Book of Abstracts

Joint IUBMB/FEBS Advanced Lecture Course

Molecular targets for anti-aging interventions

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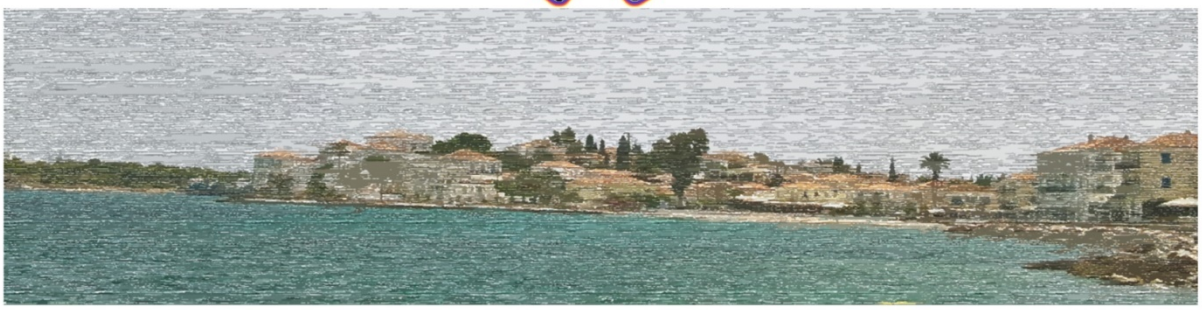

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SCIENTIFIC COMMITTEE:

Dr Aleksandra Mladenovic

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Dr Silva Katusic

Dr Alexandra Newton (IUBMB)

Dr Beata Vertessy

Dr Selma Kanazir

Dr Smilja Todorovic

Dr Marianna Kapetanou

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Dr Beata Vertessy

Dr Smilja Todorovic

Dr Marianna Kapetanou

SECRETARIES:

Dr Smilja Todorovic

Dr Marianna Kapetanou

SPEAKERS:

Aleksandra Mladenovic, Institute for Biological Research "Sinisa Stankovic", Belgrade, Serbia

Alexandra Newton, IUBMB Speaker, University of California at San Diego, La Jolla, CA, USA

Moussa Youdim, Special guest, Professor Emeritus, Rappaport Faculty of Medicine at the Technion – Israel Institute of Technology

Beata Vertessy, FEBS Speaker, Hungarian Academy of Sciences, Institute of Enzymology, Budapest, Hungary

Bertrand Friguet, Sorbonne Université, Paris, France

Cláudia Nunes dos Santos, CEDOC - Centro de Estudos de Doenças Crónicas, NOVA Medical School, Universidade Nova de Lisboa, Portugal

Claudio Franceschi, Università di Bologna, Bologna, Italy

Dan Ehninger, German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany

Efstathios S. Gonos, National Hellenic Research Foundation, Athens, Greece

Ilaria Bellantuono, UK Centre for Integrated Research into Musculoskeletal Ageing (CIMA), Sheffield, UK

Irene Diaz-Moreno, Instituto de Investigaciones Químicas (IIQ), Universidad de Sevilla – CSI, Sevilla, Spain

Jan H.J. Hoeijmakers , Dept of Molecular Genetics, Rotterdam, The Netherlands

João Laranjinha, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal

Richard Faragher, Centre for Stress and Age-Related Disease, University of Brighton, UK

Selma Kanazir, President of the Serbain Neuroscience Society

Silva Katusic Hecimovic, Rudjer Boskovic Institute, Zagreb, Croatia

Varvara Trachana, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece

Aging and Cytochrome c Phosphorylation

I. Díaz-Moreno[I]

[I]Institute for Chemistry Research, cicCartuja - University of Seville - CSIC, Seville, Spain

The response of cells to physiological changes, i.e. oxidative stress, involves the modulation of mitochondrial metabolism. Dysfunction of metabolism favors the development of hypoxia-dependent pathologies, including ischemia and cancer. A key modulator of mitochondrial activity is cytochrome *c* (C*c*), which plays a double role in cell life and death. During homeostasis, C*c* is confined to mitochondria and acts as an electron carrier within the respiratory transport chain[1]. When cells are exposed to stress, however, C*c* is released from mitochondria to interact with several targets in the cytoplasm[2] and even the nucleus[3-5].

All functions of C*c* are regulated by posttranslational modifications, which increase the functional diversity of the hemeprotein. C*c* undergoes phosphorylation *in vivo* at threonines 28[6] and 49[7], serine 47[6] and tyrosines 48[8] and 97[9]. In this lecture, we describe the structural and dynamic changes occurring in mutants mimicking C*c* tyrosine phosphorylation, as well as their implications in metabolism. We report that phosphomimetic C*c* species at positions 48 and 97 are better electron donors than WT C*c* as display a higher oxidation rate[8,9]. Moreover, these phosphomutants show decreased caspase activation ability[8,9], thereby suppressing hypoxia/reperfusion-induced apoptosis.

In summary, our findings reveal that C*c* phosphorylation sites that some increase with aging are a way to modulate hemeprotein activity in orchestrating key cellular events and thus represent an important target for the development of therapeutics approaches.

[1]Pérez-Mejías et al (2022) *Coord Chem Rev* 450: 214233
[2]Elena-Real et al (2021) *Plant J* 106: 74
[3]González-Arzola et al (2017) *Nucleic Acids Res* 45: 2150
[4]Rivero-Rodríguez et al (2021) *Redox Biol* 43: 101967
[5]González-Arzola et al (2022) *Nat Struct Mol Biol in press*
[6]Guerra-Castellano et al (2016) *BBA – Bioenerg* 1857: 387
[7]Li et al (2022) *Aging* 14: 5699
[8]Moreno-Beltrán et al (2017) *PNAS* 114: E3041
[9]Guerra-Castellano et al (2018) PNAS 116: 7955

Of mice and men: pathways and mechanisms of aging

D. Ehninger[I]

[I]German Center for Neurodegenerative Diseases, Bonn, Germany

Current concepts regarding the biology of aging are primarily based on studies aimed at identifying factors regulating natural lifespan. However, lifespan as a sole proxy measure for aging can be of limited value because it may be restricted by specific sets of pathologies, rather than by general physiological decline. We used large-scale phenotyping to analyze hundreds of phenotypes and thousands of molecular markers across tissues and organ systems to establish lifetime profiles of age-dependent phenotypic change in C57BL/6J mice. We then examined central genetic and environmental lifespan regulators (putative anti-aging interventions; PAAIs) for a possible countering of the signs and symptoms of aging. Importantly, unlike most previous studies, we included in our study design young treated groups of animals, subjected to PAAIs prior to the onset of detectable age-dependent phenotypic change. In parallel to our studies in mice, we assessed genetic variants for their effects on age-sensitive phenotypes in humans. We observed that, surprisingly, many PAAI effects influenced phenotypes long before the onset of detectable age-dependent changes, rather than altering the rate at which these phenotypes developed with age. Accordingly, this subset of PAAI effects does not reflect a targeting of age-dependent phenotypic change. Overall, our findings suggest that comprehensive phenotyping, including the controls built in our study, is critical for the investigation of PAAIs as it facilitates the proper interpretation of the mechanistic mode by which PAAIs influence biological aging.

Cell senescence causes ageing: But how, where and what can we do about it?

R. Faragher[I]

[I]Centre for Stress and Age-related disease (STRAND) University of Brighton, Brighton, United Kingdom

Cellular senescence was first reported in human fibroblasts as a state of permanent viable growth arrest following culture in vitro. Although a causal link between this phenomenon and the ageing of whole organisms was first proposed in the 1960s it proved controversial for over fifty years. However, studies on humans and rodents have now established that senescent cells exist in vivo, that they accumulate with ageing, that interventions that lengthen healthy lifespan slow their accumulation and that removing them improves health status [1].

My talk will review the main lines of evidence for cell senescence as an ageing mechanism in humans with reference to work on the premature ageing disease Werner's syndrome [2] and the variable phenotypes of senescent cells from different tissues [3]. It will deal with the potential therapeutic benefits of removing senescent cells or reverting them to normal [4]. Lastly it will consider the possibility that the contribution of cell senescence to ageing may differ widely between species as a consequence of their evolutionary history [5] and the urgent need for studies on senescent cell biology from species other than humans and rodents [6].

[1] Faragher RGA & Ostler EL (2020) Resveralogues Gerontology. 66:231-237.

[2] Cox LS & Faragher RG (2007) From old organisms to new molecules: integrative biology and therapeutic targets in accelerated human ageing. Cell Mol Life Sci. 64:2620-41.

[3] Burton DG et al. (2009) Microarray analysis of senescent vascular smooth muscle cells: A link to atherosclerosis and vascular calcification. Exp Gerontol. 44:659-65.

[4] Latorre E et al. (2017) Small molecule modulation of splicing factor expression is associated with rescue from cellular senescence. BMC Cell Biol. 18:31.

[5] Overall AD & Faragher RG (2019) Population type influences the rate of ageing. Heredity 123:273-282.

[6]. Kelly E et al. (2020) The importance of senescence in tendinopathy: New opportunities Equine Vet J. 52:349-351

Inflammaging and the Heterogeneity of the Aging Process

Claudio Franceschi[1,2]

[1] Alma Mater Studiorum University of Bologna, Italy
[2] Lobachevsky State University of Nizhny Novgorod, Russia

At a recent symposium on aging biology a marked disagreement on the most fundamental questions in the field emerged, and little consensus on anything other than the heterogeneous nature of aging processes. In this complex scenario, paying particular attention to the widely documented sex/gender gap in human health and longevity, particularly evident in centenarians, two major points/commonalities emerge: A. A major (maybe universal?) characteristic of aging is its overwhelming HETEROGENEITY at all levels of possible investigation, from populations to individuals, including men and women. In H. sapiens, owing to his unique combination of biology and culture, a major consequence of such heterogeneity is the progressive divergence between "chronological" and "BIOLOGICAL" AGE. Indeed, centenarians are consistently younger of their chronological age, and in general people of the same chronological age can be "younger" or "older" regarding a large number of different features, including biological clocks like the whole genome changes in methylation of CpG sites. Even in young/adults the different organs and systems of the body are a mosaic of different clocks, as suggested by the data obtained by measuring a total of 402 parameters on liver, gut microbiome composition, kidney, immune and metabolic system, in 4,066 individuals aged between 20 and 45 years of age. B. In 2000 I suggested to term "INFLAMMAGING" a chronic, low-grade inflammation which increases with age and is a highly significant risk factor for a large variety of age-related diseases and geriatric syndromes, all sharing an inflammatory component. I'll illustrate a recently described NEW INFLAMMATORY CLOCK OF AGING (iAge) able to track multimorbidity, immunosenescence, frailty and cardiovascular aging, also associated with exceptional longevity in centenarians.

