62° CONGRESS SIB

7-9 SEPTEMBER *FIRENZE*

POLO DIDATTICO MORGAGNI - UNIVERSITÀ DI FIRENZE

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ABSTRACT BOOK

SOCIETÀ ITALIANA DI BIOCHIMICA E BIOLOGIA MOLECOLARE - 1951 -

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INVITED SPEAKERS

Paola Branduardi, Università Milano Bicocca

Christian Cambillau, University College Cork

Serena Carra, Università di Modena e Reggio Emilia

Maria Rosa Ciriolo, Università di Roma "Tor Vergata"

Francesca Cutruzzolà, Università La Sapienza di Roma

Bart De Strooper, University of Leuven and University College London

Ivano Eberini, Università degli Studi di Milano Statale

Valeria Giosafatto, Università degli studi di Napoli Federico II

Andrea Huwiler, University of Bern

Mauro Maccarrone, Università degli studi dell'Aquila

Sandra Maceido Ribeiro, Instituto de Biología Molecular e Celular, Universidade do Porto

Mauro Magnani, Università degli studi di Urbino

Massimiliano Mazzone, KU Leuven

Saverio Minucci, Università degli studi di Milano Statale

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INVITED SPEAKERS

Mike Murphy, University of Cambridge, UK

Mario Nuvolone, Uni¬versità di Pavia

Anna Maria Porcelli, Alma Mater Studiorum Università di Bologna

Alessandro Prinetti, Università degli studi di Milano Statale

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Ildikò Szabò, Università degli studi di Padova

Saverio Tardito, CRUK Beatson Institute, Glasgow

Gian Gaetano Tartaglia, Università La Sapienza di Roma e IIT

Peter Tompa, IB-VUB Center for Structural Biology, Vrije Universiteit Brussel

Matthew G. Vander Heiden, MIT Boston, USA

Eleftheria Zeggini, Institute of Translational Genomics, Helmholtz Muni¬ch

62° SIB CONGRESS

SELECTED SHORT TALKS

Domenica Scumaci, Magnae Grecia University of Catanzaro

Simona Fontana, University of Torino

Marina Bacci, University of Firenze

Lorena Pochini, University of Calabria

Francesca Paoletti, CNR Trieste

Rosario Avolio, University of Naples Federico II

Valentina Giorgio, University of Bologna

Teresa Rossi, AUSL-IRCCS Reggio Emilia

Marta Alberti, University of Piemonte Orientale

Giulia Babbi, University of Bologna

Chiara Damiani, University of Milano-Bicocca

Rosanna Culurciello, University Naples Federico II

Greta Bianchi, University of Milano-Bicocca

Gianluca Molla, University of Insubria

Nicola Curci, IBBR-CNR Naples

Francesca Lavatelli, University of Pavia

Adele Di Matteo, University La Sapienza Rome

PROGRAMME

7th THURSDAY

11.00-13.00 REGISTRATION of PARTICIPANTS

13.00-13.10 Congress Opening and Welcome

AUDITORIUM

13.10-13.55 **"FEBS EDUCATION NATIONAL LECTURE"**
Chair: Prof. Francesco Malatesta (University of Rome La Sapienza

Teaching and Learning: focus on innovative technologies and methodologies for student engagement
Prof. Ivano Eberini – University of Milan

14.00-14.05 **Welcome Address Rettrice Università di Firenze**
Prof. Alessandra Petrucci

14.05-15.00 **"Eraldo Antonini" Lecture**
Chair: Prof. Martino Bolognesi (University of Milan)

Structure prediction of proteins and largecomplexes with AlphaFold2
Prof. Christian Cambillau -University College Cork

15.00-15.05 **FEBS Congress 2024 Announcement -** *Prof. Mauro Magnani*

15.05-15.30 **Coffee Break**

15.30-17.15 **Plenary Symposium**

CANCER METABOLISM AND EPIGENETICS
Chairs: Prof. Andrea Morandi (University of Florence)
Prof. Ferdinando Chiaradonna (University of Milan Bicocca)

AUDITORIUM

15.30-15.55 > Mitochondrial bioenergetics contribution to ovarian cancer progression
Prof.Anna Maria Porcelli – University of Bologna

15.55-16.20 > Activation of PP2A modulates the DNA damage response
Prof. Saverio Minucci – IEO and University of Milan

16.20-17.00 > Influences of metabolism on cell division and cancer progression
Prof. Matthew G. Vander Heiden – MIT Boston

Selected Short Talks:

17.00-17.15 Epigenetics meets metabolism: Phospo-DJ1 at the cross-road, a novel player in epigenetic misregulation
(Domenica Scumaci – Università di Catanzaro)

17.15-17.30 C/EBP-β splicing determines chemoresistance in non-small cell lung cancer via metabolic rewiring
(Simona Fontana – Università di Torino)

19.30 Welcome Cocktail in Giardino dei Semplici

PROGRAMME

8th FRIDAY

9.00-10.45 **2 Parallel Symposia**

SYMPOSIUM 1
NOVEL INSIGHT INTO THE ROLE OF LIPIDS IN CELL COMMUNICATION

Chairs: Prof. Paola Bruni (University of Florence)
Prof. Cesare Indiveri (University of Calabria)

ROOM A

9.00-9.25 > Sphingosine 1-phosphate in chronic kidney disease: novel mechanistic insights
Prof. Andrea Huwiler – University of Bern

9.25-9.50 > Sphingolipids orcHestrating the cross-talk between different cell populations in the repair of damaged myelin
Prof. Alessandro Prinetti – University of Milan

9.50-10.15 > Functional Interaction between Endocannabinoids and Additional Bioactive Lipids
Prof. Mauro Maccarrone – University of L'Aquila

Selected Short Talks:

10.15-10.30 Acetyl-CoA carboxylase 1 controls lipid droplets-peroxisomes metabolic axis and is a vulnerability of ER+ breast cancer resistant to estrogen deprivation
(Marina Bacci – Università di Firenze)

10.30-10.45 Emerging role of Cholesterol in membrane transporters function
(Lorena Pochini – Università della Calabria)

SYMPOSIUM 2
UNLEASHING NOVEL PROTEIN FUNCTIONS: MOONLIGHTING PROTEINS

Chairs: Prof. Paolo Paoli (University of Florence)
Dr. Riccardo Miggiano (University of Eastern Piedmont)

ROOM B

9.00-9.25 > Riboregulation of serine hydroxymethyltransferase, a novel mechanism controlling serine metabolism across cellular compartments
Prof. Francesca Cutruzzolà – University of Rome La Sapienza

9.25-9.50 > Glutamine metabolism in liver physiology and cancer
Dr. Saverio Tardito – CRUK Beatson Institute Glasgow

PROGRAMME

8th FRIDAY

Selected Short Talks:

9.50-10.05 Differential Interactions between ATP and NGF / proNGF: Chance or Necessity?
(Francesca Paoletti – CNR Trieste)

10.05-10.20 Cytosolic and mitochondrial translation elongation are coordinated through the molecular chaperone TRAP1 for the synthesis and import of mitochondrial proteins
(Rosario Avolio – Università di Napoli Federico II)

10.20-10.35 The dual role of the mitochondrial protein IF1 in cancer
(Valentina Giorgio – Università di Bologna)

10.45 – 11.15 **Coffee Break**

11.15 – 13.00 **2 Parallel Symposia**

SYMPOSIUM 1
REDOX BALANCE AND MITOCHONDRIA IN HEALTH AND DISEASE
Chairs: Prof. Luigi Palmieri (University of Bari)
Prof. Francesca Cencetti (University of Florence)
ROOM A

11.15-11.40 > Mitochondrial superoxide in ischemia-reperfusion injury
Prof. Mike Murphy – University of Cambridge (UK)

11.40-12.05 > A redox-cycler based therapeutic strategy against mitochondrial diseases
Prof. Ildikó Szabó – University of Padua

12.05-12.30 > Reprogrammed mitochondria as a central hub in cancer cell metabolism and anticancer therapeutic intervention
Prof. Maria Rosa Ciriolo – University of Rome "Tor Vergata"

Selected Short Talks:

12.30-12.45 Dihydroorotate dehydrogenase as innovative target for host and pathogen-directed therapies
(Marta Alberti – Università del Piemonte Orientale)

12.45-13.00 BRD4 inhibitors JQ1 and OTX015 promote cell apoptosis by altering mitochondria dynamics and shifting metabolisms from glycolysis to OXPHOS
(Teresa Rossi – AUSL Reggio Emilia)

PROGRAMME

8th FRIDAY

SYMPOSIUM 2
MULTI-OMICS AND SYSTEMS APPROACHES IN BIOCHEMISTRY

Chairs: Prof. Gabriella Tedeschi (University of Milan)
Prof. Marco Vanoni (University of Milan Bicocca)
ROOM B

11.15-11.40 > Unbiased functional screenings and complexomic pipelines to elucidate mitochondrial dynamics and contact sites
Prof. Luca Scorrano – University of Padua

11.40-12.05 > Translational genomics of osteoarthritis
Prof. Eleftheria Zeggini – Inst. Of Translational Genomics, Helmholtz Munich

12.05-12.30 > From interaction to function; untargeted identification of protein-metabolite interactions to learn about metabolites' functions
Dr. Aleksandra Skirycz – Max Planck Institute

Selected Short Talks:

12.30-12.45 The DAR database: mapping disease-related enzymes to Reactome pathways
(Giulia Babbi – Università di Bologna)

12.45-13.00 Challenges and advances in omics data integration in constraint-based models of metabolism
(Chiara Damiani – Università di Milano Bicocca)

13.00-15.00 **Light Lunch and Poster Exhibition**
ATRIUM

15.00-16.45 **2 Parallel Symposia**

SYMPOSIUM 1
STRESS GRANULES AND OTHER MEMBRANE AND MEMBRANELESS ORGANELLES

Chairs: Prof. Cristina Cecchi (University of Florence)
Prof. Silvio Tosatto (University of Padua)
ROOM A

15.00-15.25 > Targeting the phase separation of dipeptide repeats in ALS/FTD: a new modality in drug development
Prof. Peter Tompa – Vrije Universiteit Brussel

15.25-15.50 > Protein - RNA interactions and phase separation
Prof. Gian Gaetano Tartaglia – University of Rome La Sapienza e IIT

PROGRAMME

8th FRIDAY

15.50-16.15 > Protein quality control of stress granules: implication in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia
Prof. Serena Carra – University of Modena and Reggio Emilia

Selected Short Talks:

16.15-16.30 Stress-related role of a new angiogenin NLS mutant in the stress response of human keratinocytes (HaCaT)
(Rosanna Culurciello – Università di Napoli Federico II)

16.30-16.45 Charge clustering and LLPS: what is the link?
(Greta Bianchi – Università di Milano Bicocca)

SYMPOSIUM 2
EMERGING FIELDS IN BIOTECHNOLOGIES
Chairs: Prof. Gianna Ferretti (Marche Polytechnic University)
Prof. Chiara Schiraldi (University of Campania)
ROOM B

15.00-15.25 > New treatments for rare diseases: Engineering RBC with new metabolic pathways
Prof. Mauro Magnani – University of Urbino

15.25-15.50 > Tackling the challenge of plant secondary metabolites productions in yeasts by leveraging the potential of biodiversity and synthetic biology
Prof. Paola Branduardi – University Milan Bicocca

15.50-16.15 > From wood waste to the manufacturing of tailored bioplastics
Prof. Valeria Giosafatto – University Naples Federico II

Selected Short Talks:

16.15-16.30 Enzymatic conversion of group A red blood cells to the universal donor group O
(Nicola Curci – IBBR - CNR)

16.30-16.45 Engineered polyethylene terephthalate hydrolyzing enzymes as key enabling tools for a sustainable conversion of waste plastics to high added-value compounds
(Gianluca Molla – Università dell'Insubria)

PROGRAMME

8th FRIDAY

16.45-17.15	**Coffee Break**
17-15-18.00	**KEYNOTE LECTURE** *Chair: Prof. Paola Chiarugi (University of Florence)* **Harnessing tumor metabolism to overcome immunosuppression** *Prof. Massimiliano Mazzone – University of Leuven* **AUDITORIUM**
18.00-19.30	**SIB ASSEMBLY** **AUDITORIUM**
20.30	**Social Dinner in Citrarium Villa Le Fontanelle**

PROGRAMME

9th SATURDAY

9.30-11.30 **Plenary Symposium**
PROTEIN AGGREGATION AND DEGENERATIVE DISEASES
Chairs: Prof. Fabrizio Chiti (University of Florence)
Prof. Martino Bolognesi (University of Milan)
AUDITORIUM

9.30-9.55 > Breaking the amyloid chain with small molecule inhibitors: A path for Spinocerebellar ataxia TYPE 3 therapies
Prof. Sandra Macedo Ribeiro – IBMC, University of Porto

9.55-10.20 > Sequencing sick molecules: diagnostic and therapeutic implications
Prof. Mario Nuvolone - University of Pavia

10.20-10.45 > Ex-vivo structural biology: new insights on systemic amyloidosis
Prof. Stefano Ricagno – University of Milan

Selected Short Talks:

10.45-11.00 The KH domains of FMRP: exploring folding, aggregation properties, and pathological variants.
(Adele Di Matteo – CNR Roma)

11.00-11.15 Immunoglobulin light chain (AL) amyloidosis: towards a physiological fibrillogenesis model
(Francesca Lavatelli – Università di Pavia)

11.30-12.00 **Coffee Break**

12.00-12.45 **KEYNOTE LECTURE**
Chair: Prof. Fabrizio Chiti (University of Florence)
From basic insight to therapies in Alzheimer's Disease
Prof. Bart de Strooper – University of Leuven - University College London
AUDITORIUM

12.45-13.30 **Closing Remarks and poster/presentation prizes**

62° SIB CONGRESS

INVITED SPEAKERS and KEY LECTURES ABSTRACTS

TACKLING THE CHALLENGE OF PLANT SECONDARY METABOLITES PRODUCTIONS IN YEASTS BY LEVERAGING THE POTENTIAL OF BIODIVERSITY AND SYNTHETIC BIOLOGY

Paola Branduardi, Immacolata Serra, Valeria Mapelli, Letizia Maestroni, Riccardo Milanesi, Pietro Butti, Stefano Bertacchi, Vittorio Giorgio Senatore
IndBioTech Lab, Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, 20126, Milan - Italy
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The microbial production of complex plant secondary metabolites represents a challenge and an opportunity that in the last decade is more and more promising to move these processes from lab to pilot scale. In fact, in many cases the proof of concept of the heterologous production is achieved, and the yeast *Saccharomyces cerevisiae* is often the platform of choice, especially for complex molecules. Moving from lab-scale to market readiness level, however, requires many optimization steps. They include the assessment of diverse genes to exploit natural biodiversity and accompanying the host requirements, the monitoring and targeting of enzyme localization, the construction of stable but flexible strains. We developed the Easy Modular Integrative fuSion-ready Expression (Easy-MISE) toolkit, which is a novel combination of synthetic biology tools based on a single Golden Gate multiplasmid assembly meant to further ameliorate the rational predictability and flexibility of the process of yeast engineering. Here we show with a couple of case studies how the developed toolkit can accelerate the construction and the analysis of intermediate and final engineered yeast strains. By assessing different combination of endogenous and heterologous gene expression and by exploiting biodiversity, we demonstrated that we can better characterize the heterologous biosynthetic pathway in the final host, more rationally select the metabolic route of choice and, overall, improve the fermentation performances.

Structure prediction of proteins and large complexes with AlphaFold2

Christian Cambillau

School of Microbiology, University College Cork, Cork, Ireland
Laboratoire d'Ingénierie des Systèmes Macromoléculaires (LISM), Institut de Microbiologie, Bioénergies et Biotechnologie (IM2B), CNRS and Aix-Marseille Université UMR7255, Marseille, France
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The release of the powerful AlphaFold2 (AF2) software in mid-2021 revolutionised structural biology. AF2 makes it possible to accurately predict the structures of proteins and their complexes. We reason that AF2 may be an appropriate method to study host adhesion devices of bacteriophages (phages) belonging to the Siphoviridae family that possess a long flexible tail. The flexibility of this tail, formed of multi-domain proteins, limits structural analyses as a whole by experimental approaches such as X-ray crystallography and electron microscopy. Recently, we applied AlphaFold2 prediction to the study of different adhesion devices of phages infecting Gram-positive bacteria such as Oenococcus oeni (1), Lactobacillus (2), Streptococcus (3), Lactococcus (4) as well as the monoderm Mycobacterium (5). I will present the specific approaches to predict proteins, domains and phages' adhesion devices structures and the perspectives of predicting the whole structure of a bacteriophage.

(1) Goulet A, Cambillau C. Structure and Topology Prediction of Phage Adhesion Devices Using AlphaFold2: The Case of Two Oenococcus oeni Phages. 10. Microorganisms, 2021/10/24 ed. 9 (2021).
(2) Goulet A and Cambillau C., Present impact of AlphaFold2 revolution on structural biology, and an illustration with the structure prediction of the bacteriophage J-1 host adhesion device, Frontiers in Molecular Biosciences, 9 (2022).
(3) Goulet A, Joos R, Lavelle K, Van Sinderen D, Mahony J, Cambillau C. A structural discovery journey of streptococcal phages adhesion devices by AlphaFold2. Frontiers in Molecular Biosciences 9:960325 (2022).
(4) Goulet A, Mahony J, Cambillau C, Van Sinderen D, Exploring the structural diversity among adhesion devices encoded by lactococcal P335 phages with AlphaFold2, Microorganisms, 10 2278 (2022).
(5) Cambillau C and Goulet A, Exploring host-binding machineries of mycobacteriophages with AlphaFold2, J. Virol. e01793-22 (2023).

PROTEIN QUALITY CONTROL OF STRESS GRANULES: IMPLICATION IN AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL DEMENTIA.

Serena Carra,[a] Francesco Antoniani,[a] Marco Cimino,[a] Laura Mediani,[a] Enza M. Verde,[a] Valentina Secco,[a] Alfred Yamoah,[b] Priyanka Tripathi,[b] Maria E. Cicardi,[c] Davide Trotti,[c] Jared Sterneckert,[d] Anand Goswami[b]

[a]*Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy*

[b]*Institute of Neuropathology, RWTH Aachen University Hospital, Aachen, Germany*

[c]*Jefferson Weinberg ALS Center, Vickie and Jack Farber Institute for Neuroscience, Department of Neuroscience, Thomas Jefferson University, USA*

[d]*Center for Regenerative Therapies TU Dresden, Technische Universität Dresden, Dresden, Germany*

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TDP-43, FUS and the C9orf72-dipeptide repeat proteins accumulate in form of cytoplasmic inclusions in Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). Although the origin of TDP-43 and FUS pathological inclusions is still debated, their protein aggregation is considered a key pathogenic event.

The majority of the studies focus on understanding how cells control TDP-43 and FUS aggregation in the cytoplasm, overlooking how dysfunctions in the nucleus may influence the maintenance of protein solubility outside of the nucleus. However, protein quality control (PQC) systems that deal with aggregation-prone proteins simultaneously operate in the cytoplasm and nucleus and share players (chaperones and proteasomes). Thus an impairment of the nuclear PQC may have a negative impact on the cytoplasmic PQC, contributing to the formation of cytoplasmic inclusions.

Cells transiently store aggregation-prone proteins in deposition sites, such as Promyelocytic leukemia protein (PML) nuclear bodies (PML-NBs) in the nucleus and stress granules (SGs) in the cytoplasm. PML-NBs compartmentalize misfolded proteins, including defective ribosomal products, and recruit chaperones and proteasomes to promote their clearance. SGs sequester aggregation-prone RNA-binding proteins linked to ALS-FTD and mRNAs to attenuate their translation. We show that dysfunction of the nuclear PQC due to PML depletion promotes misfolded protein accumulation in SGs, impairing their disassembly. Moreover, PML assembly is impaired in the human brain and spinal cord of familial C9orf72 and FUS ALS-FTD cases. We propose that altered nuclear PQC due to PML-NB loss represents a novel pathomechanism in ALS-FTD that can contribute to SG accumulation and cytoplasmic protein aggregation.

Reprogrammed mitochondria as a central hub in cancer cell metabolism and anticancer therapeutic intervention

Maria Rosa Ciriolo *Department of Biology, University of Rome "Tor Vergata"*
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Several decades have passed since Otto Warburg interpreted tumor lactate secretion as an indication that mitochondrial oxidative metabolism was impaired. The today view of cancer as a metabolic disease in which cancer cells attempt to fulfill their proliferation in a microenvironment characterized by fluctuating resource availability completely changed the scenario. Oxygen and nutrient shortages are well-known characteristics of cancer that result from a mismatch between supply and use, inherent to blood perfusion abnormalities and high consumption rates[1]. At least three different evolutionary metabolic strategies allow cancer cells to cope with fluctuating resource availability: i) glucose addiction under hypoxic conditions; ii) autophagy; iii) cooperativeness – e.g. oxidative cancer cells recycle lactate, provided by glycolytic cancer cells, as fuel for citric acid cycle thus sparing glucose for hypoxic cancer cells. Central to cell metabolism is the mitochondrion not only viewed through the efficient ATP production in the catabolic pathways but as a key site for redox signaling and anabolism. The Mitochondrial electron transport chain (ETC) is the main source of reactive oxygen species (ROS), which are essential drivers of metabolic and therapeutic responses to intracellular and environmental cues. Moreover, the redox regulation is an essential function of ETC activity in proliferating cells[2]. ETC activity is fundamental for providing electron acceptors in terms of NADH oxidation necessary for the anabolic synthesis of aspartate. Consistently, a major metabolic consequence of decreased NAD+/NADH upon ETC impairment is the depletion of the amino acid aspartate. Most cells are dependent on *de novo* aspartate synthesis since aspartate is poorly permeable at physiological concentrations. Aspartate is a central metabolic node in proliferative metabolism, serving as a direct substrate for protein synthesis and as a precursor for the synthesis of other essential metabolites, including asparagine, arginine, and both purine and pyrimidine nucleobases. Thus, aspartate depletion likely impairs core metabolic processes necessary for cell proliferation[3]. Understanding how cancer cells reconfigure their metabolism to support cell proliferation and cope with ROS burst will help reveal additional metabolic roles of mitochondria and could support the development of novel therapies targeting the metabolic abilities of cancer cells.

1. Vander Heiden, M.G. and DeBerardinis, R.J. Understanding the intersections between metabolism and cancer biology. Cell 2017 doi: 10.1016/j.cell.2016.12.039.
2. Luengo, A., Li, Z., Gui, D.Y., Sullivan, L.B., Zagorulya, M., Do, B.T., Ferreira, R., Naamati, A., Ali, A., Lewis, C.A., Thomas, C.J., Spranger, S., Matheson, N.J., and Vander Heiden, M.G. Increased demand for NAD+ relative to ATP drives aerobic glycolysis. Molecular Cell 2021 doi:https://doi.org/10.1016/j. molcel. 2020.12.012, PMID: 33382985
3. De Falco, P., Lazzarino, G., Felice, F., Desideri, E., Castelli, S., Salvatori, I., Ciccarone, F., and *Ciriolo M.R*. Hindering NAT8L expression in hepatocellular carcinoma increases cytosolic aspartate delivery that fosters pentose phosphate pathway and purine biosynthesis promoting cell proliferation. Redox Biol 2022 doi: 10.1016/j.redox.2022.102585.

RIBOREGULATION OF SERINE HYDROXYMETHYLTRANSFERASE, A NOVEL MECHANISM CONTROLLING SERINE METABOLISM ACROSS CELLULAR COMPARTMENTS

Francesca Cutruzzolà[1,6*], Sharon Spizzichino[1], Federica Di Fonzo[1], Chiara Marabelli[2], Angela Tramonti[3], Antonio Chaves-Sanjuan[4,5] Alessia Parroni[3], Giovanna Boumis[1], Francesca Romana Liberati[1], Alessio Paone[1,6], Linda Celeste Montemiglio[3], Matteo Ardini[7], Arjen J. Jakobi [8], Alok Bharadwaj[8], Paolo Swuec[9], Gian Gaetano Tartaglia [10,11], Alessandro Paiardini[1], Roberto Contestabile[1], Serena Rinaldo[1], Martino Bolognesi[4,5] and Giorgio Giardina[1]

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Serine catabolism through one-carbon metabolism (OCM) is important for organismal development, cancer cell growth, and immune function [1]. In OCM, serine hydroxymethyltransferase (SHMT), the key enzyme interconverting serine and glycine in the presence of folates, can be found in the cytosol (SHMT1) and in the mitochondria (SHMT2). Serine to glycine interconversion is highly dynamic and yields serine or glycine according to nutrient availability and the cell needs.

We discovered a RNA-based regulatory mechanism of serine metabolism. We showed that SHMT's catalytic activity can be controlled by RNA [2], in a process called riboregulation, and showed in silico and in cell lines that RNA can dynamically control the levels of serine/glycine across cellular compartments [3]. Here we present a complete structural, functional, and phylogenetic analysis of the mechanism of riboregulation of SHMT1. We show that the RNA modulator competes with polyglutamylated folates and acts as an allosteric switch, selectively altering the enzyme's reactivity vs. serine. We also present data suggesting that riboregulation may have played a role in the evolution of eukaryotic SHMT1 and the need to compartmentalize one-carbon metabolism. We will also provide the proof-of-concept that RNA molecules acting as molecular switches of this metabolic pathway can be successfully employed in vitro and in vivo to control serine metabolism in cancer.

1) Amelio I, Cutruzzolá F, Antonov A, Agostini M, Melino G. Trends Biochem Sci. 2014 Apr;39(4):191-8.
2) Guiducci, et al., Nucleic Acids Research, 2019, 47:4240–4254.
3) Monti M, et al. Comput Struct Biotec 2021; 19: 30343041.

Teaching and learning: focus on innovative technologies and methodologies for student engagement

Ivano Eberini[a]

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New teaching and learning technologies and methodologies are at the basis of teaching innovation, but some resistances are present in introducing them practically, because of the necessarily reorganization of the classical frontal lesson/practical workshop paradigm, the initially steep learning curve of some digital learning environments and tools, and the growing attention towards relational and social aspects.

Biochemistry is an almost ubiquitous discipline in the academic life science and biomedical area and laboratory activities are strongly recommended to enhance and develop specific skills to be merged with knowledge to generate high quality competencies. In person laboratories cannot be replaced by multimedia, but virtual environments can strongly enhance the learning process, integrating the workshop experience of the students with highly immersive, asynchronous activities that are continuously monitored for performance and progress.

Older teaching paradigms, such as the constructivist, are progressively replaced by interactionist and enactivist ones, leading to the reduction of frontal lesson as the only teaching approach, and increasing different student-centered activities. Some digital interactive engagement tools can be exploited to increase the synchronous participation of students and to support asynchronous activities for review and reinforcement of learning through feedback.

Evidence suggests also that a positive and healthy classroom atmosphere can help achieve the teaching and learning goals of both teachers and students. Social and emotional learning approaches can also be fruitfully applied to university students, having a positive impact on the classroom environment and encouraging debates and discussions, essential for the development of a critical sense towards the subject studied.

FROM FOOD WASTE TO THE MANUFACTURING OF TAILORED BIOPLASTICS.

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The disposal of petroleum-derived plastics is highly pollutant for ground, water as well as marine life. In addition, the plastics burning releases poisonous chemicals in the air. Bioplastics produced from biodegradable molecules seem an attractive eco-friendly alternative since they can be easily degraded by the enzymes present in different microorganisms occurring in the environment[1]. The technical attitude, such as mechanical and barrier properties of the bioplastics is crucial for their industrial application, and it is highly influenced by the different additives used for film manufacture[2]. The methods for preparation of the bioplastics are different depending on their specific use since they can be applied in the agriculture and food as well in biomedical and pharmaceutical sectors. For an industrial application it is of a paramount importance to characterize the bioplastics according to their structure and biodegradability. The study of their morphological, barrier and mechanical properties is, thus, essential for making them promising environmentally friendly candidates able to replace the petroleum-derived plastics for their application in different industrial sectors (agriculture, pharma and food).

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Sphingosine 1-phosphate in chronic kidney disease: novel mechanistic insights

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Podocytes are visceral epithelial cells of the renal glomerulus. They play a critical role in the glomerular ultrafiltration barrier by interactions of neighbouring foot processes and formation of the slit diaphragm. A key protein that is needed for a tight slit diaphragm is nephrin. When nephrin is downregulated or mutated, a process called foot process effacement is initiated which leads to the leakage of larger proteins, such as albumin, into the urine. Many gene mutations encoding for glomerular podocyte proteins have been described in various nephrotic syndrome pathologies. Among these is the *Sgpl1* gene which encodes for the sphingosine 1-phosphate (S1P) lyase (SPL), an enzyme that degrades the sphingolipid S1P. These patients develop a steroid-resistant nephrotic syndrome. Based on these observations, it is hypothesized that the S1P metabolism plays a key role in podocyte physiology although the detail mechanisms remain unclear.

In this study, the molecular effects of SPL knockdown in human podocyte cell line were investigated to better understand the mechanism underlying the nephrotic syndrome in patients. A stable SPL knockdown cell line of human podocytes was generated by the lentiviral shRNA transduction method. This cell line was further studied for changes in podocyte-specific proteins and signaling factors that could be involved in the regulation of the ultrafiltration barrier. We found that nephrin is a critical factor downregulated by loss of SPL, which may directly cause podocyte foot process effacement as observed in mice and humans, leading to albuminuria. A detailed mechanism of action of S1P will be discussed.

Breaking the amyloid chain with small molecule inhibitors: A path for Spinocerebellar Ataxia Type 3 therapies

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Spinocerebellar Ataxia type 3 (SCA3) is a rare dominant neurodegenera12ve hereditary ataxia with no disease-modifying treatments. SCA3 is a gene12c disorder that arises from the expansion of a trinucleo12de CAG repeat in the ATXN3 gene. This expansion leads to the forma12on of an expanded polyglutamine (polyQ) tract in the ataxin-3 protein (Atx3). Atx3 is a modular deubiqui12na12ng enzyme with a globular cataly12c Josephin (JD) and a flexible C-terminal tail containing the polyQ tract and two or three ubiqui12n interac12ng mo12fs (UIMs). Biophysical studies have shown that both wild-type and pathogenic forms of Atx3 can assemble into amyloid-like structures in vitro. The aggrega12on-prone region in JD is responsible for ini12a12ng this process and serves as the first step towards Atx3 self-assembly. However, it is noteworthy that only when the length of the polyQ expansion reaches the pathological threshold does it trigger the forma12on of mature and SDS-resistant fibrils. Protein aggregates with the pathogenic polyQ-expanded Atx3 accumulate in degenera12ng brain regions, causing SCA3 symptoms. The exact cause of neurotoxicity is s12ll being debated, but abnormal aggrega12on of pathogenic Atx3 contributes to neuronal damage in SCA3 and other polyQ disorders. In this context, disease-modifying therapies for SCA3 and other polyQ diseases, which target aberrant Atx3 self-assembly are being ac12vely pursued.

Currently, there is a need for molecules that can target mul12ple domains of Atx3. To meet this need, we are employing various approaches to select specific small molecule inhibitors that can prevent the toxic self-assembly of Atx3. In this regard, we will briefly present our recent findings on the mechanism of ac12on of a pan-amyloid inhibitor that targets mul12ple aggrega12on-prone regions of Atx3. Addi12onally, we will discuss a set of small molecules that we have iden12fied through a drug repurposing screening to iden12fy inhibitors of Atx3 aggrega12on with known toxicological and pharmacokine12c profiles. The chosen molecules could reduce the rates of secondary nuclea12on in the forma12on of amyloid fibers and delay the emergence of disease-related characteris12cs in animal models of SCA3.

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HARNESSING TUMOR METABOLISM TO OVERCOME IMMUNOSUPPRESSION

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Anti-cancer immunotherapy has provided patients with a promising treatment. Yet, it has also unveiled that the immunosuppressive tumor microenvironment (TME) hampers the efficiency of this therapeutic option and limits its success. The concept that metabolism is able to shape the immune response has gained general acceptance. Nonetheless, little is known on how the metabolic crosstalk between different tumor compartments contributes to the harsh TME and ultimately impairs T cell fitness within the tumor. This lecture will decipher some of the metabolic changes in the TME impeding proper anti-tumor immunity. Starting from the meta-analysis of public human datasets, corroborated by metabolomics and transcriptomics data from several mouse tumors, we ranked clinically relevant and altered metabolic pathways that correlate with resistance to immunotherapy. Using a CRISPR/Cas9 platform for their functional in vivo selection, we have identified cancer cell intrinsic metabolic mediators and, indirectly, distinguished those belonging specifically to the stroma. By means of genetic tools and small molecules, we have targeted promising metabolic pathways in cancer cells and stromal cells (particularly in tumor-associated macrophages) to harness tumor immunosuppression. Finally, we went back to patient samples to assess the relevance of these metabolic networks in humans. By analyzing the metabolic crosstalk within the TME, this lecture would like to shed some light on how metabolism contributes to the immunosuppressive TME and T cell maladaptation.

Activation of PP2A modulates the DNA damage response

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We have previously shown that PP2A integrates metabolic sensing with the the DNA-Damage Response (DDR) in yeast. Through a pharmacological and genetic approach, we found that the combination of metformin (the most commonly used drug for type 2 diabetes) with glucose starvation increased PP2A activity. In Triple Negative Breast Cancer (TNBC) cell lines and patient-derived tumors, metformin and glucose starvation enhanced the efficacy of low-dose, DNA-damaging chemotherapy. We demonstrated that activation of PP2A by metformin and glucose starvation attenuated the DDR triggered by chemotherapy, thus preventing the cell cycle arrest necessary for DNA repair and increasing genomic fragmentation, which finally led to cell death. In mouse models of TNBC, metformin and cycles of intermittent fasting (which lowered blood glucose) increased the efficacy of low-dose chemotherapy and induced tumor regression. Targeting the DDR is considered an attractive therapeutic opportunity, based on the intrinsic genomic instability of tumor cells. Here we provide a metabolic strategy to mitigate the DDR at multiple levels, which is safe, tolerable and of potential clinical use.

MITOCHONDRIAL SUPEROXIDE IN ISCHEMIA REPERFUSION INJURY

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Mitochondrial redox metabolism is central to the life and death of the cell. For example, mitochondrial production of free radicals and subsequent oxidative damage has long been known to contribute to damage in conditions such as ischaemia-reperfusion (IR) injury in stroke and heart attack. More recently mitochondrial redox changes have also been implicated in redox signalling. Over the past years we have developed a series of mitochondria-targeted compounds designed to ameliorate or determine how these changes occur. I will outline some of this work, which suggested that ROS production in IR injury during stroke was mainly coming from complex I. This led us to investigate the mechanism of the ROS production and using a metabolomic approach we found that the ROS production in IR injury came from the accumulation of succinate during ischaemia that then drove mitochondrial ROS production by reverse electron transport at complex I during reperfusion. This surprising mechanism led up to develop further new therapeutic approaches to impact on the damage that mitochondrial ROS do in pathology and also to explore how mitochondrial ROS can act as redox signals. I will discuss how these unexpected mechanisms may lead to redox and metabolic signals from mitochondria in a range of conditions under both healthy and pathological conditions.

SEQUENCING SICK MOLECULES: DIAGNOSTIC AND THERAPEUTIC IMPLICATIONS

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In AL amyloidosis and other monoclonal gammopathies of clinical significance (MGCS), a small B cell or plasma cell tumor produces a patient's specific monoclonal antibody (the M protein) displaying a sequence-dependent, pathologic behavior leading to often life-threatening organ damage[1,2].
The molecular mechanisms underlying these conditions are poorly understood, mainly because of the limited number of clinically annotated, sequenced M proteins.
Our laboratory has recently developed a novel, sensitive and accurate high-throughput methodology termed Single Molecule Real-Time Sequencing of the M protein (SMaRT M-Seq) to unambiguously identify the full-length variable sequence of M protein genes[3].
This enabled us to study the potential role of N-glycosylation, a post-translational modification which recently emerged as a potential risk factor for the development of AL amyloidosis. By combining bioinformatics, biochemical, proteomics, structural and genetic analyses, we identified an N-glycosylation hotspot in immunoglobulin κ light chains that is associated with AL amyloidosis[4].
More recently, leveraging on the presence of circulating tumoral cells in patients with monoclonal gammopathies, we combined SMaRT M-Seq in the peripheral blood to sequence the circulating immunoglobulin repertoire with mass spectrometry-based analysis of the urinary proteome, enabling the identification of the full-length clonal light chain sequence in most patients with monoclonal gammopathies without bone marrow studies[5].
Overall, reliable, high-throughput sequencing of clonal immunoglobulin genes from large patient populations has the potential of deepening our mechanistic understanding of the pathophysiology of MGCS. Moreover, at the individual patient level, the identification of clonotypic immunoglobulin sequences can empower personalized medicine approaches at diagnosis and during follow-up.

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Mitochondrial bioenergetics contribution in ovarian cancer progression

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In the last years, the involvement of mitochondrial respiration has been increasingly recognized during tumor progression and resistance to chemotherapy. In particular, targeting respiratory Complex I (CI) has been proposed as a new therapeutic approach to hinder cancer growth[1,2]. Our recent work demonstrated that a severe CI impairment promoted a delay of tumor expansion but not its complete eradication of different solid cancers[3]. Indeed, after an initial lag phase, CI-defective cancer cells survive and undergo growth reactivation likely due to the triggering of adaptive molecular and atypical microenvironment responses, which in turn may support cell survival and resume proliferation[4]. This observation might be particularly relevant in the context of ovarian cancer (OC), where about 85% of patients develop relapses after standard surgical and pharmacological treatments, thus still in need for alternative therapeutic approaches. Here, we will discuss some of the adaptive responses triggered upon CI impairment, and how their dissection may lead to the identification of molecular players potentially in synthetic lethality with CI inhibition, thus providing new synergistic strategies for OC treatment.

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SPHINGOLIPID-DEPENDENT MEMBRANE ORGANIZATION AND SIGNALING ORCHESTRATING MYELIN REPAIR

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Recombinant human IgM22 (rHIgM22) binds to myelin and oligodendrocytes (OLs) and promotes remyelination in mouse models of multiple sclerosis. However, its molecular and cellular targets antigen and its mechanisms of action are still unclear.

We showed that 1) rIHgM22 binds to sulfatide and lysosulfatide, but also to phosphatidylinositol, phos-phatidylserine and phosphatidic acid; 2) changes in the composition of the lipid microenvironment of the target antigen can modulate the affinity of the antibody, suggesting that reorganization of lipid membrane microenvironment might be relevant in its biological activity [1].

rHIgM22 induced proliferation in rat mixed glial cells (MGCs), with the most significant response associated with astrocytes, and increased the production and release of sphingosine 1-phosphate (S1P). rHIgM22 treatment did not induce changes in the release of S1P in pure astrocyte or OPCs cultures, but it increased S1P release in BV-2 microglia cells, suggesting that rHIgM22 indirectly influences astrocytes proliferation via microglia-released S1P [2].

rHIgM22 had no effects on glycosphingolipids in MGCs and pure astrocytes, while in OPCs, OLs and BV-2 microglia we observed a significant increase in the levels of GM3 and GD3 gangliosides, and a significant decrease in cholesterol levels in differentiated OLs. Thus, we hypothesize that rHIgM22 myelin-repair activity could be at least in part mediated by alterations of lipid-dependent membrane organization in OPCs, OLs and microglia.

In conclusion, rHIgM22 exerts its protective effects by acting directly or indirectly on different glia populations involved in the mechanism of myelin repair, with sphingolipids being always key players.

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Ex-vivo structural biology: new insights on systemic amyloidosis

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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 protein fibrils. In this group of diseases, Light chain amyloidosis (AL) is the most common systemic amyloidosis, affecting mainly heart and kidney. AA amyloidosis has been found in humans and in many other vertebrates and typically targets kidney, spleen, and liver.

We have been employing single particle Cryo electron microscopy (Cryo-EM) to structurally characterize amyloid fibrils *ex vivo* from patients. Here we show that amyloid fibrils display a structural variability unmatched in native protein folds. The Cryo-EM structures of fibrils from different organs of the same AL patient show that amyloidogenic light chains form structurally identical assemblies in different organs [1,2]. Conversely, amyloid fibrils extracted from the heart of another AL patient display two different independent structures and none of the two are structurally similar with previously reported amyloid fibrils (unpublished data).

Moreover, we showed the extreme prevalence of AA amyloidosis in cat shelters where 60% of deceased cats were positive for AA amyloidosis strongly suggesting the possibility of a prion-like horizontal transmission between individuals [3]. The Cryo-EM structure of fibrils extracted from renal tissue of a deceased cat show no structural conservation with human and murine AA amyloids and suggests the molecular bases of the marked aggregation propensity of SAA in cats.

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Using protein–metabolite interaction networks to tap into the dark matter of metabolomes

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Functional diversity reflects the immense chemical diversity of living organisms that produce hundreds of thousands of small molecule compounds, most of which remain to be chemically and functionally characterized. Because small molecules rarely work on their own but rather *via* interactions with proteins, following the proverbial "tell me who your friends are, and I will tell you who you are," identification of protein interactors can be used to unravel the function of a metabolite. The complex and dynamic protein-metabolite interaction (PMI) network underlies all biological processes but remains under-characterized. In my group, we adapted co-fractionation mass-spectrometry (CF-MS), a well-established approach to map protein assemblies, for proteome and metabolome-wide identification of the protein-metabolite complexes. CF-MS experiments combine the separation of native complexes with MS analysis of the obtained fractions and use the similarity of elution profiles, referred to as co-elution or co-fractionation, to delineate interactors. CF-MS enables the untargeted identification of complexes without needing a protein or a metabolite bait. The resulting PMIs networks comprise tens of annotated metabolites and hundreds of unknown metabolic features. During my seminar, I will introduce novel regulatory roles of 2', 3' - cyclic nucleotides and proteinogenic dipeptides uncovered by our studies in plants and yeast. Moreover, I will discuss how we can use CF-MS to probe cell-state-specific and "inter-organismal" PMIs, exemplified by a diauxic shift transition in yeast and Arabidopsis infection with bacterial pathogen *P.syringae*, respectively.

A REDOX-CYCLER BASED THERAPEUTIC STRATEGY AGAINST MITOCHONDRIAL DISEASES

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Mitochondrial diseases result from a decreased oxidative phosphorylation (OXPHOS) that leads to a broad spectrum of incurable pathologies [1,2]. Our goal was to understand whether membrane-permeant small molecule(s) can be exploited to treat OXPHOS-related diseases as an alternative to gene therapy. Therefore, we selected some molecules for their ability to replace the redox functions of complex III and among them identified pyocyanin as a promising agent. Pyocyanin is a bacterial redox cycler that can shuttle electrons from reduced coenzyme Q to cytochrome c, acting as an electron shunt. Sub-µM dose of pyocyanin is harmless, restores respiration and increases ATP production in Ttc19-/- mouse embryonic fibroblasts as well as in fibroblasts from patients harboring pathogenic mutations in three different assembly/stabilization factors of complex III (namely, TTC19, BCS1L and LYRM7). The drug normalized the mitochondrial membrane potential, mildly increased ROS production, and triggered mitochondrial biogenesis. These in vitro effects were confirmed also in vivo, in both Drosophila melanogaster^{TTC19KO}, in Danio rerio^{TTC19KD} [3]. Here we show that pyocyanin and its derivative with enhanced life-time and tissue distribution exhibited a benefit in Ttc19 KO mouse model. Administration of low, non-toxic concentration of pyocyanin significantly ameliorated movement and coordination proficiency, without inducing toxicity. Likewise, pyocyanin, able to receive electrons from NADH, showed a beneficial effect also in the case of cells and mice with complex I disease. Our results point to exploitation of redox cyclers for therapy against diseases due to OXPHOS dysfunction.

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GLUTAMINE METABOLISM IN LIVER PHYSIOLOGY AND CANCER

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Glutamine is the most abundant amino acid in human blood circulation and glutamine-dependent reactions are involved in Krebs cycle, ammonia homeostasis, glycosylation, cofactor and nucleotide biosynthesis, directly impacting cancer initiation and progression [1]. Glutamine in mammals is synthesized uniquely from glutamate, ammonia and ATP by the enzyme Glutamine Synthetase. By applying Liquid Chromatography Mass Spectrometry-based untargeted metabolomics *in vivo*, we have demonstrated that hepatic Glutamine Synthetase produces N^5-methylglutamine, a previously-undescribed glutamine analogue [2]. Indeed, liver and circulating levels of N^5-methylglutamine, strictly depend on Glutamine Synthetase activity and its urine levels correlate with the progression of β-catenin-mutant Hepatocellular Carcinoma (HCC) in a genetically engineered mouse model. Moreover, the genetic deletion of Glutamine Synthetase from the β-catenin-mutant HCC tumours normalizes the urine levels of N^5-methylglutamine. Unexpectedly glutamine synthetase-deleted HCC tumours have an increased proliferative index and progress more rapidly to clinical endpoint. The pharmacologic inhibition of Glutamine Synthetase recapitulates this phenotype, demonstrating that its metabolic activity is detrimental for tumour progression. By applying stable isotope tracing-assisted metabolomics *in vivo* we demonstrate that Glutamine Synthetase constrains the *de novo* pyrimidine biosynthesis of tumours by reducing the availability of aspartate.
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Protein - RNA interactions and phase separation.

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Our lab combines computational and experimental approaches to investigate the interactomes of RNA molecules, which is key to unravel the complexity and functionality of mammalian genomes and could open up therapeutic avenues for the treatment of a broad range of human disorders. We predicted and experimentally validated interactions of non-coding RNAs (e.g. Xist [1]) with proteins involved in transcriptional and translational regulation as well as neurodevelopmental (FXTAS [2]) and neurodegenerative diseases (Parkinson's disease [3]). We are especially interested in understanding mechanisms whose alteration lead to aberrant phase separation and aggregation [4]. Exploiting the novel finding that non-coding RNAs can act as scaffolding molecules for protein-RNA interactions [5], we found that structural properties of transcripts control the formation of large ribonucleic assemblies and their phase separation [6]. The importance of this discovery is fundamental to unravel many aspects of cell biology, including events related to viral infections [7]. Most importantly, the principles identified in our analysis have led to the design of artificial RNAs (aptamers) that alter the ability of proteins to condensate [8].

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TARGETING THE PHASE SEPARATION OF DIPEPTIDE REPEATS IN ALS/FTD: A NEW MODALITY DRUG DEVELOPMENT

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Biomolecular condensation is a process whereby many macromolecules (proteins and RNAs) form non-stoichiometric, functional assemblies. The dominant mechanism of such biomolecular condensation is liquid-liquid phase separation (LLPS), which leads to the formation of membraneless organelles (MLOs), such as the nucleolus and stress granules, in the cell [1]. The proteins involved often have a high proportion of intrinsic structural disorder, which drive LLPS by transient, multivalent interactions. As MLOs play key roles in cell signaling, the misregulation of their formation and dissolution often leads to diseases termed "condensatopathies" [2]. In my presentation, I will outline the basic mechanisms leading to such disease states, focusing on cancer, viral infections and neurodegeneration. I will also discuss the different potential strategies for correcting these errors in cell signaling, and show through specific examples how drug candidates, "c-mods" capable of correcting MLO misregulation, can be developed [3, 4].

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TRANSLATIONAL GENOMICS OF OSTEOARTHRITIS

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Osteoarthritis is one of the leading causes of disability and pain worldwide, with over 300 million people affected. Currently no curative treatments are available. A detailed understanding of disease aetiopathology and novel drug targets are therefore urgently needed.

In this talk, I will give an overview of how we have used translational genomics approaches to enhance our understanding of the genetic aetiology of osteoarthritis, shed novel biological insights, and provide a stepping stone for translating genetic associations into osteoarthritis drug development.

62° SIB CONGRESS

SELECTED SHORT TALKS ABSTRACT

Epigenetics meets metabolism: Phospo-DJ1 at the cross-road, a novel player in epigenetic misregulation.

Marina La Chimia[1], Cristina Fontana[1], Valter Agosti[1], Maria Teresa De Angelis[1], Francesca Alessandra Ambrosio[2], Giosuè Costa[2], Giovanni Cuda[1], Domenica Scumaci[1]

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Abstract

Recently a plethora of histone non-enzymatic covalent modifications, correlating epigenome landscape and metabolic rewiring, has been described[1]. These modifications are tightly related to cells metabolic fitness and are able to impair chromatin architecture[2]. Our group demonstrated that in breast cancer cells, the high glycolytic flux induces carbonyl stress, a damaging condition that increases reactive carbonyl species making histones more susceptible to glycation. Glycation leads to the formation of advanced glycation end-products (AGEs) and induces a fatal deconstruction of histone code. DJ-1, a deglycase enzyme, found dysregulated in several human tumours, appears to be crucial for preserving the proliferative potential of cancer cells counteracting AGEs-formation.

Using omics-strategy, we identified on DJ-1 a novel threonine phosphorylation, part of a putative Akt consensus, establishing that DJ-1 pro-tumorigenic abilities are dependent on Akt-pathway[3]. In breast cancer cells, the over-activation of Akt-signaling prevents, through a functional tuning of DJ-1 proteoforms, glycation-induced histones mysregulation. To corroborate the role of DJ-1, in preserving the malignant proliferative potential, we used the novel DJ-1-proteoform as a molecular template for docking simulation and we identified a parterre of DJ-1 inhibitors able to selectively target DJ-1 glyoxalase activities.

Virtual Screening studies were done using the Food and Drug Administration (FDA)-approved drugs database. Histones glycation profiling in response to treatment with DJ-1 inhibitors showed a parterre of histone marks critical for chromatin homeostasis, an Achilles' heel that might improve targeted therapy in breast cancer. Therefore our results reinforce the notion that targeting the novel DJ-1-proteoform might be a promising therapeutic strategy.

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Categories

Tumor Biochemistry

C/EBP-β splicing determines chemoresistance in non-small cell lung cancer via metabolic rewiring

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Abstract

Cisplatin is the first-line treatment in non-small cell lung cancer (NSCLC) without actionable oncogenic drivers, even if resistance often develops. We found that chemoresistance is induced by the increased ratio between LAP and LIP, the two splicing isoforms of transcriptional factor CCAAT/Enhancer Binding Protein-β (C/EBP-β) [1]. Interestingly, the altered LAP/LIP ratio induces huge metabolic rewiring in murine embryonic fibroblasts [2]. Our aim is to investigate if C/EBP-β splicing determines chemoresistance in NSCLC by metabolic reprogramming.

Using LAP/LIP-overexpressing NSCLC cells, we found that LAP increased drug efflux ABC transporters, decreased cisplatin-induced cytotoxicity and DNA damage, while LIP produced opposite effects. Consistently, LAP was a negative predictive factor in cisplatin-treated NSCLC patients. According to metabolomic/lipidomic profiles and functional assays, LAP-overexpressing cells had more building blocks, energy-producing and anti-oxidant metabolites, increased TCA cycle, FAO, OXPHOS and mitochondrial ATP. Interestingly, cisplatin sensitivity was restored by silencing of CPT1A, the FAO pacemaker enzyme, or by FAO inhibitor etomoxir, which counteracts LAP effects on lipid metabolism. In Hu-CD34+NSG mice LAP+tumors had quantitative and qualitative differences in the metabolic profile of cancer cells and immune populations that were abrogated by etomoxir (single-cell RNA-Seq analysis).

Our study shows that an altered LAP/LIP ratio causes chemoresistance by increasing FAO flux. Targeting this pathway is a novel chemosensitizing strategy in NSCLC.

Acknowledgments: Italian Association for Cancer Research (AIRC; IG21408); Cassa di Risparmio di Torino (ID2021.05556)

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Categories

Tumor Biochemistry

Acetyl-CoA carboxylase 1 controls lipid droplets-peroxisomes metabolic axis and is a vulnerability of ER+ breast cancer resistant to estrogen deprivation.

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Abstract

Aromatase inhibition (AI) is an effective therapeutic approach for estrogen receptor-positive (ER+) breast cancer and acts by reducing estrogen levels. However, resistance limits its efficacy. We have previously demonstrated that long-term estrogen-deprived (LTED) cells, a model of AI-resistance, show enhanced metabolic plasticity. Here we report that LTED cells showed increased fatty acid (FA) synthesis and FA uptake fueling FA accumulation into lipid droplets (LD), in parallel to enhanced peroxisome content and activity. This metabolic reprogramming sustains LTED cells when challenged by nutritional stress. However, we could still identify a lipid metabolic vulnerability by targeting the Acetyl-CoA carboxylase 1 (ACC1), that selectively impaired the survival of the AI-resistant cells. Mechanistically, ACC1 targeting increased the reactive oxygen levels thus unbalancing the redox homeostasis. Malonyl-CoA produced by ACC1 is required for FA elongation and production of complex FA (i.e., VLCFA and BCFA), which require peroxisome for their catabolism. Crucially, supplementation of complex FA reverted ACC1 targeting restoring LD-peroxisome axis, while failing when peroxisomal activity is impaired. Accordingly, ACC1 targeting in vivo reduced tumor volume and proliferation of AI-resistant PDX. As revealed by IHC analysis, enhanced expression of the LD-associated protein PLIN2 correlates with high levels of Ki67 in neoadjuvant AI-treated ER+ breast cancers, suggesting that LD levels could be of AI predictive value. Our data suggest that lipid metabolic plasticity is involved in the acquisition of adaptive features of ER+ tumors resistant to estrogen deprivation and offers novel metabolic players that could be used as biomarkers or targeted for therapeutic approaches.

Categories

Tumor Biochemistry

Emerging role of Cholesterol in membrane transporters function

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Abstract

Cholesterol is an essential constituent of the mammalian plasma membrane. Recently, a crucial role of this lipid emerged in regulating some membrane transporters, thus playing a key role in cell communication. Transporters for glutamine (SLC1A5), carnitine (SLC22A5), organic cations (SLC22A2), large amino acids (SLC7A5) and the lysosomal transceptor SLC38A9, were shown to be modulated by cholesterol. We studied the effect of cholesterol on OCTN1 (SLC22A4) which is renewed for the well assessed link with inflammation despite uncertainty on its physiological ligands. Two different approaches were adopted: i) cholesterol removal by MβCD from HeLa cell membranes; ii) cholesterol addition to cholesterol-free hOCTN1 over-expressed in *E. coli*. Activity has been then assayed in proteoliposomes harboring hOCTN1 extracted from cells or recombinant hOCTN1. Transport measurement has been performed following the uptake of [^{3}H]-acetylcholine, one of the most acknowledged physiological substrates. Cholesterol removal from cells impaired transport whereas addition of cholesterol to the recombinant hOCTN1 stimulated transport; kinetic analysis revealed that cholesterol increased the Vmax with no changes in Km, indicating a direct effect on the protein. An *in silico* analysis has been performed to find possible sites of interaction of cholesterol. Indeed, OCTN1 possesses some CARC/CRAC sequence motifs which are known as cholesterol binding motifs. Then, the homology model of OCTN1 was built using the SLC22A3 structure as template. Blind molecular docking, identified cholesterol binding sites corresponding to the CRAC/CARC. These findings point to a role of cholesterol in influencing OCTN1 function and thus in modulating inflammatory response.

Categories

Membranes

Differential Interactions between ATP and NGF / proNGF: Chance or Necessity?

Francesca Paoletti[1], Franci Merzel[2], Sonia Covaceuszach[1], Alberto Cassetta[1], Antonio N. Calabrese[3], Iza Ogris[2], Urban Novak[2], Petr Konarev[4], Jože Grdadolnik[2], Doriano Lamba[1,5], Simona Golič Grdadolnik[2]

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Abstract

The prototype of the neurotrophin family, Nerve Growth Factor (NGF), is essential for neurons' development and maintenance and is crucial in immune and endocrine systems and in the pain pathway. NGF precursor, proNGF, whose pro-peptide is an intrinsically unstructured domain (IUD), is endowed with different biological properties. The binding to TrkA, $p75^{NTR}$ and sortilin receptors activates the NGF/proNGF signaling pathways. Much is known about NGF neuronal physiology. However few reports described essential endogenous ligands as modulators of NGF biology.

Recently, the binding of ATP to NGF was identified. To determine the molecular elements of this binding, we used integrative structural biology to unveil for the first time the binding cartography of ATP to NGF [1]. Isothermal Titration Calorimetry (ITC), ^{1}H Saturation Transfer Difference NMR (^{1}H STD-NMR), coupled to the determination of the 3D solution NMR structure of NGF and MD simulations, helped identifying the likely binding mode of ATP on NGF. ATP/NGF binding to the receptors was investigated through Surface Plasmon Resonance (SPR).

We also undertook a complementary biophysical study on the binding of ATP to proNGF. Our results reveal a different binding profile for mature and precursor proteins. A combination of Small Angle X-ray Scattering (SAXS), Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS) and limited proteolysis showed that ATP binding induces a change in the conformation and/or dynamics of proNGF, predominantly in the IUD pro-peptide [2].

Combined, these results suggest a functional role for ATP in modulating the biological role of proNGF/NGF in health and disease states.

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Categories

Proteins

Cytosolic and mitochondrial translation elongation are coordinated through the molecular chaperone TRAP1 for the synthesis and import of mitochondrial proteins

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Abstract

A complex interplay between mRNA translation and cellular respiration has been recently unveiled, but its regulation in humans is poorly characterized in either health or disease. Cancer cells radically reshape both biosynthetic and bioenergetic pathways to sustain their aberrant growth rates. In this regard, we have shown that the molecular chaperone TRAP1 not only regulates the activity of respiratory complexes, behaving alternatively as an oncogene or a tumor suppressor, but also plays a concomitant moonlighting function in mRNA translation regulation. Herein we identify the molecular mechanisms involved, demonstrating that TRAP1: i) binds both mitochondrial and cytosolic ribosomes as well as translation elongation factors, ii) slows down translation elongation rate, and iii) favors localized translation in the proximity of mitochondria. We also provide evidence that TRAP1 is co-expressed in human tissues with the mitochondrial translational machinery, which is responsible for the synthesis of respiratory complex proteins. Altogether, our results show an unprecedented level of complexity in the regulation of cancer cell metabolism, strongly suggesting the existence of a tight feedback loop between protein synthesis and energy metabolism, based on the demonstration that a single molecular chaperone plays a role in both mitochondrial and cytosolic translation, as well as in mitochondrial respiration.

