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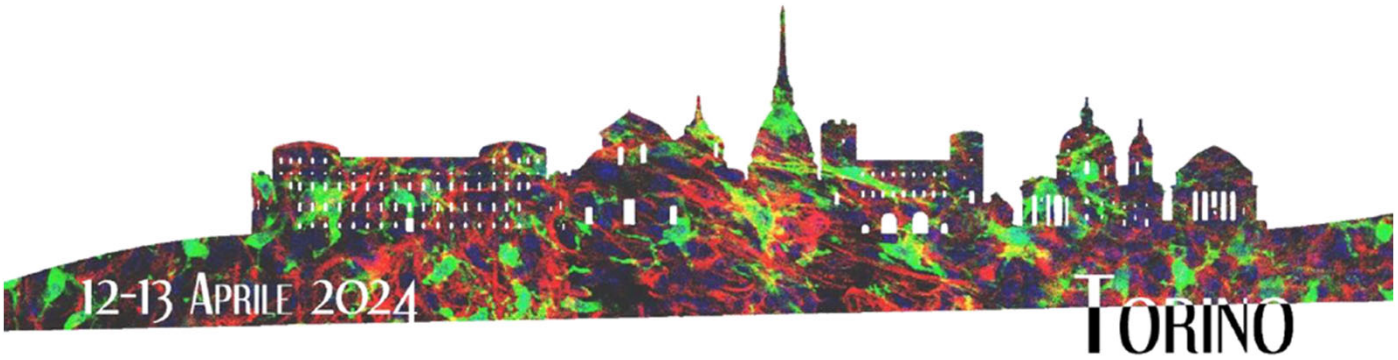

ABSTRACT BOOK

I° Edizione -
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Poster Categories

Neurosciences

Aging and degenerative diseases

Epithelial-mesenchymal transition in organogenesis and carcinogenesis

Histogenesis, functions and dysfunctions of the musculoskeletal system

Morphology, Cadaver Lab and Teaching strategies

Morphology and molecular pathology

Innovative technologies, 3D models and organoids.

Epithelial and connective tissues

Anatomy and movement

Ist POSTER SESSION, April 12th 17.00-18.30

Neurosciences

1. “Primary cilium morpho-functional alterations in human GnRH neuroblasts exposed to cadmium” Guarnieri Giulia et al.

2. “Potential neuroprotective effect of melatonin in the hippocampus of male BTBR mice” Bonetti Matteo et al.

3. “GABA signalling and metabolism (dys)regulation in spinal muscular atrophy: a new disease target at cortical level?” Menduti Giovanna et al.

4. “The protein kinase CDKL5 is required for the maturation of oligodendrocytes progenitor cells and myelination integrity in the cerebral cortex” Pizzo Riccardo et al.

5. “Evaluation of the efficacy of Glucidex membranes in supporting nerve regeneration *in vitro*” Metafune Miriam et al.

6. “Effect of Tail Pinch on BDNF and trkB expression in the hippocampus of two lines of rats with different vulnerability to stress-induced depression” Lai Ylenia et al.

7. “Study of possible glycinergic system alterations in spinal muscular atrophy” Caretto Anna et al.

Aging and degenerative diseases

8. “Brain iron dyshomeostasis and mitochondrial features’ alteration in 5xFAD mouse model in the pre-pathological phase of Alzheimer’s disease” Mezzanotte Mariarosa et al.

9. “Terpenes and Alzheimer’s disease: a study in stem cell derived models” Dallere Sveva et al.

10. “Iron and βamyloid deposition pattern in the adult 5xFAD mouse model of Alzheimer’s disease” Chicote Javier et al.

Epithelial-mesenchymal transition in organogenesis and carcinogenesis

11. “The role of miR-145 in the emergence of adaptive resistance to AKT inhibition in prostate cancer” Antolini Ludovica et al.

Histogenesis, functions and dysfunctions of the musculoskeletal system

12. “Red photobiomodulation promotes skeletal myoblast differentiation: morpho-functional evidences” Parigi Martina et al.

Morphology, Cadaver Lab and Teaching strategies

13. ““Live cadaver” model for internal carotid artery injury simulation in endoscopic endonasal skull base surgery” Pacca Paolo et al.

Morphology and molecular pathology

14. "Phenotypic and morphofunctional changes in the life cycle of chronic lymphocytic leukemia B cells during the transition between peripheral blood and secondary lymphoid organs" Mazzarello Andrea Nicola et al.

15. "Influence of antiretroviral drugs on adipocyte differentiation" Del Genio Emiliano et al.

Innovative technologies, 3D models and organoids

16. "*In-vitro* development of the microvascular network serving the central nervous tissue" Virtuoso Assunta et al.

2nd POSTER SESSION, April 13th 9:30-11:00

Neurosciences

17. "The investigation of the stress effects on amyotrophic lateral sclerosis onset and/or progression in an *in vitro* disease-predisposed condition" Rasà Daniela Maria et al.

18. "Ultrastructural effects of mTOR inhibition in iPSC-derived motor neurons from patients with *C9orf72* repeat expansion" Scotto Marco et al.

19. "Attività nutraceutica di molecole bioattive ottenute dal grano" Mengoni Beatrice et al.

20. "Composti bioattivi per il trattamento della neuroinfiammazione" Armeli Federica et al.

21. "Drug repositioning and Spinal Muscular Atrophy: in vitro validation of SMN-independent drugs" Nicorvo Ersilia et al.

22. "Maternal behavior and offspring stress axis affected by an altered maternal diet, low in protein and with added phytoestrogens" Ricci Elena et al.

23. "Chemotherapy-induced peripheral neuropathy: a focus on ER-mitochondria interactions" Tonelli Elisa et al.

24. "The biological effects of "green-therapy" on MDD" Pavarino Gianna et al.

Aging and degenerative diseases

25. "Pre-clinical analysis of intracerebroventricular hNSC cell graft in $SOD1^{G93A}$ mice as a potential treatment for amyotrophic lateral sclerosis" Ferrero Clelia et al.

26. "The complex cross-talk between lipid metabolism and inflammation: neuroprotective role of antioxidants" Fields Matteo et al.

Epithelial and connective tissues

27. "Galectin-10 and respiratory remodelling: new insights from nasal washes of SAR pediatric patients" Picone Domiziana et al.

Anatomy and movement

28. "Influence of acoustic and visual stimuli on vertical jump performance" De Girolamo Ciro Ivan et al.

Morphology, Cadaver Lab and Teaching strategies

29. "Peer-to-peer tutoring activities in human anatomy exploiting digital tools" Alberti Paola

Morphology and molecular pathology

30. "Inibizione delle deacetilasi in modelli cellulari di anemia di Fanconi: effetti sulle risposte antiossidanti e sulla morfologia e funzionalità del mitocondrio" Cossu Vanessa et al.

31. "Polylactic acid (PLA) versus polystyrene (PS) microplastics: internalization studies in different human cell lines" Marino Marianna et al.

32. LINC complex as potential mediator in bone sarcomas differentiation. Serena Truocchio et. al

Neuroscience

Composti bioattivi per il trattamento della neuroinfiammazione

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La neuroinfiammazione è implicata nell'insorgenza delle malattie neurodegenerative e si verifica principalmente come risultato dell'iperattivazione delle cellule gliali nel sistema nervoso centrale (SNC). Le cellule della microglia rispondono agli insulti attraverso il rilascio di mediatori infiammatori come citochine e chemochine. La nostra ricerca si concentra sullo studio di alcuni composti nutraceutici che promuovono la polarizzazione morfo-funzionale della microglia verso un fenotipo antinfiammatorio. Abbiamo recentemente dimostrato la presenza di composti bioattivi negli estratti di mirtillo in grado di polarizzare le cellule microgliali BV2 stimolate da LPS verso un fenotipo antinfiammatorio (De Caris et al., Blueberry counteracts BV-2 microglia morphological and functional switch after LPS challenge, 2020, *Nutrients*). Inoltre, abbiamo considerato il ruolo dell'asse intestino-cervello e della disbiosi intestinale nell'induzione di processi patologici a livello del SNC. A questo proposito, abbiamo dimostrato che un ceppo di *Saccharomyces cerevisiae* trattato con onde elettromagnetiche, reverte le cellule microgliali stimolate da LPS verso un fenotipo antinfiammatorio (Armeli et al., Milmed yeast alters the LPS-induced M1 microglia cells to form M2 anti-inflammatory phenotype, 2022, *Biomedicines*). La polarizzazione microgliale è stata valutata mediante l'analisi dell'espressione di citochine, di marcatori M1 e M2 e di enzimi antiossidanti tramite RT-PCR e immunofluorescenza. La valutazione della morfologia cellulare, l'analisi delle citochine pro- e anti-infiammatorie e dei marcatori chiave dell'attivazione microgliale (iNOS e ARG-1) espressi dalle cellule nelle diverse condizioni sperimentali, hanno dimostrato la capacità dei fattori analizzati di indirizzare l'attivazione microgliale verso uno stato anti-infiammatorio. Nel complesso, i nostri dati suggeriscono che le molecole da noi analizzate possano guidare la polarizzazione microgliale verso un fenotipo antinfiammatorio per contrastare lo sviluppo di malattie cronico-neurodegenerat