The Oxi-proteome (carbonylome) as a toolbox for monitoring protein redox homeostasis and assessing anti-ageing strategies

B. Friguet[I]

[I]Sorbonne Université, IBPS, UMR8256, Vieillissement Cellulaire Intégré et Inflammation, Paris, France

Accumulation of oxidatively modified proteins is a hallmark of organismal aging in vivo and of cellular replicative senescence in vitro. Failure of protein maintenance is a major contributor to the age-associated accumulation of damaged proteins that is believed to participate to the age-related decline in cellular function. In this context, quantitative proteomics approaches, including 2D-gel electrophoresis based methods, represent powerful tools for monitoring the extent of protein oxidative modification at the proteome level and for identifying the targeted proteins, also referred as to the “oxi-proteome” or “carbonylome”. Our previous studies have identified proteins targeted by oxidative modification during replicative senescence and/or oxidative stress-induced premature senescence of human WI-38 fibroblasts and myoblasts and have been shown to represent a restricted set within the total cellular proteome that fall in key functional categories, such as energy metabolism, protein quality control and cellular morphology. To provide mechanistic support into the role of oxidized proteins in the development of the senescent phenotype, untargeted metabolomic profiling was also performed for young and senescent myoblasts and fibroblasts. Metabolomic profiling was indicative of energy metabolism impairment in both senescent myoblasts and fibroblasts, suggesting a link between oxidative protein modification and the altered cellular metabolism associated with the senescent phenotype of human myoblasts and fibroblasts. Moreover, monitoring the extent of protein oxidative modification of a specific protein and/or at the proteome level has also recently proven to be very useful for assessing anti-aging and anti-photoaging strategies based on preventing environmental stressors induced protein oxidative damage in such organs and tissus as skin, hair or nails.

identification of genetic and environmental factors of human aging and use of innovative anti-aging approaches

S. Gonos[I]

[I]Hellenic Pasteur Institute, Athens, Greece

We have studied proteasome function in human aging (Mol Aspects Med 35, 1-71; Ageing Res Rev 23, 37-55) and we have observed reduced proteasomecontent and activities due to the down-regulation of its catalytic subunits (J Biol Chem 278, 28026-28037). In support, its partial inhibition in young cells induces premature senescence which is p53 dependent (Aging Cell 7, 717-732). Stable over-expression of catalytic subunits or POMP resulted in enhanced proteasome assembly and activities and a significant delay of senescence (J Biol Chem 280, 11840-11850; J Biol Chem 284, 30076-30086), mediated by the transcription factors Nrf2 (J Biol Chem 285, 8171-8184) and FoxO1 (Front Cell Dev Biol 9, 6257150. Likewise, proteasome activation in mesenchymal stem cells increases their lifespan and enhances stemness mediated by Oct4 (Free Rad Biol Med 103, 226-235). Finally, proteasome activation delays aging in vivo and it confers deceleration of aggregation-related pathologies, such as Alzheimer's or Huntington's diseases (FASEB J 29, 611-622). Given these findings, we have identified novel proteasome activators that decelerate aging and Alzheimer's disease progression in various in vivo models (Antiox Redox Signal 25, 855-869; Free Rad Biol Med 162, 88–103). We have also developed biobanks of donors of different ages, including healthy centenarians and long-lived siblings and we have cloned several longevity genes (Biogerontology 5, 401-409). We have also found that healthy centenarians have a functional proteasome (Exp Gerontol 35, 721-728). Moreover, we have identified specific somatic point mutations in mtDNA control region (PLoS One 5, e13395; Aging Cell 13, 101-107) and four chromosomal loci (Aging Cell 12, 184-193) that are linked with longevity. Finally, we determine the rate of aging and the efficacy of anti-aging protocols in healthy individuals. This work led to the development of personalized anti-aging protocols and innovative wellness products (Antioxidants 11, 468).

Dysfunction of the endolysosomal pathway is a common feature of neurodegenerative diseases

S. Hecimovic[I], K. Dominko[I], L. Vidatic[I], A. Rastija[I]

[I]Division of Molecular Medicine, Ruder Boškovic Institute, Bijenicka 54, HR-10000 Zagreb, Croatia

Niemann-Pick type C disease (NPC) is a rare inherited lysosomal storage disorder characterized by cholesterol accumulation leading to progressive neurodegeneration and neuroinflammation. It is intriguing that this rare monogenic disease (caused by mutations in NPC1/ NPC2 genes) shows several key features of a complex Alzheimer's disease (AD). Using NPC disease cellular and animal models our goal is to elucidate both common and specific pathways involved in neurodegeneration and/or neuroinflammation in NPC and AD. We showed that proteolysis by a key AD protease BACE1 is enhanced in NPC1-null cells/neurons as well as in NPC1-null mouse brains, and is mostly likely due to a defect within the endolysosomal transport resulting in accumulation of BACE1 and its substrates in endocytic compartments. Moreover, we detected impaired retromer function in NPC. Changes in retromer distribution in NPC1 mouse brains were observed already at presymptomatic stage (at 4-weeks of age), indicating that retromer defect occurs early in the course of NPC disease and may contribute to downstream pathological processes. Cholesterol depletion in NPC1-null cells and in NPC1 mouse brains reverted retromer dysfunction, suggesting that retromer impairment in NPC is mechanistically dependent on cholesterol accumulation. We propose that rescue of retromer impairment may represent a novel therapeutic approach against NPC, in addition to AD. The knowledge gained through this work could have a broader impact since defects of endolysosomal pathway are shared by other rare lysosomal storage disorders as well as the more common neurodegenerative disorders, such as Alzheimer's and Parkinson's disease.

DNA damage repair: understanding the process of aging and nutritional applications for medicine

J. Hoeijmakers[I,II,III]

[I]Erasmus Medical Center Rotterdam, Rotterdam, Netherlands, [II]CECAD, Univ. Cologne, Cologne, Germany, [III]Princess Maxima Center for Pediatric Oncolgy, Utrecht, Netherlands

Aging appears remarkably plastic: e.g. suppressing insulin/IGF signalling extends lifespan in numerous species. However, progeroid syndromes link with genome instability. We have generated DNA-repair-deficient mouse mutants displaying wide-spread accelerated aging. For instance, Ercc1Δ/- mice, carrying multiple repair defects show extensive multi-morbidity, limiting lifespan to 4-6 month. To counteract the premature aging these mutans also mount an anti-aging 'survival response', which suppresses growth and enhances maintenance, resembling the longevity response induced by dietary restriction (DR). Interestingly, subjecting these mutants to actual (30%) DR tripled lifespan, and drastically retarded accelerated aging, most notably neurodegeneration. We also found that DR lowered DNA damage, explaining its anti-aging effect and why repair mutants overrespond to DR. Interestingly, Ercc1Δ/- liver expression profiles showed progressive decline of transcription upon aging, preferentially affecting long genes, due to genome-wide accumulation of stochastic, transcription-blocking lesions.This transcription stress is also prominent in normal aging of many (post-mitotic) tissues and explains protein aggregation in proteinopthies. DR alleviates transcription stress and prolongs genome function. I will present phenotypes of conditional DNA repair models targeting aging to selected organs, striking parallels with Alzheimer's disease and the first remarkable results translating these concepts from mice to rare progeroid children. Our findings identify DNA damage as main cause of systemic aging, reveal untapped potential of nutritional interventions for reducing transcription stress in neurodegeneration and other aging-associated pathologies. Additionally, the 'survival response' triggered by pretreatment short-term fasting strongly reduces ischemia reperfusion injury in surgery and organ transplantation and improves chemo- radiotherapy outcome in cancer treatment.

Zoledronate: a new geroprotector.

I. Bellantuono[I]

[I]Healthy Lifespan Institute University of Sheffield, Sheffield, United Kingdom

Human life expectancy has been increasing steadily over the last century but this has resulted in an increasing incidence of age-related chronic diseases. Over 60% of people over the age of 65 will suffer from more than one disease at the same time (multimorbidity) and 25-50% of those over 80 years old develop frailty, defined as an accumulation of deficits and loss of reserve. Multimorbidity and frailty have complex medical needs and are strongly associated with disability and hospitalization. However, current treatments are suboptimal with problems of polypharmacy due to the fact that each disease is treated individually. Geroprotectors target fundamental mechanisms of ageing common to multiple age-related diseases and shows promise in delaying the onset of multimorbidity and frailty in animal models. However, there are many barriers to their clinical translation in patients due to the high level of complexity in the mode of action of geroprotectors and in the way multimorbidity and frailty develop. The talk will present preclinical data on a new geroprotector, Zoledronate, together with the most recent thoughts on how we may design a clinical trial to determine whether the encouraging results in animal models translate in patients' benefit.

Redox regulation of neurovascular coupling by nitric oxide in animal models of aging and neurodegeneration

João Laranjinha
Faculty of Pharmacy and Center for Neurosciences and Cell Biology, University of Coimbra Portugal

At glutamatergic synapses in hippocampus, nitric oxide (NO) synthesized by the neuronal isoform of nitric oxide synthase (nNOS) bridges neuronal activation to increases of cerebral blood flow (CBF), thus establishing a neurovascular-coupling axis.
On basis of in vivo and real-time electrochemical recordings of NO and O_2 coupled to CBF laser doppler flow measurements we have supported a role for nNOS derived NO as a direct mediator of neurovascular coupling (NVC). The maintenance of the functionality of NVC is critical for cognitive performance and in rodent models of hypoperfusion and neurodegeneration, a redox shift towards a more oxidizing environment during brain aging compromises the functionality of NVC by diverting NO activity from a neural messenger to a neural stressor.
Given this scenario, redox strategies to maintain NVC operative in connection with cognitive enhancement in the rodent models will be discussed.
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Dietary restriction as an antiaging intervention

S. Todorovic[I], M. Prvulovic[I], D. Milanovic[I], S. Sokanovic[I], A. Vukojevic[I], V. Simeunovic[I], S. Kanazir[I], **A. Mladenovic**[I]

[I]Institute for Biological Research "Siniša Stankovic" - National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia

Dietary restriction (DR) is the oldest and the most investigated anti-aging intervention. Since the famous McCay's study that showed that the restriction of calories without malnutrition prolongs mean and maximal lifespan in rats, thousands of papers demonstrated numerous beneficial effects of DR. DR is most famous for its proven capability to prolong life span, but what we know today is that DR prevents age-related diabetes, it decreases both incidence and progression of the different forms of cancer, protects from cardiovascular diseases, delays osteoporosis and sarcopenia. Its effects on the nervous system include preserved cognition, delayed brain atrophy, and protection from various neurodegenerative diseases.

By applying long-term dietary restrictions from the adulthood of male and female Wistar rats we demonstrated several beneficial outcomes. DR increased the level of synaptic plasticity markers and neurotrophic factors in the rat cortex and hippocampus and preserved brain cholesterol homeostasis during aging. It also suppressed apoptotic cell deaths after cortical injury and restored age-related impaired glucocorticoid receptor (GR) signalling in the brain. DR changed the expression of genes involved in AD pathology and suppressed microglial activation following cortical injury. However, recently we have been shown that the outcome of DR is highly dependent on the onset and duration. Namely, we demonstrated that short–term DR with a late-onset could have unfavorable effects on cognitive performances, anxiety level, and frailty in Wistar rats. In addition, DR could have a negative impact when introduced to transgenic AD animals. The results of our studies impose great caution when introducing CR to humans. To achieve its favorable effect DR should be introduced in humans up to the middle age.