Categories

Proteins

The dual role of the mitochondrial protein IF1 in cancer

Valentina Giorgio[1], Martina Grandi[1], Cristina Gatto[1], Simone Fabbian[2], Natascia Tiso[3], Francesco Argenton[3], Massimo Bellanda[2], Giancarlo Solaini[1], Alessandra Baracca[1]

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Abstract

The mitochondrial protein IF1 is overexpressed in many human tumors and has been shown to favor cancer growth, although its mechanism of action is still debated. It has been established that IF1 binds to the catalytic domain of the ATP synthase and inhibits ATP hydrolysis in anoxic/hypoxic tumors, avoiding ATP dissipation and promoting cell survival [1, 2]. Recently, we have shown, by immunoprecipitation and proximity ligation assay, that IF1 binds to the ATP synthase OSCP subunit in HeLa cells under oxidative phosphorylation conditions [3]. The IF1-OSCP interaction is confirmed by NMR spectroscopy analysis of the recombinant soluble proteins. The ATP5IF1 gene disruption in HeLa cells decreases colony formation in soft agar and tumor mass development in xenografts, underlining the role of IF1 in cancer. The lack of IF1 in normoxic conditions does not affect proliferation, but it sensitizes the cells to the opening of the permeability transition pore (PTP). Overall our results suggest that the IF1-OSCP interaction protects cancer cells from PTP-dependent apoptosis under normoxic condition.

In conclusion, we show that the upregulation of IF1 in cancer cells can act with a dual pro-survival mechanism under stress conditions. On the one hand, IF1 inhibits ATP hydrolysis through the canonical binding to the F1 sector, in severe hypoxic/anoxic and acidic pH conditions; on the other hand, under oxidative phosphorylating conditions, IF1 binds to the ATP synthase OSCP subunit, preventing PTP opening and apoptosis.

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Categories

Tumor Biochemistry

Dihydroorotate dehydrogenase as innovative target for host and pathogen-directed therapies

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Abstract

Mycobacterium tuberculosis (Mtb) is one of the most infectious killers causing 1.5 million deaths. Moreover, the insurgence of antibiotic resistant strains has led to worrying rise of deaths making urgent the need for innovative antitubercular drugs. Being involved in DNA and RNA formation, pyrimidine biosynthesis pathway (PBP) plays a pivotal role in more conventional pathogen-directed therapies (PTD) and in innovative host-directed-therapies (HDT) primarily due to the strong dependence of Mtb, obligate intracellular bacterium, on host nucleotides for its survival and replication. Among the enzymes involved in de novo PBP, dihydroorotate dehydrogenase (DHODH) catalyzes the rate-limiting step by converting (S)-Dihydroortotate into Orotate in a redox ping-pong reaction. In this context, *human* DHODH (*h*DHODH) has been deeply investigated as essential player for maintaining the fitness of the host[1], whose survival must be assured and eventually balanced with that of the pathogen. While *h*DHODH has been widely characterized as druggable target[2], *Mt*DHODH still lacks biochemical and structural insights. In the present study, we propose the first biochemical characterization and the previously unreleased crystal structure of the protein that allowed us to rationally screen our *in-house* chemical library identifying the first selective *Mt*DHODH inhibitor, paving the way to a further *hit-to-lead* process to reach acceptable drug-like properties for *in vivo* experiments[3]. Moreover, we demonstrated that a plant-derived compound already reported to be active on *h*DHODH, is also active on *Mt*DHODH, suggesting its potential use in a balanced combining of PTD and HDT that may result in a severe nucleotide deficiency and pyrimidine starvation.

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Categories

Proteins

BRD4 inhibitors JQ1 and OTX015 promote cell apoptosis by altering mitochondria dynamics and shifting metabolisms from glycolysis to OXPHOS

Teresa Rossi[1], Egidio Iorio[2], Mattea Chirico[2], Maria Elena Pisanu[2], Nicola Amodio[3], Francesca Colciaghi[4], Alessia Ciarrocchi[1], Antonino Neri[1], Mariaelena Pistoni[1]

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Abstract

BRD4 is a member of the BET family. Repressing BET proteins' function using bromodomain inhibitors (BETi) led to antitumor effects by regulating the transcription of genes downstream of BRD4. Here, we showed that BETi promoted the apoptosis of triple-negative breast cancer (TNBC) cells by altering mitochondrial dynamics inducing mitochondrial dysfunction, and increasing OXPHOS metabolism. We found that BETi-treatment of TNBC cells significantly downregulated the expression of BCL2 and proteins involved in mitochondrial fission, but not proteins involved in mitochondrial fusion. We also confirmed by microscopy analysis (confocal fluorescence and electronic transmission) the increase in mitochondria fusion upon BETi-treatment. Changes in mitochondrial morphology prompted alterations in mitochondrial biogenesis, tricarboxylic acid cycle (TCA), electron transport chain, reactive oxygen species, and energy metabolism, which eventually caused cell death. We demonstrated that BETi administration increased the mtDNA content and the expression and function of Succinate dehydrogenase (SDHA). The increase in TCA cycle intermediates resulted in a reduction in fatty acid synthesis without varying the redox state of the TNBC cells. To improve efficacy in reducing TNBC tumor growth, we treated cells with a combination of BETi and metformin. We found that the treatment synergistically suppressed growth, vitality, and upregulated SDHA expression. The results of this study demonstrated that BETi could affect mitochondrial structure and function by regulating mitochondrial dynamics and, ultimately, induce apoptosis and inhibit tumor growth. This study provided some clues for exploring the relationship between BRD4 and mitochondrial dynamics and apoptosis in TNBC cells.

Categories

Tumor Biochemistry

The DAR database: mapping disease-related enzymes to Reactome pathways

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Abstract

For understanding the molecular mechanisms at the basis of disease insurgence, it is important to know which biochemical pathways and reactions are involved. We aim to investigate the enzyme universe, retrieving the molecule interacting networks related to disease-associated enzymes.

To address this problem, we released a new database called DAR [1] (Diseases And Reactome), which is available on our website (https://dar.biocomp.unibo.it). DAR contains 1,494 human enzymes associated with 2,539 genetic diseases derived from OMIM, Humsavar (https://www.uniprot.org/), Clinvar (https://www.ncbi.nlm.nih.gov/clinvar/) and Monarch (https://monarchinitiative.org/). Disease names are standardized following the Mondo ontology (https://mondo.monarchinitiative.org/). In DAR, we mapped disease-associated enzymes into biological processes using the Reactome pathways (https://reactome.org/), taking advantage of their hierarchical organization of 2580 pathways into 29 main roots. By mapping into Reactome pathways the set of enzyme-associated diseases (described with MONDO code), we found a Reactome-disease association for 1525 pathways. The possibility of resuming the gene-disease mapping by Reactome roots highlights the complex relationships among different pathways, establishing links among diseases and possible co-morbidities. A search in DAR allows to characterize the disease-gene-pathway/s association, helping in understanding the biochemical/molecular biology of the disease across different pathways. This can help in the annotation of pathogenetic gene variants, particularly in the case of rare diseases, 75% of the collected diseases have an Orphanet identifier (https://www.orpha.net/).

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Categories

Computational and Systems Biology

Challenges and advances in omics data integration in constraint-based models of metabolism

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Abstract

The regulation of metabolism is relevant in various fields, such as biotransformations, wellness, and health. A thorough understanding of metabolic processes requires knowledge of metabolic fluxes, which can be challenging to determine directly, especially at the single-cell level. Researchers often rely on other omics data, such as transcriptomics, proteomics, and metabolomics, to study metabolic fluxes [1,2]. However, a single omics approach may not provide a complete characterization of fluxomics. As a result, integrating multiple omics data sources and utilizing mathematical tools capable of simulating metabolic fluxes is necessary.

To address this challenge, we developed a computational approach called INTEGRATE [3]. This approach combines a genome-scale metabolic model (GEM) with transcriptomics and metabolomics data to create a predictive model of metabolic fluxes. The pipeline enables the prediction of which reactions are purely controlled by metabolic control, rather than by gene expression or a combination of the two, in a steady-state metabolic model of central carbon metabolism. This information is valuable in a clinical setting to develop personalized therapies for patients with multifactorial diseases such as cancer.

We demonstrated the effectiveness of the INTEGRATE pipeline using a set of immortalized normal and cancer breast cell lines. The ability to determine the regulatory level at which a given metabolic reaction is controlled is crucial in developing targeted and truly personalized therapies for cancer patients.

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Categories

Computational and Systems Biology

Stress-related role of a new angiogenin NLS mutant in the stress response of human keratinocytes (HaCaT)

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Abstract

Human angiogenin (hANG) is the most studied stress-induced RNase. In altered cell conditions, hANG is actively involved in the translational arrest and the recruitment of stress granule (SGs), transient cytosolic ribonucleoprotein complexes containing mRNAs encapsulated in stalled translation pre-initiation complexes (1). The role of hANG in stressed cells is directly related to its intracellular localization and ability to cleave tRNA in halves, known as tiRNAs (stress-derived tRNA fragments), which play a key role in the SGs recruitment (1). In physiological conditions hANG is active only in nucleoli, where it promotes rDNA transcription and thus biogenesis of ribosomes and cell proliferation. In all other compartments hANG is strongly limited by RNH1 to which it is closely associated. In stressed cells, hANG loses its binding to RNH1 and concentrates in the cytoplasm where it promotes the production of tiRNAs and the subsequent assembly of SGs (2).

The involvement of hANG in the stress response mechanism is widely validated in many cells but practically unknown in the skin, the protective shield of the human body against many external stressors. Recently, we highlighted significant stress response related properties of hANG also in human keratinocytes (3) and our findings led us to the design of an interesting ANG variant in which we deeply altered its nuclear localization sequence. This variant, produced and extensively characterized both for its structural features and for its potential effects on keratinocytes subjected to oxidative stress, opens an interesting scenario on future studies concerning the enhancement of skin defences by RNases.

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Categories

Proteins

Charge clustering and LLPS: what is the link?

Greta Bianchi[1], Marco Mangiagalli[1], Diletta Ami[1], Junaid Ahmed[2], Silvia Lombardi[1], Noemi Destefani[1], Sonia Longhi[3], Antonino Natalello[1], Peter Tompa[2], Stefania Brocca[1]

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Abstract

The discovery of liquid biomolecular condensates has changed the way biologists conceive cell organization. Indeed, compartmentalization via liquid-liquid phase separation (LLPS) gives rise to a variety of so-called membraneless organelles (MLOs). MLOs form in a spatially and temporally regulated manner, bringing in proximity interactors that will bind directly, or isolating specific components from the cytoplasmic environment, to quench or dampen their function/signal[1,2]. Flexible biopolymers such as nucleic acids and intrinsically disordered proteins (IDPs) are broadly involved in LLPS, due to their multivalency. The rules of protein-protein interactions giving rise to MLOs still elude our knowledge, albeit we know that electrostatic forces strongly contribute to the phenomenon. Interestingly, it has been assessed that the patchiness of interacting elements impact on the rheology of condensates, their cellular function and fate[3]. In this context, we aimed at understanding how the distribution of charged residues impacts LLPS[4,5]. For this purpose, we selected as a model the highly charged N-terminal domain (hNTD) of the human topoisomerase I (hTOP1), an enzyme capable of relaxing supercoiled DNA. First, we demonstrated the ability of hNTD to undergo LLPS in vitro through electrostatic interactions. Then, using permutants of hNTD, we investigated the impact of different charge distributions on hNTD LLPS. We observed that an increased charge clustering of oppositely charged residues affects the sensitivity of condensates to salts and RNA. This suggests that there is an optimal charge distribution favoring in-vitro LLPS and that an extreme charge segregation hinders protein phase separation, leading to atypical biocondensates.

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Categories

Proteins

Enzymatic conversion of group A red blood cells to the universal donor group O

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Abstract

Worldwide demand for blood supplies is constantly increasing and solutions to stabilize these stocks are needed. Incorrect blood transfusions could lead to fatal consequences, making the availability of the correct blood group critical in those conditions where a shortage of blood supply may occur[1]. The immunity recognition system of the ABO blood groups is defined by the presence or absence of specific antigens on the red blood cells (RBCs) surfaces, represented by oligosaccharides linked to the erythrocyte cell membranes' glycolipids and glycoproteins[2]. Glycoside hydrolases (GHs) are the principal actors in the breakdown of complex carbohydrates and glycoconjugates. Thanks to their high specificity, they are often exploited for biotechnological purposes playing a key role in developing new applications[3]. Some GHs can convert A and B-type blood to produce group O universal donor blood, giving a biotechnological breakthrough to developing a universal blood production technology. It has been demonstrated that α-N-acetylgalactosaminidases belonging to the GH109 family can convert blood group A to group O by removing the immunodominant determinant N-acetylgalactosamine sugar moiety from RBCs surface oligosaccharides[4]. Our work describes the biochemical characterization of three novel GH109 enzymes to explore their ability to produce enzymatic converted RBCs (ECO-RBC). The three enzymes showed superior specificity on pNP-α-N-acetylgalactosamine, compared to previously reported GH109 enzymes, the ability to act on purified antigen-A trisaccharide and produce ECO-RBC from human donors' blood. In particular, one of the enzymes was able to convert blood group A more efficiently compared to the commercially available enzyme, previously used for this application.

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Categories

Biotechnology

Engineered polyethylene terephthalate hydrolyzing enzymes as key enabling tools for a sustainable conversion of waste plastics to high added-value compounds

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Abstract

Due to its large and ubiquitous use and recalcitrance to natural degradation, accumulation of postconsumer plastics is generating environmental and health issues [1].

Under a circular economy perspective, the enzymatic biodegradation of polyethylene terephthalate (PET), with the production of terephthalic acid (TPA) and ethylene glycol (EG), simultaneously allows its elimination from the environment (bioremediation) and its conversion to high added-value compounds (upcycling). Although several natural enzymes can, in principle, catalyse this process (named PET hydrolyzing enzymes, PHEs), currently only two of them show properties suitable for an industrial application: the PET hydrolase from Ideonella sakaiensis (IsPET) and the thermostable leaf-branch compost cutinase (LCC).

Using a modular workflow for the evolution of PHEs, set up in our research group, several improved variants of IsPET and LCC were identified [2,3]. Specifically, we produced a thermostabilized and most active variant of IsPET (W159H/F238A/S121E/D186H-ΔIsPET, TS-ΔIsPET) and a variant of ΔLCC (S101N/F243T-ΔLCC) more efficient at moderate temperature. Both variants showed significantly superior performances as biocatalyst for the biodegradation of PET. TS-ΔIsPET showed a ~5 °C higher Tm and a hydrolytic efficiency on the substrate PET (~3.3-fold higher in comparison to the wild-type) [3]. The double variant of LCC was able to depolymerize fully 1.3 g of untreated postconsumer PET waste, in less than 3 days and using 1.25 mg of enzyme at a moderate temperature (55 °C).

Thus, evolved PHEs variants represent novel valuable tools for the design of novel integrated, multidisciplinary, and transversal biotechnological processes for the recycling and upcycling of PET

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Categories

Biotechnology

The KH domains of FMRP: exploring folding, aggregation properties, and pathological variants.

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Abstract

K homology domains (KH) are conserved RNA binding domains in various proteins that play essential roles in RNA metabolism and regulation. They interact with specific RNA motifs, participating in mRNA splicing, translation, and localization, which is crucial for the control of gene expression.

Fragile X Mental Retardation Protein (FMRP) is vital for normal brain development and functioning. FMRP deficiency or mutations cause fragile X syndrome (FXS), a genetic disorder characterized by intellectual disability, behavioral problems and developmental delays [1].

Structurally, FMRP consists of i) an N-terminal domain, ii) two typical KH domains (KH1 and KH2), and iii) an unstructured C-terminal region with an RGG box. The N-terminal domain contains a degenerate KH0, which lacks the GxxG region, usually involved in nucleic acid binding [2].

Among others, R138Q and G266E, located in KH0 and KH1, significantly impact FMRP neuronal development and roles of synaptic function and have been associated with FXS [2].

Using a battery of biophysical techniques, we investigated the stability, folding mechanism and aggregation propensity of KH0 and KH1 and their pathological variants R138Q and G266E. We found that the KH0 domain folds through a 3-state mechanism while KH1 apparently folds via a two-state folding mechanism. Furthermore, KH0 has the propensity to form amyloid-like aggregates under mild conditions in vitro, and the R138Q mutation leads to a general destabilization of the protein and an increased propensity for fibrillogenesis [3]. In contrast, KH1 does not aggregate under physiological conditions and the G266 to E mutation completely deconstructs the KH1 domain.

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Catalano F., Santorelli D, Troilo F., Angelucci F., Fata F., Astegno A., Favretto F., D'Abramo M., De Sciscio M.L., Del Giudice A., Federici L., Demitri N., Giardina G., Travaglini-Allocatelli C., Di Matteo A.

Categories

Proteins

Immunoglobulin light chain (AL) amyloidosis: towards a physiological fibrillogenesis model

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Abstract

Background. AL amyloidosis is a life-threatening disease caused by deposition of immunoglobulin light chains (LCs). The amyloidogenic mechanism in vivo is highly debated, through theories and models highlighting the role of the tissue environment, or of the individual LCs' structural propensity to amyloid conversion. We have specifically addressed the role of major truncated forms of amyloidogenic LCs in determining the kinetics of fibril formation in a bio-compatible environment, thus providing a novel model of LC fibrillogenesis suitable for mechanistic studies, and a new tool for drug discovery.

Methods. Distinct LCs proteoforms were produced, both unlabeled and isotopically labeled, as recombinant proteins1. Fibrillogenesis kinetics, cross-seeding, aggregate structure and molecular dynamics are being studied by biochemical (ThT fluorescence, gel-based studies), electron microscopy and spectroscopic (FTIR) approaches, and high resolution nuclear magnetic resonance (NMR).

Results. A full-length amyloidogenic LC (FL-LC) and fragments thereof of different length were purified. In contrast to FL-LC, the fragments are amyloidogenic in vitro under physiological conditions, but with different kinetics and differently sensitive to the effect of seeding in kinetics and structure of the aggregates. In particular, we have identified, within the population of truncated LCs, a major player in the formation of fibrils, mimicking structural features of natural amyloid.

Conclusions. The role of proteolysis in LC fibrillogenesis represents an ancestral but still crucial issue concerning the pathophysiology of AL amyloidosis2-3. Our data can provide new clues for a theory reconciliating both the role of LC structure and the contribution of the natural biochemical activity of affected tissues.

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Categories

Proteins

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POSTERS

B I O T E C H N O L O G Y

Design Of Experiments (DOE) approach to obtain biotechnological products exploiting buffalo whey: a biorefinery for food, nutraceutical and cosmeceutical applications.

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Abstract

A recently arising aim in the circular economy approach is to convert a potential pollutant and difficult to manage wastewater in a resource to be exploited with potential nutraceutical, cosmeceutical and food applications (1). In this direction, the present study focused on the implementation of a downstream process (2) through DOE experiments in order to optimize membrane based processes parameters such as pressure, flow rate, cut-off (in ultra and nanofiltration) in order to obtain diverse value added fractions from whey. Specifically a fraction rich in lactose from nanofiltration, usable for the fermentation of lactic acid bacteria. The latter was exploited in medium formulation to grow LAB in bioreactors, towards lactic acid and biomass (starter/probiotic) production. Also the parameters of fermentation process, such as nitrogen sources, stirring, microaerophilic conditions, gas flow rate, feed quantity and profile in fed-batch processes were optimised using DOE experiments. In particular, we aimed at improving growth and study the physiology and metabolism of Lactococcus Lactis I7 using Plunket-Burman Model for screening in DOE. The data obtained prompted further studies to analyze the growth features of the strain aiming at the production of biotechnologically relevant molecules (exopolysaccharides, bacteriocins..). In fact besides targeting gastrointestinal disorders probiotics (often belonging to LAB) have been reported to improve immunomodulation and protection of the host against infections caused by viral and bacterial pathogens. Furthermore, in the proposed downstream another fraction was recovered and characterized rich in proteins that proved able to form protein-based films for food applications (3).

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Categories

Biotechnology

Advanced biomaterials engineered for the production of sustainable compound.

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Abstract

Among the components that constitute tyre, nowadays there are silica and carbon black used as filler system. The goal of the project is to develop materials from renewable, non-petroleum-related raw source in order to replace them and reduce the ecological impact of the composites themselves throughout their life cycle.

Cellulose and chitosan are the selected biomaterials, thanks to their structure and features. The key challenge is to identify a biocatalytic approach that functionalizes them in order to promote their dispersibility and compatibility with Natural Rubber. Methods and preliminary results will be presented.

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Categories

Biotechnology

Therapeutic Potential of Antarctic fungal extract in dampening the aggregation propensity of α-Synuclein

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Abstract

Parkinson's disease (PD) is a neurodegenerative disorder associated with the amyloid aggregation of the presynaptic protein α-Synuclein (α-Syn) and its pathological mutants, like E46K found to be associated with early onset of disease. The pathogenesis is not completely clear but oxidative stress seems to contribute to the formation of α-Syn fibrillar aggregates.

New strategies for PD treatments are focused on decreasing α-Syn neurotoxicity by reduction of its aggregation propensity or by interfering with oxidative stress in the brain. Indeed, there is growing interest in exploring alternative therapeutic approaches based on natural products, such as polyphenols.

We characterized three unexplored Antarctic fungal species in terms of Total Phenolic Count (TPC), Total Flavonoid Count (TFC), and antioxidant activity. The effect of fungal extracts on the aggregation propensity of α-Syn as well as E46K was also investigated using spectroscopic techniques. Our results indicate that they influence the aggregation pathway of both proteins. In particular, the methanol extract of Trametes sp. acts as a fibrillation inhibitor of E46K while the aqueous extract of Sistotrema sp. favors the acquisition of an α-helical secondary structure.

Further studies are underway to identify active compounds endowed with the α-Syn aggregation inhibition activity within alcoholic and aqueous extracts. This study highlights the possibility of using the extracts of Antarctic fungi as a potential source of new functionally relevant metabolites to be used as a therapeutic strategy for the prevention of amyloid aggregation and treatment of PD.

Categories

Biotechnology

A critical balance between conductivity and osmolarity in buffer for CTCs isolation in Dielectrophoretic Experiments

Paolo Giuseppe Bonacci[1], Samuele Moscato[2], Massimo Camarda[2], Andrea Ballo[2], Salvatore Petralia[3], Ludovica Maugeri[3], Stefania Stefani[1], Nicolò Musso[1]

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Abstract

Dielectrophoresis (DEP) is currently used to determine the electrical properties of cells, which are then analyzed and exploited for manipulation and selection from a suspension mixture [1]. One of the major limitations of this technique is the buffer composition that must not interfere with cellular biochemical parameters, in conditions of low conductivity/osmolarity, in order to minimize parasitic effects that could reduce the dielectrophoretic potential difference [2]. In this work, we have reproduced a buffer reported in the literature [3] with some modification, and ordinarily used by us for Dielectrophoresis experiments on Colorectal Cancer Cells (Caco-2 ATCC HTB-37™), and we have evaluated its impact on cellular metabolism by modifying both conductivity and osmolarity. The buffer composition is Sucrose 9,5%; Dextrose 0,1 mg/mL; 0,1 % Bovine Serum Albumin, 2%, 1X Phosphate Buffered Saline pH 7.0, 1%; (CH3COO)2Ca 0,1 mM. The conductivity is adjusted at 33 mS/m with KCl 4%. The cells were incubated for 5 and 24 hours with the aforementioned buffer and with dilution of the original one at 10, 1 and 0,1 mS/m. To evaluate the effect on metabolism, a (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed. The result was a noticeable decrease in cell metabolic activity (about 55%) already after 5 hours for the least diluted buffer (10 mS/m). This result confirms how the preparation of a buffer suitable for cell maintenance is a critical step in the management of a dielectrophoretic experiment, and how minimal variations can lead to a considerable metabolic disomeostasis.

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Categories

Biotechnology

A critical balance between conductivity and osmolarity in buffer for CTCs isolation in Dielectrophoretic Experiments

Paolo Giuseppe Bonacci[1], Samuele Moscato[2], Massimo Camarda[2], Andrea Ballo[2], Salvatore Petralia[3], Ludovica Maugeri[3], Stefania Stefani[1], Nicolò Musso[1]

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Abstract

Dielectrophoresis (DEP) is currently used to determine the electrical properties of cells, which are then analyzed and exploited for manipulation and selection from a suspension mixture [1]. One of the major limitations of this technique is the buffer composition that must not interfere with cellular biochemical parameters, in conditions of low conductivity/osmolarity, in order to minimize parasitic effects that could reduce the dielectrophoretic potential difference [2]. In this work, we have reproduced a buffer reported in the literature [3] with some modification, and ordinarily used by us for Dielectrophoresis experiments on Colorectal Cancer Cells (Caco-2 ATCC HTB-37™), and we have evaluated its impact on cellular metabolism by modifying both conductivity and osmolarity. The buffer composition is Sucrose 9,5%; Dextrose 0,1 mg/mL; 0,1 % Bovine Serum Albumin, 2%, 1X Phosphate Buffered Saline pH 7.0, 1%; (CH3COO)2Ca 0,1 mM. The conductivity is adjusted at 33 mS/m with KCl 4%. The cells were incubated for 5 and 24 hours with the aforementioned buffer and with dilution of the original one at 10, 1 and 0,1 mS/m. To evaluate the effect on metabolism, a (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed. The result was a noticeable decrease in cell metabolic activity (about 55%) already after 5 hours for the least diluted buffer (10 mS/m). This result confirms how the preparation of a buffer suitable for cell maintenance is a critical step in the management of a dielectrophoretic experiment, and how minimal variations can lead to a considerable metabolic disomeostasis.

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Categories

Biotechnology

Design of Nanodiamonds – DAAO nanosystems for potential antitumor applications: studies of activity, citotoxicity and biocompatibility

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Abstract

In late 1900, the oxidation therapy, based on the controlled production of Reactive Oxygen Species directly into the tumor site, was introduced as alternative antitumor approach. For this purpose, D-amino acid oxidase (DAAO) from the yeast Rhodotorula gracilis, an enzyme able to efficiently catalyze the production of hydrogen peroxide from D-amino acids1, was adsorbed onto nanodiamonds (NDs), carbon allotropes with a diamond inner core (sp3 bonded carbon atoms) and a graphitic outer shell (sp2 layers covering the core), used in nano-oncology as excellent vehicles for drug delivery2. NDs were previously functionalized with hyaluronic acid (HA)3, able to increase both the biocompatibility and the CD44 receptor mediated phagocytosis of nanoparticles in many cancer cell lines, polyethylene glycol (PEG)4, creating drug carriers with prolonged circulation time, and poly(glycerol monomethacrylate) (PGMA)5, characterized by low toxicity and stealth properties.

In vitro activity and ROS measurement assays demonstrated that DAAO-functionalized NDs (f-NDs-DAAO) are able to produce H2O2. Cytotoxicity tests and cells viability assays showed the ability of f-NDs-DAAO systems to induce toxic effects on selected tumor cell lines.

After incubation in human plasma, the protein corona was investigated by SDS-PAGE and mass spectrometry analysis. Both the functionalization and the DAAO adsorption generally seemed to influence the composition of the protein corona.

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Categories

Biotechnology

Natural compounds modulate inflammatory state in in-vitro model of Preeclampsia

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Abstract

Preeclampsia (PE) is a disorder affecting approximately 2-8% of pregnant women characterized by hypertension and proteinuria. It is the leading cause of maternal and perinatal mortality and morbidity. The pathology manifests itself at 20 weeks of pregnancy, activating a series of pro-inflammatory cytokines. Currently, the treatment of PE includes rest and blood pressure control. In light of this, our study focused on: the generation of an in-vitro PE model; testing the cytotoxicity of 4 biocompounds; evaluating the protective role of natural substances against inflammation; study the epigenetic modifications in the PE model.

We generated an in-vitro model of PE using trophoblast cell line HTR8-SVneo treated with TNF-α for 24h at a concentration of 50 ng/mL; after we verified by cell viability assay that the compounds were not cytotoxic. We also evaluated by MTT whether pre-treatment with the compounds for 24h could protect against inflammation caused by TNF-α.

In addition, we monitored IKB-α at the protein level showing a significant accumulation in the cells subjected to pre-treatment and in the same samples by qPCR a down-regulation of IL-8 and Cox-2 was highlighted.

Finally, we evaluated by western blot two histone modifications: H3k4me2 (opening) and H3k9me3 (closing). Our data showed for the first time that pre-treatment with biocompounds increased H3k4me2 protein level and decreased H3k9me3; the trend reversed in cells treated with TNF-α alone.

This preliminary study allowed us to generate an in-vitro PE model and verify the efficiency of some biocompounds in the modulation of inflammatory targets and histone proteins.

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Brancaccio et al. https://doi.org/10.3390/genes13101781

Categories

Biotechnology

Blockade of BAG3 protein impairs adverse remodeling after myocardial injury

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Abstract

Most myocardial diseases result in cardiac fibrosis, that is associated with adverse outcome. In many cases, fibrosis results from a reparative process following cardiomyocyte injury. Evidence in non-cardiac context indicate a pro-fibrotic activity of BAG3 protein. BAG3 plays a key role in cardiomyocyte intracellular homeostasis and is also released by stressed cardiomyocytes. We evaluated the effects of anti-BAG3 mAb treatment on cardiac fibrosis in a murine model of cryoinjury-induced myocardial infarction. Infarcted animals showed heart fibrosis after 6 weeks, along with severe impairment of heart hemodynamic and morphological parameters. In the same timespan anti-BAG3 mAb treatment reduced fibrosis and preserved the ejection fraction, fractional shortening and left ventricular internal diameter in diastole. These results could pave the way through the therapeutic use of the anti-BAG3 mAbs in cardiac fibrosis.

Categories

Biotechnology

Transcriptomic analysis of the left and right ventricles in a rat model of pulmonary hypertension

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Abstract

Pulmonary hypertension is a chronic, progressive and fatal pulmonary vascular disease that leads to right ventricular hypertrophy, RV failure and ultimately death. Asynchronous ventricular activation, a condition that occurs in heart failure or pulmonary hypertension, can alter the normal pump function of the heart by increasing the electromechanical delay (EMD).

This study investigated the change in EMD in the cardiac tissue of male rats (Sprague-Dawley strain) in which pulmonary hypertension was induced through an intraperitoneal injection of monocrotaline[1] (60 mg/kg i.p., MCT group), or an equivalent volume of saline solution (as control group). Cardiac chamber dimensions, heart and lung weights were measured and several samples were collected for histological and molecular analysis. Our results have confirmed that pulmonary hypertension causes cardiac changes mainly on the right side of the heart. In addition, a structural remodelling of the left ventricle was observed, still preserving the ejection fraction.

To gain insights into the molecular drivers underlying this phenotype, RNA was extracted from the right and left ventricles of five rats with pulmonary hypertension and heart failure and from five control rats. Libraries for RNA sequencing (Illumina HiSeq) were generated using the QuantSeq 3' mRNA-Seq gene expression technology. A preliminary analysis will focus on the expression of genes encoding voltage-gated ion channel and biomarkers associated with cardiac hypertrophy and tissue remodelling. Then, the transcriptomic profiles will be correlated to the phenotypic traits, to obtain a molecular portrait in the different ventricles under pathological conditions.

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Categories

Biotechnology

Biochemical characterization of three bacterial glucuronoyl esterases from Dyadobacter fermentans NS114

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Abstract

Glucuronoyl esterases (GEs) are serine-type hydrolase enzymes belonging to the carbohydrate esterase family 15 (CE15) which play a central role in the reduction of recalcitrance in plant cell walls, by cleaving the ester bonds between carbohydrates and lignin in the lignocellulosic matrix[1]. Recent studies have suggested that bacterial CE15 enzymes are more heterogeneous in terms of sequence, structure, and substrate preferences than their fungal counterparts. However, the sequence space of bacterial GEs has still not been fully explored, and further studies on diverse enzymes could provide novel insights into new catalysts of biotechnological interest[2]. To expand our knowledge of this family of enzymes we have investigated three putative GEs from Dyadobacter fermentans NS114[3], a Gram-negative bacterium found endophytically in Zea mays. The encoded CE15 genes are dissimilar, with ≤ 35% sequence identity to each other, and were cloned into a modified pET-28a vector and expressed in Escherichia coli BL21(λDE3). The resulting recombinant proteins DfCE15A, DfCE15B and DfCE15C were purified and biochemically characterized by using synthetic substrates. By comparing the biochemical properties of these three GEs, we both expand the existing knowledge on CE15 members and, support the recent thesis that diverse GEs encoded by a single microorganism may have evolved to fulfill diverse physiological functions[2].

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Categories

Biotechnology

RNAi-Mediated Reduction of the Transcription Factor Nrf-2 Blocks the Positive Effects of Dimethyl Fumarate on Metabolic Stress-Induced Cell Death

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Abstract

The rate of obesity in the world is quickly increasing and profoundly impacting our society; indeed, it is considered one of the leading causes of death. Added sugar consumption is one of the major culprits for these events. In addition to obesity, increased consumption of added sugar is linked to a higher risk of heart disease, diabetes, and brain disorders such as Alzheimer's disease (AD). To this end, too much sugar can increase brain inflammation and oxidative damage, two neu-ropathological hallmarks of AD. Dimethyl fumarate (DMF) is an orally bioavailable methyl ester of fumaric acid with potential neuroprotective and immunomodulating activities. In addition, DMF activates the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf-2), a master regula-tor of cells' antioxidant response system. The present work aimed at evaluating the potential bene-ficial effects of DMF in an in vitro model of metabolic stress induced by high sugar levels. We found that DMF rescued the detrimental effects of high and low glucose exposure on SHSY5Y cell viability and oxidative stress. Mechanistically, the effects of DMF were mediated by Nrf-2. Indeed, we found that DMF increased the expression of manganese superoxide dismutase (MnSOD) and heme-oxy-genase-1 (HO-1), two genes whose expression is regulated by Nrf-2. More importantly, we found that reducing the expression of Nrf-2 prevented the beneficial effects of DMF. Together our results indicate that DMF could represent valuable support for therapies aimed at metabolic disorders.

Categories

Biotechnology

Design of human lactate dehydrogenase (*h*LDH-A) based biosensors for the potential screening of anticancer drugs

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Abstract

Despite the progress made by research in cancer treatment, this is the second leading cause of death worldwide. The overexpression of lactate dehydrogenase (LDH-A) is one of the main factors promoting cancer cell proliferation[1]. This enzyme produces lactate, a signaling molecule that allows aerobic glycolysis to occur[2]. The development of selective LDH-A inhibitors is reported to be an efficient strategy to decrease cancer cell proliferation and increase the sensitivity to traditional chemotherapies[3]. This research focuses on the preparation of a stable and active biocatalyst that can be employed in a biosensor for a simpler and cost-effective screening of LDH-A inhibitors. Human LDH-A (*h*LDH-A) is covalently immobilized on mesoporous silica following different strategies. Mesoporous silica is functionalized post-synthesis to provide the functional groups necessary to perform the immobilization. The surface chemistry and the porous structure of the supports prepared are characterized with complementary analyses. The tested immobilizations reach yields higher than 80% while the best retained activity of the enzyme achieved is as high as 24.2%. The presence of the enzyme on the silica surface is proved using Fluorescence microscopy analysis. The best immobilized *h*LDH-A is further investigated, its highest activity is reached at pH 5 and 45 °C, while for the free enzyme, the maximum activity is achieved at pH 8 and 45 °C. The thermal stability is tested by incubating the enzyme at 45 °C for at least 64 hours. These results pave the way to develop stable and active LDH-based sensors.

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Categories

Biotechnology

The interplay between uremic toxicity and vascular calcification in Chronic Kidney Disease: role of lanthionine, as a novel uremic toxin.

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Abstract

Chronic Kidney Disease (CKD) is becoming the fifth leading cause of death worldwide by 2040. Cardiovascular (CV) events are the main mortality cause in CKD (CV risk is about 10 times higher than that occurring in the healthy subjects). Metastatic calcification, calcium salt deposition in the vessel wall, is frequent in CKD. Patients also accumulate retention solutes (uremic toxins; UT), which may contribute to CV risk. As only a few UT have been considered for their effects on vascular calcification (VC), we assessed the potential role of lanthionine, a transsulfuration side-product, as a novel UT acting on VC. We selected a group of CKD patients, including hemodialysis subjects. Lanthionine levels increased proportionally to the severity of CKD, particularly in the hemodialysis group. Circulating lanthionine raised with the increase of Total Calcium Score (TCS), according to the Agatston score. Then, we evaluated the effects of lanthionine at concentrations in the range actually detected in CKD patients, alone or in combination with calcium and phosphate, in a Zebrafish embryo model. Using the ZFIN network, we analyzed some calcification-related markers. Results showed that lanthionine alone or under pro-calcifying condition increased the expression of Bone Morphogenetic Protein 2b/4 (*BMP2b/4*), Runt-related transcription factor 2a/2b (*RUNX2a/b*), Osteopontin (*SPP1*) and Alkaline phosphatase (*ALPL*) as early calcification markers, as well as the expression of Osteocalcin (*BGLAP*), as a late marker. Results set the basis for assessing the role of lanthionine as an UT contributing to VC, by inducing the expression of markers involved in the mineralization process.

Bibliographic references

Categories

Biotechnology

Renewable biopolymers SEC-TDA hydrodynamic characterization as a powerful tool towards the optimization of their biomedical application.

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Abstract

Alginate(Alg), made of repetitive dimers of D-Mannuronic-acid and L-Guluronic-acid, obtained by bio-fermentation or by extraction from algae, is among the most used renewable biopolymers in biomedical-field. In these applications it's often used in form of hydrogel obtained exploiting Alg ability to gel/crosslink in presence of calcium-ions.

Although Alginate molecular-weight(MW)distribution and conformational-features rationally have a great impact on final hydrogel performance, these property of such hydrogels are rarely adequately characterized, in most cases,informations like "low/high-viscosity from brown-algae" are provided ,and in few cases, size-exclusion-chromatography coupled to multi-angle light-scattering(SEC-MALS) characterization is supplied.[1]

Indeed data on the hydrodynamic-parameters of alginates and on their correlation with biophysical-properties of hydrogels are still lacking.

On this basis, several commercially alginates were characterized using Size-exclusion-chromatography coupled with triple-detection(online laser light-scattering, refractometry, viscometry) (SEC–TDA).[2]

The weight and number average MW (Mw;Mn), polydispersity-index(Mw/Mn), hydrodynamic-radius(Rh), and intrinsic-viscosity([η]) were derived. The dynamic-viscosity of alginates solutions were measured. Hydrogels were prepared, following conventional protocols described in literature.

Their morphology, porosity, hydration mechanical-properties, stability under different conditions were evaluated.

The correlation among all the properties studied and biopolymer hydrodynamic-parameters was investigated providing valuable data for the optimization of alginate performance in biomedical-field.

Alg samples will be selected (e.g. high/low MW) to evaluate the effect of the biopolymer size, viscosity and hydrodynamic-features on the final performance in relation to encapsulation of probiotics for preservation of viability and also to evaluate other emerging-applications , like tissue-repair,wound-healing.[3]

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Categories

Biotechnology

Novel probiotic formula containing hydroxyectoine for preserving the viability and enhance the biological activity of probiotics on enterocyte based *in vitro* model

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Abstract

Dysbiosis is commonly detected in patients with inflammatory bowel disease (IBD), supporting the concept that a dysregulated immune reaction to bacterial antigens has a pathogenic role in the development of intestinal inflammation (Aragon, G et.al 2010). We have investigated the beneficial effects of a novel probiotic formulation assembled by combining three probiotics (Lactobacillus fermentum, Lactobacillus brevis and Bifidobacterium lactis) with hydroxyectoine (HOE), which is a compatible solute that has a potent cryopreservation, anti-inflammatory, and non-toxic properties (Bethlehem L et al 2020). Briefly the three probiotics strains were lyophilized after the addition of HOE solution, and their viability were assessed after lyophilization, storage up to 6 months and after the exposure to simulated gastrointestinal juices. The anti-inflammatory ability of this formula was also investigated using differentiated enterocytes. These were challenged with LPS to induce cell inflammation alone or in the presence of a mixture of the three probiotic strains formulated with HOE. The result showed the ability of HOE + probiotics in preserving the viability of probiotics during lyophilization, subsequent storage and during the exposure to simulated gastrointestinal fluids. Moreover, adding HOE to probiotics improved the beneficial effects of the bacterial therapy by reducing expression of pro-inflammatory mediators , and counteracting the loss of Zonulin. In summary, we have shown that a novel three probiotics strains probiotics combined with HOE exerts beneficial effects on preserving probiotic`s viability during production and simulated digestion. Also this formula showed their potentialities as nutraceuticals to alleviate intestinal inflammation and improve mucosal barrier function.

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Categories

Biotechnology

Bacterial biofilms from *Staphylococcus* sp. trigger activation of coagulation: a possible link between infections and cardiovascular diseases

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Infective endocarditis (IE) is an infection of the cardiac endothelium. It has an annual incidence of 3–10/100,000 of the population with a mortality of up to 30%[1]. Staphylococcus aureus is the most prevalent cause of IE[2]. After endothelial injury, bacterial colonisation is facilitated, thus triggering additional endothelial injury and thrombus formation. Production of a biofilm assists bacterial persistence and contributes to antibiotic tolerance[3].

Since thrombotic complications are often associated with IE, here we explore the possibility that *S.aureus* (coagulase+) and *S.epidermidis* (coagulase-) biofilms, could induce fibrin generation in human plasma and investigate their ability to convert directly fibrinogen into fibrin.

S.aureus and *S.epidermidis* were cultured, added to a microtiter plate and incubated, leading to the formation of bacterial biofilms. To test fibrin generation in plasma, diluted human plasma was added to each biofilm and to empty wells as a blank experiment. To test fibrin generation from purified fibrinogen, a solution of fibrinogen (0.15mg/mL) was added to each biofilm and to empty wells as a control. Fibrin generation was monitored by turbidimetry.

The data obtained indicate that: both biofilms of *S.aureus* and *S.epidermidis* efficiently and similarly induce fibrin clotting in human plasma; both biofilms of *S.aureus* and *S.epidermidis* do not convert isolated fibrinogen solutions into fibrin.

This study's results prove that bacterial biofilms can trigger blood coagulation, thus providing the molecular basis for explaining the positive relationship between IE, biofilm formation and increased thrombotic risk. Further studies are need to elucidate the biochemical mechanisms underlying biofilm-induced activation of blood coagulation.

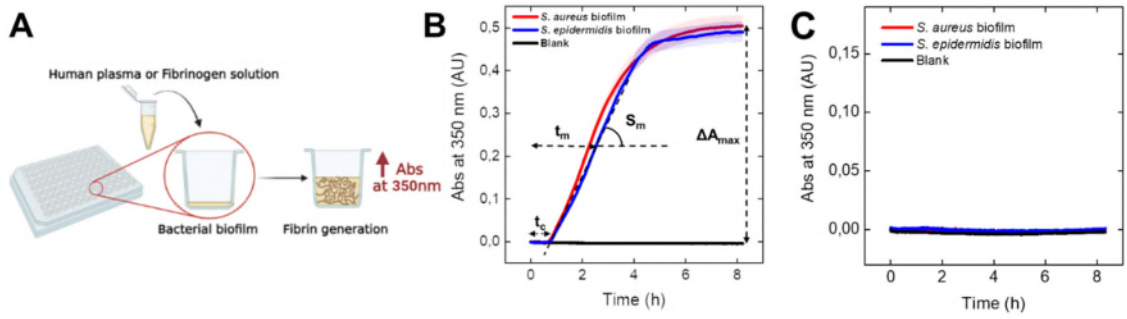

Figure 1. Fibrin generation in human plasma induced by *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms (B), as indicated (A) and fibrinogen conversion in fibrin in presence of *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms (C).

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Categories

Biotechnology

SARS-CoV-2 Main protease (M^{pro}) activates blood coagulation: a possible link between viral infection and thrombotic complications in COVID-19

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Abstract

Severe/fatal thrombotic complications are often associated with COVID-19. However, the mechanisms linking infection and thrombosis are not yet well understood. Here, we hypothesise that the Main protease (M^{pro}) of SARS-CoV-2 could trigger coagulation by proteolytically activating coagulation factors at specific Arginine residues. Notably, M^{pro} is necessary for viral duplication, as it cleaves the viral polyprotein at Glutamine-X bonds, to release the capsid-forming subunits.

- Investigate whether Mpro can trigger plasma coagulation by activating one or more coagulation factors;
- unveil the mechanism of activation.

Turbidimetric assays and Survival Analysis[1] were used to access plasma clotting activity of MPro. Activation of isolated coagulation factors by Mpro was detected by specific enzymatic assays. The Mpro cleavage sites on coagulation factors were identified by Mass Spectrometry (MS), using the TAILS protocol[2]. Mpro substrate specificity was determined using the HTPS method[3].

• The “Survival Analysis” of plasma form 20 healthy donors treated or un-treated with Mpro (50-100nM), showed a 2.8-fold increase of the clotting probability for the Mpro-treated group compared to the control group (p-value: 0.022).

• Enzymatic assays TAILS analysis showed that Mpro can activate both FVII and FXII and that FVII activation occurs after cleavage at Arg212-Ile213 bond.

• HTPS analysis confirmed that Mpro has a secondary substrate specificity for Arg-X bonds.

We demonstrate for the first time that addition of Mpro to human plasma leads to clot formation by proteolytic activation of both FVII and FXII, at the beginning of the intrinsic or extrinsic pathways, thus shifting the pro-coagulant-anticoagulant equilibrium toward thrombosis in COVID-19 patients.

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Categories Biotechnology

Engineering PET-hydrolysing enzymes for display on *E. coli* outer membrane: a promising approach for plastic waste management

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Abstract

Humans dominate the Earth, changing it in ways that menace their and other animals' survival [1]. Particularly, the massive use of plastics causes many worries due to their accumulation in nature. The most used plastic is polyethylene terephthalate (PET) for its versatility and durability. These advantageous properties also constitute the reason why it is not biodegradable, enhancing the demand for biological solving from the local to the global scale [2].

For industrial purposes, due to problems of thermostability and solubility, many efforts have been done to improve the production of PET-hydrolysing enzymes (PHEs). In particular, PETases and MHETases convert this polymer into lower environmental impact building blocks [3]. To date, the preferred expression host is *Escherichia coli*, the most frequently used one for surface display. The fusion of catalysts with anchoring motifs indeed represents a promising device for large-scale production [4].

Here we present an approach for PHEs display *in vivo* on *E. coli* surface, exploiting the utilization of these degrading enzymes by their fusion to the innovative Anchoring-and-Self-Labelling-protein-tags (ASL^{tag}) [5]. The final aim is to employ an enzymatic cascade of *in vivo* anchored enzymes able to efficiently degrade PET. We show here that known PHEs are successfully expressed and displayed on the *E. coli* outer membrane and active on chromogenic ester substrates. These promising results lay the foundations also for the development of a versatile PHEs screening system, with the final purpose of PET waste valorisation.

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Categories

Biotechnology

SERPINA3/SerpinA3n in prion diseases: a novel clearance mechanism

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Abstract

To investigate the molecular mechanisms underlying prion diseases, Barbisin and colleagues conducted an extensive transcriptional analysis in prion-infected cynomolgus macaques. Among the various biomolecules examined, SERPINA3 exhibited the most significant level of overexpression.

SERPINA3, the mouse homolog of which is SerpinA3n, belongs to the serine protease inhibitor family of acute-phase proteins. Our hypothesis is that in prion diseases, SERPINA3/SerpinA3n inhibits target protease/s involved in the clearance of prions (PrPSc) or the degradation of the cellular prion protein (PrPC) itself, thereby exacerbating the prognosis of prion diseases.

To test this hypothesis, we employed an in silico-designed small molecule named "compound 5" to inhibit SerpinA3n in various prion-infected cell lines, resulting in a decrease in prion load. Furthermore, treatment of the prion-infected neuroblastoma line (ScN2a cell line) with bioactive recombinant SerpinA3n protein in its native conformation led to an increase in prion load.

Presently, we are focused on producing and characterizing the first commercially available monoclonal anti-SerpinA3n antibody.

The availability of a reliable anti-SerpinA3n antibody would facilitate a more comprehensive study of this molecular pathway and aid in identifying the target proteases involved.

Categories

Biotechnology

Biochemical characterization of an extract from the macroalga Chaetomorpha linum

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Abstract

Algal biomass from the Orbetello Lagoon, a coastal area in the south-west of Tuscany, has high ecological importance as well as significant historical and socioeconomic value. However, for decades it has been vulnerable to repeated cycles of eutrophication, leading to environmental and economic disasters for lagoon activities. The macroalgae that infest the Lagoon of Orbetello, with an average biomass of 5,000 t/year, are disposed of as special biodegradable waste, incurring enormous costs for the affected municipalities and the Tuscany Region.1. *Chaetomorpha linum* is the most prevalent species and is studied not just from an ecological standpoint, but also for biotechnological applications, such as the use of its extracts in animal disease control or the cosmetics industry.2.

In this study, an extract of the Orbetello lagoon-collected macroalgae *Chaetomorpha linum* was shown to be exceptionally rich in fatty acids, particularly palmitic, myristic, oleic, and linoleic acids. The extract was tested in cell cultures and the anti-inflammatory activity was found to be the strongest. As a result, our work aims to transform what is commonly considered waste into a resource.

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Categories

Biotechnology

Clove Essential Oil Encapsulated by Chitosan-Based Systems: A Future Outlook

Sondos Hejazi[1], Valeria Scala[2], Nicoletta Pucci[2], Mohammed Sabbah[3], Loredana Mariniello[1], C. Valeria L. Giosafatto[1]

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Abstract

Environmental pollution caused by the excessive use of pesticides is a growing concern for the planet. One of the serious threats to olive trees and olive oil production is Xylella fastidiosa subsp. pauca (Xfp). Xfp is the causal agent of a novel devastating disease, causing the olive quick decline syndrome. Xfp can switch from a planktonic stage, useful to spread within xylem vessels, to a sessile stage, in which the pathogen forms a biofilm that eventually blocks the xylem sap flux. Therefore, we investigated the effect of clove essential oil (CEO) against Xfp and preliminary results showed its powerful activity for inhibiting the growth of Xfp suggesting CEO potential role as a management treatment. To enhance CEO stability, it was encapsulated into chitosan particles (CHPs) obtained by ion gelation. The CHPs functionalized with different amounts of CEO (CHP: CEO = 1:0.04, 1:0.08, 1:0.16, 1:0.32) were characterized in terms of size, charge, encapsulation efficiency and loading capacity. The results showed that the particle size increased by increasing the amount of CEO, while zeta potential analyses confirmed the stability of dissolved particles being around +20 mV. The amount of CEO in the functionalized systems also influenced the encapsulation efficiency and loading capacity since the obtained CHP: CEO = 1:0.08 presented the highest values, around 22% and 35%, respectively. Current investigations are exploring the release of CEO from the encapsulated CHPs for their application in agriculture as a green pesticide to improve the plant disease control strategies.

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Categories

Biotechnology

Gaining insights into biotechnological applications of antimicrobial peptides as emerging therapeutics agents

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Abstract

Antimicrobial peptides (AMPs) have a great interest as potential next-generation therapeutics, since they are natural peptides, produced by all organisms, active against bacteria, viruses, yeasts and protozoa. As they show a broad spectrum of activity against multidrug resistant bacteria, strong efforts are in progress to bring AMP-based drugs into clinical use, in order to counteract the rapidly increasing resistance to classical antibiotics. AMPs are also currently being investigated as anticancer agents that potentially overcome tumor resistance to conventional chemotherapy. In this context, we found an AMP, named chionodracine (Cnd), produced by an Antarctic fish that lives in an extreme environment. Based on its scaffold, we designed a mutant (KHS-Cnd) active against ESKAPE pathogens, and some shorter peptides (added with a lipid tail) effective against *Candida* species [1,2]. We investigated the anti-virulence potential of KHS-Cnd against bacterial clinical isolates from cystic fibrosis patients [3].

Our recent results highlight the effect of KHS-Cnd on protease activity of selected Gram-negative/positive pathogens and on biofilm formation/disaggregation in different tested clinical strains. Moreover, we demonstrated that the myristoylated short peptides are cytotoxic against HeLa cervical cancer cells but sparing healthy ones. The anticancer activity could be attributed to the N-myristoylation effect on cell membrane [4] since no cytotoxic effect of correspondent non-myristoylated peptides was observed on HeLa cells.

Given the importance of identifying new drugs of biotechnological interest, our data look promising, because, starting from a natural AMP, we used its scaffold for developing strategies to increase peptide selectivity toward specific cell targets.

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Categories Biotechnology

Regenerative medicine approach in the treatment of chronic skin wounds

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Abstract

Chronic wounds, named ulcers, are a serious social and health problem, affecting millions of people every year and costing the world's healthcare system huge sums of money. They originate from the interference of internal and external factors with the physiological healing processes and heal slowly or not at all. Among these, diabetic ulcers have attracted the scientific community for their prevalence. The goal is to find a therapeutic approach using regenerative medicine to treat skin ulcers, especially diabetic ones. Fibroblasts isolated from Wistar rat skin have been seeded into PLLA (Poly-L-Lactic Acid) scaffolds treated with collagen fibrils to improve cell adhesion. Furthermore, microvascular fragments have been extracted from adipose tissue (ad-MVFs) of the same rats to be cultured in this system. In this context, fibroblasts cultured in the PLLA scaffold will promote ECM regeneration; while, the ad-MVFs interconnecting with the circulatory system of the host will avoid the hypoxic condition that generally occurs in the ulcer and will allow the right transport of nutrients and gases to the regenerating tissue cells. This construct will be tested in in vivo systems for the treatment of skin ulcers and Streptozotocin diabetes-induced ulcers and analyzed by biochemical, molecular biology and imaging assays. This multifactor approach could be a huge step forward in the treatment of ulcers of different nature.

Categories

Biotechnology

Traumatic Brain Injury Alters Cerebral Concentrations and Redox States of Coenzymes Q9 and Q10 in the Rat

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Abstract

To date there are no information on the changes in brain CoQ levels and variations in its redox state following TBI. In this study, we induced graded TBIs (mild TBI, mTBI and severe TBI, sTBI) in male rats, using the weight-drop closed-head impact acceleration model of trauma. At 7 days post-injury, CoQ9, CoQ10 and α-tocopherol were measured by HPLC in brain extracts of the injured rats, as well as in a group of control sham-operated rats. In the controls, total CoQ was predominantly in the form of CoQ9 and the oxidized/reduced ratios of CoQ9 and CoQ10 were, respectively, 1.05 ± 0.07 and 1.42 ± 0.17. No significant changes were observed in rats experiencing mTBI. In the brains of sTBI-injured animals, an increase in reduced and a decrease in oxidized CoQ9 produced an oxidized/reduced ratio of 0.81 ± 0.1 ($p < 0.001$ compared with both controls and mTBI). A concomitant decrease in both reduced and oxidized CoQ10 generated a corresponding oxidized/reduced ratio of 1.38 ± 0.23 ($p < 0.001$ compared with both controls and mTBI). An overall decrease in the concentration of the total CoQ pool was also found in sTBI-injured rats ($p < 0.001$ compared with both controls and mTBI). Besides suggesting potentially different functions and intracellular distributions of CoQ9 and CoQ10 in rat brain mitochondria, these results demonstrate that sTBI alters the levels and redox states of CoQ9 and CoQ10, thus adding a new explanation to the mitochondrial impairment affecting ETC, OXPHOS, energy supply and antioxidant defenses following sTBI.

Categories

Biotechnology

One-step conversion of citrus waste into lactic acid using the thermophilic biocatalyst *Weizmannia coagulans* MA-13

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Abstract

Agri-food residues represent an excellent source for lactic acid production through microbial fermentation. In particular, citrus processing industry plays an important role in the agro-industrial sector, producing solid/semisolid residues up to million tons every year [1]. The conversion of agri-food residues into value added by-products generally requires chemical pre-treatments, which have severe impact on the environment. Therefore, sustainable strategies focused on waste reduction and its valorisation, are urgently needed [1]–[4]. In this context, *Weizmannia coagulans* MA-13 is a robust thermophilic biocatalyst for production of added-value chemicals in circular economy-based processes [5]–[8]. Indeed, it was proven to ferment efficiently lignocellulosic biomass into L-lactic acid and to tolerate high concentration of biomass-derived inhibitors that usually hamper the microbial fermentation performance. In this study, we assessed the feasibility of using *W. coagulans* MA-13 to valorise untreated citrus waste and produce L-lactic acid in one-step conversion. The use of a thermophilic enzymatic cocktail along with the hydrolytic repertoire of MA-13 enhanced the biomass degradation up to 62% and L-lactic acid production reached 44.8 g/L in fed-batch fermentation. These results highlight the ability of MA-13 to convert the whole glucose amount into L-lactic acid, can be efficiently extended also to agri-food wastes.

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Categories

Biotechnology

Novel amylose-based bioplastics containing argan byproducts derived proteins modified by the means of transglutaminase

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Abstract

The circular economy aims to valorize byproducts that otherwise would get wasted. In this work, we investigate the possibility of producing novel bioplastics, made up of argan seed proteins (APs), extracted from argan oilcake, and amylose (AM), obtained from barley plants through an RNA interference technique. Argan is a plant widespread in arid regions of Northern Africa, where it plays a fundamental socio-ecological role. Argan seeds are used to obtain a biologically active and edible oil, producing a byproduct, the oilcake, that is rich in proteins, fibers, and fats, and is generally used as animal food. Recently, argan oilcakes have been attracting attention as a waste to be recovered to obtain high[1]added-value products. Here, APs were chosen to test the performance of blended bioplastics with AM, because they have the potential to improve the properties of the final product. High-AM-starches present attractive features for use as bioplastics, including a higher gel-forming capacity, a higher thermal stability, and reduced swelling compared to normal starch. It has already been demonstrated that pure AM-based films provide more suitable properties than normal starch-based films. Here, we report on the performance of these novel blended bioplastics in terms of their mechanical, barrier, and thermal properties; and the effect of the enzyme microbial transglutaminase as a reticulating agent for AP's components was also studied. These results contribute to the development of novel sustainable bioplastics with improved properties and confirm the possibility of valorizing the byproduct, APs, using them as a new raw material.

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Categories

Biotechnology

Characterization of Trematocine derived antimicrobial peptides with enhanced antimicrobial activity againstantibiotics resistant bacteria.

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Abstract

Antimicrobial resistance (AMR) is a huge problem for public healthcare system. In fact, infections due to resistant pathogens causes every year more than 700.000 deaths worldwide and this number should be around 10 million in 2050 [1]. A way to contrast this phenomenon is the development of new therapeutic agents, like antimicrobial peptides. These molecules usually display a net positive charge, a high hydrophobicity and are produced by the innate immunity system of all organisms.

This work aims to study the effects of increasing the net positive charge of a natural peptide, the Trematocine, isolated from Trematomus bernacchii [2], on its antimicrobial activity and selectivity towards bacterial membranes.

We designed two mutant peptides (KH-Trem and KHS-Trem) and studied their interaction with two membrane systems, one anionic and one zwitterionic, their capability to permeabilize the outer membrane of

Gram-negative bacteria and the plasmatic membrane of Gram-positive bacteria, their cytotoxic and haemolytic activity against mammalian cells and their antimicrobial activity against ESKAPE pathogens.

Also, we performed in vivo toxicity studies using Galleria mellonella larvae.

