-liquid interface (ALI). Several inflammatory and remodeling mediators released by cells exposed to whole smoke (WS) and total particulate deprived smoke (vapor phase; VP) were evaluated. We were able to replicate the results obtained in the original cytotoxicity studies, confirming that nearly 80% of the cytotoxic effect of smoke is due to the vapor phase of smoke. Our results confirmed a significant reduction in the cytotoxic effects of ENDS aerosol compared with cigarette smoke. Overall, the data obtained independently in different laboratories clearly confirm the reduced cytotoxicity of ENDS products compared with smoking, thus providing a valuable tool for harm reduction strategies in smokers.

#34

David Israel Escobar Marcillo • Rome

Obesity and genomic instability: a human longitudinal study after bariatric surgery

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The higher cancer susceptibility observed in obese patients and the relationship among diet, obesity and gut microbiota are well established. However, the molecular mechanisms that underlie this crosstalk are still unknown. To study the potential role of genomic instability to the obesity-related metabolic dysfunctions, we carried out a longitudinal study on 36 severe obese patients who had to undergo bariatric surgery (BS). Blood, stool, and saliva samples were collected before and 6 and 12 months after BS. Analyses of clinical parameters, inflammatory markers, 16S rRNA gene sequencing, serum metabolic profiles and reverse phase protein array of specific DNA damage response (DDR) players were carried out. Altogether the data clearly show a restoration of a healthier profile after BS. Furthermore, activation of DDR/senescence markers were found in visceral adipose tissue biopsies in obese patients compared to controls, suggesting that the accumulation of senescent cells can contribute to the deleterious obesity-related long-term effects. To gain insight into the molecular mechanisms that underlie the obesity-related comorbidities remission, a correlation network will be discussed.

#35

Giulia Fanelli • Rome

Role of MdtEF and AcrAB multidrug resistance efflux pumps in macrophage intracellular life of Adherent-Invasive Escherichia coli strain LF82

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Efflux pumps (EPs) are inner membrane proteins involved in the transport process. EPs mediate the transport of a wide range of molecules, including several antibiotics, contributing to the emergence of resistance to multiple antibiotics (MDR). We studied the role of MDR EPs in the intracellular life of LF82, the prototype strain of Adherent Invasive Escherichia coli (AIEC). AIEC is a group of enteropathogenic E. coli, capable of colonizing the ileal mucosa of patients with Crohn's disease (CD). AIEC strains are able to invade and replicate in epithelial cells and macrophages without inducing cell death. The results of MDR EP expression during LF82 infection revealed that their transcription is highly modulated during the bacterial intracellular life. We found that that lack of MdtEF and AcrAB-TolC are responsible for a severe decrease in the macrophage survival. The results show how the MDR efflux pumps of the AIECs are involved in the host-pathogen interaction and highlight the contribution of MdtEF and AcrAB to the survival of LF82 in the macrophage environment.

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#36

Luigi Fattore • Rome

Systemic delivery of miRNA-loaded nanoparticles blunts resistance to therapy in BRAF-mutant melanoma

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BRAF-mutated melanoma relapsing after targeted therapies is an aggressive disease with unmet clinical need. Hence the need to identify new combinations able to overcome drug resistance. miRNAs have emerged as orchestrators of non-genetic mechanisms adopted by melanoma cells to challenge therapies. We previously identified a subset of oncosuppressor miRNAs downregulated in drug-resistant melanomas. Here we demonstrate that lipid nanoparticles delivering two of them, miR-199-5p and miR-204-5p, inhibit tumor growth both in vitro and in vivo in combination with target therapy and block the development of drug resistance. Mechanistically they act by directly reducing melanoma cell growth and also indirectly by hampering the recruitment and reprogramming of M2 macrophages. Molecularly, we demonstrate that the effects on macrophages are mediated by the dysregulation of a newly identified miR-204-5p-miR-199b-5p/CCL5 axis. Finally, we unveiled that M2 macrophages programs are molecular signatures of resistance and predict response to therapy in patients. Overall, these findings have strong translational implications to propose new combinations making use of RNA therapeutics for melanoma.

#37

Maria Grazia Ferraro • Naples

Fighting triple negative breast cancer aggressive phenotype with novel Ru(III)-based nucleolipidic compounds

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The triple negative breast cancer (TNBC) is the most aggressive phenotype and has, to date, limited treatment options. Our research group is focusing on a newly synthesized anticancer Ru(III) complex – named AziRu – incorporated in different nucleolipid nanostructures to improve its stability and in vivo delivery. Among our compounds, HoThyRu/DOTAP is the most effective against TNBC in vitro model represented by MDA-MB-231 cells. We investigated its capability to trigger multiple cell death pathways i.e., apoptosis and autophagy, both in vitro and in xenograft mice model in vivo. Moreover, we focused on the metastatic potential of TNBC demonstrating that HoThyRu/DOTAP can inhibit migration and invasion of MDA-MB-231 cells, as proved by different functional assays and by the modulation of principal EMT related proteins. Finally, we showed the efficacy of HoThyRu/DOTAP in vivo exploiting MDA-MB-231 xenograft model. The induction of different cell death pathways by multi-target Ru-based formulations and the reduction of metastatic potential could represent a valid and appealing chemotherapeutic option for breast cancer treatment.

#38

Giuseppe Forlani • Ferrara

Antiproliferative and phytotoxic effects of P5C reductase inhibitors do not rely upon proline starvation

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The amino acid proline is synthesized from glutamate or ornithine in short pathways sharing the last step, catalysed by d1-pyrroline-5-carboxylate (P5C) reductase. P5C is also formed during the mitochondrial degradation of proline by the sequential action of proline dehydrogenase (ProDH) and P5C dehydrogenase (P5CDH). The occurrence of a shortcut in which the P5C released by ProDH is not further oxidized by P5CDH, but is reduced back to proline by P5C reductase, has been demonstrated in mammals and hypothesized in plants. Such apparently futile proline-P5C cycle may provide the cell with a mechanism for transferring reducing equivalents from the cytosol to the mitochondrion, and to fuel the respiratory chain. Moreover, ProDH activity may alternatively lead to ROS production, which can trigger in turn the apoptotic mechanism, or increased ATP synthesis for protective autophagy. Consistently, many tumors show P5C reductase overexpression. P5C reductase thus represents a promising target for both, the suppression of tumor growth and weed control. Here we report that P5C reductase inhibitors limit the growth of human cancer cells and plant seedlings without causing proline starvation.

#39

Maria Franza • Rome

The CHK1 kinase inhibitor, MK-8776, induces the degradation of the oncogenic protein PML-RARα in retinoic acid-resistant acute promyelocytic leukemia cells

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Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia, in which more than 90% of patients express the PML-RARα oncoprotein that causes myeloid differentiation blockage. APL is managed by the administration of all-trans retinoic acid (atRA) and arsenic trioxide, which allow the degradation of PML-RARα. However, therapy resistance and side effects require the development of new therapies. Here, we characterized the possible efficacy of the selective CHK1 inhibitor MK-8776 in two atRA-resistant and one atRA-responsive APL cell line. Treatment of APL cells with MK-8776 for 48-96 h causes a significant decrease of PML-RARα levels compared to controls by a proteasome-dependent mechanism. Notably, we reported that also caspase-3 and -1 are involved in PML-RARα stability. Results obtained by Affymetrix microarray showed that MK-8776 modulates pathways and upstream modulators involved in the inflammatory response and cell cycle control. Overall, results obtained reveal that CHK1 inhibition induces PML-RARα degradation in APL, representing a promising target for APL treatment cells.

#40

Lorenzo Germelli • Pisa

Translocator Protein (TSPO, 18 kDa)-mediated neurosteroidogenesis regulates microglial functional phenotype

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Dysregulated microglia activation has been associated with neurodegenerative diseases related to neuroinflammation. We herein demonstrate the neurosteroidogenic capacity of a human microglial cell line that increases during neuroinflammatory conditions. On the contrary, the inhibition of neurosteroidogenesis leads to a hyperreactive microglia phenotype. To identify a possible target for the regulation of neurosteroidogenesis, our attention was directed to the protein TSPO, as it has been implicated in the step that influences neurosteroid biosynthesis. Thus, experimental approaches consisting of TSPO gene silencing and pharmacological stimulation were undertaken. The results showed a key role of TSPO both in maintaining the homeostatic signature of microglia and in fine-tuning the dynamic changes leading to the acquisition of functional phenotypes. Of note, TSPO pharmacological stimulation led to a specific neurosteroid production profile associated with an anti-inflammatory phenotype.Overall, the results support the concept of a key role of neurosteroidogenic function of TSPO in autocrine/paracrine regulation of microglial secretion of soluble pro- and anti-inflammatory mediators.

#41

Luisa Gesualdi • Rome

Microgravity exposure induces antioxidant barrier deregulation and mitochondrial structure alterations in TCam-2 cells

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One of the hallmarks of microgravity-induced alterations in several cell models is an alteration of oxidative balance. Notably, also male germ cells, sensible to oxidative stress, have been shown susceptible to changes of gravitational force. To gain more insights into the mechanisms of male germ cell response to altered gravity, a 3D cell culture model was established from TCam-2 cells, a seminoma cell line, and the only available in-vitro model to study mitotically active human male germ cells. TCam-2 spheroids were cultured for 24 hours under unitary gravity (UG) or simulated microgravity conditions (SM), which were obtained using the Random Positioning Machine (RPM). The ultrastructural analysis by Transmission Electron Microscopy revealed that SM drastically altered mitochondria, that appeared swollen and, in some cases, disrupted. In SM treated samples, gene expression levels (evaluated by Real-time PCR) of the main enzymes of the antioxidant barrier showed a reduced expression of GPX1, CYBB and TNX, an increased expression of CAT and HMOX. SOD, XDH, CYBA, NCF1-2 and TXNRD were not affected. These preliminary data confirm the susceptibility of human germ spheroids to SM.