Protein Kinase C: Molecular Target in Neurodegeneration

A. Newton[I]

[I]Department of Pharmacology, University of California San Diego, San Diego, United States of America

Protein Kinase C (PKC) isozymes are tightly regulated Ser/Thr kinases that transduce a myriad of signals from receptor-mediated hydrolysis of membrane phospholipids. Considered as oncogenes for three decades, recent studies showing cancer-associated PKC mutations are generally loss-of-function, coupled with clinical trial data showing that PKC inhibitors have worsened patient survival, have reframed PKC as a tumor suppressor. In contrast, mounting evidence indicates that PKC activity is enhanced in neurodegeneration. Gain-of-function mutations in PKCα are associated with Alzheimer's disease and mutations that impair autoinhibition of PKCγ cause spinocerebellar ataxia type 14. Thus, aberrant PKC signaling may be a general hallmark of neurodegenerative diseases, poising PKC iosyzmes as new biomarkers and molecular targets in these devastating disease. Given the high druggability of protein kinases, PKC would present itself as a viable target, particularly since inhibitors for this kinase that were ineffective in cancer clinical trials (where activity should be restored, not inhibited) could be repurposed.

Bioavailable polyphenols metabolites: brain uptake and effectors for attenuating neuroinflammation

Cláudia Nunes dos Santos[1,2,3**]

[1] NOVA Medical School, Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Lisboa, Portugal. [2] iBET, Instituto de Biologia Experimental e Tecnológica, Av. da República, Apartado 12, 2781-901 Oeiras, Portugal, [3] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Av. da República, 2780-157 Oeiras, Portugal

Age-associated pathophysiological changes such as neurodegenerative diseases are multifactorial diseases with increasing incidence and no cure. Prevention and treatment of neurodegeneration, will require novel multi-targeted therapeutic strategies, targeting different disease key points. The possibility of altering the progression and development of these diseases through diet is an emerging and attractive approach with accumulating supporting data. Studies with (poly)phenols arising from our diet have been shown their multipotent and pleiotropic ability to modulate several cellular and molecular pathways and in that sense, can emerge as an alternative, with potential to be further explored. However, the precise contribution of dietary (poly)phenols and circulating (poly)phenol metabolites to human health is still in the beginning of being elucidated. Absorption and blood concentrations of some (poly)phenols is quite low, which can hamper the research in terms of understanding their effects in specific biomarkers of disease.

The difficulty in demonstrating (poly)phenols true effects can be justified by their uncertain metabolic fate. In fact, it is necessary to identify the bioavailable metabolites resulting from (poly)phenol ingestion through the diet. Low molecular weight (poly)phenol metabolites are produced from colonic microbiota metabolism. Due to their smaller size have the potential to overcome cellular barriers and reach target tissues, such as the brain.

For this reason, it will be discussed the current knowledge on low molecular weight polyphenol metabolites in circulation, their ability to cross the blood-brain barrier and the possible mechanisms behind their role on modulating neuroinflammation one central hallmark common in neurodegenerative diseases.

MITOTIC ERRORS-DRIVEN SENESCENCE IS CONTROLLED BY AUTOPHAGY

V. Trachana[I]

[I]Faculty of Medicine, University of Thessaly, Larissa, Greece

Senescence onset has previously reported to be accompanied by several defects, such as aneuploidy or erroneous mitosis features. Evidence is provided that these defects could result from an age-associated spindle assembly checkpoint (SAC) attenuation. New results prove that SAC weakening upon aging is escorted by expression modifications of SAC main components, and especially of BubR1. BubR1 has been reported to decrease naturally upon aging and, we now demonstrate that autophagy is the molecular entity responsible for its degradation. This provides a missing explanation about its downregulation, especially since it is well established that the proteasome function decays as cells age. Hence, these data not only agree with the previously reported notion of a shift from proteasome to autophagy-dependent degradation upon aging, but also provides a mechanistic insight for mitotic errors-driven senescence. Even more, the homeostatic role of autophagy is emphasized, as more evidence is provided regarding its function towards the establishment of senescence as a barrier to cellular transformation and subsequent carcinogenesis.

Future Of Parkinson's and Alzheimer's Diseases Treatment; Why Multi-Target Drugs

M. Youdim[I]

[I]Technion-Israel Institute of Technology , Faculty of Medicine, Haifa, Israel

In Parkinson's (PD)and Alzheimer's (AD) diseases, no drugs are currently approved to prevent the neuronal cell loss characteristic in the brains of patients. Both PD and AD subjects have a predisposition to depressive illness and a significant percentage also have dementia. At best the present mono-target drug therapy has symptomatic activity. Due to the complex etiology of PD and AD, no "magic bullet" is expected to be developed to prevent the various cascade of neurotoxic events associated with the diseases. Thus, we have hypothesized and developed an innovative novel approach toward neuroprotection and neurorestoration (neurorescue) in these disorders with the development of multi-target drugs that target an array of pathological pathways, each of which is believed to contribute to the cascade that ultimately leads to neuronal cell death. The compounds discussed originate from synthetic chemistry. In our presentation, we shall discuss examples of novel multi-target ligands (eg. M30, M30P, HLA-20, and ladostigil) . These drugs combine cholinesterase and monoamine oxidase inhibitory and neuroprotective moieties into iron chelator-radical scavenger compounds. They also possess neuroprotective and neurorestorative activities, which may have potential as neuroprotective-neurorescue therapeutics in PD and AD. We have determined their neuropharmacological activities in cell cultures and in several established animal models of PD and AD. The major actions of these drugs are their ability to induce HIF (hypoxia inducing factor) which regulates the neurotrophins the BDNF, GDNF, VEGF, and erythropoietin and the cell cycle at G0 G1, with neurons differentiation and neuroplasticity, cognitive enhancement and anti depressant activities.

An advanced skin delivery system from Vitis vinifera leaves and propolis – enhanced skin fibroblasts functionality

S. Athanasopoulou[I], E. Spanidi[I], M. Gkika[I], K. Gardikis[I]

[I]APIVITA S.A., Markopoulo Attikis, Greece

Agricultural by-products are usually sources of valuable bioactive ingredients the exploitation of which is essential for sustainable agriculture but also for the biological properties it can impart to cosmetics, pharmaceuticals and food. The vine (Vitis vinifera L.) is considered one of the most important fruit crops in the world, covering over 7.5 Mha of the world area (FAO 2016) and producing 80 million tons in 2018 (FAOSTAT 2020).
Vine leaves are a bulky by-product that is disposed of and treated as waste in the wine production process, with applications being limited, at best, to a limited number of edible products and at worst to the creation of a soil enhancer. In the present study polyphenols from vine leaves were extracted and simultaneously encapsulated in a new combinatorial system consisting of liposomes and cyclodextrins. In parallel, propolis polyphenols from Mount Olympus were encapsulated in cyclodextrins resulting in a colloidal suspension that releases polyphenols in a time-controlled way, the rate of which depends on the ratio of the materials. The result is a raw material that exhibits antioxidant and ECM protective effects when administered in skin fibroblasts (NHDFs). Treatment of NHDFs with the combinatorial delivery system for vine extract and propolis polyphenols promoted collagen end elastin synthesis and deposition in normal conditions and upon induced external stress, as assessed by in vitro transcriptomic and proteomic analysis. Therefore, this liposome-cyclodextrin encapsulated polyphenol complex represents a novel bioactive ingredient with promising skin applications.

New advanced skin delivery systems that add value to bee products – molecular targets for in vitro bioactivity evaluation

S. Athanasopoulou[I], E. Spanidi[I], K. Gardikis[I]

[I]APIVITA S.A., Markopoulo Attikis, Greece

Natural products are rich sources of diverse classes of valuable bioactive molecules (e.g., phenols, flavonoids, carotenoids, alkaloids) that exhibit significant biological activity (e.g., antioxidant, anti-inflammatory, antimicrobial, etc.). Their bioactivity is usually hindered by factors such as low stability, poor solubility, and bioavailability – making their food, cosmetics and pharmaceutical applications difficult. To overcome these issues several strategies have been developed, with delivery systems being one of them. Depending on their design such systems may increase the stability of natural bioactive compounds upon processing, storage, or application, can enhance their solubility, mask undesirable flavors as well as efficiently deliver them to the target tissues where they can exert their biological activity.
In this context, advanced drug delivery nanosystems (aDDNSs) that consist of the combination of more than one different biomaterials (e.g., lipids, phospholipids, chitosan, dendrimers, etc.) may offer several advantages: they can modify the release profile of the entrapped bioactive molecule, alter its pharmacokinetic profile and consequently improve its biodistribution, absorption and metabolism.
In the present study several advanced skin delivery systems incorporating plant or bee product biomolecules are presented. The carriers used consist of liposomes and cyclodextrins and have been proven to provide stability, controlled release, increase of solubility and significant in vitro efficacy – for skin applications.
The development of such applications is of utmost importance as significant value can be provided to precious bioresources that, in such formulations, can be exploited to their full potential in an environmentally friendly way.

EVALUATION OF 4-AMINOQUINOLINES AS POTENTIAL MTDL LIGANDS FOR THE TREATMENT OF ALZHEIMER'S DISEASE

M. Bartolić[I], A. Matošević[I], M. Spasić[II], D. Opsenica[III], A. Bosak[I]

[I]Institute for Medical Research and Occupational Health, Zagreb, Croatia, [II]University of Belgrade, Faculty of Chemistry, Belgrade, Serbia, [III]University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Belgrade, Serbia

Derivatives of 4-aminoquinolines have been present in medicine for decades thanks to their antimalarial properties. They also show anti-inflammatory, anti-infective, antithrombotic, as well as antitumoral properties which candidates them for repurposing in treatment of other diseases. Their ability to pass the blood-brain barrier and inhibit the activity of cholinesterases makes them promising agents for the treatment of neurodegenerative diseases, whose aetiology encompasses the decrease of neurotransmitter acetylcholine levels.

The aim of our study was to determine whether 4-aminoquinolines have the potential to be used as multi-target directed ligands (MTDLs) in treating Alzheimer's disease acting as inhibitors of acetylcholinesterase (AChE) and/or butyrylcholinesterase (BChE), and as inhibitors of β-secretase (BACE1). BACE1 is the aspartic protease responsible for the cleavage of the amyloid precursor protein (APP) and formation of self-aggregating amyloid β peptides and senile plaques. Our results showed that all of the 12 tested 4-aminoquinoline derivatives reversibly inhibited the activity of both AChE and BChE with dissociation constants of the enzyme-aminoquinoline complex (Ki) in low micro- to nanomolar range, the most potent being DO250 and DO251. It was determined that our compounds display certain inhibitory potential against BACE1, the most potent being DO250, DO251, DO256 and DO266 which showed an up to 18% decrease in BACE1 activity for 1.0 μM compounds. Based on these results, we conclude that compounds DO250 and DO251 could be promising leads for further evaluations and structural refinements in the search for potent MTDLs for treating Alzheimer's disease.