The results showed that the modifications made on Trematocine scaffold increased the peptides interaction with membrane model systems, with a slight selectivity towards the anionic model membrane, and highly enhanced their antimicrobial activity. KH-Trem and KHS-Trem revealed a low haemolytic and cytotoxic activity at the concentrations needed to kill pathogens and no any toxicity during the in vivo experiments.

These results are a promising starting point for the development of peptides candidates with the aim to fight

AMR.

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Categories

Biotechnology

New human ATM variants are able to regain ATM functions in Ataxia Telangiectasia disease.

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Abstract

Ataxia Telangiectasia (A-T) is a rare neurodegenerative disease caused by biallelic mutations in the Ataxia Telangiectasia Mutated gene (ATM). No cure is currently available for these patients but positive effects on neurologic features in A-T patients have been achieved by dexamethasone administration through autologous erythrocytes (EryDex) in phase II and phase III clinical trials, leading us to explore the molecular mechanisms behind the drug action. During these investigations new ATM variants, which originated from alternative splicing of ATM messenger, were discovered, and detected *in vivo* in the blood of A-T patients treated with EryDex. Some of the new ATM variants, alongside an *in silico* designed one, were characterized and examined in A-T fibroblast cell lines. Conventional investigations and multi-omics approaches were achieved. ATM variants were capable of rescuing ATM activity in A-T cells, particularly in the nuclear role of DNA DSBs recognition and repair, and in the cytoplasmic role of modulating autophagy, antioxidant capacity and mitochondria functionality, all of the features that are compromised in A-T but essential for neuron survival. These outcomes are triggered by the kinase and further functional domains of the tested ATM variants, that are useful for restoring cellular functionality. The *in silico* designed ATM variant eliciting most of the functionality recover may be exploited in gene therapy or gene delivery for the treatment of A-T patients.

Categories

Biotechnology

Fusion constructs for outer membrane vesicles (OMVs) surface functionalization with antibody portions. Single Chain Fragment Variable (scFv) vs. Heavy Chain Variable (VHH) domains.

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Abstract

Outer Membrane Vesicles (OMVs) are bacterial small proteo-liposomes naturally derived from the bulging of the outer membrane in several Gram-negative bacteria. Enriching OMVs with specific membrane proteins makes them optimal candidates for the development of selective drug delivery systems. In this context, particularly promising is the use of antibodies to achieve nanocarriers cell-specific targeting. Here, OMVs surface functionalization with different antibody portions is described. We developed different chimeras composed by the monomer of the membrane protein cytolysin A (ClyA) as a membrane scaffold, fused at the C-terminus either to a scFv or to a VHH camelid nanobody domain. Both constructs were recombinantly expressed in Escherichia coli (E. coli) and their presence was screened in the purified OMVs. Results highlighted a significant difference in terms of recombinant production and protein processing efficacy, depending on the different antibody domain fused at the C-terminus of the chimera. Indeed, VHH constructs were efficiently targeted to E. coli OMVs, as inferred by Western Blot analysis of the purified vescicles. On the other hand, the scFv fusion construct was mainly found intracellularly; however, the modification of the leader peptide sequence of the scFv construct allowed the successful localization of the fusion construct on OMVs. The correct exposure was confirmed through a partial Proteinase-K proteolysis assay on OMVs, and an immunofluorescence experiment on E. coli recombinant cells. Finally, to evaluate whether scFv and VHHs expressed on OMVs preserve the correct functional features, preliminary binding experiments were carried out through surface plasmon resonance.

Categories

Biotechnology

Identification and characterization of antibacterial compounds targeting the DNA polymerase III holoenzyme

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Abstract

Antibiotic resistance is one of the main causes of death, and antibiotics currently in use sometimes fail to counteract this problem. Protein-protein interactions are essential in microbial physiology due to their central role in many physiological processes and they represent new targets for antimicrobial drug discovery[1,2]. The interaction between α subunit and β-sliding clamp of DNA polymerase III holoenzyme represents a promising target for replication inhibition.

The Bioluminescence Resonance Energy Transfer (BRET) assay is an excellent method to monitor protein-protein interactions. We have developed an *in vivo* yeast-based version of BRET (yBRET[3–6]) reproducing the *Escherichia coli* β-sliding clamp - α subunit interaction, that was used to screen a total of 11.530 compounds. Of these, 6596 came from an untargeted library, while 4934 were selected by a structure-based *in silico* screening.

Six hits and 28 structural analogues were tested for their binding to β-sliding clamp using a Protein Thermal Shift assay to evaluate the specificity of the molecule toward the target. Their activity was also confirmed in vitro by competitive ELISA assay and in the DNA Synthesis Inhibition assay[7]. In addition, the molecules displayed antimicrobial activity against Gram-negative (*E. coli*) and Gram-positive (*Bacillus subtilis*) bacteria.

Efforts are currently being made to validate the interaction between these molecules and the β-sliding clamp through crystallization assays and to identify the most important features that enable the binding of molecules to β-sliding clamp with the aim of supporting the optimization of the hit compounds thus identified.

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Categories

Biotechnology

Preliminary investigation of antiproliferative activity of *Astragalus spuneri* growing in Albania.

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Abstract

Finding new anticancer agents that would show a noticeable selective antiproliferative effect against abnormal cells, as well as minor toxic effect against normal untransformed cells is hugely important for the development of new drugs in oncology. Hence, there is a no information about likelihood of antiproliferative activity of Astragalus spuneri plant extracts. Determination of inhibitory potential, chromosome aberration and disturbance in the mitotic cycle of A. spuneri was performed by Allium cepa squash method. Excised tips were fixed in aceto-orcein for 48 hrs and the cellular and nuclear changes were examined under Motic light microscope, B1 series. Allium cells exposed to different concentrations water plant extracts (125, 300, 600, 1000 µg/ml) showed clearly increasing inhibitory growth change with elevation in concentrations, reaching a maximum of 48.67%. The mitotic index values were differed significantly at $p< 0.05$ than the control with the highest significant decrease observed is 52.1% at 1000 µg/ml water extract. Stickiness is more common than c-mitosis, bridges, laggard chromosomes and apoptosis with increasing in water extract concentration. Based on the results of cytotoxicity, it is possible to assume that the pronounced inhibitory activity of underexploited A. spuneri extracts can affect cellular division and thus lead to a decrease in cell proliferation being a helpful candidate towards anti-cancer property.

Keywords: antiproliferative potential, plant, extracts, *Astragalus spuneri*.

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Categories

Biotechnology

Immuno-HUB: development of new, effective glycoconjugate vaccines for tuberculosis

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Abstract

Tuberculosis (TB) is still a major cause of ill health and one of the leading causes of death worldwide [1]. At present, the only available vaccine is the old and scarcely effective Bacillus Calmette-Guérin (BCG). Thus, a new, efficacious vaccine is urgently needed.

The use of glycoconjugate vaccines is a successful strategy employed to fight various pathologies [2]. Starting from results obtained up to now [3], our work is aimed at the design and production a series of subunit vaccines produced from selected recombinant antigenic proteins of Mycobacterium tuberculosis (TB10.4 and Ag85B) and from their fusion (chimeric) proteins. In order to combine the antigenic properties of the proteins with the antigenic and/or immunogenic characteristic of the linked oligosaccharides (double hit approach), proteins have been engineered to remove unwanted glycosylation sites and to improve their solubility before to proceed with chemical glycosylation. Protein engineering is driven by bioinformatics approaches (e.g. prediction of protein solubility and of sequential and structural epitopes) and protein production is optimized using appropriate expression systems and conditions for each variants. Glycosylation is then conducted employing different sugars (mainly arabinomannans), based on approaches already set up [4].

This project, based on the rational production of semi-synthetic glycoproteins, represents a new strategy for developing TB vaccines, that shows the potential for application to other pathologies.

Funding: This work was supported by the Italian Ministry of Health (Project Immunoterapia: cura e prevenzione di malattie infettive e tumorali (Immuno-HUB), project number T4-CN-02).

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Categories

Biotechnology

Biotechnological production of melanin by *Streptomyces nashvillensis* by using carob pods as substrates

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Abstract

Melanin is a natural pigment occurring in animals, plants, and microorganisms whose color changes from yellow to black. As melanin is a good UV-visible light absorber, free radical scavenger, antioxidant, and metal ion chelator, it has potential applications in food packaging, cosmetic and textile industries. Nowadays it is mainly produced by extraction from marine animal tissues with expensive and not sustainable processes. The possibility to biotechnologically produce melanin by *Streptomyces* strains have been poorly investigated so far, as well as the use of raw lignocellulose wastes as substrates for its production. In this research, 96-h experiments were performed to explore for the first time the possibility to produce melanin by *Streptomyces nashvillensis*. The optimal conditions of bacterial growth and melanin production were investigated by testing different pH and temperature parameters. Results showed that the strain was able to produce melanin in a range of temperature between 26 and 28 °C, and of pH between 6.0 and 7.0, when grown on a yeast extract, malt extract-based medium at 250 rpm. Furthermore, carob pods, a waste from the food industry, were investigated as potential substrates to be supplemented in the growth medium to increase the melanin synthesis. An addition of carob pod pods between 1.0 and 5.0 g/L enhanced the pigment production up to 3-folds, compared to the control. The produced melanin was purified by acidic precipitation from the clarified harvested broth, analyzed by UV absorbance and for its antioxidant, photo-protective and heavy metal chelator properties.

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Categories

Biotechnology

Spike-mediated viral membrane fusion is inhibited by a specific anti-IFITM2 monoclonal antibody.

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Abstract

The early steps of viral infection involve protein complexes and structural lipid rearrangements which characterize the peculiar strategies of each virus to invade permissive host cells. Members of the IFITM protein family have been described as inhibitors of the entry of a broad range of viruses into the host cells. Recently, it has been shown that SARS-CoV-2 is able to hijack IFITM2 for efficient infection. Here, we report the characterization of a newly generated specific anti-IFITM2 mAb able to impair Spike-mediated internalization of SARS-CoV-2 in host cells and, consequently, to reduce the SARS-CoV-2 cytopathic effects and syncytia formation. Furthermore, the anti-IFITM2 mAb reduced HSVs- and RSV-dependent cytopathic effects, suggesting that the IFITM2-mediated entry mechanism might be shared with other viruses besides SARS-CoV-2. These results show the specific role of IFITM2 in mediating viral entry into the host cell and its candidacy as a cell target for antiviral therapeutic strategies.

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Categories

Biotechnology

Systems Biocatalysis for renewable biomasses valorization: dream and/or reality?

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Abstract

Developing a sustainable bioprocess to convert low-value natural substrates to high-value products is an increasingly attractive strategy due to the lower ecological footprint as compared to chemical synthesis. Lignin is the second most abundant polymer in Nature, also widely generated during biomass fractionation in lignocellulose biorefineries. Lignin is heterogeneous in composition and recalcitrant to degradation: at present, 98% of lignin is burnt although it represents the richest natural source of aromatics [1]. Moreover, wheat bran is an agricultural inexpensive by-product, usually used as livestock feed.

Recently, we developed an efficient green process for producing *cis,cis*-muconic acid (ccMA), a building block for the synthesis of plastics [2,3], based on: a) the optimization of the extraction procedure of vanillin from lignin and of ferulic acid from wheat bran; b) the genetic engineering of an *E. coli* strain expressing up to seven recombinant enzymes [4]. The engineered *E. coli* strain converted lignin-derived vanillin into ccMA with a productivity of 4.2 mg of ccMA/g of Kraft lignin in 30 min. Starting from the wheat bran-derived ferulic acid, ccMA was produced with a >95% conversion yield in 10 h, corresponding to 0.73 g of ccMA/g of ferulic acid, and 2.2 mg of ccMA/g of wheat bran. The optimized whole-cell biocatalysts could be combined to create a biosynthetic platform of relevant chemicals.

The proposed bioconversion system represents a sustainable, cost-competitive process for producing high value-added products obtained by combining metabolic engineering and the use of renewable biomasses.

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Categories

Biotechnology

Characterization of a novel thermophilic xylanase from *Alicyclobacillus mali* FL18 and its heterologous expression in *Sulfolobus acidocaldarius*

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Abstract

Lignocellulosic residues are the most abundant renewable feedstock in nature and can be considered a sustainable and low-cost source of useful compounds for the biochemical and biofuel industries. Xylan is the main component of hemicellulose, it consists of a xylose backbone with β-1,4 linkages, and other compounds, such as arabinose, linked to the backbone. Its complete degradation requires an array of several enzymes, including endo-β-1,4-xylanase (EC 3.2.1.8) and β-xylosidase (EC 3.2.1.37) [1]. A novel endo-β-1,4-xylanase (*Am*Xyn) from the thermo-acidophilic bacterium *Alicyclobacillus mali* FL18 [2] was biochemically characterized: it hydrolyzes xylan into fermentable sugars exhibiting a peculiar thermophilicity and stability in a wide range of pH, as well as a high tolerance to many chemicals. In addition, it shows a good degree of synergy in xylan digestion with the previously characterized β-xylosidase (*Am*βXyl) from *A. mali* FL18 [3].

Our attention has also been focused on the heterologous expression of *Am*Xyn in *Sulfolobus acidocaldarius*, a thermo-acidophilic archaeon that could be a good candidate for hemicellulose saccharification since it is able to transport pentose sugars [4]; however, it lacks enzymes capable of degrading hemicellulose and in particular xylan. The genetic manipulation system with no mobile elements [5] is being developed to obtain genomic integration of AmXyn in *S. acidocaldarius*.

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Categories

Biotechnology

Engineered *Saccharomyces cerevisiae* for the upcycling of polyethylene terephthalate (PET) monomers

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Abstract

Plastic has become an indispensable material in many fields, with production increasing every year; however, most of the plastic waste is still incinerated or landfilled and, only 10% of the new plastic is recycled even once. Among all plastics, PET is one of the most produced polyesters worldwide (56 Mt/year), and recently, its enzymatic hydrolysis has been proven at industrial level. Hence, the availability of new strategies for the convenient exploitation of PET-monomers is now increasingly relevant.

This work focuses on the development of new microbial routes for the upcycling of PET-derived monomers (terephthalic acid (TPA) and ethylene glycol (EG)) into industrially relevant organic acids (protocatechuic acid and glycolic acid). The combined use of the optimized synthetic biology tool (Easy-MISE toolkit)[1] and metabolic engineering approaches allowed us to create *Saccharomyces cerevisiae* strains harboring *ad-hoc* designed pathways for the above-mentioned bioconversions.

The promising outcomes that resulted from fruitful Design-Build-Test-Learn cycles will be here illustrated.

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Categories

Biotechnology

Ferritin nanocages loaded with Indocyanine Green-Loaded for fluorescence-guided detection of cancer tissues

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Abstract

Ferritin nanocages (HFn) offer new therapeutic options, potentially allowing the specific drug delivery to cancer cells, as well as the possibility of exploiting them as nanotracers for tumor detection [1-2]. HFn are protein-based nanoparticles displaying a natural tumor recognition thanks to their capability to interact with the Transferrin receptor 1, which is overexpressed in most of solid cancers. We assessed the feasibility of indocyanine green-loaded (ICG) ferritin nanoformulation (HFn-ICG) as tumor specific nanotracer in image-guided surgery approaches. After confirming its ability to accumulate at the primary tumor, we are evaluating its capability to detect even metastatic cells by improving the nanotracer's performances in terms of stability and circulation time. To this end, we developed a ferritin variant named HFn-PAS via recombinant production in E. coli. A Proline-Alanine-Serine sequence was genetically inserted at the N-terminus of each ferritin subunit followed by a cleavable site recognized by tumor metalloproteinases to ensure the release of PAS sequences and to restore the interaction with tumor cells in the tumor microenvironment [3]. Then, ICG was encapsulated into HFn-PAS and injected in vivo in a murine model of breast cancer. The evaluation of fluorescence at tumor obtained by optical imaging revealed a significantly higher accumulation of the nanotracer compared to ICG free and a superior retention until one week. Moreover, by analyzing the signal-to-noise ratio, we observed a prominent advantage of HFn-PAS-ICG, thus improving the lesion signal identification and reducing the background noise which is essential to discriminate tumor cells from healthy tissues.

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Categories

Biotechnology

Age-Associated Alterations in Sialylation Affect Nav1.5 Channel Function and Contribute to Cardiac Dysfunction

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Abstract

The proper functioning of cardiac muscle cells relies on the voltage-gated sodium channel Nav1.5, which mediates their depolarization. Sialylation is essential for the functionality of Nav1.5 due to its negative charge, and its alteration has been observed in various cardiovascular diseases, including Chagas disease and congenital disorders of glycosylation affecting the heart. Sialylation status changes human adult life, impacting the brain and muscles. Cardiac arrhythmias increase in prevalence with age and contribute to higher morbidity and mortality in older people; we aimed to investigate the impact of age-related changes in sialylation on Nav1.5 channels in cardiac cells. This study analyzed the levels of the Nav1.5 protein and sialic acid (α-2,3 and α-2,6) in heart tissue from an aging mouse model. In-vitro experiments using cell lines expressing Nav1.5 channels and induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) to examine the role of sialic acid in Nav1.5 channel biosynthesis, trafficking, degradation, and recycling and patch clamp analysis to investigate changes in sodium ion current. Our results revealed a positive correlation between Nav1.5 protein levels and the amount of sialic acid in aged mice. In-vitro, the reduction in sialylation status seems to induce Nav1.5 channel internalization that is reversed by adding exogenous mannosamine. In conclusion, our findings suggest that age-related decreases in sialylation status in cardiac tissue induce Nav1.5 channel internalization. Modulating sialic acid levels could be a promising therapeutic strategy to prevent cardiac dysfunction in the elderly.

Categories

Biotechnology

Combined Treatment of Cancer Cells Using Allyl Palladium Complexes Bearing Purine-Based NHC Ligands and Molecules Targeting MicroRNAs miR-221-3p and miR-222-3p: Synergistic Effects on Apoptosis

Chiara Tupini[1], Matteo Zurlo[1], Jessica Gasparello[1], Irene Lodi[1], Alessia Finotti[1,2], Thomas Scattolin[3], Fabiano Visentin[4], Roberto Gambari[1,2], Ilaria Lampronti[1,2]

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Abstract

To develop novel and more effective anticancer therapy strategies, combined treatments using lower dosages of different drugs are studied. In this regard, new findings from our research group have demonstrated the efficacy of peptide nucleic acids able to target miR-221 in triggering apoptosis in a variety of tumor cells, including glioblastoma and colon cancer cells. Recently, we also described a series of new palladium allyl complexes showing strong antiproliferative activity on different tumor cell lines [1]. The present study was aimed at investigating the biological effects of the most active compound tested in combination with antagomiRNA molecules targeting two miRNAs, miR-221-3p and miR-222-3p. The results show that the combination therapy using these antagomiRNAs and the palladium allyl complex 4d determines synergistic effects in inducing apoptosis, supporting the concept that the combination treatment of cancer cells with antagomiRNAs targeting specific upregulated oncomiRNAs and metal-based compounds represents a promising therapeutic strategy while reducing side effects at the same time. The combined treatments of colon cancer HT29 and glioblastoma U251 cells based on compound 4d (4d + miR-221-3p inhibitor and 4d + miR-222-3p inhibitor) and on the known Cisplatin (CisPt + miR-221-3p inhibitor and CisPt + miR-222-3p inhibitor) led to an apoptosis induction that was found to always be higher than the sum of the different treatments. In conclusion, the combined use of metal-based anticancer agents (compound 4d) and inhibitors of upregulated "oncomiRNAs" seems to be a promising method in the field of developing efficient anticancer therapies, according to our results [2].

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Categories

Biotechnology

Development of new biological drugs for the treatment of fungal infections

Tania Vanzolini[1], Tomas Di Mambro[2], Gianluca Morroni[3], Simona Fioriti[3], Francesco Barchiesi[3], Mauro Magnani[1,2]

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Abstract

Fungal infections represent a serious threat to the global health. The spread of resistance and the appearance of species intrinsically less susceptible to the drugs currently available make the hunt for new antifungal agents extremely urgent[1,2]. Different therapeutic strategies were considered, and, among them, the use of biological drugs is an interesting approach. Despite the several bottlenecks that led to the failure of several studies, herein we present a successful humanized monoclonal antibody (mAb) called Dia-T51[3,4]. Dia-T51 is the result of a humanization process of the murine monoclonal antibody 2G8. Both antibodies selectively recognize β-1,3 glucans, vital components of the fungal cell wall, but with an affinity that is higher for the humanized mAb compared to the murine parental. Dia-T51 resulted efficient in controlling alone fungal infections *in vitro*, inhibiting the growth and the adhesion of *C. auris*. As a full-length antibody, following fungal cell opsonization, Dia-T51 positively interacts with different components of the immune system promoting the eradication of the pathogen through phagocytosis, CDC, and ADCC mechanisms. Finally, and surprisingly Dia-T51 enhances the activity of echinocandins and amphotericin B when used in combination against different *candida* species, including resistant strains. In conclusion, considering the encouraging preclinical results obtained, Dia-T51 can be considered a highly promising drug candidate for the treatment of fungal infections and particularly, candidiasis. We are optimistic about its rapid move to clinical phases and about its efficiency in treating fungal infections *in vivo* both alone and in combination with other antifungal drugs.

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Categories

Biotechnology

Development of new "lab-on-a-chip" devices for the evaluation of CKD progression markers by the analysis uremic toxins and inflammatory markers in biological matrices

Angela Vastante[1], Mario Barra[2], Pasquale D'Angelo[3], Giuseppe Tarabella[3], Bartolomeo Della Ventura[4], Alessandra Perna[5], Diego Ingrosso[1], Margherita Borriello[1]

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Abstract

Lab-on-a-chip (LOC) have a wide application range, including clinical biochemistry. LOC circumvents several steps in laboratory setting, resulting in a faster turnaround time. Provider access to rapid results allows prompt medical decision making, improving patient outcomes, operational efficiencies, and cost savings. These features are particularly attractive for healthcare settings with complicated patients, such as chronic kidney disease (CKD). CKD is compared to diabetes, for impact, and consequences on well-being. CKD is a syndrome delineated as alterations in kidney function and/or structure lasting than 3 months. Cardiovascular disease (CVD) is the major cause of death in CKD. Between the factors that correlate CVD to CKD there are uremic toxins and inflammation. Interestingly, a set of cytokines are found increased in CKD patient with vascular calcification (VC), compared to those not affected by VC. Thus, inflammatory cytokines could represent a signature of novel biomarkers for early CV detection in CKD. With the aim to quantify inflammatory cytokines, exploiting LOCs advantages, two LOC systems were selected: screen printed electrode- (SPE) and lateral flow assay- (LFA). Commercial SPE were coated with TEGO by 3D printing and their efficiency to detect cytokines was verified by cyclic voltammetry and impedance spectroscopy. LFAs are optical LOCs. Compared to the commercialized LFA, these sensors are coated with gold nanoparticles (AuNPs) that increase the sensitivity of the device. Accordingly, LFAs resulted able to detect various inflammatory mediators both in PBS and serum. Results are compelling and represent a starting point for the application of LOCs in cytokines detection.

Categories

Biotechnology

COMPUTATIONAL AND SYSTEM BIOLOGY

Big-data-driven computational study of type-1 and -2 Cannabinoid Receptors SNPs among 730.000 samples of exome and genome sequences

Alessandro Berghella[1], Annalaura Sabatucci[1], Beatrice Dufrusine[1], Alexander S. Hauser[2], Enrico Dainese[1]

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Abstract

The endocannabinoid system plays a crucial role in regulating various physiological processes by activating CB1 and CB2 cannabinoid receptors. These receptors influence functions such as anxiety, depression, obesity, and immune system. While three drugs targeting the CB1 receptor have been approved by the FDA and are used for epilepsy, inflammation, and chronic pain, no CB2 drugs have advanced beyond phase 2 clinical trials. Spontaneous adverse event reports have suggested potential associations between genetic variations in the coding regions of these receptors and pathological conditions. To explore this further, a data-driven computational approach was developed to evaluate the impact of genetic variations on the functional sites of CB1 and CB2 receptors and their potential link to specific phenotypes. The pipeline used in this study analyzed a vast number of samples from genetic repositories, examining somatic mutations and germline variations, while employing both sequence-based and structure-based workflows. By integrating genetic data, computational analysis, and knowledge of the endocannabinoid system, this research provides valuable insights into the interplay between genetic variations in receptor coding regions and their impact on specific phenotypes. The findings contribute to a better understanding of the complex nature of the endocannabinoid system and may facilitate the development of more effective therapeutic approaches in the future.

Categories

Computational and Systems Biology

PhD-SNP^{g}: updated tool for predicting pathogenic variants in coding and noncoding regions.

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Abstract

One of the primary challenges in human genetics is determining the functional impact of single nucleotide variants (SNVs) and insertion and deletions (InDels), whether coding or noncoding. In the past, methods have been created to detect disease-related single amino acid changes, but only some can assess the influence of noncoding variations [1]. CADD [2] is the most commonly used and advanced algorithm for predicting the diverse effects of genome variations. It employs a combination of sequence conservation and functional features derived from the ENCODE project data. To use CADD, a large set of pre-calculated information must be downloaded during the installation process. To streamline the variant annotation process, we developed PhD-SNP^{g} [3,4]. a machine-learning tool that is easy to install and lightweight, relying solely on sequence-based features. Here we present an updated version, trained on a larger dataset, that can also predict the impact of the InDel variations. Despite its simplicity, PhD-SNP^{g} performs similarly to CADD, making it ideal for rapid genome interpretation and as a benchmark for tool development.
Availability: https://snps.biofold.org/phd-snpg/

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Categories

Computational and Systems Biology

PathLay: a novel graphical server for -omics integration and interpretation

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Abstract

Over the recent years -omics studies have provided valuable information for depicting the global properties of biological systems and how they respond to stimuli. Differently from traditional approaches, -omics offer the opportunity to capture coordinated alterations that usually take place at the System level. Although hard to be explored, the system may be properly addressed at the level of biological pathways with tools able to properly integrate them.

Here we present PathLay, a freely accessible platform for multi-omics data interaction and integration directly on graphical pathway maps. Users may associate up to 6 different -omics to an experiment: transcripts, proteins, microRNAs, metabolites, methylation status and the accessibility of chromatin with a built-in feature that can recognize transcription factors from transcripts or proteins and assign them to their targets.

PathLay will take care of associating every input data to appropriate nodes in graphical maps derived from KEGG and WikiPathways and represent such integrated information as intuitive interactive symbols.

The PathLay user intuitively plays with such symbols using a battery of interactive methods that interact with each other and with the map viewer, which in turn displays relevant information in real-time. Such methods allow displaying specific players or groups of them, or players that are expressed with a specific trend or that see their actions localized in specific cellular compartments.

PathLay allows rapid refocusing of the experimental perspective with biological lenses,allowing to intuitively follow paths within and through maps without being distracted by technicalities, with great benefit for data interpretation.

Categories

Computational and Systems Biology

Computational estimation of the effects of amino acid mutations on protein-ligand interactions using Alphafold and Molecular Dynamics Simulation

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Abstract

Amino acid mutations can affect protein's structure stability and functionality, with important biological effects especially when at the level of key residues. Molecular dynamics simulation (MDS) is an effective tool to study the effects of these mutations and, in association with computational methods for estimating binding free energies, is a popular approach to evaluate mutation's effects on protein-ligand interaction [1]. An essential element for this analysis is the availability of the three-dimensional structures of the wild-type (wt) and mutated protein isoforms. With unavailable crystallographic models, homology modeling has been used to obtain protein tridimensional structures. Nowadays, with AlphaFold2 it is possible to make predictions with high model accuracy [2]. Here, an automatized computational workflow to compute the difference in the binding free energy between the wt and the mutated proteins using AlphaFold2 is proposed, and a comparison with the binding affinity predictions using crystallographic models is made. Briefly, the tridimensional structures of wt proteins and their mutated isoforms were predicted using AlphaFold2, complexed with their ligands, and interactions were simulated with MDS. Then, the binding free energy was calculated, and the mutation effect on binding affinity was inferred. To validate the method, a benchmark of 15 mutant proteins was selected, the analysis was conducted also using their crystallographic structures, and a comparison with the predictions' performances using AlphaFold's models was made: the workflow based on AlphaFold's proteins had an accuracy in predicting affinities comparable to that obtained by using crystallographic structures.

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Categories

Computational and Systems Biology

Hybrid computing approach to estimate the impact of amino acid mutations on protein-ligand interactions.

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Abstract

The development of quantum computing (QC) methods can positively contribute to the study of biochemical systems, promising advantages over classical computing in the solution of complex problems. However, currently available computational resources are limited, thus combining a classical approach with a QC method can be convenient. Here, a hybrid classical-quantum workflow to identify ligands binding to mutated protein isoforms is proposed. The method is applied on three different proteins: Translocator Protein (TSPO), Tropomyosin receptor kinase A (TrkA), and Apolipoprotein E (ApoE). TSPO ligands are of interest for neuroinflammation diagnostic purposes [1]; TrkA, implicated in the insurgence of congenital insensitivity to pain, is an example of a protein bearing multiple mutations [2]; and, finally, the ApoE cholesterol binding capability is dependent on the protein isoform [3]. Several classical or candidate ligands able to bind the protein are identified with a classical computational screening method, followed by quantum machine learning (QML) analysis to predict the impact of mutations on the protein-ligands interactions. Briefly, a docking procedure for the initial virtual screening of a ligand dataset is conducted, and the most promising compounds are selected for a subsequent molecular dynamics simulation to measure their affinity [4]. Then, the identified lead compounds, the wt protein structure, and the selected amino acid mutations are used for predicting the mutations' impact on ligands binding affinity with a physical-statistical classifier QML approach. Thus, compounds able to interact with mutated isoforms of the analyzed protein can be identified.

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Categories

Computational and Systems Biology

METABOLIC AND PHYSIOLOGICAL REARRANGEMENTS IN BLADDER CANCER CELLS TRANSITIONING FROM ADHERENT TO SPHEROID CULTURES

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Abstract

Bladder cancer is one of the most common malignancies worldwide. Therefore, identifying new markers contributing to patients' stratification is crucial to direct them to more effective and less toxic targeted treatments, improve prognosis, and avoid relapses[1]. Energy metabolism reprogramming is an established cancer hallmark, and altered metabolic pathways can represent attractive clinical targets exploitable in new therapeutic strategies[2].

Here we use 3D cultures (spheroids), that better simulate the architectural complexity of a tumor mass in vivo, to characterize the metabolic and physiological rearrangements induced by spheroid formation in a panel of six bladder cancer cell lines at different stages and grades. Using a systems metabolomic approach, integrating omics analyses, and morpho-functional assays (including analysis of metabolic fluxes by Seahorse technology[3]) with mathematical models of metabolism[4], we show that 3D growth induces a profound stage- and grade-independent gene expression rearrangement indicative of a proliferative rate decrease, differentiation, EMT transition, and alteration of sensing processes. These changes correlate with a significant downregulation of folate metabolism, biosynthesis of purines and pyrimidines, serine and glycine metabolism, and alteration of the tricarboxylic acid cycle. However, cell line- and stage-specific differences accompany the transition from 2D to 3D: notably, in two cell lines, glycolysis is down-regulated in spheroids compared to adherent-growing cells. A mathematical model of metabolism integrates different omics data[5] highlighting the possible regulatory layers controlling metabolic rewiring in spheroids, with the final aim to contribute to the identification of novel clinical targets for precision medicine[6].

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Categories

Computational and Systems Biology

Multi-Omics Analysis Reveals the Metabolic and Polygenic Basis of Brugada Syndrome

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Abstract

Brugada syndrome (BrS) is an inherited disorder that can lead to sudden cardiac death. Candidate genes primarily include those encoding the sodium channel, whereas other genetic variants affecting other channels, signaling, sarcomere, and mitochondrial proteins are controversial, leaving the genetic architecture of BrS largely unknown.

The aim of this work was to improve the BrS diagnosis, further understanding its molecular basis by performing the first multi-omics study in patients with and without BrS.

Transcriptomics, proteomics, metabolomics, lipidomics, and whole-genome sequencing analyzes were performed on PBMCs and plasma samples of 293 BrS patients and 295 controls. These datasets were integrated to provide a comprehensive view of the molecular pathways and genetic factors underlying BrS.

WGS showed that polygenic factors contribute to BrS and that there is genetic overlap with arrhythmogenic, cardiovascular, and noncardiac pathologies. This complex genetic background includes pathogenic mutations in *SCN5A* and in other genes that regulate metabolic functions related to energy production. Transcriptomics and proteomics analyses of PBMCs independently confirmed dysregulation of pathways, indicative of metabolic abnormalities. This was reflected in changes in metabolomics and lipidomics, including downregulation of the TCA cycle, decreased ATP production, and evidence of a shift from oxidative phosphorylation to glycolysis.

Multi-omics ntegration and analysis of patients' peripheral blood cells revealed dysregulation of ubiquitous metabolic pathways, revealing that BrS is not limited to the heart. This challenges the traditional BrS classification as a cardiac channelopathy and suggests a possible role of mutations in metabolic genes and pathways in the disease pathophysiology.

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Computational and Systems Biology

ISPRED-SEQ: Deep Neural Networks and Embeddings for Predicting Interaction Sites in Protein Sequences

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Abstract

Protein-Protein Interactions (PPI) are crucial in many biological processes. Identifying PPI sites is key to understanding a protein function in the context of cell complexity.

Computational tools can identify PPI sites on protein structures or sequences when experimental evidence is missing. Much effort has focused on methods exploiting structural information. Nonetheless, sequence-based prediction is a more challenging task and few methods are available, achieving limited prediction performance.

Here we present ISPRED-SEQ, a web server for identifying PPI sites in protein sequences freely available at https://ispredws.biocomp.unibo.it.

The method stands on a deep architecture combining convolutional blocks and three cascading fully connected layers. ISPRED-SEQ is trained on a dataset of 6,066 protein structures, comprising 285,751 binding and 1,471,545 non-binding residues. The input is generated using two state-of-the-art language models, ESM-1b and ProtT5, and avoids the need of computing hand-crafted features such as sequence profiles or physicochemical properties. Each residue is considered in a window of 31 residues centered around the target.

We benchmark ISPRED-SEQ on a dataset comprising 448 proteins. We adopted a stringent homology-reduction procedure to guarantee that all proteins included in the training dataset have less than 25% sequence similarity with sequences used for benchmarking.

Results show that ISPRED-SEQ significantly outperforms other state-of-the-art methods, reporting a MCC of 0.39, surpassing by 7 percentage points the second best-performing method, PITHIA. Moreover, thanks to the adoption of protein embeddings instead of routinely adopted encodings based on sequence profiles, the method is extremely time-efficient.

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Categories

Computational and Systems Biology

CoCoNat: computational prediction of coiled-coiled regions from sequence using protein language models

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Abstract

Coiled-coils domains (CCDs) are found in proteins in all kingdoms of life. They perform a wide range of important cellular functions, acting as molecular spacers and influencing the organization of organelles within the cell. In some enzymes, CCDs have function of molecular rulers, constraining the geometry of the catalytic site. Moreover, CCDs are the basic unit of transmembrane ion channels, forming bundle-shaped structures. Canonical Coiled-Coil Domains (CCD) consist of intertwined alpha helices containing heptad repeats (labeled abcdefg, the so-called registers) with constraint pairing. CCDs are classified according to the number and orientation of the α-helices involved, i.e, by their oligomerization state. The importance of CCDs demands computational methods for predicting the presence and localization of CCDs, including registers, and their oligomerization state. Here we present CoCoNat (web server available at https://coconat.biocomp.unibo.it), a novel deep-learning based computational method for predicting CCD regions, registers and oligomerization state. Our method, for the first time, adopts a sequence encoding based on two state-of-the-art protein Language Models (pLMs): ProtT5 and ESM1-b. The pLMs embedding are processed by a three-step architecture including a deep network, a conditional random field and single-layer feed forward network. We trained CoCoNat on a dataset comprising 2191 proteins containing CCDs and 9040 proteins not endowed with CCD. When tested on a blind test set comprising 429 CCD and 278 non-CCD proteins, CoCoNat overpassed the current state-of-the-art both for residue-level and segment-level CCD detection, register annotation as well as oligomerization state prediction.

Categories

Computational and Systems Biology

E D U C A T I O N

Where Do We Come From? What Are We? Where Are We Going? - A portrait of the SIB Education Community

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Abstract

The SIB Education Group (GD, Gruppo Didattica SIB) was established in 2018 and in 2019, as its first international activity, it organized the FEBS Education Meeting: "Excellence in Learning and Teaching Biochemistry" [1] with the participation of more than seventy people and the intervention of five international invited speakers, including Donald H. Voet, who sadly passed away recently [2]. In 2020, the GD was formalized as a cross-sectional group of the SIB Society [3], with membership of over 100 people. In 2021, the 61° SIB Conference virtual edition hosted the GD panel discussion "Education and Mentoring in Biochemistry and Molecular Biology."

The first annual meeting of the Group's members was held on September 19, 2022. On that occasion, we prepared a questionnaire to conduct a survey on the teaching activities carried out by the group members at the university level. About 50 participants agreed to answer the various questions. Analysis of the responses allowed us to obtain a picture of our community, the teachings entrusted to it, and the main interests and challenges currently facing the teaching of biochemistry. Valuable insights for future activities of the group also emerged from this analysis. We have decided to make this data available to the entire SIB community so that we can reflect on the present and look for new ways in which the teaching of biochemistry, at all levels, can become more impactful and effectively address the new challenges that science confronts us with.

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Note: The title of our abstract is an homage to Gauguin's masterpiece: "D'où venons-nous? Que sommes nous? Où allons-nous?" Oil on canvas, Tahiti, 1897-1898. Tompkins Coll. Museum of Fine Arts, Boston, Ma, USA. https://www.mfa.org/gallery/impressionism-and-beyond

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[3] https://sib-biochemistry.it/about-us/sib-group/education/

Categories

Education

M E M B R A N E S

Identification of a N-terminal-cleaved form of Cyclophilin D: a new player in an old game?

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Abstract

Cyclophilin D (CyPD), the only mitochondrial cyclophilin, is a master regulator of the mitochondrial permeability transition pore (PTP), a Ca2+-dependent, high-conductance channel whose opening can lead to cell death. We have already provided evidence that CyPD binds to the OSCP subunit of ATP synthase, one of the most promising candidates for generating the PTP [1]. Different post-translational modifications of CyPD have been found to affect CyPD binding to OSCP and PTP opening [2]. Here we report that two forms of CyPD can be identified in various tissues. Mass spectrometry analysis showed that one form is ~1 kDa shorter at the N-terminus, suggesting that CyPD can be cleaved by a mitochondrial protease. In vitro studies using the human full-length CyPD (FL-CyPD) and a N-terminal truncated form (ΔN14-CyPD) revealed that the latter more avidly interacts with OSCP than FL-CyPD. Consistently, when expressed in HEK 293 mitochondria, ΔN14-CyPD rapidly triggers PTP opening in response to Ca2+ treatment. Nuclear Magnetic Resonance (NMR) spectroscopy revealed that the CyPD N-terminal tail is highly flexible, in sharp contrast with the remaining globular rigid part. NMR analysis showed that KCl selectively affects the gatekeeper region of the active site, involving a significantly larger patch of residues in FL CyPD than in ΔN14-CyPD. Overall, our data suggest the N -terminal-cleavage generates a CyPD with unique properties, opening new perspectives on how the PTP is regulated by CyPD.

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Categories

Membranes

Glucose-derived glutamate drives neuronal differentiation

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Abstract

Neuronal differentiation is the phase during which neurons acquire their final characteristics in terms of morphology, electrical activity, and metabolism. However, little is known about the metabolic pathways governing neuronal differentiation. Here, we investigate the contribution of the main metabolic pathways, namely glucose, glutamine, and fatty acid oxidation, during the differentiation of primary rat hippocampal neurons. Blunting glucose oxidation through the genetic and chemical inhibition of the mitochondrial pyruvate transporter revealed that this protein is critical for the production of glutamate, which is required for neuronal arborization, proper dendritic elongation, and spine formation. Glutamate supplementation in the early phase of differentiation restores morphological defects and synaptic function by rescuing synaptic local translation. Fatty acid oxidation does not affect neuronal differentiation, whereas glutamine metabolism is important for mitochondria, but not for endogenous glutamate production. Our results provide new insights into the role of glucose-derived glutamate as a key player in neuronal differentiation.

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Membranes

Analyses of frequent and conserved intron positions shed light on the evolution of the mitochondrial carrier family SLC25

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Abstract

Mitochondrial carriers (MCs) constitute a family of mostly mitochondrial proteins that transport different specific substrates, such as cofactors, nucleotides, amino acids, dicaboxylates and inorganic anions, across the inner membrane. MCs have characteristic triplicated protein sequence repeats that are reflected in the three-fold symmetrical structure of their six-transmembrane α-helical transporter domain. These common sequence features have been used to identify MC genes in various eukaryotic genomes. We have mapped and analyzed the positions of the introns in MCs of highly diversified organisms. The results show that many MCs have introns at the same specific positions within the MC transporter domain and that several of these positions are three-fold symmetric. Moreover, many of these frequently occurring intron positions are particularly common in orthologs of specific MC subfamilies, which transport similar substrates. These findings imply that the present day MCs have partially conserved the gene architectures of ancestral MCs. Based on this reasoning the frequent and conserved intron positions were used to reconstruct a phylogenetic tree that also included evolutionary relationships between distant MC homologs with low sequence similarities. Furthermore, the structural locations of the intron positions suggest that exon shuffling and intron sliding may have contributed to the substrate specificity diversification in the evolution of the MC family.

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Categories

Membranes

The antiapoptotic URG7 protein increase the prosurvival re-sponse to tunicamicin er stressed of the human neuroblastoma cell line SH-SY5Y

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Abstract

URG7 is a liver protein localized in the endoplasmic reticulum (ER), over-expressed following HBV infection. Its activity has been related to the attenuation of ER stress resulting from viral infection, promoting protein folding and ubiquitination and overall reducing cell apoptosis. [1]

While the antiapoptotic activity of URG7 in HBV infected cells is deleterious as it could promote neoplastic transformation, this effect could be exploited positively in the context of proteinopathies, such as neurodegenerative diseases, characterized at molecular level by protein misfolding, mitochondrial dysfunction, oxidative stress, dysregulation of calcium homeostasis, promoting cell death. Preliminary data, obtained on SH-SY5Y cells over expressing URG7 and treated with tunicamycin, a toxin capable of triggering ER stress, demonstrated that URG7 is able to modulate several markers of the UPR in favor of cell survival. Based on these assumptions, a panel of experiments was performed, using the same in vitro model, aimed at further characterizing the activity of URG7.

Analysis of the protein expression of several apoptotic biomarkers, as well as the reduction of calcium release from the ER to the cytosol together with an overall reduction of intracellular ROS, confirmed the pro-survival activity performed by URG7. Finally, since an imbalance of cellular Ca2+ concentrations and redox state can reduce the protein folding capacity of chaperones within the ER, resulting in protein accumulation and aggregation, it was evaluated whether URG7 was able to affect the levels of unfolded proteins: the results confirmed that URG7 protein overexpression promotes ubiquitination and reduces protein aggregation. [2] [3]

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Membranes

Mutations in RFVT2 and their biochemical consequences in experimental models from a RTD2 patient

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Abstract

Vitamin B2, or riboflavin (Rf), in its active forms, FMN and FAD, provides important functions in mitochondrial terminal metabolism, protein folding and other cell regulatory functions [1]. Rf cellular uptake in humans occurs via three transporters (SLC52As or RFVTs), showing different tissue distributions, with *SLC52A2* expression being relevant for the brain. *SLC52A*2 variations are correlated with Rf Transporter Deficiency 2 (RTD2, OMIM #614707), a rare Rf-responsive neurodegenerative disorder [1].

In the frame of resuming all *SLC52A2* pathogenic variants reported so far, we addressed the molecular consequences of altered RFVT2 structure/function relationships by using different models: recombinant mutant transporters reconstituted in proteoliposome, fibroblasts and induced pluripotent stem cells (iPSCs) and derived motor neurons (MNs) obtained from a compound heterozygous RTD2 patient.

With respect to the WT protein mutants performed a slightly altered Vmax and a significantly higher Km, consistent with the favourable outcome shown in RTD2 patients after Rf treatment and with the undiminished level of flavins measured in patient' fibroblasts and MNs. The observed increased SLC52A1 protein amount in MNs might also explain the undiminished cellular flavin levels.

Nevertheless, despite the normal content of flavins and certain flavoproteins, patient's fibroblasts showed a cell growth rate delay and altered mitochondrial morphology, along with a reduced content of tubulin in MNs, according to published results [2]. Thus, rather than being directly linked to transport deficiency, SLC52A1 responses and mitochondrial derangements in RTD2 patients could be triggered either by redox or unfolded protein sensors, as observed in a worm model [3].

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Membranes

N U C L E I C A C I D S

Analysis and biochemical impact of the alteration of miRNA expression involved in different Disorders of Consciousness by digital PCR.

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Abstract

The "intrinsic regulator Gene Expression" role of miRNAs is important: in this study the dysregulation of five miRNA has been studied as clinical marker and as epiphenomenon for the altered biochemical pathways. We found that severe disturbs of consciousness (DoC) cause a reorganization of miRNAs expression that is: (I) dependent by the time since injury, (II) etiology-independent, (III) etiology-specific. Patients with Trauma Brain Injury (TBI) have lower levels of miRNAs 150-5p, 132-3p, and 23-b-3p at baseline, while patients with Hypoxic-ischemic brain injury (HIBI) have lower levels of miRNA 150-5p at baseline and 6 months post-injury and a reduction of miRNA 451a at baseline. Higher levels of miRNA 132-3p and miRNA 23b-3p are associated with better outcomes in patients with TBI. miRNA-150-5p and miRNA 451a dysregulation are involved in microglial activation after severe TBI and HIBI, inhibiting the release of proinflammatory cytokines such as interleukine-1β, interleukine-6, and tumor necrosis factor-α by binding the protein kinase AKT3. miRNA 132 has been linked to various pathways, including astrocyte-related inflammation, synaptic structure and plasticity and cholinergic signaling. The expression of miRNA 23-b-3p showed a similar pattern to 132-3p, with a decrease in patients with TBI at baseline. miRNA 23-b-3p regulates neuronal apoptosis and is involved in various cellular pathways; in particular this miRNA has a different temporal expression (1-3 months post-injury vs 6 months). This is significant because it provides new comprehensions to the dynamic changes in miRNA 23-b-3p levels after TBI and highlights its potential as biomarker for post-traumatic DoC.

Categories

Nucleic Acids

Small Non-coding RNA in Plants: From Basic Science to Innovative Applications

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Abstract

Plants possess an arsenal of different classes of small RNAs (sRNAs) of variable size, which play a regulatory role in a multitude of physiological and pathological processes via tran- scriptional or post-transcriptional gene silencing. The hard challenges that agriculture will face in the next few decades, such as an increasing demand for agrifood production related to the global in- crease in population, have stimulated the development of innovative biotechnological approaches in agriculture. In this regard, the use of artificial sRNAs has already been exploited successfully for many purposes, including control of severe plant diseases, improvement of genetic and agronomic traits of cultivated species, and increasing the nutritional value of plant foodstuffs. This strategy relies on the application of synthetic sRNA molecules to induce specific physiological responses by triggering appropriate RNA silencing pathways. This review contextualizes the use of artificial sRNAs in consideration of the huge diversity of RNA silencing mechanisms in plants. Additionally, the discussion also examines microRNAs from edible plants and exosome-like vesicles, also known as plant-derived edible nanoparticles (ENPs), which themselves can act as micronutrients.

Categories

Nucleic Acids

Impaired formation of a stable RPA/RNAseH1 complex in senescent cells leads to uncontrolled processing of R-loops and unsuccessful DNA repair

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Abstract

The maintenance of genome stability ensures cellular fitness. Cells are constantly exposed to DNA-damaging agents and DNA integrity is maintained by successful activation of the DNA damage response (DDR). Senescent cells accumulate unrepaired DNA due to a defective DDR. Non-canonical chromatin structures (NCSs) enable the maintenance of genome integrity by controlling genome replication, transcription and DNA repair. Among NCSs, R-loops are three-stranded DNA-RNA hybrids that have been reported to recruit repair factors and maintain successful DNA repair when formed *in trans* and to increase genome instability when formed co-transcriptionally *in cis*.

Here, we used BJ/hTERT-RAS-ER as an inducible cellular model of oncogene-induced senescence (OIS) to investigate the contribution of R-loops to the maintenance of genome stability. Cells undergoing OIS accumulate more R-loops compared to proliferating fibroblasts, but only a small fraction of them support BRCA1 loading. By crossing DRIP-seq and DRIVE-seq data, we found that impaired loading of DDR proteins at double-strand breaks (DSBs) correlates with unsuccessful recruitment of RNAseH1 by the RPA complex to R-loops. By adopting the LacO/LacR tethering system, we found that forced recruitment of both catalytically active and inactive RNAseH1 to DSBs delays DNA repair, suggesting that both premature and delayed removal of R-loops affects DDR.

Finally, our research identified phosphorylation of RPA32 as the signal that controls the release of RNAseH1 from DSBs. Hyperphosphorylation of the RPA complex observed in pre-senescent cells alters this mechanism, leading to further accumulation of irreparable damage and onset of senescence.

Categories

Nucleic Acids

Inflammatory and immunological basis of obsessive-compulsive disorder

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Abstract

Obsessive-compulsive disorder (OCD) is a complex and multifactorial mental health condition. Genetic, inflammatory and other environmental factors play together a key role in its etiology and epigenetic mechanisms have been suggested underlying the cross-talk among these factors. Recently, we identified a new protein originally described as Testis-Development Related Protein (TDRP) and re-named Immuno-*mood*ulin (I*mood*), significantly more expressed in T-cells of OCD subjects compared to controls [1]. Here, we evaluated the transcriptional regulation through DNA methylation of I*mood*. Moreover, we analysed the gene expression of different modulators of inflammation as well as the telomeres length to evaluate the hypothesis that mental disorders might have a deeper impact on our physiology and aging than it was previously supposed [2]. We highlighted a significant decrease in DNA methylation at one specific CpG site of I*mood* promoter region in OCD subjects. Moreover, the proinflammatory genes TNF-α, interleukin-2, interleukin-4, interleukin-6 and interleukin-10 were found overexpressed in patients compared to controls. Finally, a guanine-rich region was identified in the proximity of the transcription start site of the I*mood* gene promoter. Our data confirm the relevance of peripheral inflammation in OCD and support the role of I*mood* as potential peripheral biomarker.The guanine-rich region suggests the possible formation of secondary structures known as G-quadruplexes (G4s), binding sites for transcription factors (TF) and involved in transcription and epigenetic regulation [3]. Since TF binding I*mood* are well known modulators of immune/inflammatory response, decreased methylation of this region in OCD patients might induce an increased expression of I*mood* during inflammation.

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Categories

Nucleic Acids

Metabolism and DNA repair: clues from the role of human serine hydroxymethyltransferase

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Abstract

Serine Hydroxymethyltransferase (SHMT) is a metabolic enzyme involved in the reversible conversion of serine and tetrahydrofolate, into glycine and N5,N10- Methylenetetrahydrofolate. This tetrameric protein is PLP dependent enzyme, that works as a dimer of obligate dimers.SHMTs is a part of a complex network of metabolic pathways, the one carbon metabolism (OCM) that fuels cancer cells proliferation. Therefore SHMTs are overexpressed in several types of tumors, being an interesting target for cancer therapy. In humans, there are two genes encoding SHMT, a cytosolic (SHMT1) and a mitochondrial (SHMT2) isoform(1). In addition to having a catalytic activity, it has shown SHMT1 also has nucleic acids binding affinity. In cytosol SHMT1 binds SHMT2 transcript 5'UTR, through which negatively regulates SHMT2 expression and in turn serine to glycine reaction, catalyzed by SHMT1, is negatively ribo-regulated by the 5'UTR mRNA(2). Furthermore, SHMT1 translocates into the nucleus during S and G2/M phase or in response to DNA damage, to form a protein complex with DHFR and TYMS for de novo thymidylate biosynthesis *in situ*(3)(4). To understand how SHMT is involved in DNA repair, we characterized the molecular basis of SHMT1-DNA interaction *in vitro* and *in cellulo*. *In vitro* SHMT1 binds preferentially ssDNA, which affect the enzyme catalytic activity. Cell studies show how SHMT1 knock out leads to an increased interferon-β expression and a higher phosphorylation of H_2AX histone. Moreover, preliminary studies show it leads to an uracil accumulation in the genome and to an elevated content of fragmented DNA.

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Categories

Nucleic Acids

Antisense Peptide Nucleic Acids (PNAs) targeting the -1 programmed ribosomal frameshift of SARS-COV-2: effects on SARS-CoV-2 infection and expression of pro-inflammatory genes in bronchial epithelial Calu-3 cells

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Abstract

Background: Among the large variety of pharmaceutical strategies, the antisense approach is of great interest for SARS-CoV-2 RNA targeting. One of the most interesting SARS-CoV-2 target sites is the well-known and highly conserved SARS-CoV-2 frameshifting element that allows to produce protein 1a and 1b using two shifted ORFs. Peptide Nucleic Acid (PNA) are of great interest for the development of highly efficient antisense reagents. In addition to SARS-CoV-2 infection, the so called “cytokine storm” is a key clinical feature of COVID-19. (2) **Methods:** Production of SARS-CoV-2 genomes in infected Calu-3 cells and expression of pro-inflammatory genes have been studied by RT-qPCR, for transcription factor production and by Western blotting for proteins investigations. (3). **Results:** In this study we demonstrate that SARS-CoV-2 replication and SARS-CoV-2 mediated activation of NF-kB is inhibited by a PNA targeting the SARS-CoV-2 pseudoknot (PNA-RFSE). Of relevance is the fact that, following treatment of SARS-CoV-2 infected cells with the PNA(RFSE), inhibition of the production of mRNA for the N protein is rapidly obtained. This might trigger downstream effects, including lower induction of NF-kB expression and NF-kB regulated genes. (4). **Conclusions:** We demonstrate that a PNA targeting the SARS-CoV-2 pseudoknot has several effects on Calu-3 infected cells: (a) inhibition of SARS-CoV-2 replication; (b) inhibition of the NF-kB transcription factor and (c) decrease in the expression of the pro-inflammatory proteins IL-1beta, IL-6, IL-8, G-CSF and TNF-alpha.

This work was funded by the MUR-FISR COVID-miRNAPNA Project (FISR2020IP_04128) (to R.G., R.C. and A.F).

Categories

Nucleic Acids

Differentially expressed microRNAs in lipedema tissue: data from microarray analysis

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Abstract

Lipedema is a chronic disorder mainly present in women. It is characterized by symmetric enlargement of nodular, often painful subcutaneous adipose tissue in the limbs, sparing the feet, trunk, and hands.

Multidisciplinary teams manage lipedema but delays in diagnosis and misdiagnosis often as general obesity shame subjects. The physical appearance of lipedema is distressing and reduces emotional and social functioning. Therefore, having a biomarker of this condition can be beneficial for lipedema nutritional treatment and mental health status. In this view, microRNAs (miRNAs) are promising biomarkers because they circulate in plasma/serum. This study used subcutaneous adipose tissue (SAT) from liposuction in subjects with lipedema. First, we performed a microarray using the n-counter flex platform. We identify differentially expressed miRNAs by comparing expression miRNAs in inflamed and normal SAT.

Bioinformatics-based approaches, particularly differential miRNAs expression analysis, a tool that effectively identifies potential prognostic and diagnostic biomarkers. We identified a miRNAs signature that was further analyzed by in silico approach recognizing their target gene. The plugin “BiNGO” of Cytoscape applied Gene Ontology enrichment analysis. The key genes mainly enriched in the biological process of cellular regulation were linked to endocrine resistance with possible receptor upregulation, steroidogenesis, and cell cycle regulation with a potential increase in proliferation. Lastly, the protein-protein interaction and co-expression network were analyzed by the STRING and GeneMANIA plugins of Cytoscape, respectively.

Key words: Lipedema; Microarray; miRNAs

Categories

Nucleic Acids

Role of miR-15b-5p/SIRT4 axis in endothelial dysfunction under in vitro septic conditions

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Abstract

Sepsis is related to severe endothelial impairment leading to organ damage and death. Dysfunctional endothelium and dysregulated microRNAs (miRNAs) are critical players for the septic inflammatory outcome. Evidence described the role of miR-15b and SIRT4 in sepsis, although precise molecular mechanism in endothelial dysfunction during septic conditions are still not fully elucidated. Here, the role of miR-15b-5p on inflammatory pathways, programmed cell death mechanisms and altered redox metabolism has been evaluated in endothelial cells (HUVEC and TeloHAEC) treated with lipopolysaccharide (LPS). Results indicated that stimulation with LPS increased miR-15b-5p levels and decreased SIRT4 protein expression. Inhibition of miR-15b-5p (i-miR-15b) by antagomir transfection upregulated SIRT4 expression ($p<0.05$) and ameliorated sepsis-induced inflammatory mechanisms ($p<0.01$). Moreover, i-miR-15b counteracted mitochondrial oxidative stress ($p<0.01$) and opposed induction of apoptosis, autophagy ($p<0.01$) and NLRP3-sustained pyroptosis ($p<0.01$). These results by unveiling the relationship between miR-15b-5p and SIRT4 during septic endothelial damage suggest a key role of miR-15b-5p/SIRT4 axis in sepsis-related endothelial dysfunction [PSR – Regione Campania, B68H19005200009 - STRABUF].

Categories

Nucleic Acids

NUTRITION AND ENVIRONMENT

Evaluation of the neuroprotective potential of Indicaxanthin from *Opuntia ficus indica* fruit against dysmetabolism-related neurodegeneration in high-fat, diet-fed mice.

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Abstract

Indicaxanthin (Ind) is a bioavailable phytochemical able to cross the Brain Blood Barrier and to modulate specific, redox-dependent signal transduction pathways both *in vitro* and *in vivo*. Accordingly, the pigment has been demonstrated to exert significant anti-oxidative, anti-inflammatory and anti-dysmetabolic effects. Along these lines, we here investigated its protective effects, and the underlying mechanisms, in an *in vivo* model of dysmetabolism-related neurodegeneration, i.e. the high-fat, diet-fed mice. Our results clearly show that Ind-treatment significantly reduces brain oxidative stress (evaluated as RONS, malondialdehyde and NO levels, SOD-2 protein expression and Nrf-2 activation), neuro-inflammation (in terms of COX-2, iNOS protein expression and NF-κB activation) and neuro-apoptosis (assessed as Fas-L, Bim, P27, Bcl-2 and BDNF gene expression). These evidences are integrated by *in vitro* data showing that Ind protects SH-SY5Y neuroblastoma cells from methylglyoxal-induced neurodegeneration *via* a mechanism involving the amelioration of the insulin signalling axis (from IRS-1 to AKT and GSK-3). As a whole, our results demonstrate that Ind treatment modulates the expression of crucial genes and proteins involved in the insulin resistance-related, oxidative stress-dependent, inflammatory reaction underlying the obesity-induced neurodegeneration.