#42

Chiara Giacomelli • Pisa

COVID-19 and plasma level of the hypoxia inducible factor 1 subunit alpha (HIF1α): effect of ACE2 inhibition on bronchial and alveolar epithelial cells

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) enters into host cells by binding to angiotensin converting enzyme 2 (ACE2). The increased expression of the hypoxia inducible factor 1 subunit alpha (HIF1α) contribute to COVID-19 pathophysiology. However, there is no direct evidence of the relationship between HIF-1a overexpression and ACE2 inhibition. Herein, the plasma level of HIF-1a were quantify in twenty-five COVID-19 positive patients. The results highlighted a direct correlation between the plasma HIF-1a levels and the disease severity, indicated by respiratory failure. To investigate the link between ACE2 inhibition and alterations of HIF-1a expression, alveolar (A549) and bronchial epithelial cells (16HBE) were employed. Then, an ACE2 protein inhibitor was used to model the SARS-CoV-2 infections. The preliminary results showed that the inhibition of ACE2 increased AT1R protein levels and increased HIF-1α mRNA expression. Overall, the data highlighted a correlation between the alteration of physiological HIF-1a expression and a direct ACE2 inhibition, occurring during SARS-CoV-2. This research project is funded by Tuscany Region “Bando ricerca covid-19”.

#43

Barbara Giordani • Bologna

Nanocarriers for the delivery of Lactobacillus crispatus BC1 biosurfactant: alternative strategies to fight Candida biofilms

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The growing problem of drug resistance claims the need for new strategies to treat vulvovaginal candidiasis. In this regard, biosurfactants (BS) produced by lactobacilli have gained increasing interest for their proven anti-adhesive properties, safety profile and natural origin. Moreover, the development of an appropriate nanocarrier is crucial to overcome the problem of biofilm-associated resistance. In the present study, the BS isolated from the vaginal strain Lactobacillus crispatus BC1 was delivered by two different nanocarriers: (i) chitosan nanoparticles (CH-NP-BS); (ii) conventional liposomes (LP-BS) and hyalurosomes (HY-LP-BS), obtained through ionic gelation and thin-film hydration method, respectively. CH-NP-BS and LP-BS/HY-LP-BS displayed size suitable for vaginal delivery, good mucoadhesive properties and stability over the storage period. CH-NP-BS retained the good anti-adhesive properties of free BS towards Candida spp. clinical isolates. More interestingly, HY-LP-BS were able to improve both the capability of BS to inhibit the fungal biofilm formation and to eradicate the pre-formed biofilms and can represent a promising anti-Candida strategy.

#44

Sarah Giulietti • Viterbo

Elucidation of the Arabidopsis NPK1-related protein kinases (ANPs) role in cell wall biosynthesis and signalling

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The plant cell wall (CW) is a resilient barrier against environmental stresses. It is built to resist pathogenic attacks and is the place where many danger-related signals exert their action. Local mechanical damage of the CW leads to systemic protection response with an important role played by jasmonic acid (JA) and ROS. The MAP triple kinases (MAP3K) named Arabidopsis NPK1-RELATED PROTEIN KINASES (ANP1-3) are involved in this process. Loss of ANPs leads to a CW with a lower content of cellulose, and a compromised response to CW-derived damage-associated molecular patterns (DAMPs), with alteration of ROS and JA accumulation. A strong parallelism has been observed between the response to the cellulose biosynthesis inhibitor isoxaben (ISX), and the phenotype associated with the lack of ANPs. The aim of this study is providing knowledge of the molecular mechanisms underlying the action of ANPs (and ISX) on the cell wall, investigating also the associated subcellular dynamics. Moreover, since JA and ROS have a pivotal role in the response to the depletion of cell wall cellulose, we are investigating the role of these signals in the alterations observed in the anp mutants.

#45

Roberta Grillo • Naples

S-Adenosylmethionine inhibits colorectal cancer cell migration by upregulating the expression of miRNA-34/449 superfamily

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Colorectal cancer (CRC) is a highly aggressive tumor with a strong proliferative capability and a high risk of metastasis. The acquisition of invasive properties is driven by aberrant activation of EMT, a highly regulated biological process responsible for tumor invasion and metastasis. Therefore, targeting EMT might offer promising opportunities to restrain metastatic spreading and to improve the outcome of CRC. In this context, S-adenosyl-L-methionine (AdoMet), the universal biological methyl donor, has emerged as a promising anticancer agent capable of modulating the expression of oncogenic miRNAs.

Here, we demonstrated that AdoMet inhibited cell migration in HCT-116 and Caco-2 CRC cell lines and that this effect was associated with reduced expression of migration- and EMT-related protein markers. We found that AdoMet upregulated tumor-suppressive miRNA-34a/34c/449a which have been shown to act as mediators of AdoMet’s antimigratory effects. Taken together, our findings strongly support the notion that AdoMet may be considered as an anti-metastatic agent in virtue of its ability to inhibit EMT and migration of CRC cells representing a good candidate for future drug development.

#46

Luisa Guttieri • Rome

Role of FGFR2c/PKCε signaling in the control of MCL-1-mediated cell survival and invasion in pancreatic ductal adenocarcinoma cells

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The pancreatic ductal adenocarcinoma (PDAC) is a lethal malignancy characterized by KRAS activating mutations and aggressive phenotype. Since we recently identified the high aberrant expression of the mesenchymal isoform of FGFR2 (FGFR2c) and its downstream PKCε signaling as responsible for the enhancement of EMT profile, here we investigated their involvement in the establishment of additional tumorigenic features. Using stable protein depletion by shRNA in PDAC cell lines expressing divergent levels of the FGFR2c, we found that FGFR2c/PKCε axis is responsible for FGF2-mediated cell invasion and for anchorage-independent growth, while in vitro clonogenic assays, coupled to cleaved PARP1 check by Western blot, highlighted its involvement in cell viability. Finally, monitoring of MCL-1 expression and SRC phosphorylation suggested that a FGFR2c/PKCε-mediated control of cell migration/invasion via MCL-1/SRC-dependent reorganization of actin cytoskeleton. The identification of PKCε as hub molecule downstream FGFR2c at the crossroad of signaling networks governing the main malignant tumor hallmarks could represent an important advance towards innovative target therapies overcoming RAS.

#47

Ciro Iaccarino • Sassari

LRRK2 and the exocyst complex: possible therapeutic option for Parkinson's disease?

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Parkinson’s Disease (PD) is the second most common neurological disorder after Alzheimer disease. Mutations in LRRK2 gene are the main genetic causes of PD. Up to date, the LRRK2 patho-physiological function is still cryptic although different experimental evidence underlines a prominent role of LRRK2 in the control of vesicle trafficking, and, interestingly, alteration in synaptic vesicle dynamics seems a common pathological mechanism in both familial and idiopathic PD. We have previously demonstrated that LRRK2 interacts with Sec8, a member of exocyst complex and regulates the exocyst complex formation. The exocyst complex is an evolutionarily conserved multisubunit protein complex mainly implicated in tethering secretory vesicles to the plasma membrane. Extending the mentioned results, we demonstrate that Sec8 over-expression rescue the LRRK2 pathological phenotype in continuous neuronal cell line and drosophila models. Moreover, endosidin2, an inhibitor of exocyst complex partially but significantly rescues the LRRK2 G2019S negative effect on neuronal cell differentiation strongly suggesting the vesicle trafficking as a possible therapeutic target for PD treatment.

#48

Giuseppina Iacomino • Portici, NA

Biological effects of biochar-derived smoke-water on insects and plants

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The pyrolysis of organic feedstock yields the solid fraction biochar, bio-oils, and a volatile fraction that can be reused for energetic purposes or technological applications in agro-ecosystems in the form of smoke-water (SW). In this study, we tested SW effects on Spodoptera littoralis, a notorious agricultural pest worldwide , and on strawberry seedlings. We characterized SW by extensive application of mass spectrometry coupled with liquid and gas chromatography, which showed different compounds, including cresol, guaiacol, catechol, syringol and phenol. As for the applications to strawberry plants, the results showed a better yield in terms of fruit weight only at 1% of application. Treatments at 5% and 10% resulted in a reduction of yield. Regarding S. littoralis eggs, no survival with topic application was observed at 100% and 75%. Furthermore, at the same concentrations, no mortality was observed for the L1 larval stage. In conclusion, SW, depending on the concentration used, show notable biological activities and potential applications for both plant growth promotion and insects control. Further studies are planned on the Aphis fabae and on the Bactrocera oleae.

#49

Matilda Iemmolo • Palermo

Alzheimer patients cerebrospinal fluid effects on neurons/astrocytes/microglia co-culture system

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Alzheimer's disease (AD) is the most common form of dementia characterized by the accumulation of β-amyloid plaques, tau tangles, neuroinflammation, and synaptic/neuronal loss, the latter being the strongest correlating factor with memory and cognitive impairment. Through an in vitro study on a neuron-astrocyte-microglia co-culture system, we have analyzed the effects of cerebrospinal fluid (CSF) samples from healthy and AD continuum patients. Morphologically, treatment with CSF from AD patients showed a loss of neurofilaments and spheroids. Suggesting the presence of elements destabilizing the neurofilaments, cellular adhesion processes, and intercellular contacts. Immunofluorescence assays showed an increased expression of p38 and fraktaline by AD CSF compared to the non-AD patients and not treated co-cultures. By Zymography, the expression of proteolytic enzymes was valued in cell extracts and the co-cultures conditioned medium; results indicated MMPs cascade activation by elements present only in the CSF obtained by AD patients. In Q-PCR assays, the expression of the inflammatory transcription factor, MMPs, and other Alzheimer's-related factors, show exciting differences.