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Mitochondria damaged during UVB-induced senescence of human dermal fibroblasts are eliminated by NIX-dependent mitophagy and extracellular vesicles-based mitochondria excretion

M. Cavinato[I], I. Martic[I], S. Wedel[I], M. Schosserer[I], M. Bobbili[I], J. Haybaeck[II], G. Pierer[II], C. Ploner[II], M. Hermann[II], N. Romani[II], M. Schmuth[II], J. Grillari[III], P. Jansen-Dürr[I]

[I]IBA/University of Innsbruck, Innsbruck, Austria, [II]Medical University of Innsbruck, Innsbruck, Austria, [III]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria

Skin aging results from two types of aging, “intrinsic aging” an inevitable consequence of physiologic and genetically determined changes, and “extrinsic aging” which is dependent on external factors like exposure to sunlight, smoking, and dietary habits, among others. UVB causes skin injury through the generation of free radicals and other oxidative byproducts, also contributing to DNA damage. Appearance and accumulation of senescent cells in the skin are considered one of the hallmarks of aging in this tissue. Mitochondria play an important role in the development of cellular senescence, in particular stress-induced senescence of human cells. However, many aspects of mitochondrial physiology relevant for cellular senescence and extrinsic skin aging remain to be unraveled. Here we demonstrate that damaged mitochondria generated by UVB are eliminated by NIX-dependent mitophagy and that this process is important for cell survival under these conditions. Additionally, UVB irradiation of human dermal fibroblasts (HDF) induces the shedding of extracellular vesicles (EVs) and this process is significantly enhanced in UVB-irradiated NIX-depleted cells. Our findings establish NIX as the main mitophagy receptor in the process of UVB-induced senescence and suggest the release of EVs as an alternative mechanism of mitochondrial quality control in HDF.

The Changes in the p53 Protein across the Animal Kingdom Point to Its Involvement in Longevity

J. Cerven[I]

[I]University of Ostrava, Ostrava, Czech Republic

Recently, the quest for the mythical fountain of youth has produced extensive research programs that aim to extend the healthy lifespan of humans. Despite advances in our understanding of the aging process, the surprisingly extended lifespan and cancer resistance of some animal species remain unexplained. The p53 protein plays a crucial role in tumor suppression, tissue homeostasis, and aging. Long-lived, cancer-free African elephants have 20 copies of the TP53 gene, including 19 retrogenes (38 alleles), which are partially active, whereas humans possess only one copy of TP53 and have an estimated cancer mortality rate of 11-25%. The mechanism through which p53 contributes to the resolution of the Peto's paradox in Animalia remains vague. Thus, in this work, we took advantage of the available datasets and inspected the p53 amino acid sequence of phylogenetically related organisms that show variations in their lifespans. We discovered new correlations between specific amino acid deviations in p53 and the lifespans across different animal species. We found that species with extended lifespans have certain characteristic amino acid substitutions in the p53 DNA-binding domain that alter its function, as depicted from the Phenotypic Annotation of p53 Mutations, using the PROVEAN tool or SWISS-MODEL workflow. In addition, the loop 2 region of the human p53 DNA-binding domain was identified as the longest region that was associated with longevity. The 3D model revealed variations in the loop 2 structure in long-lived species when compared with human p53. Our findings show a direct association between specific amino acid residues in p53 protein, changes in p53 functionality, and the extended animal lifespan, and further highlight the importance of p53 protein in aging.

Nutrition, ageing and prostate cancer: an experimental approach using Drosophila

M. Emery[I], S. Wigby[I], D. Bennett[I], C. Wilson[I], M. Simons[I]

[I]University of Liverpool, Liverpool, United Kingdom

Prostate cancer (PCa) is the most prevalent cancer of ageing men, with 1/3 of cases in the UK affecting those over 75 years old. It can be a slow growing disease where optimised, preventative actions could be effective as long-term treatment. Nutrition is a risk factor of high importance, thought to be linked to mis-regulated mTOR signalling in the mTORC1 nutrient sensing pathway. Nutrition and mTOR are known evolutionarily conserved regulators of ageing and age-related disease, and mTOR plays a role in tumour-promoting exosome production. However, there is little data to illustrate how these mechanisms interact to cause cancer in vivo and whether nutrient/mTOR interventions could prevent or reverse PCa.

Using the fruit fly, *Drosophila melanogaster*, as a model organism the impact of ageing, nutrition and the mTORC1 pathway on prostate-like cell growth and genome replication will be investigated. The secondary cells (SCs) of *D. melanogaster* share many functional similarities to the human prostate, such as seminal fluid production, exosomes, and growth regulation by steroid-dependent and independent signals. Environmental manipulations of male *D. melanogaster* will be applied through diet, genetic mutations and treatment with lifespan-extending drugs. With confocal microscopy, SCs can be observed for changes in growth and endoreplication.

This project benefits from an in vivo, whole animal system, where dietary and environmental changes are made in a controlled setting. It will provide opportunities for novel or improved interventions, to prevent or ameliorate prostate cancer, contributing to the wider geroprotection against the multimorbidities of ageing.

TP53 mutation-associated and copy-dependent KDM7A-DT expression affects DNA repair and promotes invasive breast cancer progression

Antonis Giannakakis[1,2,3], Margaritis Tsifintaris[1], **Vasileios Gouzouasis[1]**, Charisios Triantafyllou[1], Ghim Siong Ow[2], Csaba Papp[4], Anna V. Ivshina[2] and Vladimir A. Kuznetsov[2,4]

[1]Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupolis, Greece.
[2]Bioinformatics Institute, A-STAR, Singapore
[3]University Research Institute for the Study of Genetic & Malignant Disorders in Childhood, National and Kapodistrian University of Athens, Athens 11527 Greece.
[4]Department of Urology, Department of Biochemistry and Molecular Biology, 750 East Adams St, SUNY Upstate Medical University, Syracuse, NY 13210, USA.

Abstract
The expression of specific short-lived long non-coding RNAs (lncRNA) is induced in response to acute oxidative stress in non-malignant cells, with the highest levels at promoter regions in antisense coding gene orientation. KDM7A Divergent Transcript (KDM7A-DT) is one of such stress-induced lncRNAs. Our experiments demonstrate that KDM7A-DT biogenesis is complex, involving at least one cleaved and abundantly expressed intermediate-size lncRNA with a cytoplasmic localization. In contrast, the full-length transcript is detected both in the nucleus and the cytoplasm of BC cells. We show that stable overexpression of KDM7A-DT in non-malignant cells up-regulates TP53, CDKN1A, and γH2AX signaling, resulting in a prolonged cell growth retardation phenotype. Importantly, KDM7A-DT induction by acute oxidative stress on semi-transformed fibroblasts is TP53-dependent. However, its expression is significantly enriched in cells carrying the TP53-mutation, such as T47D luminal B breast cancer (BC) cells and basal-like (BL) and HER2 BC clinical samples, controlling pro-oncogenic metabolic and oncogenic pathways associated with DNA damage response (DDR) mechanisms. Our study reveals that KDM7A-DT overexpression in MRC5 fibroblasts upregulates DNA repair, G2/M checkpoint arrest, and oxidative phosphorylation and downregulates apoptosis and glycolysis. Also, KDM7A-DT knock-down in T47D BC cells upregulates apoptosis and downregulates G2/M checkpoint arrest and oxidative phosphorylation, activating the non-homologous end-joining but not the homologous recombination pathway. Our results suggest that KDM7A-DT copy number alterations, aneuploidy, and TP53 missense mutations provide a genetic basis for chronic stress-like KDM7A-DT overexpression, which modulates metabolic pathways, activates epithelial-mesenchymal transition pathways, suppresses DDR and apoptosis, and contributes to poor clinical BL BC outcome.

Keywords:
KDM7A-AS1, JHDM1D-AS1, Stress-induced promoter-associated antisense lncRNA, Breast cancer, Oxidative stress, DNA damage and repair, Apoptosis, TP53 mutation, Gene alteration

Induction of the senescence phenotype in equine tendon derived cells by dexamethasone and rescue by small molecule senescence inhibitors

N. Heidari[I], J. Dudhia[I], R. Smith[I], R. Faragher[II]

[I]Royal Veterinary College, London, United Kingdom, [II]University of Brighton, Brighton, United Kingdom

Tendon injuries in humans are a major healthcare concern but they also occur spontaneously in other species including horses. The superficial digital flexor tendon (SDFT) of the horse is a highly relevant model for studying tendinopathies. Previous studies with cyclical loading of tendon explants have revealed that injured SDFTs produced high levels of pro-inflammatory cytokines and 10-1000-fold overexpression of matrix metalloproteinases with impaired resolving mechanisms, especially in older horses. In combination, these markers are hallmarks of senescent cells. Senescent cells are generated by many pathways (both cell division dependent and independent) all of which produce viable cells that (a) overproduce pro-inflammatory cytokines collectively known as the Senescence Associated Secretory Phenotype (SASP) and (b) actively remodel matrix, all of which are implicated in age-related degenerative changes that predispose to tendinopathies. Corticosteroids are frequently used clinically to treat tendinopathies to control inflammation but also may cause cellular senescence and interfere with tendon repair, contributing to the high re-injury rate. The aim of this study is to investigate the effects of dexamethasone on senescence induction in tenocytes and rescue by small molecule senescence inhibitors.

This study showed that dexamethasone at clinically relevant doses induced senescence in equine tenocyte cells which explains potential adverse effects of using corticosteroids for the treatment of tendinopathies. Dexamethasone-induced senescence may be prevented by polyphenolic compounds such as resveratrol. The model will enable investigations of novel candidate molecules that can slow or stop the degenerative process by inhibiting senescence in vitro, which could be used to improve the clinical benefits of corticosteroids via its anti-

Healthy Longevity with Rapamycin (LONGER) - study design

A. Hodzic Kuerec *[I], D. Lee *[I], A.B. Maier *[I]

[I]Centre for Healthy Longevity (CHL), Yong Loo Lin School of Medicine, National University of Singapore (NUS), Singapore, Singapore

Rapamycin, an inhibitor of the mammalian target of rapamycin (mTOR), extends lifespan in various animal models. It is approved by the Food and Drug Administration (FDA) for preventing immune reactions after kidney transplantation, treating fibrous skin tumors of tuberous sclerosis, and treating pulmonary lymphangioleiomyomatosis. Therefore, rapamycin is a potential repurpose drug candidate for geroprotection in humans. LONGER is a double-blinded randomized control trial (RCT) of 6mg/week rapamycin versus placebo given to 40-60-year-old healthy volunteers for 12 weeks. The primary outcome is the change in C-reactive Protein (CRP) levels. The secondary outcomes are other inflammatory and metabolic parameters in blood, leg extension strength, six-minute walking test, and arterial stiffness.

* The authors marked with an asterisk equally contributed to the work.

The mTOR 2 complex signaling and functions in neurotoxicity induced by 1-methyl-4-phenylpyridinium (MPP+) and 6-hydroxydopamine (6-OHDA)

M. Jeremic[I], M. Jovanovic-Tucovic[I], D. Stevanovic[II], V. Trajkovic[II], I. Markovic[I]

[I]Institute of Medical and Clinical Biochemistry, Faculty of Medicine, University of Belgrade, Belgrade, Serbia, [II]Institute of Immunology, School of Medicine, University of Belgrade, Belgrade, Serbia

Parkinsonian neurotoxins, 1-methyl 4-phenyl 1-pyridinium (MPP+) and 6-hydroxydopamine (6-OHDA) are commonly used to investigate mechanisms of neurodegeneration. mTOR is a serine/threonine protein kinase, which forms two complexes, mTORC1, and mTORC2. The role of mTORC1 is well known, whereas the function of mTORC2 is still insufficiently elucidated. Our ongoing study aims to investigate the role of mTORC2 signaling pathway in the neurotoxicity caused by MPP+ and 6-OHDA.