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Categories

Nutrition and Environment

The oxidation of oleocanthal in oleocanthalic acid during extra virgin olive oil (EVOO) storage is crucial for the loss of its anti-neuroinflammatory activity

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Abstract

Neuroinflammation is a key feature of neurodegenerative diseases involving the over-activation of microglia that leads to the release of pro-inflammatory mediators, resulting in neuronal death[1].Oleocanthal (OL), an EVOO phenolic component, is known for its “ibuprofen-like” activity, strongly inhibiting the cyclooxygenase enzymes[2]. During EVOO shelf-life, OL is oxidized to oleocanthalic acid (OA), and this process is accelerated by inappropriate storage conditions[3]. To date, the biological effects of OA are unknown, and the anti-neuroinflammatory activity of OL has not been characterized. The aim of this study was to investigate the potential anti-inflammatory activity of OA and OL in an in vitro model of neuroinflammation. BV-2 cells were treated with OL and OA for 2 h and then activated with 100 ng/ml LPS for 24 h. OL, but not OA, significantly reduced NO production, measured by Griess. Furthermore, OL significantly decreased the gene expression of iNOS, NLRP3, COX2, IL-1b, IL-6, and TNF-a and up-regulated anti-inflammatory mediators like IL-4 and CD206. In addition, the protein levels of iNOS, COX-2, and NLRP3, analyzed by immunoblotting, were significantly reduced by OL but not by OA. Interestingly, OL completely inhibits p38 MAPK phosphorylation induced by LPS suggesting that the reduction of neuroinflammation by OL could be, at least partially, due to the modulation of this signaling pathway. Our data clearly demonstrate that OL efficiently counteracts microglia activation, and this effect is lost when oxidized to OA. EVOO appropriate storage conditions, as well as the consumption of a fresh product, are crucial for its beneficial health effects.

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Categories

Nutrition and Environment

TRPM2 is fundamental for adipose tissue and liver thermogenic response promotion during cold exposure

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Abstract

BACKGROUND:

During thermogenesis, adipose tissue (AT) becomes more active and enhances oxidative metabolism. The promotion of this process in white AT (WAT) is called “browning” and, together with the brown AT (BAT) activation, is considered as a promising approach to counteract obesity and metabolic diseases. Transient receptor potential cation channel, subfamily M, member 2 (TRPM2), is an ion channel that allows extracellular Ca^{2+} influx into the cytosol, and is gated by adenosine diphosphate ribose (ADPR), produced from NAD^+ degradation.

AIM:

To investigate the relevance of TRPM2 in the regulation of energy metabolism in BAT, WAT, and liver during thermogenesis.

METHODS:

Wild Type (WT) and $Trpm2^{-/-}$ mice were exposed to cold and BAT, WAT and liver were collected to evaluate mRNA, protein levels and ADPR content. Furthermore, O_2 consumption, CO_2 production and energy expenditure were measured in these mice upon thermogenic stimulation. Finally, the effect of the pharmacological inhibition of TRPM2 was assessed in primary adipocytes, and evaluating the response to ß-adrenergic stimulation.

RESULTS:

Trpm2$^{-/-}$ mice displayed lower expression of browning markers in AT and lower energy expenditure in response to thermogenic stimulus, compared to WT animals. *Trpm2* gene overexpression was observed in WAT, BAT and liver upon cold exposure. In addition, ADPR levels and mono/poly-ADPR hydrolases expression were higher in mice exposed to cold, compared to WT mice, likely mediating ADPR generation.

CONCLUSION:

Our data indicate TRPM2 as a fundamental player in BAT activation and WAT browning. TRPM2 agonists may represent new pharmacological strategies to fight obesity.

Categories

Nutrition and Environment

In vitro digested fractions of Phaseolus vulgaris protect colon cells from inflammation and oxidative stress

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Abstract

In recent years, the consumption of nutraceuticals is increased due to their beneficial health effects. The Mediterranean diet includes high-level consumption of nutraceuticals, which exert protective biological effects against many types of pathologies, such as metabolic, cardiovascular, and neurodegenerative diseases and different types of cancer. Among foods rich in bioactive nutrients, Phaseolus vulgaris L., known as common bean, is a key Mediterranean diet component. Beans are rich in unsaturated fatty acids, minerals, dietary fibers, vitamins, and phenolic compounds. Previous studies have been conducted on an aqueous extract of "Fagiola di Venanzio" (FV), a Tuscan variety of beans, which showed to be rich in protein and polyphenols with antioxidant and anti-inflammatory activity [1]. Based on these encouraging results, we mimicked the traditional cooking procedures of beans and set up an in vitro gastrointestinal digestion of FV and other two different varieties of beans, Cannellino and Piattellino. Then we collected soaking and cooking water and the bioaccessible fraction of the digestion process and demonstrated that the digestion fraction of Cannellino and Piattellino beans significantly reduces colon cancer cell growth [2]. In addition, all the fractions of FV showed anti-inflammatory effects by inhibiting COX-2, NOX1, and antioxidant activity by reducing the levels of ROS. Overall, this study supports the beneficial effect on human health deriving from the presence of beans in the diet and stimulates the research of the molecules responsible for these health properties.

Aknowledgements: This work was supported by PNRR 2022-2025 Tuscany Health Ecosystem (THE), spoke 6.

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Categories

Nutrition and Environment

Nutraceuticals on heart failure: molecular mechanisms in iPSC-derived cardiac organoids

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Abstract

Diet-derived bioactive compounds with beneficial effects on human health (nutraceuticals) may exert interesting cardioprotective actions; therefore, our study is focused on elucidating the underlying molecular mechanisms. In order to have an in vitro model of heart failure, we differentiated human induced pluripotent stem cells (iPSCs) into self-assembled cardiac organoids, and we stimulated them with endothelin-1 (ET1). This treatment promoted cardiac hypertrophy, as demonstrated by increased expression of hypertrophy markers Natriuretic Peptides A and B and Actin Alpha 1, as well as protein misfolding and the deposition of pre-amyloid oligomers. Since these events lead to cellular proteotoxicity in heart failure, we evaluated the ability of selected nutraceuticals (epigallocatechin gallate, spermidine, oleuropein, and quercetin) to interfere with them. Given the structural difference between these compounds, we hypothesized that they might modulate the proteostasis of cardiac organoids by several mechanisms, including autophagy promotion, inhibition of protein misfolding and aggregation, and enhancing antioxidant systems. Thus, we have investigated these cellular processes by detailing the effect of the various nutraceuticals. Our work, funded by the Fondazione Carisbo, has provided new insights into the bench research of nutraceuticals in heart failure.

Categories

Nutrition and Environment

Micro and nano plastics induce metabolic rewiring and signal transduction alteration in normal human colon cells: a risk factor for human health

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Abstract

The metabolome represents the collection of the intermediates and end products of cellular processes, and is the most proximal reporter of the body's response to environmental exposures and pathological processes. Metabolomics is a powerful tool for studying how organisms interact with their environment and how these interactions shape diseases related to pollutant exposure. Polystyrene is a thermoplastic polymer widely used in commercial products. Like all plastics, polystyrene can be degraded into microplastic and nanoplastic particles and ingested via food chain contamination. Although the ecological impact due to plastic contamination is well known, there are no studies indicating a carcinogenic potential of polystyrene microplastics (MPs) and nanoplastics (NPs). Here, we evaluated the effects of the MPs and NPs on normal human intestinal CCD-18Co cells. Our results show that internalization of NPs and MPs induces metabolic changes under both acute and chronic exposure by inducing oxidative stress, increasing glycolysis via lactate to sustain energy metabolism and glutamine metabolism to sustain anabolic processes. Along with metabolic rewiring, different molecular pathways related to stress response, such as NRF2-HIF1alpha axis, are altered after plastics exposure We also show that these evidence mirror the effect of the potent carcinogenic agent azoxymethane and HCT15 colon cancer cells, carrying out the typical strategy of cancer cells to optimize nutrients utilization and allowing metabolic adaptation to environmental stress conditions. Taken together our data provide new evidence that chronic NPs and MPs exposure could act as cancer risk factor for human health.

Categories

Nutrition and Environment

Sprouted bean flour as a novel functional ingredient for the formulation of bakery products

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Abstract

Due to their nutritionally balanced nutritional profile legumes are widely consumed as human food. However, legumes as a staple diet is hampered by the presence of anti-nutritional components and low digestibility di/oligosaccharides. Many biotechnological approaches have been tested to overcome one or more of the aforementioned restrictions. Among them, sprouting has been used to alter the macromolecular composition of certain grains and legumes, to decrease the content in anti-nutritional elements. Among legumes, cowpea (Vigna unguiculata) is a versatile crop, and represents a good candidate for developing novel products. This study aims to investigate the impact of short time sprouting (48 and 72 hours) on the biomolecular features and the antioxidant and antinutritional factors in cowpea seeds. Short sprouting modifies the protein pattern, with an improvement in the features required for formation of a protein network. Sprouting also leads to a decrease of anti-nutritional factors and of low digestibility oligosaccharides while improving the content in phenolics with conceivable antioxidant activity. Although the maximum effect on phytate and oligosaccharides content and protein hydrolysis are observed after 48h, the highest decrease of trypsin inhibitor activity was observed after 72h. These findings suggest that sprouted cowpea flour may be a food ingredient with enhanced nutritional and technological value. A characterization of wheat bread containing 25% of 72h-sprouted cowpea flour is currently undergoing to support this assumption.

This investigation is partially supported by National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 - Call for tender No. 341 of 15/03/2022.

Categories

Nutrition and Environment

The power of MALDI-TOF mass spectrometry in the exploration of lentil bioactive peptides.

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Abstract

Lentil (*Lens culinaris*) is a healthy food rich in proteins, vitamins and fibers, that can be consumed as it is or transformed into flour for food fortification. Moreover, hydrolysis of lentil proteins generates bioactive peptides to be included in functional foods. Lentil flour extracts were provided by our scientific partner (A. Costantino & C. S.p.A.) after hydrolysis with alkaline protease under two agitation speeds. Sample aliquots were taken immediately after the addition of the enzyme and at different hydrolysis time points. The antioxidant activity was assayed with the ABTS and DPPH tests. The polypeptide profiles were assessed with SDS-PAGE and MALDI-TOF mass spectrometry following the methods reported previously[1]. The ABTS radical scavenging activity of extracts was higher compared with the DPPH results. However, the ABTS activity of hydrolyzed samples was similar irrespective of hydrolysis time or agitation speed, while the DPPH activity increased between the first and the second time point of hydrolysis, possibly due to the release of hydrophobic molecules. Protein masses mainly decreased within the first 60 minutes, as highlighted by SDS-PAGE analysis. MALDI-TOF mass spectrometry allowed to examine in depth the hydrolysis process, confirming the increase of peaks in the low mass range with the progression of hydrolysis time and detecting peculiar peaks, especially at 1927 and 1252 m/z. Hydrophobic peptides sharing the same m/z and showing antioxidant activity have been already identified in lentil seeds[2], suggesting a similar nature of those observed in this study. This work was supported by progetto NUTRAcore - POR FESR 2014/20[3].

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Categories

Nutrition and Environment

Effect of sustainable feeding strategy with olive oil pomace in Holstein lactating cows on bioactive peptides derived from milk proteins.

Costanza Cicchi, Simone Luti, Paolo Paoli, Luigia Pazzagli

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Abstract

In addition to being a rich source of carbohydrates, fatty acids, minerals and vitamins, milk is a good source of proteins and bioactive peptides that may have a beneficial effect on human health, including anti-inflammatory and anti-proliferative activity. Recently, several research lines are investigating sustainable strategies of animal feed. In this work, Olive oil pomace (OOP) produced during olive-oil milling has been added to the diet of Holstein lactating cows.

In this work, milk bioactive peptides from traditionally fed cows and OOP fed cows were obtained by simulating gastrointestinal digestion of milk and fractionated by RP-HPLC.

The application of 0.05 µg/µL peptides on RAW 264.7 cells decreases intracellular ROS content and expression of COX2 and iNOS, showing anti-oxidant and anti-inflammatory activity in agreement with literature.

In addition to these activities, anti-proliferative effect of peptides was highlighted on RKO cells with 0.1 µg/µL peptides, acting in synergy with 5-fluorouracyl to further reduce cell proliferation. Finally, peptides impact on cellular differentiation was observed in Caco-2 cells by assaying alkaline phosphatase.

These results confirmed the anti-oxidant and anti-inflammatory activity of milk-derived peptides and highlighted other biological activities, such as anti-proliferative and pro-differentiating potential. However, all experiments show that OOP supplementation does not affect either the composition or the biological activity of the peptides. Nonetheless, it was demonstrated that agro-industrial waste products may be successfully included in the feeding strategy of dairy cows.

Categories

Nutrition and Environment

Dysfunction of cellular proteostasis in human primary chondrocytes.

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Abstract

Local deposits of amyloid have been described in the superficial layers of osteoarthritis (OA) and normal aged cartilage tissue. Osteoarthritis (OA) is the most prevalent joint disease and aging is its major risk factor. An efficient cellular proteostasis is crucial to prevent aggregate deposition and is maintained by different quality control systems, including selective autophagy, ubiquitin-proteasome system (UPS) and molecular chaperones.

Aim of this study has been to investigate the role of the main pathways implicated in the correct protein folding in OA pathology. Amyloid deposition in OA human knee cartilage was determined by Congo-Red and Thioflavin-T stainings. Interestingly, amyloid was also observed in middle and deeper layers, close to the pericellular matrix of chondrocytes, thereby indicating an endogenous protein aggregation. This finding supports our hypothesis that alterations in cellular homeostasis could contribute to amyloid deposition and thus, to OA onset and progression. In order to better understand the implication of autophagy and UPS pathways, epigallocatechin gallate (EGCG), lipopolysaccharide (LPS), a pro-inflammatory stimulus, and chloroquine (CQ), a treatment able to inhibit the major proteostasis control systems, were administrated to OA chondrocytes. Our findings suggest that EGCG is able to reduce LPS and CQ-induced aggregates and this effect could be mediated by the modulation of autophagy and chaperone system.

A better comprehension of the inflammatory modifications affecting cellular proteostasis, as well as the knowledge of the mechanisms underlying these alterations, might lead to the discovery of novel therapeutic targets for OA.

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Categories

Nutrition and Environment

Interplay between Wnt/β-catenin and Nrf2/HO-1 pathways in renal ischemia/reperfusion injury in diabetic mice

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Abstract

Diabetes condition is an important risk factor in renal damage which makes this organ significantly more susceptible to ischemia/reperfusion damage. However, the pathophysiological mechanisms underlying renal ischemia/reperfusion injury (RIRI) in diabetic conditions is still not well understood. Nowadays, there is considerable interest in the use of medicinal herbs and natural products in oxidative and inflammatory disorders. Saffron extracts have shown anti-inflammatory, antioxidant and hypoglycemic properties. Therefore, the aim of this study was to evaluate the potential molecular mechanisms of saffron in an experimental model of RIRI. Non-diabetic and diabetic mice were subjected to bilateral renal ischemia for 30 min followed by 6h of reperfusion, and Saffron (60 mg/kg, o.s.) was administered 15 min before release of the end of ischemia. Our results showed that saffron was able to counteract histological damage and the increase in kidney dysfunction markers induced by RIRI equally in non-diabetic and diabetic mice, despite a worse baseline condition induced by hyper-glycemia. These effects could be explained at the molecular level through modulation of WNT pathway, oxidative stress and the apoptotic process. Additionally, saffron decreased H2O2-induced intracellular ROS generation in Vero cell cultures. These results suggest that Saffron could represent a nutritional product that could limit the progression of the disease and diabetic comorbidity.

Categories

Nutrition and Environment

Extra Virgin Olive Oil polyphenols exert antioxidant and anti-inflammatory effects on peripheral blood mononuclear cells of rheumatoid arthritis patients.

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Abstract

Numerous food, as fruits and vegetable, contain bioactive phytochemicals, such as polyphenols, capable of exerting anti-inflammatory, antioxidant and anti-tumoral effects [1,2]. The Mediterranean Diet is characterized by a high consumption of extra virgin olive oil (EVOO), a food rich of nutritive and nutraceutical compounds. Thanks to the high content of polyphenols, EVOO exerts beneficial effects against several autoimmune and chronic inflammatory diseases. Our study focused on evaluating the antioxidant and anti-inflammatory effects of EVOO on rheumatoid arthritis (RA) patients. RA is a systemic autoimmune disease primarily affecting the synovial joints and defined by a concomitant systemic inflammation characterized by autoantibodies and pro-inflammatory cytokines production [3]. We extracted polyphenols from EVOO (PE-EVO) and demonstrated the antioxidant capacity of these components using the DPHH assay. Notably, peripheral blood mononuclear cells (PBMC) of RA patients showed a significant reduction of intracellular pro-inflammatory cytokines (TNFα and IL-1β) when they were pre-treated in vitro with PE-EVO for 48 and 72h, compared to the same cells incubated in culture medium alone. The efficacy of PE-EVOO was also evaluated on PBMC of healthy controls activated by inflammatory stimuli, such as Ionomycin-PMA and LPS. Pre-treatment of RA patients PBMC with PE-EVOO increased the production of anti-inflammatory cytokines IL-4 and IL-13, drastically reducing ROS content. Taken together, these preliminary results demonstrate that PE-EVOO possess antioxidant and anti-inflammatory effects on PBMC of RA patients. Further experiments will be performed to identify the molecular mechanisms underlying these PE-EVOO effects.

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Categories

Nutrition and Environment

In vitro and in silico methods to assess modulation of digestive proteases by food-derived phenolics

Sara Margherita Borgonovi[1], Florinda Perugino[2], Lorenzo Pedroni[2], Andrea Pinto[1], Sabrina Dallavalle[1], Stefania Iametti[1], Luca Dellafiora[2], Gianni Galaverna[2], Mattia Di Nunzio[1]

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Abstract

The interaction of food components with digestive enzymes can modify the digestion process and affect human health. Although polyphenols (PPs) have a beneficial impact, they are often referred to as anti-nutritional factors, having been reported to inhibit proteolytic digestive enzymes. However, studies on this topic are often contradictory, highlighting the necessity of using standardized methods. In this study, the effects of selected food-derived PPs were assessed "in vitro" on pepsin, trypsin, and chymotrypsin using albumin, gluten, and hemoglobin as substrates. Results show that PPs may affect proteolytic activity in opposite ways, depending on the substrate/enzyme combination. Therefore, a selection of PPs was further investigated via “in silico” approaches to assess a possible structure/activity relationship on the ovalbumin/chymotrypsin system. After searching for putative binding pockets, molecular docking and dynamics allowed to point out the possible capability of PPs to interact in stable fashion with either ovalbumin or chymotrypsin. Interestingly, the most potent proteolytic digestion inhibitors according to the “in vitro” results was found to correspond to those best interacting PP within the enzyme binding site. Conversely, digestion-promoting PPs were best interacting with the substrate’s binding pockets. These studies indicate that structural features have a role in eliciting specific effects of PPs binding affinity, orientation, and geometry. The evidence gathered here suggests the possibility of considering some PPs as “digestion-promoting agents” in the formulation of functional foods.

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Categories

Nutrition and Environment

ADHERENCE TO MEDITERRANEAN DIET AS AN ANTIOXIDANT: THE IMPACT ON NEURODEGENERATIVE DISEASES

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Abstract

Alzheimer's disease (AD) is the most common neurodegenerative disease in the elderly population. AD is a multifactorial pathology. However, nutrient deficiency has been associated with dementia onset. The Mediterranean Diet (MD) is a healthy dietary pattern related to a lower risk of certain chronic diseases, including neurodegenerative diseases. MD is characterized by some bioactive compounds that are beneficial to health. Researchers agree on the positive role of polyphenols, main constituent of the MD. In fact, polyphenols can cross the blood-brain barrier, scavenge pathological concentrations of reactive oxygen and nitrogen species, and chelate transition metal ions. Thus, we aimed to investigate in a sample of 92 elder people, affected by AD, the adherence to the MD. We administered the Medi-Lite questionnaire, meant to collect frequency and portion of food consumption of groups characterizing MD. The final adherence score comprised nine food categories with a score ranging from 0 point (lowest adherence) to 18 points (highest adherence). Due to the remarkable neuroprotective activity of polyphenols, we evaluated the plasma Total Phenolic Content (TPC), Total Flavonoids Content (TFC) and Oxygen Radical Absorbance Capacity (ORAC), in all enrolled subjects. Results were compared to a control group, constituted by healthy volunteers. Our findings highlight a decrease in antioxidants in AD patients compared to controls. These data correlate also with the score obtained in the Medi-Lite questionnaire, which is lower in AD population. In conclusion, we can affirm that a higher adherence to the MD could be associated with a reduced risk of developing AD.

Categories

Nutrition and Environment

Understanding the influence of clinical variables on neonatal metabolic profiling for an improved interpretation of newborn screening results

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Abstract

Introduction: Newborn screening (NBS) is a public health preventive medicine program with the aim of early detection of different inborn errors of metabolism (IEMs) to prevent adverse outcomes. The advent of tandem mass spectrometry (MS/MS) allowed an impressive expansion of NBS through a multiplexing approach. Italian NBS program is actually screening for 40 IEMs on dried blood spot (DBS) samples. Although NBS methods are characterized by high specificity, a positive result cannot be considered a diagnosis, and follow-up and confirmatory testing are required. In this context, the application of second-tier tests (2-TT) can minimize false positives. 2-TTs allow a better interpretation of abnormal results and improve the positive predictive value of NBS. In this study, we reviewed the NBS data (from the first and second-level tests carried out after alteration of propionyl-carnitine metabolism) of approximately 26,000 newborns screened in Abruzzo region between 2019 and 2023; 12% of the total samples required the execution of 2-TT.

Methods: DBS were extracted with the NeoBase2 Non-derivatized MSMSkit and analyzed with MS/MS platform RenataDX-ScreeningSystem. For 2-TT two DBS disks were extracted for the simultaneous determination of methylmalonic acid, methylcitric acid and homocysteine by LC-MS/MS analysis.

Results: The data obtained show a significant correlation between first level results and clinical data. NBS programs worldwide tend to apply post-analytical interpretive tools, which allow to adjust NBS data using a variety of clinical variables, such as birth weight, gestational age, gender, time at blood collection, antibiotic and/or cortisone treatment and type of nutrition.

Categories

Nutrition and Environment

EFFECTS OF COMBINED PHYSICAL EXERCISE ON FUNCTIONALITY OF HIGH DENSITY LIPOPROTEIN IN OBESE SUBJECTS

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Abstract

Dysfunctional high-density lipoproteins (HDL) and oxidative stress are involved in the development of several complications and metabolic disorders associated to obesity, so they represent a possible therapeutic target. The results of many studies reported a beneficial impact of physical exercise on HDL-C levels in obesity. Aim of our study was to investigate the effect of exercise on HDL functional properties in obese subjects. 18 obese subjects (9 F and 9 M, BMI=30.3±3kg/m2) attended supervised training (ET) consisted of seven weeks, including combined resistance and conditioning training, four to five times each week. Before and at the end of the intervention the activity of the antioxidant HDL-associated enzyme paraoxonase-1 (PON1) and HDL redox activity and HDL antioxidant properties were evaluated in serum samples of subjects. Moreover, serum MPO levels and biochemical markers of oxidative stress (ox-LDL and total antioxidant capacity) were studied.

At the end of the intervention, an increase of PON1 activity ($p<0.001$) and a decrease of MPO levels ($p<0.001$) were observed in serum of obese subjects, with a decrease of the MPO/PON1 ratio. Moreover, HDL isolated from serum of obese subjects after exercise training showed a lower redox activity and higher antioxidant activity. A significant correlation was established between serum MPO/PON ratio and HDL redox activity and HDL antioxidant activity, confirming that MPO/PON1 ratio is a marker of HDL functionality.

In conclusion our results that demonstrate that in obesity, exercise training improved HDL functionality in absence of significant modification of anthropometric variables and of plasma lipids levels

Categories

Nutrition and Environment

Identification and characterization of the allergen Arginine Kinase from the edible insect Hermetia illucens

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Abstract

Insects represent alternative protein sources to traditional products with numerous advantages in terms of respect for environmental sustainability. Hermetia illucens (Black Soldier Fly, BSF) is one of the most promising insects for human consumption due to its composition in fatty acids, proteins and minerals. In this perspective it is crucial to evaluate the risks of allergenicity, meant as primary sensitization and cross-reactivity with known allergens. A well-known invertebrate pan-allergen is arginine kinase (AK), which has been identified as a major allergen in crustaceans and mites. Several studies show its allergenic potential and its cross-reactivity with other invertebrates. In this work, the sequence coding for AK was identified in the genome of BSF using bioinformatics analysis and AK was produced as a recombinant protein in E. coli. The stability of AK to increasing temperature and at different pH values was also evaluated by using SDS-PAGE and CD spectroscopy. The data showed that AK is unstable when incubated in acidic conditions or at temperatures above 50 °C. In vitro digestion assays combined with high-resolution mass spectrometry also led to the identification of peptides released after the digestion process. IgE-immunoblotting assays using sera from patients with allergy to shrimps and/or to mites demonstrate a cross-reactivity of AK with shrimps and mites and highlight the importance of further analysis to investigate AK stability and its allergenicity under different conditions.

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Categories

Nutrition and Environment

Immunomodulatory effect of environmental disruptors exposure on myocarditis

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Abstract

An inflammatory cardiac disorder known as myocarditis is the primary cause of heart failure in young adults [1]. Its etiology is ascribed to various issues including bacterial or viral infections, toxins or drugs or endocrine disruptors (EDs) exposure, as well as autoimmune processes [2]. Tebuconazole (TEB), which is a member of the triazole fungicide family, is utilized to safeguard agricultural crop plants against fungal pathogens. The information about how it induces toxic effects through various pathways, in particular, in autoimmune diseases are still limited [3]. Thus, the aim of this paper was to evaluate the effect of TEB exposure in autoimmune myocarditis (AM). To induce AM, rats were immunized with porcine cardiac myosin and exposed to TEB for 21 days. Thereafter, animals were sacrificed and histological, biochemical and molecular analyses were performed. TEB exposure increased heart weight, systolic blood pressure and heart rate already augmented by AM. From the histological point of view, TEB exacerbates the histological damage induced by AM and increased fibrosis and collagen deposition [4]. TBE exposure strongly increased prooxidants levels (O2-, H2O2, NO2-, lipid peroxidation) and reduced antioxidant enzymes levels, already dysregulated by AM. Additionally, TBE increased NOX-4 expression and the TGFβ1-Smad pathway already activated by AM. Overall, our results showed that TEB exposure strongly aggravated the damage induced by AM [5].

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Categories

Nutrition and Environment

The RNA cargo in small extracellular vesicles from chicken eggs are bioavailable in humans and contribute toward spatial learning and memory in mice

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Abstract

Small extracellular vesicles (sEVs) and their cargos are not exclusively obtained from endogenous synthesis but may also be absorbed from dietary sources. Little is known about the content and biological activity of sEVs in foods of animal origin other than milk. Here we tested the hypothesis that sEVs in chicken eggs facilitate the transfer of RNA cargo from an avian species to humans and mice and their dietary depletion elicits phenotypes. sEVs were purified from raw egg yolk by ultracentrifugation and authenticated by transmission electron microscopy, nano-tracking device, and immunoblots. The miRNA profile was assessed by RNA-sequencing. Bioavailability of miRNAs in humans was assessed by egg feeding study, and by culturing human peripheral blood mononuclear cells (PBMCs) with fluorophore-labeled egg sEVs ex vivo. Bioavailability in C57BL/6 Jmice was tested by administering fluorophore-labeled miRNAs encapsulated in egg sEVs by oral gavage. Phenotypes of sEV RNA cargo depletion were assessed by feeding egg sEV and RNA-defined diets to mice and using spatial learning and memory in the Barnes and water mazes as experimental readouts. Egg sEVs contained eighty-three distinct miRNAs. Human PBMCs internalized sEVs and their RNA cargo. Egg sEVs in mice accumulated primarily in brain, intestine and lungs. Spatial learning and memory (SLM) was compromised in mice fed on egg sEV- and RNA-depleted diet (ERD) compared to controls (ERS). Eighty-eight genes were differentially expressed in mice hippocampi, high-confidence gene networks included pathways implicated in learning and memory. We conclude that sEVs and their RNA cargo are bioavailable in human and mice.

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Categories

Nutrition and Environment

Environmental stress and food chain: proteomic evaluation of biotic and abiotic stress effects on beebread, a potential long term surveillance matrix

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Abstract

An animal model, that has proved particularly useful for the purpose of monitoring and characterizing metabolic responses implemented by organisms exposed to both biotic and abiotic stress, is represented by bees. Honeybee play an essential role in plant pollination and provide precious service to the ecosystem, indeed one-third of the world food production relies on insect pollinators. Among bee products, beebread results from the transformation of plant pollen by biochemical processes caused by the enzymes in the saliva and gastric fluid of the bee. It contains pollens gathered throughout the year by bees; therefore, it can be used as a long-term surveillance matrix. Beebread was collected from March to September 2022 from 4 honeycombs in the University of Milano beehive, close to the city of Lodi. Samples were then analyzed by adapting the proteomic protocol used on pollen in Yin S et al. (2022) [1], on beebread, for the first time. Briefly, 50 mg of freeze-dried beebread was homogenized using a Potter homogenizer and ultrasonicated in lysis buffer. After proteins precipitation, pellet was resuspended in urea and proteins were then reduced, alkylated and digested. The proteolytic digest was desalted and directly inject into LC-ESI-MS/MS. MS spectra were searched against a homemade reference Uniprot sequence database, containing Apis Mellifera, plant species typical of the sample collection area, flowering during the collection and yeasts involved in the maturation of pollen to beebread, as reported in Detry R et al. (2020). Statistical and functional analysis are ongoing.

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Categories

Nutrition and Environment

Callus cultures from the pulp of Malus domestica 'Mela Annurca Campana': first chemical characterization and biological properties investigation

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Abstract

In this study, an innovative method to produce secondary metabolites from Malus pumila Miller cv. Annurca was set up using an in vitro technique based on the production of callus cultures from fruit pulp cells. Annurca apple ripe pulp was used as starting material and two callus cultures, one grown in the dark and the other in the 16-hour photoperiod, were obtained. The qualitative and quantitative content in phenolic and triterpenic acids from calli hydro-alcoholic extracts were elucidated by GC–MS, GC, and HPLC-DAD-ESI-MSn. The calli extract' radical scavenging and antioxidant activity were investigated through cell-free assays including DPPH, ABTS, ORAC assays, and lipoxygenase inhibition activity. The genoprotection was assessed by nicking assay. All the analyses performed were compared with peel and pulp hydroalcoholic extracts. Compared to the peel and pulp, the triterpene content of the calli was significantly more abundant, while the polyphenolic content, especially of the dark-grown callus, was lower. Both calli extracts showed radical scavenging and lipoxygenase inhibition activity, lower and higher respectively, when compared to pulp and peel. Nicking assay demonstrated that callus grown in the dark can protect DNA to a greater extent than callus grown in the light, although the greatest protection is provided by the pulp.

The data obtained led us to further studies aimed at using the callus as a bio-source of secondary metabolites of therapeutic interest.

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Categories

Nutrition and Environment

Estrogens-dependent TRX2 activation reverts oxidative stress and subsequent non-alcoholic fatty liver disease

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is the general name for a broad spectrum of liver damaging conditions. Oxidative stress is key in the pathology progression and NAFLD prevalence is lower in pre-menopausal women than in men. Therefore, this study explores the role of estrogens in the metabolic and antioxidant response that occurs during NAFLD progression in a preclinical relevant model of NAFLD using hepatocyte-like cells (HLCs) derived from human male WA01 and female WA09 embryonic stem cells (hESCs). HLCs NAFL induction, by treatment with sodium lactate, sodium pyruvate and octanoic acid (LPO), was confirmed by lipid droplets (LD) accumulation in parallel to increase mitochondrial ROS levels in both HLCs exposed to LPO. In line with our hypothesis, LD and ROS levels were significantly reduced when estrogens were administrated in both male and female NAFL-induced samples, suggesting that the "gender-relevant phenotype" was related to the presence of estrogen rather than to the genetic background of the HLCs. Interestingly, our data showed that the protective effect of estrogens in mediating ROS reduction could be explained through the upregulation of mitochondrial thioredoxin-2 (Trx2), an antioxidant system under the control of the estrogen receptor alpha. Importantly, the antioxidant effect induced by estrogens was reverted by the administration of auranofin, a compound capable of disrupting the Trx2-system. Our data confirm that the mitochondrial Trx2 system, activated by estrogens, can reduce the oxidative stress associated with NAFLD and suggests that the estrogen-mediated activation of specific antioxidant mechanism could prevent or delay the progression of the disease.

Categories

Nutrition and Environment

Mechanism of Action of Natural Compounds in Peripheral Multiorgan Dysfunction and Hippocampal Neuroinflammation Induced by Sepsis

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Abstract

Bacterial sepsis induces the production of excessive pro-inflammatory cytokines and oxidative stress, resulting in tissue injury and hyperinflammation. Patients recovering from sepsis have increased rates of central nervous system (CNS) morbidities, which are linked to long-term cognitive impairment. Here we evaluate the effects of Coriolus versicolor administration to treat polymicrobial sepsis. Rats underwent cecal ligation and perforation (CLP), and Coriolus versicolor (200 mg/kg in saline) was administered daily by gavage. Survival was monitored, and tissues from vital organs that easily succumb to infection were harvested after 72 h to evaluate the histological changes. Twenty-eight days after CLP, behavioral analyses were performed, and serum and brain (hippocampus) samples were harvested. Coriolus versicolor increased survival and reduced acute tissue injury: it reduced the release of pro-inflammatory cytokines in the bloodstream, leading to a reduced inflammation. In the hippocampus, Coriolus versicolor restored tight junction expressions, reduce cytokines accumulation and glia activation. It also reduced TLR4 and nNOS and NLRP3 inflammasome components expression. Coriolus versicolor showed antioxidant activities, restoring GSH levels and catalase and SOD activities and reducing lipid peroxidation, nitrite and ROS levels. Importantly, Coriolus versicolor reduced APP, p-Tau, PHF1, AT8, IFITM3 expression, and β-amyloid accumulation induced by CLP. Indeed, Coriolus versicolor restored synaptic dysfunction and behavioral alterations. This research shows the effects of Coriolus versicolor administration on the long-term development of neuroinflammation and brain dysfunction induced by sepsis: counteracting the degenerative process.

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Categories

Nutrition and Environment

Taste sensitivity and saliva antioxidants in anorexia nervosa adolescent female patients at onset and after 6 months of integrated therapy

Valentina Membrino[1], Alice Di Paolo[1], Sonila Alia[1], Tiziana Di Crescenzo[1], Laura Nanetti[2], Lucia Emanuela Svarca[2], David Mazzoni[2], Michele Severini[2], Arianna Vignini[1]

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Abstract

Anorexia nervosa (AN) is a complex disorder affecting mainly, but not only, teenage women. It is a mental illness characterized by disturbances in eating behaviors, involving food restriction and severe weight loss. The consequences of calorie restriction can have a negative impact on bone density, growth, and brain maturation, especially in children and adolescents. Multiple concomitant medical complications occur throughout the body and become more pronounced as the severity of the illness increases. Restriction of foods consumption can impact on human health in many ways. The flavor of a food determines its acceptability and modulates its intake. Thus, we aimed to investigate if there could be an alteration in taste perception in subjects affected by AN able to influence their food intake. We performed the administration of Taste Strip Test on 47 AN patients at t0 (time at enrollment), t1 (after 3 months of integrated therapy) and t2 (after 6 months of integrated therapy). We compared the results to understand if there would be a test score increase with the disease remission. We also determined the differences in antioxidant levels in saliva samples between t0, t1 and t2. The Taste Strip Test score correlates very well with the levels of antioxidants and with the course, response to treatment and severity of the disease. In conclusion, we can affirm that altered taste sensitivity could represent a component in the wider altered taste processing observed in anorexia nervosa.

Categories

Nutrition and Environment

Toxic Effects of Endocrine Disruptor Exposure on the immune system

Francesco Molinari[1], Ylenia Marino[2], Alessia Arangia[2], Livia Interdonato[2], Gianluca Antonio Franco[1], Roberta Fusco[2], Rosanna Di Paola[1], Salvatore Cuzzocrea[2]

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Abstract

Endocrine disruptors (EDs) are chemical substances that can disrupt the normal functioning of the endocrine system, affecting organ development and physiological processes [1, 2]. Bone tissue, which undergoes complex hormonal regulation, is particularly susceptible to the effects of these disruptors [3]. This research aimed to investigate how exposure to EDs impacts the immune profile, inflammatory response, and oxidative stress following an immunological challenge using an in vitro model called Peripheral Blood Mononuclear Cells (PBMC).

In the study, PBMC exposed to EDs were stimulated with LPS, a substance that activates the immune response and triggers the release of pro-inflammatory cytokines. The researchers used ELISA tests to evaluate inflammation and oxidative stress by measuring cytokines such as IFN-g, interleukins (IL), IL-6, IL-1β, IL-17A, TNF-α, as well as MPO and MDA assays.

When blood cells are simultaneously exposed to LPS and endocrine disruptors, their effects on the immune response and inflammation can be different. The results of the study demonstrated that certain endocrine disruptors can enhance the production of pro-inflammatory cytokines in response to LPS stimulation. This heightened inflammatory response can contribute to chronic inflammation, which has been associated with various health issues including cardiovascular disease, metabolic disorders, and immune dysfunction.

Furthermore, EDs can interfere with the normal functioning of immune cells by altering the expression of hormone receptors or signaling pathways. This disruption can impair immune cell activation, proliferation, and differentiation, ultimately leading to a compromised immune response. Therefore, it is important to limit exposure to EDs to mitigate their negative effects.

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Categories

Nutrition and Environment

Proteomic changes driven by water pollution in the unicellular microalga *Chlamydomonas reinhardtii*

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Abstract

Heavy metals are one of the most toxic contaminants of the aquatic ecosystems. Increasing industrialization and anthropogenic activities are causing an increasing pollution in soils and water.

In this context, proteomics has emerged as a powerful tool for a better understanding of the metabolic responses, tolerance and detoxification mechanisms in microalgae under metal stress. *Chlamydomonas reinhardtii* is a robust microalga with the capacity to grow in a broad range of environmental conditions, including industrial or urban wastewater.

This microalga tolerates high amounts of different heavy metals, but there is no proteomics study about *C. reinhardtii* grown in presence of a heavy metal mixtures.

In the present work, *C. reinhardtii* cells were growth both in absence of heavy metals and in presence of CuSO4, ZnCl2, NiCl2, PbCl2 at the final concentration equal to legal limits for groundwaters. After 7 days of treatment, cells were collected and analyzed by a typical shotgun and label free proteomic approach to identify and quantify proteins differentially expressed among the two different conditions.

Our results show that there is correlation between the expressed protein pattern and the environmental conditions, confirming the findings of others proteomics works carried out on different aquatic marine organisms[1-3].

Overall, studying the proteome expressed in *Chlamydomonas reinhardtii* is important for understanding many different biological, physiological and ecological aspects that can be useful to understand the biological effects of environmental pollution and climate change.

This research has been financially supported by Piano di Sostegno alla Ricerca SEED_2020 (Università degli Studi di Milano)

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Categories

Nutrition and Environment

Mercury Chloride Affects Band 3 Protein-Mediated Anionic Transport in Red Blood Cells: Role of Oxidative Stress and Protective Effect of Olive Oil Polyphenols

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Abstract

Mercury is a toxic heavy metal widely dispersed in the natural environment. Mercury exposure induces an increase in oxidative stress in red blood cells (RBCs) through the production of reactive species and alteration of the endogenous antioxidant defense system. Recently, among various natural antioxidants, the polyphenols from extra-virgin olive oil (EVOO) have generated growing interest. Here, we examined the potential protective effects of hydroxytyrosol (HT) and homovanillyl alcohol (HVA) on an oxidative stress model represented by human RBCs treated with HgCl2 (10 µM, 4 h of incubation). Morphological changes as well as markers of oxidative stress, including thiobarbituric acid reactive substance (TBARS) levels, the oxidation of protein sulfhydryl (-SH) groups, methemoglobin formation (% MetHb), apoptotic cells, a reduced glutathione/oxidized glutathione ratio, Band 3 protein (B3p) content, and anion exchange capability through B3p were analyzed in RBCs treated with HgCl2 with or without 10 µM HT or HVA pre-treatment for 15 min. Our data show that 10 µM HT or HVA pre-incubation impaired both acanthocytes formation, due to 10 µM HgCl2, and mercury-induced oxidative stress injury and restored the endogenous antioxidant system. Interestingly, HgCl2 treatment was associated with a decrease in the rate constant for SO42− uptake through B3p as well as MetHb formation. Both alterations were attenuated by pre-treatment with HT and HVA. These findings provide mechanistic insights into benefits deriving from the use of naturally occurring polyphenols against oxidative stress induced by HgCl2 on RBCs. Thus, dietary supplementation with polyphenols might be useful in populations exposed to HgCl2 poisoning.

Categories

Nutrition and Environment

POTENTIAL EFFECTS OF DIFFERENT ANTIOXIDANTS IN THE CO-TREATMENT OF DUCHENNE MUSCULAR DYSTROPHY

Cecilia Prata[1], Luca Pincigher[1], Maria Cristina Barbalace[2], Silvia Zecchini[3], Roberta Ottria[3], Harcel Djaya Mbissam[3], Marco Malaguti[2], Clara De Palma[4], Emilio Clementi[3], Cristiana Perrotta[3], Silvana Hrelia[5]

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Abstract

Duchenne muscular dystrophy (DMD) is a genetic disorder characterized by progressive muscle degeneration and weakness due to the alterations of a protein called dystrophin that helps keep myocytes intact. Unfortunately, a definite cure has not yet been identified. Corticosteroids are widely used because they delay the progression of the disease, however they lead to severe side effects, therefore highlighting a pressing need for an alternative therapeutic strategy for DMD.

Since oxidative stress and inflammation are commonly features of skeletal muscle in DMD mouse model, this study aims to evaluate the potential effects of selective serotonin reuptake inhibitors (SSRI) and/or antioxidants on the expression of several enzymes involved in the antioxidant defence system in dystrophic mdx mice. In particular, in addition to the SSRI Fluoxetine or Sertraline, known to have anti-oxidative/inflammatory properties, two molecules with different antioxidant mechanisms were selected: Plumbagin, an inhibitor of NOX activity, and quercetin, that not only is able to directly scavenger reactive oxygen species (ROS) but also to induce phase II enzymes.

Results show that mouse treated with SSRIs and/or antioxidants are endowed with a higher antioxidant defence system compared to the untreated mdx mouse model. We identified Fluoxetine and Plumbagin as the most efficient in their class, notably due to their ability to activate the Nrf2 pathway, which plays a pivotal role in the anti-oxidant/inflammatory response and muscle regeneration. The combination of these two molecules was tested in order to verify potential additive/synergistic activities and the obtained results are promising.

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Categories

Nutrition and Environment

Characterisation of Nero Antico di Pretalucente Wine: An Expression of Abruzzo Region Cultivar Heritage

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Abstract

In recent years, the cultivation of a limited number of highly productive and widely adapted grape cultivars has led to a loss of biodiversity, mirroring the trend observed in many other crops. However, consumers are nowadays considering local foods as a way to enhance the sustainability by reducing carbon and water footprints and creating new sales prospects, particularly for small to medium-sized local wineries. This study aims to characterise the biochemical, genetic, and epigenetic profiles of *Vitis vinifera L.* cv. Nero Antico di Pretalucente, a black grape variety cultivated in the Gessopalena village of the Abruzzo region. The findings provide valuable insights into the unique characteristics of this ancient and autochthonous grapevine variety. Furthermore, the cultivation of these grape varieties contributes to environmental biodiversity and fosters sustainability in viticulture. The characterization of genotypes using SSR markers plays a crucial role in identifying synonyms, homonyms, and parentage relationships among grapevine varieties. In the case of Nero Antico di Pretalucente, the analysys of SSR regions revealed a distinctive genetic profile, suggesting its classification as a new cultivar. The presence of homozygous loci indicates natural self-fertilization events associated with the grapevine's geographic isolation. By deepening our understanding of the polyphenolic content and their healty effects we valorised the wine characteristics of this ancient grapevine variety, as well as providing to consumers novel information and knowledge of a wine possessing unique qualities and a connection to its origin.

Categories

Nutrition and Environment

Phytochemical Indicaxanthin from Opuntia ficus-indica (L. Mill) fruit at nutritional relevant plasma concentration inhibits eryptosis induced by cigarette smoke extract

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Abstract

Eryptosis is a programmed death mechanism that eliminates damaged RBCs before natural senescence[1]. Due to the adhesiveness of apoptotic RBCs to the vascular cells or platelets, excessive eryptosis is associated to inflammatory pathologies of the cardiovascular system such as atherosclerosis, thrombosis and heart failure[2]. Our recent studies have reported that cigarette smokers have higher levels of eryptotic erythrocytes than non-smokers[3] and that the cytotoxic mechanism of the cigarette smoke in RBCs is mediated by p38 MAPK-induced extrinsic apoptotic signaling pathway[4].

Indicaxanthin (Ind) from Opuntia ficus-indica fruits is a phytochemical highly-bioavailable in humans. After 4 hours from ingestion of four fruits of cactus pear, Ind reaches micromolar plasma concentrations and incorporates in RBCs enhancing their defence against a number of adverse stimuli[5, 6].

In this in vitro study we show that Ind, at plasma concentrations of nutritional relevance, inhibits cigarette smoke extract (CSE)-induced RBCs programmed death. Human isolated RBCs were pre-treated for 1 hour with 1-5 µM Ind before a 4 hours treatment with CSE. In comparison with RBCs pre-treated with medium alone, a significant dose-dependent reduction of the hallmarks of the CSE-induced eryptosis were evident. Through flow cytometry, immunoprecipitation and immunoblotting, confocal fluorescence microscopy and enzyme assays, our data show that Ind is able to inhibit the assembly of the death-inducing signaling complex, ceramide release, the cleavage of caspases 8/3, caspase 3 activity, phosphorylation of p38 MAPK and ATP depletion. Opportunities of dietary intervention with prickly pear fruit in smokers may be considered to protect RBCs from smoke-induced cytoxicity.

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Categories

Nutrition and Environment

CD300e as a novel biomarker in obesity: crosstalk between immune system and adipose tissue.

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Abstract

Obesity is a multifactorial pathology associated with metabolic dysfunction. Most obese patients (OPs) develop insulin resistance (IR) and have an increased risk of developing type 2 diabetes (T2D). Recently, it has been demonstrated that both T1D and T2D patients are seropositive for the self-antigen CD300e, a surface immune receptor expressed by myeloid cells. We revealed that OPs were also seropositive for CD300e, and the anti-CD300e antibody titre declined after weight loss following bariatric surgery. The improvement of anti-CD300e titre after weight loss correlated positively with the improvement of insulin sensitivity.

We aimed to explore the role of CD300e in modulating glycemia by interfering with the signalling cascade triggered by insulin.

By using human monocytes, we revealed the activation of CD300e hindered the insulin-stimulated phosphorylation of AKT. In accordance, internalization of glucose was hampered. Next, we revealed that monocytes from OPs expressed significantly more CD300e than monocytes from normal weight subjects, and this positively correlated with their fasting glycemia. ROC curve analysis revealed the serum level of anti-CD300e antibodies before weight loss can predict the beneficial effect of weight loss on the improvement of insulin sensitivity.

Our data suggest CD300e might contribute to glycaemic control, by negatively modulating the insulin-triggered pathway and the titre of anti-CD300e antibodies in OPs might become a predictive biomarker for the improvement of insulin sensibility after weight loss.

Categories

Nutrition and Environment

Up-cycling of agricultural food waste: pomegranate peels and tomato skin extracts to counteract oxidative stress, inflammation, and bacteria in the oral mucosa

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Abstract

Given the serious environmental impact of food waste, the development of new functional applications for its upcycling is highly demanded. Agricultural food waste and by-products (e.g., peels and seeds) are rich in high-added-value compounds that can positively affect human health[1]. This study aims to characterize the biological activities of tomato skin (T) and pomegranate peel (P) extracts on oral mucosa evaluating their use as ingredients in mouthwashes.

The safety of the extracts at different concentrations [0.5%-3%] and the final mouthwash formulation (F) which contains both extracts at 3% was assessed through a spectrophotometric assay in Human Gingival Epithelial cells (GECs). Next, antioxidant and anti-inflammatory activities were analyzed by a cell-based chemiluminescent assay for intracellular H_2O_2 detection[2] and real-time PCR in GECs, injured with 25ug/ml lipopolysaccharide. After 24 hours of treatment with the extracts [0.5%-3%], and the final formulation, a good antioxidant activity ($IC50_P$:0.51±0.01µg/mL; $IC50_T$:0.57±0.02µg/mL, $IC50_F$:0.04±0.02µg/mL) and an increased Superoxide Dismutase-1 expression ($p<0,0001$) were observed. Moreover, these treatments significantly decreased the expression of inflammatory biomarkers such as Tumor Necrosis Factor α ($p<0,001$) and Monocyte Chemoattractant Protein-1 ($p<0,0001$).

The antibacterial activity regarding *S. mutans* and *S. sanguinis* was evaluated by broth microdilution method and agar diffusion test for the extracts and the final formulation, respectively.

The solutions showed good antibacterial activity on the reference strains ($MIC_{P,T}$=10% $Ø_F$=24±1mm for *S. mutans* and $MIC_{P,T}$=5% $Ø_F$=18±1mm for *S. sanguinis*). Thus, extracts are worthy of investigation for their use as bioactive ingredients to counteract oxidative stress, inflammation, and pathogenic oral bacteria.

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Categories Nutrition and Environment

Snail Extracted Mucus and Cosmeceutical Applications: Strategies To Increase Its Beneficial Effects

Valeria Sorrenti[1], Valeria Consoli[2], Maria Gulisano[1], Salvatore Petralia[1], Ludovica Maugeri[1], Angela Castellano[3], Luca Vanella[1]

[1]Department of Drug and Health Sciences, Catania, Italy. [2]Department of Drug and Heath Sciences, Catania, Italy. [3]Mediterranean Nutraceutical Extracts (Medinutrex), Catania, Italy

Abstract

Nowadays, several studies have highlighted the ability of snail mucus in maintaining healthy skin conditions due to its emollient, regenerative and protective properties. In particular, mucus derived from H. aspersa muller has been reported to have beneficial properties such as antimicrobial activity and wound repair capacity1-3. In order to enhance beneficial effects of snail mucus two different approaches were exploited: enrichment with antioxidant compounds derived from edible flowers wastes (Acmella oleacera, Centaurea Cianus, Tagetes erecta, Calenda officinalis, Moringa Oleifera) and development innovative nanoparticles with added therapeutic value. Thus, UVB-damage and LPS-induced inflammation were used as models to investigate in vitro the cytoprotective effects of both extracts and nanoparticles. Firstly, the green method ultrasound assistited extraction, was used to obtain an Edible flower wastes extract. Results demonstrated that flowers waste extract's polyphenols boosted antioxidant activity of snail mucus providing cytoprotective effects in keratinocytes exposed to UVB . Additionally, GSH content, ROS and LOOH levels were reduced following snail mucus and edible flowers wastes' extract combination treatment. Secondly, to enhance antioxidant activity of snail mucus, it was extracted in a hydroalcoholic solution and subsequently freeze-dried. Snail mucus extract was then used to develop snail mucus-coated gold nanoparticles (SM-NP) which exhibited anti-inflammatory properties. Co-treatment with LPS and SM-NP significantly reduced pro-inflammatory cytokines transcription and release. We demonstrated that flowers wastes can be considered valid candidates for cosmeceutical applications to enrich the snail mucus based anti-age products already present in the market. Moreover, we proved that snail mucus is suitable for creating innovative formulations.

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Categories

Nutrition and Environment

Snail Extracted Mucus and Cosmeceutical Applications: Strategies To Increase Its Beneficial Effects

Valeria Sorrenti[1], Valeria Consoli[2], Maria Gulisano[1], Salvatore Petralia[1], Ludovica Maugeri[1], Angela Castellano[3], Luca Vanella[1]

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Abstract

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Categories

Nutrition and Environment

A green path to the recovery of bioactive molecules from buckwheat husk

Anna Ramona Speranza, Giuditta Heinzl, Alessio Scarafoni, Sara Limbo, Alberto Giuseppe Barbiroli, Stefania Iametti

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Abstract

Buckwheat is a summer growing pseudocereal that has attracted considerable interest thanks to its excellent nutritional value and low environmental impact. Buckwheat requires low doses of fertilizers and does not need pesticides. Noteworthily, buckwheat is a healthy alternative to gluten-containing grain. The first step of buckwheat processing is husk removal through decortication, a process that produces a significant amount of byproducts. Buckwheat husk has shown to be a rich source of bioactives as well as of various other materials, including cellulose and lignocellulose fractions. The recovery of these species can be beneficial to human nutrition or exploited in the context of many industrial applications. The objective of this study is to propose a “green” valorisation method for the bioactive recovery from buckwheat husk, by exploiting different extraction methods, such as Ultrasound Assisted Extraction (UAE) and Microwave Assisted Extraction (MAE). These procedures have been evaluated by using water-based media as the solvent, and results have been compared to one of the traditional extraction methods, involving the use of acidified methanol. The bioactive molecules extracted in both conditions were characterized in terms of profile, relative abundance, and antioxidant activity, showing improved overall yields and a higher content of relevant bioactive species in the “green” extracts. The potential protective effects of bioactive compounds on cell inflammation was studied on human Caco-2 cells, indicating significant effects of the “green” extracts on selected inflammation biomarkers. Information from these studies will allow to define the possible use(s) of individual materials in food and non-food products.

Categories

Nutrition and Environment

Protective effect of astaxanthin on Olfactory Ensheathing Cells exposed to amyloid-β: biological and molecular investigation

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Abstract

Alzheimer's disease (AD) is characterized by agglomerated proteins constitued by amyloid-beta (Aβ). Aβ possesses neutoxic effect and is also a substrate of tissue transglutaminase (TG2), an ubiquitarian protein

that plays a key role in AD. Findings indicated that in AD is involved oxidative stress and that the treatment with antioxidants mitigates the effects of oxidative stress in the central nervous system. In particular, astaxanthin, an antioxidant with antiflammatory properties, could have an important therapeutic role in AD. Herein, we have evaluated the effect of astaxanthin pretreatment on olfactory ensheathing cells (OECs) exposed to the native peptide of Aβ(1-42) or its fragments Aβ(25–35) and Aβ(35–25). OECs are glial cells located in the olfactory system, the first to show a deficit in neurodegenerative diseases. Vimentin, GFAP, nestin, cyclin D1, TG2 expression and the activation of the apoptotic pathway were assessed through immunocytochemical techniques. The percentage of cell viability and ROS levels were detected. In addition, to monitor the mitochondrial status, we used delayed luminescence (DL). We found that astaxanthin was able to reduce TG2 expression up-regulated by Aβ, reducing also GFAP and Vimentin levels. Astaxanthin pre-treatment stimulates cellular repair increasing cyclin D1 and nestin levels and inhibing apoptotic pathway activation. We observed a significant change of DL intensity in OECs exposed to the toxic fragment Aβ (25-35), that completely disappear when OECs were pre-incubated with astaxantin. Therefore, we can suggest that astaxanthin pretreatment could represent an innovative mechanism to counteract the aberrant TG2 overexpression in AD.

Categories

Nutrition and Environment

Perfluorinated compounds impact bone cells homeostasis in a zebrafish model for dominant osteogenesis imperfecta

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Abstract

Perfluorinated compounds (PFCs), widely used in industrial field, are chemicals characterized by the replacement of all hydrogen atoms on the carbon chain with fluorine. They are poorly biodegradable and accumulate in the environment. Excessive exposure to PFCs is known to be toxic for healthy individuals and it could have even worst effect on more fragile people. Particularly, we aim to investigate the impact of PFCs on individuals affected by osteogenesis imperfecta (OI), a rare brittle bone genetic disease mainly characterized by mutations in genes encoding collagen I, and representing a model for juvenile osteoporosis. OI individuals are characterized by defect in bone cell differentiation associated to poor mineralization, resulting in fragile and misshaped bones. To investigate the impact of PFCs on OI bone we exploited the zebrafish Chihuahua (*Chi/+*), a well validated model for dominant OI. *Chi/+* crossed with the transgenic line *Tg(OlSp7:nlsGFP)* expressing GFP under the early osteoblast marker Osterix were used to follow *in vivo* bone cell differentiation. *Chi/+;Tg(OlSp7:nlsGFP)* larvae were treated with perfluorooctanoic acid (PFOA) for 6 days. Following fixation and bone specific staining with alizarin red, osteoblast differentiation and bone mineralization were evaluated. qPCR analysis of proliferation, apoptosis, osteoblast and adipocyte differentiation markers was performed. PFOA exposure reduced mineral deposition in mutants and wild type compared to controls and impaired pre-osteoblasts differentiation in *Chi/+* larvae. Furthermore, an upregulation of the pro-apoptotic marker *bcl-2* was observed upon PFOA exposure. Our data represent a first warning sign, suggesting a negative impact of PFCs exposure on skeleton.

Categories

Nutrition and Environment

P R O T E I N S

Enzymatic properties and interaction network of the Mycobacterium tubercolosis rhodanese-like protein SseA

alvatoreS Adinolfi[1], Mauro Marengo[2], Alex Fissore[1], Edoardo Salladini[1], Simona Oliaro-Bosso[1], Giulia Di Napoli[1]

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Abstract

Tuberculosis remains a global health issue and one of the leading causes of death worldwide. In this frame, the appearance of multi-drug resistance strains has further emphasized the need to identify new targets for diagnostics, drugs and vaccines.

Although its physiological role in Mycobacterium tubercolosis (Mtb) has not been clarified yet, the putative thiosulfate-sulfurtransferase SseA appears as a potential drug target candidate since its to involvement in Mtb macrophage infection and oxidative stress resistance pathways has been widely demonstrated.

In particular, this project aims at gathering knowledge on the biochemical and biophysical properties of the rhodanese-like protein SseA. Since bioinformatics allowed the identification, as a neighbouring sseA gene, of a sequence that encodes for a yet uncharacterized SufE-like protein (SufE), and highlighted the co-expression of putative homologs for the genes encoding the two proteins, this project also aims at providing computational and experimental evidence of the interaction between SseA and SufE.