#50

Angela Karimpour Ghahnavieh • Rome

GOLPH3 protein controls organ growth by interacting with TOR signaling proteins in Drosophila.

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Golgi phosphoprotein 3 (GOLPH3) is a phosphatidylinositol 4-phosphate effector, mainly localized to the Golgi apparatus and required for Golgi architecture and vesicle trafficking. Human GOLPH3 is highly amplified in several solid tumor types and is associated with enhanced signaling downstream of mTOR (mechanistic target of rapamycin). However, the molecular link between GOLPH3 and mTOR remains elusive.

Our work demonstrates that Drosophila GOLPH3 (dGOLPH3) physically interacts with the mTOR signaling factors Lst8, Rheb, Tctp and 14-3-3ζ and is required for Rheb localization to the Golgi apparatus. In the absence of dGOLPH3, Rheb association to Tctp is reduced and mTOR activation is impaired. Moreover, RNAi-mediated knockdown of dGOLPH3 leads to wing and eye growth defects and enhances the phenotypes associated with depletion of other mTOR components.

Consistent with dGOLPH3 involvement in Rheb-mediated mTORC1 activation, depletion of dGOLPH3 also reduces levels of phosphorylated ribosomal S6 kinase, a downstream target of mTORC1. Overall, our data provide the first in vivo demonstration that GOLPH3 regulates organ growth by directly associating with mTOR signaling proteins.

#51

Rossella La Grotta • Rende, CS

Circulating miR-181a as a novel potential plasma biomarker for multimorbidity burden in the older population

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Chronic low-level inflammation plays a role in many age-related diseases and contributes to multimorbidity and disability in the elderly. In this scenario, inflamma-miRs, a subset of miRNA able to regulate inflammation molecules, appear to be key players. This study evaluated plasma levels of inflamma-miR-181a in relation to age, parameters of health status and indices of multimorbidity in a cohort of 244 subjects aged 65-97 years. Correlation tests and multivariate regression analyses were applied on gender-stratified groups. MiR-181a levels resulted to increase with age in men, and significantly correlated with worsened blood parameters of inflammation (low levels of albumin and bilirubin and high lymphocyte content) in females. Furthermore, miR-181a was positively correlated with the overall multimorbidity burden, measured by CIRS Comorbidity Score, in both genders. These data support a role of miR-181a in age-related chronic inflammation and development of multimorbidity in older adults, indicating that the routes through this miRNA influence health status are likely to be gender specific. We suggest miR-181a as a promising biomarker of health status of the older population.

#52

Lucia Longhitano • Catania

Lactate modulates microglia polarization via IGFBP6 expression and remodels tumor microenvironment in glioblastoma

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Lactic acidosis has been reported in TME including glioblastoma. In TME, signaling molecules, growth factors and metabolites have been identified to induce immune escape. IGFBP6 is expressed during tumor development, and it is involved in immune-escape and inflammation, providing an attractive target for GBM therapy. Here, we studied the crosstalk between lactate and IGFBP6 in TME of GBM. Our results showed that microglia exposed to lactate or IGFBP6 significantly increased MCT1 expression and genes involved in mitochondrial metabolism. We, observed an increase in M2 markers, suggesting a role of lactate/IGFBP6 metabolism in immune-escape. GBM cells lactate-exposed also showed increased levels of IGFBP6 and vice-versa. Such a phenomenon was coupled with a IGFBP6-mediated SHH signaling increase. We, used a GBM zebrafish model, showing an increase in microglia cells and igfbp6 gene expression after lactate exposure. Our results were confirmed by the transcriptomes analysis and by human GBM biopsies, suggesting the existence of a lactate/IGFBP6 crosstalk in microglia, and IGFBP6 expression is regulated by lactate production in GBM cells which in turn modulates microglia polarization.

#53

Francesco Loreto • Florence

Proteome and volatilome profile changes induced in Phaseolus vulgaris by Colletotrichum lindemuthianum infection

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First phases of pathogen infection cause deep changes into the plant. Even if not immediately conducible to phenotype level, plant changes are evident at molecular level, propagating from gene to phenome level. The aim of this work was the characterization, by using high-throughput profiling techniques, of the early changes induced by Colletotrichum lindemuthianum, a major pathogen of common bean, in the proteome and volatilome profiles of a tolerant (cv. Flor de Junio Marcela, FJM) and a susceptible (landrace Negro St. Louis, NSL) bean genotype. Both analyses highlighted differences between genotypes when responding to the infection. Infected FJM accumulated proteins specifically involved in the first line of defense against pathogen such as cell wall reinforcement and protein delivery to plasma membrane, also showing an alteration of proteins involved in stomatal opening. Volatile organic compounds (VOC) emission was 30% lower than in all other plants, may be due to the stomatal closure induced by pathogen invasion. Infected NSL activated a wider range of defensive biochemical responses, several of which could involve release of VOCs priming defensive responses, and showed higher emission of green leaf volatiles (C6 alcohols, aldehydes, and acids), usually representing massive damage in leaves and likely correlated with widespread fungal infection.

#54

Mauro Magnani • Urbino

Superparamagnetic nanoparticles encapsulated in red blood cells as novel contrast agents for diagnostic techniques in the biomedical field

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The application of Superparamagnetic Iron Oxide Nanoparticles (SPION) in drug delivery, magnetic resonance imaging (MRI), hyperthermia and cell labelling and tracking has been well addressed thanks to their inducible magnetic properties (superparamagnetism). SPIONs are rapidly cleared from the circulation by the reticuloendothelial system (RES), with uptake dependent on factors such as the hydrodynamic diameter, electrical charge and surface coating. The aim of the research is the synthesis of new SPIONs, precisely Iron Oxide Nanoparticles (IONPs) and Zinc Manganese Ferrite Nanoparticles (Zn-Mn FNPs) which have the appropriate chemical-physical properties to allow an efficient encapsulation in human Red Blood Cells (RBCs) and the appropriate magnetic properties for use in diagnostic techniques. The RBC encapsulation process aims to obtain SPION-RBC constructs, with a long blood circulation lifetime, which could be promising intravascular imaging contrast agents for MRI and functional MRI (e.g. for measuring cerebral blood volume). Here we present the preliminary results obtained by using this strategy of SPION encapsulation into RBCs.

#55

Andrea Magrì • Catania

NHK1 peptide counteracts mitochondrial accumulation of SOD1 mutant and improves the respiratory profile of a cellular ALS model

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Mutations in Cu/Zn Superoxide Dismutase (SOD1) gene account for about 20% of familial amyotrophic lateral sclerosis (ALS) cases, a fatal neurodegenerative disease that affects motor neurons (MNs). SOD1 G93A mutants aggregate onto the cytosolic surface of mitochondria by binding the Voltage-Dependent Anion Channel 1 (VDAC1). VDAC1 controls apoptosis and the metabolic exchanges of ions, small metabolites and ADP/ATP across the outer membrane. However, in affected MNs, SOD1 G93A impairs VDAC1 activity and the physiological interaction with the glycolytic enzyme Hexokinase 1 (HK1), promoting organelle dysfunction and cell death.

Here we show that a small synthetic HK1-based peptide (NHK1, patent no. EP3430026) is able to counteract cell death in a dose-response manner in ALS cells, and the defective mitochondrial respiration profile, especially the oxygen flows relative to ADP phosphorylation. This correlates with a reduction of SOD1 G93A at the mitochondrial level and an unexpected increase of VDAC1 expression.

Overall, these results provide new insights into the development of interfering peptides to fight mitochondrial dysfunction in ALS and in other neurodegenerative diseases.

#56

Claudia Martini • Pisa

The role of HIF-1α to predict cognitive disorders in OSAS patients: a pilot study

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Obstructive sleep apnea syndrome (OSAS) is a sleep-disordered breathing characterised by fluctuations in blood oxygen levels and often associated with the onset of mild cognitive impairment (MCI). This study aims to investigate possible biomarkers that can discriminate between patients with OSAS and OSAS in comorbidity with MCI. Thus, OSAS patients were recruited, subjected to neuropsychological evaluation and blood sampling. Plasma was used to measure the levels of HIF-1α and neurodegenerative disorders (NDs)-related proteins including β-Amyloid (Aβ), t-Tau and p-Tau. The results obtained show a positive correlation between plasma HIF-1α levels and SatO2 90. Also, HIF-1α positively correlates with Aβ and p-Tau. We then compared the protein levels of HIF-1α and NDs -related proteins between OSAS and OSAS+MCI groups, the latter showing significantly higher plasma levels of these biomarkers. Overall, the current work shed light on the putative role of HIF-1α and NDs-related proteins as candidate markers in contributing to predict MCI in OSAS patients.

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#57

Stefania Martucciello • Fisciano,SA

Tbx1 interacts with Vegfr3 to regulate cardiac morphogenesis

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Tbx1 is the major gene implicated in 22q11.2 deletion syndrome (22q11.2DS), the most common known genetic cause of congenital heart disease (CHD). The clinical phenotype is recapitulated in Tbx1 mouse mutants, including cardiovascular abnormalities affecting the aortic arch, ventricular septum and cardiac outflow tract (OFT). Recent studies have correlated rare variants of VEGFR3, a gene that is regulated by TBX1, to CHD including Tetralogy of Fallot, the most common cardiac defect found in 22q11.2DS patients. We have shown that in mice, TBX1 regulates Vegfr3 in endothelial cells and the two genes interact strongly during cardiac lymphangiogenesis thereby ensuring the correct number, morphology and growth of cardiac lymphatic vessels. We hypothesize that TBX1 and VEGFR3 play essential roles in endothelial-mesenchymal transition, a key process in OFT formation. To test this, we first performed a histological analysis of hearts of Tbx1 conditional Vegfr3 homozygous embryos at E18.5. The results show that Tbx1-driven inactivation of Vegfr3 causes intracardiac anomalies, including defects of the OFT, providing proof of a genetic interaction Tbx1-Vegfr3 in cardiac morphogenesis.