Potential neuroprotective effect of melatonin in the hippocampus of male BTBR mice

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder identified by impairments in common social interactions and repetitive behaviors. In ASD patients, substantial morphological alterations have been observed in the hippocampus, which represents an important region for the development of social skills. Melatonin, commonly found in many foods and plants, is produced by the pineal gland. This indolamine, known to regulate the circadian rhythm, shows antioxidant and anti-inflammatory properties. We therefore hypothesized that melatonin may improve oxidative stress and inflammation in the hippocampus of ASD patients. We explored our hypothesis using the BTBR mouse, a well-regarded murine transgenic model for ASD. Immediately after weaning, male BTBR and C57BL/6 mice underwent an 8-week treatment with melatonin or vehicle. Later, through immunohistochemistry of brain sections and immunoblotting analysis of the hippocampal protein lysate, we evaluated overall expression and cellular localization of Nrf2 and SOD1, two enzymes involved in the oxidative stress response. Similarly, we evaluated NLRP3 and NFkB, two mediators of inflammation, and GAD67, an enzyme responsible for the synthesis of GABA. Ultimately, we addressed melatonin's potential to regulate iron metabolism through a DAB-enhanced Perls reaction assay. Results showed melatonin potential for modulating the analyzed markers in BTBR mice, suggesting a potential neuroprotective effect in ASD patients.

Study of possible glycinergic system alterations in spinal muscular atrophy

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Spinal Muscular Atrophy (SMA) is a neurodegenerative disease due to SMN1 gene mutation causing motor neuron (MN) loss in brainstem and spinal cord and some peripheral district alterations. Although the available gene-based treatments have improved SMA course, they still show important limitations and the identification of novel therapeutic strategies remains needed.
Recently it has been observed that SMA and other neurodegenerative diseases share mitochondria alterations, suggesting that these organelles can be considered promising target for SMA therapy. Through a bioinformatic search, we identified 8 mitochondrial genes significantly physiologically SMN1-anticorrelated. To investigate if any of them could be related to SMA, we performed RT-PCR on SMNdelta7 mouse tissues. We found that Gcsh, a gene encoding a mitochondrial subunit of the glycinergic cleavage system (GCS), is significantly upregulated in the lumbar spinal cord of SMA pups at an early symptomatic stage (postnatal day 5, P5). Western Blot analysis on tissues and on Smn-siRNA silenced NSC34 cells revealed an upregulation of the corresponding protein. Furthermore, RNA-Scope ISH showed a significant Gcsh increase in SMA MN soma. Consistently, IF staining on spinal cord sections confirmed a significant higher expression of GCSH in MN soma from SMA mice.
To further analyze alterations of the glycinergic phenotype, we did a morphological analysis of Renshaw Cells, the glycinergic interneurons involved in MN recurrent inhibition observing a shrinkage of their cell body that worsens between P5 and the late P12 stage in the SMNdelta7 mouse. These findings raise the possibility of an SMA-associated higher glycine degradation, with a possible reduction of the recurrent inhibition resulting in MN hyperexcitability and loss. Further GCS analysis (in MNs and astrocytes) and on glycinergic pathway could be instrumental for a better understanding of the mitochondrial role in SMA and to identify new molecules for SMA complementary therapy.

The protein kinase CDKL5 is required for the maturation of oligodendrocytes progenitor cells and myelination integrity in the cerebral cortex

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CDKL5 deficiency disorder (CDD) is an X-linked neurodevelopmental disorder caused by mutations in cyclin-dependent kinase-like 5 (CDKL5) and characterized by early-onset drug resistant epilepsy, intellectual disability, autistic-like features and sensory impairments [1]. CDKL5 is necessary for the integrity of neuronal connectivity, synaptic transmission and plasticity underlying complex sensorimotor and cognitive functions [2]. However, the understanding of the specific processes contributing to atypical brain circuits maturation occurring in the absence of CDKL5 remains limited. Myelination integrity, by favoring fast and reliable axon potential propagation, allows efficient neural communication underlying motor, sensory and cognitive functions [3]. CDKL5 is expressed in both oligodendrocyte precursor cells (OPCs) and myelinating oligodendrocytes (OLs) [4], but its role in myelin organization has not yet been investigated. To fill this gap, we evaluated myelin deposition and axonal injury in primary sensory cortices of young and adult CDKL5-KO mice. Morphological investigations using immunofluorescence and confocal microscopy revealed severe alterations of myelination integrity in both S1 and V1 cortex of mutant mice. Next, we assess if impairments of whiskers-mediated tactile perception shown by CDKL5-KO depends on parvalbumin interneurons (PV^+ IN) myelination integrity, as suggested by recent studies [5]. We analyzed 3D reconstructed confocal images and found a that lack of CDKL5 produces a significant decrease of myelin volume surrounding PV^+ axons of the barrel cortex (BC). Moreover, a significant different number of putative synaptic contacts between PV^+ IN and OPCs was found in the BC of mutant mice, indicating that CDKL5 regulates GABAergic inputs onto OPCs, a critical step for their maturation into OLs [6]. In sum, our findings show that CDKL5 is required for the maintenance of grey matter myelination integrity thus revealing a novel important CDKL5-dependent process underlying brain maturation, likely of great importance for CDD. Moreover, our data disclosed that, by mediating PV^+ axons myelination, CDKL5 expression is essential for the cortical responses underlying tactile-related stimuli processing.

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Primary cilium morpho-functional alterations in human GnRH neuroblasts exposed to cadmium

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The primary cilium (PC) is a sensory antenna protruding on the surface of most mammalian cells, involved in signal transduction and cell migration. This organelle was found in both developing and adult neurons, including the hypothalamic gonadotropin-releasing hormone (GnRH) neurons, which regulate reproductive functions. Recently, defects in PC have been linked to hypogonadotropic hypogonadism, however, data in humans are limited. Here, we analyzed the presence of the PC in developing GnRH neurons isolated from the human olfactory placode (FNCB4) to evaluate the effect of the environmental endocrine disruptor Cadmium (Cd) on PC. The FNCB4 cell phenotype was confirmed by the expression of GnRH and Kisspeptin receptor (KISS1R). Moreover, we found that 54% of cells expressed the PC. Immunocytochemistry analysis revealed that Cd exposure (10 µM, 24h) significantly reduced by half the number of ciliated FNCB4 cells (25.4%±6%; $p<0.005$). In addition, Cd-treated FNCB4 showed PC morphological changes, such a reduction of both length and area ($p<0.05$). Accordingly, the expression of genes involved in PC formation, such as IFT88 and KIF3A, was significantly decreased in FNCB4 after Cd exposure ($p<0.001$). As demonstrated in animal models, neuronal PC is highly enriched by G-protein coupled receptors, such as KISS1R. Accordingly, we demonstrated for the first time the localization of KISS1R at the PC membrane in sections from human fetal olfactory placode. Interestingly, Cd-treated FNCB4 exhibited a significant reduction of both protein and gene KISS1R expression ($p<0.001$; $p<0.05$, respectively). Finally, as functional effect, we demonstrated that Cd significantly reduced FNCB4 migration ($p<0.01$). Overall, our findings demonstrated that Cd exposure affects PC in developing human GnRH neurons, reducing their number and size. This may affect crucial events implicated in GnRH maturation and function, such as migration and KISS1R expression, thus suggesting a link between environmental pollution and reproductive defects.