Recombinant SseA and SufE were produced in E. coli as well folded monomeric protein, as highlighted by circular dichroism and size-exclusion chromatography measurements. Enzymatic measurements demonstrate the ability of SseA to transfer sulfur from low-MW thiols to cyanide as an acceptor molecular and that its enzymatic activity shows up to a 4-fold increase in the presence of SuFE. ITC and MST measurements demonstrated that this increase results from a direct protein-protein interaction and allowed to measure binding affinities. Ongoing studies are addressing the interaction stoichiometry and mechanism, also by using conveniently designed mutants of the two proteins.

Categories

Proteins

Proteome alterations contributing to microglial dysfunction in Alzheimer Disease

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Abstract

Accumulating evidence reveals a pivotal role of dysfunctional microglia in the pathogenesis of Alzheimer Disease (AD). The acquisition of a peculiar "pro-AD" phenotype by microglial cells exacerbates the β amyloid (Aβ) cascade, which in turn contributes to microglial activation and shift towards pathogenetic hallmarks. From a molecular point of view, there is an urgent need to characterize these CNS primary immune cells, when contributing to AD onset. The aim of the present study was the proteomics characterization of primary microglial cells, challenged or not with lipopolysaccharide (LPS), isolated from neonatal Tg2576 (Tg) and wild-type (WT) mice. This allowed us to evaluate the proteome alterations due to the exposure of microglia to soluble forms of Aβ peptides within the brain of Tg mice during intrauterine life. The four groups of samples (WT Ctrl, WT LPS, Tg Ctrl, Tg LPS) have been analyzed in a shotgun LC-MS/MS experiment on a Synapt G2-Si Mass spectrometer (Waters). PCA evidenced a full segregation of Tg cells from WT cells. Moreover, LPS treatment in vitro induced a marked change in Tg cells, while a milder effect was observed on the proteome of WT cells. By comparing WT LPS and Tg LPS samples, over-representation analysis and Gene Set Enrichment Analysis evidenced lipid metabolism and trafficking, pattern recognition receptors biosignaling, and cellular senescence as enriched categories. In conclusion, these mechanisms (and related proteins) may be those that are mainly altered in the "pro-AD" microglial phenotype and contribute to AD pathogenesis.

Categories

Proteins

Fighting antibiotic tolerance by targeting the H_2S-producing enzymes in the superbug *Pseudomonas aeruginosa*

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Abstract

*Pseudomonas aeruginos*a (Pa) represents a major clinical and public health threat owing to the increasing prevalence of community and hospital-associated infections. Pa converts to a persistent antibiotic-tolerant form in which traditional treatments are powerless. Persister cells, which are abundant in biofilms, are responsible for recalcitrant chronic infections; therefore, targeting persisters is a promising approach in the fight against multi-drug resistance. It has been recently shown that the signaling molecule hydrogen sulfide (H_2S) plays a critical role in antibiotic tolerance, and genetic disruption of H_2S-producing enzymes sensitizes a wide range of pathogens to antibiotics [1, 2]. In this study, we have addressed the kinetic and structural characterization of the two enzymes responsible for bulk H_2S production in Pa, i.e., cystathionine beta-synthase (*Pa*CBS) and cystathionine gamma-lyase (*Pa*CGL), which are pyridoxal 5'-phosphate (PLP)-dependent enzyme that work in the reverse transsulfuration pathway to produce L-cysteine. The obtained biochemical and crystallographic data provided new clues about the regulation of the two Pa H_2S-producing enzymes and revealed significant structural and mechanistic differences between the human and bacterial enzymes. This information is of key importance for the rational development of inhibitors able to selectively block the source of bacterial H_2S that can sensitize Pa persisters to low doses of conventional antibiotics.

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Categories

Proteins

FAD synthase as a target for cancer therapy

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Abstract

FAD synthase (FADS, EC 2.7.7.2) catalyses the last step of the biosynthesis of flavin cofactors from riboflavin and is therefore essential for flavoproteome biogenesis. In humans, *FLAD1* (OMIM: 610595) generates different isoforms of FADS by alternative splicing. The key role of FADS in human health has been highlighted in patients suffering from a neuromuscular disorder described as LSMFLAD (Lipid Storage Myopathy due to Flavin Adenin Dinucletide Synthase Deficiency) (OMIM: 255100) (1).

Recently, altered expression of *FLAD1* has been associated with different tumors, such as breast and gastric cancer (2,3). Using pancreatic ductal adenocarcinoma (PDAC) cell lines, we found an increased expression/activity of FADS in the tumor parenchymal cells (PANC-1) and even more in their derived cancer stem cells (CSCs) with respect to the normal HPDE cells (4).

With the aim of proposing FADS as a novel target for therapy, we tested the effect of three compounds already known to inhibit bacterial FADS (5) on the FAD synthase activity performed by the best characterized human isoform of FADS (FADS2), overexpressed in *E. coli*, and purified. Chicago Sky Blue (CSB) resulted the most effective inhibitor, showing an IC_{50} of approximately 1.5 µM.

The effect of CSB was then tested on FAD formation catalysed by extracts from the PDAC cell lines, where an IC_{50} in the µM range was confirmed. More importantly, CSB selectively affected the viability of the highly chemoresistant PANC-1 CSCs, showing its strong ability to inhibit cell growth, confirming FAD synthase as a novel pharmacological target for cancer therapy.

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Categories

Proteins

Evaluation of the residence time of new 4-HPPD inhibitors as starting point to provide novel strategies for AKU treatment

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Abstract

Alkaptonuria (AKU) is an ultrarare disease caused by the deficiency in homogentisate 1,2-dioxygenase (HGD) activity in the tyrosine catabolic pathway, resulting in the accumulation of homogentisic acid (HGA). Over time, HGA accumulation leads to the formation of an ochronotic melanin-like pigment, a dark deposit irreversibly bound to connective tissue. Ochronosis usually causes severe osteoarthropathy and heart complications, resulting in significant disability[1]. In the late decade, scientific research focused on the development of inhibitors of 4-Hydroxyphenylpiruvate dioxygenase (4-HPPD), the enzyme-producing HGA. The story of HPPD inhibitors began with the serendipitous discovery of natural herbicides produced by plants for defense, all having triketone structure[2,3]. Based on this inhibitory ability, a brilliant intuition arose to use Nitisinone, NTBC, a synthetic triketone, to treat type 1 Tyrosinemia and AKU. Since the administration of NTBC can result in severe side effects, as a consequence of increasing tyrosine concentrations caused by the treatment, the identification and development of new potential HPPD inhibitors is a priority to improve the treatment of AKU patients[4].

In this dramatic scenario, a screening of several analogs of NTBC has been performed, and their toxicological and pharmacokinetics profiles evaluated: cell toxicity, tyrosine accumulation, ADME.

Moreover, we provide an innovative integrated in vitro and in silico protocol to deeply explore the 4-HPPD/inhibitor binding, and define the residence time of our compounds.

Our study provides the determinations of triketones activity in human AKU cells and suggests the use of alternative compounds as promising scaffolds for developing new therapeutic strategies for the treatment of AKU.

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Categories

Proteins

Evaluation of peripheral compensatory mechanism in SARS-CoV-2 infection: 2,3-bisphosphoglycerate accumulation in RBCs of COVID-19 patients.

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Abstract

Angiotensin-converting enzyme 2 (ACE2) represents a key receptor on cell surfaces through which SARS-CoV-2 enters the host cells [1]. During SARS-CoV-2 infection, the ACE2 activity inhibition contributes to pulmonary injury and a hypoxemia state characterized by peripheral acid-base alteration [2]. Peripheral oxygenation is affected by several factors which alter the oxygen affinity for haemoglobin, such as the 2,3-Bisphosphoglycerate (2,3-BPG) concentration, which represents an activating compensatory mechanism. Herein, the current study aimed to evaluate the 2,3-BPG concentration in red blood cells of 75 COVID-19 patients and the variation of its levels during acid-base alterations. First of all, patients were divided into two groups according to the average partial arterial oxygen pressure value (pO2). The trends of 2,3-BPG and the lung damage parameter CALIPER ILD percentage obtained from TAC analysis were then evaluated. Subsequently, patients were divided into three groups (no-alkalosis, metabolic-alkalosis and respiratory-alkalosis) according to pH, partial arterial carbon dioxide and bicarbonate levels, and 2,3-BPG trends were assessed. Patient's lung injury inversely correlated with pO2 values (hypoxemia state); however, no correlation was found with parameters used for defining respiratory alkalosis (pH, pCO2 and bicarbonates). Moreover, patients with respiratory alkalosis showed higher 2,3-BPG levels and hospitalized later compared to the others.

Overall, these results highlight an increase of 2,3-BPG levels in COVID-19 patients with acid-base alterations, which could explain several episodes of silent hypoxia described in SARS-CoV-2 infection. (This research project is funded by Tuscany Region "Bando ricerca covid-19")

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Categories

Proteins

Enzymatic characterization of human succinic semialdehyde dehydrogenase, a NAD^+ dependent enzyme involved in a neurodevelopmental rare disorder

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Abstract

Glutamate plays a pivotal role in neurotransmission and its metabolism is finely tuned by converting it to γ-aminobutyric acid (GABA), which is sequentially oxidized at first to succinic semialdehyde and then to succinic acid by succinic semialdehyde dehydrogenase (SSADH), a mitochondrial NAD^+ dependent enzyme. Mutations in SSADH *ALDH5A1* gene cause SSADH deficiency, with toxic accumulation of GABA and its metabolites, causing a wide range of neurological and motor symptoms.

Surprisingly, human SSADH has not been characterized in an extensive manner regarding structure-function relationship. Here, we report preliminary data on characterization of recombinant wild-type (WT) human SSADH together with active site variants that are employed to dissect the enzymatic mechanism. The enzyme is a homotetrameric protein from 5 mg/ml to 0.1 mg/ml. Secondary structure determination, thermal stability at 222 nm, near UV circular dichroism, and intrinsic fluorescence reveal us that NAD^+ binds to the active site of SSADH forming a complex that causes a conformational change and renders the enzyme more thermally stable. The kinetic mechanism of the enzyme proceeds through a ternary complex (enzyme-NAD^+-SSA) of Bi-Bi ordered type with NAD^+ binding before the aldehyde. The measured catalytic parameters have been measured as a function of pH suggesting that a still not identified group with pKa of about 6.8 is responsible for catalysis. Some site-directed variants of residues proposed to be involved in catalysis have been cloned, expressed, purified and characterized. Results are interpreted in terms of enzymatic mechanism, useful to properly address structural and functional defects exhibited by pathogenic variants.

Traditional Cigarette Smoke Condensate induces phenotypic switch in smooth muscle cells: a Holistic Overview

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Abstract

Atherosclerosis is the leading cause of myocardial infarction and stroke and the major cause of death in the Western world. Among the environmental factors that may contribute to cardiovascular risk, incidence, and severity, cigarette smoking (CS) is one of the biggest threats to current and future world health. The CS condensate (CSC), from the particulate phase of the CS aerosol, contains lipophilic components that may pass the respiratory membranes and reach the blood stream, thus representing a cardiac and vessel-systemic risk factor.

Here we evaluated the effects of CSC on aortic smooth muscle cells (SMC) phenotypic switch by applying biochemical, transcriptional and differential-proteomic approaches. In SMC, CSC increased the expression of inflammatory markers (IL-1beta, *IL-6*, *IL-8*, *LGALS-3*) and reduced that of contractile ones (*ACTA2*, *CNN1*, *KLF4*, *MYOCD*). The LC-MS/MS analysis further reveled a CSC-induced deregulation of proteins active in signalling-pathways related to pro-inflammatory cytokine and IFN expression, inflammasome assembly and activation, cytoskeleton regulation and SMC-contraction, mitochondrial integrity and oxidative stress response, proteostasis control, cell proliferation, and epithelial-to-mesenchymal transition. Then, the bioinformatics functional-processing of the CSC-affected proteins indicated EIF2AK2/PKR as the main relevant factor involved in SMC phenotypic plasticity. The functional combination of gene expression and differential proteomics data successively led to the generation of a hybrid network where all the experimental CSC-affected factors were highly integrated under the direct control of KLF4. The hybrid network evidenced a tight functional cross-talk between PKR and KLF4, hence suggesting that CSC may orchestrate vascular SMC phenotypic plasticity through PKR and KLF4.

Bibliographic references

Categories

Proteins

Novel promising CSF biomarkers of Alzheimer's disease based on protein misfolding, protein aggregation and proteotoxicity

Alessandra Bigi[1], Giulia Fani[1], Valentina Bessi[2], Liliana Napolitano[1], Lorenzo Neri[1], Roberta Cascella[1], Paolo Matteini[3], Cristina Cecchi[1], Fabrizio Chiti[1]

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Abstract

Alzheimer's disease (AD) is a devastating neurodegenerative condition and the most common form of dementia. Its diagnosis is actually based on the use of various applications of neuroimaging and molecular biomarkers in the cerebrospinal fluid (CSF), including the low concentration of the $A\beta_{42}$ peptide and the high levels of total and phosphorylated tau protein. Despite the utility of currently available CSF biomarkers, we need to identify additional valuable biomarkers to strengthen our diagnostic accuracy of AD. Importantly, failed proteostasis is a cardinal feature of AD, leading to the abnormal aggregation of a great number of proteins, including $A\beta_{42}$ and tau, giving rise to an array of misfolded assemblies that increase, in turn, the propensity of many others to aggregate into neurotoxic misfolded species.

In this study, we compared CSF samples from non-AD and AD cases performing an array of biochemical, biophysical and cellular analyses.

We show that CSF samples from non-AD and AD subjects are distinguishable for important parameters, including the average tryptophan exposure of the proteome, indicating protein misfolding, the presence of large protein species, indicating protein aggregation, and the presence of species prompting the destabilization of the neuronal membrane of cultured cells, evoking an influx of calcium ions from the extracellular space to the cytosol, indicating neurotoxicity. Scatter plots produced by plotting two of these parameters, following an approach commonly used for identifying biomarkers in the AD field, provide well defined separations between non-AD and AD cases, indicating that they may represent valuable biomarkers of AD.

Bibliographic references

Alessandra Bigi and Giulia Fani contributed equally to the work.

Categories

Proteins

Identification of a hypofunctional phosphoserine phosphatase variant in the brain of Alzheimer's disease patients

Valeria Buoli Comani[1], Francesco Marchesani[1], Ivan Arisi[2,3], Alessio Peracchi[4], Stefano Bruno[1], Andrea Mozzarelli[5], Loredano Pollegioni[6], Barbara Campanini[1]

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Abstract

In human astrocytes phosphoserine phosphatase (PSP) catalyzes the removal of the phosphoryl group of 3-phosphoserine, the last step of the de novo biosynthesis of L-Ser. Defects in PSP are associated with low levels of L-Ser in the cerebrospinal fluid and serious neurological disorders.[1,2]

The aim of the work was the identification and characterization of single nucleotide polymorphisms (SNPs) of PSP in hippocampus samples of Alzheimer's disease (AD) patients. The SNPs were identified by Targeted Genotyping by NGS on post-mortem brain samples of AD subjects (6 females/5 males) and age-matched controls (5 females/5 males) obtained from the London Neurodegenerative Diseases Brain Bank.[3] Two point mutations (chr7:56088825 T>A and chr7:56088811 T>C coding for R27S PSP and D32G PSP) were identified in brains from AD patients (three females and one male), only. These two variants are reported by gnomAD to form a haplotype with total allele frequency of 0.06, with higher frequencies in African/African American and East Asian populations.

We expressed in *E. coli* and purified the single and double variants and characterized their functional properties and stability. The R27S/D32G variant shows a 40-fold reduction in catalytic efficiency and a 2-fold increase in the IC_{50} for L-Ser, that are mostly accounted for by the D32G substitution. The R27S/D32G substitution does not affect protein stability.

We conclude that the reduced activity of the PSP variant, in heterozygotes, is unlikely to severely affect L-Ser concentration in CNS but might have more subtle effects, possibly influencing AD development and progression.

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Categories Proteins

Identification of a hypofunctional phosphoserine phosphatase variant in the brain of Alzheimer's disease patients

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Abstract

In human astrocytes phosphoserine phosphatase (PSP) catalyzes the removal of the phosphoryl group of 3-phosphoserine, the last step of the de novo biosynthesis of L-Ser. Defects in PSP are associated with low levels of L-Ser in the cerebrospinal fluid and serious neurological disorders.[1,2]

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This work was supported by PRIN 2017 2017H4J3AS "Dissecting serine metabolism in the brain".

Categories Proteins

Biochemical characterization of bacterial Tdm for the detection of TMAO in solution produced by human FMO3

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Abstract

Trimethylamine n-oxide (TMAO) is currently being considered as a potential biomarker for predicting cardio-metabolic and chronic kidney diseases. While it is known that in humans TMA is oxidized to trimethylamine n-oxide (TMAO) by flavin-containing monooxygenase 3 (hFMO3) in the liver, a clear reason for having higher circulating levels of TMAO is yet to be established. Nevertheless, the measurement of TMAO is often hampered by the absence of a quick assay to quantify the amount the metabolite produced by the monooxygenation reaction. In order to bypass this problem in this work bacterial trimethylamine N-oxide (TMAO) demethylase, Tdm, was characterized biochemically. Indeed it was previously reported that bacterial Tdm uses TMAO as substrate and carries out a demethylation reaction resulting in the formation of dimethylamine and formaldehyde that can be furtherly detected and quantified using formaldehyde dehydrogenase (FALDH). A 3d-model generated by Alphafold reveals the presence of 2 distinct domains in the structure of Tdm. The enzyme was expressed in E.coli and purified to a high degree of purity. Differential scanning calorimetry studies of Tdm in the absence and presence of physiological ligands confirms the presence of two domains that fold independently. Ligand binding thermodynamics was tested by isothermal titration calorimetry. Calorimetric data were integrated with temperature and pH dependent activity assays leading to the identification of the optimal conditions to exploit the bacterial enzyme as a biosensor of TMAO.The final outcome of the work integrates 3 enzymes in a cascade reaction: hFMO3, TdM and FALDH to quantify TMAO in solution.

Categories

Proteins

In silico and in vitro investigation of interactions between active compounds and target proteins

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Abstract

The study of how compounds with potential pharmacological actions interacting with target proteins is one of the most exciting topics in medical science. The use of mass spectrometry techniques applied to isolated biomolecules and/or complexes, in particular, can provide structural information. The current project developed in collaboration with Dompé Farmaceutici S.p.A., is devoted to studying the interaction between proteins and drugs, mainly by using proteomics and mass spectrometry-based approaches.

In particular, one of the main focuses, funded by the project Exscalate 4Cov, concerned the investigation of the interactions of the main protease involved in SARS-CoV-2 replication, 3CLpro (also known as M-pro) with molecules showing potential inhibitory activity. 3CLpro, a cysteine protease involved in the proteolytic maturation of SARS-CoV-2 polyprotein, is known to be catalytically active as homodimer. Therefore, numerous pharmacological anti-Covid19 drugs have been developed with the goal of inhibiting its catalytic activity, by affecting its quaternary structure and/or covalently modifying the catalytic cysteine residue. In this field, the elucidation of the inhibitory mechanism and the mapping of the interacting regions are the main goals of this research activity for development of drug design or repurposing.

Categories

Proteins

Pharmacological activation of the HIF-1α pathway regulates satellite cells' fate in adult mice through histone lactylation

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Abstract

Sarcopenia is a multifactorial disease characterized by progressive loss of skeletal muscle mass and function during aging. It is associated with decreased muscle satellite cells (SCs) and impaired skeletal muscle regeneration, especially in muscle fibers with glycolytic metabolism. In this context, hypoxia-inducible factor-1α (HIF-1α) plays an essential role in the cellular response to oxygen levels by determining the switching of the glucose pathway from oxidative phosphorylation to glycolysis. In this context, we have previously reported that the HIF-1α pathway is strongly downregulated in human skeletal muscle biopsies from sarcopenic patients.

This study aims to determine the role of pharmacological activation of HIF-1α, using the prolyl-hydroxylase inhibitor FG-4592, on the fate of mice SCs during sarcopenia and establish whether this treatment can counteract the switch of SCs metabolism to glycolysis. The results showed that treatment with FG-4592 promotes an increase in lactate production associated with the activation of anaerobic glycolysis. The main effect of this metabolic change is a decrease in the proliferation rate of treated SCs, which was induced by an epigenetic modification in chromatin. The results showed that lactate can act as a second messenger that promotes histone lactylation, thereby altering gene expression and the fate of SCs. Specifically, treatment with FG-4592 stimulates the expression of PAX7, the main marker of SCs.

In conclusion, these results support the notion that pharmacological activation of HIF-1α may counteract the development of sarcopenia by increasing the self-renewal of SCs, which in turn may activate muscle regeneration.

Categories

Proteins

AMYLOTHROMBOSIS AS A NOVEL PATHOGENIC MECHANISM OF THROMBOTIC COMPLICATIONS IN AMYLOIDOSIS: THE CASE OF TRANSTHYRETIN (HTTR)

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Abstract

Background:hTTR amyloidosis is characterized by amyloid deposition in several organs, especially in heart chambers.It is associated with cardiomyopathy and heart failure, intracardiac thrombosis, with a prevalence of 1–3% in elderly people >75 years of age. However, the biochemical pathways leading to intracardiac thrombosis are unknown.

Aims:Demonstrate the ability of amyloid fibrils to induce blood coagulation, and establish the molecular mechanisms resulting in the activation of the coagulation cascade.

Methods:Natural fibrils were isolated from autopsies of patients with SSA.Full-length hTTR and hTTR(49-127) were obtained as recombinant species in E.coli, and allowed to fibrillate.Fibrils were characterized by thioflavin-T, DLS, TEM, then added to plasma or blood, monitoring fibrin generation by turbidimetry,thrombin generation, thromboelastometry.Role of coagulation factors was established by incubating fibrils either with factor-depleted plasma, or with isolated zymogens.Binding of coagulation factors to fibrils was quantified by ELISA.Histopathological images of cardiac amyloid infiltrations were from endomyocardial biopsies of patients with thrombosis in ATTR-CM.

Results:Histopathological investigation reveals exposure of amyloid fibrils in the heart chambers and localization of a small intracardiac thrombus nearby.Data obtained by assays on plasma and blood indicate that hTTR fibrils induce clotting, while hTTR in a non-fibrillar state does not.Fibrils induce autoactivation of factor XII,and increase efficiency of prothrombin activation by FXa of 40 times.

Conclusions:Amyloid fibrils could represent an ordered surface on which coagulation factors can anchor and become activated.These mechanisms explain the hypercoagulable state that lead to the formation of thrombi observed in patients affected by cardiac amyloidosis.

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Categories

Proteins

Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS): an Emerging Tool in Structural Biology

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Abstract

Background

HDX-MS is a powerful tool for investigating protein conformation/dynamics and ligand-protein interactions, at a spatial resolution of 3-6 amino acids and with tiny sample amounts (20-100µg). HDX-MS is based on the notion that peptide backbone amide hydrogens at exposed/flexible sites exchange much more rapidly with deuterium in D_2O than those that are buried in the protein interior or at ligand-receptor interfaces.

Methods

Measurements were run on a Xevo G-2S Q-TOF mass spectrometer (Waters), connected to an Acquity-M UPLC and an Automation 2.0 robotic platform. To the best of our knowledge, this is the first automated HDX-MS system present in an academic institution in Italy.

Results

Due to space limitations, here we report the macromolecular systems studied by HDX-MS in our laboratory (to be published). Each system will be more widely described in the Poster/Oral Communication that will be (hopefully) presented:

- Elucidating the effect of pathogenic mutations in human transthyretin on the susceptibility to proteolysis and generation of amyloidogenic fragments.
- Identification of perfluoroalkyl substances binding site in human transthyretin.
- Analysis of the conformational metamorphosis of Prothrombin zymogen after proteolytic activation to mature α-Thrombin in blood coagulation.
- Molecular mapping of effector binding proteins to α-Thrombin.
- Identification of the binding site of novel inhibitors of SARS-Cov-2 Main Protease.
- Conformational analysis of wild-type Protein Disulphide Isomerase and its pathogenic mutants.
- Identification of membrane binding regions in coagulation factor zymogens and amyloidogenic proteins.

Conclusion

These results put forward HDX-MS as a promising tool in structural biology, drug discovery and molecular medicine.

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Categories

Proteins

Krabbe disease carriers: emerging evidence for an increased risk in neurodegeneration

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Abstract

Krabbe disease (KD) (#245200) is a rare autosomal recessive disorder caused by mutations in the galactocerebrosidase gene (*GALC*). Defective GALC leads to aberrant metabolism of galactolipids, present almost exclusively in myelin, with consequent demyelination and neurodegeneration of the central and peripheral nervous system (NS).

Despite the very low incidence of KD (1:100.000), heterozygous carriers are estimated to be really frequent in Caucasian (1:150) and are suggested having a risk factor in neurological disorder onset higher than $GALC^{+/+}$ subjects, although they are not clinically ill. In order to identify biochemical defects that may increase the probability KD carriers develop NS affections, we took advantage of the KD murine-model twitcher (Twi).

We performed a proteomic differential analysis on whole brains from 33-old day Twi ($galc^{-/-}$), heterozygous (Het) ($galc^{+/-}$), and wild-type ($galc^{+/+}$) mice. At this age, the Twi mouse displays a severe phenotype but is still alive, while no defects are evident in Hets. Our data and their predictive functional processing highlighted the Het mice to differ from both Twi and wild-type animals by showing deregulation of several multifunctional factors, e.g. vimentin, RACK1 (P68040), MBP (P04370), CNP (P16330), VCP (Q01853), and NDRG1 (Q62433). These proteins are involved in (mechano)signaling, intracellular trafficking, proteostasis, lipid metabolism, mitochondrial failure, and energy supply balancing. Since they are critical in NS homeostasis and function, defects in such processes are likely to make KD carriers more vulnerable to other risk factors, even environmental or genetic, thus exposing them to a greater likelihood of developing neuropathies.

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Categories

Proteins

A potential and dynamic active Gal-3 inhibitor DHI derivative

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Abstract

Galectins, a family of lectin proteins, bind glycans containing disaccharides with β-galactoside bonds, such as N-acetyl-lactosamine. Gal-3 represents the most peculiar member of this family, combining its C-terminal Carbohydrate Recognition Domain with a non-lectin N-terminal part, responsible of its oligomerization. It has been reported an increased concentration of Gal-3 in different cancers, proposing this protein as an important therapeutic target[1]. Actually, there are several galectin inhibitors that show interesting functional profiles[2]. Melanins, a well-known class of insoluble dark pigments, based on their significant role in photoprotection and in other cell processes that ensure homeostasis, together with their high biocompatibility, represent ideal candidates for biomedical applications. Thus, starting from 5,6-dihydroxyindole (DHI), a key intermediate in eumelanin biogenesis, it was synthetised its thioglycosylated derivative, here proposed as new potential galectin inhibitors. The functionalisation of the DHI results in increasing the solubility of the starting molecule, as previous studied[3]. This outcome, together with the natural attitude of DHI to undergo on spontaneous oxidative polymerization, represents the base to produce a biocompatible scaffold exposing multiple glycans. The property to spontaneously form polymers, characterized by high degree of complexity, renders this DHI derivative a dynamically active inhibitor. Therefore, we here report a detailed binding study between recombinant Gal-3 and thioglycosylated DHI, both as monomer and polymer; this analysis was carried out using different techniques, as dynamic light scattering, isothermal titration calorimetry and bio-layer interferometry. These collected results could represent a new emerging way to drive the rational design for dynamic Gal-3 inhibitor.

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Categories

Proteins

Correlation between iron dyshomeostasis and 5-lipoxygenase post-transductional activation

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Abstract

5-lipoxygenase (5-LOX) is a non-heme iron-containing dioxygenase mainly expressed in immune cells and responsible for the synthesis of leukotrienes, bioactive lipids involved in numerous inflammatory states. We recently reported that iron content modulates 5-LOX intracellular localization in macrophages by increasing the ability of the enzyme to bind to nuclear membranes, thus activating the 5-LOX-mediated inflammatory processes. Dysregulated levels of iron-related protein are associated with various pathological conditions including cancer and pulmonary disease. Interestingly, the "cytokine storm" that occur during COVID involves the activation cascade of auto-amplifying cytokines, some of them directly affected by iron dyshomeostasis. This knowledge has led the investigation of the 5-LOX activation due to iron altered levels in the overlapping outcomes of cancer inflammation with pulmonary virus-induced inflammation. We analyzed the alteration of proteins related to iron metabolism induced by SARS-CoV-2 in patients' immune cells and plasma. To characterize the post-acute infection phase, we extended our analysis to long-COVID patients with persistent symptoms after acute infection. Overall, our findings suggest that iron dyshomeostasis occurs during COVID-19 and causes an increase in the oxidative stress and the hemolytic process, which in turn can increase free iron and heme levels, as well as cellular iron overloading and the post-translational activation of 5-LOX.

Categories

Proteins

Comparison of heme extraction from human hemoglobin by *Staphylococcus aureus* hemophores IsdB and IsdH

Valeria Buoli Comani[1], Monica Cozzi[2], Omar De Bei[2], Francesco Marchesani[2], Marialaura Marchetti[2], Luca Ronda[2,3], Serena Faggiano[1,3], Barbara Campanini[1], Stefano Bettati[2,3]

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Abstract

Staphylococcus aureus is one of the six highly virulent and antibiotic-resistant bacteria forming the so-called "ESKAPE" group, which represents a global threat for public health. This Gram-positive bacterium requires iron for host invasion and infection and exploits different mechanisms for iron acquisition, including siderophores and hemophores[1,2,3]. Hemoglobin (Hb) is the main iron source and heme iron is acquired from cell-free Hb by an iron-responsive surface determinant (Isd) system, able to extract heme, degrade it and release iron into the microorganism.

The first step of heme capture by *S. aureus* Isd system is carried out by two surface proteins, IsdB and IsdH. IsdB can only extract heme from Hb, while IsdH can capture it from both free Hb and Hb-haptoglobin complex. IsdB seems to be necessary for the virulence and proliferation while IsdH does not appear to be overexpressed during host infection. It is still not fully clear which is the functional difference between these two hemophores, given that they exhibit 85% sequence identity and have a similar role in heme extraction.

In order to decipher the functional significance of the presence of two different surface hemophores in *S. aureus*, we have performed a comparative characterization of Hb binding, heme extraction kinetics and heme oxidation rates for IsdB and IsdH. Furthermore, we have adapted an ELISA platform for the validation of inhibitors of IsdB/IsdH:Hb interactions as potential antibacterial drugs.

This work was supported by PRIN 2020AE3LTA "Defeat antimicrobial resistance through iron starvation in *Staphylococcus aureus* (ERASE)".

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Categories

Effects of Non-Immunosuppressant Cyclosporin A Analogues on *Toxoplasma gondii* Cyclophilins

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Abstract

Cyclophilins (Cyps) are a class of ubiquitous enzymes that catalyze the *cis-trans* isomerization of prolyl bonds in peptides and proteins. They were firstly identified as the molecular targets of the drug cyclosporin A (CsA), a cyclic undecapeptide used as immunosuppressant in the field of organ transplantation [1].

It has been shown that CsA possesses a significant parasiticidal activity against many parasite species, including *Toxoplasma gondii*, the causative agent of toxoplasmosis [2]. Despite its parasiticidal activity has not been fully understood, parasite Cyps are obvious drug targets and offer an attractive therapeutic approach to combat parasitic infections.

We previously characterized two *T. gondii* Cyps (TgCyps), named TgCyp23 and TgCyp18.4, at a structural and functional level, as well as their interaction with CsA [3]. Herein, we describe the interaction of TgCyp23 with two non-immunosuppressant analogues of CsA, namely NIM811 and dihydrocyclosporin A. By employing biophysical and biochemical methodologies, including spectrophotometric assays, Isothermal Titration Calorimetry (ITC), Nuclear Magnetic Resonance spectroscopy (NMR) and X-ray crystallography, we successfully characterized the interaction at an atomic level. Our findings revealed that TgCyp23 binds both compounds with a nM affinity and that the residues involved in the interaction overlap with those responsible for the binding of CsA. Moreover, both compounds inhibit TgCyp23 enzymatic activity. Our results demonstrate that CsA-based inhibitors hold significant potential as effective alternatives for tackling toxoplasmosis and pave the way for the development of novel therapeutic strategies to address parasitic diseases.

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Categories

Proteins

Influence of Tylotoin peptide on human skeletal muscle degeneration in an *in vitro* model of sarcopenia

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Abstract

The decline of skeletal muscle mass and strength that leads to sarcopenia is a pathology that might represent an emergency healthcare issue in future years.

Sarcopenia is a multifactorial process, characterized by inflammation, oxidative stress, motor neuron loss, a change in endocrine function and age-related loss of muscle mass and function, due to an increase in muscle protein degradation and reduced protein synthesis. In sarcopenic muscle, a reduction in the number of myofibers and hypotrophic myofibers, as well as infiltration into adipose and fibrotic tissue has also been observed.

Tylotoin, a peptide of 12 amino acid residues (KCVRQNNKRVCK), extracted from the skin of the salamander Tylototriton verrucosus, plays a key role in increasing cell motility and proliferation; however, its role in skeletal muscle function is unknown.

We investigated the potential role of Tylotoin on an experimental model of sarcopenia.

Human skeletal muscle myoblasts (HSMM) were differentiated in myotubes, and sarcopenia was induced by dexamethasone (DEXA) treatment. Differentiation and sarcopenia were evaluated by both real-time PCR and immunofluorescent techniques.

Data show that myosin heavy chain 2 (MYH2), troponin T (TNNT1), and miogenin (MYOG) were expressed in differentiated myotubes. Tylotoin significantly reduced muscle atrophy induced by DEXA.

These preliminary findings identify Tylotoin as a regulator of skeletal muscle degeneration. More data are necessary to investigate this results.

Categories

Proteins

The AlphaFold and crystal structures of human hydroxyproline dehydratase reveal a regulatory catalytic mechanism

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Abstract

We report an integrated structural investigation that combines the AlphaFold and crystal structures of human *trans*-3-Hydroxy-l-proline dehydratase, an enzyme involved in hydroxyproline catabolism and whose structure had never been reported before, identifying a structural element, absent in the AlphaFold model but present in the crystal structure, which was subsequently proved to be functionally relevant. Although the AlphaFold model lacked information on protein oligomerization, the native dimer was reconstructed using template-based and *ab initio* computational approaches. Moreover, molecular phasing of the diffraction data using the AlphaFold model resulted in dimer reconstruction and straightforward structure solution. Our work adds to the integration of AlphaFold with experimental structural and functional data for protein analysis, crystallographic phasing, and structure solution.

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Eugenio Ferrario, Riccardo Miggiano, Menico Rizzi, Davide M. Ferraris,

The integration of AlphaFold-predicted and crystal structures of human trans-3-hydroxy-l-proline dehydratase reveals a regulatory catalytic mechanism,

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Categories

Proteins

Biochemical and Bioinformatic Studies of Mutations of Residues at the Monomer-Monomer Interface of Human Ornithine Aminotransferase Leading to Gyrate Atrophy of Choroid and Retina

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Abstract

Deficit of human ornithine aminotransferase (hOAT), a mitochondrial pyridoxal-5'-phosphate (PLP) enzyme, leads to gyrate atrophy of the choroid and retina (GA). Although 70 pathogenic mutations have been identified, only few enzymatic phenotypes are known (1). Unlike the majority of the PLP-dependent enzymes, the dimeric units of human OAT are assembled in a homotetrameric structure (2). Here, we report biochemical and bioinformatic analyses of the G51D, G121D, R154L, Y158S, T181M, and P199Q pathogenic variants involving residues located at the monomer–monomer interface. All mutations cause a shift toward a dimeric structure, and changes in tertiary structure, thermal stability, and PLP microenvironment. The impact on these features is less pronounced for the mutations of Gly51 and Gly121 mapping to the N-terminal segment of the enzyme than those of Arg154, Tyr158, Thr181, and Pro199 belonging to the large domain. These data, together with the predicted ΔΔG values of monomer–monomer binding for the variants, suggest that the proper monomer–monomer interactions seem to be correlated with the thermal stability, the PLP binding site and the tetrameric structure of hOAT. The different impact of these mutations on the catalytic activity was also reported and discussed on the basis of the computational information. Together, these results allow the identification of the molecular defects of these variants, thus extending the knowledge of enzymatic phenotypes of GA patients.

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Categories

Proteins

Targeting human aldehyde dehydrogenase 1A3 for new drugs and diagnostics tools development against solid tumours

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Abstract

ALDHs activity correlates with poor outcome for solid tumours, sustaining cell proliferation and chemoresistance of CSCs. Accordingly, potent and selective inhibitors of ALDH enzymes may represent a novel CSC-directed treatment paradigm for $ALDH^+$ cancer types. Isoenzyme ALDH1A3 belongs to the enzymatic superfamily of 19 different isoforms involved in the oxidation of many aldehydes to the respective carboxylic acids, through a $NAD(P)^+$-dependent reactions. We focused on two different types of tumour that have low survival rate at five years from diagnosis. Glioblastoma, that is the most aggressive primary brain tumour, and malignant pleural mesothelioma (MPM), for both of which effective treatments and efficient tools for early-stage diagnosis are lacking. Thanks to the rational structural analysis of human ALDH1A3 model, we identified potent and selective inhibitors towards ALDH1A3[1-5]. Our best selected compound, NR6, hinders Cancer Cell Growth, Invasiveness and Stemness[6]. We synthesized a curcumin-based fluorescent molecule, Probe10, that illuminates CSCs thanks to its selective ALDH1A3 binding. In-vivo, Probe10 selectively accumulates in glioblastoma cells, allowing for the identification of the growing tumour mass[7]. We treated MPM spheroids with NR6, revealing an accumulation of toxic aldehydes, induced DNA damage, CDKN2A expression and cell growth arrest skewed cell fate from senescence to apoptosis. NR6 induce IL6 expression, abolished CXCL8 expression and IL-8 release, preventing both neutrophil recruitment and generation of neutrophil extracellular traps[8]. Our results demonstrate that selective targeting of the ALDH1A3 enzyme is a promising approach for improving precise tumour diagnosis and treatments outcomes of patients affected by ALDH1A3-positive cancers.

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Categories

Proteins

Development of new dual VGFR2/MTA inhibitors for treatment of drug refractory/metastatic cancer forms

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Abstract

Although most cancer cells are sensitive to classical antineoplastic drugs, tumors often hide small populations of inherently resistant cells that are generally responsible for tumor relapse. These cells can avoid drug-induced apoptosis through various mechanisms and continue to proliferate regardless of the antitumor therapeutic protocol adopted. Furthermore, most resistant cancer cells are able to evade attacks from immune system cells and spread to healthy tissues, promoting metastatic dissemination.

Activation of VEGFR2, which stimulates the formation of new intratumoral vessels, is considered an essential process for promoting metastatic dissemination. For this reason, VEGFR2 inhibitors are often used to treat metastatic forms of cancer in combination with microtubule-targeting agents (MTAs). These drugs exhibit different pharmacokinetics and can induce unsustainable side effects when administered at the same time. To overcome this problem, we propose the development of novel dual VEGFR2/microtubule inhibitors that are expected to possess similar cytotoxic activity but lower non-specific toxicity. The new molecules are engineered by linking some well- known MTAs to Axitinib, a potent and highly selective VEGFR2 inhibitor.

The obtained compounds were initially analyzed to evaluate their ability to induce cell cycle arrest and to evaluate their IC50 value on different types of melanoma cell lines. Preliminary results showed that some of the proposed compounds have the typical dual function behavior suggesting that this strategy has some potential for further anti- cancer multi-target drug development and in this moment, VEGFR2 activity assays are in progress.

Categories

Proteins

Cryo-EM-based structural investigation of Mycobacterium tuberculosis Nucleotide Excision Repair pathway.

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Abstract

During its entire life cycle, *Mycobacterium tuberculosis* (MTB) deals with toxic agents altering its genomic stability, mounting a DNA repair response in which the Nucleotide Excision Repair (NER) has a key role in counteracting the harmful potential of oxidation and alkylation damages.[1]

The first steps of NER refer to the UvrA and UvrB proteins, which are part of a multi-step pathway in which the dynamic assembling of protein complexes is required for the lesion sensing and removal activities.[2] UvrA is thought to scan the DNA searching for the damage, either alone or in complex with UvrB: interestingly, there are evidences in literature of the formation of UvrA-UvrB complex[3], but both the stoichiometry and the functional dynamics of the complex are still under debate.

We present here a Cryo-EM-based structural investigation of the UvrAUvrB complex and of the UvrA dimer, both in complex with damaged DNA. Our analyses reveal new insights in the DNA binding mode of UvrA as well as an alternative conformation of some crucial regions involved in DNA coordination. Moreover, at supramolecular level, we obtained a structural snapshot of the different oligomers (namely A_2B_1 and A_2B_2) which alternate during the early stages of damage recognition, shedding light on the scientific debate regarding the stoichiometry of the protein assemblies that lead the system to the formation of the UvrB-DNA pre-incision complex. Structural information on key events in MTB NER will drive the rational design of active molecules interfering with DNA repair pathway in MTB, possibly acting as “anti-evolution” drugs.

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Categories

Proteins

Structural insights into mutations of myelin protein zero linked to Charcot-Marie-Tooth disease.

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Abstract

Myelin protein zero (MPZ) is a transmembrane protein of Schwann cells whose role is to promote the formation of the multiple wraps of the myelin sheath in peripheral nervous system. Several mutations in MPZ are associated with the onset of Charcot-Marie-Tooth (CMT) disease, a set of demyelinating or dysmyelinating neuropathological disorders involving the degeneration of motor neurons and peripheral sensory neurons. The MPZ-dependent forms of CMT result from mutations mainly affecting its extracellular (EC) domain. The structural changes by which such substitutions cause the disease are not well understood. The present study aims to obtain a biophysical characterization of the wild-type EC domain and compare the results with those obtained on a set of disease-involved mutants, to highlight the structural changes that lead to CMT and map the hot-spots for possible misfolding events. We are currently analysing histidine-tagged EC domains by circular dichroism in the far-UV in order to assess the secondary structure of the different variants. Wild-type and mutant EC domains are being investigated by means of dynamic light scattering and thermal and chemical denaturation to study conformational stability and aggregation propensity. The role of myelin sheath adhesion is played by the EC domain via oligomerization between the domains themselves. In relation to this aspect, we are investigating the tendency of the EC domain to form tetrameric structures and how individual mutations affect adhesion properties. The results obtained in the present work will provide us with information on the etiopathogenesis of CMT concerning particularly the failure of myelin packing.

Categories

Proteins

STRUCTURAL CHARACTERIZATION OF ALPHA SYNUCLEIN FIBRILS FROM PATIENTS AFFECTED BY MSA AND PARKINSON'S DISEASE

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Abstract

Synucleinopathies, such as Multiple System Atrophy (MSA), Parkinson's Disease (PD), and Dementia with Lewy bodies (DLB), are neurodegenerative diseases characterized by the presence of abnormal protein aggregates of α-synuclein (SNCA) in brain cells. SNCA is a an intrinsically disordered protein, but it can adopt different structures when associated with synaptic vesicles, playing a role in vesicle trafficking. Mutations in the SNCA gene and post-translational modifications (PTMs) can affect the aggregation and toxicity of α-synuclein. Protease cleavage can also modulate α-synuclein aggregation. This study focused on characterizing α-synuclein fibrils extracted from the cerebellum and frontal lobe of MSA and Parkinson's patients using limited proteolysis coupled with mass spectrometry (MS) analysis. The samples were treated with proteinase K under controlled conditions, and the resulting fragments were analyzed in a mass mapping strategy. Different proteolytic patterns were observed, suggesting different conformations of α-synuclein in the initial fibrils depending on the brain region. MS analysis was used to identify the specific features of the released α-synuclein fragments. The gel electrophoresis profiles of the fibrils from the cerebellum and frontal lobe exhibited differences, indicating conformational variability of the protein depending on the brain district. The MS analysis confirmed the presence of various truncated forms, some of which were specific to certain brain regions. These findings support the idea that α-synuclein fibrils exhibit conformational polymorphism and provide insights into the prion-like nature of α-synuclein. Understanding the mechanisms leading to accelerated α-synuclein aggregation is crucial for developing diagnostic and therapeutic strategies for synucleinopathies.

Categories

Proteins

Catechol-induced covalent modifications modulate the aggregation tendency of α-synuclein: an *in-solution* and *in-silico* study

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Abstract

Parkinson's Disease (PD) is a neurodegenerative disorder characterized by the formation of neuronal cytoplasmic inclusions known as Lewy Bodies (LBs) in dopaminergic neurons. LBs contain many proteins including α-Synuclein (α-Syn) aggregates. Targeting α-Syn aggregation is a possible approach to combat PD.

Recently, we demonstrated that 3,4-dihydroxyphenylacetic acid (DOPAC) and 3,4-dihydroxyphenylethanol (DOPET) hinder the formation of α-Syn fibrils by interfering with the aggregation process. These compounds bind non-covalently the protein and induce the generation of off-pathway oligomers, harmless species that do not grow into fibrils.

Mass spectrometry (MS) analysis and proteolysis studies performed on α-Syn incubated in the presence of DOPAC suggest that the protein is also covalently modified in a specific region, but the exact involved residue is unclear. Molecular dynamics simulations were used to investigate how the DOPAC-induced covalent modification may affects α-Syn aggregation mechanism. Our results suggest that the presence of an adduct on the side chain of certain residues increases the fibril flexibility without inducing destabilization of its secondary structure. Further, in the monomeric form of the protein, the modified residue establishes novel bonds, promoting alteration in the long-range interactions occurring between the N- and C-termini of the protein.

Collectively, our data suggest that the presence of an adduct in this region of α-Syn may be responsible for an overall change in the entropy of the system.

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Categories

Proteins

TMEM65 controls mitochondrial activity through respiratory complex I assembly and calcium homeostasis

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Abstract

The transmembrane protein 65 (TMEM65) is an orphan protein localized within the inner mitochondrial membrane. Few years ago, a homozygous loss-of-function mutation in *TMEM65* was identified in a patient with a syndromic early-onset encephalomyopathy, with a clinical presentation resembling a mitochondrial disorder [1]. Knock down (KD) of TMEM65 expression in human fibroblasts was shown to severely affect mitochondrial content and respiration [1], but the exact mechanism remains elusive. Interestingly, by performing a quantitative proteomic screening we have found TMEM65 accumulated in cells lacking respiratory complex I (RCI), suggesting a possible role as assembly factor. Thus, in order to get a deeper understanding of the function of the protein we have analyzed the effects on mitochondrial function of both the downregulation and overexpression of TMEM65 in different human cell lines. On one hand, ablation of TMEM65 resulted in a mild oxidative phosphorylation deficiency, associated with lower amounts of fully assembled functional RCI and abnormal accumulation of low molecular weight subcomplexes, suggesting an assembly defect. On the other hand, we have compelling evidence showing that TMEM65 controls mitochondrial Ca^{2+} homeostasis, since its overexpression dramatically enhances mitochondrial Ca^{2+} efflux, while its silencing decreases organelle Ca^{2+} extrusion, thus causing a mitochondrial overload. Intriguingly, a direct relationship between the stability of RCI and the mitochondrial calcium uniporter (MCU) has been recently reported [2]. This would support a role for TMEM65 linking the regulation of RCI biogenesis and mitochondrial Ca^{2+} homeostasis in the fine-tuning of organelle bioenergetics, which we will continue to investigate.

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Categories

Proteins

PF-04691502, a PI3K/mTOR dual inhibitor, improves learning deficits in APP/PS1 mice.

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Abstract

Aging is the greatest risk factor for several neurodegenerative disorders, including Alzheimer's disease (AD). Overwhelming evidence indicates that reducing mTOR signaling improves health span and lifespan in a multitude of organisms. PI3K is a key regulator of mTOR activity; the PI3K/mTOR signaling pathway regulates several key biological mechanisms related to cell development, cell survival, protein synthesis, autophagy, metabolism, and learning and memory. To this end, up-regulation of the PI3K/mTOR signaling contributes to AD neuropathology and causes neurodegeneration and learning and memory deficits. In this study, we sought to determine the molecular correlates of memory deficits in APP/PS1 mice, a widely used animal model AD. 18-month-old APP/PS1 and WT mice were dosed orally with 1 mg/Kg PF-04691502, an ATP-competitive PI3K/mTOR dual inhibitor, for 12 weeks. At the end of the treatment, we assessed changes in spatial learning and memory using the Morris water maze. We then processed their brains for neuropathological and biochemical assessment of amyloid-β (Aβ). We found that PF-04691502 improved learning and memory in APP/PS1 mice. Currently, we are processing the tissue to assess potential changes in brain Aβ deposits and soluble and insoluble Aβ levels. We will also assess the effects of reducing PIK3/mTOR signaling on inflammation. These results provide preclinical data indicating that PF-04691502 may be a valid therapeutic approach for AD and other neurodegenerative disorders associated with aging and mTOR hyperactivity.

Categories

Proteins

A multi-layered approach for the identification of EIF2A as protein target of cannabidiolic acid in glioblastoma cell line

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Abstract

Phytocannabinoids have shown encouraging activities against tumoral cells. The present research was focused on the study of the mechanism of action of cannabidiolic acid (CBDA) in U87MG glioblastoma cell line by a multi-layered approach. First, by chemical-proteomics we identified the Eukaryotic Translation Initiation Factor 2A (EIF2A)[1] as the main protein target of CBDA. We validated this molecular interaction by CETSA assay, which showed that CBDA confers a thermal stabilization to EIF2A, and measured a KD of 6.3 mM for the EIF2A/CBDA by SPR. Moreover, using a combination of limited proteolysis experiments and molecular dynamics calculations we showed that CBDA engages stable interactions with a stretch of residues in the 460-480 portion of EIF2A and the adjacent C-terminal helix, acting as a bridge between them. Since EIF2A is an initiator factor of the minor-pathway of the translation process we investigated the impact of CBDA-EIF2A interaction on protein synthesis. We observed that treatment of U87MG with CBDA and EIF2A-siRNA produced similar remodeling of the nascent proteome, thus confirming EIF2A as a functional target of the cannabinoid. In particular, after treatment with CBDA, the synthesis of proteins involved in translation initiation and ER stress response was reduced. Notably, studying the EIF2A interactome, we observed that after CBDA binding the protein showed an increased affinity for the same proteins previously identified as under-expressed. Overall, these results suggested that CBDA interacting with EIF2A could reduce the bioavailability of proteins responsible for the protection of cancer cells from ER stress through a dual mechanism.

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Categories

Proteins

Deletion of VDAC1 in HAP1 cells affects mitochondrial respiration impacting on complex I activity

Cristiana Lucia Rita Lipari[1], Giuseppe Battiato[1], Salvatore A.M. Cubisino[1], Stefano Conti Nibali[2], Andrea Magrì[1], Vito De Pinto[1], Angela Messina[1]

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Abstract

Voltage-Dependent Anion-selective Channel isoform 1 (VDAC1) is the most expressed pore-forming protein of the outer mitochondrial membrane (OMM) in eukaryotes. VDAC1 promotes the communication between the cytosol and the mitochondrion, allowing the passive diffusion of ions, ATP/ADP, NAD+/NADH and small metabolites feeding Krebs' cycle. In this work, we investigated the impact of VDAC1 genetic deletion in near-haploid human cell line HAP1 by High-Resolution Respirometry (HRR). Our data indicate that VDAC1 knockout affects the overall profile of mitochondrial respiration: if, in one hand, a dramatic reduction of oxygen fluxes related to the main respiratory states and the respiratory reserves was expected, on the other hand, we noticed a significant increase in the specific contribution of complex I to the maximal capacity, a possible compensatory effect due to the absence of the main mitochondrial porin. In conclusion, albeit VDAC1 is not directly involved in oxidative phosphorylation, our findings highlight the importance of VDAC1 as a general regulator of the mitochondria functionality.

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Categories

Proteins

The importance of improving the FAIRness of structural data: a case report about spike analysis

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Abstract

During the COVID-19 pandemic event, the structural biology community has spent a tremendous effort in determining the structures of SARS-CoV-2 proteins. The attention of scientists has been particularly focused on spike protein, for which more than 3000 structures have been determined in less than 3 years.

Recently, we performed an in silico study to predict the influence of mutations of different SARS-CoV-2 variants on antibody binding to the spike protein. We encountered unexpected problems in automatizing these analyses because of the lack of standardization of the structural information contained in the PDB and PDBx/mmCIF files describing these structures. A first problem was related to the lack of standardization with which the name of the same structural entity is reported in the various files in which it is contained. Another issue concerned the lack of uniformity of chain identifiers, which are not homogeneous in the different entries with the same chains, and of the order in which they are reported. The difficulties that emerged during the analysis, due to the non-heterogeneity of the information in the files of the complexes, forced us to manually check each individual file before starting the automated analyses.

We acknowledge the important work of harmonization and data FAIRification made by the structural biology community; we hope that this case report will suggest further rules to better standardize the description of additional information in structural files.

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Categories

Proteins

Understanding the cross-talk between iron acquisition and energetic metabolism in *Staphylococcus aureus*

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Abstract

Staphylococcus aureus belongs to the so-called ESKAPE group of bacteria, able to develop resistance against most of the available antibiotics. *S. aureus* relies on iron to grow and induce infections, therefore the bacterium developed different strategies to acquire iron from the host. Staphyloferrin A and B are secreted carboxylate-type siderophores, able to chelate non-hemic iron and allow its internalization. The biosynthesis of staphyloferrin B relies on the expression of 9 enzymes belonging to the *sbn* gene cluster, which is regulated by intracellular concentration of both iron and heme[1]. During the last decade, a link between virulence and energetic metabolism in *S. aureus* has been proposed[2], with citrate playing a major role, being the precursor of both staphyloferrin A and B and also being able to regulate different enzymes of the staphyloferrin B biosynthetic pathway[3]. We recently characterized the kinetic mechanism of SbnA, a pyridoxal 5'-phosphate dependent enzyme which uses L-O-phosphoserine and L-glutamate to produce N-(1-amino-1-carboxy-2-ethyl)-glutamic acid (ACEGA), the first intermediate in staphyloferrin B biosynthesis. SbnA shows a ping-pong catalytic mechanism with substrate inhibition by L-glutamate. Citrate modulates the activity of SbnA through a mixed-type inhibition mechanism with an IC_{50} close to its cellular concentration (1 mM), suggesting a physiologically relevant role in the modulation of staphyloferrin B biosynthesis. We plan to investigate the effect of citrate also on other enzymes of the *sbn* gene cluster, in order to gain insight into the cross-talk between metabolism and iron acquisition in *S. aureus* and validate new targets for antimicrobial therapy.

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Categories

Proteins

Influence of female sex hormones on salivary protein secretion and brain-heart interplay in test anxiety

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Abstract

Social anxiety is a psychological disorder derived from the fear of being judged by others that leads to a strong state of stress. The physiological response to an altered emotional state, due to a stressful source, occurs mainly through the involvement of the endocrine, nervous and immune systems. In particular, the hypothalamic-pituitary-gonadal and hypothalamus-pituitary- adrenal axis are involved.

This study aims to investigate the modulation, by female sex hormones, of salivary protein secretion and brain-heart interplay during an acute psychological stress. Twenty-four healthy females recruited either in the pre-ovulatory or post-ovulatory phase of the menstrual cycle, characterized, respectively, by low and medium-high levels of estradiol and progesterone, participated to a test anxiety task. The task simulated an oral exam and consisted of 3 phases: relaxation, study of a written text, and oral exposition in front of a “professor” of the studied text. During the test, 4 saliva samples were collected: a basal sample (T1) before starting the test, and samples were collected after the relaxation time (T2), the exam simulation (T3), and 20 minutes after the exam end (T4). To monitor anxiety perception participants were required to fill out questionnaires during the various periods. Comparative proteomic analysis of saliva samples was performed by 2DE/MS. Significant differences of protein expression was observed at different times both in pre-Ovulatory and post-Ovulatory groups suggesting a different response to acute stress linked to sex hormone levels. Changes of expression, validated by western blot analysis, were observed in post-Ovulatory for alfa-amylase, IgA, profilin and prolactin.

Categories

Proteins

Structural and functional effect of hPHGDH pathological SNPs on L-serine synthesis by the phosphorylated pathway

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Abstract

In the brain, the phosphorylated pathway (PP) is responsible for the synthesis of L-serine from the glycolytic intermediate 3-phosphoglycerate: it plays a major role in the metabolism of eukaryotic cells and in the development and function of the central nervous system. Moreover, L-serine is converted into D-serine and glycine, the two co-agonists of NMDA receptors [1]. In humans, the enzymes of the PP, namely phosphoglycerate dehydrogenase (hPHGDH), phosphoserine aminotransferase (PSAT), and phosphoserine phosphatase (PSP) are organized in the cytosol as a metabolic assembly, the "serinosome" [2]. hPHGDH deficiency is a pathological condition characterized by reduced L-serine levels in plasma and cerebrospinal fluid and severe neurological impairment [1].

Here, three hPHGDH variants corresponding to known SNPs (V261M, V425M and V490M) have been produced in E. coli and ectopically expressed in U251 cells with the aim to clarify the effect of the substitutions on the structure-function relationships of the enzyme, its cellular distribution, and the PP functionality. All the substitutions deeply altered hPHGDH secondary, tertiary, and quaternary structure resulting in a significantly reduced protein solubility, stability, and activity. At the cellular level, the variants were partially mistargeted to the nucleus and induced the formation of aggregates containing PSAT and PSP too. Moreover, the expression of the pathological variants led to a decrease in L-serine and glycine cellular concentrations.

Our results suggest alternative therapeutic approaches, in addition to the commonly used L-serine supplementation, for the treatment of hPHGDH deficiency-affected patients.

This project was founded by ”PRIN-2017 - Dissecting serine metabolism in the brain”.

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Categories

Proteins

Modelling the hyperexcitability preceding Alzheimer's disease by treating neuronal cells with sub-threshold concentrations of amyloid-β oligomers and glutamate

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Abstract

Alzheimer's disease (AD) is the most common form of dementia in the elderly. This condition is strictly related to the aberrant deposition of amyloid-β (Aβ) peptide aggregates in the extracellular space of the central nervous system resulting in cognitive impairment. Aβ oligomers are able to induce the activation of mechanosensitive N-methyl-D-aspartate (NMDA) receptors and elicit neuronal pre-synaptic and astrocytic release of glutamate, the main neurotransmitter and NMDA receptor agonist, leading to Ca2+ ions influx and neuronal hyperexcitability that has recently been suggested as a potential very early indicator of AD. Indeed, several observations on animal models of AD and familial cases of the disease indicate hyperexcitability as an early feature in AD which manifests even before the formation of amyloid plaques occurring in the preclinical phase of the disease. In this study, we focused on this very early stage of AD, mimicking it by treating SH-SY5Y neuroblastoma cells with sub-threshold Aβ oligomers and glutamate concentrations, at which Ca2+ ion entry is not observed. Using Ca2+ pump inhibitors its influx becomes significant, indicating an active influx effectively faced by the pumps. The treatment with sub-threshold concentrations did not cause cell loss and downregulation of membrane NMDA receptors, but caused an enhancement of ROS production determined with confocal scanner microscopy. All together, these results suggest the use of our experimental approach as a good model system to study the very early stage of AD and how neuronal cells may behave in response to this condition of hyperexcitability.