#58

Maria Benedetta Mattei • L'Aquila

Characterization of a novel member of the Arabidopsis berberine bridge enzyme-like protein family that specifically oxidizes cellulose oligomers and mixed-linked β-1→3/ β-1→4-glucans

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Endogenous elicitors of plant defenses, referred to as damage-associated molecular patterns (DAMPs), include fragments derived from the plant cell wall such as oligogalacturonides (OGs), cellodextrins (CDs), xyloglucans, arabinoxylans (AX) and also the newly identified mixed-linked β-1→3/ β-1→4-glucans (MLGs).

Oxidases of these oligosaccharides that dampen their elicitor activity have been recently discovered in Arabidopsis, in particular four OG oxidases and one CD oxidase [CELLOX]. These enzymes belong to the FAD-binding berberine-bridge enzyme-like (BBE-like) family, and the challenge is to understand their substrate specificity and their physiological role.

The activity of the product encoded by the closest CELLOX paralogue BBE23 was characterized upon heterologous expression, showing that its activity is similar to that of CELLOX; thereby the enzymes were renamed CELLOX1 and CELLOX2. Both enzymes oxidize not only CDs but also MLGs, reducing DAMP elicitor activity.

Moreover, a reverse genetic approach was used to investigate the role played by both CELLOX1 and CELLOX2 in immunity against the fungal pathogen B. cinerea.

#59

Luigia Meo • Fisciano (SA)

Characterization of a urokinase receptor variant

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The receptor (uPAR) of the urokinase-type plasminogen activator (uPA) promotes pericellular proteolysis and cell invasion, and, by interacting with vitronectin, integrins and receptors for formylated peptides (FPRs), regulates cell adhesion, proliferation and survival. uPAR expression is strongly up-regulated and is a negative prognostic factor in various cancers.

We demonstrated that the uPAR-3'UTR acts as competitive endogenous RNA (ceRNA) in leukemia cells. We identified three uPAR mRNA variants containing 3'UTR and showed that the uPARΔ5 variant, lacking exon 5, acting as a ceRNA promotes the expression of pro-tumoral factors and increase biological activities in leukemia cell. We are investigating whether the uPARΔ5 mRNA can be translated in protein.

We show that uPAR-negative HEK-293 cells, transfected with uPARΔ5 cDNA, express the uPARΔ5-variant protein, which lacks a integrin binding-site and still exposes the uPA binding domain and the FPR binding site. The expression of uPARΔ5 protein influences the adhesion of HEK-293 cells to ECM components.

The characterization of uPARΔ5 protein will elucidate whether it can contribute to a malignant phenotype as the corresponding mRNA.

#60

Paola Minghetti • Milan

Conditional marketing authorisation: a “lifeboat” for unmet medical needs

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Effective and authorised medicines to be used in emergency situations, such as the Covid-19 pandemic, or for life-threatening pathologies, rare diseases or cancer may not be readily available on the market. To deal with such events, the European legislator has set up regulatory pathways to facilitate the marketing authorisation of medicines of interest to both public health and individuals. One such early access route is represented by the conditional marketing authorisation (CMA), through which, under certain conditions and specific requirements, medicines can be authorised although some data are missing at the time of submission of the dossier. In any case, the risk-benefit assessment of the medicinal product must be positive.

During the pandemic, the CMA has proved, even more, to be a useful and successful tool in addressing unmet medical needs, as it has allowed a faster route to the MA for 6 different Covid-19 vaccines, although some quality and clinical data, such as manufacturing data and vaccination coverage, were missing due to the emergency situation. Applicants fulfilled the obligations set down by EMA, so that most of vaccines’ CMAs have been renewed for another year.

#61

Vittoria Monaco • Naples

Spike S1 domain interactome in non-pulmonary systems: possible novel roles beyond the receptor recognition

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The SARS-CoV-2 virus became rapidly a pandemic event. Spike is the viral glycoprotein that owns the role to recognize and mediate the viral entry into the host cells. It is known that the subunit S1 can bind the host receptor ACE2. It also has been demonstrated that this virus can infect cells expressing low levels of ACE2, indicating the existence of alternative receptors. The investigation of these alternative receptors can better elucidate the pathogenicity of SARS-CoV-2, also in other tissues than lungs, but also highlight other S1 intracellular functions. We investigated the Spike S1 subunit interactomes in different cell lines HK-2, NCM460D, and Caco-2 employing an AP-MS approach. After the pulldown experiment, a shotgun approach was employed for protein identification. The lists of S1 interacting proteins have been filtered by CRAPome software obtaining a final number of putative partners proteins of S1 selected of 80, 67, and 81 proteins for HK-2, NCM460D, and Caco-2, respectively. The proteins were then classified by their functions and biological processes. These preliminary studies highlighted possible novel S1 receptors and also novel intercellular roles of S1.

#62

Shirin Navacci • Rome

The CRL3REN/KCTD11/SALL4/HDAC1 axis promotes Hedgehog-dependent medulloblastoma through GLI1 deacetylation

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Medulloblastoma (MB) is the most common pediatric brain tumor that arises from an aberrant cerebellum development. The Sonic Hedgehog subgroup (SHH-MB) is characterized by mutations in crucial components of SHH signaling and cytogenetic alterations, such as chromosome 17p deletion. The Cullin3 BTB-containing adaptor RENKCTD11 (REN) is a tumor suppressor that maps on 17p. REN is a negative regulator of SHH signaling promoting ubiquitylation and degradation of HDAC1, an activator of the pathway. We identified the Spalt-like transcriptional factor 4 (SALL4) as a new REN interactor. SALL4 is a stemness factor and its overexpression in human malignancies is often associated with poor prognosis. CRL3REN induces SALL4 ubiquitylation and proteasome-mediated degradation. SALL4 binds GLI1 and works in complex with HDAC1 to promote GLI1 deacetylation thus inducing its transcriptional activity. Knockdown of SALL4 inhibits SHH-MB growth both in vitro and in vivo. Our findings highlight the relevance of a key interplay between ubiquitylation and acetylation in the regulation of the SHH pathway and identify SALL4 as a novel CRL3 substrate and promising target for SHH-MB therapy.

#63

Valeria Nele • Naples

Self-Assembling Nanoparticles for RNA Delivery in the Treatment of Cancer

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The use of coding or non-coding RNA has recently emerged as a novel approach for cancer treatment. Among non-coding RNA, microRNAs (miRNA) have been shown to function either as onco-suppressors or as oncogenes; however, systemic delivery of miRNAs requires the use of nanoparticles. We developed self-assembling nanoparticles (SANP) with a calcium phosphate core enclosed by a lipid shell as a novel miRNA delivery platform for the treatment of glioblastoma and metastatic melanoma. SANP can be prepared immediately before use, enabling the encapsulation of miRNAs tailored to the need of a specific patient and paving the way to personalized RNA therapies. We optimized the lipid composition and mixing ratios to achieve miRNA-loaded SANP, which showed hydrodynamic diameters below 200 nm, high miRNA encapsulation, good colloidal stability in serum, and low hemolytic activity. In vitro, SANP were able to effectively deliver miRNAs to different cell lines; furthermore, SANP were shown to efficiently deliver miRNA in various animal models of tumor. These results demonstrate the potential of the SANP technology for RNA delivery, with applications spanning from cancer treatment to vaccines.

#64

Vittoria Nicolis di Robilant • Rome

Inactivation of the nijmegen breakage syndrome protein (NBS1) abrogates SHH-driven cerebellar development/tumorigenesis through the primary cilium

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Medulloblastoma (MB) is the most common pediatric tumor of the developing cerebellum. Of note, mutations in DNA Damage Response (DDR) genes have been associated with MB insurgence. We have recently demonstrated a dual function of NBS1, essential for the maintenance of genomic integrity, in MB development which is contingent to its genetic dosage. Homozygous deletion of NBS1 abrogates cerebellar SHH-driven development and tumorigenesis, suggesting an epistasis of NBS1 function on the SHH pathway.

Given the emerging link between DDR and centrosomes, we raised the hypothesis that NBS1 regulates the SHH pathway through a new uncanonical role on ciliogenesis. We demonstrated that NBS1 loss exclusively in granule cell progenitors (GCPs) abrogates Ptch1-dependent MB formation and impairs the SHH pathway in a cell autonomous manner. Mechanistically, we show that NBS1-deficient cells consistently display severe morphometric alterations in the Primary Cilium (PC). Collectively, our results uncover a possible novel role of NBS1 in regulating ciliary morphology, through which it could influence the activity of the SHH pathway and impact on SHH-driven cerebellar development/tumorigenesis.

#65

Marco Rinaldo Oggioni • Bologna

Ex vivo perfusion of the human spleen to study infection

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The spleen is the main organ involved in clearance of encapsulated bacteria. We have recently shown that invasive infection starts after replication of bacteria in the CD169-positive tissue macrophages (Ercoli Nature Microbiology 2018; Carreno eBioMedicine 2021). Despite a plethora of models, we have an insufficient knowledge of what really happens in the human spleen.

We have approval for normothermic human spleen ex vivo perfusion, where we infect the organ with bacteria and monitor the interaction between the bacteria and tissue macrophages.