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Effect of Tail Pinch on BDNF and trkB expression in the hippocampus of two lines of rats with different vulnerability to stress-induced depression

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One of the most validated genetic models for the study of fear/anxiety- and stress-related behaviors is provided by the Roman rat lines, a model designed to investigate the impact of genetic and environmental factors on the neural substrates of depression. They were selected for rapid (RHA) vs. extremely poor (RLA) acquisition of active avoidance, in a shuttle-box. It has been shown that emotional reactivity is the most prominent behavioral difference between the two lines, with the RLA rats being more fearful/anxious than their RHA counterparts. Different forms of stress-induced depression-like symptoms impair the signaling of neurotrophins like the Brain Derived Neurotrophic Factor (BDNF) and induce alterations of synaptic plasticity. We have previously reported behavioral and immunochemical data in the dorsal (dHC) and ventral hippocampus (vHC) of Roman rats exposed to the forced swimming (FS) as acute robust stressor. Here we extend the Roman rat brain characterization by investigating the effects of tail pinch (TP), a mild stressor, on BDNF/trkB neuronal signaling in the same regions. Using western blot (WB) and immunohistochemistry assays, we show that TP induces distinct changes in the levels of BDNF and trkB proteins of RHA and RLA rats. In particular, the WB assays showed that TP increases BDNF and trkB levels in the dHC of both lines but induces opposite changes in the vHC, decreasing BDNF levels in RHA rats and trkB levels in RLA rats. These results suggest TP may enhance plastic events in the dHC and hinder them in the vHC. In keeping with WB data, immunohistochemical labelling revealed that, in the dHC, TP increases BDNF-like immunoreactivity (LI) in the CA2 sector of the Ammon's horn of both Roman lines and in the CA3 sector of the Ammon's horn of RLA rats while, in the dentate gyrus, TP increases trkB-LI in RHA rats. In contrast, in the vHC, TP elicits only a few changes, represented by decreases of BDNF- and trkB-LI in the CA1 sector of the Ammon's horn of RHA rats. Collectively, these results provide additional evidence that the genotypic/phenotypic features influence the effects of an acute stressor, even as mild as TP, on the basal BDNF/trkB signaling, eliciting different changes in the dorsal and ventral subdivisions of the HC and influencing the direction and subregional distribution of the adaptive plastic responses of BDNF/trkB signaling in the HC.

Key-words: BDNF; trkB; tail pinch; stress; depression; Roman high- and low-avoidance rats; hippocampus; western blot; immunohistochemistry.

Attività nutraceutica di molecole bioattive ottenute dal grano

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I più recenti risultati sperimentali hanno individuato nella neuroinfiammazione le cause principali dello sviluppo delle malattie a livello del sistema nervoso centrale (SNC), come il morbo di Alzheimer e il morbo di Parkinson. L'obiettivo della medicina complementare è quello di fornire un supporto alla farmacologia tradizionale, limitando la progressione delle malattie mediante trattamenti anti-infiammatori. Abbiamo recentemente dimostrato in estratti di grano la presenza di composti bioattivi in grado di polarizzare le cellule microgliali stimolate da LPS verso un fenotipo anti-infiammatorio. Abbiamo valutato l'attività nutraceutica di estratti di grano duro appartenente all'antica cultivar "Senatore Cappelli" sulla polarizzazione microgliale analizzando l'espressione dell'mRNA di marcatori M1 e M2, quali iNOS, COX2, ARG-1 e CD206 e di citochine pro e anti-infiammatorie come IL-1β, TNF-α, IL-6 e IL-10. Sono stati condotti esperimenti su cellule BV2 di microglia murina pre-trattate con estratti derivati da campioni di dell'antica cultivar "Senatore Cappelli" alle concentrazioni ottimali ottenute da curve di citotossicità in presenza e in assenza di LPS. Attraverso esperimenti di immunofluorescenza abbiamo valutato in cellule BV2 la capacità degli estratti di grano duro di revertire il fenotipo pro-infiammatorio M1 nel fenotipo anti-infiammatorio M2. I nostri studi preclinici sottolineano la possibilità di isolare composti bioattivi dai prodotti di scarto delle lavorazioni agricole e industriali. Le molecole da noi analizzate hanno mostrato di possedere proprietà fitochimiche e nutraceutiche atte a contrastare l'infiammazione nel SNC.

Evaluation of the efficacy of Glucidex membranes in supporting nerve regeneration *in-vitro*

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The objective of the present study is to evaluate an innovative membrane with the aim to apply it for repairing somatic and autonomic peripheral nerves in case of traumatic or iatrogenic lesions. Starch-derived (GLUCIDEX) hyper-crosslinked polymers with suitable mechanical properties were electrospinned as membrane and tested *in vitro* using immortalized Schwann Cells (RT4-D6P2T cells), for cell survival and proliferation to evaluate the biocompatibility and biomimetic nature of the scaffolds.
RT4-D6P2T cells were cultured i) in direct contact with the membrane, to investigate the interaction with the substrate and ii) in the presence of membrane dissolution products, to test the effect of the material components on cell proliferation and organization.
i) Concerning to the adhesion assays, the actin cytoskeleton results more organized in the control group, however, after 24 hours, the density and the area occupied by RT4-D6P2T increased.
ii) Several analyzes were conducted using the dissolution products, obtained by incubating Glucidex membranes 7, 14 and 28 days in high glucose DMEM. The proliferation assay revealed that after 1, 4 and 7 days of culture, cells maintain proliferative behavior under all conditions tested, although a slight decrease, compared to the control, is observed at the first two time points. The study of cell morphology and the actin cytoskeleton profile revealed that cells cultured in conditioned medium have a high organization and generate membrane protrusions, *lamellipodia*, correlated to cell migration, an important feature of glial cells in support of peripheral nerve regeneration.
Investigating apoptosis and the specific cellular alterations due to Bax, pro-apoptotic protein, and Bcl-2, anti-apoptotic protein, our study revealed that the dissolution products of the membrane are not related with cell death, contrarily, they are associated with good survival.
Further investigations are underway to deepen the effect of the dissolution products on the expression of genes involved in the regulation of nerve regeneration by Schwann cells.

Drug repositioning and Spinal Muscular Atrophy: *in vitro* validation of SMN-independent drugs

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Introduction. Spinal Muscular Atrophy (SMA) is the most common genetic cause of infant mortality. Due to the mutation/deletion of Survival Motor Neuron 1 (*Smn1*) gene and the consequent lack of SMN protein, SMA is characterized by the progressive degeneration of lower motor neurons (MNs). FDA and EMA have approved three revolutionary SMN-dependent treatments that are focused on increasing the production of the SMN protein. However, these approaches present some limitations: therefore, the identification of alternative/synergistic therapeutic strategies is strongly needed.

Methods. Thanks to a drug screening performed on a *Caenorhabditis elegans* SMA model, we identified new FDA-approved drugs able to rescue the neurodegeneration in the SMA-affected worms. The most effective molecules were then tested *in vitro* on primary cortical neurons from SMA delta7 mice (a severe SMA model) and on *Smn*-silenced NSC-34 cells (a MN cell line). Different analyses (MTT assay, neurolucida reconstructions, immunofluorescence reactions) were performed to assess cell viability and morphology. Moreover, to evaluate the drug efficacy and mechanism of action (MoA), live-cell analysis and WB were used to measure further morphological and functional parameters (including SMN expression, apoptosis and autophagy cascades, mitochondrial network/functionality).

Results. Compared to untreated primary cortical cells, most of the compounds exerted positive effects, by significantly improving cell viability, increasing soma area and length/branching of neuronal processes, and influencing the synaptic vesicle distribution. On the contrary, gross morphological differences were not detected in treated vs untreated NSC-34 cells. By WB analysis, we also evaluated the SMN expression levels: since no differences were observed among groups, we suggest that the tested compounds act via a SMN-independent MoA. Preliminary results also show a modulation of the mitochondrial activity and of autophagic pathway, in both *in vitro* models.