Categories

Proteins

THE ROLE OF SIALIC ACID IN THE BIOLOGICAL ACTIVITY OF LACTOFERRIN

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Abstract

Bovine lactoferrin (bLf), a milk-derivative glycoprotein, is constituted by 689 amino acids and folds into two distinct lobes, each bearing a high affinity iron-binding site. BLf exerts a number of biological functions, including antiviral, antioxidant and anti-inflammatory activities. Five N-linked glycosylation sites are present on bLf but only four sites are invariably glycated. BLf possesses two oligomannosidic type and three biantennary N-acetyllactosamine type glycans, partially fucosylated and sialylated. Sialic acid, usually present in a ratio of about three residues per molecule, could affect the functionality and bioavailability of bLf, however, little efforts have been made so far to unravel its influence on the various functions of the glycoprotein.

Here, the role of sialic acid on the biological activity of bLf was investigated. For this purpose, a desialylated version of bLf was produced and tested for its antioxidant, anti-inflammatory and antiviral activities. Antioxidant and anti-inflammatory activities were tested in phorbol-12-myristate 13-acetate (PMA)-challenged human leukemic cell line (THP-1), whereas the antiviral activity was evaluated in human bronchial epithelial cells (16HBE14o-) by using a Spike-decorated pseudovirus.

The data show that the effects of bLf on cell antioxidant response and on expression of pro-inflammatory cytokines in PMA-treated THP-1 cells are impaired when bLf is desialylated. Moreover, desiaylated bLf was less efficient than the native form in inhibiting pseudoviral cell fusion, thus suggesting a crucial role for sialic acid in the multifunctionality of the glycoprotein.

Categories

Proteins

A multi-omics integrated analysis of serine metabolism in astrocytes differentiation and in Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is a neurodegenerative disorder affecting different brain regions, in particular the hippocampus, an area that is critical for learning and memory. Multi-omics investigations of hippocampus samples from healthy controls and AD patients, showed profound differences between males and females in terms of up- and down-regulated metabolic pathways during aging and pathological conditions. The serine metabolism (generating the NMDA receptor coagonist D-serine) is significantly modulated, in keeping with previous findings showing that dysfunctions in serine metabolism are associated with neurological and psychiatric disorders (1).

Astrocytes are the major source of L-serine in the brain. To understand the cellular processes timely involved in the differentiation program and to clarify the potential of L-serine role in pathologies, we recently analyzed by an integrated multi-omics approach the profiles of NSC-derived human astrocytes at different times of differentiation. The results underline metabolic changes during astrocytes differentiation, highlight that D-serine synthesis is restricted in differentiated astrocytes, and provide a valuable model for developing potential novel therapeutic approaches to address selected brain diseases (2).

By exploiting all these multi-omics data, we are now focusing on their integration by means of clustering methods, such as Ingenuity Pathway Analysis IPA, to identify the most altered pathways related to serine variation, to uncover the possible upstream regulators and to find key drivers of pathogenesis. The final aim is to shed light on the role played by the serine metabolism in brain differentiation and in AD.

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Categories

Proteins

Evaluation of flavone C-glycoside vicenin-2 biological functions and potentialities.

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Abstract

Vicenin-2 (6,8-di-C-β-D –glucopyranosylapigenin) is a flavone C-glycoside characteristic of the genus Citrus (like fruits and juices), but also of the genus Ocimum, Perilla frutescens, Artemisia capillaries, Peperomia blanda, Potentilla discolor and several flowers [1-6]. It shows several interesting biological functions and recently it has been reported its identification in the blood [3,7]. The preliminary citotoxicity tests reveal a very good tolerability of the compounds to cells in culture, well above 150 mM. Starting from this evidence we performed an in deep analysis of its antioxidant activity, tested its binding ability to human serum albumin and evaluated its activity on selected cells line incubated with 0-100 mM of the compounds. Vicenin-2 does not show remarkable antioxidant activity in almost all the performed assays. It binds with high affinity to human serum albumin that spreads the compounds around the body. Vicenin-2 ability to activate/blocks signal cascade and key enzymes for metabolic changes of nutrients' flux make necessary a special attention to their biochemical characteristics for the modulation of pathways inside living organisms. The evaluation of lactate dehydrogenase release, caspase 3 activation, intracellular ROS detection, protein carbonylation and modulation of antioxidant enzymes let us to shed some light on its biological potential and the ability to avoid the deleterious effects of tert-butyl hydroperoxide on mononuclear cells. Moreover, it shows also the potentiality to avoid protein fibrillation and aggregation on human serum albumin heat treated. The obtained results highlighted potentials of vicenin-2 utilization and its implication in the modulation of specific metabolic pathways.

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Categories

Proteins

Molecular studies toward the comprehension of the role of the polyalanine expansion in the aggregation of PHOX2B: a significant contribution by NMR structural characterization

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Abstract

The detection in about 90% of patients with congenital central hypoventilation syndrome (CCHS) of poly-alanine triplet expansions in the coding region of the transcription factor PHOX2B makes this protein an intriguing target for understanding the onset of the syndrome and for designing a new therapeutic approach[1]. Therefore, our study primarily focused on the biochemical characterisation of PHOX2B variants containing the correct C-terminal tract (20 alanine), and one of the most frequent poly-alanine expansions (+7 alanine)[2]. Comparison of the different variants by means of a multidisciplinary approach revealed the aggregation propensity of the PHOX2B variant containing the poly-alanine expansion (+7 alanines) especially in the presence of DNA. Furthermore, and unexpectedly, fibril formation was only revealed for the pathological variant, suggesting a plausible role of such fibrils in the onset of CCHS. As structural information on PHOX2B is important to obtain clues to elucidate the onset of the alanine expansion-related syndrome and also to define a viable therapy, we focused on the structural characterisation by NMR spectroscopy of the homeodomain (HD) and the HD + C-terminus PHOX2B protein also in the presence of the target DNA. The structural model obtained of the PHOX2B-DNA interaction opens up an interesting scenario aimed at elucidating the basis of the onset of CCHS. This study paves the way for the rational design of therapeutic drugs, suggesting as a possible therapeutic route the use of specific anti-aggregating molecules capable of preventing the aggregation of the variant and possibly restoring the DNA-binding activity of PHOX2B.

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Categories

Proteins

Identification of selective AKR1B10 inhibitors in Zolfino bean extracts

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Abstract

AKR1B10 (also known as aldose reductase-like protein) is a NADPH-dependent reductase belonging to Aldo-Keto Reductase superfamily, involved in cell proliferation and differentiation, through its role in the homeostasis of retinoic acid1. Its overexpression in many tumours increases resistance to oxidative stress to cancer cells, due to its detoxifying ability on cytotoxic aldehydes derived from lipid peroxidation1. Due to its ability to reduce anthracyclines to their corresponding alcohols, AKR1B10 is involved in chemoresistance and in the onset of side effects of anthracycline anticancer therapy. Therefore, AKR1B10 is considered a target for antineoplastic treatments1. AKR1B10 shares kinetic and structural features with the ubiquitous AKR1B1, which plays a role in cell detoxification. Inhibitors that discriminate between AKR1B10 and AKR1B1 and minimize the interference with the detoxifying role of AKR1B1, may be relevant to develop novel treatment for cancer. Previous studies reported that Zolfino beans extracts are a natural source rich in AKR1B1 inhibitors2.

Here we report results on isolation and characterization of selective inhibitors of AKR1B10. The workflow includes fractionation of a crude extract on a C18 column, evaluation of inhibitory ability of the fractions, and LC-MS characterization of relevant sets of fractions. Correlation between results from LC-MS and kinetic analyses allowed identification of promising candidates for selective enzyme inhibition.

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Categories

Proteins

The emerging cardioprotective role of sialidase Neu3 against Ischemia and Reperfusion Injury

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Abstract

Coronary reperfusion techniques are life-saving approaches to restore blood flow to cardiac tissue after myocardial infarction. However, they also lead to the ischemic and reperfusion injury (IRI). Despite the urgent need for cardioprotective strategies against IRI, none has yet been introduced into clinical practice, suggesting that a more detailed characterization of the pathophysiology of IRI is essential. In this regard, our group has shown that the sialidase Neu3 is modulated during IRI in vivo and that its constitutive overexpression in rat cardiomyoblasts is sufficient to counteract the deleterious effects of IRI through activation of reperfusion injury salvage kinase (RISK) and the HIF-1α pathway.
In this study, we developed an inducible model of Neu3 overexpression in human cardiac cells and in mice to further elucidate the role of this protein in promoting cardioprotection.
Inducible upregulation of Neu3 significantly increased cell resistance and decreased oxidative stress in cells exposed to IRI in vitro. These beneficial effects were related to Neu3-mediated maintenance of mitochondrial membrane potential, which was altered in control cells.
We also generated for the first time αMHC-Cre/LSL-Neu3 mice by crossing the tamoxifen-inducible αMHC-Cre mouse with a mouse carrying a floxed-transcriptional STOP sequence upstream of Neu3. These mice were characterized for cardiac morphology and functionality and showed no significant differences compared with wild-type mice. They will undergo surgical induction of IRI to test the beneficial effects of Neu3.
Based on our results, Neu3 may hold therapeutic promise in increasing cardioprotection and reduce the incidence and severity of myocardial infarction.

Categories

Proteins

New pharmacological targets for the treatment of cardiovascular diseases, from hypertension to ischemia-reperfusion injury.

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Mitochondrial proteins represent a group of promising pharmacological targets in the search of new molecular targets and drugs to counteract the onset of cardiovascular diseases (CVDs). Indeed, several mitochondrial pathways result impaired in CVDs, showing ATP depletion and ROS production as common traits of cardiac tissue degeneration. Thanks to the advances in omics approaches, to a greater availability of mitochondrial crystallized proteins and to the development of new computational approaches for protein 3D-modelling and drug design, it is now possible to investigate in detail impaired mitochondrial pathways in CVDs. Furthermore, it is possible to design new powerful drugs able to hit the selected pharmacological targets in a highly selective way to rescue mitochondrial dysfunction and prevent heart failure. The role of mitochondrial dysfunction in the onset of CVDs appears increasingly evident, as reflected by the impairment of proteins involved in lipid peroxidation, mitochondrial dynamics, respiratory chain complexes, and membrane polarization maintenance in CVD patients. Conversely, little is known about proteins responsible for the cross-talk between mitochondria and cytoplasm in cardiomyocytes, such as mitochondrial transporters of the SLC25A family, which are responsible for the translocation of nucleotides, amino acids, organic acids, and other cofactors between the mitochondrial and cytosolic compartments. In this context, mitochondrial carriers, leading metabolic pathways such as the malate/aspartate shuttle, the carnitine shuttle, the ATP export from mitochondria, and the regulation of permeability transition pore opening, crucial for cardiomyocyte viability, emerge as an interesting class of new possible pharmacological targets for CVD treatments.

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The serinosome: a novel human multienzyme metabolic assembly for L-serine biosynthesis

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Abstract

De novo L-serine (L-Ser) biosynthesis in the mammalian astrocytes proceeds through the phosphorylated pathway (PP) made by 3-phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase (PSAT) and phosphoserine phosphatase (PSP) [1]. L-Ser plays a major role in the development and function of the human CNS: various severe, infantile, neurological disorders have been linked to its deficiency. L-Ser is the precursor of the neuroactive signaling molecules glycine and D-serine, which modulate the activity of N-methyl-D-aspartate receptors. So far, little is known about control mechanisms allowing the PP to meet cellular needs.

Using the proximity ligation assay and confocal microscopy, we demonstrated that in iPSC-derived differentiated human astrocytes the three enzymes of the PP co-localise in cytoplasmic clusters, which size is similar to the one reported for other metabolons (i.e. the purinosome). The in vitro generation of a stable complex using the recombinant human PHGDH, PSAT and PSP was not observed (i.e. by native PAGE, size exclusion chromatography and cross-linking experiments). Anyway, kinetic studies of the reconstituted pathway (at physiological enzymes and substrates concentrations) supported the production of an enzymatic agglomerate: PHGDH catalyzes the rate-limiting step and PSP reaction is the driving force for the whole pathway.

We propose that human PHGDH, PSAT and PSP can cluster in a transient metabolic assembly, the putative “serinosome” [2], providing a channeling solution for the pathway intermediates and delivering a relevant level of sophistication to the control of L-Ser biosynthesis.

This project was founded by ”PRIN-2017 - Dissecting serine metabolism in the brain”.

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Categories

Proteins

Proteins playing fundamental roles in plant-microbe interactions: tomato-*Beauveria bassiana* as a case study

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Abstract

Throughout their lives, plants can benefit from associations with other organisms that boost their fitness and resistance to (a)biotic stresses. The beneficial fungus *Beauveria bassiana* has a wide range of plant hosts, including tomato (*Solanum lycopersicum* L.), a globally cultivated species of considerable economic importance. It is widely recognized that B. bassiana promotes plant growth and acts as a pathogen/pest biocontrol agent. However, little is known about the plant molecular mechanisms that regulate *B. bassiana* interactions. Using a proteomic approach, we found that during the establishment of the plant-fungus relationship, tomato exhibited altered molecular pathways. During the early stages of colonization, proteins associated with defense responses against the fungus were down-regulated, while proteins associated with calcium transport were up-regulated. At later stages, up-regulation of molecular pathways linked to protein/amino acid metabolism and to biosynthesis of energy compounds suggested a growth-promoting interaction. At this later stage, the profile of leaf hormones and related compounds was also examined, with a focus on the over-production of those involved in plant growth and defense. Additionally, *B. bassiana* colonization was found to increase plant resistance to the pathogenic fungus *Botrytis cinerea*, which impacts the oxidative protein machinery of plants. Further -omics analyses are underway to gain insight into the tripartite tomato-beneficial fungus-pathogen interaction.

Categories

Proteins

Completely human antibodies as potential vaccines against *Staphylococcus aureus* infections

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Abstract

Staphylococcus aureus is a human commensal bacterium; however, its pathogenic form is responsible for a great variety of infections including endocarditis, osteomyelitis, and bacteremia. *S. aureus* has been treated efficiently with antibiotics until the emergence of resistance to them[1]. Since antibiotic resistance is spreading both in clinical and non-clinical settings, there is a clear need to find new drugs to treat staphylococcal infections. The aim of this work was to biochemical, biophysical and biological characterize human monoclonal antibodies (mAbs), selected through antibody-phage display, against the staphylococcal collagen-binding adhesin (CNA). 18 unique mAbs were selected and they were characterized with Enzyme Linked Immunosorbent Assay (ELISA) and Surface Plasmon Resonance (SPR) technique. The activity was evaluated on recombinant purified adhesins and on adhesin-expressing bacteria. The results on the antibody-antigen binding demonstrated that they not only bind to CNA but one of them can also recognize an adhesin expressed from another Gram-positive bacterium since it is structurally similar to CNA[2]. We discovered that 2 mAbs were able to neutralize the *in vitro* infection (either by inhibition or displacement) of adhesins-expressing bacteria. The epitopes of the mAbs of interest were *in silico* and experimentally mapped. In conclusion, fully human monoclonal antibodies have been selected for the first time against the staphylococcal collagen binding adhesin (CNA) and 2 antibodies showed an interesting neutralization activity against *Staphylococcus aureus* and *Enterococcus faecium* bacteria. Therefore, they could be promising candidates for the development of a vaccine to treat staphylococcal and other Gram-positive infections.

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Categories

Proteins

Deciphering the mtDNA replication machinery through the identification of novel regulatory factors by BioID2 proximity labelling approach

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Abstract

Mitochondria are organelles responsible for many cellular functions and they have been related to several diseases. mtDNA is replicated asymmetrically and unidirectionally by a set of proteins and only a small fraction of the initiation events gives rise to full-length mtDNA; most of them result in the formation of a short nascent DNA fragment (7S DNA) that remains annealed to the parental strand, forming the D-loop structure. The mechanism that decides if replication should proceed or not is still unknown. Mitochondrial genome maintenance exonuclease 1 (MGME1) could be a possible regulator of mtDNA replication at the level of D-loop expansion as part of a not yet identified termination complex assembling at the end of the D-loop. To unravel MGME1 regulatory role in mtDNA replication, we searched for novel interacting partners by using the proximity-dependent biotin identification (BioID2) method. We used as a bait both full length MGME1 and mutants with altered nuclease activity in N- and C-termini. Data showed that MGME1 interacts with the catalytic subunit of the mtDNA polymerase (POLGA) using the N-terminal portion. We also revealed the presence of Exonuclease 3'-5' domain-containing protein 2 (EXD2) only in the enriched proteome of the full length and C-terminus deleted version, suggesting that N-terminus is essential for interaction with EXD2, similarly to POLGA. We generated stable cell lines expressing EXD2-FLAG and EXD2-BioID2HA to be employed in FLAG CoIP and reverse BioID2 experiments, respectively, to analyse the involvement of EXD2 in mtDNA replication.

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Categories

Proteins

The catechol 3,4-Dihydroxyphenylacetic acid affects the aggregation and the lipid-binding properties of α-synuclein and its mutant E46K at different extent: a biophysical study

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Abstract

α-Synuclein (α-Syn) is an intrinsically disordered protein, highly abundant in pre-synaptic neurons, where it is involved in synaptic vesicle trafficking and turnover. It populates an ensemble of conformations in vivo and it floats in equilibrium between the free random coil and the membrane-bound α-helical structure. α-Syn was found in the Lewis Body inclusions in the form of amyloid fibrils, present in the brain of patients suffering of Parkinson's Disease. E46K is a pathogenic mutant form of α-Syn, and the mutation accelerates the formation of fibrils. Moreover, the presence of a lysine residue in position 46 affects several structural properties of the mutant including its interaction with membrane and in particular the rate of association-dissociation on lipid surface.

Recently, we have shown that 3,4-dihydroxyphenylacetic acid (DOPAC), a dopamine metabolite, not only hampers α-Syn to form fibrils, interfering with the protein aggregation process, but also alters the ability of the protein and its aggregates to interact with cell membranes. Here, to understand the mechanism of such alteration, we studied the interaction of α-Syn and its mutant E46K with biological membranes in the presence of DOPAC by Circular Dichroism and Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS). These measurements allowed the identification of the regions involved in the protein binding to the catechol and to lipid membranes and to provide a dynamic model of interaction able to explain the different effect of DOPAC on lipid binding properties of α-Syn and E46K.

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Categories

Proteins

Characterisation of ADAMTS9 proteoglycanase activity

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Abstract

A Disintegrin and Metalloproteinase with Thrombospondin-like motif 9 (ADAMTS9) is a secreted metalloprotease widely expressed during mouse embryo development where it plays to a crucial role in regulating levels of cardiovascular proteoglycans. Reduced proteoglycan cleavage due to *Adamts9* haploinsufficiency is associated with cardiac and aortic anomalies, whereas complete *Adamts9* knockout is embryonically lethal. Despite its pivotal role in cardiac development, the proteolyglycanase activity of ADAMTS9 has not been biochemically characterised.

Here, we report a detailed characterisation of ADAMTS9 proteoglycanase activity and compared to that other ADAMTS family members such as ADAMTS1, 4 and 5. Due to difficulties in expressing full-length ADAMTS9 (MW:216kDa), FLAG-tagged ADAMTS9 truncated after the spacer domain (MDTCS, MW:75kDa) was expressed in HEK293T cells and purified using anti-FLAG chromatography. We then characterised ADAMTS9 ability to cleave the proteoglycans versican, aggrecan and biglycan.

ADAMTS9 was able to cleave all three proteoglycans, although with different potency. ADAMTS9 versicanase activity was quantitatively determined using an in-house developed sandwich-ELISA detecting the major versican cleavage fragment. ADAMTS9 cleaved full-length versican with a specificity constant (k_{cat}/K_m) of $6\times10^3 M^{-1}s^{-1}$. This was approximately 600-fold lower than ADAMTS5 and 35-fold lower than ADAMTS4, but 2-fold higher than ADAMTS1.

These results indicate that ADAMTS9 proteoglycanase activity resembles in potency and substrate repertoire that of other ADAMTS family members. Since the phenotype of *Adamts1*, *Adamts4* and *5* mice has been reported to be not as severe as that of *Adamts9*, it is likely that ADAMTS9 proteoglycanase activity contributes significantly to proteoglycan remodelling during early cardiac development.

Categories

Proteins

Fatty Acid Amide Hydrolase (FAAH) Inhibition downregulates Aβ42 Production in Tg2576 Primary Neurons

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Abstract

The endocannabinoid (eCB) system is a lipid signalling system with important pro-homeostatic functions, consisting of at least two receptors (CB1 expressed by neurons and CB2 by immune cells), their endogenous ligands (N-arachidonoylethanolamine, AEA, 2-arachidonoylglycerol, 2-AG) and distinct eCB metabolic enzymes. Recent studies by our group indicate that eCB levels and eCB metabolic enzymes change in an age-dependent manner in both animal models of Alzheimer's disease (AD) and in patients with mild AD. Furthermore, inhibition of the major fatty acid amide hydrolase (FAAH) enzyme has beneficial effects in AD mice. Here, we explored the fundamental role of FAAH inhibition in the mechanism of APP processing and Aβ42 production in primary neurons of Tg2576 (AD-like model) mice. First, we assessed whether the Tg2576 neurons could produce the amyloid peptide, and as we reported, the transgenic cells compared to wild-type produced a significant amount of Aβ42 ($p=0.001$). To evaluate the role of FAAH in the Aβ42 production process, we treated Tg2576 with the selective FAAH inhibitor: URB597. Interestingly, we found a downregulation of Aβ42 ($p=0.0255$) and a reduced expression of its main biosynthetic enzyme, BACE-1 ($p=0.0221$). To assess the key mechanism by which URB597 exerts its action, we co-administered the CB1 receptor antagonist SR141716 with the FAAH inhibitor and analyzed the same readout as previously described. In particular, we showed that the CB1 antagonist reverted the URB-mediated effect on both BACE-1 expression and Aβ42 production. Taken together, these results suggest that inhibition of FAAH could play a key role in the amyloidogenic cascade.

Categories

Proteins

The zinc finger couple ZNF639/ZBTB2 recruits chromatin remodelling multiprotein complexes to targeted genomic loci

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Abstract

ZBTB2 is a transcription factor containing a POZ domain at the N-terminus and four zinc-finger domains at the C-terminus. Two of these domains are Krüppel-like zinc-fingers, whereas the other two show an atypical zinc-coordination sphere (C2HC) [1]. ZBTB2 is correlated with cancer [1] and binds GC-rich regions in human genome [1, 2]. By affinity purification mass spectrometry approach and co-immunoprecipitation experiments, we found that ZBTB2 is a new subunit of the NuRD complex [3] involved in chromatin remodelling. Our data also reveal that ZBTB2 interacts with the transcription factor ZNF639 [3].

ZNF639 is a protein identified in esophageal squamous cell carcinomas. It contains nine Krüppel-like zinc-finger domains at the C-terminus, whereas little is known about the N-terminus [4]. ZNF639 has a role in cancer [4] just like it has been reported for ZBTB2.

We obtained the interactome map of ZNF639 that confirms the interaction with ZBTB2 and shows many partners involved in chromatin remodelling, suggesting a role of ZNF639 in this function. Our data, together with those already published, lead us to propose a model in which ZNF639 targets genomic loci, while ZBTB2 works as a bridge interacting with ZNF639 and HDAC1, recruiting multiprotein complexes on genomic loci.

We performed ChIP-seq of ZBTB2 and ZNF639 to uncover a DNA consensus sequence and genomic loci where the two proteins recruit chromatin remodelling multiprotein complexes.

Our results will reveal the molecular mechanism in which ZNF639/ZBTB2 couple plays its role leading to alteration of gene expression and cancer.

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Categories

Proteins

MucR/Ros family of nucleoid-associated proteins from α-proteobacteria

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Abstract

The MucR/Ros protein family comprises prokaryotic zinc finger proteins with N-terminal domain implicated in oligomerization and C-terminal domain allowing interaction with DNA. The prokaryotic zinc finger domain possesses a peculiar fold of three β-strands and two α-helices, stabilized by extensive hydrophobic core, which is different from the classical ββα topology of the eukaryotic counterparts. The prokaryotic zinc finger is believed to be an ancestral motif, from which the common eukaryotic C_2H_2 zinc finger has evolved[1].

Bacterial genome is generally organized by a range of different conformations, such as bends and bridges, mediated by NAPs (nucleoid-associated proteins)[2]. We study MucR from *Brucella abortus* and *Sinorhizobium meliloti* as prototypes of the MucR/Ros family of global transcriptional regulators involved in pleiotropic functions such as virulence, symbiosis, and various physiological processes. MucR is a heat-stable protein which forms oligomeric structure[3] - features shared by histone-nucleoid structuring proteins, a particular type of NAPs from another bacterial classes.

The higher-order oligomeric structure of the recombinant protein MucR was analysed by Cryo-EM experiments. MucR purified from *S. meliloti*, a strain naturally expressing this protein, was used to confirm the DNA-binding activity and the quaternary structure. Proteomic analyses of protein samples purified from *S. meliloti* allowed us to identify a putative new member of MucR/Ros family. Moreover, we focus our attention on DNA-bridging activity and the interaction network to demonstrate MucR is a convergently evolved NAP in α-proteobacteria and to gain new insight into the compaction of bacterial DNA.

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Categories

Proteins

The ABA/LANCL1-2 hormone receptors system controls ROS production in cardiomyocytes through ERRα

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Abstract

Rat H9c2 cardiomyocytes overexpressing the abscisic acid (ABA) hormone receptors LANCL1 and LANCL2 have an increased mitochondrial proton gradient, respiration and vitality after hypoxia/reoxygenation. Our aim was to investigate the role of the ABA/LANCL1-2 system in ROS turnover in H9c2. H9c2 cells were retrovirally infected to induce overexpression or silencing of LANCL1 and LANCL2, without or with the concomitant silencing of the transcription factor ERRα. Enzymes involved in radical production or scavenging were studied by qPCR and Western blot. Mitochondrial proton gradient and ROS were measured with specific fluorescent probes. ROS generating enzymes decreased, ROS-scavenging enzymes increased and mitochondrial ROS were reduced in LANCL1/2-overexpressing vs. control cells infected with the empty vector, while the opposite occurred in LANCL1/2-silenced cells. Knock-down of ERRα abrogated all beneficial effects on ROS turnover in LANCL1/2 overexpressing cells. Taken together, these results indicate that the ABA/LANCL1-2 system controls ROS turnover in H9c2 via ERRα. The ABA/LANCL system emerges as a promising target to improve cardiomyocyte mitochondrial function and resilience to oxidative stress.

Categories

Proteins

Characterization of zebrafish Tgds protein, a model to study the pathogenesis of Catel-Manzke syndrome.

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Abstract

Catel-Manzke syndrome (CMS) is a rare recessive disorder characterized by skeletal and heart malformations, due to mutations in the TGDS gene (1). TGDS is annotated in the databases as dTDP-glucose 4,6 dehydratase, due to its homology with the bacterial enzymes involved in dTDP-L-rhamnose biosynthesis; however, this pathway was never reported in vertebrates. CMS clinical presentation led to the proposal that TGDS could have a role in glycosaminoglycan formation (2), but biochemical evidences are lacking and TGDS function remains unknown.

In the perspective of developing a model to study CMS, we have identified and characterized zebrafish Tgds, both as mRNA isoform expression in tissues and during development and at the protein level. When expressed in *E. coli*, Tgds exhibited the dehydratase activity, specific on UDP-D-glucose; no activity was observed on UDP-D-glucuronic acid, thus excluding a role on UDP-D-xylose production. Mutant Tgds harboring the mutations observed in CMS patients showed reduced catalytic activity and stability; the damaging effects were further confirmed by Alphafold2 structural modeling.

Studies are ongoing to elucidate the role of Tgds in vertebrates and CMS pathogenic mechanisms, testing three hypotheses: i) Tgds is an enzyme in a pathway never described in higher animals, ii) the enzymatic activity is a remnant and during evolution the protein acquired another function, i.e. a regulatory role , iii) both the enzymatic activity and regulatory properties are essential, making TGDS a “moonlight” protein, as observed for other enzymes involved in nucleotide sugar metabolism such as GMDS, the dehydratase of the GDP-L-fucose pathway (3).

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Categories

Proteins

Multi-omic analyses of hiPSC-derived astrocytes during differentiation

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Abstract

Astrocytes are essential players in brain development and functions, being particularly relevant as regulators of energy metabolism, ionic homeostasis, and synaptic transmission. They are also the major source of L-serine (L-Ser) in the brain, which is synthesized from the glycolytic intermediate 3-phosphoglycerate through the phosphorylated pathway (PP), which comprises 3-phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase (PSAT) and phosphoserine phosphatase (PSP). L-Ser is the precursor of the two main co-agonists of the N-methyl-D-aspartate receptors, glycine and D-serine. Remarkably, astrocytes also contribute to neurodegenerative disorders by various mechanisms, including metabolic alterations [1].

Here, we generated human mature astrocytes from pluripotent stem cells (hiPSC) to get a picture of the changes that occur during astrocytes differentiation. By using an integrated multi-omics approach, we tracked astrocytes at different times of differentiation, showing that up to 30 days axon guidance processes, folate cycle, pyrimidine and amino acid metabolism were prevalent, along with sphingolipid synthesis. Consistent with the proliferation and cellular maturation processes that are taking place, also metabolites related to the serine pathway showed the same biosynthetic time course [2].

We have recently reported that the levels of the enzymes of the PP are increased in Alzheimer's disease brains [3]. Following this observation, we overexpressed PHGDH, PSAT or PSP in the hiPSC-derived astrocyte model and significant metabolic alterations were apparent. These results provide a valuable model for developing potential novel approaches to address brain diseases, especially those related to serine metabolism alterations.

This work was supported by PRIN 2017 2017H4J3AS-Dissecting serine metabolism in the brain.

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Categories Proteins

Characterization of lymphocytes protein cargo in Covid-19: unveiling the impaired coagulation

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Abstract

Background. SARS-CoV-2 is a highly transmissible pathogenic beta-coronavirus that caused Covid-19 pandemic since 2019. The striking variability of symptoms, with lymphopenia, cytokine storm, neurodegeneration and hypercoagulopathy, highlighted the urgence to characterize the molecular networks involved. In this context, peripheral lymphocytes bring the cellular basis of adaptative immune responses, thus playing a considerable role as predictors of Covid-19 outcomes.

Methods. Fluorescence-activated cell sorting (FACS) was combined with mass spectrometry (MS) to purify and analyze the protein cargo of 100,000 T and 100,000 B cells from pooled plasma of Covid-19 patients (I), recovered (R) and healthy subjects (H). Quantified proteins were processed with Ingenuity Pathway Analysis (IPA) for functional enrichment. Validation of putative proteins was performed on plasma specimens from expanded cohorts of I and H.

Results. 221 (I), 165 (R) and 234 (H) proteins were quantified in T cells; 205 (I), 118 (R) and 161 (H) proteins in B cells. I pool was devoid of lung healing proteins while expressed proteins of cytoskeleton remodeling, inflammation, proteasomal activity, virus entry, coagulation, and metabolic switch. IPA suggested the interplay between inflammation, viral infection, and coagulation cascade by confirming the acute phase response signalling, vascular dysfunction, and the infection by RNA-viruses. Interestingly, we report that coagulation is impaired as shown by the overexpressed levels of the platelet factor 4 (PF4) in patients, that were confirmed in the validation set. These data provide useful tools in the assessment of response to Covid-19 and to predict the outcomes.

Categories

Proteins

NAD(P)H regeneration via renewable hydrogen: here comes a robust [FeFe]-hydrogenase (with a little help from redox friends).

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Abstract

NAD(P)H cofactors are crucial for bio-catalysis and in turn can generate higher value compounds such as regio- and stereo-selective intermediates or final products for fine chemicals and pharmaceutics. Many regeneration systems proposed suffer from drawbacks such as accumulating by-products, acidification [1] and requirement for purification of the product of interest for which the cofactor regeneration is ancillary.

Alternatively, the use of gaseous hydrogen allows efficient recycling of NAD(P)H without the formation of by-products that could alter the pH or complicate product recovery. Also, the availability of cheap hydrogen from renewable energy sources such as solar and wind powered electrolysis or dark fermentation of wastes [2,3] can ensure the sustainability of the process and be in line with a circular economy paradigm.

Here we demonstrate an artificial and stable two-component protein system able to sustain a good NADPH regeneration rate from hydrogen, based on the modular combination of non-physiological partners as already successfully employed in other “Molecular Lego" approaches [4-9]. We exploit the very robust, highly active and oxygen resilient [FeFe]-hydrogenase CbA5H from *Clostridium beijerinckii*, previously identified in our group [10-11] combined with a reductase (BMR) from *Priestia megaterium*. The system shows a good stability as evaluated by DSC and it was demonstrated to reach up to 28±2 nmol NADPH regenerated s^{-1} mg of hydrogenase^{-1} (TOF: 126±9 min^{-1}) and 0.46±0.04 nmol NADH regenerated s-^{1} mg of hydrogenase^{-1} (TOF: 2.1±0.2 min^{-1}).

The system is first of its kind based on a [FeFe]-hydrogenase.

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Categories

Proteins

New selective N-acylethanolamine-hydrolyzing acid amidase inhibitors: design, synthesis, and biological effects.

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Abstract

The endocannabinoid system (ECS) is a lipid signaling system involved in a wide range of physiological and pathological processes like appetite's control, stress and maintenance of homeostasis, immune and inflammatory response, cell proliferation and migration. Latest studies propose the inhibition of the EC degradative enzymes, like monoacylglycerol lipase (MAGL), fatty acid amide hydrolase (FAAH) and N-acylethanolamine-hydrolyzing acid amidase (NAAA), as a pharmacological tool to modulate the endogenous ECs tone. The EC palmitoylethanolamide (PEA) is an endogenous potent anti-inflammatory agent and its activity is regulated by N-acylethanolamine acid amidase (NAAA) [1]. Here we report the design, synthesis and characterization of new PEA mimics, in which the amide moiety is replaced by a urea group, as N-acylethanolamine-hydrolyzing acid amidase (NAAA) inhibitors. The inhibitory ability was also evaluated by a fluorescence high-throughput screening method on human recombinant NAAA that allowed to select more active compounds. We also demonstrated the ability of the selected molecules to reduce inflammation and oxidative stress in a Parkinson's disease cellular model due to the ECS role in neuro-inflammation and oxidative stress prevention [2,3]. Together our results suggest the new proposed molecules and NAAA enzyme as promising pharmacological tool against neurodegenerative disease.

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Categories

Proteins

Exploring the molecular evolution of ovothiol biosynthesis

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Abstract

Ovothiols are 5-thiohistidines endowed with antioxidant and anti-inflammatory properties and synthesized by marine invertebrates, protists, and bacteria. Ovothiol biosynthesis is catalyzed by two enzymes: 5-histidylcysteine sulfoxide synthase (OvoA) and pyridoxal phosphate-dependent lyase (OvoB) (1). Previous bioinformatics analyses have highlighted that OvoA is highly conserved in Metazoa, despite the loss of the ovoA gene in Ecdisozoa and Vertebrata. Additionally, two cases of horizontal gene transfer (HGT) have been identified in Rotifera Bdelloidea and in Hydrozoa (2). The objective of this study is to deepen evolutionary aspects and the distribution of this metabolic pathway within the metazoan lineage by focusing on the Cnidaria phylum. Cnidarians are particularly intriguing for this investigation due to their early branching position in the animal kingdom and their close relationship with bilaterians. Moreover, they represent an extraordinary example of molecular diversification of ovothiol biosynthesis, as Anthozoa conserved the canonical OvoA structure, Medusozoa lost OvoA, while Hydrozoa reacquired it through HGT (2). In this study, by genomic analysis, we found that the hydrozoan Clytia hemisphaerica displays a single transcript that combines ovoB and ovoA coding regions. We cloned ovoB and ovoA and produced the recombinant proteins to replenish the enzymatic activities necessary for ovothiol biosynthesis in vitro. Finally, we have identified the cellular thiols in key cnidarian species by LC-MS. Overall our results shed light on the origin and diversification of ovothiol biosynthesis, giving new insights into the relationship of marine organisms with their environment and food habits.

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Categories

Proteins

TUMOR BIOCHEMISTRY

ROLE OF DIACYLGLYCEROL KINASES IN ACUTE MYELOID LEUKEMIA

Teresa Gravina[1], Chiara Maria Teresa Boggio[1], Elisa Gorla[1], Silvia Polidoro[1], Sara Centonze[1], Daniela Ferrante[2], Monia Lunghi[3], Andrea Graziani[4], Davide Corà[1], Gianluca Baldanzi[1]

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Abstract

Diacylglycerol kinases (DGK) play a role in cell transformation but also in immunosurveillance against tumors. Cancer expression databases highlight a strong overexpression of several DGK isoforms in acute myeloid leukemia (AML) tumor tissue and specifically of DGKA, DGKD and DGKG, without a correlation with specific AML subtypes. Interestingly, in some database, high expression of DGKA and DGKE negatively correlates with survival, while high DGKG expression leads to a more favorable prognosis. In line with this, the functional analysis of the genes co-expressed with the different isoforms evidence divergent genetic programs between DGKA and DGKG.

To verify the suitability of DGK as therapeutic targets we treated the HL-60 and HEL cellular models with DGK inhibitors and compared them to healthy donor's lymphocytes. We observed a specific sensitivity to R59022 and R59949, two poorly selective inhibitors, which promote cell accumulation in the S phase. Conversely, the DGKA specific CU-3 and AMB639752 are nearly ineffective.

In the search for novel therapeutic targets for AML, those data indicate that DGK play a relevant and isoform-specific role. In particular, the DGKA isoform appears relevant although its inhibition is not sufficient to impair AML cell viability.

Categories

Tumor Biochemistry

IF1, the endogenous regulator of ATP synthase, does not inhibit oxidative phosphorylation in cancer cells and promotes proliferation after anoxia-mimicking stress conditions.

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Abstract

Many cancer cells overexpress IF1, the endogenous protein that binds to the catalytic domain of ATP synthase and inhibits ATP hydrolysis when the mitochondrial membrane potential (ΔμH+) falls, as occurs in the anoxic areas of solid tumors [1]. IF1 has also been reported to play a role in mitochondrial cristae structure organization and resistance to apoptosis [2, 3]. Here, we investigated the role of IF1 in regulating bioenergetics under physiological and stress conditions. To this end, we generated IF1-silenced clones of osteosarcoma (143B) and colon carcinoma (HCT116) cells. Analysis of mitochondrial membrane potential, ATP synthesis, and oxygen consumption rate revealed no significant changes of these bioenergetic parameters in silenced clones compared to parental cells, demonstrating that IF1 does not affect ATP synthesis and therefore oxidative phosphorylation (OXPHOS) in cancer cells. We then investigated the ability of IF1 to promote survival and proliferation after stress conditions by exposing cells to FCCP, an uncoupler that collapses ΔμH+. Under this condition, IF1-expressing cells showed higher energy charge than IF1-knockdown cells. Interestingly, we could also observe an increase in mitophagy in parental cells, which was balanced by mitochondrial biogenesis. After uncoupler withdrawal, the growth of IF1-expressing colon cancer cells that mainly rely on OXPHOS for energy was faster than the IF1-knockdown clones. Overall, our study demonstrates that IF1 does not inhibit the physiological activity of ATP synthase, but by inhibiting its ATP hydrolytic activity it can confer a proliferative advantage to cancer cells exposed to anoxic-mimicking stress conditions.

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Categories

Tumor Biochemistry

The protein corona changes the biological effect of gold nanoparticles in breast cancer cells

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Abstract

Gold nanoparticles (AuNPs) are emerging as elective candidates for selective breast cancer damage (1). However, their use in clinical practice remains limited due to an incomplete understanding of the factors at the bio-nano interface both in vitro and in vivo. Upon exposure to biological fluids, proteins rapidly adsorb to AuNPs and form protein corona (PC), which can modify the biological identity of NPs. To this end, we hypothesized that the PC governs AuNPs-breast cancer interactions (2). To test this hypothesis, PC was formed by incubating AuNPs (sphere- or star-shaped) in the cell culture medium (supplemented with 10% fetal bovine serum) of SK-BR-3 (HER2 over-expressing breast cancer cell line) at 37°C and at different incubation times. PC formation was assessed by dynamic light scattering (DLS), zeta potential measurements, UV-Vis spectrophotometry, SDS-PAGE electrophoresis, Cryo-EM, and bicinchoninic acid (BCA) assay. Mass spectrometry (MS)-based proteomic analysis was used to assess PC composition. Both differently shaped gold nanoparticles without PC significantly reduced the viability of SK-BR-3 cells by altering the expression of apoptotic proteins. Interestingly, PC reversed these effects. Similarly, the presence of PC affected the uptake of AuNPs by decreasing the level of internalization in breast cancer cells. Preliminary proteomic data revealed unique protein patterns based on the shape of AuNPs. Bioinformatic analyses are underway to identify proteins related to the uptake and biological effects of AuNPs. These findings are expected to have implications for the future development of AuNPs-based anticancer therapies.

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Categories

Tumor Biochemistry

The naturally occurring estrogen receptor-activating mutation ESR1Y537S revealed enhanced susceptibility to ferroptosis induction in breast cancer cells with acquired resistance to estrogen deprivation

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Abstract

Most breast cancers are estrogen receptor-positive (ER+) and treated with endocrine therapy. However, resistance limits its efficacy, and ER mutations associate with endocrine-resistant cancers. Of note, we have evidence that fatty acids (FA) metabolic reprogramming and storage are involved in the response and adaptation to long-term estrogen deprivation (LTED), a condition that mimics AI resistance.
Here we characterized FA metabolism in a LTED cell model harboring a naturally occurring ER-activating mutation (ESR1Y537S). We found that FA synthesis and uptake, together with the subsequent accumulation into lipid droplets (LD) are features of LTED, independently of ER status. However, LTED-ESR1Y537S cells succumb when challenged with nutritional stress, whereas LTED-ESR1WT cells show enhanced metabolic plasticity and resilience, thanks to LD mobilization. FA intracellular availability could be exploited as a metabolic vulnerability that exposes cells to ferroptosis, a form of cell death caused by iron-dependent peroxidation of polyunsaturated fatty acids (PUFA). Since LD can sequester PUFA to protect cells from ferroptosis, we hypothesized that the inability of the LTED-ESR1Y537S cells to adapt their lipid metabolism could sustain sensitivity to ferroptosis. Indeed, we observed that LTED-ESR1Y537S cells are sensitive to the ferroptosis inducer RSL3 and display high lipid peroxidation, increased reactive oxygen species (ROS) levels together with enhanced expression of acyl-CoA synthetase long-chain family member 4 (ACSL4), an essential component for ferroptosis execution. Crucially, the treatment with RSL3 resensitized cells to the endocrine agent Fulvestrant, indicating that the increased sensitivity of LTED-ESR1Y537S cells to ferroptosis can be exploited to overcome resistance to conventional endocrine therapy.

Categories

Tumor Biochemistry

Linking epithelial-mesenchymal transition and hexosamine synthesis pathway in pancreatic adenocarcinoma

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer deaths and will be the second cause by 2030. Nearly 80% of patients are diagnosed at advanced stages and chemotherapy, based on gemcitabine (GEM) alone or in combination with other drugs, is the main treatment [1]. PDAC develops chemoresistance to GEM so alternative therapeutic regimens should be investigated [2]. PDAC shows metabolic alterations that could lead to resistance to chemotherapy. In particular, an upregulation of the hexosamine biosynthesis pathway (HBP) is recurrent in PDAC. The FR054 inhibitor strategically targets the PGM3 enzyme in the HBP, affecting both N and O-glycosylation. Previous studies have shown that the inhibition of growth and viability induced by GEM alone are significantly enhanced by the combined treatment with FR054 both in vitro and in vivo [3]. Epithelial-to-mesenchymal transition (EMT) is responsible for the invasive phenotype of cancer cells and chemoresistance. Our aim is to investigate how FR054 inhibits HBP and modulate the EMT. The experiments are carried out on MIAPaCa2 and BXPC3 pancreatic cell lines in 2D and 3D spheroids treated with FR054 alone or in combination with GEM. Modulation of genes (RNAseq) and proteins (western blot and immunofluorescence), confirmed the FR054 ability to inhibit the HBP in 2D and 3D, inducing a decrease in cell proliferation, alone or combined with GEM. Furthermore, FR054 modulates the levels of important proteins in EMT both in 2D cultures and in spheroids, attributing to FR054 an effect on important cancer hallmarks: invasion and metastasis.

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Categories

Tumor Biochemistry

Ribosomal protein uL3 status affects translation efficiency in colorectal cancer cells

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Abstract

mRNA translation is critical for gene expression regulation (1). A growing body of evidence suggests that changes in translational efficiency help activate key oncogenic signaling pathways. Translational control of specific subsets of mRNAs, in particular, promotes cancer cell survival and invasion, as well as chemotherapeutic resistance (2,3). Ribosomal protein L3 (uL3) is a component of cytosolic ribosomes that plays a crucial role for both ribosome structure and function. Our research group has identified uL3 as stress sensing molecule essential for cellular response to certain chemotherapeutics in colorectal cancer cells lacking functional p53 (4,5). Specifically, uL3 status is associated with chemoresistance, epithelial-mesenchymal transition program alteration, increased cell migration and proliferation, inhibition of apoptosis, enhancement of autophagy, and overexpression of drug efflux transporters (6-9).

The goal of this study was to determine the role of uL3 in the regulation of translational efficiency in p53-depleted colorectal cancer cells and a derivative cell line stably silenced for uL3 and resistant to 5-FU. The influence of uL3 on “translatome” and associated mRNAs has been investigated by using polysome profiling technique and qPCR analysis of ribosome-associated mRNAs. Results from these experiments will be presented.

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Categories

Tumor Biochemistry

Sirtuin 6 inhibition as a pharmacological approach in cutaneous Squamous Cell Carcinoma

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Abstract

Sirtuin 6 (SIRT-6) has a critical role in cutaneous Squamous Cell Carcinoma (cSCC): SIRT6 silencing in skin SCC cells has pro-differentiating effects and SIRT6 deletion abrogated DBMA-induced skin tumorigenesis in mice. On the other hand, SIRT6 acts as tumor suppressor in SCC by enhancing glycolysis in tumor propagating cells.

Our aim was to pharmacologically modulate SIRT6 deacetylase activity in cSCC, with S6 (inhibitor) and MDL-800 (activator). In cSCC cells, S6 recreated the pro-differentiating effects of SIRT-6 silencing, as the levels of Keratin-1, Kerati-10 and Loricrin were upregulated compared to controls.

Next, the effects of SIRT-6 pharmacological modulation was evaluated in a DMBA-TPA-induced skin cancer model. Mice treated with the SIRT-6 inhibitor S6 in a preventive approach, at the beginning of the promotion stage, presented reduced number and size of papillomas, compared to the controls, whereas MDL-800 displayed an opposite trend. The hyperproliferation marker Keratin 6 and the cSCC marker Keratin 8 were less abundant when SIRT-6 was inhibited. In S6-treated lesions, the Epithelial-Mesenchymal Transition (EMT) markers Zeb1 and Vimentin were less expressed compared to untreated lesions.

In a therapeutic approach, the treatment started after papilloma appearance: the S6 group presented reduced papillomas (number and size), whereas MDL-800-treated mice displayed an opposite trend. Keratin 6 was less expressed in S6-treated lesions, EMT was less advanced and a delayed carcinogenesis in the S6 group was indicated by higher E-cadherin/Vimentin ratio and decreased Keratin 8 expression.

These results suggest that SIRT-6 pharmacological inhibition may decrease skin carcinogenesis in cSCC.

Categories

Tumor Biochemistry

Bile acids in the onset of colorectal cancer: the possible role of Notch signalling

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Abstract

Colorectal cancer (CRC) ranks as second among the causes of tumor death worldwide, therefore understanding its molecular and cellular mechanisms becomes crucial. Recent studies suggest a pivotal role of altered Notch signaling activation in CRC, which is physiologically involved in various cellular processes. In addition, perturbations of bile acids (BAs) synthesis and composition are related to colorectal oncogenic signals, even if a clear link between BAs and the Notch pathway in CRC has not yet been fully elucidated [1].

In this context, we analyzed biological samples (stools and specimens) from subjects with different intestinal tumor lesions (hyperplastic polyps, low- and high-grade adenomas, and cancer lesions). Fecal BAs content was assessed through HPLC-mass spectrometry analysis [2] showing that secondary BAs (deoxycholic acid: DCA, and lithocholic acid: LCA) are altered in pathological conditions. On the human specimens, Notch pathway components were analyzed through immunohistochemistry, observing that Notch3 and the ligand Jagged1 are highly expressed in CRC patients compared to hyperplastic polyps and adenomas. In parallel, proteins and RNA were extracted from human colorectal adenocarcinoma cells (Caco-2 and HT29) treated with secondary BAs (10 μM for 24 h). Western blot and RT-PCR analysis showed that DCA and LCA treatment increases the expression and the activation of the Notch 3 pathway.

Taken together our results indicate an existing crosstalk between the Notch signaling pathway and BAs in the onset and progression of CRC, suggesting a wide range of potential clinical applications including new screening and therapeutic approaches.

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Categories

Tumor Biochemistry

EXPLORING THE ROLE OF PARAOXONASE-2 IN HUMAN CLEAR CELL RENAL CELL CARCINOMA: *IN VITRO* EFFECT OF SHRNA-MEDIATED GENE SILENCING ON CELL PROLIFERATION AND CHEMOSENSITIVITY

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Abstract

Renal cell carcinoma (RCC) represents the most lethal urological neoplasm. Among various subtypes, clear cell RCC (ccRCC) accounts for about 85% of all tumor forms. One-third of RCC patients are diagnosed with metastatic disease, thus displaying around 12% 5-year survival rate. Despite progress in research focused on immunoactive and target molecules, the prognosis of patients with advanced RCC remains poor, mainly due to the lack of sensitivity to chemotherapy and radiation therapy [1]. The identification of new molecules that can be used for early detection and the development of effective targeted therapies is therefore crucial. In this work, we focused on paraoxonase-2 (PON2), an intracellular membrane-bound enzyme ubiquitously expressed in human tissues. PON2 upregulation was reported in a variety of solid tumours, thus suggesting its possible role in cancer cell survival and proliferation, due to its antioxidant and anti-apoptotic activity [2,3]. To investigate PON2 involvement in tumor cell metabolism, human ccRCC cell lines were transfected with plasmid vectors coding short harpin RNAs targeting PON2 transcript. Efficiency of enzyme knockdown was assessed by Real-Time PCR and Western blot. The impact of PON2 silencing on cell viability, migration and response to chemotherapeutic treatment was then explored. Our results showed that PON2 downregulation was able to trigger a decrease in proliferation and migration of ccRCC cells, as well as an enhancement of cell sensitivity to chemotherapy. Data reported in this study suggest that the enzyme may represent an interesting therapeutic target for ccRCC.

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Categories

Tumor Biochemistry

Smart functionalized polyvinylpyrrolidone nanogels for drug and siRNA delivery in solid tumours

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Cancer is one of the leading causes of death in the world. Traditional therapies, including chemotherapy, induce remarkable side effects due to poor solubility, non-specific targeting and therefore systemic toxicity. The development of engineering nanoparticles (NPs) adopted as drug delivery systems (DDSs) can overcome these limitations because they can be functionalized to obtain controlled and targeted drug release. In this contest, polyvinylpyrrolidone (PVP) nanogels (NGs) have been synthesized by e-beam irradiation, an innovative technique that permits to obtain, in only one step of the synthesis, sterile and functionalized (with carboxyl or amino groups) NGs with a specific size. PVP NGs are biocompatible and able to enter the cells through the micropinocytosis pathway. The specific tumour targeting was obtained by conjugating addressing molecules like folic acid or the antibody against integrin αvβ3, as suggested by confocal microscopy and flow cytometric analysis. On the other hand, the anticancer drug doxorubicin or the proapoptotic siRNA Bcl2 were conjugated through a glutathione-sensitive spacer for a controlled release in the tumour mass.

Therefore, the possibility to synthesize sterile and size-controlled PVP nanogels and functionalize them for specific tumour targeting and selective controlled drug release makes them optimal candidates for the treatment of different types of solid tumours.

Essential oil from *Sicilian Origanum vulgare* (L.) induces cytotoxic effects in Breast Cancer cells

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Abstract

Essential oils (EO) extracted from medicinal plants and culinary herbs have gained particular interest due to their cytotoxic and anticancer effects. Of particular interest seems to be oregano EO due to its antitumor, antioxidant and anti-lipogenic properties. In the present study, we evaluated the effect of EO extracted from Origanum vulgare L (EOO) in MDA-MB-231 and MCF7 cultured breast cancer cells. The composition of EOO was analyzed by gas chromatography-mass spectrometry, highlighting the presence of p-cymene, terpinene and thymoquinone. We have shown that EOO causes a drop in cell viability in both cell lines examined. Caspases and the mitochondria seem to be involved in the mechanism of death induced by EOO. In particular, western blotting analysis showed activation of pro-caspase-9 and -3 and fragmentation of PARP, down regulation of Bcl2 and BclxL and increase in Bax. The use of fluorochrome JC-1 highlighted the loss of the mitochondrial membrane potential. The effect of EOO on MDA-MB231 and MCF7 seems to be mainly due to thymoquinone, one of its main constituents.

Categories

Tumor Biochemistry

NME4-driven mitochondrial reshape signals to the nucleus and promotes pancreatic carcinogenesis

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Abstract

Understanding how mitochondrial function and morphology impact tumor progression could unravel metabolic and signaling vulnerabilities, enabling novel therapeutical approaches. In fact, PDA is one of the deadliest cancers worldwide and it is projected to be the second highest contributor to cancer-related deaths by 2030. To dissect the contribution of mitochondria to pancreatic cancer progression, we combined organelle immunopurification with LRF mass spectrometry to examine changes in the mitochondrial proteome of human PDA cell lines compared to premalignant, oncogene-expressing cells. We found upregulation of mitochondrial proteins involved in nucleotide synthesis and processing, such as NME4. This protein is a nucleotide diphosphate kinase involved in the regulation of mitochondrial morphology and able to increase GTPase activity of the master regulator of cristae shape and biogenesis, OPA1. In line, mitochondrial cristae width significantly decreases during pancreatic cancer progression, as observed both in multiple human cell lines and in mice expressing oncogenic KRAS in the pancreas (KC mice). Notably, NME4 is upregulated in neoplastic lesions in vivo and OPA1 overexpression in KC mice accelerates pancreatic cancer progression, causing the formation of high grade dysplasia. This phenotype is associated with an increased cellular proliferation and histone hyperacetylation, both in the normal pancreas and during pancreatic cancer progression. In cancer cells, this determines significant genomic instability. This work documents dynamic changes of mitochondrial ultrastructure during carcinogenesis, defines molecular underpinnings and suggests the possibility that OPA1 promotes tumor progression through the regulation of the epigenome.

Categories

Tumor Biochemistry

Mutant p53 stimulates mitochondrial fragmentation in pancreatic ductal adenocarcinoma (PDAC)

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Abstract

Mutation in p53 tumor suppressor gene occurs in about 75% of pancreatic ductal adenocarcinoma (PDAC). These mutations are often hotspot missense mutations, which provide new oncogenic functions. In detail, they promote cancer progression by enhancing the aggressive abilities of cancer cells. Given the pivotal role of mitochondria in cancer cells, to improve their fitness and maintain redox balance we investigated the mutant p53 (mut-p53)-dependent regulation of mitochondrial dynamics in PDAC. We analysed mitochondria morphology with Transmission electronic microscope (TEM) that revealed a higher aspect ratio, as index of mitochondrial length, in PANC-1 cells after transient knock down of mut-p53 compared to the scramble control. Further live cell imaging evaluations confirmed that, when mut-p53 gene is silenced, mitochondria appear more elongated than mitochondria present in the control. On the contrary, in wild type PDAC cells an opposite trend is observed when p53 is silenced. The analyses of mitochondrial length and network formation were performed by using the Mitochondrial Network Analysis (MiNA) macro tool, that allowed us to analyse 3D images and revealed longer mitochondria and a more branched network after knock down of mutant p53. Lastly, data obtained in our lab suggest that p53 silencing can modulate key proteins involved in mitochondria morphology and dynamics. In fact, we found that the protein OPA-1, which is involved in mitochondria fusion processes, is upregulated by mut-p53 silencing in PDAC cells. Taken together, these data indicate a direct correlation between mutant p53 and mitochondrial dynamics, thus highlighting mitochondria as promising target in PDAC.

Categories

Tumor Biochemistry

Functional multi-omics investigation reveals potential molecular mechanisms involved in chemoradiotherapy response prediction in locally advanced rectal cancer

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Abstract

Treatment response assessment of rectal cancer patients is a critical component of personalized cancer care and allows to identify suitable candidates for organ-preserving strategies[1].In this study, we combined Radiomics and Metabolomics features to predict treatment response at staging, in 24 responder (R) versus 11 non responder (NR) patients. A machine-learning classifier was used to analyze MRI-based radiomics and untargeted metabolomics data.Metabolomics investigation was performed on serum of patients before the start of therapy, obtaining more than 4000 compounds have been quantified, of which 1324 have been identified. Bioinformatics investigation of the endogenous compounds identified, quantified, and mapped in the KEGG and HMDB databases, highlighted a possibly down-regulation of "Cell viability of cancer cells" (p-value= 2.09 E10-3; z-score= -1.86) biological function in R patients, even before starting therapy. Moreover, "Oxidative stress response of cells" (p-value= 2.97 E10-4; z-score= +1.98) and "Release of L-Glutamic acid" (p-value= 6.15 E10-5; z-score= +1.17) resulted up-regulated in R patients. Contextually, the expression of specific serum metabolites in responders have highlighted a significant implication in the response to oxidative stress.Oxoproline accumulation coupled with low levels of gamma-glutamyl-tyrosine in NR patients may suggest a dysregulation in glutathione degradation, and therefore a worse response to oxidative stress[2]. Here, oxoproline levels were positively correlated with the maximum diameter of the tumor measured on axial MR images. To conclude, the integration of radiomics and metabolomic data could help in the future therapeutics directions by improving the understanding of biological mechanisms involved in therapy-response susceptibility.

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Categories

Tumor Biochemistry

Aptamer-Based Therapeutic Targeting for Glioblastoma: suicide gene therapy and Aptamer-Chimera

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Abstract

The intrinsic heterogeneity of GBM cells defines an urgent need to find targets available for an efficient drug-targeting approach; in this contest aptamers offer a great opportunity. They are small nucleic acid molecules that can specifically bind to molecular targets. Nucleolin and EGFRvIII represent two important extracellular targets.