Microscopy analysis of seven perfused spleens shows that Streptococcus pneumoniae is rapidly taken up by the spleen and removed from the perfusate. CD163+ red pulp macrophages are associated with 99% of bacteria. We show that CD169+ perifollicular capillary sheath-associated macrophages contain the largest bacterial area, in-keeping with intracellular replication of bacteria as previously shown in mice.

This is the first human-specific study of bacterial interaction with splenic tissue macrophages. The results emphasise the significance of CD169+ macrophages, which we hypothesise playing a significant role in the onset of invasive disease.

#66

Giulia Oliveri • Alessandria

Alessandria biobank: origins, evolution and future scenarios

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A biobank is described as a facility aimed at the collection, management, preservation and distribution of biomaterials and related data for genetic research purposes.
Biobanks represent a bridge between the evolution of personalized medicine and the preservation and improvement of public health in light of the role they play in allowing precisely the preservation of a significant amount of both biomaterial and related data necessary for the advancement of biomedical research.
At the Research Training Innovation Infrastructure of the Azienda Ospedaliera SS Antonio e Biagio e Cesare Arrigo is located the Centre for Biological Collection (CRMB) which includes the collection of malignant mesothelioma, the collection of breast carcinoma, and the biorepository established in 2016 to ensure high levels of quality and safety in the field of storage and preservation of biological samples required by protocols of clinical trials and/or intended for research activities.
The study aims to describe the history of biobanking and the evolution of Alessandria Biobank, the institutionalization and the results achieved to increase biobank’s knowledge and its magnitude.

#67

Simona Paladino • Naples

Endosomal trafficking and synaptojanin 1 (Synj1): a dangerous liaison

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Endosomal trafficking is essential for cellular homeostasis. Synaptojanin 1 (Synj1; 21q22.11 locus) is ubiquitous inositol-phosphatase acting on various phosphoinositides, key regulators of membrane trafficking.

We have recently shown that Synj1 plays a crucial role in the homeostasis and functions of early endosomes (EEs) in different human cell types, including neuronal cells. By applying imaging and biochemical approaches we showed that the knockdown of Synj1 or the pathogenic R258Q mutation, causative of the Parkinson disease 20, leads to the enlargement of EEs and impairment of recycling trafficking. Interestingly, the structure and dynamics of EEs are altered in Down syndrome (DS) fibroblasts, where Synj1 is overexpressed. Moreover, the recycling trafficking is markedly compromised in these cells. Importantly, Synj1 overexpression recapitulates the alterations observed in DS cells, thus highlighting its role in DS pathogenesis.

Overall, our data pointed out that proper levels of Synj1 are critical for cellular physiology. They also suggest a pivotal role of endosomal trafficking and Synj1 for neuronal functionality, highlighting a dangerous liaison with brain disorders.

#68

Gaetana Paolella • Fisciano,SA

Peculiar regulation of ER-stress and autophagy in cells from celiac patients

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Celiac disease (CD) is an inflammatory intestinal disease caused by the ingestion of gluten-containing cereals by genetically predisposed individuals. Constitutive differences between cells from CD and control subjects, including alterations of vesicular trafficking, Ca2+ homeostasis, regulation of type 2 transglutaminase, have been reported. In this work we investigated constitutive differences between CD and control cells regarding the response to the stress agent thapsigargin, an inducer of ER-stress and of autophagy. We employed cultures of skin-derived fibroblasts from CD and control subjects, a suitable model to study differences independent from gluten exposure. When we investigate ER-stress response, we found a slightly higher occurrence of ER-stress markers, GRP78 and spliced XBP1, in CD cells than in controls. Autophagic markers levels (LC3 and p62) suggested that in CD cells, treated with thapsigargin, autophagy was delayed or blocked, whereas normal cells presented a regular autophagic flux. Overall, our findings indicate that CD cells are less capable to adapt to a stress condition and contribute to the comprehension of molecular features of CD cellular phenotype.

#69

Marina Papaianni • Portici, NA

Biological properties of essential oils from Eucalyptus cinerea and Eucalyptus nicholii by-products

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Eucalyptus by-products are rich in bioactive compounds that can be recovered and potentially used in agriculture. This study focuses on E. cinerea F. Muell. ex Benth. (EC) and E. nicholii Maiden & Blakely (EN), analyzes the macro and micromorphological features of the leaves, the chemical composition of essential oils (EOs) and biological activities on microorganisms in vitro. The antimicrobial activity of EO was tested on plant pathogens, bacteria Xanthomonas campestris pv. camprestis (Xcc), Enterobacter cloacae, and Citrobacter freundii, and fungus Fusarium oxysporum. The antibacterial activity depended on the concentration of the EO used, differences were noted depending on the plant species origins, the Minimum inhibitory concentration (MIC), and the target bacteria tested: EO from EC had a MIC of 10-4 (EO dilution) on all three bacteria, whereas EO from EN had a MIC of 10-3 (EO dilution) for Xcc and E. cloacae, and a MIC of 10-2 (EO dilution) for C. freundii. Antifungal activity on Fusarium, was interesting showing that a 10-3 dilution of EO from EN had the highest antifungal activity in comparison to those from EC, demonstrated 90% and 65% of inhibition growth respectively.

#70

Carola Parolin • Bologna

Candida spp. biofilm formation is inhibited by vaginal Lactobacillus supernatants

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Candida spp. infections, including vulvovaginal candidiasis (VVC), are caused by fungal overgrowth and are related to the development of virulence factors. Among these, the formation of biofilm by Candida spp. affects VVC clinical impact and response to antifungal treatments. Lactobacilli, generally dominating healthy vaginal microbiota, can prevent Candida spp. adhesion and growth by various mechanisms.

A panel of Lactobacillus strains of vaginal origin were tested towards Candida albicans and non-albicans clinical isolates. In particular, cell-free culture supernatants (CFS) collected from lactobacilli grown in planktonic (pk-CFS) and adherent form (bf-CFS) were used to challenge Candida isolates. Candida biofilms were analyzed by different and complementary methodologies, i.e. crystal violet staining, MTT assay and confocal microscopy.

CFS recovered from L. crispatus and L. plantarum strains were the most effective in inhibiting Candida spp. biofilm. In particular, bf-CFS from L. crispatus showed the best inhibition profile, highlighting the role of such species as a hallmark of vaginal eubiosis and prompting its employment in the development of fourth-generation probiotics.

#71

Martina Pasqua • Rome

Investigating the relevance of the multidrug resistance efflux pump AcrAB-TolC during the invasive process of Shigella flexneri

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AcrAB-TolC is a multidrug resistance efflux pump (MDR EP), belonging to the resistance nodulation cell-division family, that represents the major efflux pump in Escherichia coli and in other Enterobacteriaceae. AcrAB-TolC functions as a tripartite complex and consists of the inner membrane transporter (AcrB), a periplasmic adaptor (AcrA) and the outer membrane channel (TolC).

Besides extruding a wide range of antibiotics, contributing to the emergence of multidrug resistance phenotypes, AcrAB is known to take part in other relevant processes, including pathogenicity of several bacterial pathogens. For instance, in E. coli and Salmonella AcrAB is involved in the adaptation and colonization of the intestinal tract.

Considering the emerging role of AcrAB in bacterial virulence, we asked whether AcrAB might be involved also in the invasive process of Shigella flexneri, the causative agent of human bacillary dysentery. By analyzing the behavior of mutants defective in acrA and/or acrB, we demonstrate that a functional AcrAB EP is required for S. flexneri to survive and replicate within macrophages and intestinal epithelial cells.

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#72

Valentina Pastore • Rome

Inhibition of colistin resistance in Pseudomonas aeruginosa strains

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Colistin is a cationic peptide that targets electrostatically the lipid A component of lipopolysaccharide. Colistin resistance can occur through lipid A modifications that, by changing its negative charge, inhibit colistin binding. In Pseudomonas aeruginosa the addition of 4-amino-4-deoxy-L-arabinose to lipid A, mediated by ArnT, is the prevalent mechanism of resistance to colistin. Previously, the diterpene BBN149 (10.1093/jac/dkaa200) has been identified as a putative inhibitor of ArnT and demonstrated to act as colistin adjuvant against colistin-resistant P. aeruginosa planktonic cultures. As biofilms are very relevant for the persistence of bacterial infection, we test the colistin adjuvant activity of BBN149 and its derivatives in P. aeruginosa biofilms. Biofilms treated with different combinations of colistin and adjuvants were quantified by crystal violet, metabolic assay and viable cell counting. P. aeruginosa biofilms of reference and clinical strains were tested. The results showed a good activity of colistin against biofilms, which was further improved by the compounds. Interestingly, the adjuvant activity of the compounds was more effective against the clinical strain.

#73

Annalisa Pecoraro • Naples

Novel therapeutic strategy combining β-carotene with 5-FU to overcome uL3-mediated chemoresistance in colorectal cancer cells

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One of the major obstacles for the clinical use of 5-FU is the acquired multidrug resistance (MDR) that frequently occurs during colorectal cancer (CRC) chemotherapy. Our previous data demonstrated that ribosomal protein uL3 status is strictly associated to 5-FU chemoresistance. Carotenoids have shown the ability to improve cancer cell response to cytotoxic drugs providing a safer choice to defeat MDR. The analysis of a cohort of 594 CRC patients revealed a correlation between uL3 expression and both progression-free survival and response to treatment. uL3 low transcriptional state was found to be associated with increased expression of specific ATP-binding cassette (ABC) genes. By using 2D and 3D models of 5-FU resistant CRC cells p53 deleted and stably silenced for uL3, we investigated the effect of a novel therapeutic strategy by combining β-carotene and 5-FU. Our results demonstrated that the combined treatment induced cell cycle arrest and apoptosis and significantly reduced the expression levels of analyzed ABC genes. In conclusion, this study may provide a more effective therapeutic approach to treat CRCs lacking functional p53 and expressing low levels of uL3.