We performed all experiments on the human neuroblastoma SH-SY5Y cell line. The activation status of mTOR, Rictor, sin1, AMPK, Akt, and ERK following short-term (2, 4, 8, and 12h) and long-term (3, 5, and 7 days) MPP+ or 6-OHDA treatment was determined by immunoblotting. The role of mTORC2 in neurotoxin-induced cell demise and its relation to other signaling pathways was assessed using pharmacological or genetic modulation, and cell viability was detemined using the MTT test.

We showed that treatments with both MPP+ and 6-OHDA led to an increase in mTORC2 components' (Rictor, sin1), AMPK, Akt, and ERK activity. Genetic inhibition of mTORC2 or AMPK increased sensitivity of SHSY5Y cells' to the effect of neurotoxins (MPP+ or 6-OHDA). On the other hand, ERK inhibition led to a higher survival rate of cells treated with neurotoxins in comparison to control cells. The time-kinetic analysis revealed that AMPK activation preceded increase in mTORC2 members activation. Also, we showed that neurotoxins led to sustainable activation of mTORC2, up to 5 days of treatment.

These data demonstrate that mTORC2 plays an important role in MPP+ and 6-OHDA-induced neurotoxicity.

Treatment with hyperbaric oxygen reduces death of immature neurons in the adult hippocampal dentate gyrus following brain injury

R. Jeremic[I], P. Brkic[I], M. Djelic[I], S. Pekovic[II], S. Dacic[III]

[I]Institute of Medical Physiology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia, [II]Department of Neurobiology, Institute for Biological Research "Siniša Stankovic", University of Belgrade, Belgrade, Serbia, [III]Institute of Physiology and Biochemistry, Faculty of Biology, University of Belgrade, Belgrade, Serbia

Objectives: Growing number of data suggests that hyperbaric oxygenation (HBO) can influence the activity of adult neural stem cells (NSCs). Since the role of NSCs during the recovery after brain injury is still unclear, the aim of this study was to investigate the effects of sensorimotor cortex ablation (SCA) and hyperbaric oxygenation (HBO) upon processes of neurogenesis in the adult Dentate gyrus (DG), a region of hippocampus (Hippo) with a significant role in the process of adult neurogenesis.

Methods: The experiments were conducted on 10 weeks old male Wister rats. Animals were organized into following groups (n = 10 per group): Control (C) intact animals, Control + HBO (CHBO) intact animals subjected to HBO treatment, Sham control (S) animals that underwent surgical procedure without damaging the brain tissue, Sham control + HBO (SHBO), Lesion group (L) – the right sensorimotor cortex was removed by suction ablation and Lesion + HBO (LHBO). HBO protocol: pressure applied 2.5 absolute atmospheres (ATA), for 60 minutes, once daily for 10 days. Effects of HBO treatment were monitored using immunohistochemistry, double immunofluorescence and were verified with Western blot analysis.

Results: SCA caused a significant loss of neurons in DG compared to the control. Newborn neurons from subgranular zone (SGZ), inner, and partially middle granular cell layers were predominantly affected by SCA. HBO treatment reduces the number of immature neurons undergoing degeneration.

Conclusions: Our results point to protective effect of HBO in decreasing vulnerability of immature neurons in the adult DG to SCA injury.

Short term fish oil supplementation changes glial cells function in 5XFAD mice

M. Jovic[I], A. Mladenovic[II], D. Milanovic[I], S. Todorovic[I], M. Prvulovic[I], A. Vukojevic[I], V. Simeunovic[I], S. Sokanovic, N. Loncarevic[III]

[I]Institut for Biological research Sinisa Stankovic, Belgrade, Serbia, [II]Institute for Biological Research „Sinisa Stankovic“, National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia, [III]Molecular Nutrition and Health Lab CEDOC - Centro de Estudos de Doenças Crónicas NOVA Medical School / Faculdade de Ciências Médicas UNIVERSIDADE NOVA DE LISBOA, Lisboa, Portugal

The presence of large amounts of amyloid beta (Aβ) oligomers, amyloid plaques formation, and inflammation in the brain are one of the neuropathological characteristics of Alzheimer's disease (AD). The use of supplements with omega-3 fatty acids has been associated with reduced risk and lessened AD pathology. The purpose of this study was to elucidate whether such a treatment could affects glial cells and macrophages behavior in the early phase of the disease.

We examined influence of fish oil (FO) treatment in 5xFAD mice, an animal model which rapidly recapitulates major hallmarks of AD amyloid pathology. Three-month old female 5xFAD mice received FO (100µl/animal/day) via oral gavage during 3 weeks period. Histological analysis was used to detect changes in pathological features of AD in parietal cortex of 5xFAD mice. Aβ peptide, macrophages, microglial cells and astrocytes were detected by anti-Aβ42-, anti-Iba-1, anti-TMEM119- and anti-GFAP-antibody, respectively. Immunostaining was observed by confocal microscopy. Quantification was done by ImageJ. FO supplementation alters the behaviour of macrophages prompting them to establish a physical barrier around amyloid plaques and leads to changes in number of over all astrocytes and microglial cells.

These results confirmed and extended previous findings suggesting that FO supplementation suppresses brain aging and has a typical pleiotropic effect, suggesting that FO in combination with other drugs could be good approach for long-term treatment in AD suppression.

Antioxidant and Antiaging Properties of a Novel Synergistic Nutraceutical Complex: Readouts from an In Cellulo Study and an In Vivo Prospective, Randomized Trial

M. Kapetanou[I], S. Athanasopoulou[I], N. Mougolia[I], E. Gonos[I]

[I]National Hellenic Research Foundation, Institute of Chemical Biology, Athens, Greece

Aging is a dynamic procedure that is developed in multiple layers and characterized by distinct hallmarks. The use of biomarkers that target different hallmarks of aging is substantial in predicting adverse outcomes during the aging process, implementing specifically designed antiaging interventions and monitoring responses to these interventions. The present study aimed to develop a novel composition of plant extracts, comprising identified active ingredients that synergistically target different hallmarks of aging in cellulo and in vivo. The selected single extracts and the developed composition were tested through a powerful set of biomarkers that we have previously identified and studied. The composition of selected extracts simultaneously increased cellular lifespan, reduced the cellular oxidative load and enhanced antioxidant defense mechanisms by increasing proteasome activity and content. In addition, the combination prevented telomere attrition and preserved optimum DNA methylation levels. Remarkably, biomarker profiling of healthy volunteers who received the identified combination in the form of a nutritional supplement within the frame of a prospective, randomized, controlled 3-month trial revealed an unprecedented antioxidant capacity in humans. In conclusion, our results support the notion that interventions with specifically designed combinations of natural compounds targeting multiple hallmarks of aging represent an effective way to improve healthspan and well-being.

FoxO1 Is a Novel Regulator of 20S Proteasome Subunits Expression and Activity

M. Kapetanou[I]

[I]National Hellenic Research Foundation, Institute of Chemical Biology, Athens, Greece

Proteostasis collapses during aging resulting, among other things, in the accumulation of damaged and aggregated proteins. The proteasome is the main cellular proteolytic system and plays a fundamental role in the maintenance of protein homeostasis. Our previous work has demonstrated that senescence and aging are related to a decline in proteasome content and activities, while its activation extends lifespan in vitro and in vivo in various species. However, the mechanisms underlying this age-related decline of proteasome function and the down-regulation in expression of its subunits remain largely unclear. Here, we demonstrate that the Forkhead box-O1 (FoxO1) transcription factor directly regulates the expression of a 20S proteasome catalytic subunit and, hence, proteasome activity. Specifically, we demonstrate that knockout of FoxO1, but not of FoxO3, in mice severely impairs proteasome activity in several tissues, while depletion of IRS1 enhances proteasome function. Importantly, we show that FoxO1 directly binds on the promoter region of the rate-limiting catalytic β5 proteasome subunit to regulate its expression. In summary, this study reveals the direct role of FoxO factors in the regulation of proteasome function and provides new insight into how FoxOs affect proteostasis and, in turn, longevity.

Mitochondrial genome landscape characterization in induced-MSCs (iMSCs) derived from bone marrow MSCs (BM-MSCs) of aged OA and healthy donors

V. Konteles[I], A. Destouni[I], E. Mourmoura[I], E. Sfougataki[II], A. Mertzanian[II], T. Brantzos[III], M. Poulou[III], V. Trachana[I], M. Tzetis[III], E. Goussetis[II], A. Tsezou[I]

[I]University of Thessaly, Faculty of Medicine, Laboratory of Cytogenetics and Molecular Genetics, Larissa, Greece, [II]Stem Cell Transplant Unit, Aghia Sophia Children's Hospital, Athens, Greece, [III]Department of Medical Genetics, Medical School, NKUA, Athens, Greece

Mesenchymal stem cell-based treatments are intensively being tested in patients with osteoarthritis (OA). OA is a degenerative disease which progresses with advancing age. Recent studies have shown that heteroplasmic mitochondrial variants accumulate with advancing age, impairing respiration and cell metabolism. Moreover, it was recently shown that cell reprogramming to iPSCs "purifies" heteroplasmic mt variants, in primary cells. Induced MSCs (iMSCs) derived from BM-MSCs which were first reprogrammed to iPSCs are an alternative to MSCs because of their higher proliferation and regenerative potential. The present study aims at characterizing the mitochondrial genome landscape in BM-MSCs, iPSCs and iMSCs from OA and healthy donors. Linearization of mtDNA, ultra-deep NGS and mtDNA analysis were used to determined homoplasmic and heteroplasmic SNVs and short deletions. Homoplasmic variants were not different between donors and cell types, while heteroplasmic variants were higher in OA-MSCs compared to healthy-MSCs. Differences were also observed between OA-MSCS, iPSCs and iMSCs. Unsupervised hierarchical clustering showed a "purifying effect" for the heteroplasmic variants in OA patients during reprogramming to iPSCs. The decreased variant number remained stable during iPSCs differentiation while the majority heteroplasmic variants in OA and healthy donors are coding. In OA donors, the variants with the highest heteroplasmic fraction (HF~10%) are located in the control region (DLOOP1, DLOOP2), whereas in healthy donors(HF>90%) in ATP6 and a t-RNA gene TQ. The majority of the heteroplasmic variants fall in the r-RNA gene followed by ND1, ND2 genes in both groups.The study characterized the mtDNA landscape during the generation of iMSCs from OA and healthy MSCs donors suggesting a decreased heteroplasmic variant load in OA-iPSCs which remains stable during differentiation in iMSCs. The findings indicate that iMSCs might be valuable alternative to cell-based OA treatment.