Conclusions. Although additional analyses are necessary, our results suggest that the repositioned drugs identified are neuroprotective and could be combined with SMN-dependent treatments, to develop synergistic pharmacological strategies and improve the efficacy of the available ones.

The biological effects of “green-therapy” on MDD

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In recent years, human-nature interactions are becoming a fundamental issue. Indeed, spending time in nature reduces stress and promotes mental well-being; however, the underlying biological mechanisms of action are still elusive. Major depressive disorder (MDD), one of the most prevalent and debilitating psychiatric diseases, is characterized by severe symptoms that negatively influence health perception. Unfortunately, even after antidepressant treatments, a high percentage of patients do not achieve a personal full recovery. Therefore, we decided to i) evaluate the biological, molecular and epigenetic impact of spending time in the greenery on MDD patients, and ii) highlight the importance of urban “green-therapy” on depressive symptoms. For this purpose, we enrolled MDD patients (recommended to walk regularly in urban parks, at least 40 minutes 3/4 times per week, for 6 months) and healthy control subjects. Then, we evaluated serological markers of inflammation (e.g. cortisol, CRP, IL-6) and epigenetic markers (i.e. miRNAs and post-translationally modified histone proteins) that correlate with MDD. Our results, in MDD patients, over time (from baseline to 6 months), after “green-therapy”, clearly show that: IL-6 level significantly decreases returning to healthy control levels; the expression of the tested miRNAs seems to be completely restored in PBMCs; the levels of some post-translational modified histone proteins analysed in PBMCs are significantly restored. Therefore, we provide new scientific evidence about beneficial effects of green environment on depressive symptoms, and propose greenness-related activities as a potential combinatorial treatment to reduce pharmacological administration and achieve a personal full recovery.

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The investigation of the stress effects on amyotrophic lateral sclerosis onset and/or progression in an *in vitro* disease-predisposed condition

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During our life we constantly expose to physical, social and environmental stressors able to trigger several cellular alterations that similarly characterize the pathogenesis of many neurodegenerative diseases, including Amyotrophic Lateral Sclerosis (ALS). ALS is a motor neuron (MN) disease characterized by progressive degeneration of both upper and lower MNs: the patients show weakness, muscle atrophy and die prematurely.
The aim of this study is clarifying how stressors can contribute to the onset and progression of ALS in disease-predisposed individuals through an *in vitro* experimental model. It has been set-up using naive NSC-34 cells (MN-like cells) and NSC-34 cells expressing hSOD1 (WT or G93A) gene under the control of a doxycycline-inducible promoter. The cells have been differentiated in mature MNs with 20µM of retinoic acid (RA) for 4 days, while the hSOD1 expression has been induced adding doxycycline for 24 hours into culture medium. To mimic a stress condition, cells have been undergone to oxygen and glucose deprivation adding CoCl2 as hypoxic agent and reducing the glucose of the medium. Cell damage was validated studying mitochondria with MitoTrackerTM Red CMXRos and evaluating protein levels of specific stress markers (HIF1α, caspase3): mutated cells seem less able to counteract stress conditions compared to WT.
Afterwards, we studied the expression of some ALS-related genes by qRT-PCR using pre-designed plates: Ang, Casp1, Cldn5, Col4a2, Gsk3b, Hdac7, Hspb1, Igf1, Il6 and Tgfb1 genes are resulted significantly de-regulated in hSOD1 G93A stressed cells compared to WT. Thus, Gene Ontology and Pathway Enrichment analyses have been performed to clarify the possible correlations among the identified genes acting on common biological pathways.
Although additional investigations are needed, we are confirming that stressful events could influence ALS onset and progression and we are highlighting the molecular pathways involved.

Maternal behavior and offspring stress axis affected by an altered maternal diet, low in protein and with added phytoestrogens

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Offspring health can be affected by maternal nutrition in pregnancy, such as a low-protein diet that may present a greater risk to their development, physical and neurological. Therefore, dietary supplements such as soybeans, that are rich in phytoestrogens, in particular Genistein (GEN), are recommended for these incorrect diets during pregnancy. GEN belongs to the class of endocrine disruptors for its ability to bind estrogen receptors, affecting various estrogen-sensitive neural systems, such as the stress axis.
In this work, we studied in Female Sprague Dawley rats the effects of a maternal chronic low-protein diet (8%) with and without the phytoestrogen GEN on maternal and feeding behavior, as well as on both the stress axis and offspring development.
The results showed that the effect of the low-protein diet with genistein alone leads to an anxiolytic effect, which is better observed in the OF test. With pregnancy, however, all animals showed anxiogenic behavior, more pronounced in those treated. At birth of the pups, we analyzed the plasma corticosteroid levels by ELISA assay, which were found to be lower in pups of mothers treated with the low-protein diet, compared with controls. Moreover, the expression of the glucocorticoid receptor and CRH, analyzed in the brain by RT-PCR, decreased in both female-treated groups compared with controls. Pups born to these treated mothers never resumed their normal development, always remaining underdeveloped in weight and size. In conclusion, these data indicate that maternal diet is critical for maintaining the balance of the stress axis in the offspring at birth and normal development, but also for maternal anxiety-like behavior.

Ultrastructural effects of mTOR inhibition in iPSC-derived motor neurons from patients with *C9orf72* repeat expansion

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The use of iPSC promoted to differentiate into motor neurons (iPSC-MNs), is now more and more important in dissecting at cellular level the cell pathology occurring in a variety of disorders, including Amyotrophic Lateral Sclerosis (ALS). In the present study we used iPSC-MNs, obtained from patients carrying a repeat expansion in C9orf72, a common genetic cause of familial ALS, to test whether specific cell pathways, such as the autophagy machinery and the formation and release of exosomes, were altered. In neurodegeneration, autophagy impairment may alter the clearance of pathological aggregates, which can be transmitted from cell to cell. This could promote the spreading of pathological elements, such as pathological proteins and exosomes, contributing to disease progression.
The gene coding C9ORF72 protein is involved in the autophagy machinery, interacting with Unc-51 Like Kinase 1 (ULK1) in autophagy initiation. The autophagy activator lithium when administered to patients carrying either ULK1and C9orf 72 mutation, delays disease course. This suggests that autophagy plays a key role in the degeneration of motor neurons. According to this evidence, in the present study, iPSC-MNs were treated with rapamycin, which activates autophagy by inhibiting the mTOR complex. In these cells we carried out quantitative morphometry of TDP-43 protein, that contributes to the formation of pathological aggregates in C9orf-associated ALS and two exosomal markers: ALIX and TSG-101. These are involved in exosome biogenesis from multi-vesicular bodies. Moreover the autophagy markers LC3 and p62/sequestosome 1 were also analyzed. Rapamycin significantly reduces the massive amount of TDP-43, which accumulates within C9orf72 iPSC-MNs both within the cytosol and nucleus. Concomitantly, rapamycin suppresses TSG-101 and ALIX within C9orf72 iPSC-MNs. These findings suggest that mTOR inhibition occurring in the course of autophagy-dependent specific ALS phenotype improves the disease course.

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Chemotherapy-induced peripheral neuropathy: a focus on er-mitochondria interactions.

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Chemotherapy (CT)-induced peripheral neuropathy (CIPN) is a common adverse effect of the treatment with different classes of chemotherapeutic agents and it manifests as a set of sensory-related symptoms. It is estimated that around 60-70% of patients undergoing CT experience these symptoms, that might be so disabling to force them to CT dose reduction or treatment withdrawal. At the cellular level, CT is well known to exert neurotoxic effects over sensory neurons and peripheral glial cells, but the precise molecular mechanisms by which it induces damage to these cells are poorly understood. Noteworthy, mitochondria have been reported as targets of several chemo-based regimens, both in terms of their morphology/motility and of their specific functionality, essential for the maintenance of cellular homeostasis. In the context of mitochondria, growing body of evidence highlights the relevance of ER-mitochondria interaction, which takes place at the level of complex morpho-functional units known as mitochondria-ER contact sites (MERCS). Therefore, a promising strategy to unveil CIPN molecular mechanisms could be represented by a focused analysis of the possible effects of antineoplastic drugs at the level of MERCS in both sensory neurons and glial cells. To achieve this goal, we are investigating MERCS structure and functionality upon treatment with different classes of chemotherapeutic agents, firstly in F11 and Msc80 cell lines (chosen as models for sensory neurons and peripheral glial cells, respectively) and next in primary cells. Here we provide preliminary insights of dramatic alterations observed in mitochondria and MERCS distribution in Msc80 after treatment with bortezomib (BTZ), despite the mechanism by which the drug exerts these effects still needs to be fully elucidated. Then, once identified possible mechanisms altered by CT, we will try to correct them by employing neuroactive steroids, that have been reported to have beneficial effects over neurons in different subtypes of peripheral neuropathies.