#74

Maria Pelullo • Rome

The activation of Jagged1 signaling by chemotherapeutic agents counteracts the Oxaliplatin/5FU-induced anti-cancer effects: a novel mechanism of drug resistance in CRC

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Colorectal cancer (CRC) is a leading cause of mortality worldwide, characterized by metastasis and unresponsiveness to standard therapy, due to intrinsic or acquired drug-resistance.
Recently, we demonstrated that Krasmut drives the activation of Jag1-ICD oncogene, which favours tumor development and aggressiveness, through a non-canonical mechanism.
Herein, we evaluate the effects of OXP, 5FU and GSIs alone or in combination, on Jagged1 processing, in CRC cell lines. We demonstrate that the anticancer drugs, OXP and 5FU, lead to a massive Jag1-ICD activation that results in the selection of a drug-resistant subpopulation protected from apoptosis, via the activation of Jag1-ICD-dependent pro-survival targets. In addition, GSIs induce the proliferation of Jag1-ICD positive CRC cells, functioning as tumor-promoting agents. Finally, the Jagged1 abrogation in OXP- or 5FU-resistant subpopulations is enough to restore the sensitivity to chemotherapy, confirming that drug resistance is Jag1-ICD-dependent.
Therefore, we suggest Jagged1 as molecular predictive biomarker for the outcome of chemotherapy in CRC patients bearing Krasmut and Jagged1 over-expression.

#75

Riccardo Perriera • Palermo

Nonsense suppression in human cystic fibrosis organoids (CFTRW1282X/dele2,3) by a new translational readthrough inducing drug (NV848) combined to CFTR modulators.

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Cystic Fibrosis (CF) is one of the most widespread autosomal recessive genetic disease caused by mutations in CFTR gene. Nonsense mutations affect about 20% of CF patients in Italy (RIFC-Report 2018), they are due to single-nucleotide substitutions in gene codifying sequences, leading to the formation of premature termination codons (PTC) in the mRNA, and causing the production of truncated and non-functional proteins. A strategy to rescue nonsense mutations is the induction of PTC-readthrough generating a ribosome misreading and the synthesis of a full-length protein. Previously, we claimed that NV848 molecule suppress the presence of nonsense codons in various kind of CF in-vitro models.
To investigate the activity of the NV848 molecule in advanced preclinical CF system, it was tested on human intestinal CF organoids harbouring the W1282X stop mutation. Functional assays showed that NV848 rescues CFTR activity when combined with CFTR modulators.
Finally, we studied the stability of this compound in human liver microsomes in order to evaluate the amount of metabolised molecule, demostrating a good metabolic stability of NV848 molecule respect to the positive control (Ataluren).

#76

Marialuisa Piccolo • Naples

Ruthenium(III) complex chemotherapy efficacy boosting by interfering with cellular iron metabolism

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Iron, an essential nutrient for life, can be considered as double-edge sword. Indeed, this metal is involved in all important physiological activities. Imbalance iron metabolism, on the other hand, is considered an important hallmark of cancer and in particular of breast cancer. Particularly, spatio-temporal accumulation of iron in the tumor microenvironment/niche was related to an increased cancer risk and poor outcome. Fe can stimulate cancer metabolism, alter cellular redox balance, which increases organelle damage, loss of tumor suppressors, oncogene expression and triggers pro-oncogenic signaling. Bearing this concept in mind and knowing that modulation of iron metabolism can significantly change responsiveness of cancer cells to chemotherapeutics, herein, we analyzed the effects of Fe deficiency and/or overload on the effectiveness of anticancer chemotherapy with a new ruthenium-based compound (HoThyRu/DOTAP) in different models of breast cancer in vitro. Moreover, preclinical investigations on the effects of a combination therapy consisting of Ru-based nanosystems and Fe modulators (chelators/donors) on tumor intake/growth as well as on tumor migration were performed.

#77

Angela Pironti • Portici, NA

Treatments of Trichoderma harzianum strain M10 and benzothiazole derivatives on tomato plants (Solanum Lycopersicum) as biostimulant and plant protection products.

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Scientific research looks for new strategies to stimulate plant growth and protect plants from phytopathogens in agricultural production. An alternative strategy can combine the use of Systemic Resistance Inducers (SRI) and beneficial microorganisms from the soil. This study evaluates T.harzianum strain M10 combined with three benzothiazole derivatives CH, BTH (BION, Syngenta) and BTHWA as treatments to improve plant production. Compatibility was determined between the fungus and compounds in vitro, then a greenhouse test was conducted on tomato plants with single and/or combined treatments of M10 (soil irrigation) and compounds (foliar spray). Plant growth promotion was confirmed for plants treated with BTHWA+M10 treatments compared to controls, and these plants were also more resistant to the attack by the bacteria Pseudomonas syringae than those treated with BTHWA alone. A metabolome analysis conducted by LC-MS QTOF, on leaves treated with BTHWA+M10 indicated a higher presence of metabolites involved in plant defence pathways. These results are promising for the development of bioformulations with beneficial microbes and SRI as plant protection products or plant biostimulants.

#78

Daniela Pontiggia • Rome

Oligogalacturonide Oxidases (OGOXs) play a role in plant immunity and development

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Plant immunity can be activated by endogenous elicitors referred to as damage-associated molecular patterns (DAMPs). Typical DAMPs are the oligogalacturonides (OGs), released from the cell wall pectin upon pathogen infection or mechanical injury or during the physiological cell wall remodeling. Four OG oxidases, named OGOX1-4, that belong to the Berberine Bridge Enzyme-like (BBE-like) family have been identified in Arabidopsis. BBE-l enzymes play a role in immunity, likely preventing over-accumulation of DAMPs and consequent deleterious effects on plant health (hyper-immunity). The oxidation reaction produces hydrogen peroxide (H2O2) as a secondary product. To study the role of OGOXs, transgenic plants over-expressing OGOX1 and CRISPR-Cas9 edited single and double ogox1 and ogox2 mutants have been generated. The characterization of these mutants shows that OGOXs play a role in the homeostasis of OGs and in the growth-defense trade-off.

#79

Giuseppe Quaratiello • Pisa

Hyperspectral detection of temporal and spatial development of wheat blast disease

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Reported in Brazil in 1985, wheat blast (WB) disease, caused by the fungus Magnaporthe oryzae pathotype (pt) Triticum, represents a threat to wheat production. Although spike symptoms are distinct, leaf ones are usually inconspicuous and their development is not well understood. This study aimed to elucidate the capability of reflectance spectroscopy (400-2400 nm) to detect the temporal (0-5 days) and spatial (old-young leaves) development of WB on leaves. Plants of wheat cv Bobwhite were inoculated at Feekes 6 and 10 stages with M. oryzae pt Lolium, used as a pt Triticum surrogate, at 0, 1,000, 20,000, and 100,000 spores ml-1 concentrations. At Feekes 6, leaf spectral profiles were statistically different (PERMANOVA, $P < .05$) for the 'inoculum concentration × day' combinations. At Feekes 10, a significant 'inoculum concentration × leaf × day' effect was even observed, and older leaves infected by the highest spore concentration were mostly discriminated already at three days from inoculation (PLS-DA, accuracy: .85), even in the absence of visible symptoms. These differences were confirmed by physiochemical alterations displayed by spectral indices or traits predicted from spectra.

#80

Marta Ranesi • Portici, NA

Beauveria bassiana biocontrol of fungal diseases is independent of rhizosphere acidification

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The biocontrol entomopathogenic fungus Beauveria bassiana (Bb) plays multiple roles in natural agroecosystems including endophytism, plant growth promotion and disease control. Rhizosphere pH alkalinization represents a renowned pathogenicity mechanism of fungal pathogens including F. oxysporum (Fol), the causal agent of tomato vascular wilt disease. To understand if Bb biocontrol activity against fungal pathogens might be related to rhizosphere pH modulation, we characterized the pH acidifying and biocontrol activity in vitro and in vivo of ten Bb isolates. Nine isolates were able to acidify the culture medium and six of them produced a more intense acidification halo around the colony. However, when biocontrol activity was tested in vitro only three highly-acidifying and one non-acidifying isolate inhibited Fol growth. Unexpectedly, when Bb biocontrol activity was evaluated in vivo against Fol, all isolates similarly protected tomato plants from wilting, suggesting that rhizosphere acidification is only one of the biocontrol mechanisms used by Bb in vivo. Further experiments are required to determine which additional mechanisms are involved in Bb biocontrol activity.

#81

Stefano Ricagno • Milan

Ex-vivo structural biology: new insights on systemic amyloidosis

Antonio Chaves-Sanjuan[1], Tim Schulte[2], Giulia Mazzini[3], Valentina Speranzini[1], Francesca Lavatelli[3], Paolo Swuec[1], Eric Zini[4], Mario Nuvolone[3], Giovanni Palladini[3], Giampaolo Merlini[3], Martino Bolognesi[1], Stefano Ricagno[1, 2]

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Systemic amyloidoses is a group of diseases whereby amyloid aggregation targets one or several internal organs. Amyloid deposits localize in the extracellular space and consist of cross-β misfolded protein assemblies. Among this group of diseases, Light chain amyloidosis (AL) is the most common systemic amyloidosis, and cardiac involvement is the most life-threatening form of the disease. AA amyloidosis has been found in humans and in several animal species and typically targets kidney, spleen, and liver.