A novel suicide gene therapy approach was tested. We describe the use of aptamer AS1411 sequence embedded in a plasmid to deliver the gene of the plant toxin saporin into glioblastoma cells. With this plasmid "APTSAP", the gene encoding ribosome-inactivating protein saporin is driven intracellularly by AS1411 that binds to cell surface-exposed nucleolin and efficiently kills target cells. The results indicate a toxic activity of APTSAP in the low concentration range in contrast with a AS1411 effect (IC50 APTSAP 1.30 × 10−8 M; IC50 APT 4.30 × 10−6 M).

Another approach is the use of chimeric aptamers consisting of two sequences namely U2 and AS1411 binding EGFRvIII and nucleolin respectively in GBM cells. Through the chimera it is possible to simultaneously reach two targets for tumor proliferation. We generated four different chimeras, poly-A and poly-T with different lengths were used as linkers. The most potent is U2-A18-AS1411 (IC50 5X10-7M). We are furthermore planning to combine this chimera with the drug DM3, a chemical derivative of maytansine.

Our findings highlight the potential value of APTSAP and aptamer chimeras as a multifunctional therapeutic strategy for killing brain tumor cells.

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Categories

Tumor Biochemistry

Heme oxygenase dictates ferroptosis sensitivity in breast cancer cells

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Abstract

Heme oxygenase (HO) is an intracellular enzymatic system responsible for heme degradation in stoichiometric amounts of ferrous iron, carbon monoxide and biliverdin. Among the two main isoforms studied so far, HO-1 is strongly induced under cellular stressful conditions and its expression is transcriptionally regulated by the Nrf2-Keap1 axis. HO-1 has been demonstrated to be a useful target for the treatment of pathologies related to an unbalanced oxidative stress status. Moreover, HO-1 can exert either a cytoprotective or a detrimental action in cancer[1,2], depending on the specific cellular conditions. Recent findings suggest a potential role of HO-1 induction in ferroptosis, a newly discovered form of cellular death triggered by iron accumulation and lipid peroxidation[3]. Herein we report biological evaluation on breast cancer cells of different HO-1 inducers such as hemin, curcumin, caffeic acid phenethyl ester (CAPE) and novel CAPE analogs. HO-1 siRNA and enzymatic inhibitors were used in order to understand the mechanism behind HO-1 participation in ferroptosis process. Moreover, since sigma receptor modulation was shown to affect HO-1 expression levels, we investigated the role of agonists/antagonists as novel therapeutic agents able to increase cellular sensitivity to ferroptosis. Analysis of HO expression, subcellular localization and enzymatic activity together with assessment of main oxidative stress and ferroptosis features were performed in MCF-7 and MDA-MB 231 breast cancer cells. Preliminary data showed significant HO-1 contribution in ferroptosis onset, highlighting the potential use of its modulation as a novel therapeutic strategy for cancer treatment.

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Categories

Tumor Biochemistry

Orobanche crenata extracts exerts antitumor activity “in vitro”

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Abstract

Colorectal cancer (CRC) is the second deadliest cancer, causing 900 000 deaths worldwide.

Despite progress in diagnosis and treatment, chemotherapy significantly compromises the quality of life of oncologic patients. Therefore, the use of adjuvants endowed with anticancer property could be considered a good strategy to reduce the dose of chemotherapy improving the health conditions of oncologic patients.

Thus, in the present work, we investigated the anticancer activity of Orobanche crenata leaf extract against two different colorectal cancer cell lines Caco-2 and HCT-116. The activity of the aqueous extract was compared to the standard drug Cisplatin.

Human colon cancer cells (Caco-2 and HCT-116) were treated with O. crenata aqueous extracts (10 - 160 µg/ml) or with cisplatin (0.03 – 30 µg/ml) for 24, 48 and 72h. Human dermal fibroblasts were used as control cell line. After treatments, we evaluate cytotoxicity by MTT assays and apoptosis (by Annexin V/Propidium Iodide assays) and both BAX and Bcl-2 protein levels (by western blot analysis).

Extract exhibits cytotoxicity in Caco-2 in a dose and time-dependent manner significantly reducing cell viability. Co-treatment with 40 µg/ml of extract for 48h potentiated the sub-toxic effect of cisplatin. Hoechst and Annexin V/PI staining showed the induction of apoptosis by extract in Caco-2 cells; this data were confirmed by increased levels of Bax/Bcl-2 ratio. These findings suggest that O. crenata aqueous extracts exert an anti-cancer effect enhancing apoptosis in Caco-2 cells thus considering a promising therapeutic adjuvant against various cancer cell types.

Categories

Tumor Biochemistry

FLASH irradiation spares bronchial epithelial cells, limits fibrotic marker, and increases the mortality of lung adenocarcinoma cells.

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Abstract

Radiation therapy (RT) is one of the most effective strategies used for cancer treatment. Unfortunately, the major drawback of conventional RT (CONV RT) is the impossibility of delivering high doses of radiation due to both short- and long-term damage to normal healthy tissues, which can result in a non-complete eradication of the tumor, leading to an increase in the chances of relapses. FLASH-RT is an emerging technique that consists in the deliverance of pulsed radiation at dose/rates higher than the ones used clinically and is able to overcome the collateral damages caused by CONV-RT by sparing healthy cells, through a process that is not fully understood, known as the “FLASH effect”. Concerning lung cancer, RT treatment can trigger the generation of fibrotic scars in the healthy tissue that can lead even to death in severe cases.

Human lung adenocarcinoma (A549) and normal human bronchial epithelial (16HBE) cell lines were irradiated with CONV- or FLASH-RT at different doses, ranging from 2 to 20 Gy with an electron linear accelerator. Irradiated cells were tested for survival, clonogenic assay, apoptosis, cell cycle assessment and for the expression of vimentin, an early fibrotic marker.

In healthy cells, a FLASH effect was observed when cells were irradiated with lower doses of FLASH-RT (2-4 Gy), meanwhile, at the same irradiation dose, tumoral cells showed higher mortality when exposed to FLASH- compared to CONV-RT, apoptosis and cell cycle arrest; lastly, vimentin’s expression was significantly higher both in tumoral and healthy cells irradiated with CONV-RT.

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Categories

Tumor Biochemistry

IRAK1: a novel target to overcome the chemo-immuno-resistance in NSCLC

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Abstract

The high expression of ABCB1 (P-glycoprotein) and ABCC1 (Multidrug Resistance-related Protein 1), both involved in the efflux of chemotherapeutic drugs, and the low expression of ABCA1, a transporter promoting the immuno-recognition of the tumour determine a chemo-immuno-resistant phenotype in non-small cell lung cancer (NSCLC). By screening 28 NSCLC cell lines, we found that ABCB1/ABCC1highABCA1low cells are resistant to chemotherapy and immune killing, while ABCB1/ABCC1lowABCA1high cells are chemo-immuno-sensitive [1], but the underlying molecular pathways are unknown.

To investigate the molecular circuitries involved, we built a CRISPR KO kinome library on NCI-H2228 chemo-immuno-resistant cell line. The Interleukin 1 receptor associated kinase 1 (IRAK1) was in the top 6 kinases that shifted the phenotype from ABCB1/ABCC1highABCA1low into ABCB1/ABCC1lowABCA1high. IRAK1-silencing in chemo-immuno-resistant NCI-H2228 cells transcriptionally decreased ABCC1 and ABCB1, and increased ABCA1, while IRAK1-overexpression in chemo-immuno-sensitive NCI-H1975 cells produced the opposite effects. IRAK1-silenced NCI-H2228 tumours implanted in NSG Hu-CD34+ mice were re-sensitized to cisplatin compared to wild-type tumours. Moreover, the transcriptomic profile of 110 NSCLC patients, subjected to cisplatin-based chemotherapy, revealed that IRAK1 was significantly more expressed in tumours than in non-tumoral lung tissues. Moreover, patients categorized as IRAK1high had worse progression free survival after chemotherapy and overall survival.

Our preliminary results unveil the role of IRAK1 as a new biomarker of chemo-immuno-resistance in NSCLC and as a predictive factor of chemoresistance and poor outcome in patients.

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Categories

Tumor Biochemistry

Mutant p53 (mutp53)-driven HMGA1 secretion promotes pancreatic ductal adenocarcinoma (PDAC) proliferation and chemoresistance

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive and lethal cancers. It has been found that mutant p53 (mutp53) modulates the secretion of signalling molecules, thus reprogramming the tumour microenvironment (TME) to drive cancer invasion. Importantly, the characterization of cancer secretome may lead to the identification of druggable targets. Therefore, we investigated how mutp53 modulates the secretome released by PDAC cells and affects the TME. We demonstrated that mutp53-driven secretome exerts an oncogenic role in PDAC cells. In detail, we performed mass-spectrometry analysis to detect secreted proteins modulated by mutp53 and, among them, the nuclear high mobility group A1 (HMGA1) was chosen for further studies. HMGA1 is an architectural transcription factor involved in several cellular processes, whose high intracellular expression levels are correlated with poor prognosis of PDAC patients. Interestingly, our data indicate that mutp53-induced secretion of HMGA1 promotes PDAC cells hyperproliferation and resistance to gemcitabine (GEM) treatment, thus suggesting a critical role of this protein in tumour aggressiveness. Furthermore, we found that GEM increases HMGA1 secretion in mutp53-PDAC cells. Since we previously demonstrated that GEM aberrantly stimulates mutp53 activity in PDAC, we assume that mutp53-driven HMGA1 secretion could be exploited as a mechanism of chemoresistance to GEM. Therefore, HMGA1 can be considered a therapeutic target in mutp53-PDAC cells. Taken together, our data indicate that mutp53-driven secretion of HMGA1 may stimulate key anabolic and oncogenic pathways. Moreover, we demonstrated that HMGA1 hypersecretion induces hyperproliferation and invasiveness of mutp53-PDAC cells, thus representing a promising secreted target in aggressive PDAC.

Categories

Tumor Biochemistry

The epi-drug ITF2357 (Givinostat) affects nuclear BRAF and oncogenic p53 interaction in melanoma cells

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Abstract

A high percentage of melanomas bear the V600E oncogenic BRAF mutation, which is responsible for high proliferation and malignancy. Using BRAFV600E-mutated SK-MEL-28 and A375 melanoma cells, we found oncogenic BRAF localization in the nucleus by either immunofluorescence or western blot of nuclear and cytosolic fractions. Hypothesizing that nuclear BRAF accounts for melanoma aggressiveness, we focused on a possible interaction with oncogenic p53. The two melanoma cells lines used for this study differentially display oncogenically mutated p53 (SK-MEL-28) and wild type p53 (A375). Immunoprecipitation confirmed BRAF interaction with oncogenic p53, which was found entirely in the nucleus of SK-MEL-28 cells. This interaction was weaker in A375 cells, suggesting that BRAF preferentially binds to the p53 oncogenic form. Interestingly, the HDAC inhibitor ITF2357 (Givinostat), an epi-drug with a potent anti-tumor activity (1), dramatically reduced BRAF and oncogenic p53 levels in SK-MEL-28 as well as their interaction. The compound also targeted BRAF in A375 cells but not (wild-type) p53, which conversely increased most likely promoting ITF2357-induced apoptosis. P53 Silencing experiments confirmed that the response to the compound in BRAF mutated melanoma cells depends on p53 status, thus suggesting a rationale for a possible ITF2357-based melanoma targeted therapy.

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Categories

Tumor Biochemistry

Mitochondrial citrate carrier (SLC25A1) is NOT essential for KRAS-mutated pancreatic ductal adenocarcinoma (PDAC) cell growth

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Abstract

Cytosolic citrate maintenance is ensuring by mitochondria transporter SLC25A1 or by extracellular import across the plasma membrane belonging to the SLC13 family transporters. PDAC is characterized by KRAS mutations, which continuatively stimulate effector pathways inducing tumor growth. Indeed, in Panc-1 and Patu8988T cells characterized by $KRAS^{G12D}$ and $KRAS^{G12V}$ mutations respectively, glutamine metabolism increases NADPH production. Since SLC25A1 is involved in the production of NADPH and in lipids synthesis, we hypothesized its important role in KRAS mutated PDAC cell growth. By silencing SLC25A1 in KRAS wt (Bx-PC3) and KRAS mutated (Panc-1 and Patu8988T) cells, we evaluated the cell growth. Surprisingly, we demonstrated that SLC25A1 is only necessary for KRAS wt PDAC cell growth. Secondary, we hypothesized that extracellular citrate or similar substrates, could avoid the Bx-PC3 shSLC25A1 cell death. Indeed, citrate, succinate, and α-ketoglutarate partially restore the growth defect induced by SLC25A1 silencing in Bx-PC3 cells. RT-qPCR data showed a drastic increase of SLC13A3 expression in Bx-PC3 shSLC25A1 cells. This result suggests that Bx-PC3 cells rely on cytosolic citrate for their proliferation, while SLC25A1 is silenced; SLC13A3 is overexpressed trying to supply cytosolic citrate and other substrates for their survival. Although the induced $KRAS^{G12V}$ mutation in Bx-PC3 cells seems to induce an increase expression of SLC13A3, this latter does not seem to be responsible for the cytosolic citrate independence. Finally, according to literature data, KRAS mutations improve cellular survival by promoting glutaminolytic pathway and cellular metabolic reprogramming that, probably, make KRAS mutated PDAC cells independent by cytosolic citrate.

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Categories

Tumor Biochemistry

Lactate accumulation reshapes the myelofibrotic microenvironment metabolic profile

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Abstract

Primary myelofibrosis (PMF) is characterized by ineffective clonal hematopoiesis, splenomegaly, and bone marrow fibrosis. This outcome is also given by the microenvironment changes where malignant cells rely on glycolysis as a primary metabolic pathway, thus producing lactate as a byproduct. The aim of our work is to dissect the role of lactate shuttling as a novel target in PMF treatment. For this purpose, we supplemented lactate to healthy mesenchymal stromal cells measuring their proliferation. Furthermore, we assayed the accumulation of different fibrotic and inflammatory markers by western blot analysis and multiplex array. Our results were corroborated by using a TPOhigh Zebrafish (*Danio rerio*) model. We unveiled that PMF patients' CD34+ cells are characterized by an increased expression of lactate shuttling channels MCT1 and MCT4. Furthermore, the incubation of peripheral blood mononucleated cells with PMF serum enhanced Treg and Myeloid-derived suppressor cell expansion. Corroborating the central role played by lactate in this context, this effect was reverted by MCT1 selective inhibitor AZD3965. Therefore, lactate supplementation increased cancer-associated fibroblast markers a-SMA, FAP1, and TGFb. Interestingly, both lactate and PMF sera triggered extracellular matrix remodeling, calcium accumulation, and histone lactylation, which were reverted by AZD3965. Similar results were also shown on the whole kidney marrow of our TPOhigh Zebrafish model. Overall, our results unveil lactate shuttling as a possible target for PMF treatment.

Bibliographic references

Categories

Tumor Biochemistry

SELECTIVE ANTICANCER EFFECT OF S-ADENOSYLMETHIONINE ON HUMAN GLIOBLASTOMA CELL LINES VIA CELL CYCLE ARREST, MITOTIC CATASTROPHE, APOPTOSIS AND INHIBITION OF DNA REPAIR

Roberta Grillo[1], Roberta Veglia Tranchese[1], Roberta Arpino[1], Francesca Cadoni[1], Cristina Pagano[2], Giovanna Navarra[2], Laura Mosca[1], Giovanna Cacciapuoti[1], Marina Porcelli[1]

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Abstract

Glioblastoma multiforme (GBM) is the most common highly invasive primary tumor in the brain with a median survival ranging from 14 to 30 months [1]. Despite aggressive multimodal treatments, prognosis for patients with GBM continues to be poor. A growing body of evidence indicates that natural compounds exhibit effective anticancer activity and might be used in the treatment of GBM. Among them, recent research identified the naturally-occurring sulfonium compound S-adenosyl-L-methionine (AdoMet), the universal methyl donor and one of the most studied epigenetic regulators, as a modulator of multiple signaling pathways important for carcinogenesis, including proliferation, apoptosis and autophagy via different signaling pathways [2]. Several clinical studies have demonstrated that, at pharmacological doses, AdoMet has a low incidence of side effects with excellent tolerability and no toxic or antiproliferative effects in normal, non-tumorigenic cells. In agreement with this, we found that AdoMet selectively and efficiently causes a time- and dose-dependent inhibition of GBM cell viability, without affecting non-tumorigenic cells growth.
In the current study, we reported the selective anticancer effects of AdoMet on human GBM U87MG, U343MG, and U251MG cell lines and we explored the underlying mechanisms. We provided evidence that AdoMet inhibited GBM cell viability and proliferation by inducing cell cycle arrest and apoptosis. We also demonstrated that AdoMet targeted DNA repair, cell cycle checkpoint activation, and spindle assembly processes leading to mitotic catastrophe-induced cell death. Taken together these findings suggest that AdoMet may be a promising adjuvant for the development of new targeted therapies against GBM.

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Categories

Tumor Biochemistry

Metabolic-driven immune changes in melanoma in a gender-dependent manner

Marta Iozzo[1], Giuseppina Comito[1], Luigi Ippolito[1], Giada Sandrini[2], Elisa Pardella[1], Carlo Catapano[2], Paola Chiarugi[1], Elisa Giannoni[1]

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Abstract

The epidemiology of melanoma has highlighted sexual dimorphism with a higher incidence and mortality rate in the male population. Gender disparity in immune response has been highlighted as a key point in cancer. Alterations of metabolism in the tumor milieu have been shown to affect the balance between immune surveillance and immune escaping. In general, melanoma shows an increased glycolytic metabolism. We hypothesize that fluctuating levels of metabolites, differentially enriched in the environment of male and female melanoma, can regulate and support different immune responses related to sex. We aim to better investigate the gender dependence of metabolic regulation on the immune component.

We performed a metabolic analysis of representative male and female melanoma cell lines. Male cell lines showed high levels of HKII/LDH-A/MCT4, accompanied by a more pronounced secretion of lactate. IHC analysis performed on TMA obtained from melanoma patients showed high LDH-A expression in male samples, accompanied by a more pronounced CD4+ infiltration. We observed a different composition of the immune infiltrate in melanoma cell lines, appreciating a significant increase in %Treg upon exposure of CD4+ cells with SkMel28 male melanoma cells. The impairment of LDHA in SkMel28 cells by genetically and pharmacological (FX-11) approaches showed reduced Treg percentage. We also observed a significant enrichment of Treg in male melanoma specimens. Our data show an increase in LDH-A expression in male melanoma and suggest a role for lactate in eliciting an immunosuppressive landscape with high Treg infiltration.

Categories

Tumor Biochemistry

Stromal lactate sustains a tumor cell-derived collagen signature through the activation of P4HA1-DDR1 axis in prostate cancer cells

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Abstract

Increasing evidence highlighted the role of cancer cell-derived extracellular matrix (ECM) in the acquisition of aggressive features. Recently, non-cell autonomous metabolic reprogramming in tumors can provoke striking transcriptional and metabolic changes within tumor cells enhancing their malignancy. Lactate sustains a prostate cancer cells (PCa)-cancer-associated fibroblasts (CAF) crosstalk. However, how lactate can impact on the function of tumor-derived ECM remodeling is poorly addressed.

GSEA analysis on PCa cells exposed to lactate reveals that lactate conditioning promotes the expression of several collagens. Importantly, lactate promotes an enhanced activity of the collagen α-ketoglutarate-dependent prolyl-4-hydroxylase 1 (P4HA1) and the inhibition of MCT1 lactate inward transporter blocks P4HA1 expression and activity. Also, we found that P4HA1 targeting is detrimental for lactate-induced invasiveness and transendothelial migration in vitro and in vivo. Interestingly, we identified a non-integrinic collagen I receptor – discoidin-domain collagen receptor 1 (DDR1) – as responsible for triggering tumour collagen-dependent signaling, leading to a lactate-induced invasiveness and stem-like features achievement. Also, we found that DDR1 activation is confined by tumour, not CAF collagens. Ultimately, we highlighted that DDR1 acts via STAT3 activation, whose inhibition results in a decreased collagen I expression and inefficient prostaspheres formation in lactate-treated cells. Finally, we showed that tumor xenografts from DDR1- or P4HA1-silenced PCa cells are impaired to lung metastasize in mice experiencing CAF/lactate exposure.

Overall, these findings uncover a new aspect of tumour metabolic reprogramming driven by CAF-derived lactate, which sustains PCa cell ability to disseminate and metastasize through the autocrine activation of P4HA1-collagen-DDR1 axis.

Categories

Tumor Biochemistry

Isolation of rat primary breast carcinoma cells to study the synergic action of nano-constructs on the microenvironment and antitumor efficiency

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Abstract

Breast carcinoma in rats was generated with a single dose of 7,12-Dimethylbenzatracene (DMBA), intraperitoneally administered in 5 weeks old female Wistar rats. After 24 weeks, a solid tumor mass was generated and surgically removed. The extracellular matrix (ECM) was removed from the tumor mass using dissecting microsurgery and the tumor tissue treated with a mixture of ultrapure (>99%) recombinant collagenases (Class I and Class II) and thermolysin. Selected characterized population of primary breast carcinoma cells were used to generate three-dimensional multicellular tumor spheroids/organoids, to evaluate *in vitro* the effects of the recombinant collagenases (in the best formulation) for the digestion of the ECM and the subsequent target and efficiency of Nanoparticles (NPs) functionalized with tumor targeting molecules. Furthermore, primary cells-induced tumor in female Wistar rats will be treated with recombinant collagenases to reduce the thick and dense collagen matrix around the mass, making it more accessible to NPs.

Therefore, standardized isolation protocol for primary cancer cells is fundamental to preserves important cell characteristics and properties and to obtain reliable sources for *in vitro* and *in vivo* studies for cancer treatment.

Categories

Tumor Biochemistry

FADS1/2-mediated lipid metabolic reprogramming drives ferroptosis sensitivity in metastatic triple-negative breast cancer

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Abstract

Triple-negative breast cancer (TNBC) is frequently refractory to therapies and patients experience recurrence with metastases. Metabolic flexibility is crucial for tumor progression and metastatic cascade, and lipid metabolism is often altered in TNBC and therefore exploitable for therapeutic and diagnostic purposes. Genome-wide profiling together with an array of metabolic techniques applied to TNBC cells with different metastatic abilities revealed enhanced de novo fatty acid (FA) biosynthesis and content (i.e., lipid droplets, LD) in the more aggressive models. Since targeting lipid metabolism with different agents was ineffective in reducing survival, we postulated that the increased FA synthesis could be relevant as it alters lipid composition and complexity. Indeed, metastatic cells showed increased expression of FA desaturases (FADS1 and 2) and subsequent alteration in the content of polyunsaturated FA (PUFA) that emerged as a metabolic vulnerability since they are peroxidation substrates for ferroptosis execution, a form of iron-dependent cell death. Accordingly, aggressive TNBC were sensitive to ferroptosis inducers and displayed increased ROS levels and accumulation of toxic lipid peroxides. Mechanistically, targeting FADS1/2 prevented cell death, whereas inhibition of stearoyl-CoA desaturase 1 (SCD1) failed since this enzyme catalyzes the rate-limiting step in the production of monounsaturated FA (MUFA). To note, altering PUFA/MUFA ratio by increasing the availability of exogenous MUFA exerted an anti-ferroptosis effect. Moreover, inhibiting LD formation and turnover suppressed the LD buffering capacity and potentiated cell death. Clinically, high levels of FADS1/2 correlate with worse prognosis and highlight a potential metabolic liability useful for synthetic lethality therapeutic approaches in TNBC.

Categories

Tumor Biochemistry

PDL-1 promotes proliferation of glioblastoma cells

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Abstract

Glioblastoma frequently expresses PDL-1 causing a defective host anti-tumoral immunity. We previously found that in the course of a cell culture, PDL-1 increased concomitantly to cyclin-D on G1/S transition, to progressively decrease until cell confluency, when proliferation ceased. We found a correlation between CCND1 and PDL-1 gene expression levels. Herein, we investigated whether PDL-1 could play a direct role in the proliferation of this tumor. Using different glioblastoma cell lines, we show that PDL-1-silencing impaired proliferation, measured in flow cytometry and immunoblot exploiting Ki67 and PCNA proliferation markers. Conversely, PDL-1 ectopic expression showed opposite results with increased proliferation levels of overexpressing cells in comparison with control cells. As FKBP51s regulates the expression of PDL-1 serving as cochaperone, we investigated the effect of FKBP51s depletion on glioblastoma cell proliferation. Using FKBP51s siRNA, we downmodulated the expression of FKBP51s and, as expected, PDL-1 was impaired. Such an effect was accompanied by reduced proliferation of silenced-glioblastoma cells, compared with non-silenced cells. Our study provides preliminary results that PDL-1 positively regulates glioblastoma cell growth, adding a further aspect of malignancy to this molecule involved in tumor immune evasion.

Categories

Tumor Biochemistry

PHGDH heterogeneity is a key driver of 5-Fluorouracil resistance in colorectal cancer

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Abstract

Cancer cells are strongly dependent on serine availability to support biosynthetic pathways and fast proliferation by fuelling one-carbon metabolism. Phosphoglycerate dehydrogenase (PHGDH) is the rate-limiting enzyme in the de novo serine synthesis pathway (SSP), a highly regulated pathway overexpressed in several cancer types. Recently, it emerged that PHGDH expression is dynamically regulated during different stages of tumor progression, promoting cancer aggressiveness. In our laboratory, we demonstrated that high serine availability, due to both increased exogenous uptake or SSP activation, supports resistance of colorectal cancer (CRC) cells to 5-Fluorouracil (5-FU) administration. The present study aims to investigate whether PHGDH heterogeneity may act as critical determinant of 5-FU response in human CRC. Interestingly, western blot and immunohistochemistry analysis of CRC patients-derived cancer tissues revealed a great heterogeneity among patients in PHGDH protein levels, as well as a strong spatial heterogeneity within tumor tissues, hinting PHGDH as a possible driver of different 5-FU responsiveness among CRC patients. By means of distinct colon cancer cell lines, we demonstrated that high PHGDH expression correlates with reduced 5-FU sensitivity with respect to low expression of the enzyme. The modulation of PHGDH expression levels, with specific inhibitor or through protein overexpression, correlates with altered sensitivity to 5-FU treatment in CRC cells. Finally, we demonstrated that modulating PHGDH levels interferes with intracellular content of metabolic intermediates of the Krebs Cycle, particularly succinate and α-ketoglutarate, two known epigenetic regulators.These data underline a strong correlation between PHGDH levels and 5-FU response and open new possibility therapeutic options for patients.

Categories

Tumor Biochemistry

Circulating tumor cells detection by Raman spectroscopy

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Abstract

Circulating tumor cells (CTCs) detach from solid tumors and circulate in the bloodstream of cancer patients. CTC are a precious tool in clinic since their number correlates with patient prognosis and they can be used ex-vivo to study the primary tumor characteristic. Nevertheless, the current CTC detection methods rely on the expression of surface markers and are not universal for all tumor types. Thus, efficient and universal CTC detection method are urgently required. Here, we used deuterium as vibrational tag to develop a new CTCs detection method based on Raman microspectroscopy (RS) and Warburg effect, the cancer cell capability to internalize glucose faster than healthy cell. As model normal and cancer prostatic cells (PNT2, PC3), and hepatocarcinoma (HepG2) cells were used. Cells were cultured in presence of deuterated glucose and then analyzed by RS. The deuterium Raman band was present only in the cancer cell spectra. These results indicate the presence of Warburg effect in our cellular model and that cancer cells can be differentiated from normal cells following glucose metabolism. To simulate the CTC presence in blood, PC3 and HepG2 cells were co-cultured with white blood cells o healthy donors in presence of deuterated glucose. The deuterium Raman signal was observable only in PC3 and HepG2 cell spectra. Our data demonstrate that cancer cells can be distinguished from healthy cells exploiting the glycolytic metabolism also when they are in the same media. These results shed a light on the possibility to develop new CTCs detecting methods based on RS.

Categories

Tumor Biochemistry

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

Laura Marrone, Valeria Di Giacomo, Simona Urzini, Mariapaola Gammella, Marialuisa Alessandra Vecchione, Chiara Malasomma, Andrea Cerullo, Maria Fiammetta Romano, Simona Romano

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Abstract

Tumor-associated-macrophages (TAMs) play a pivotal role in promoting tumor progression and therapy resistance; their targeting has recently emerged as a promising strategy for cancer defeat. Current approaches focus on reprogramming TAMs from the M2 pro-tumoral to the M1 anti-tumoral phenotype to kill cancer cells. Unfortunately, in-depth knowledge of TAMs and a signature that can reliably identify them still needs to be improved. We identified a splicing isoform of the FKBP5 gene, FKBP51s, exploited by cancer cells to suppress undesired immunity and highly expressed in circulating monocytes of cancer patients resistant to immunotherapy. Aim of this study is to decipher the role of FKBP51s in TAM biology to identify new potential therapeutic targets to their reprogramming. Alternative-macrophage polarization showed typical features of M2, such as STAT1 downregulation in favor of STAT3/6, and a shift towards arginase1 metabolism and scavenger receptors expression. Interestingly, FKBP51s levels strongly increased in M2-macrophages, thus suggesting that FKBP5 alternative splicing occurs in TAMs. FKBP51s silencing restored STAT1 activity, increased the secretion of pro-inflammatory cytokines, while impairing the anti-inflammatory cytokines. The depletion of the splice isoform also impacted on TAMs migration, invasiveness, and T-cells proliferation. Finally, FKBP51s silencing also impaired OXPHOS, typical of M2 macrophages, and restored the glycolytic activity, an M1 feature. Results from this study suggest a relevant role for FKBP51s in promoting the pro-tumoral activities of TAMs and highlight this splice isoform as a new potential therapeutic target to reprogram TAMs towards an anti- tumoral macrophage phenotype, thus overcoming the immune suppressive tumor microenvironment.

Categories

Tumor Biochemistry

Human ABCC6 protein comes into play in mechanisms which control the aggressiveness of hepatocarcinoma cells

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Abstract

Multidrug Resistance-Associated Protein 6 (MRP6, also known as the ABCC6 protein) is a member of the superfamily of ATP-binding cassette (ABC) proteins which is encoded by the human Abcc6 gene. Mutations in the Abcc6 gene are associated with Pseudoxantoma elasticum (PXE), an autosomal recessive condition marked by a gradual ectopic calcification of elastic fibers in cutaneous, ocular and vascular tissues.

ABCC6 contributes to the efflux of ATP outside of the cell. In the extracelluar environment the ATP concentration is maintained by ecto-nucleotidases, such as CD39 and CD73, which hydrolyze released ATP rapidly to ADP, AMP and then adenosine. In the physiological contest ABCC6 helps both to the production of pyrophosphate thus preventing ectopic calcification and contributes to modulate the purinergic signaling, a cell communication system which regulate different physiological and pathological cellular functions including cancer (1).

Recent researches suggested that knock-down or pharmacological inhibition of ABCC6 in hepatocarcinoma HepG2 cells lead to down-regulation of CD73, a protein associated with more aggressive tumor phenotype. In addition, ABCC6 is also involved in the cytoskeleton's reorganizations and cell motility (2,3).

The clonogenic assay showed a less aggressive phenotype of Abcc6-silenced HepG2 cells. Silenced cells have high levels of epithelial marker E-caderin and low levels of mesenchymal markers N-cadherin and vimentin, proteins involved in the epithelial-mesenchymal transition (EMT). Compared to control cells, they are also less able to invade the extracellular matrix. Therefore, ABCC6 may provide a viable target for anticancer therapy in tumors where is overexpressed.

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Categories

Tumor Biochemistry

Molecular pathways involved in the biological effect of lemon essential oil in an *in vitro* experimental model of resistant human acute lymphoblastic leukaemia

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Abstract

Cancer is one of the most concerning health issues for the developed world, with around 17 million new cases and over 8 million deaths in 2020, a condition exacerbated by the rising appearance of chemoresistance to standard therapy. Essential oils (EOs) are phytocomplexes, whose effects have been widely studied for their pharmacological value, including against chemoresistance. The EOs of *Citrus* spp. have stood out among others for their significant anti-cancer properties, along with those of their individual constituents. This research was therefore designed to study the anti-proliferative effects of *Citrus limon* essential oil (LEO) in an *in vitro* experimental model of resistant human acute lymphoblastic leukaemia, and to evaluate its mode of action.

In this study, LEO and its furocoumarin-free fraction, LEO-FF, were evaluated in human leukaemic T-lymphoblasts (CCRF-CEM) and their doxorubicin-resistant counterpart (CEM/ADR5000).

Treatment of CCRF-CEM and CEM/ADR5000 cells with increasing concentrations of LEO and LEO-FF produced a significant anti-proliferative effect in both cell lines, associated with an increase of both early and late apoptosis, without alteration of cell cycle progression. Investigation of a broad spectrum of genes associated with cancer showed that treatment with LEO enhanced both nuclear factor erythroid 2-like 2 (Nrf2) and eukaryotic initiation factor 2 (eIF2) signalling pathways, underlining the presence of a strong oxidative stress thus leading to the activation of the ferroptosis machinery.

Our results suggest that LEO and LEO-FF could be valuable anti-cancer agents, both in sensitive and resistant leukaemic cells, through potentially inducing both apoptosis and ferroptosis, triggered by oxidative stress.

Categories

Tumor Biochemistry

Development of an oral mucosa model useful to characterize drug delivery systems for the Oral Lichen Planus treatment

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Abstract

Oral lichen planus (OLP) is a chronic mucocutaneous inflammatory disease with a malignant transformation rate of 1.5%.Current therapeutic strategies involve the administration of immunosuppressive drugs (like Dexamethasone -DEX-) through a topical treatment characterized by the difficulty of obtaining preparations able to adhere to the mucosa without interfering with the patient's normal activities. Hence, developing and validating in vitro models of the oral mucosa to determine both the release of the drug in contact with the mucosa and its ability to cross it is fundamental. Thus, the aims of the project are:

1) Setup of an in vitro oral mucosa model.

2) Development of specific DEX Delivery Systems.

To obtain a 3D model of the oral mucosa, a bioreactor (IV Tech) and PLA scaffolds were used: after evaluating different conditions to mimic salivary flow and allow cell proliferation, it was observed that fibroblasts attached to the scaffold with a flow rate of 100 µl/min and proliferated with a flow rate of 400 µl/min. Chitosan-coated PLA fibers and PLGA nanoparticles are two drug delivery systems currently under investigation. Spectrophotometric techniques were used to measure the drug release kinetics, and HPLC was used to assess the drug recovery from both the cell culture medium and the cell lysate.The MTT test was used to detect cytotoxicity, while SEM was used to investigate morphology.These findings provide valuable insights for further research in the development of an in vitro 3D model of the oral mucosa and specific drug carriers for the treatment of OLP.

Categories

Tumor Biochemistry

Inactive mutated VEGFR2 promotes tumor growth interacting with wild-type receptor

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Introduction: In cancer, the activation of the VEGF/VEGFR2 pathway regulates both stromal and parenchymal cell biology and metabolism, supporting tumor progression. For this reason, VEGFR2-targeted tyrosine kinase inhibitors (TKi) are widely used in the clinic to treat different cancer types. However, primary or acquired resistance often occurs, likely due to the acquisition of novel mutations. Here we studied the mechanism of action of the most frequent non-synonymous mutation R1032Q of VEGFR2. Although this substitution entails a loss of function of the receptor, the expression of $VEGFR2^{R1032Q}$ in cancer cells promotes tumor growth.

Material and Methods: By using protein-protein interaction assays, molecular imaging (FRAP, FLIM/FRET) and enzymatic assays we studied the dimerization, membrane dynamics and activation of mutated receptor. Next, we set up a melanoma model with heterozygous R1032Q mutation of VEGFR2 which was exploited to characterize the pro-tumorigenic effects and drug response of $VEGFR2^{R1032}{}_{Q}$.

Results and discussions: $VEGFR2^{R1032Q}$ forms functional heterodimers with wild-type VEGFR2, altering the membrane dynamics of the wild-type receptor and increasing the VEGFR2-associated intracellular signaling in the absence of exogenous VEGF-A stimulation. In a melanoma model, heterozygous $VEGFR2^{R1032Q}$ triggers pro-oncogenic events modifying gene expression, cell metabolism, and increasing cell growth and metastasis *in vitro* and *in vivo*. Also, the expression of $VEGFR2^{R1032Q}$ increases melanoma cell resistance to the VEGFR2-targeted TKi linifanib and vatalanib.

Remarkably the R1032Q substitution of VEGFR2 occurs in a hot-spot residue of the kinase domain which is recurrently mutated in many other receptor tyrosine kinases (RTKs), including EGFR, KIT, FLT3, FLT4 and PDGFRA, among others. This underscores the importance of mutations found at this position. Moreover, mutations of corresponding residues across different proteins elicit similar effects and may be similarly targeted. Therefore, our results anticipate the effects and druggability of all other uncharacterized mutations corresponding to the substitution R1032Q of VEGFR2.

Conclusion: Our data reveal a possible ligand-independent inter-receptor kinase activation of $VEGFR2/VEGFR2^{R1032Q}$ heterodimers which drives tumor progression. This novel mechanism of activation of VEGFR2, which may be shared by other RTKs, could be exploited to develop new therapeutic approaches to treat tumors harboring the $VEGFR2^{R1032Q}$ mutation and possibly all other corresponding ones.

Patterns of immunoproteasome subunit expression and assembly into proteolytic particles in gastric cancer cells with different histotypes: implications for cancer metastasis

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Abstract

Gastric adenocarcinoma which represents 90% of gastric cancers remains an aggressive and poorly understood malignancy with a heterogeneous presentation and tumor biology. Understanding the molecular basis of this variability is important for effective treatment. Due to its role in controlling protein homeostasis, the proteasome is considered an attractive drug target in cancer. More recently, its variant called the immunoproteasome has been implicated in the biology of different types of tumors and its expression correlated to disease outcome. Neverthless, relevance of the immunoproteasome in neoplasia is poorly understood. It has been recently appreciated that besides expression, proteasome subunit assembly into the catalytic 20S core and association of this particle with different regulators have important implications in tumorigenesis and response to therapy. Based on this evidence, we have characterized proteasome patterns in gastric cancer cells representative of different histotypes (Lauren's classification): epithelial (23132/87), epithelial/diffuse (MKN45) and diffuse (KATO III). Native PAGE analysis followed by western immunoblotting and in gel activity assays with fluorogenic substrates demonstrated that cells with diffuse-type components express higher levels of immunoproteasome subunits which are preferentially incorporated into active 19S-capped regulatory particles. Inhibition of immunoproteasome activity with ONX 0914 significantly affects cell migration in diffuse-type gastric cell lines. Analysis of differentially expressed proteins in KATO III cells by LC-HR-MS-based proteomics highlighted that ONX 0914 affects several signaling pathways involved in tumor invasion and metastasis which are under investigation. These results suggest that pharmacological inhibition of the immunoproteasome may be useful in treating metastatic gastric cancers.

Categories

Tumor Biochemistry

Natural compounds against tumor dormancy, a potential therapeutic target in tumor recurrence and metastasis prevention

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Abstract

Tumor dormancy is a state of some cancer cells' life characterized by mitotic cell cycle arrest in G0/G1 phase. The dormant cells exhibit a stem-like phenotype, survive in a quiescent state, and wait for appropriate environmental conditions to begin proliferation again giving rise to metastasis. Moreover, dormant cancer cells appear to be responsible for tumor initiation, sustained growth, and their presence is believed to play an important role in tumor metastasis and resistance to chemotherapy or radiation therapy.

In a previous paper we demonstrated that chronic hypoxia (1% O2) selects MDA-MB-231 population that presents the cancer stem-like phenotype with the expression of CD24-/CD44+/ESA+ and spheroid forming capacity. In this model of dormant state, we show that the vegetable extract CC is able to decrease the expression of CD44 marker leading to cells death. These data suggest that this vegetable extract CC could have an inhibitory effect on the transition to cancer stem-like phenotype suggesting its further applications as a therapeutic approach.

Categories

Tumor Biochemistry

Gene-expression variation and analogies with 5-Fluorouracil of two novel ethylene heteroaryl compounds

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Abstract

During the pharmacological research of new compounds with promising antitumor activity, our research team focused on the metabolic and biochemical impact of two new Heteroaryl-Ethylenes molecules, PB4 and GC-VI-70, which synthesis and Toxicity impact are already reported by this research group in the literature [1,2]. We evaluated their impact on gene expression, using 5-Fluorouracil (5-FU) as a comparison model. The two compounds were tested for 24 hours on the Colorectal Cancer cells line Caco-2 ATCC HTB-37™ at a concentration below their IC50 at 24 hours: 0.15 µM for PB4 and 1 µM for GC-VI-70, both calculated with a Linear Regression Model. Then, RNA was extracted, and an Amplicon mRNA was conducted using a Custom Panel comprising 56 genes, involved in Oxidative stress, immunoregulation and inflammation, apoptosis and other pathways involved in different cellular process such as: metalloproteinase, nAChr, ATP Binding Protein. PB4 shows a dramatic gene silencing impact on most genes, probably depending on its acute toxicity, except for TXN2 and CHRNA2 (Oxidative stress and nAChr). GC-VI-70 instead has a similar trend to 5-FU, reporting a variation of gene expression analogous to 5-FU, especially for TGFB1, IL8 (immunoregulation and inflammation), NOX1, KEAP1, CAT (Oxidative stress) and ABCC1 (ATP Binding Cassette). These results encourage further investigation to match new compounds synthesis and the molecular pathway they impact. CG-VI-70 show an apparent 5FU mimicry; this behavior and the modulation of the cellular metabolism make it an interesting candidate for further studies for pharmaceutical purposes to take action on altered biochemical pathway.

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Categories

Tumor Biochemistry

Role of BPGM in regulating cell proliferation

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Abstract

Bisphosphoglycerate mutase (BPGM) has both mutase activity that converts 1,3-BPG, to 2,3-BPG and phosphatase activity, which converts 2,3-BPG to 3-phosphoglycerate (3-PG). These enzymatic reactions are known as Luebering-Rapoport pathway or shunt.

BPGM has a well-known role in red blood cells where it is highly expressed and controls the oxygen binding to hemoglobin, while little is known about its role in maintaining the glycolytic flux.

We have hypothesized that BPGM can be involved in the regulation of the metabolism of proliferating cells. Shunting the glycolytic pathway, indeed, reduces the ATP production efficiency allowing proliferating cells to increase the glucose influx to the rate that is necessary to produce anabolic metabolites such as 3-PG.

We have investigated the role of BPGM in regulating the proliferation of DU145 and PC3 cancer cell lines and of normal human dermal fibroblasts (HDFs). We observed that BPGM mRNA and protein levels were higher when these cells were cultured in presence of serum compared to a condition of serum starvation.

In addition, we found that the silencing of BPGM in these cells increased the efficiency of the glycolytic pathway in term of ATP production, but it drastically reduced both the glucose uptake and the cell proliferation rate. In conclusion, our data suggest that BPGM is a key enzyme of the metabolic setting of proliferating cells.

Categories

Tumor Biochemistry

FAD synthesis and delivery to lysine demethylase 1: a crucial step in cancer epigenetics

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Abstract

Riboflavin (Rf) is an essential dietary component and the precursor of FMN and FAD, the redox enzymatic cofactors involved in a broad spectrum of biological processes like mitochondrial terminal metabolism and nuclear epigenetics (1). Alterations in flavin homeostasis are associated with several pathological conditions, among which cancer (1).

In the frame of the demonstration of an altered expression of Rf transporters in colorectal cancer, we recently proposed that cancer cells are greedy for Rf and its derived cofactors (2). To further confirm this idea, we compared the expression levels of FAD synthase (EC 2.7.7.2, FADS) in HPDE cells expressing wt-p53 with those of two pancreatic ductal adenocarcinoma cell lines, PANC-1 and MiaPaca2, carrying the R273H and the R248W mutations in p53, respectively (3). A significant increase in FADS expression was found in tumour cells and even higher in their derived cancer stem cells (CSCs) (4).

Since the increment in FADS is presumably demanded by the increased levels of “client” flavoproteins, we also evaluated the expression levels of the nuclear FAD-dependent lysine demethylase 1 (LSD1). Western blot and qPCR experiments confirmed the LSD1 increase. Previously performed dot blot and immunoprecipitation experiments (5) allowed us to propose a physical interaction between the FADS and LSD1. Here, we further confirm that the most abundant isoform of FADS, i.e., FADS2, directly associates with LSD1 by the *Gaussia princeps* Complementation Assay (GPCA), performed in HEK293T cells. Our data enforce the concept that FAD synthesis and delivery to LSD1 are crucial processes for cancer epigenetics.

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Categories

Tumor Biochemistry

A HYDROALCOHOLIC EXTRACT FROM SICILIAN GRAPE POMACE AS MODULATOR OF CANCER CELL DEATH

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Abstract

In recent years more interest has been shown on the possibility of using the waste, such as grape pomace obtained from winemaking industries, as a source of molecules beneficial to human health. In this scenario we have characterized a hydroalcoholic extract (HE) obtained from Sicilian grape pomace from the last harvest. The HPLC-DAD analysis revealed the presence of phenolic compounds, anthoxanthins and stilbenes. HE induced a time and dose-dependent reduction of viability of colon cancer HCT116 and breast cancer MDA-MB231 cells, being HCT116 cells more responsive. The effect was accompanied by oxidative stress induction, as demonstrated by the increase of ROS level and antioxidant enzymes. HE-induced death seemed to be related to a canonical apoptotic pathway in HCT116 cells with caspase-3 activation and the appearance of 89 kDa-PARP fragment. In MDA-MB231 cells, HE induced a reduction of pro-caspase-3 and the appearance of the 55kDa-PARP fragment, usually related to lysosome proteases. Since autophagy lysosomal pathway is the main mechanism for degrading molecules, we analyzed two specific markers, p62 and LC3. In HCT116 the level of these markers was significantly high at 24 h, probably indicating an initial autophagy commitment. Differently, in MDA-MB231 cells p62 was accumulated only after 48 h of treatment. Moreover, since the two cell lines show different p53 status (wild type and mutated, respectively) we hypothesize that this protein could play a crucial role for determining the autophagy/apoptosis balance. Studies are in progress to evaluate this hypothesis.

Categories

Tumor Biochemistry

New analogues of marine alkaloid Nortopsentin with antitumoral activity on breast cancer cells

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Abstract

Natural extracts constitute an important source of bioactive molecules. Numerous marine sponge-derived products have attracted considerable attention because of their unique biodiversity, structural differences from terrestrial natural products and antineoplastic activity. Nortopsentin, an alkaloid isolated from Spongsorites ruetzleri has been shown to have strong antiproliferative activity against neoplastic cells and is a major lead compound for the synthesis of new anticancer agents. Therefore, the synthesis of such naturally-derived Nortopsentin inspired compounds is of considerable interest. A library of Nortopsentin-inspired compounds was synthesized and assayed for cytotoxicity on a panel of several cell lines with variable level of transformation. MF164 analogue compound showed highly selective activity against malignant MCF-7 cell line when compared to non-tumoral MCF-10A cell line. Remarkably, MF164 is not cytotoxic for MCF-10A cells at the same concentration which is fully cytotoxic for MCF-7. We also evaluated the migratory capacity by wound healing technique and antiproliferative capacity by colony assay. Results showed relevant activity for MF164 compound in both cases. Moreover, as several studies showed that Nortopsentin activity is mediated by an action on Cdk-1, we started to evaluate through flow cytofluorimetry the effect of MF164 compound on cell cycle and our data show an arrest in G1/S phase. To better evaluate implicated pathways, further analyses are in progress to assess the expression of other possibly involved factors and identify specific molecular targets of MF164. We conclude that our compound has relevant perspectives in the development of antineoplastic drugs.

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Categories

Tumor Biochemistry

Overcoming drug-resistance in advanced prostate cancer by bifunctional inhibitors

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Abstract

Androgen deprivation therapy is the mainstay of treatment for advanced prostate cancer. However, following a period of remission, most patients develop a more aggressive castration resistant prostate cancer (CRPC) due to adaptive response in tumour.

Although second-generation therapies like Abiraterone (a CYP17A1 inhibitor) and Enzalutamide (an androgen receptor-AR antagonist) benefit patients with CRPC, drug resistance frequently occurs, eventually resulting in therapy failure. The overexpression of AKR1C3, the enzyme responsible of the synthesis of intratumoral androgens, is one of the most common mechanisms of drug resistance [1].

Treatment of abiraterone-resistant cells with AKR1C3 inhibitors like indomethacin, overcomes resistance and enhances abiraterone therapy both in vitro and in vivo by reducing the levels of intracrine androgens and diminishing AR transcriptional activity [1].

Recently, AKR1C3 inhibitors showed a bi-functional effect on the AR signalling, also exhibiting an AR antagonistic activity [2].

Starting from our potent and selective AKR1C3 inhibitors previously developed [3,4] and from the pharmacophore scaffold of either CYP or AR, new compounds were synthetized and tested for their ability to dually target AKR1C3 and either CYP or AR .

In vitro enzymatic and binding assays on purified proteins and cell-based assays were performed. The results obtained highlight the potential usefulness of bifunctional inhibitors targeting AKR1C3 and either CYP or AR to treat advanced prostate cancer.

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Categories

Tumor Biochemistry

New evidence on KRIT1 role in tumor biology and therapy

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Abstract

Melanoma is a common tumor characterized by high mortality consequent to its metastatic capability and resistance to therapy. In the last years, specific targeting of drivers of melanoma on cell proliferation and invasion, such as BRAF (vemurafenib), has been developed. However, successful treatment is limited by the early development of drug resistance. Very recently we provided the first indications about the role of KRIT1 in the growth and migration of melanoma by using KRIT1 knockdown melanoma cells and human melanoma specimens. We detected a significant and progressive decrease in KRIT1 protein expression which is correlated with tumoral aggressiveness [1].

We investigated the potential role of KRIT1 in the modulation of molecular signals following treatment with vemurafenib and in the development of drug resistance. In melanoma cells treated with vemurafenib and in vemurafenib-resistant cells, we observed a decrease in KRIT1 expression, indicating that KRIT1 may be involved in the activity of vemurafenib and in the process of drug resistance. It is known that KRIT1 is located inside the cell in a complex structure connected to the cytoskeleton and to β-tubulin. Vemurafenib treatment induces a dissociation between KRIT1 and b-tubulin suggesting that vemurafenib is able to determine a change in the intracellular localization of KRIT1. Furthermore, resistant cells showed an F-actin redistribution and a change in morphology.

The role of KRIT1 in cancer has never been investigated before. Our novel data are promising and encourage more in-depth studies to further characterize the impact of KRIT1 signaling in tumor biology and therapy.

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Categories

Tumor Biochemistry

Lactic acid secreted by cancer associated fibroblasts supports ferroptosis resistance in prostate carcinoma

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Abstract

Cancer cells display an elevated flexibility in the utilization of nutrients available in the tumor microenvironment to acquire aggressive traits. In prostate cancer (PCa), cancer associated fibroblasts (CAFs) reprogram their metabolism, upon contact with tumor cells, resulting in enhanced secretion of lactic acid. Besides, PCa cells exploit CAF-derived lactic acid to sustain OXPHOS metabolism and lipid anabolism, leading to lipid droplet (LD) accumulation and utilization of acetyl-CoA for epigenetic reprogramming, ultimately supporting an invasive phenotype. Several non-cell autonomous mechanisms are known to regulate cancer cell sensitivity to ferroptosis. This form of cell death is driven by extensive peroxidation of phospholipids containing polyunsaturated fatty acids (PUFAs) at cell membranes and is mediated by accumulation of intracellular redox active iron. We found that either conditioned media collected from CAFs or exogenous lactic acid similarly protects PCa cells from cell death promoted by two ferroptosis inducers (RSL3, Erastin) and inhibits lipid peroxidation. TMA analysis of tumor tissues from PCa patients revealed a positive correlation between high Gleason grade and GPX4 protein levels, an antioxidant defense enzyme strongly regulating ferroptosis. Remarkably, targeting of DGAT1/2, involved in LD accumulation, rescues ferroptosis sensitivity, suggesting that lipid accumulation into LDs mediates the observed resistance mechanism. Moreover, inhibition of MCT1 or targeting of the extracellular pH regulators, carbonic anhydrase IX/XII, reverts ferroptosis resistance. Overall, our data highlight the role of stromal lactic acid in modulating ferroptosis sensitivity in PCa, identifying lactic acid uptake as a promising therapeutic target to sensitize PCa cells to ferroptosis inducers.

Categories

Tumor Biochemistry

Tissue metabolite composition drives metastatic organotropism in prostate cancer

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Prostate cancer (PCa) is the second-leading cause of cancer-associated death in men. Because of recent advances in primary tumor treatment, mortality in PCa is now increasingly related to metastatic disease. PCa differentially metastasizes to various organs with a propensity to bone and lung. Understanding the molecular adaptations driving the remarkable tropism of PCa cells for these secondary organs may be crucial for designing new successful cancer treatments.
Growing evidence has demonstrated that cancer-associated fibroblasts (CAFs) in primary tumors facilitate the progression of metastatic disease by rewiring cancer cell metabolism and remodeling the extracellular matrix. In particular, by RNA-seq and GC-MS analysis, we demonstrated that CAF-derived lactate modifies the transcriptional and metabolic profile of PCa cells, finally fostering their metastatic potential.
Here we investigated how lactate pre-conditioning in the primary tumor preselects for properties that subsequently support cancer cells fitness in specific distant organs. Using a spheroid assay, we found that treating PCa cells with lactate stimulates 3D but not 2D growth, suggesting an enhanced ability to proliferate in the metastatic niche. Remarkably, by measuring organ metabolite composition using mass spectrometry on different tissues from healthy mice, we identified a set of metabolites particularly enriched in the bone and lung. Modulating the availability of these metabolites is sufficient to interfere with PCa cells' ability to grow in low-adhesion conditions mimicking cellular outgrowth in the metastatic niche.
This data provides evidence of a nutrient environment-dependent regulation of PCa metastatic organotropism opening new possibilities for therapeutic strategies in PCa patients.

METHIONINE GAMMA-LYASE: AN ENZYME FOR NUTRIENT STARVATION IN CANCER THERAPY. Addressing challenges through molecular mechanistic studies and a novel delivery strategy.

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Abstract

The exploitation of specific peculiarities of cancer cell metabolism is a promising approach for cancer treatment. Consequently, enzymes capable of limiting the supply of essential nutrients have gained increased recognition as potential anticancer drugs. In this context, methionine gamma-lyase (MGL), a bacterial methionine-degrading enzyme, represents an attractive antineoplastic candidate that targets methionine dependence.

Building on our previous study of the effects of MGL-mediated depletion of methionine (a precursor of the major methyl donor in methylation reactions) on the post-translational modifications of canonical H3 and H4 histones [1], the present investigation analyzes the modifications on histone variants (H1, H2A, H2B and H3.3) in HT-29 cancer cells upon MGL treatment [2]. This study - based on a high-resolution mass spectrometry pipeline - helps to shed light on how variant histones may contribute to fine-tuning chromatin organization and function and may unveil mechanistic details and potential biomarkers.

Concurrently, spherical citrate-capped gold nanoparticles (AuNPs) have been explored as nanocarriers for MGL to devise an effective strategy for enzyme delivery. Nanoparticle size, shape, functionalization yield and retention of MGL enzymatic activity and structure integrity were investigated by a panel of biochemical and biophysical techniques to deepen our understanding of the interactions between the inorganic matrix of AuNPs and the protein cargo.

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4-Methyl umbelliferone effects on cell proliferation in human glioma cells.

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Abstract

Background. 4-Methyl umbelliferone (4-MU) is a derivative of coumarin and already an established therapeutic currently used in humans mainly for its choleretic and biliary antispasmodic activity. 4-MU has been shown to inhibit HA production in multiple cell lines and tissue types both in vitro and in vivo. Different studies have been shown that 4-MU inhibits the proliferation, migration, and invasion of multiple cancer cell types, both in vitro and in vivo.

Aim and methods. Aim of this study was to evaluate the effects of 4-MU on glioma cells of different grades. Glioma cells were treated with 4-MU at different doses (from 0 to 1 mM) for 24 or 48 hours and its effects on proliferation and apoptosis were evaluated.

Results. 4-MU decreased cells proliferation in a dose dependent manner over the dose of 0-1 mM. We also evaluated the expression of Akt, caspase-3, bcl-2 and cyclin D. 4MU significantly reduced the expression of cyclin D, Akt and birc1 while enhanced the expression of the proapoptotic caspase.

Conclusions. In conclusion our results demonstrated that 4-MU is able to reduce the proliferation rate of glioma cells through the modulation of cell cycle and apoptosis signals. Given these results more studies are needed to evaluate the potential of 4-MU as new therapeutic tool in the treatment of gliomas.

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Tumor Biochemistry

Mitochondrial impairment induced by new tamoxifen-derived metal complexes in breast cancer cells

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Abstract

Tamoxifen is an anticancer drug inhibiting the pro-proliferating activity of estrogen on breast tumors. However, tamoxifen cytotoxicity also involves mitochondrial targeting, suggesting multiple mechanisms of action. Despite its wide use as hormone adjuvant therapy, the development of resistance in initially responsive breast tumors is a recurring issue.

Therefore, basing on tamoxifen backbone, we designed and synthesized new gold(III) and copper(II) complexes with the aim of generating multitarget compounds with an enhanced efficacy against breast cancer. The choice of coordinating these specific metals stems from the fact that several gold complexes are known to inhibit thioredoxin reductase (TrxR), a selenoenzyme often overexpressed in cancer cells, and that copper compounds affect cellular redox homeostasis promoting oxidative stress.

The complexes have been tested on breast cancer cell lines either expressing or not the estrogen receptor (ER). Firstly, we analyzed their cytotoxicity, which resulted similar to the parental compound and even greater for the copper complex. Then, through an in silico analysis, we found comparable ER binding energy of tamoxifen and its derivatives. In order to dissect the target of the new complexes, we assessed their effect on TrxR and on the overall cellular redox balance. The gold complex was the only one inhibiting TrxR by alkylating TrxR's active site. However, in cancer cells, also the copper complex induced an increased production of reactive oxygen species and a decrease of thiol levels. Moreover, the two complexes elicited a decrease of both cellular respiration and the mitochondrial membrane potential indicating mitochondrial damage.