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Aging and degenerative diseases

Terpenes and Alzheimer's disease: a study in stem cell derived models

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Alzheimer's disease (AD) is the leading cause of dementia during aging. While its pathological hallmarks have been identified, disease-modifying treatments are not yet available. Therefore, there is increasing interest in studying lifestyle interventions that could help in the prevention and mitigation of the symptoms. In this context, we aim to study the effect of green exposure and particularly of phytochemicals inhalation, focusing on terpenes, specifically pinene, widespread in Italy. For this purpose, we set up two stem cell derived models of AD. The first is NE-4C, a murine neural stem cell line that upon retinoic acid stimulation differentiates at 7 days in neurons and at 14 days in a mixed population of neurons and astrocytes. In this model Aβ1-42 monomers (0.1-5 μM) 48h administration in both undifferentiated and differentiated cells results in increased cell death (assessed through MTT assays), while pinene (250 μM) co-administration enhances viability. Moreover, in NE-4C Aβ1-42 oligomers (whose assembly has been verified through WB) seem to be more toxic compared to monomers. Following monomers or oligomers treatment we also evaluated apoptotic pathway activation through C-Caspase3 staining, whose level are significantly decreased with pinene co-treatment. As a second model, we successfully differentiated cortical neurons from hiPSCs using Autar K. et al. protocol (2022) and we are currently repeating the same experiments in a human background. This study clarifies the mechanisms underlying the benefits derived from green exposure, thus representing the scientific basis to promote the diffusion of lifestyle interventions to prevent and treat AD.

Pre-clinical analysis of intracerebroventricular hNSC cell graft in SOD1^{G93A} mice as a potential treatment for amyotrophic lateral sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disorder characterized by the impairment of the upper and lower motor neurons (MNs), leading to muscles atrophy, weakness, and finally death due to respiratory failure.
The potentiality of neural stem cells (NSCs) as a therapeutic approach to improve the state of the MNs and motor performances in neurodegenerative disorders have been evaluated in pre-clinical studies. Moreover, phase I clinical trials (NCT01640067, NCT01348451) have proven the safety and feasibility of human Neural Stem Cell (hNSC) transplantation in spinal cord of ALS patients. However, this administration route presents several disadvantages.
The aim of this project is to analyse the potentiality of a new way of administration of hNSCs in SOD1G93A mice, a well-known ALS model.
For this purpose, at postnatal day 70 (P70; presymptomatic stage) the mice received an intracerebroventricular injection of hNSCs. Following the graft, the motor performances were evaluated until sacrifice (at P110 or end-stage). To support cell survival, mice underwent a transient (15 days) or an extended (40 days) immunosuppression protocol. At sacrifice, brain, spinal cord, muscles and blood were collected: we are currently evaluating the biodistribution of the hNSCs, the rate of neuroinflammation, the number of upper and lower MNs and the muscular atrophy.
Our preliminary results suggest that the treatment might delay the decline of motor performances. However, cell survival is not optimal (in particular with the transient immunosuppression protocol), and the reduced sample size prohibits any definitive conclusion.
However, we are confident that this administration route could be an effective approach to increase cell dosage, to encourage a broader spread of transplanted cells and released factors throughout the motor neuraxis by exploiting the CSF circulation, and to possibly target also the motor cortex.

The complex cross-talk between lipid metabolism and inflammation: neuroprotective role of antioxidants

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Neurodegenerative diseases comprise a wide spectrum of pathologies characterized by progressive loss of neuronal functions and structures. Despite having different genetic background and aetiology, in recent years, several studies have shown a point of convergence in the mechanisms that lead to neurodegeneration. Mitochondrial dysfunction and oxidative stress have been observed in several pathologies, and their harmful effects on neurons contribute to the exacerbation of the pathological phenotype at various levels of severity. In this context, increasing relevance has been acquired by antioxidant therapies, with the aim of restoring mitochondrial functions to revert the neuronal damage. Indeed, in the last decades, new antioxidant compounds targeted at mitochondria have been developed and studied to address the need to counteract the oxidative stress and restore energy supply and membrane potential in neurons. This category includes Mitoquinone (MitoQ) which is characterized by a high bioavailability, and previous studies have demonstrated its neuroprotective potential.
The present study aims at examining the effect of MitoQ on the activation of the inflammatory pathway in a neuronal cell line (DAOY) treated with a specific inflammatory stimulus. It was also assessed whether the addition of MitoQ could modulate oxidative stress. To mimic cholesterol deregulation, DAOY cells were exposed to lovastatin (10uM), a statin that biochemically blocks the cholesterol pathway, also known as the mevalonate pathway. To investigate the effect of MitoQ, cells were treated with MitoQ 1 hour before lovastatin administration.
The lovastatin treatment was able to induce a statistically significant decrease in the normalized values of the cellular index, an index of cell proliferation, compared to untreated cells, at 12 hours and 48 hours after treatment. The results obtained at 12 hours show the protective effects of pretreatment with MitoQ, while this effect was no longer observable at 48 hours after stimulation. Interestingly, the effect of MitoQ does not seem to be limited to its overall antioxidant action, since treatment with another powerful and well-recognized ROS scavenger, N-acetylcysteine (Nac), failed to protect DAOY cells from statin-induced cell death, thus showing a specific MitoQ effect that can prevent cytotoxicity caused by the block of the mevalonate pathway.
The lovastatin treatment was able to induce overexpression of NLRP3, CASP-1 and OPA-1 compared to the control condition; at the same time the pretreatment with MitoQ was able to significantly counteract this trend at 12 hours after lovastatin administration. The cytokine profile also showed a statistically significant modulation in the production of several analytes (IL-1β, IL-2, IL-4, IL-6, IL-8, IL-17, IFN-γ and TNF-α) at 12 hours after administration of lovastatin compared to the untreated condition. The pretreatment with MitoQ was able to reduce the secretion of all cytokines and in particular the decrease was appreciable for IL-1β, IL-2, IL-4, IFN-γ and TNF-α.
All the results obtained suggest that MitoQ can be an effective adjuvant for the treatment of autoinflammatory diseases characterized by a deregulation of the cholesterol pathway affecting mitochondrial homeostasis.

Iron and βamyloid deposition pattern in the adult 5xFAD MOUSE model of Alzheimer's disease

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Iron deposition has been described to naturally occur in the brain during aging and other neurological disorders, including different forms of dementia as Alzheimer's disease (AD). The dysregulation of iron metabolism can cause oxidative stress and inflammation. However, the mechanisms by which iron accumulates in these conditions are yet to be determined.
In this work we combined histological techniques, as Prussian blue PERL's + DAB and thioflavin-T staining, on brain sections of 5xFAD (AD model) and WT mice at 4 and 12 months of age. We detected an important accumulation of non-heme iron in many of the regions of 5xFAD brains' mice compared to WT: i.e., cortex, third ventricle, striatum and the hippocampus. Interestingly, these are the main brain areas affected by amyloid pathology, already at 4 months of age. Consistently, this accumulation increased progressively until the maturity of the animals at 12 months. At a cellular level, older 5xFAD showed important amounts of cytosolic iron assimilated to the protein Ferritin-L, detected by IHC, confirming the iron overload that proceeds with age. Moreover, thioflavin-T, upon binding amyloid fibrils, allowed us to observe the pattern of distribution of βamyloid in the brain and, consistently, it revealed that there is colocalization with iron deposits at the different ages studied. Furthermore, inflammatory markers, such as IBA-1 and GFAP, showed a severe glial activation already at 4 months of age in the 5xFAD background.
These data suggest a correlation between the two main types of deposits studied and stress the relevance of iron accumulation during the amyloidosis process that the brain undergoes prior to neurodegeneration. We point this evidence as a novel pathophysiologic target to treat dementia.