In vivo study of the effect of microglial BIN1 deletion following LPS-induced neuroinflammation

M. Margariti[I], I. Thanou[I], E. Papadimitriou[I], M. Avloniti[I], V. Kyrargyri[I], M. Costa[II], D. Thomaidou[I]

[I]Hellenic Pasteur Institute, Athens, Greece, [II]Institut Pasteur de Lille, Lille, France

Aging is the primary risk factor of Late Onset Alzheimer's Disease (LOAD), with its prevalence exponentially increasing with age. Genome-Wide Association Studies have identified several Single Nucleotide Polymorphisms (SNPs) strongly associated to increased risk of developing LOAD, many of which are related to microglia activation. SNPs in the locus harboring Bridging Integrator 1 (BIN1) gene show the strongest association with AD, after Apolipoprotein E. BIN1 is a member of the Bin/Amphiphysin/Rvs (BAR) family of adaptor proteins implicated in cell membrane modelling dynamics. Although, its role in neurons has been studied both in vitro and in vivo, the role of BIN1 in microglial activation state and its contribution in LOAD pathology remains to be clarified. To this end we have developed a transgenic Cx3CR1 Cre-ERT2//Bin1 fl/fl mouse, in which BIN1 is knocked-out in microglial cells. Furthermore, we have challenged BIN1-KO mice with LPS, to investigate the effect of microglia-specific Bin1 deletion on mouse brain under homeostatic and inflammatory conditions. Besides forebrain molecular phenotype characterization, we are analyzing the transcriptomic profile of all brain cell populations by snRNA-Seq to reveal novel targets related to microglial BIN1. Importantly, following recent literature suggesting that microglia in our mouse model exhibits an aging phenotype, we are investigating the expression levels of senescence markers under homeostatic and inflammatory conditions by real time RT-PCR and immunofluorescence. We anticipate that our analysis will allow us get insight into the effect of microglial BIN1 deletion on microglia activation state and appearance of senescence phenotype.

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THE ROLE OF MELANOCYTES IN SKIN PIGMENTATION, SENESCENCE, AND SKIN AGING INDUCED BY EXPOSURE TO ENVIRONMENTAL STRESSORS

I. Martic[I], P. Jansen-Dürr[I], M. Cavinato[I]

[I]Inst. f. Biomedical Aging Research, University of Innsbruck, Innsbruck, Austria

Introduction: Extrinsic aging of human skin is mainly a result of exposition to environmental factors such as sunlight, air pollution, and cigarette smoke. Melanocytes become less active and senescent with aging and environmental factors can lead to premature aging and pigmentation disorders. However, the mechanisms activated upon exposition of melanocytes to environmental stressors are not fully understood.

Material & Methods: We treat melanocytes and skin biopsies with UV (UVA+UVB), urban particulate matter (UPM) or a combination of these two stressors (UV+UPM), in order to understand how these environmental stressors affect skin biology and, in particular, melanocytes' homeostasis. Following treatment, we investigate several morphological and physiological parameters such as cell proliferation, senescence status, apoptosis, pigmentation, and DNA damage.

Results: Preliminary results using UV or UPM alone as well as the combination of both stresses have demonstrated that melanocytes respond diversely to each different type of stress in terms of senescence markers, cell survival and pigmentation. Accordingly, all three types of treatments induced different changes in *ex vivo* skin biopsies, indicating that an understanding of the underlying molecular processes activated in response to these treatments are vital to estimate the impact of exposure to such environmental factors on the progression of skin aging and health in general.

Conclusion: Taken together, this new experimental setup will allow us to perform further research on mechanisms of extrinsic skin aging, including the role of melanocytes in this process and could give rise to the development of new therapeutical targets for pigmentation disorders and premature skin aging.

Design, synthesis and biological evaluation of biscarbamates as potential selective butyrylcholinesterase inhibitors for the treatment of Alzheimer`s disease

A. Matošević[I], A. Knežević[II], A. Zandona[I], N. Maraković[I], Z. Kovarik[I], A. Bosak[I]

[I]Institute for Medical Research and Occupational Health, Zagreb, Croatia, [II]Ruder Boskovic Institute, Zagreb, Croatia

Alzheimer's disease (AD) is a multifactorial neurodegenerative disease that affects more than 50 million people worldwide. Currently, the treatment of AD is based on increasing the concentration of the neurotransmitter acetylcholine in the brain by inhibiting enzymes responsible for its hydrolysis, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Recent findings on the role of BChE in the symptoms progression and pathophysiology of Alzheimer's disease indicated that selective inhibition of BChE can represent a promising pathway in treating AD. With the aim to determine new drug candidates for the treatment of AD as selective inhibitors for BChE, we used bambuterol, a selective inhibitor of BChE , as a structural basis and synthesized 25 biscarbamates with different substituents at the carbamoyl and hydroxyaminoethyl chain and evaluated their inhibition potency toward AChE and BChE. Their cytotoxic profile, ability to cross the BBB by passive transport and to chelate biometals were also evaluated. All biscarbamates proved to be very potent inhibitors of AChE and BChE with inhibition rate constants up to 10^6 M^{-1} min^{-1}, with generally higher preference to BChE. The inhibition potential and selectivity were analysed by molecular docking as well. For three biscarbamates, it was determined that they should be able to pass the BBB by passive transport, while for nine biscarbamates this ability was slightly limited. Twenty-one biscarbamates were neither hepatotoxic, nephrotoxic nor neurotoxic. All biscarbamates have the ability to chelate at least one of biometals (Zn, Fe and Cu) and thus they could be able to reduce the neurotoxic effects of biometal imbalances. This study pointed out biscarbamates as a promising structural motif for the development of more effective drugs for the treatment of middle and late stages of AD.

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Long-Term Sildenafil Treatment Improves Expression Of Genes That Regulate Mitochondrial Dynamics In Aged Miocardium

M.. Medar[I], M. Andrejic[II], S. Andric[II], T. Kostic[I]

[I]University of Novi Sad, Faculty of Sciences Novi Sad, Department of Biology and Ecology, Laboratory for Reproductive Endocrinology and Signaling, Novi Sad, Serbia, Novi Sad, Serbia, [II]Laboratory for Chronobiology and Aging, Laboratory for Reproductive Endocrinology and Signaling, Centre of Excellence CeRES, Faculty of Sciences, Univ. of Novi Sad, Novi Sad, Serbia., Novi Sad, Serbia

Multiple mitochondrial functions important for cardiac function, ranging from energy and reactive oxygen species production to Ca2+ homeostasis and cell death are modulated by mitochondrial dynamics. The relevance of altered mitochondrial dynamics in aging-associated heart failure and cardiac hypertrophy is recognized. In this study, we analyzed the effect of long-term treatment with sildenafil (inhibitor of phosphodiesterase 5) on age-associated changes in expression of main markers of mitochondrial dynamics (mitochondrial biogenesis, fission/fusion, and mitophagy) in rat myocardium. Middle-aged (12 month-old) Wistar rats were treated orally for 6 months till old-age (18 months-old) with sildenafil (1.25 mg/kg body weight per day) while a control group of rats received water.

RQPCR analysis reviled increased transcription of Ppargc1a (main regulator of mitochondrial biogenesis and function) in myocardium obtained from old compared to adult (3 month-old) rats, while sildenafil-treatment prevented the age-associated increase of *Ppargc1a*. However, aging as well as sildenafil-treatment did not change expression patterns of its downstream genes *Nrf1, Tfam, and Nrf2*. In high energy-demanding cardiomyocytes balance between mitochondrial fusion and fission is of crucial importance because imbalance causes cardiomyocyte dysfunction and death. Aging stimulates expression of main genes that constitute machinery promoting mitochondrial fusion (*Mfn1, Mfn2, and Opa1*) and possibly the formation of enlarged mitochondria. This aging-induced stimulation of profusion genes was prevented by sildenafil-treatment. Aging interfered with mitochondrial quality control and increased transcription *Pink1* (a marker of mitophagy) while sildenafil-treatment normalized expression to level observed in adult rats.

Taking together, our results suggest that pharmacological targeting of cGMP-signaling could affect mitochondrial dynamics in the myocardium during aging.

A systematic pre-clinical (ex vivo) study of anti-cancer properties of olive oil polyphenolic secoiridoid derivatives

A. Papakonstantinou *[I,II], P. Koumarianou *[I], E. Frakolaki[III], N. Vassilaki[III], E. Chavdoula[IV,V], E. Melliou[II,VI], P. Magiatis[II], H. Boleti[I]

[I]Department of Microbiology, Hellenic Pasteur Institute, Athens, Greece, [II]Department of Pharmacy, National and Kapodistrian University of Athens, Athens, Greece, [III]Molecular Virology laboratory, Hellenic Pasteur Institute, Athens, Greece, [IV]Biomedical Research Division, Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, Ioannina, Greece, [V]Biomedical Research Foundation, Academy of Athens (BRFAA), Athens, Greece, [VI]World Olive Centre for Heath, Athens, Greece

Olive oil consumption has been associated by epidemiological studies with prevention of many human cancers. This activity has been attributed to olive oil polyphenols (OOPs) such as hydroxytyrosol and tyrosol, along with their derivatives Oleuropein aglycone (OPA), Ligstroside aglycone (LIGA), Oleacein (OLA), Oleomissional (OM), Oleocanthalic acid (OA) and Oleocanthal (OLC).

We performed a systematic ex vivo study assessing the anti-proliferative/cytotoxic effects of six OOPs as single compounds or in combinations on sixteen human cancer cell lines representing eight different tissue origins. Cells were treated with increasing OOPs' concentrations and viability was determined by an adenosine-triphosphate (ATP)-based luminescence assay. Half-maximal effective concentrations (EC_{50}) were calculated after 72h treatment. Combinations of two OOPs and natural extracts were examined for synergistic effect. Finally, the anti-proliferative effect was studied by EdU-based Fluorescence Assay and the induction of apoptosis by Flow cytometry using Annexin-V and PI staining.

All OOPs reduced cell numbers in a dose- and time-dependent manner. The relative bioactivity strength of the tested OOPs was: OLC>OPA>LIGA>OLA>OM>OA. OOPs' in double combinations showed strong synergistic effect with OM and OLA standing out for their synergistic bioactivity with the other OOPs. With respect to the total extracts tested, their bioactivity was comparable to that of Oleocanthal when the latter was used alone on almost all cell lines tested. However, OOPs' extracts presented significantly higher activity than OPA, LIGA, OLA, OM and OA when used alone. Finally, each single OOP tested reduced cell numbers by either inhibiting cell proliferation or inducing cancer cell apoptosis or by a combined effect

* The authors marked with an asterisk equally contributed to the work.

Combination of Dasatinib and Quercetin improves cognitive abilities in aged Wistar rats

A. Krzystyniak *[I], M. Wesierska[II], **G. Petrazzo** *[I], A. Gadecka[II], M. Dudkowska[II], A. Bielak-Zmijewska[I], G. Mosieniak[I], I. Figiel[I], J. Włodarczyk[I], E. Sikora[I]

[I]Instytut Biologii Doswiadczalnej im. Marcelego Nenckiego PAN, Warszawa, Poland, [II]Instytut Biologii Doswiadczalnej im. Marcelego Nenckiego PAN, Warsaw, Poland

Introduction. Neurons and other glial cells have the potential to acquire senescent characteristics that could lead to defect in neuronal plasticity and alteration of cognition which negatively impact quality-of-life of elders. Eliminating senescent cells that accumulates with age, using senolytics drugs, has proven to be effective in alleviating symptoms of aged-related diseases.

Hypothesis. Combination of Dasatinib and Quercetin senolytics (D+Q) might prevent cognitive decline observed in aged rats.

Objectives. Quantify systemic inflammation level since inflammaging is a key component of unhealthy aging. Assess synaptic plasticity in hippocampal structures that are principally involved in memory processing, spatial processing and navigation. Investigation epigenetic and senescence hallmarks to shed light on relevant molecular pathway affected by D+Q treatment.