Recently, single particle Cryo electron microscopy (Cryo-EM) allowed a substantial leap forward in the understanding of the structural properties of amyloid fibrils. Cryo-EM can determine fibrillar structures to nearly atomic resolution not only of fibrils prepared in vitro in research labs but also of fibrils extracted ex vivo from the targeted organs of affected patients (Swuec et al. 2019, Schulte et al. 2022).

Here we show three examples of Cryo-EM structures of ex vivo fibrils from the autoptic hearts of two AL patients and from the kidney of a cat died of AA amyloidosis in a cat shelter.

#82

Anastasia Ricci • Urbino

New human ATM variants are able to regain ATM functions in ataxia telangiectasia disease

Anastasia Ricci, F. Biancucci, G. Morganti, M. Magnani, M. Menotta

Ataxia Telangiectasia (AT) is a rare neurodegenerative disease caused by biallelic mutations in the Ataxia Telangiectasia Mutated (ATM) gene. No cure is currently available for these patients. However, the occasionally discover of positive effects of dexamethasone administered through autologous erythrocytes (EryDex) in a phase II and phase III clinical trials, on neurological symptoms of AT patients, led to a new hope for a plausible treatment. The drug was able to induce an alternative splicing of the native ATM messenger producing shorter transcripts named ATM variants, detected in vivo, in the blood of treated AT patients. In the light of these findings, the use of ATM variants in AT fibroblast cell lines, was studied. ATM variants were capable of partially rescuing ATM activity in AT cells, particularly in the nuclear role of DNA repair, and in the cytoplasmic role of modulating autophagy, antioxidant capacity and mitochondria functionality, all features compromised in AT and essential for neuron survival. Consequently, they may be applicable for the development of innovative therapies, for both gene therapy and gene delivery, for the treatment of AT patients.

#83

Lara Russo • Pisa

Biomimetic scaffolds for tendon/ligament regeneration

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Tendon injury is a clinical problem; after surgical intervention, the tendon-to-bone interface, “the enthesis”, often does not regenerate leading to high numbers of rupture recurrences. Tissue engineering techniques are pursued to improve its regeneration. Herein, we aim to develop an implantable graft using biomimetic scaffolds as a skeleton to improve the mesenchymal stem cells (MSCs) differentiation. Bioresorbable natural (gelatin) or synthetic (PLLA, PLGA or PCL) materials were evaluated. All the scaffolds supported the MSC adhesion and growth even if to different degrees. Interestingly, PLGA per se enhanced tenogenic differentiation. Hence, PLGA and PCL were used to create two inductive scaffolds as a basis for the enthesis creation. The 3D printed PCL grids supported osteoblast differentiation as demonstrated by the increase of mineralized sites stained with alizarine red. The tendon-like pattern was replicated on electrospinning PLGA scaffold as confirmed by the increase of collagen deposition. Overall, the results highlight the possibility to use these scaffolds to improve the tendon/bone interface engineering. This research project is funded by Tuscany Region.

#84

Alessandra Sacchi • Rome

Myeloid-derived suppressor cells from SARS-CoV-2 infected patients contribute to platelets activation and extracellular traps release

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Platelet activation and thrombosis characterize severe COVID-19. The expansion of myeloid-derived suppressor cells (MDSC) was observed during COVID-19 and was associated with disease severity. MDSC are potent suppressors of immune functions. In this work, we evaluated the role of MDSC from COVID-19 patients in platelet activation and extracellular traps (ET) formation.

A lower plasmatic L-arginine level was observed during COVID-19 compared to healthy donors, which correlated with MDSC frequency. L-arginine can control platelets activation as shown by the inverse correlation between L-arginine level and PAC-1 platelet expression. MDSC were able to induce PAC-1 expression in vitro by reducing L-arginine, indicating a direct role of MDSC in platelet activation. Accordingly, we found a positive correlation between ex vivo platelet PAC-1 expression and MDSC frequency. We also found that MDSC were able to extrude ET when cultured with autologous plasma and this effect was independent from platelets.

Overall, we show the involvement of MDSC in platelet activation and ET formation during COVID-19, highlighting a novel role of MDSC in modulating coagulation and micro thrombi generation.

#85

Bernardina Scafuri • Fisciano

Computational strategy to find pharmacochaperones for GALT enzyme

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Severe galactose-1-phosphate uridyltransferase (GALT) deficiency causes classic galactosemia, a rare inborn error of metabolism for which, to date, there is no pharmacological treatment. Recently, a new therapeutic strategy for this disease has been hypothesized involving the use of pharmacochaperones (PCs) that are able to stabilize variants of the human GALT enzyme. According to this hypothesis, we applied a computational approach to identify candidate PCs for classic galactosemia among known PCs and other active molecules.

We selected 5 known PCs and we evaluated their interactions with wild-type and mutant p.Gln188Arg GALT enzyme, both in the active sites and in the central cavity at the interface of the two subunits forming the enzyme, where we inferred the presence of two possible allosteric sites by means of computational predictors. These results were used to extract pharmacophore models, which allowed us to select further 19 possible PCs, with which molecular docking simulations on the two allosteric sites were performed, with promising results. Experimental studies are ongoing to confirm our predictions and to further expand our search.

#86

Rosario Luigi Sessa • Rome

Role of autophagy in Fibrodysplasia Ossificans Progressiva (FOP) disease

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Fibrodysplasia Ossificans Progressiva (FOP) is a rare disease caused by a recurrent heterozygous activating mutation of activin receptor A, type I/activin-like kinase 2 (ACVR1/ALK2), a bone morphogenetic protein (BMP) type I receptor. The canonical FOP mutation (R206H) exhibits loss of auto inhibition of BMP signalling, that results in constitutive active ACVR1 signalling and correlates with enhanced chondrogenic differentiation. Although gain-of-function ACVR1 mutations are identified as the sole genetic cause of FOP, the molecular mechanism involved in the effect of the mutant ACVR1 is still under investigation. Autophagy is an essential pathway necessary to maintain cartilage homeostasis, in particular in the hypoxic environment necessary for chondrocytes' growth in vivo. Herein, we provide, for the first time, evidences of a dysregulation of autophagy signalling in ATCD5 cells expressing mutant FOP receptor. Moreover we demonstrate that reactivation of autophagy could deregulate chondrocytes differentiation of ATCD5 cells expressing mutant receptor. Overall, our data support the idea that modulation of autophagy could be beneficial for FOP patients.

#87

Chiara Siniscalchi • Caserta

Human microRNAs binding to SARS-CoV-2 sequences: computational analysis and experimental validation

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MicroRNAs (miRNA) play an emerging and important role in the interplay between viruses and host cells. This study started by a bioinformatics prediction of cellular miRNAs potentially targeting viral RNAs; then, a number of criteria also based on experimental evidence and virus biology were applied, giving rise to eight promising binding miRNAs. Their interaction with viral sequences was experimentally validated by transfecting luciferase-based reporter plasmids carrying viral target sequences into lung cell lines, leading to select five out of the eight potential binding sites for their responsiveness to endogenously expressed miRNAs. Co-transfection of the reporter plasmids along with miRNA mimics further supported the interaction between miR-219a-2-3p, miR-30c-5p, miR-378d, miR-29a-3p, miR-15b-5p, and SARS-CoV-2 viral sequences. miR-29a-3p and miR-15b-5p were also able to repress plasmid-driven Spike expression. Importantly, the viral target sequences are fully conserved in Beta, Delta and Omicron SARS-CoV-2 variants. The evaluation of possible anti-viral activity of the selected miRNAs is now ongoing on an in vitro cell models for virus replication.

#88

Chiara Stefani • Verona

Increased prevalence of HLA-C unstable variants in HIV-1 rapid progressor patients

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Based on previous studies showing that less expressed/unstable HLA-C variants are associated with poor HIV-1 control and increased HIV-1 infectivity, we investigated whether there is a correlation between different stages of HIV-1 progression and presence of specific HLA-C allotypes. HLA-C genotyping was performed by allele-specific PCR by analysing a treatment-naïve cohort of 96 HIV-1 infected patients from USA, Canada, and Brazil. HIV-1-positive subjects were classified according to their disease progression status as Progressors (n = 48), Long-Term Non-Progressors (n = 37) and Elite Controllers (n = 11). HLA-C variants were classified as stable or unstable based on their binding stability to β2-microglobulin. Our results showed a significant correlation between rapid progression to AIDS and the presence of two or one HLA-C unstable variants (p-value: 0.0078, p-value: 0.0143, respectively). These findings strongly suggest a link between unstable HLA-C variants at both genotype and at alleles level and rapid progression to AIDS. This work provides further insights into the impact of host genetic factors on AIDS progression.

#89

Simona Titoli • Palermo

Exploring specific antisense oligonucleotides (ASOs) fused to ADARs recruiting domain to restore CFTR nonsense mutation

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Cystic Fibrosis (CF) is an autosomal recessive disease, caused by loss-of-function mutations of the CFTR gene. About 10% of CF patients worldwide carry CFTR nonsense mutations generating a premature termination codon (PTC) in the mRNA, responsible for a shortened, non-functional protein. Currently, there is no pharmaceutical able to restore the nonsense mutations. In this regard, we are investigating the possibility of rescuing CFTR nonsense mutations by sequence-specific RNA editing. In this approach, specific antisense oligonucleotides (ASOs) are designed to be complementary to the mutated region of the CFTR mRNA except for a cytidine opposite the target adenosine causing the stop mutation. The generated mismatch promotes the recruitment of endogenous Adenosine Deaminases Acting on RNA (ADAR) that edits the target adenosine to inosine, which is decoded as guanosine by ribosomes, thus converting the PTC UGA into UGG. By transfecting CFF-16HBEge CFTR W1282X and CFTR G542X cells with specific ASOs, our results show an increase of CFTR in respect to the untreated mutated cells, suggesting that this system is able, in different cell lines, to restore the CFTR full-length protein.