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Brain iron dyshomeostasis and mitochondrial features' alteration in 5xFAD mouse model in the pre-pathological phase of Alzheimer's disease

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Iron is essential for neuronal activity, neurotransmitters' synthesis and energy homeostasis, in particular for ATP production by the electron transport chain. Several evidence reported that iron dyshomeostasis impacts mitochondrial function in neurodegenerative diseases, such as Alzheimer's disease (AD), leading to energy failure and contributing to neuronal death. However, how iron-induced mitochondrial dysfunction participates to AD remains unknown.
Here, we investigated if iron homeostasis in the 5xFAD mouse model, expressing human APP and PSEN1 transgenes with five AD-linked mutations, is altered during the pre-pathologic phase of the disease (1 and 2 months).
At 1 month of age, we observed that in the hippocampus and cortex of 5xFAD mice there is no iron deposition, verified by Brain Iron Content and Prussian blue Perl's staining. Moreover, the Hepcidin/Ferroportin pathway involved in systemic iron regulation was not active. Although we observed no differences between 5xFAD and wild-type mice in iron income measured by protein levels of the Transferrin Receptor 1 and storage (Ferritins), we found an initial perturbation affecting mitochondria. Indeed, the activity of mitochondrial Aconitase (mAco2), the enzyme involved in the Krebs cycle for the generation of NADH for the respiratory chain, was altered. Consistently, we observed decreased protein levels of the Complex V of the OXPHOS respiratory chain. In 2 month old 5xFAD, we found the presence of iron deposition in the hippocampus and striatum, in the absence of amyloid plaques. At this stage, the Hepcidin/Ferroportin pathway is still not active. Interestingly, we observed a clear mitochondrial phenotype with a significant increase of Tom20, mAco2 and all the complexes of the respiratory chain, indicative of mitochondrial content increment.
Overall, our data show initial alterations in brain iron metabolism together with mitochondria perturbation in 5xFAD mice suggesting a close correlation between these two phenomena in worsening amyloid pathology.

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Morphology and molecular pathology.

Inibizione delle deacetilasi in modelli cellulari di anemia di Fanconi: effetti sulle risposte antiossidanti e sulla morfologia e funzionalità del mitocondrio

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L'Anemia di Fanconi (FA) è una malattia genetica rara caratterizzata da anomalie nella riparazione del DNA e da un accumulo di stress ossidativo dovuto ad un difetto del metabolismo aerobico associato ad un'alterazione dell'organizzazione della rete mitocondriale. Questo disequilibrio redox non è contrastato dalle difese antiossidanti endogene, le cui espressioni e attività sono inferiori rispetto alle cellule sane. Poiché la ridotta espressione di queste proteine potrebbe dipendere dall'ipoacetilazione dei rispettivi geni, abbiamo indagato l'effetto di alcuni inibitori delle deacetilasi (i-HDAC) sull'espressione e sull'attività di enzimi coinvolti nelle risposte antiossidanti e sulla morfologia e funzionalità dei mitocondri in modelli cellulari di FA. Linfoblasti e fibroblasti mutati per il gene FANC-A sono stati trattati con 3 diversi i-HDAC: acido valproico (VPA), β-idrossibutirrato (OHB) o EX527 (un inibitore di Sirt1) in condizioni basali e a seguito di induzione di stress ossidativo. In condizioni basali, il trattamento con VPA ha aumentato l'espressione e l'attività degli enzimi coinvolti nella risposta antiossidante, ha corretto il difetto metabolico, risultando in un miglioramento dello stato energetico e in una riduzione del danno ossidativo. Questi miglioramenti potrebbero dipendere da un ripristino della dinamica mitocondriale dovuta ad un calo dell'espressione di DRP1, che ha comportato una riorganizzazione dei mitocondri e, quindi, un miglioramento della loro efficienza funzionale. Al contrario, il trattamento con OHB ha peggiorato il difetto della funzionalità mitocondriale, contribuendo all'aumento dello stress ossidativo. Infine, EX527 non ha mostrato alcun effetto.
I diversi i-HDAC hanno mostrato gli stessi effetti anche in presenza di un insulto ossidativo a seguito dell'aggiunta di H_2O_2.
In conclusione, i dati suggeriscono che il VPA potrebbe essere un farmaco promettente per modulare l'espressione genica nelle cellule di FA, confermando che la modulazione della risposta antiossidante svolge un ruolo centrale nella patogenesi della FA, poiché agisce sia sui livelli di stress ossidativo che sulla funzionalità e sull'organizzazione mitocondriale.

Influence of antiretroviral drugs on adipocyte differentiation

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The aim of the study is to understand the possible influence of antiretroviral drugs on adipocytes differentiation by using an *in vitro* adipogenesis model. The focus was on four integrase inhibitors (INSTIs) (DTG, CAB, DOR, RIL), administered individually or in combination, alongside to two nucleoside reverse transcriptase inhibitors (NRTIs) (TAF and TDF), used in combination with some INSTIs.
The adipogenesis model was developed using 3T3-L1 cell line [1]. The differentiation was carried out by switching multiple media for eight days [1, 2]. The drugs, at a concentration of 30 µg/ml, were added to the differentiation medium on the first day and administered daily until the fourth day [4]. The outcome was evaluated by Red Oil O staining, estimating the accumulation of intracellular lipids, and by Western Blot, determining the expression levels of differentiation markers (PPARɣ and C/EBPα).
Each INSTIs induced adipocyte differentiation: there was an increase in the lipid droplets formation and the upregulation of both PPARɣ and C/EBPα, compared to the control group. The combination of INSTIs proved to have a synergistic effect on the phenotypic shift, resulting in enhanced differentiation. Alternatively, the combination of INSTIs and NRTIs showed opposite effects: the RIL-TAF combination resulted in an inhibition of the differentiation, with a reduction in the lipid droplets formation and the downregulation of PPARɣ and C/EBPα, compared to the control group. A similar, but lesser effect, occurred in the DOR-TDF combination.
In conclusion, INSTIs can significantly induce adipocyte differentiation in 3T3-L1 cell line. Combination treatment has proved to achieve greater results than single drug treatment, implying a synergistic effect. Interestingly, both NRTIs (TAF, TDF) acted as antagonists to adipocyte differentiation in combination with RIL and DOR. These findings emphasized the complexity of the antiretroviral drugs effects on the adipose tissue biology and highlighted the need for further investigation to clarify the mechanism underlying this interplay.
Keywords: antiretroviral therapy; adipocytes; differentiation; INSTIs; NRTIs; PPARɣ and C/EBPα; lipid droplet.

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Polylactic acid (PLA) versus polystyrene (PS) microplastics: internalization studies in different human cell lines

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Microplastics (MPs) and nanoplastics (NPs) have become ubiquitous in the environment due to extensive use of plastic and poor waste management. These pollutants contaminate ecosystems and can reach the human body through inhalation, ingestion, and dermal contact representing a potential human health risk [1]. Recently, the concerns surrounding plastic's environmental and public health implications have led to the development of biodegradable polymers, such as polylactic acid (PLA) [2]. Although biodegradable polymers like PLA may offer environmental benefits such as being derived from plants, recyclable, compostable, and easily degraded by microbes [3], their biosafety cannot be guaranteed due to the lack of research in this field. This background prompted us to investigate the potential harmful impact of PLA by assessing its potential to cross the cellular membrane and accumulate in the cells. To achieve this objective, we exposed human dermal fibroblast (HDF), and human colon adenocarcinoma cells (HT29, and Caco-2) mimicking the dermal and ingestion human exposure routes to different concentrations of PLA MPs. Additionally, we also evaluated the cellular uptake ability of polystyrene (PS)-MPs, a non-biodegradable plastic, used at the same concentrations and treatment times, to compare the effects of biodegradable versus non-biodegradable plastics. Commercially available green fluorescent PS-MPs (Fluoro-Max Dyed Green Aqueous Fluorescent Particles, Thermo Scientific™, G0100) and PLA-MPs conjugated with Rhodamine synthesized in our lab through a microfluidic-assisted nanoprecipitation were used. HDF, Caco-2, and HT29 cell lines were exposed to increasing concentrations of PLA-MPs and PS-MPs for different time points. Flow cytometry analysis demonstrated that PS-MPs were taken up by approximately 20-50% of the cell population in all tested cell lines. In contrast, the cellular uptake of PLA-MPs was more notable than that of PS-MPs, especially in intestinal cell lines. Cellular internalization studies have been also corroborated by immunofluorescence analyses showing a perinuclear and intracytoplasmic localization of both PLA- and PS -MPs in treated cells. These preliminary findings suggest that PLA and PS may penetrate cell membranes, potentially impacting the functioning of cellular organelles with specific outcomes that could be influenced by MPs particle size, polymer type, and exposure time.