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ENHANCED EXPRESSION OF NICOTINAMIDE N-METHYLTRANSFERASE IN HUMAN OSTEOSARCOMA: POTENTIAL ROLE IN TUMOR GROWTH AND CHEMORESISTANCE

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Abstract

Osteosarcoma (OS) is the most frequent primary malignant tumour of bone, mainly arising in children and adolescents. The 5-year survival rate of OS patients ranges between 70% (localized disease) to 20% (metastatic of relapsed forms). Due to chemosresistance and high tendency to metastasize, OS patients often display a poor prognosis (1). Therefore, the identification of new therapeutic biomarkers for effective OS treatment is urgently needed.
Among molecules potentially affecting OS cell phenotype, we speculated about the contribution of the S-adenosyl-L-methionine-dependent cytosolic enzyme nicotinamide N-methyltransferase (NNMT), catalyzing the N-methylation reaction of nicotinamide and structural analogs (2). NNMT was reported to be overexpressed in many solid neoplasms, promoting proliferative and invasive capacities, as well as drug resistance (3).
Immunohistochemistry was used to analyze NNMT expression in normal and tumor bone tissue specimens, obtained from OS patients. Subsequently, OS cell lines (U2OS and Saos-2) were transfected with plasmid vectors coding for short hairpin RNAs leading to NNMT transcript degradation. The efficiency of enzyme knockdown was then determined by Real-Time PCR and Western blot analyses. Further cell-based assays were performed to evaluate the effect of NNMT silencing on cell viability, proliferation, migration and chemosensitivity.
Obtained results showed a significant enzyme upregulation in OS compared with healthy bone tissue. Moreover, NNMT downregulation in OS cell lines was significantly associated with decreased proliferation and migration, as well as to enhanced sensitivity to chemotherapeutic treatments.
These data reasonably propose NNMT as an interesting diagnostic and therapeutic biomarker for human osteosarcoma

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Stromal-derived lactate activates amoeboid motility by engaging the Endocannabinoid receptor GPR55 in a prostate cancer model

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Abstract

Many studies sustain the impact of the endocannabinoid system (ECS) in the interplay between cancer cells and tumor microenvironment. In a prostate cancer model (PCa), we demonstrated the establishment of a metabolic cross-talk between PCa cells and cancer-associated fibroblasts (CAFs), in which lactate released by CAFs impacts the metabolic and epigenetic rewiring of PCa cells, culminating in the improvement of metastatic potential. To understand the impact induced by CAFs on the ECS components, we initially focused on the modulation of the cannabinoid receptors (CBRs) expression in PCa cells, upon exposure to CAF conditioning or lactate treatment. We observed that the classical CBRs (CB1,CB2) are not regulated by stromal conditioning, while the non-canonical GPR55 and TRPV1 are up- and down-regulated, respectively. Remarkably, lactate is able to activate the ERK1/2 pathway downstream GPR55, suggesting an unexplored role of lactate as GPR55 ligand. Through the engagement of GPR55, lactate sustained tumor cell migration/invasion and some molecular traits characteristic of the amoeboid phenotype, such as the sub-membrane reorganization of actin filaments, RhoA activation and MLC2 signaling. To reinforce the role of lactate in promoting a GPR55-mediated amoeboid motility, we observed that i) the selective inhibition of GPR55 with ML193 prevented the acquisition of amoeboid traits; ii) lactate-driven PCa invasiveness cells is sensitive to RhoA inhibition by CT04, but unresponsive to MMP impairment by Marimastat. Overall, these data propose a novel contribution of lactate to PCa malignancy, by directly activating a GPR55/RhoA signaling which endows PCa cells with amoeboid-based invasive skills.

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Biochemical analysis of a selective compound library for aldehydes dehydrogenase1A3

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Abstract

The aldehyde dehydrogenases superfamily includes 19 $NAD(P)^+$ dependent enzymes that catalyse the oxidation of a variety aldehydes into the corresponding carboxylic acids. The ALDH1As as key molecules involved in cell proliferation, survival, and chemo/radio-resistance of cancer stem cells (CSCs). The ALDH1A3 was associate whit a poor prognosis in a lot of tumours, such as, breast cancer, gliomas, and pleural malignant mesothelioma (MPM). We focused our work of one compound derives on the patented fluorescent probe 10 (PCT/IB2022/053216) with a selective inhibitory activity on ALDH1A3 named CD2272. Based on preliminary results of this compound, a library of 24 compounds was synthesized. Through the analysis of residual activity, we isolate 13 potential candidates, and we decide to test their effect on MPM cells, and we selected the three most promising inhibitors. To complete the inhibitory kinetics analysis, IC_{50} and K_I parameters were calculated to confirm the effective potency and evaluate the affinity between the protein of interest and the target of our best compounds. We try to simulate the binding of the molecules in the active site of ALDH1A3 using modelling. This simulation highlights that all three compounds bind in the active site at the end of the catalytic tunnel in proximity of catalytic cysteine and the aminoacid involved in the binding are the same ones that stabilize the original substrate retinaldehyde. The ALDH1A3 is a good new target in MPM, and this approach could give a breakthrough in MPM therapy as current therapeutic solutions are poor and ineffective.

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Tumor Biochemistry

ADAPTIVE MECHANISMS TRIGGERED BY PHARMACOLOGICAL COMPLEX I INHIBITION IN OVARIAN CANCER

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Abstract

In the last years the involvement of mitochondrial respiration has been increasingly recognized as pivotal player during tumor progression and chemoresistance. In particular, targeting respiratory Complex I (CI) has been proposed as a new therapeutic approach to hinder cancer growth. In this context, we have demonstrated that a severe CI impairment promotes a delay of tumor expansion but not its complete eradication. Indeed, over time CI-defective cancer cells survive and reactivate their proliferation, allowing us to speculate that adaptive mechanisms occur to overcome the metabolic and molecular consequences of mitochondrial impairment. This scenario might be relevant in ovarian cancer (OC), where about 85% of patients develop relapses after standard surgical and pharmacological treatments. Here, we show that under metabolic stress condition the activation of AMPK allows CI-defective cancer cells to experience the downregulation of mTORC1 activity and the block of protein synthesis which could explain their proliferation slowdown. The survival of CI-defective quiescent cells may be supported by an upregulation of a matricellular protein SPARC associated with the cytoskeleton remodeling likely mediated by PKCa activation. The dissection of such different pathways may offer potential molecular players in synthetic lethality with CI inhibition, thus providing new synergistic strategies for cancer treatment and, in particular, for OC.

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Identification of metabolic vulnerabilities in ER+ breast cancer cells resistant to estrogen deprivation using RNA interference screening.

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Abstract

Aromatase inhibitors (AI) represent the first-line endocrine therapy for postmenopausal women with estrogen receptor positive (ER+) breast cancer. Unfortunately, resistance limits their clinical efficacy and metabolic reprogramming plays a major role. In this study, we wanted to uncover innovative metabolic vulnerabilities using a loss-of-function shRNA screening in cells exposed to long-term estrogen deprivation (LTED), a condition that mimics AI resistance. The library includes 5 shRNA lentiviral vectors targeting 2,752 genes encoding all known human metabolic enzymes and transporters that we obtained by cross-referencing maps of metabolic pathways with the KEGG database, plus 45 control genes that we know to be important for endocrine therapy response. By adapting and applying the Model-based analysis of genome-wide of CRISPR/Cas9 Knockout algorithm, we identify 58 selected genes whose silencing reduced LTED cell survival. Over Representation Analysis (ORA), using Cluster Profiler Analysis and EnrichR Analysis, showed that negatively selected genes were enriched in many fundamental pathways, including arginine and proline metabolism, purine metabolism, and membrane lipid metabolic process. Indeed, Protein-Protein Interaction Networks Functional Enrichment Analysis (STRING) of the essential genes, revealed a network cluster consisting of proteins involved in proline biosynthesis, including the Aldehyde Dehydrogenase 18 family member A1 (ALDH18A1). Interestingly, analysis of publicly available clinical datasets showed that higher ALDH18A1 expression correlates with poor survival in ER+ breast cancer. Collectively, our preliminary data suggest that proline biosynthesis could confer advantages for ER+ breast cancer cells survival under estrogen deprivation and will offer novel potential predictive biomarkers and/or therapeutic targets implicated in AI resistance.

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NAMPT in melanoma: linking NAMPT-dependent metabolic reprogramming and immune regulation.

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Abstract

Resistance to treatment dramatically impacts on the survival of metastatic melanoma (MM) patients. BRAF(i)nhibitors-resistant MM cells showed increased amounts of NAD, an essential redox cofactor, supporting their metabolic adaptations. This was obtained selectively overexpressing the rate-limiting NAD-biosynthetic enzyme nicotinamide phosphoribosyltransferase (NAMPT). NAMPT-NAD axis becomes a driver of melanoma progression and resistance to BRAFi1-3. Its inhibition can be therapeutically exploited1.

Results show that mutations in the BRAF oncogene positively correlate with NAMPT expression. NAMPT is overexpressed and genetically amplified in a region on the 7q22 chromosome that includes a co-amplification of PIK3CG among other 39 genes. NAMPT and PIK3CG expression is positively correlated, and NAMPT overexpressing MM cell lines show overactivation of the PI3K pathway. We are starting to evaluate cellular responses to NAMPTi/PI3K-γi combination, thinking to a synergistic effect in melanoma model.

A second set of preliminary data revealed a positive correlation between NAMPT expression and interferon signaling, including upregulation of PD-L1, IRF1, STAT1 genes analyzing TCGA melanoma cohort. Treatment of MM cells with interferon up-regulates NAMPT that is directly correlated with PD-L1 levels, suggesting a potential common mechanism of regulation via interferon. Lastly, data from NAMPT immunoprecipitation/mass spectrometry in MM cellular extract revealed a physical interaction between NAMPT and proteins involved in the regulation of immune response.

The multiple roles of NAMPT as intracellular and soluble protein are known; here we speculate that NAMPT could have a role in regulating signaling (i.e., PI3K and interferon) involved immune responses, with a possible impact on ICIs activities.

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Interaction of lipid metabolism and ferroptosis in myeloid leukemia cells over-expressing the short isoform of GATA-1 (GATA-1_S)

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Abstract

Ferroptosis is a recently recognized form of regulated cell death involving lipid peroxidation. Recently, interest has grown in ferroptosis induction as a potential strategy to overcome drug resistance in hematological malignancies. GATA-1 is a key transcriptional factor controlling hematopoiesis-related gene expression. Two GATA-1 isoforms, the full-length protein (GATA-1_{FL}) and a shorter isoform (GATA-1_S), are described. A balanced GATA-1_{FL}/GATA-1_S ratio helps to control hematopoiesis, with GATA-1_S overexpression acting as a pro-leukemic factor[1]. Based on the evidence that lipid metabolism is closely related to cell sensitivity to ferroptosis, we asked whether the two GATA-1 isoforms may differently regulate lipid metabolism. Interestingly, a lower content of long chain-PUFAs along with reduced lipid peroxidation was found in in K562 myeloid leukemia cells overexpressing GATA-1_S[2]. Accordingly, gene expression studies in these cells also showed down-regulation of ACSL4 and DEGS1, two pro-ferroptosis enzymes involved in the production of long chain-PUFAs, along with increased levels of glutathione peroxidase 4 (GPX4), a key anti-ferroptosis player[3]. Studies on GPX4 inhibition revealed that GATA-1_S over-expression prevents K562 cells from ferroptosis. These findings were corroborated by in silico analysis of a publicly available leukemia dataset showing that four genes related to lipid metabolism (ASAH1, STAT1, LPCAT3 and GPX4) were significantly upregulated in cells over-expressing GATA-1[4]. This study provides the first evidence that GATA-1_S over-expression prevents K562 cells from ferroptosis through modulation of lipid metabolism to promote pro-proliferative and survival pathways in hematopoietic cells and could shed light on new therapeutic targets to induce cell death and overcome chemoresistance in hematological malignancies.

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Effect of Curcumin on Prostate Cancer Prevention and Treatment: Molecular and Metabolic Mechanisms

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Abstract

Prostate cancer (PCa) is the second most common cancer in men. Although epidemiological studies indicate that curcumin (CUR) reduces the risk and incidence of PCa, the anticancer mechanisms have not been fully understood yet. Our results showed that CUR inhibits PC-3 cell viability by inducing apoptosis and ROS production. Also, we demonstrated the ability of CUR to inhibit cell migration and reverse the EMT pathway. Then, we focused on PCa metabolism and we found that CUR can restore cellular energetic metabolism and inhibits several enzymes and proteins involved in lipid metabolism, restoring their expression to normal levels, and so further reducing tumor progression. Despite CUR's therapeutic value, several factors limit its use in clinical practice. Thus, we compared the biological effectiveness of CUR with that obtained with a dextran/curcumin (DEX/CUR) conjugate. The effect of the conjugate was to increase the uptake of CUR inside the cancer cells and consequently increase its active bioavailable share. Lastly, the conjugate was used as a tool to encapsulate and vectorize doxorubicin into PC-3 cells. The combination treatment made possible the improvement of the therapeutic efficacy of PCa, together with a significant reduction in the doses of the drug used, and, therefore, in the toxic effects on healthy tissues. In conclusion, although in vivo studies are required to prove the actual therapeutic performance of the proposed nanosystem, our preliminary in vitro data can be considered a proof-of-principle for the use of targeted nanotechnology-based CUR for the treatment of this deadly disease.

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PGM3 inhibition shows cooperative effects with erastin inducing pancreatic cancer cell death via activation of the unfolded protein response.

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive cancer with a poor patient prognosis[1]. Remarkably, PDAC is one of the most aggressive and deadly tumor types and is notorious for its resistance to all types of treatment. PDAC resistance is frequently associated with a wide metabolic rewiring, particularly the glycolytic branch named hexosamine biosynthetic pathway (HBP) whose final product is the uridine-5'- diphospho-N-acetyl-Dglucosamine (UDP-GlcNAc), the main substrate for O- and N- protein glycosylation. These post-translational modifications play critical roles in several protein features such as folding, activity, and function. Therefore, we have recently developed and tested a novel compound, FR054, capable of diminishing the HBP flux by targeting the HBP's enzyme PGM3[2,3]. Here, we performed transcriptional and bioinformatic analysis to obtain further information about HBP inhibition. Moreover, we used cell count, western blot, HPLC, and metabolomics analysis to determine the impact of the combined treatment between FR054 and erastin (ERA), a recognized ferroptosis inducer, on PDAC cell growth and survival. Of note, the combined treatment applied to PDAC cell lines induces a significant decrease in cell proliferation and a concurrent enhancement of cell death. Furthermore, this combined treatment induces unfolded protein response (UPR), NFE2-like BZIP transcription factor 2 (NRF2) activation, a change in cellular redox state, a greater sensitivity to oxidative stress, a major dependence on glutamine metabolism, and finally ferroptosis cell death. Our study discloses that HBP inhibition enhances, through UPR activation, the ERA effect and therefore might be a novel anticancer mechanism to be exploited as PDAC therapy.

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Tumor Biochemistry

Immunological targeting of tumor cells: a novel approach

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During early stages of carcinogenesis, cancer cells become invisible to the immune response. From this moment on, the evolution of the tumor depends essentially on the genotype of the original cancer cells and on their subsequent genetic drift. Therefore, a clinical treatment that is capable of restoring the expected anti-tumor role of the immune system could prove to be an effective weapon against cancer.

We found that the activation of antigen presenting cells (APCs) by inflammatory cytokines induced the secretion of microvesicles that transfer the class I major histocompatibility (MHC-I) to the surrounding cancer cells. We have exploited this ability of APCs to develop a therapeutical approach for the treatment of solid cancers. The novelty of the proposed treatment relies on loading the class I MHC molecules with commonly used vaccine antigens (VAs) that can quickly activate the cytotoxic response of memory $CD8^+$ T lymphocytes in immunized patients. We have tested this approach on immunocompetent BALB/c mice that were immunized against ovalbumin protein (OVA). We found that the injection of OVA-specific APCs in immunized tumor-bearing mice reduced tumor growth compared to the injection of OVA alone or PBS. These findings pave the way for utilizing commonly used antigens as vaccine in cancer immunotherapy.

M I S C E L L A N E O U S

Evidence for autologous cell therapy in diabetic retinopathy: protective effects of human pericyte-like adipose-derived mesenchymal stem cells on human retinal endothelial cells.

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Abstract

Diabetic retinopathy (DR) is characterized by morphological and metabolic alterations of endothelial cells (EC) and pericytes (PC) of the blood-retinal barrier (BRB). Loss of interendothelial junctions, increased vascular permeability and loss of PC from the vascular bed are the main features of DR.

Previous results demonstrated that, under high glucose concentrations, human adipose stem cells (ASCs) and their differentiated pericyte-like phenotype (P-ASCs), exhibited high rate of proliferation and remarkable migratory capacity [1].

In in vitro co-culture we demonstrated that (i) P-ASCs, more resistant to high glucose, modulate the HREC response to hyperglycemia and (ii) some molecular mechanisms activated in both cell types in a cross-talk that could counteract the retinal damage in DR.

In co-culture with HREC, P-ASCs induced a reduction of the release of TNF-α, IL-1β and MMP-9 and the angiogenic factor VEGF. Furthermore, P-ASCs counteracted the HG-induced activation of the phospho-ERK1/2/phospho-cPLA2/COX-2 pro-inflammatory pathway [2].

Assuming that endothelial cells secrete PDGF-BB [3] and prolonged hyperglycemia causes the chronic imbalance in PDGFR signaling, we found that the crosstalk between HRECs and ASC or P-ASC is based on the PDGF-B/PDGFR-β axis.

Furthermore, in high glucose, the specific activity of cPLA2 in P-ASC/HREC co-cultures and VEGF-A and PGE2 levels were markedly reduced compared to co-cultures of undifferentiated HREC/ASC. Finally, P-ASCs in contact with HRECs showed a reduction of TNF-α, IL-6, IL-1β and MMP-9 at the mRNA level.

In conclusion, our data indicate that human P-ASCs can be considered good candidates to protect the functional characteristics of BRB in DR.

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Categories Miscellaneous

Boosting NAD in senescent microglia to fight neurodegeneration?

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Abstract

In neurodegenerative disorders and senescence, microglia, the brain immune cells, acquire a disease-associated microglia signature that may favor tissue repair in early disease state but at late stages lose its protective homeostatic functions. Senescent microglia exhibit a secretory associated senescence phenotype, deficits in phagocytosis, and impaired metabolism, with depletion of NAD, which plays a central role in genome integrity and cell metabolism. Emerging evidence highlighted lower levels of NAD in senescence and neurodegenerative diseases, with consequent impairment of sirtuins' activity.

The aim of this study was to investigate changes that occur during senescence in microglia developing an in vitro model of chronically exposure (up to 30 days) to high iron concentration. Initially, iron treatment induces microglia to proliferate more, enhances phagocytosis, and increase NAD levels suggesting microglia activation. After 30 days of treatment microglia acquired a senescent-like phenotype characterized by proliferation arrest, decreased phagocytosis, upregulation of SASP markers with a significant increase in EVs production. Biochemical analyses showed decreased levels in NAD content in iron-treated microglia, concomitantly to an increased expression of CD38 (the major NAD consuming enzyme). Moreover, the levels and activity of Sirtuin 6, which is downregulated in aged/senescent cells, were strongly reduced compared to control microglia. Senescent microglia co-cultured with healthy microglia induced senescent traits in healthy cells, as revealed by a significant increase in SA-ß-Gal and p21 positive cells and in reduced levels of NAD. We are currently trying to understand the molecular mechanism underlying senescence propagation to healthy cells.

Categories

Miscellaneous

Multiomics approaches for therapeutic developments in ataxia telangiectasia.

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Abstract

Ataxia Telangiectasia (A-T) is a rare autosomal multisystemic disorder caused by biallelic mutations in the ATM gene, which codes for a kinase protein (PIKK family) named ATM that is implicated in a plethora of biochemical pathways. Currently not any cure is available but positive effects of neurologic features in AT patients have been achieved by dexamethasone (dex) administration through autologous erythrocytes (EryDex) in a phase II and phase III clinical trials. The ATM splicing variants named ATMv1 ATMv2 and miniATM, observed in A-T patients during the clinical trials, and concurrent in “a silico” designed ATM variant, named ATMv3, led us to investigate their role in an A-T cellular model and their possible ability to reversing A-T phenotype. To this purpose, a metabolomic and proteomic investigation was performed by HR-MS. Hundreds of compounds and proteins were found as modulated by ATM variants, thus recovering some of the molecular functions impaired in A-T; in particular, it was demonstrated the greater capability of ATMv3 in recovering intermediates of glycolytic pathway and Krebs cycle, especially Acetyl CoA recovery. In addition, in redox homeostasis and nucleotide levels, ATMv3 was able to improve the typical A-T impairment. These results have shown the possibility to choose the most prominent ATM variant to be employed in a next gene therapy or gene delivery for the treatment of A-T.

Categories

Miscellaneous

STAT3-loaded Extracellular Vesicles: basis of a new possible therapeutic approach to restore STAT3 signalling deficiency

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Abstract

Recently, extracellular vesicles (EVs) emerged as a promising drug delivery tools for their intrinsic ability to cross biological barriers, protect their cargo, and target specific cells or tissues (1). Here we propose the use of EVs as platform to deliver a functional form of STAT3 to restore its signalling in the Autosomal Dominant Hyper-IgE syndrome, disease characterized by negative mutations in the stat3 gene (2).

A novel recombinant fusion construct of STAT3 tagged with EGFP was produced using a baculovirus-based expression system and characterized from a biochemical and biophysical point of view. EVs were isolated from RO cells conditioned medium by ultracentrifugation, EGFP-STAT3 was encapsulated using a saponin-assisted method followed by Size Exclusion Chromatography (3). The obtained EVs were characterized by Nanoparticle Tracking Analysis (NTA), Transmission Electron Microscopy (TEM), immunoblotting and fluorescence detection.

NTA and TEM analyses revealed that EGFP-STAT3 EVs presented size range of 80-150 nm, in accordance with previous report (4). CD63, EVs surface marker, as well as STAT3 were detected by immunoblotting, and densitometric analysis of the obtained bands indicates that 2.7 ± 0.9 µg of EGFP-STAT3 were associated with the EVs. Confocal images confirmed that the EVs successfully delivers STAT3 protein into PBMCs.

Our data represent an interesting starting point for the development of new therapeutic strategy to restore STAT3 signalling.

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Categories

Miscellaneous

Gene knockout of *DDC* in SH-SY5Y cells using CRISPR/Cas9: A promising neuronal model for aromatic amino acid decarboxylase (AADC) deficiency.

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Abstract

DDC gene encodes aromatic amino acid decarboxylase (AADC), the pyridoxal 5-phosphate-dependent enzyme that catalyzes the last step of dopamine and serotonin synthesis. Mutations in this gene cause a severe form of infantile Parkinsonism known as AADC deficiency. AADC-deficient patients show a reduced synthesis of serotonin and dopamine, leading to a complex movement disorder and global neurodevelopmental delay. Currently, there is no effective treatment for AADC deficiency and no suitable models to investigate the molecular mechanisms of the disease. In this study, we established single-cell-derived knockout clones of *DDC* from SH-SY5Y cells taking advantage of the CRISPR/Cas9 technology. The presence of an inserted cassette or indel mutations was verified at each cut site by Sanger sequencing. *DDC* KO clones displayed a reduced expression of *DDC* at mRNA level, while the absence of the protein was confirmed by western blot. Likewise, the activity of AADC was null in *DDC*-KO clones. The expression of the proteins involved in the dopaminergic pathway was evaluated by qPCR and western blot, while dopamine and serotonin metabolites were measured using mass spectrometry. Notably, SH-SY5Y cells can differentiate into cells with morphological and biochemical characteristics of mature neurons by sequential exposure to retinoic acid and brain-derived neurotrophic factor. Therefore, *DDC*-KO SH-SY5Y cells may represent a novel *in vitro* model to investigate the intracellular effects of pathogenic AADC variants and could provide further insights into the molecular basis for the metabolic phenotype of homozygous and compound heterozygous patients, a prerequisite to develop pharmacological interventions for AADC deficiency.

Categories

Miscellaneous

Succinic Semialdehyde Dehydrogenase Deficiency: from molecular insights towards an organoid based disease model.

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Abstract

Succinic semialdehyde dehydrogenase (SSADH) is a key enzyme in γ-aminobutyric acid (GABA) metabolism. Its main role is to connect the TCA cycle with the catabolic cascade of GABA degradation.

Alterations in the *ALDH5A1* gene lead to an impaired GABA degradation, which is typical of SSADH-deficiency (OMIM #271980), a rare disease in which the toxic accumulation of GABA and its catabolites, mainly γ-hydroxybutyrate (GHB), causes a wide range of neurological and motor symptoms [1].

In order to obtain a deeper insight into the pathophysiology, we used HeLa cells overexpressing SSADH wildtype and disease-associated variants to determine its enzymatic activity, cellular localization, and post-translational processing. Subsequently, iPSCs generated from SSADH deficient patients carrying the variants W204X, G409D and R412X were analyzed. Bioinformatic analyses based on the crystal structure [2] revealed that W204X and R412X are results of stop codon mutations and they would not synthesize functional SSADH, while G409D would affect the catalytic domain. The obtained iPS cell lines were differentiated in 2D and 3D, *i.e.* neuronal progenitor cells (NPCs) and cerebral organoids respectively, and analyzed for differences in growth pattern and differentiation trajectories. The assessment of enzymatic activity, cellular localization, and viability assays was performed in 2D cultures of transfected HeLa cells.

HeLa cells transfected with disease-associated variants show a specific pattern for transit peptide processing which leads to a different mitochondrial enzyme uptake. Also, the enzymatic capability is affected. NPCs and Cerebral organoids highlighted differences in growth pattern which are consistent with a premature differentiation trajectory for disease-associated cells.

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Categories

Miscellaneous

Comparison of effects exerted by smoke extracts generated from heated and burned cigarettes on human bronchial epithelial cells and on their released extracellular vesicles.

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Abstract

The cigarette combustion generates bioactive substances that can alter the cellular redox system and promote the occurrence of highly oxidative conditions that, in turn, can affect the release of extracellular vesicles (EVs). Recently, we demonstrated that treatment of human bronchial epithelial cells (BEAS-2B) with burned cigarette smoke extract (bCSE) modified the phospholipid fatty acid composition and increase the carbonylated protein cargo of their released EVs (1). Over the last years, the consumption of heated tobacco has increased even if the effects are not yet completely defined. However, their effects on cellular metabolism as well as on the release and features of EVs are poorly investigated, so far.

In the present study, we compared the effects induced by smoke extracts generated from heated (hCSE) and burned cigarettes (bCSE) on BEAS-2B cells and on their released EVs. Cells were exposed to various concentration of hCSE and bCSE, according to previous studies (1,2). Both hCSE and bCSE reduced cell viability and induced an increase of ROS and carbonylated protein levels, in a dose-dependent manner. Increased levels of antioxidant enzymes have been also observed. However, the effects of hCSE were less pronounced compared to those exerted by bCSE. EVs released by hCSE- and bCSE-treated cells have been also biochemically characterized. Overall results evidenced that both CSEs affect either oxidative status of the cells and the biochemical content of released EVs, even if hCSE seems to be less pronounced.

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Categories

Miscellaneous

Semaphorin3A induces a novel cancer restraining signature in cancer-associated fibroblasts of pancreatic ductal adenocarcinoma

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Abstract

Pancreatic Ductal Adenocarcinoma (PDAC) has a unique tumor microenvironment (TME) characterized by desmoplastic stroma and overactivated cancer-associated fibroblasts[1,2] (CAFs). To date, targeting TME in PDAC is highly challenging and novel therapeutic targets are needed to efficiently hamper tumor growth.

We aimed to investigate the changes occurring in the TME of an orthotopic PDAC model upon Semaphorin3A (Sema3A) treatment. It has been shown that Sema3A, binding to its receptor PlexinA4, regulates immune system, angiogenesis and tumor progression[3,4,5]. We performed single cell RNA-sequencing (scRNA-seq) coupled with Spatial Transcriptomics (ST) to identify molecular changes in the TME of PDAC induced by a mutated form of Sema3A[5] (mut-Sema3A) able to bind PlexinA4 with higher affinity.

We observed that mut-Sema3A reverted the percentage of pro-tumoral CAFs, reprogramming them towards a novel cancer restraining CAF subtype. We defined a unique gene signature characterizing this unconventional class, called Sema-Associated Fibroblasts (SemAFs), that included up-regulated genes such as Pdgfra, Islr, Mmp2, Cygb, Thbs2, Cdh11 and mut-Sema3A receptor PlexnA4. Many of them, such as Islr-Meflin protein, have been correlated with improved survival and prognosis of PDAC patients[6,7]. Notably, mut-Sema3A treatment of ex-vivo purified activated fibroblasts enhanced the expression of SemAF genes and inhibited their chemo-invasion potential. Remarkably, the SemAF gene signature, and particularly Islr and PlexinA4, have been also identified by ST in mut-Sema3A-treated tumors.

These data suggest that Sema3A is a potent agent able of re-shaping the CAF population and it may represent a good candidate to be coupled with pre-existing therapies to more efficiently block PDAC progression.

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Categories

Miscellaneous

Obstructive sleep apnoea and Mild cognitive impairment: an insight on intermittent hypoxia effects and role of plasma biomarkers in prediction of cognitive decline insurgence.

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Abstract

Obstructive sleep apnoea (OSAS) is a sleep-breathing disorder affecting in particular elder population; it is related to the insurgence of mild cognitive impairment (MCI). Since MCI is considered a prodromal stage for Alzheimer's disease and a temporal window useful to slow down the progression of cognitive decline to dementia, a focus on mechanisms involved in MCI insurgence and new tools for early clinical intervention could be useful. Thus, focusing on possible link between Intermittent Hypoxia (IH) at tissues level caused by OSAS and MCI insurgence, this study was carried out with two main aims: (1) the development of a new in vitro model to mimic IH conditions on cell cultures to study effects on central nervous system (CNS) cell line; (2) the investigation of new possible peripheral biomarkers in plasma of OSAS patients to discriminates patients with MCI (OSAS+MCI) from patients without clinical signs of cognitive decline (OSAS-MCI). Obtained data showed that the IH condition led microglial cells to assume a "primed" phenotype, as demonstrated by the increase of priming markers (HLA-DRα, CX3CR1, CD86) and by the cellular overreaction in inflammation markers, following a mild inflammatory stimulus, suggesting the role for primed microglia in OSAS-driven neuroinflammation. Parallelly, data obtained from analysis of OSAS patients plasma suggested that HIF-1α and p-Tau could represent promising peripheral biomarkers to distinguish OSAS+MCI from OSAS-MCI patients, as their levels increased in OSAS+MCI group and positively correlated with the clinical parameter T90 (the cumulative time with oxygen saturation below 90% during sleep).

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Categories

Miscellaneous

Resolvins signaling modulation in an in vitro model of human inflamed keratinocytes

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Abstract

Resolvins of E- (RvE) and D-series (RvD) represent a class of bioactive lipids that belong to specialized pro-resolving mediators (SPMs) and derive from the polyunsaturated fatty acids (PUFA) omega-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Anti-inflammatory properties of RvD and E have been shown in different in vitro and in vivo studies, and alterations of their metabolism are involved in multiple diseases characterized by chronic inflammation. In this context, the evaluation of resolvins metabolism in skin inflammatory diseases, such as itch and dermatitis, seems noteworthy. Thus, in this study, an experimental model of inflamed human keratinocytes (HaCaT cells) was set up by using lipopolysaccharide (LPS) at different concentrations (2.5, 5.0, 7.5, 10.0, 12.5 µg/mL) at 24h. The best inflammatory condition (LPS at 10 µg/mL) was selected by evaluating the increase of the pro-inflammatory markers IFNγ and IL-6 gene expression. In this model, all the main resolvins system components have been evaluated at gene level, by quantitative Real Time Polymerase Chain Reaction (RT-qPCR). mRNA expression results showed a decrease of RvD1 and RvE1 receptors (ALX/FPR2 and ChemR23) and of 12-LOX enzymes (responsible for RvD3 synthesis), while there was an increase of 15-LOX (RvD1/2 synthesis). Results obtained suggest an involvement of both E- and D-series resolvins in skin inflammation, that needs further investigations in order to better evaluate their role in the resolution of skin inflammation and their potential exploitation in skin therapeutic approach.

Categories

Miscellaneous

Semaphorin receptor PlexinB1 inhibition reprograms immune cells hampering tumor growth and metastatic dissemination in mouse models of triple negative breast cancer

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Abstract

Semaphorin-Plexin signaling have a crucial role in the tumor microenvironment (TME). For instance, the class IV Semaphorin4D (SEMA4D) promotes tumor growth and metastasis (1,2). Nevertheless, the role of its high-affinity receptor PlexinB1 (PLXNB1) in the TME was not yet characterized. To this aim, we targeted PLXNB1 in the TME of mouse models of triple-negative breast carcinoma (TNBC) to investigate its relevance in tumor progression. We found that *Plxnb1*$^{-/-}$ mice displayed a strong reduction of primary tumor growth and metastasis. PLXNB1 depletion in the TME did not alter tumor vessel density but increased vessel normalization by improving pericyte coverage and decreasing intra-tumoral hypoxia. Moreover, *Plxnb1*$^{-/-}$ mice tumor associated macrophages (TAMs) displayed a shift in their polarization status towards a pro-inflammatory M1 phenotype. Interestingly, we observed an increased infiltration of cytotoxic T lymphocytes and of antigen presenting cells, together with a shift towards the Th1 phenotype in CD4^{+} T-cells. Furthermore, *Plxnb1*$^{-/-}$ tumor infiltrating lymphocytes displayed increased activation and improved anti-tumor gene signature. We next evaluated the translational relevance of targeting PLXNB1 in the TME re-programming in the response to immunotherapy. Interestingly, the efficacy of anti-PD-1 blocking antibodies was strongly enhanced in the absence of PLXNB1, significantly reducing tumor size and distant metastasis. Remarkably, pharmacological PLXNB1-blockade by systemic treatment with an antagonistic Fc-based engineered protein with a PLXNB1-binding peptide moiety (3), efficiently hampered TNBC growth in a preclinical model. Altogether, these results highlight PLXNB1 as promising therapeutic target for metastatic breast cancers for its signaling regulates the anti-tumor immune response in the TME.

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Categories

Miscellaneous

MESENCHYMAL STEM CELL IN ENTHESIS REGENERATION: TRANSLOCATOR PROTEIN 18 KDA (TSPO) AS A DRIVING DIFFERENTIATION MECHANISM

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Abstract

Mesenchymal Stem Cells (MSCs) have arisen as a pivotal tool in regenerative medicine. After tendon injury, the tendon-to-bone interface, “the enthesis”, often does not regenerate leading to high numbers of rupture recurrences [1]. First, we developed an implantable graft using biomimetic scaffolds as a skeleton to improve MSC differentiation. 3D-printed PCL and electrospinning PLGA were used to create a multiscale scaffold as a basis for the enthesis creation. To improve the MSC differentiation we deeply investigated new mechanisms as potential targets for scaffold engineering. Translocator Protein 18 kDa (TSPO) is a mitochondrial protein that plays a key role in mitochondria functionality and steroid synthesis by its association with the CYP11A1 enzyme [2]. Despite it has been involved in neuronal differentiation, less is known about its role in MSC. We demonstrated that TSPO expression was not altered during tenocyte differentiation, conversely, it increased during osteoblast differentiation suggesting the need for TSPO activation in the first phase of osteoblastogenesis. The treatment with the highly steroidogenic TSPO ligand PIGA1138 [3] induced an increase of CYP11A1 during osteoblast differentiation in parallel with an increase of the alkaline phosphatase activity, a known marker of osteoblast differentiation. Overall, these results suggest that TSPO could play a role in MSC differentiation, probably mediated by steroid production. Despite further investigations being needed, TSPO could represent a new mechanism in tuning osteoblastogenesis opening the way to its use as a target in regenerative medicine. (This research project is funded by Tuscany Region “Bando Ricerca Salute 2018”)

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Categories

Miscellaneous

Blood brain barrier damage in ischemic stroke: modulation by graphene/noble metal nanoparticles nanocomposites.

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Abstract

Ischemic stroke causes severe brain tissue damage and has become a leading cause of death globally. Currently, thrombolysis is the gold standard primary treatment but the short therapeutic window of opportunity limits thrombolysis utility [1]. The blood–brain barrier (BBB) impedes drug transport into the brain due to tight junctions among endothelial cells [2]. Noble metal nanoparticles (NPs), including gold (Au), silver and palladium, have good biocompatibility, which makes them very attractive for nanomedicine application of brain diseases. Particularly, NPs are able to cross the BBB, therefore can be used as drug delivery systems [3-4]. In this study, Au, Ag and Pd NPs, with size ranging from 20 to 100 nm, were prepared and their characterization was assessed by UV-visible spectroscopy, AFM, DLS and zeta potential, to estimate the nanoparticle optical diameter, the morphology, the hydrodynamic size and the surface charge, respectively. In order to mimic in vitro the condition of hypoxia related to the pathological situations of the ischemic stroke, we used the human brain microvascular endothelial cells (BMECs), which are the principal components of the BBB [5]. The cells were incubated with NPs and cytotoxicity was inspected via MTT assay. The cell migration was investigated by the wound scratch assay, while the cellular uptake and the organelle perturbation was scrutinized by confocal microscopy. Moreover, the levels of inflammatory cytokines such as IL-1, IL-6, IL-8, TNF-1, and HIF1 and VEGFA were detected. In conclusion, NPs, as given their anti-inflammatory intrinsic properties, could be used as nanomedicines themselves.

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Categories

Miscellaneous

Unraveling the Impact of Muscle Heme Metabolism on Tumor Microenvironment and Pre-metastatic Niche

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Abstract

Extensive metabolic changes in cancer lead to systemic inflammation and muscle mass loss. We hypothesized that muscle wasting could impact tumor development by providing metabolic fuel, and our research on Lewis Lung Carcinoma (LLC) tumor-bearing mice revealed lower levels of iron and heme in skeletal muscle. This was due to increased transcription of the heme exporter FLVCR1a in tumor-bearing muscles. To investigate muscle-derived heme's relevance in tumor progression, we created a skeletal-muscle specific knockout mouse model for the heme exporter (FLVCR1a$^{fl/fl}$; MyoD Cre$^{+/-}$ C/57) and inoculated LLC cells subcutaneously to assess tumor growth and metastatic potential. While there was no difference in tumor growth, silencing FLVCR1a in skeletal muscle significantly reduced pulmonary metastatic nodules. This suggests that muscle-derived heme could influence the tumor microenvironment, facilitating cancer cell intravasation and invasion. Analysis of fluorescence-activated cell sorting (FACS) and white blood cell (WBC) count revealed a specific decrease in neutrophils within the primary tumor and bloodstream of FLVCR1a knockout mice compared to wild-type mice. Interestingly, these findings align with suppressed levels of TIMP-1 in the serum, a cytokine known to promote metastasis by triggering Neutrophil Extracellular Traps (NETs) formation. Overall, our observations indicate that muscle FLVCR1a expression and/or function regulate local inflammation and likely systemic immunity, ultimately promoting cancer progression to metastasis. This study sheds light on the novel metabolic crosstalk between skeletal muscle and tumor progression and suggests that targeting this interaction holds promise for impeding cancer metastasis.

Categories

Miscellaneous

Identification of genes that regulate the internalization of α-synuclein pre-formed fibrils

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Abstract

Synucleopathies are a heterogeneous group of neurodegenerative diseases characterized by templated misfolding, aggregation and accumulation of the monomeric alpha-synuclein protein (α-syn) into soluble oligomeric assemblies and insoluble filamentous inclusions. Apart from neurons, pathological α-syn can be internalized by neighboring glial cells which, once activated, contribute to neuronal cell death associated with synucleopathies. Up-to-date very few glial and neuronal receptors are known for their involvement in extracellular α-syn uptake.

To contribute to the repertoire of molecular players and to determine to what extent is the human microRNAome involved in the internalization of α-syn pre-formed fibrils (PFFs) our group performed a genome-wide miRNA screen encompassing 2042 human miRNA mimics.

Uptake of α-syn PFF was probed in U87-MG glioblastoma cell line and the results of the primary screen suggest several miRNAs as either up- regulators or down-regulators of α-syn PFF uptake. Some of our “hits” were also previously found dysregulated in body fluids of patients afflicted by synucleopathies or other neurodegenerative diseases. To revalidate the high-throughput screening data; hit miRNAs were probed in different cellular models with two orthogonal approaches – fluorescence microscopy and flow cytometry.

With this approach we identified a strong downregulator of α-syn PFF uptake.

Categories

Miscellaneous

Role of endocannabinoid receptor TRPV1 in myogenic differentiation induced by sphingosine 1-phosphate in murine myoblasts

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Abstract

Skeletal muscle regeneration after trauma or during myopathies depends on myogenic differentiation process that involves cell cycle arrest and fusion of myoblasts into myofibers. Among different signals, sphingosine 1-phosphate (S1P) and endocannabinoids affect this process. S1P is a bioactive sphingolipid that exerts a plethora of biological functions mainly via the engagement of five specific G protein-coupled receptors, $S1P_{1-5}$. Specifically, $S1P_2$ receptor and the selective S1P transporter Spinster homolog 2 are known to be involved in the myogenic program induced by the sphingolipid[1]. Endocannabinoid system appears to regulate myogenic differentiation, since Cannabinoid receptor 1 (CB1) antagonism was shown to enhance the expression of myogenic markers in human satellite cells[2]. A functional interplay between the two bioactive lipid systems has been found in C2C12 murine myoblasts. S1P, indeed, increased TRPV1 expression and counteracted the effect on the mitochondrial membrane potential[3] of methanandamide (mAEA), a stable analogue of the endocannabinoid anandamide. On this basis, we investigated the effect of mAEA on myogenic differentiation, following the expression of myogenic markers by Realtime PCR, western blot and confocal immunofluorescence analysis and studied the involvement of TRPV1 in myogenesis using pharmacological approaches and RNA interference. Preliminary results indicate a dose dependent anti-myogenic action of mAEA. Instead, the TRPV1 agonist capsaicin, appears to exert a positive effect on myogenic differentiation which is attenuated by TRPV1 antagonism, but is not restored by S1P. The molecular characterization of the innovative crosstalk between S1P and endocannabinoids will possibly lead to new pharmacological approaches against skeletal muscle disorders.

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Categories

Miscellaneous

Analysis of the effects of Bisphenol AF (BPAF) on muscle and liver cells metabolism

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Abstract

Bisphenol A (BPA) is a well-known endocrine-disrupting chemical (EDC) whose use in the industry has been restricted worldwide. This circumstance led to the introduction of several bisphenol analogues into the market as a replacement for BPA such as bisphenol AF (BPAF). The structural similarity of these analogues intimates the potential similar or even more potent toxic and estrogenic effects as BPA but information about them are still limited. Previous studies have shown that BPAF has estrogen-dependent responses in vivo and in vitro via binding to estrogen receptors, ERα and ERβ, [1,2] and obtainable data suggest that BPAF might be a more potent endocrine disruptor and toxic than BPA. Thus, in the present work, we wanted to investigate the metabolic impact of BPAF on two types of eucaryotic cells, HepG2 and C2C12, focusing on the possibility to interfere with the insulin signalling pathway. We observed that BPAF influenced cellular proliferation and differentiation and altered glucose homeostasis. Indeed, cells treated with BFAF showed an increase of the oxygen consumption rate and extracellular acidification rate compared to control group. Furthermore, we investigated the impact of BPAF on the lipid metabolism and we highlighted an increase lipid accumulation in treated cells compared to control group. Our results may suggest that BPAF could function as an EDC by interfering with hormone action and altering hepatic and muscle cells metabolism. Further studies are needed to examine the effects of BPAF exposure on eukaryotic cells and in vivo models.

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Categories

Miscellaneous

Sirtuin 6 Regulates the Activation of the ATP/Purinergic Axis in Endothelial Cells

Cecilia Astigiano[1], Francesco Piacente[1], Maria Elena Laugieri[1], Andrea Benzi[1], Christian Di Buduo[2], Carolina Miguel[2], Debora Soncini[1], Michele Cea[1], Antonella Antonelli[3], Mauro Magnani[3], Alessandra Balduini[2], Antonio De Flora[1], Santina Bruzzone[1]

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Abstract

Sirtuin 6 (SIRT6) is a member of the mammalian NAD+-dependent deac(et)ylase sirtuin family. SIRT6's anti-inflammatory roles are emerging increasingly often in different diseases and cell types, including endothelial cells. In this study, the role of SIRT6 in pro-inflammatory conditions was investigated by engineering human umbilical vein endothelial cells to overexpress SIRT6 (SIRT6+ HUVECs). Our results showed that SIRT6 overexpression affected the levels of adhesion molecules and sustained megakaryocyte proliferation and proplatelet formation. Interestingly, the pro-inflammatory activation of the ATP/purinergic axis was reduced in SIRT6+ HUVECs. Specifically, the TNFα-induced release of ATP in the extracellular space and the increase in pannexin-1 hemichannel expression, which mediates ATP efflux, were hampered in SIRT6+ cells. Instead, NAD+ release and Connexin43 expression were not modified by SIRT6 levels. Moreover, the Ca2+ influx in response to ATP and the expression of the purinergic receptor P2X7 were decreased in SIRT6+ HUVECs. Contrary to extracellular ATP, extracellular NAD+ did not evoke pro-inflammatory responses in HUVECs. Instead, NAD+ administration reduced endothelial cell proliferation and motility and counteracted the TNFα-induced angiogenesis. Altogether, our data reinforce the view of SIRT6 activation as an anti-inflammatory approach in vascular endothelium.

Categories

Miscellaneous

Protein and metabolic markers of oxidative and inflammatory stress are useful for delineating the biochemical profile of human follicular fluids in a pathophysiological context

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Abstract

Diagnosis of female infertility is often difficult, and in some cases, it needs invasive tools, so the search for non-invasive and early biomarkers is considered a challenging issue. Follicular fluid (FF) is the in-vivo oocyte environment that fills the cavity or antrum of a mature follicle containing a variety of autocrine and paracrine factors responsible for the regulation of folliculogenesis, oocyte development, and ovarian function (1); it is a superfluous product, easily available during oocyte pick-up in in IVF.

Biochemical analysis of FF has proved to be a useful approach to find specific biomarkers related both to female infertility and to evaluate the effect of different drugs used for final maturation triggering of oocytes (2).

Immunochemical analysis of protein markers of oxidative stress and inflammation used in combination with an NMR-based metabolomics approach give useful information on the potential metabolic differences in FFs of COVID-19 vaccinated women and patients infected by SARS-CoV2 (5) as well to evaluate the impact of cancer on the quality of the oocytes that are cryopreserved in awaiting fertilization (3).

Biochemical profiling of FF suggests some useful biomarkers for predicting oocyte developmental potential and subsequent outcome.

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Categories

Miscellaneous

Biochemical markers and mobility function change in response to a High-Intensity Interval Training in MS patients

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Abstract

The benefits of physical activity in terms of its anti-inflammatory and neuroprotective effects, are already recognized in the medical field, especially in neuropathological conditions. Multiple sclerosis (MS) is a neurodegenerative inflammatory disease, the aetiology of which is currently not fully elucidated.

The aim of our study was to evaluate the possible benefits of a specific type of training (high intensity interval training, HIIT) on haematological biomarkers and neuromotor abilities in a group of MS patients.

From a cohort of 130 subjects diagnosed with relapsing-remitting MS at the neurology unit of the 'Paolo Giaccone' University Hospital, 16 patients who met the inclusion criteria were enrolled in the study. The patients were randomly assigned into a control group, performing no physical activity or exercise group, that performed the HIIT protocol. The training program was administered bi-weekly for 12 weeks.

Haematochemical and neuromotor evaluations were carried out at the beginning of the study (T0) and at the end of the study (T1).The results showed a change in the lipid profile and in bone metabolism; in deep, a reduction in total and LDL cholesterol levels ($p=0.02$) and an increase in osteocalcin and vitamin D levels

($p<0.05$) were detected. With regard to neuromotor tests, an improvement in the wall squat test was shown ($p<0.002$).

Given the important implications of these preliminary results in terms of the management of disease-related problems, future investigations will be needed by increasing the sample size and extending the intervention period.

Keywords: Lipid profile, Multiple Sclerosis, Bone metabolism, High-intensity interval training,

SPHINGOSINE 1-PHOSPHATE SIGNALING AXIS MEDIATES NEUROPEPTIDE S-INDUCED INVASION OF ENDOMETRIOTIC CELLS

Maryam Raeispour[1], Matteo Prisinzano[1], Isabella Seidita[1], Margherita Rossi[1], Felice Petraglia[2], Caterina Bernacchioni[1], Chiara Donati[1]

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Abstract

Endometriosis is a gynecological disorder characterized by endometrial cell invasion of the extra-uterine milieu and associated with pelvic pain and infertility. Therapy relies on symptomatic drugs however the complex molecular mechanisms underlying the pathogenesis of endometriosis is unclear.

The signaling of the bioactive sphingolipid sphingosine 1-phosphate (S1P) is dysregulated in endometriosis. Indeed, the enzyme responsible for its synthesis, sphingosine kinases (SKs) and three of S1P receptor isoforms (S1P1, S1P3 and S1P5) were more expressed in endometriotic lesions respect to healthy endometrium.

Since variants of the gene encoding the receptor for neuropeptide S (NPSR1) have been reported to be associated with endometriosis in humans, in this study it has been characterized the biological effect of neuropeptide S (NPS) in endometriotic epithelial 12-Z cells and the possible involvement of S1P signaling axis. We demonstrated that NPS potently induced cell invasion and actin cytoskeletal remodeling. The neuropeptide increased S1P levels through activating both isoforms of the enzymes, SK1 and SK2. Noteworthy, NPS-induced invasion and cytoskeletal remodeling were dependent on SK1, SK2 as well as S1P1 and S1P3. Indeed, NPS biological actions in endometriotic cells were impaired when SK1 or SK2 were pharmacologically inhibited or specifically silenced, or the signaling of S1P1/3 blocked. Furthermore, Rho/Rho kinase signaling was found critically implicated in invasion and cytoskeletal remodeling elicited by NPS in endometriotic cells.

These findings add new information to the understanding of the molecular mechanisms implicated in endometriosis pathogenesis and establish the rationale for the exploitation of innovative non-hormonal therapeutic targets for its treatment.

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Categories

Miscellaneous

Phosphoproteins and inflammation molecules as new therapeutic targets for Shwachman-Diamond syndrome (SDS).

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Abstract

Shwachman-Diamond syndrome (SDS) is a rare genetic disease. It is mainly caused by mutations in the SBDS gene that is involved in the biogenesis of rRNA [1,2]. As recently reported, SDS may also be related to mutations in other genes involved in the ribosome assembly process like DNAJC21, SRP54, and EFL1 [3]. For this reason, the syndrome is considered a multiorgan ribosomopathy and, among the symptoms, it causes various hematological disorders. Between these, bone marrow failure and myelodysplastic syndrome (MDS), which in turn leads to increased risk of Acute Myeloid Leukemia (AML), represent the major causes of mortality in SDS. In these cases, hematopoietic stem cell transplantation remains the unique therapeutic option, and currently there is no specific therapy for SDS [3].

Recent studies highlighted the hyperactivation of mTOR and STAT3 pathways in SDS, two transcription factors involved in the control of cancer cell division [4]. Considering this, we investigated through Luminex technology the phosphorylation levels of proteins involved in mTOR and STAT3 pathways in samples derived from patients and healthy donors. These analyses showed that GSK3α, GSK3β, ERK1/2 and AKT phosphoproteins are dysregulated in patients, supporting the mTOR and STAT3 participation in SDS pathophysiology. Since the activation of these two pathways are strongly influenced by the inflammatory environment [5], the expression profile of inflammatory cytokines and chemokines was investigated too. In conclusion, the results obtained in this study allowed to underline part of the molecular mechanisms of SDS, favouring the identification of potential targets for therapeutic strategies.

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Categories Miscellaneous

Regulation of human D-aspartate oxidase

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In the human brain the flavoenzyme D-aspartate oxidase (hDASPO) controls the level of D-aspartate, a molecule acting as agonist of NMDA receptors and modulator of AMPA and mGlu5 receptors [1]. hDASPO-mediated D-aspartate degradation prevents age-dependent deterioration of brain functions and has been related to psychiatric disorders such as schizophrenia and autism. Notwithstanding this crucial role, little is known about hDASPO regulation [1]. Here, we addressed this issue by combining *in vitro* and cellular studies. We investigated the role of post-translational modifications of cysteine residues: hDASPO can be nitrosylated, while no evidence of sulfhydration was apparent. The modification affected to a limited extent the kinetic and FAD binding properties of the flavoenzyme. Most notably, hDASPO interacted with the primate specific protein pLG72, a well-known negative chaperone of human D-amino acid oxidase, the enzyme deputed to D-serine degradation [2]. The interaction yielded a ~114 kDa complex, likely made by 2 hDASPO and 2 pLG72 monomers, with a micromolar dissociation constant. *In vitro* studies showed that pLG72 binding increased the rate of the flavoenzyme inactivation. At the cellular level, pLG72 and hDASPO generated a cytosolic complex and the expression of pLG72 negatively affected the hDASPO level by reducing its half-life. Based on these findings, pLG72 binding may represent a protective mechanism aimed at avoiding the accumulation of H2O2 (produced from the hDASPO enzymatic degradation of D-aspartate) and the consequent cell insults.

This project is founded by ”PRIN-2020 - Biochemical modulation of D-aspartate metabolism in brain functions”.

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Imbalance of the Endocannabinoid System in Alzheimer's Disease–Associated Retinal Inflammation

Annamaria Tisi[1], Giulia Carozza[1], Lucia Di Re[1], Giacomo Giacovazzo[2], Lucia Scipioni[1,2], Sergio Oddi[2,3], Rita Maccarone[1], Mauro Maccarrone[1,2]

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Abstract

Accumulated evidence supports that Alzheimer's disease (AD) develops extra-cerebral manifestations in the retina, which is then considered a "window to the brain". Recently, the dysregulation of the endocannabinoid system (ECS) in AD brain has been highlighted. Here, we explored gliosis and ECS alterations in retinae of twelve month-old Tg2576 (TG) mice, an AD model characterized by the over-expression of amyloid precursor protein (APP). Gliosis was investigated by immunofluorescence on retinal cryosections, showing a significant increase of IBA1 (+) microglia cells in TG versus wild type (WT) ($p=0,002$), and an increase in GFAP immunostaining. Western blotting of the ECS revealed the up-regulation of cannabinoid receptor 2 (CB2) in TG retinas (1.5 folds over WT; $p=0.032$) and this result was consistent with plot profile graphs and fluorescence intensity in anti-CB2 immuno-stained cryosections. No statistically significant differences were found for the other enzymes and receptors of the ECS, though linear regression analysis for individual animals showed a significant correlation between CB2 and fatty acid amide hydrolase (FAAH) ($r=0.787$; $p=0.021$), diacylglycerol lipase α/β (DAGLα/β) (α: $r=0.740$; $p=0.036$ – β: $r=0.927$; $p<0.001$), and APP ($r=0.681$; $p=0.063$). Overall, these findings suggest that the ECS play a role in AD-associated retinal inflammation, resembling the AD brain, and further support the correlation between retinal dysregulation and AD.

This investigation was supported by the Italian Ministry of University and Research (MUR) under the competitive grant PRIN 2017-487 2017BTHJ4R to S.O. and M.M. and by intramural competitive grants of University of L'Aquila (Progetti di Ateneo 2023) to A.T.

Categories

Miscellaneous

INTRANASAL ADMINISTRATION OF KYCCSRK PEPTIDE RESCUES BRAIN INSULIN SIGNALING ACTIVATION AND REDUCES AD-LIKE NEUROPATHOLOGY IN A MOUSE MODEL FOR DOWN SYNDROME

Antonella Tramutola[1], Simona Lanzillotta[1], Giuseppe Aceto[2,3], Sara Pagnotta[1], Gabriele Ruffolo[4,5], Pierangelo Cifelli[6], Federico Marini[7], Cristian Ripoli[2,3], Eleonora Palma[4,5], Claudio Grassi[2,3], Fabio Di Domenico[1], Marzia Perluigi[1], Eugenio Barone[1]

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Abstract

Down syndrome (DS) is the most frequent genetic cause of intellectual disability and a genetic form of Alzheimer's disease (AD). Brain insulin resistance greatly contributes to AD development in the general population and previous studies from our group showed an early accumulation of insulin resistance markers in DS brain, already in childhood, and even before AD onset. Among the strategies to ameliorate the activation of brain insulin signaling, the intranasal delivery of biologics, such as peptides, via the nose-to-brain route is of increasing interest due to their high potency and selectivity. We tested the effects promoted by the intranasal administration of the KYCCSRK peptide in Ts2Cje mice (a murine model for DS). KYCCSRK is known for its ability to foster insulin signaling activation by directly interacting and activating the insulin receptor (IR) and the AKT protein. Therefore, the KYCCSRK peptide might be a promising molecule to overcome insulin resistance. Our results show that KYCCSRK rescued insulin signaling activation and increased mitochondrial complexes levels (OXPHOS) in the brain of Ts2Cje mice. Moreover, we uncovered novel characteristics of the KYCCSRK peptide, including its efficacy in reducing DYRK1A (triplicated in DS) and BACE1 protein levels, which resulted in reduced AD-like neuropathology in Ts2Cje mice. At last, the peptide elicited neuroprotective effects by ameliorating synaptic plasticity mechanisms that are altered in DS due to the imbalance between inhibitory vs excitatory currents. Our results represent a step forward in searching for new molecules useful to reduce intellectual disability and counteract AD development in DS.

Categories

Miscellaneous

Downregulation of zebrafish neuraminidase neu3.2 affects skeletal muscle development: new insight into the biochemistry of muscle pathologies

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Abstract

Sialidases are glycohydrolytic enzymes that remove terminal sialic acids residues from non-reducing ends of glycoconjugates. They have been recognized as catabolic enzymes that, working within different subcellular compartments and can ensure the proper turn-over of glycoconjugates. Four mammalian sialidases exists, namely Neu1, Neu2, Neu3 and Neu4 with different subcellular localization, pH optimum and substrate preferences. In zebrafish the scenario is more complicated because seven different sialidases, with significant identity to their mammalian counterparts, have been identified. Zebrafish neu3.2 behaves as the human cytosolic sialidase NEU2 which, in turn, is involved in skeletal muscle differentiation and exhibits a broad substrate specificity toward gangliosides and several glycoproteins. NEU2 may facilitate the process of myogenesis by ensuring the correct turn-over of glycosylated proteins through the processing of free oligosaccharides or misfolded glycoproteins that are released into cytosol. In zebrafish neu3.2 is expressed during somites development and the enzymatic activity of the encoded protein has been detected in skeletal muscle and heart of adult animals. Using splice-blocking morpholino severe embryonic defects can be observed, mainly in somites, heart and anterior-posterior axis formation. Appropriate controls confirmed the specificity of the observed phenotype and coinjection of mRNA encoding the rat Neu2 ortholog in morphants rescued the phenotype. Myog and myod expression, two transcriptional factors regulating myoblasts differentiation, was altered in morphants. Altered musculature formation was associated with defective locomotor behavior. These data are consistent with the features of some pathologies related to muscle formation and support the use of zebrafish to investigate theirs biochemical pathogenesis.

Categories

Miscellaneous

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