Methods. Young (3-month-old) and naturally aged male Wistar rats (18-/22-month-old) were treated with D+Q for eight weeks and tested in the active allothetic place avoidance task. Arterious blood was collected to assess cytokines level. Fresh hippocampal slices were stained with Dil to analyze dendritic spine morphology. Epigenetic and senescence markers were quantified from fixed hippocampal slices or lysates.

Results. We confirmed the cognitive decline of aged rats compare to younger animals. We observed in aged but not young rats treated with D+Q a reduction in systemic inflammation and an alleviation of aged-related learning deficits and memory impairments associated with changes in synaptic plasticity and epigenetic but not senescence markers. Furthermore, D+Q treatment retains long lasting effects up to six weeks after treatment.

Conclusion. Our study brings new insights on the effects of D+Q senolytics in alleviating age-associated cognitive dysfunctions.

* The authors marked with an asterisk equally contributed to the work.

Calorie restriction changes long-term and short-term memory in rats in an onset-, duration- and sex-dependent manner

M. Prvulovic[I], S. Todorovic[I], D. Milanovic[I], A. Vukojevic[I], V. Simeunovic[I], M. Jovic[I], S. Sokanovic, A. Mladenovic[I]

[I]Institute for Biological Research „Sinisa Stankovic“ - National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia

Calorie restriction (CR) is known as a potent intervention to prolong lifespan and healthspan. However, in recent studies it was shown that its effect is not universally beneficial, but it can vary from protective to detrimental depending on age when implemented and its duration. Herein, we tried to examine the effect of CR on short-term and long-term memory.

Ad libitum (AL) fed animals were used as controls. Wistar rats of different age (adult, middle-aged and aged) were exposed to CR (60% of AL), to examine the effect of early-onset CR (EOCR) and late-onset CR (LOCR). Novel object recognition test (NORT) was used to assess short-term (STM) and long-term memory (LTM) performance.

Different pattern of changes was observed in males and females. While preserved memory was only measured in young AL males, in females both EOCR and LOCR managed to preserve STM till 18 months of age, while LTM was preserved only with EOCR. In 24-month-old females LOCR failed to preserve memory and even worsened STM and LTM performance in comparison to the age matching AL controls, while in males neither EO- nor LOCR had effect on memory performance. EOCR succeeded to preserve STM and LTM in females, but till certain point in life. LOCR however seemed to have diametrically different effect depending on whether it was implemented at middle age or old age. Memory performance of male rats in NORT seems to be insensitive to CR treatments, since only young animals had ability to discriminate novel from old object.

Endothelial dysfunction markers predict short-term mortality in patients with severe alcoholic hepatitis

T. Rubio-Tomás *[I], **T. Rubio-Tomás**[II], D. Blaya *[II], D. Rodrigo-Torres[II], J. Lozano[II], P. Sancho-Bru[II]

[I]School of Medicine, University of Crete and Molecular Biology and Biotechnology of the Foundation for Research and Technology Hellas (IMBB-FORTH), Heraklion, Greece, [II]Institut d'Investigacions Biomèdiques August Pi Sunyer (IDIBAPS), Barcelona, Spain

Objectives: Ageing is a well-established risk factor for liver disease, since it increases risk and poor prognosis of a wide range of liver diseases. Here we focus on alcoholic hepatitis (AH), a severe condition characterized by a marked inflammatory response and high short-term mortality. Endothelial dysfunction (ED) is an early event in vascular and inflammatory disorders. The aim of this study is to evaluate ED in AH patients.

Methods: Prognostic value of ED biomarkers was evaluated in patients with severe AH (n = 67), compensated alcoholic cirrhosis (n = 15), heavy drinkers without liver disease (n = 15) and controls (n = 9), and in a validation cohort of 50 patients with AH. Gene expression of ED markers was analyzed in liver tissue.

Results: Plasma levels of ED markers such as vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), E-selectin and von Willebrand factor (vWF) increased along alcohol-related liver disease (ALD) progression. Intergroup analysis showed a significant increase of these markers in AH patients. In addition, VCAM-1 showed a positive correlation with Maddrey, MELD and ABIC scores and inflammation parameters (i.e. C-reactive protein and LPS levels). Importantly, levels of VCAM-1 were higher in patients with increased mortality and were independently associated with short-term survival (90-day) when adjusted by ABIC score. These results were confirmed in an independent cohort of AH patients. In addition, severe AH patients showed altered hepatic expression of ED markers.

Conclusions: In this study we show that advanced ALD and particularly severe AH is associated with an increase of ED biomarkers, which correlate with patient outcomes. These results suggest that ED may be a pathogenic event in AH and highlight endothelial factors as potential biomarkers in AH.

* The authors marked with an asterisk equally contributed to the work.

Impaired Natural Antibody levels in the elderly and their implication in disease onset

I. Sarrigeorgiou[I]

[I]Hellenic Pasteur Institute, Athens, Greece

Naturally-occurring antibodies (NAbs) are defined as germ-line encoded antibodies that exist in all healthy organisms animal species without prior exogenous antigenic stimulation. NAbs are polyreactive with the ability to bind self and non-self components, such as nucleic acids, proteins and haptens, which is directly related to their multiple biological functions, such as first-line immune defense against infections, maintenance of tissue and immune homeostasis. NAbs are secreted spontaneously by B-1 cells that are among the first B-cells to develop during ontogeny.

NAbs belong to all immunoglobulin classes although IgM NAbs are predominant with major beneficial role. It has been demonstrated that advancing age exerts a strong influence in circulating antibody composition mainly leading in less protective antibody production. Such age-related deficits in the immune system have been associated with an increased incidence of infections, autoimmune and neurodegenerative diseases as well as cancer. For example in neurodegenerative diseases such as Alzheimer's disease, levels of NAbs recognizing the beta amyloid target antigen, are significantly decreased compared to healthy controls. Moreover, decreased levels of NAbs against a-synuclein have been observed in patients with Parkinson's disease and vascular dementia. Finally, decreased levels of NAbs with certain specificities have been also described in cancer. As significantly low levels of NAbs to specific target antigens may be correlated with the onset and prognosis of the disease, they could be exploited as potential immune biomarkers or even treatment strategies.

Overall, NAbs repertoire of healthy aged individuals differs significantly from that of healthy young individuals, as it becomes more restricted in terms of specificity. NAbs involvement in different pathologies further supports that measurement of their levels could prove of a great value in precision medicine and targeted immunotherapy.

The effect of the duration of dietary restriction on insulin signaling pathway in the hippocampus of male Wistar rats

V. Simeunovic *[I], A. Mladenovic *[I], D. Milanovic *[I], M. Prvulovic *[I], M. Jovic, S. Sokanovic, S. Todorovic *[I]

[I]University of Belgrade, Institute for Biological Research Sinisa Stankovic, Belgrade, Serbia

It has been shown that insulin has an important role in many processes in the brain, like upholding the nutritional homeostasis in the hypothalamus and synaptic plasticity in the hippocampus. Insulin exerts its effects by acting through the insulin signaling pathway. During aging, chronic activation of this pathway can occur, leading to insulin resistance, which is in the basis of many neurodegenerative diseases. Numerous environmental factors, such as dietary restriction (DR), can postpone and / or slow down many of the age-related processes. It is assumed that DR exerts its effect on insulin resistance through the insulin signaling pathway.

In this experiment we studied the effect of 40% DR (60% of ad libitum daily intake) on the expression of insulin, as well as on the amount of total (IRβ) and active (pIRβ) form of insulin receptor in the hippocampus of male Wistar rats. We examined the effect of three different types of DR: DR1, which started at 6 months of age and lasted up to 18 and 24 months, DR2, which lasted 6 months (12-18 months and 18-24 months of age), and DR3, which lasted 3 months (15-18 months and 21-24 months of age).

Our results showed that long-term DR1 led to a decrease in activity of insulin signaling pathway in the hippocampus of both 18- and 24-month-old male Wistar rats, which can further play a role in the prevention of neurodegenerative diseases.

* The authors marked with an asterisk equally contributed to the work.

Single or long-term treatment with sildenafil citrate disturbed mitochondrial dynamic markers in spermatozoa of aged rats

I. Starovlah[I], S. Radovic Pletikosic[I], T. Kostic[I], S. Andric[I]

[I]Laboratory for Reproductive Endocrinology and Signaling, Laboratory for Chronobiology and Aging, Centre of Excellence CeRES, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia., Novi Sad, Serbia

Mitochondria are the key organelle for many cellular processes such as energy production, oxidative stress, apoptosis, calcium homeostasis, steroid biosynthesis and many others. It is known that there is a close relationship between mitochondria fitness and sperm motility and that the mitochondria are important organelles for the evaluation of sperm quality. However, the transcriptional pattern of the mitochondrial dynamic markers in spermatozoa has never been investigated. The aim of this study was to determine the transcriptional profile of the mitochondrial dynamic markers in spermatozoa of aged rats treated with single or long-term treatment with sildenafil citrate (Viagra®). Aged rats, from 18 to 21 months old, were treated orally with sildenafil citrate once (2h treatment) and for 4 months, with a dose of 1.25 mg/kg body weight, while the control group of rats received water. Results showed that long-term treatment with sildenafil increased the number of spermatozoa in aged rats comparing to control. RQ-PCR results revealed that transcription of the main mitochondrial biogenesis markers *Ppargc1a*, *Ppargc1b*, *Nrf1* and *Nrf2* decreased in spermatozoa isolated from aged rats treated once with sildenafil citrate. In the same spermatozoa samples transcription of main markers of mitochondrial architecture *Opa1*, *Mfn1*, *Mfn2* was decreased, while long-term treatment decreased only the transcription level of *Mfn2*. Long-term treatment with sildenafil citrate decreased the level of mitochondrial biogenesis and function markers *Ppargc1b*, *Cox4i2* and *Ppard*, while increased the level of mitochondrial transcription factor *Tfam*. Taken all together, the transcriptional pattern of the mitochondrial dynamic markers in spermatozoa may serve as the potential biomarker for evaluation of the spermatozoa quality linked with male (in)fertility.

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Mechanisms of alpha-synuclein mediated neurodegeneration

E. Taoufik[I], E. Akrioti[I], O. Apokotou[I], K. Segklia[I], F. Papastefanaki[I], P. Koutsoudaki[I], S. Chavaki[I], R. Matsas[I]

[I]Hellenic Pasteur Institute, Athens, Greece

Alpha-synuclein (αSyn) dysregulation leads to profound neurodegeneration, however our knowledge of the molecular/biochemical events that initiate disease-related processes remains limited. Therefore there is an imminent need to understand how αSyn triggers degeneration, how pathology evolves, what primary pathways are impaired, and ideally if these mediators can serve as biomarkers or disease intervention points. In our team we integrate knowledge derived from p.A53T αSyn mutant mice and 2D/ 3D cellular systems derived from patients bearing the p.A53T mutation to determine early events in αSyn pathology. We have selected to exploit systems that express the p.A53T-αSyn mutation, as they show accelerated aggregation kinetics and a number of early distorted molecular pathways in neurons. Transcriptomics and proteomics approaches have revealed alterations in core cellular metabolic pathways including RNA metabolism, lipid and protein biosynthesis, proteostasis and synaptogenesis. In follow-up studies, we report that both mouse and human p.A53T neurons display aberrant connectivity, alterations in the numbers of excitatory and inhibitory synaptic contacts, whilst monosynaptic tracing using a modified rabies virus revealed a largely compromised network. The early occurrence of these defects is further supported by the partial inability of p.A53T-αSyn neurons to form artificial synaptic connections with non-neuronal cells ectopically expressing postsynaptic excitatory or inhibitory molecules (Biederer & Scheiffele, 2007). Moreover, extensive impairment in proteasomal activity and autophagy was detected in brain tissues from young mutant mice that correlated with the disturbances observed in human neurons at early stages of differentiation. Altogether, the combinatorial analysis of cellular and in vivo models of p.A53T-αSyn pathology allows us to gain a better insight in the spatiotemporal events of synaptic and proteostatic failure in synucleinopathies.