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Phenotypic and morphofunctional changes in the life cycle of chronic lymphocytic leukemia B cells during the transition between peripheral blood and secondary lymphoid organs

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Chronic lymphocytic leukemia (CLL) cells can be divided into subsets based on time since last cell division. This can be determined by patients drinking deuterated water (2H2O) and then using flow cytometry to identify intraclonal subpopulations with reciprocal surface levels of CXCR4 and CD5.
We redefined the kinetics of CLL fractions and provide novel insights about their functional dynamics. *Ex-vivo* CLL cells from patients who drank 2H2O were sorted by CXCR4/CD5 relative densities, 5 franctions. Using 2H-DNA as determinant of age, a unidirectional path of phenotypic change could be defined, with the bigger and complex active cells transitioning to the small, quiescent status.
Since BCR signaling is fundamental for CLL proliferation, we analyzed the densities of smIgM, smIgD (smIGs) and smCD19, finding higher densities on fractions with higher 2H-DNA incorporation. Notably, these findings were not consistent with cell division being uniquely initiated by BCR engagement. Chronological combinations of stimuli via TLR9 and smIGs showed that increased IG density required TLR9 stimulation before or concurrently with the latter. Thus, recently-divided cells might have experienced multifactorial stimulation.
The fractions sorted before and during ibrutinib treatment *in-vivo*, displayed diverse intraclonal changes in smIG densities, cell size and complexity, and metabolic activation, with 2H-enriched and higher smIG density cells being more affected.
These data defined additional CXCR4/CD5 subpopulations of divergent ages, phenotypes, and sensitivities to treatment, suggesting that CLL B-cell kinetics are more complex than the current model describes. This complexity originates in secondary lymphoid organs, where stimulation by the BCR and other pathways generates the young CLLs. In the blood, CLLs age to the quiescent fraction. Since each cell within a clone appears to traverse these stages, the unique biologic features at each phase represent novel processes for therapeutic targeting.

LINC complex as potential mediator in bone sarcomas differentiation

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Changes in lamin levels and nuclear lamina-associated proteins are associated with poor prognosis in several cancers[1]. The interaction between lamins and LINC (linker of nucleoskeleton and cytoskeleton) complex proteins mediates the transmission of mechanical stimuli from the extracellular matrix to the nucleus, resulting in chromatin reorganization and gene expression regulation[2,3]. This interaction modulates epigenetic mechanisms which controls cell differentiation. Several evidences identified the LINC complex as a crucial factor involved in mesenchymal stem cells (MSC) commitment[2,4], while lamins are already known to have a role in inducing MSC differentiation towards osteogenic lineage[5,6].
For these reasons our aim was to investigate the role of LINC complex in normal and pathological differentiation in bone sarcomas. We already demonstrated that lamin A levels were significantly reduced in osteosarcoma (OS), compared to differentiating osteoblasts[7], and that the overexpression of lamin A pushes Ewing sarcoma cells to a more differentiated phenotype, rescuing LINC complex[8].
Here, we assessed the expression and localization of LINC complex proteins during human osteoblast differentiation, compared to osteosarcoma. We found a significant increase in lamin A expression in both models during differentiation, accompanied by a strong increase in the expression of SUN1, Nesprin 2, and Emerin. Interestingly, these modulations were observed already after 24 hours of differentiation in normal osteoblasts, while in OS cells, the increase of SUN1 and Emerin was evident only after 7 and 14 days of differentiation, with a partial rescue of LINC complex. The comprehension of how to repristinate physiological nuclear envelope dynamics will be particularly relevant for these tumors, aberrantly stopped in their process of differentiation and where differentiation-based non-toxic strategies are urgently needed.

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Morphology, Cadaver Lab and Teaching strategies

Peer-to-peer tuturing activities in human anatomy exploiting digital tools

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Since Academic Year 2023/2024 first year medicine students were provided with the opportunity to exploit interactive tools for macroscopic anatomy teaching activities via peer-to-peer tutoring: fourth- and fifth-year students in Medicine and Surgery were trained to take care of this.

First year medical students (from the Italian [n=160] and International Course [n=40]) were divided into groups (10-12 students each); they group met with a tutor for at least 3 sessions lasting 2 hours with a predefined topic: thorax (topic 1), abdomen/pelvis (topic 2), neuroanatomy (topic 3). Moreover, each group participated in a session without a predefined topic during which they freely explored the teaching tool under supervision. This was not a compulsory activity and was carried out in students' free hours during the 2nd semester; a satisfactory questionnaire was collected at the end of the initiative; satisfaction for each time was rated on 0-10 scale (0: no satisfaction at all; 10: the highest possible satisfaction).

75 students decided to participate overall and a total of 47 complete questionnaires were available for evaluation. More than 70% of students rated the whole initiative as high satisfactory (i.e., score higher than 6) and 85% of students would have suggested others to participate. Topic 3 was the one receiving the highest scores. More than 85% of students replied that this initiative increased their willingness to use the Anatomy room for independent study.

For the present Academic Year the initiative is being further expanded: students are having more sessions without a predefined topic, on top of the same topics assigned last year. More students decided to opt for this adjunctive opportunity (120/170 for the Italian course and 40/48 for the international course). We are collecting more detailed information on satisfaction and effectiveness and currently the response rate to questionnaires is more than 90%; preliminary results are even more positive than last year.

“Live Cadaver” Model for Internal Carotid Artery Injury Simulation in Endoscopic Endonasal Skull Base Surgery

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Introduction: Intraoperative injury of the internal carotid artery (ICA) is the most dreaded complication in endoscopic endonasal surgery (EES) of skull base. Training for ICA injury is practically impossible in live operative settings. In this study, we evaluate a pulsatile perfusion-based live cadaveric model for ICA injury simulation in a laboratory setting. The major emphasis of the study was to evaluate various means of controlling acute bleeding and evaluating the practical utility of this model for training purposes.
Methods: Five embalmed uninjected cadaveric heads were prepared for study by connecting to a pulsatile perfusion pump system filled with artificial blood solution. EES approaches were used to evaluate different types of ICA injuries similar to operative scenarios. Various methods of managing ICA injuries such as packing, clipping, trapping, were evaluated. The educational advantages of the live cadaver model were assessed using questionnaires given to participants in a hands-on dissection course.
Results: The trainee was faced with several scenarios similar to those encountered during an actual intraoperative ICA injury. Packing, clipping and trapping of the ICA injury was successfully achieved in all segments of the ICA. Clip-based reconstruction techniques were successfully developed. All trainees reported gaining new knowledge, learning new techniques. The responses to the questionnaire confirmed the significant educational value of this model.
Conclusions: The live cadaver model presented here provides real life experience with major vessel injury during EES in a laboratory setting. This model could significantly improve current training for management of intraoperative vascular injuries during EES.