#90

Paola Trono • Monterotondo, RM

hMENA isoforms regulate cancer specific Type I IFN signaling and extrinsic mechanisms of resistance to immune checkpoint blockade

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The actin cytoskeleton regulatory protein hMENA, along with its isoforms, is a key signaling hub in different solid tumors, and when expressed in tumor cells with the epithelial specific hMENA11a isoform, identifies NSCLC patients with prolonged disease free survival.

Herein we defined a new role for hMENA11a in controlling Type I IFN signalling, lately emerged as determinant of ICB resistance, when chronically stimulated in cancer cells. NSCLC cell lines specifically depleted for hMENA11a produce different inflammatory mediators including IFNβ via RIG-I (retinoic acid-inducible gene I), and this sustains the increase of tumor PD-L1 levels and favors a pro-tumor behavior of macrophages. A gene signature was derived identifying a unique subset of macrophages associated with poor survival in LUAD patients.

Notably, in a clinical setting of NSCLC-ICB treated patients, low expression of hMENA11a and high expression of IFN target genes identify fast progressor patients.

Overall we found that the actin cytoskeleton regulator hMENA11a affects cancer cell intrinsic IFN signaling and modulates tumor microenvironment, providing insight into novel mechanisms of resistance to ICB in NSCLC.

#91

Romualdo Varricchio • Rome

Human serum albumin protective role in SARS-CoV-2 infections

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Numerous clinical studies have shown that COVID-19 patients with plasma human serum albumin (HSA) levels below 35 g/L (hypoalbuminemia) display high severity and mortality risk. Here we studied whether HSA could recognize and bind the spike protein expressed on the surface of SARS-CoV2 virus. Molecular docking results suggest the interaction of domain I and III of HSA with RBM region of spike, region also involved in the interaction with ACE2 receptor. Biochemical assays confirmed a direct interaction between HSA and spike, with a Kd value in the micromolar range. These results suggest that HSA compete with ACE2 for the recognition with spike. We also investigated if HSA could regulate virus entry into human cell by controlling ACE and ACE2 receptor levels. We observed that HSA induces a significant increase in the expression levels of ACE2, AT2 and MAS, i.e., the components of the alternative RAS pathway responsible for the activation of an anti-thrombotic, anti-inflammatory and vasodilator response, in contrast to the SARS-CoV-2-dependent effects. The alteration of those protective mechanism are responsible for the severity and mortality of COVID-19 in hypoalbuminemic condition.

#92

Mariavittoria Verrillo • Portici, NA

Antiflammatory activity and potential dermatological applications of characterized humic acids from a lignite and a green compost

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Some natural organic derivatives such as humic acids (HA) can be used to treat inflammatory processes due to their recognized antiviral, antimicrobial, profibrinolytic, anti-inflammatory and estrogenic properties. In this study, two different HAs isolated from lignite (HA-LIG) and composted artichoke waste (HA-CYN) were characterized by NMR spectroscopy, infrared spectrometry, TMAH-GC-MS pyrolysis, and molecular exclusion liquid chromatography (HPSEC), while the evaluation of anti-inflammatory properties was carried out following treatment of HaCat cells with atmospheric particulate matter. The evaluation of gene expression of the cytokines IL-6 and IL-1β showed a significant decrease following treatment with HA-LIG, suggesting a greater anti-inflammatory power of the latter material compared to HA-CYN. The specific combination of (i) hydrophobic protective HA components, (ii) metastable conformational arrangements, and (iii) releasable bioactive molecules, suggests a novel application of humic extracts in the dermatological field as skin protectants and/or as anti-inflammatory agents in response to stress induced by atmospheric particulate matter.

#93

Daniele Viavattene • Turin

STAT3-mediated regulation of ER calcium release and apoptosis in triple negative breast cancer cells, downstream of mTOR and upstream of IP3R3

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We have recently described STAT3 ability to control ER Ca2+ release and Ca2+-mediated apoptosis in STAT3-dependent Triple Negative Breast Cancer (TNBC) cells localizing to the Endoplasmic Reticulum (ER), where it interacts with the Ca2+ channel IP3R3. Here STAT3, when phosphorylated on Serine 727 (S-P), triggers IP3R3 degradation. Accordingly, STAT3 and IP3R3 protein levels are inversely correlated in basal-like BC, where STAT3 is often constitutively activated.

To investigate the mechanisms regulating STAT3 S-P at the ER, we searched for STAT3 interactors known to localize to the ER, and selected mTOR as the most promising one. STAT3-mTOR interaction was confirmed in human TNBC cells, and we could show that pan-mTOR inhibition prevents both IP3R3 degradation and Ca2+-mediated apoptosis, correlating with impaired maintenance of STAT3 S-P at late time points. mTOR thus acts as a late STAT3 serine kinase, while ERK is involved in early phosphorylation event. Understanding the role of mTOR complexes in ER-STAT3 activation and Ca2+-mediated apoptosis may reveal novel targeted approaches disrupting apoptotic resistance in STAT3-dependent TNBC cells.

#94

Francesco Vieceli Dalla Sega • Lugo, RA

Clonal hematopoiesis of indeterminate potential is associated with increased immune infiltration in the calcified aortic valve

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Clonal hematopoiesis of indeterminate potential (CHIP) is defined as the presence of clones of blood cells carrying cancer related mutations in the absence of symptomatic hematological disease. Recent findings have shown that CHIP increases the risk of atherosclerosis and cardiovascular mortality. The present study aimed to evaluate the possible association between CHIP and aortic valve stenosis and to investigate the pathological mechanism of calcific aortic valve disease (CAVD) in CHIP carriers. We screened 168 CAVD patients undergoing aortic valve replacement for the presence of CHIP by sequencing DNA of the blood cells and investigated the effect of CHIP on 1-year mortality. In addition, to reveal possible mechanisms linking CHIP to CAVD, we compared the aortic valve transcriptome of patients with or without CHIP. We found that CHIP is common in patients with CAVD, high immune infiltration in the aortic valve frequently occurs in CHIP patients, and it is associated with a poor prognosis. Our results suggest that excessive inflammatory response in CHIP patients may participate in CAVD pathophysiology and point to B cells as possible new players in CHIP-induced inflammation.

#95

Stefania Vitale • Portici, NA

Carbon and nitrogen sources regulate extracellular pH modulation and biocontrol activity in Trichoderma

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Trichoderma spp are widely used biocontrol agents in agriculture. To understand if environmental conditions influence extracellular pH modulation, production of the antimicrobial metabolite harzianic acid (HA) and biocontrol activity, the Trichoderma harzianum isolate M10 was grown in different media. Here, we found that in rich but not in poor media M10 initially induces a slight extracellular acidification followed by a strong alkalinization at later time points. Since rhizosphere pH alkalinization is a renowned virulence mechanism used by plant pathogens, we decided to test if different concentrations of carbon sources (glucose/sucrose) or different nitrogen sources (nitrate/ammonia) influence M10 extracellular alkalinization and bicontrol activity in vitro. Our results suggest that M10 mediated alkalinization is independent of the carbon source but not of the nitrogen source supplemented to the medium. Indeed, ammonia supplements reverted extracellular alkalinization to acidification. Importantly, ammonia-mediated acidification also results in higher biocontrol activity. Further experiments are required to correlate M10 HA production, pH modulation and biocontrol activity.

#96

Tiziana Zotti • Benevento

Peptides to discriminate individual antibody response: lessons from COVID-19 pandemic

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The antibody (Ab) response to SARS-CoV-2 antigens may affect clinical severity, serological diagnosis, vaccine efficacy and long-term protection to COVID-19. Recently, we have developed an Enzyme-linked Immunosorbent Assay (ELISA) by selecting 7 synthetic peptides derived from the Spike, Membrane, and Nucleocapsid protein sequences of SARS-CoV-2. This assay is effective in detecting specific Abs raised following natural infection. More interestingly, 4 of the S-derived peptides have been used to evaluate the Ab response at distinct time points after vaccination in individuals that received specific vaccines. Altogether, the peptide-based assays detect different reactivity to the viral peptides in the humoral response mounted by single subjects, proving that both infection and vaccination elicit a heterogeneous polyclonal response with individual features. Also, consistently with other findings, we have observed that sera from individuals who received the single dose vaccine are less reactive towards peptides from S1 subunits respect to fully vaccinated individuals, which, instead, show a broader reactogenicity pattern towards S1-derived peptides and a higher neutralizing capacity.

#97

Guendalina Zuccari • Genoa

Fenretinide-loaded mesenchymal stem cells-derived extracellular vesicles as novel drug carriers against human neuroblastoma

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Fenretinide (4-HPR) is a synthetic retinoid with little adverse effects, whose antitumor efficacy has been widely demonstrated. Unfortunately, clinical responses both in adult and pediatric patients are partial, revealing a limited activity of 4-HPR against existing disease. The underlying causes of this slight therapeutic efficacy consist in its poor water solubility, low bioavailability and high first-pass hepatic effect. As a result, new formulations are required to improve clinical outcomes. To this end, we propose a new 4-HPR formulation based on mesenchymal stem cells derived extracellular vesicles (EVs) as endogenous carriers for drug delivering. After isolation from human umbilical cord, the mesenchymal stem cells were treated with high doses of 4-HPR to achieve a passive drug loading. The resulting 4-HPR-EVs were collected, purified by ultracentrifugation and characterized for size, concentration. The drug amount encapsulated into the vesicles was determined by HPLC and to estimate the analyte recovery the internal standard N-(4-ethoxyphenyl)-retinamide was synthetized by us. We evaluated the EV uptake and efficacy on neuroblastoma cell lines cultured both in 2- and 3-D.