Frailty: a way to measure aging

Mladenovic A., Prvulovic M., Jovic M., Simeunovic V., Sokanovic S., Vukojevic A., **S.Todorovic**
Institute for Biological Research "Sinisa Stankovic"-National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia

Frailty is a multidimensional syndrome in aging, allowing us the identification of the most vulnerable subset of older adults. In the past few decades, medium life expectancy is increased without an appropriate increase of healthspan. This phenomenon refers to an increased life expectancy without a proper increase of healthspan, the period of life during which the organism is healthy and free of serious disease. Since healthspan is declining, there are more individuals with morbidities and consequently an increasing number of frail individuals. When an individual is frail, even a minor complication can create a chain of events that can give a rise to a disability or even death. Nevertheless, there are old individuals who are not frail, so being old does not necessarily mean being frail. That is why the concept of frailty was created, to explain the heterogeneity in clinical outcomes between older patients, so it can be determined who is more likely to fall into the longevity trap.
The origin of frailty is based on the combination of genetic, biological, physical, psychological, social and environmental factors[3]. It is important to keep in mind that frailty is a dynamic condition, and it is potentially reversible so the treatments, such as calorie restriction, can increase lifespan and concomitantly reduce age-related disease[5]. Optimal aging would then depend on conditions that both promote long life and compress morbidity to achieve greater healthspan. Model organisms have been at the forefront in the aging research, giving us a wealth of information regarding different pathways to regulate aging. That is the reason why in the past decade, an effort was made to link the biology of aging with frailty in aging animal models. To achieve that, two major frailty models, "Fried frailty phenotype" and "Rockwood frailty index" have been adapted and validated in animals.

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Path to healthy aging: assessment of different dietary restriction protocols through behavioral and frailty measurements

S. Todorovic[I], A. Mladenovic, M. Prvulovic, D. Milanovic, V. Simeunovic, M. Jovic, S. Sokanovic, A. Vukojevic

[I]Institute for Biological Research “Sinisa Stankovic”, National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia

Aging is an inevitable, complex and dynamic process of natural change, leading to deleterious age-related modifications. Still, the aging process can be modified by lifestyle interventions, like dietary restriction (DR). Although DR is a proven experimental paradigm for lifespan and healthspan extension, the impact of the type, onset and duration of DR is still debatable. In order to test different dietary types, we used frailty as a tool for detecting DR outcomes throughout aging. In this report, we describe that different dietary protocols have various impact on age-related behavioral parameters and frailty status.

We exposed male Wistar rats of various age to ad libitum (AL) or DR (60% of AL daily intake) feeding regimens with different onsets. The effect of DR on locomotor activity, memory and learning was examined in 12-, 18- and 24-month-old animals using open field (OF) and Y maze test. We used behavioral data to create unique frailty score (FS) and determine frailty status in those animals.

Our results indicated that various durations and onsets of DR can alter the course of aging, with the life-long DR responsible for the most profound effect. Shorter duration/later onset of DR had minor or in some cases even detrimental impact on behavior and frailty during aging.

Mitochondrial dynamics markers could be potential anti-aging biomolecular targets in human spermatozoa

T. Tomanić[I], I. Starovlah[I], S. Radovic Pletikosic[I], T. Kostic[I], S. Andric[I]

[I]Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia

With the delay of parenthood becoming more common and an increase in the number of infertile men at age of 30, urgent solutions are required. Since previous studies reported significantly more DNA breaks in spermatozoa of men older than 35 years and mitochondrial dynamics markers were not explored, our study aimed to examine the transcriptional profile of mitochondrial dynamics markers in spermatozoa. Men participating in the national program of *in vitro* fertilization were divided into two groups: younger than 35 (4 participants) and aged 35 and over (11 participants). Results showed a significant increase in the level of mitochondrial biogenesis markers *PPARGC1A* and *TFAM*, mitofission marker *DRP1* and mitophagy marker *PINK1* in the group of the older patients, compared to younger. The levels of mitofusion markers *MFN1* and *OPA1* tended to decrease in the older group of patients, but not significantly. The levels of transcripts for other analyzed markers (*PPARGC1B*, *NRF2*, *MFN2*, *FIS1* and *PRKN*) remained unchanged. It is important to point out that these results should be considered preliminary since a small number of participants were included in the analysis. However, results suggest a shift of mitochondrial fusion/fission balance, potentially changing mitochondrial morphology towards a fragmented shape. Since it is known that overexpression of DRP1 and exceeded mitochondrial fission, not balanced with fusion, lead to mitochondrial membrane potential loss, reduced ATP production and increased ROS formation, manipulating mitochondrial network towards fusion and inhibiting fission could be a potential therapeutic approach, preventing ROS increase and overcoming age-related (sub/in)fertility.

Ribosome profiling of Ercc1Δ/− progeroid mice liver and the effects of dietary restriction.

I. van Galen[I,II], R.A. Ozinga[I,II], S.A. Engels[I], J.T. van Dinter[I], D.A. Hofman[I], K. Smit[I,II], S. van Heesch[I], W.P. Vermeij[I,II], J.H.J. Hoeijmakers[I,II,III,IV]

[I]Princess Maxima Center for Pediatric Oncology, Utrecht, Netherlands, [II]Oncode Institute, Utrecht, Netherlands, [III]dept. Molecular Genetics, Erasmus MC, Rotterdam, Netherlands, [IV]CECAD, Cologne, Germany

Aging is a complex multifactorial phenomenon that is influenced to a great extent by genomic instability. This became apparent from situations with elevated levels of DNA damage e.g., after chemotherapy exposure when ex-cancer patients suffer from accelerated aging induced by their life-saving treatment or during conditions of DNA repair deficiency as seen in many progeroid syndromes (e.g., Werner or Cockayne syndrome). The Ercc1$^{\Delta/-}$ mouse model is an example of the latter. In these mice multiple DNA repair pathways are disrupted (including nucleotide excision repair, transcription coupled repair, interstrand cross-link repair, and homologous recombination) leading to accelerated wide-spread aging and a lifespan of only 4-6 months. However, when mice are put on a 30% dietary restriction diet, which we define as a reduction of all dietary components without malnutrition, their health and lifespan are significantly extended. To expand our knowledge on the preventive capacity of dietary restriction against DNA damage and aging, we studied translation dynamics and the translational landscape of Ercc1$^{\Delta/-}$ mice fed ad libitum or following a dietary restriction diet. Using ribosome profiling on liver tissues of these mice, we found that the expression of ribosomal proteins and some initiation & elongation factors increased in Ercc1$^{\Delta/-}$ mice and that this effect was enhanced during dietary restriction. Furthermore, we observed distinctive changes in codon occupancy, ribosome stalling, and translational efficiency linked to dietary restriction.

The role of adenosine monophosphate-activated protein kinase (AMPK) in the neurotoxicity of extracellularly secreted α-synuclein in rat pheochromocytoma PC12 cells

M. Vukojevic[I], M. Jeremic[I], A. Djuranovic[I], M. Dulovic[I], I. Markovic[I]

[I]Institute of Medical and Clinical Biochemistry, Faculty of Medicine, University of Belgrade, Belgrade, Serbia

The accumulation of wild-type (wt) alpha-synuclein (ASYN) in susceptible neurons is regarded as essential in Parkinson's disease pathogenesis. ASYN, although mostly regarded as purely intracellular protein, was recently also detected extracellularly. Having in mind that ASYN can affect mitochondrial function, as well as high sensitivity of neurons to changes in cellular energy level; AMPK, a key intracellular energy sensor, is consider to play a role in neurotoxic effect of ASYN.
The aim of this study was to investigate the role of AMPK in the neurotoxicity of extracellularly secreted ASYN.
All experiments were conducted in rat pheochromocytoma PC12 cells, exposed to secreted ASYN, present in conditioned medium (CM) from alpha-synuclein overexpressing cells, in presence or absence of pharmacological AMPK activators (AICAR, metformin). Activated (phosphorylated) forms of AMPK, Raptor and mTOR were monitored using immunoblotting. Cell viability was assesed using acid phosphatase and crystal violet assays.
Application of CM containing secreted ASYN caused cell death of recipient PC12 cells, as well as a decrease in activation of AMPK/Raptor signaling pathway, whereas application of AICAR and metformin abolished those effects and caused significant increase in cell number of PC12 cells. The reduced neurotoxicity was associated with AMPK activation, in mTOR-independent manner.
Extracellularly secreted ASYN induced cell death of PC12 cells, accompanied with a decrease in AMPK and Raptor phosphorylation. Pharmacological activation of AMPK by AICAR and metformin improved cell survival, indicating a protective role of AMPK in extracellularly secreted ASYN neurotoxicity, in mTOR-independent manner.

Protective role of fermented food in LPS-induced inflammation in C57BL/6 mice

A. Vukojevic[I], M. Prvulović[I], S. Todorović[I], A. Mladenović[I], M. Jović[I], I. Jovanović Macura[I], M. Perović[I], D. Milanović[I]
[I]Institute for Biological Research „Sinisa Stankovic", University of Belgrade, Belgrade, Serbia

Non-transmittable chronic diseases are largely driven by chronic inflammation, which can be connected to poor diet and toxic products of commensal gut microbiome. Diet intervention can influence gut microbiota function and composition. Fermented foods are specifically known to have anti-inflammatory and immunomodulatory properties, attributed to their high antioxidant content and lactic acid-producing bacteria (LAB)
In this study we examined the effects of sauerkraut brine (SB) on physiological and behavioral responses to systemic inflammation induced by lipopolysaccharides (LPS) in a mouse model. C57BL/6 mice 90 postnatal days old were used in this study. They were divided into 2 groups and treated with either 150 ml of sauerkraut brine and pasteurized sauerkraut brine (PSB) for 5 weeks (via oral gavage). Control animals (CON) were receiving an equivalent amount of water. During the final week of treatment, animals received 5 injections of LPS (0.5 mg/kg, i.p.). Behavior of animals was assessed before and after LPS administration, using the open field test, light-dark box, Y-maze, tail-suspension and rota-rod test. Analysis of pro-inflammatory brain cytokines has been performed subsequently via Western blot and PCR. Food consumption and body weight were measured throughout the experiment.
SB and PSB treatments did not influence body weights and behavior compared to CON mice. LPS treatment led to the weight loss and decreased food intake in all experimental groups. The fastest recovery and a reduced inflammatory response was detected in the SB group. Behavioral analysis revealed differences between three groups in responses to the LPS challenge.

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