Epithelial-mesenchymal transition in organogenesis and carcinogenesis

The role of miR-145 in the emergence of adaptive resistance to AKT inhibition in prostate cancer

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The tumor-suppressor miR-145 regulates different cellular processes in prostate cancer (PC) and its loss is involved in the transition from localized to metastatic disease progression. Moreover, low expression of miR-145 is able to predict poor survival of PC patients (Coradduzza et al., 2022). A recent and promising therapeutic strategy for PC associates AKT inhibitors to drugs blocking androgen synthesis such as abiraterone. However, AKT inhibition is very well known to trigger a rapid development of resistance due to the interruption of negative feedback circuits or perturbation of pathway homeostasis (Wright SCE, et al., 2021). Interestingly, in our laboratory we found that AKT modulates miR-145 expression in the prostate cancer cell line PC3. In particular, we observed that AKT inhibition not only down-regulates miR-145 but also evokes a dramatic increase (~20 folds) in the expression of a gene target of miR-145, namely the oncogene RAS. Since RAS is a potent activator of the PI3K/AKT pathway, we hypothesized that AKT inhibition could result in AKT paradoxical reactivation. Confirmation that the observed drop of miR-145 triggers an increase of RAS, both in terms of mRNA and protein, was obtained using PC3 cells engineered by us to transiently silence the 145-5p guide strand of miR-145 following exposure to doxycycline. We further demonstrated that pharmacological inactivation of AKT with capivasertib leads to overexpression not only of RAS but also of another GTPase family member, Rab5, a key regulator of early endosomes formation and maturation to late endosomes (Hutagalung and Novick, 2011). This finding is particularly interesting as RAB-5 is a direct target of miR-145 (Wen X. et al., 2023). Moreover, the overexpression and activation of this protein is considered a switch in the process of tumor cell migration, which also involves targets of miR145 such as N-cadherin and β-catenin (Jamieson C. et al., 2014). Our finding that N-cadherin and β-catenin expression and intracellular localization is affected by capivasertib is therefore in good agreement with our previous results and pave the way to the identification of markers characterizing patient subgroups that will derive maximal benefit from PI3K/AKT targeting.

Keywords:
miR-145; prostate cancer; AKT inhibitors; adaptive resistance; RAS; RAB-5

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Anatomy and movement

Influence of acoustic and visual stimuli on vertical jump performance

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The ability to perform maximal vertical jumps (VJs) significantly affects athletic performance in several sports, such as volleyball[1]. A few studies analyzed the effects of different sensory stimuli on VJ performance. Visual stimuli are processed in the visual cortex located in occipital lobe while acoustic stimuli involve temporal lobe regions called auditory cortices, which receive direct afferents from the auditory thalamus[2, 3].
To the best of our knowledge, no study quantitatively assessed the variations of spatial-temporal and kinetic parameters during Countermovement Jump-Free Arms (CMJ-FA) execution in different acoustic and visual conditions.
The aim of the present study is to explore the effects of the incentive and disincentive sensory stimuli (visual and acoustic) on CMJ-FA performance via an Inertial Measurements Unit.
Twenty male volleyball athletes were assessed using “Baiobit” sensor. Five sessions, each with 3 CMJ-FA trials, were performed without sensory stimulus (NS), with incentive (IAS) and disincentive (DAS) acoustic stimulus, and with incentive (IVS) and disincentive (DVS) visual stimulus. Eight spatial-temporal and four kinetic parameters were evaluated.
Results showed in DVS condition with respect to NS a significant decrease for Mean Time of Flight Phase, Mean and Peak Jump Height, and a significant increase for Impact Index. Moreover, a significant decrease for Mean Time of Flight Phase, Mean and Peak Jump Height was found in DAS condition with respect NS.
These findings highlighted the interference of disincentive conditions on jump performance, in particular for visual stimuli. In fact, in humans the dominant sense that controls movement is vision[4]. In addition, an increase of force during landing phase in DVS may predispose to muscle-skeletal lower limb injuries. These knowledges could be useful to the sports trainers for improving athletes’ control, in order to desensitize them from disincentive conditions, keeping a good performance and decreasing the risk of injuries during the competition.

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Histogenesis, functions and dysfunctions of the musculoskeletal system

Red photobiomodulation promotes skeletal myoblast differentiation: morpho-functional evidences

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Background: adult skeletal muscle regenerates lost damaged tissue mainly thanks to the activity of a small population of resident stem cells, namely satellite cells (SCs). In the case of chronic or severe damage, SCs' functionality may be compromised by the occurrence of an aberrant fibrotic reparative response. Strategies aimed to improve the muscle intrinsic regenerative capacity while limiting the excessive deposition of fibrotic tissue may be promising. In this perspective, photobiomodulation (PBM) (i. e. application of light with 400-1100 nm wavelength using different laser or LED devices, power density less than 100 mW/cm2 and energy density less than 10 J/ cm2 at target) may represent a valid option based on its well-known pro-regenerative effects and increasing evidence of its antifibrotic potential. However, PBM's effects on skeletal muscle are controversial, and univocal guidelines for its use are missing.
Aim: to evaluate the effects of different treatments of red PBM (laser diode 635±5 nm, energy density: 0.4, 4 and 8 J/cm2, single exposure) on murine myoblasts undergoing differentiation and on differentiated myotubes.
Methods and Results: MTS cell viability assay, morphological analyses (myotube formation, confocal immunofluorescence analysis of the expression of myogenic markers and of PGC1-α/ mitochondria biogenesis) and electrophysiological investigations (cell membrane passive properties and ion currents) revealed that red PBM with 4 J/cm2 energy density promoted myoblast differentiation process and did not alter differentiated myotube viability and features.
Conclusion: this *in vitro* study contributes to provide experimental background to support the pro-myogenic effect of red PBM and cues for further investigations.
Future Investigations: to explore red PBM's effects on cells cultured in the presence of cell-damaging or anti-myogenic agents, on human skeletal myoblasts and on myoblasts induced to differentiate on a liquid crystalline network used as a cell instructive scaffold to support a correct myogenic differentiation.

Innovative technologies,3D models and organoids

In-vitro development of the microvascular network serving the central nervous tissue

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The microvasculature underlies the supply networks that support neuro-glial activity within heterogeneous brain regions, contributing in central nervous system (CNS) health and diseases. Brain microvascular endothelial cells (BMEC) are a central element of the microvasculature, forming the infrastructure for blood-brain barrier (BBB) and shielding the brain against toxins and immune cells via paracellular, transcellular, transporter, and extracellular matrix (ECM) proteins. Functional microvasculature remains a major challenge to engineering tissue replacements and restoring function in many neurological disorders. The adequate and efficient functioning of the microcirculation requires vessels for transport, geometrical settings of the molecular components in the ECM, and an efficient distribution of convective blood flow. Our project (THOR – European Innovation Council-Pathfinder Programme, Grant Agreement number 101099719) aims at accomplishing this challenge. We used high-performance functionalized silk fibers (10-20 micrometers) with customized geometries as a scaffold to seed C57BL/6 Mouse Embryonic Brain Endothelial Cells. Silk fibers were functionalized with either vascular endothelial growth factor (VEGF) or the laminin alpha-1 chain Ile-Lys-Val-Ala-Val (IKVAV) peptides. We studied the cells response at different time points. After 5 days of incubation (DIV 5), we observed the formation of tube-like structures expressing the blood-brain barrier (claudin-5) and pericytic biomarkers (CD13, PDGFRbeta). The obtained endothelium produced basal lamina proteins (collagen IV, laminin) as the cells were anchored, parallel, or perpendicular to the silk fibers. We observed how diverse peptides bound to the silk can stimulate the embryonic cells to self-organize differently within the engineered frame. The current results prompt further studies for the *in-vitro* development of complex microvasculature networks and regenerative medicine approaches targeting the CNS.

Epithelial and connective tissues

Galectin-10 and respiratory remodeling: new insights from nasal washes of SAR pediatric patients

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The glycoprotein Galectin-10 (Gal-10) has been identified as a type 2 inflammation biomarker in eosinophilic diseases, such as asthma, where it crystalizes in Charcot-Leyden crystals (CLCs), promoting inflammation and tissue damage. However, the precise role of Gal-10 in inflammation and tissue remodeling remains unclear. To address this, we conducted a two-phase study with the following objectives: (1) to evaluate Gal-10 levels and their correlation with pro-inflammatory cytokines in nasal washes of pediatric patients with seasonal allergic rhinitis (SAR), and (2) to investigate the role of Gal-10 in respiratory mucosal remodeling using an *ex-vivo* three-dimensional culture model stimulated with nasal washes.

In the first phase, we collected nasal lavages from pediatric SAR patients and analyzed inflammation markers, including Gal-10, MUC-5AC, IL-5, IL-17, IL-23, and INF-γ. We stimulated 3D outgrowths with nasal washes in the second phase for two weeks and assessed epithelial-mesenchymal transition using immunohistochemical and biomolecular investigations. Our study sheds light on the potential role of Gal-10 in inflammation and respiratory mucosal remodeling, providing insights into the pathogenesis and treatment of eosinophilic respiratory diseases.