Chairman: Stathis Gonos

(National Hellenic Research Foundation)

Contact: Ippolyti Karvouni

Email: febsiubmb2016@eie.gr

National Hellenic Research Foundation (NHRF),

Institute of Biology, Medicinal Chemistry & Biotechnology

48 Vas. Constantinou Ave., Athens 11635, Greece.

Sponsors

The Organizing Committee gratefully acknowledges support towards the FEBS/IUBMB Advanced Lecture Course on “Molecular basis of human diseases: 50 years anniversary of Spetses summer schools” from the following sources:

Sponsors	
Federation of European Biochemical Societies http://www.febs.org	
International Union of Biochemistry and Molecular Biology http://www.iubmb.org	
Hellenic Society of Biochemistry and Molecular Biology http://www.eebmb.gr	
http://www.febs.org/our-publications www.febsjournal.org	
http://www.febs.org/our-publications www.febsletters.org	
http://www.febs.org/our-publications www.moloncol.org	
http://www.febs.org/our-publications www.febsopenbio.org	

Molecular & Cellular Ageing Programme
Institute of Biology, Medicinal Chemistry and Biotechnology

National Hellenic Research Foundation

Programme

Friday May 27th

19:30-20:00	Welcome
20:00-21:00	"IUBMB lecture": G. Petsko, USA, "The Molecular Basis of Neurodegenerative Diseases: How Parkinson's Disease starts – and how it might be stopped"
21:00	welcome reception

Saturday May 28th

9:00-10:00	"M. Grunberg-Manago lecture": P. Cossart, France, "The Bacterial Pathogen Listeria Monocytogenes: a Multifaceted Model in Biology"
10:00-11:00	"T. Evangelopoulos lecture": D. Thanos, Greece, "Stochastic and Deterministic Mechanisms of Cellular Reprogramming"
11:00-11:30	coffee break
11:30-12:30	S. Gonos, Greece, "Genetic and Environmental Factors of Human Ageing and Longevity"
12:30-14:00	lunch
14:00-16:00	free time
16:00-17:30	Poster session 1 (Nos: 1-26)
17:30-18:00	coffee break
18:00-19:00	"Ceremonial lecture": H. Feldmann, Germany, "1966-2016: A brief History of Spetses Summer Schools"
19:00-20:00	"B. Clark lecture": D. Rhodes, Singapore, "Telomeres in Ageing and Cancer"
20:30	special dinner

Sunday May 29th

9:00-10:00	A. Azzi, USA, "Ataxia from Vitamin E deficiency: A Disease still in Search for a Mechanism but already with a Straightforward Therapy"
10:00-11:00	T. Langer, Germany, "Mitochondrial proteases in health and disease"
11:00-11:30	coffee break
11:30-12:30	J. Guinovart, Spain, "Regulation of Neuronal Glycogen Metabolism in Health and Disease"
12:30-14:00	lunch
14:00-16:00	free time
16:00-17:30	Poster session 2 (Nos: 27-53)
17:30-18:30	Tutorial 1 (P. Cossart, S. Gonos, N. Kartal-Ozer, G. Petsko, D. Thanos)
18:30-19:00	coffee break
19:00-20:00	J. Frey, Switzerland, "Role of Type III secretion system effectors of Aeromonas in undermining the host's immune response"
20:30	dinner

Monday May 30th

9:00-10:00	N. Kartal-Ozer, Turkey, "High Cholesterol Induced Cardiac Failure and Unfolded Protein Response"
10:00-11:00	P. Kristensen, Denmark, "Toward single cell proteome analysis using recombinant antibodies"
11:00-11:30	coffee break
11:30-12:30	Tutorial 2 (A. Azzi, J. Frey, T. Langer, H. Poulsen, D. Rhodes)
12:30-14:00	lunch
14:00-20:30	free afternoon
20:30	dinner

Tuesday May 31st

9:00-10:00	H. Poulsen, Denmark, "Oxidation of DNA and RNA in Chronic Diseases"
10:00-11:00	B. Demeneix, France, "Thyroid Hormone Disruption and the Increased Incidence of Autism Spectrum Disorders"
11:00-11:30	coffee break
11:30-12:30	Tutorial 3 (B. Demeneix, H. Feldmann, J. Guinovart, P. Kristensen)
12:30-14:00	lunch
14:00-16:00	free time
16:00-17:00	"Round Table Discussion": A. Azzi, USA, "Sliding doors and turning points: events that may shape the future of a young scientist"
17:00-17:30	coffee break
17:30-19:30	Young scientists' presentations: Y. Bakakina, Belarus, P. Hemandez, Uruguay, M. Jimenez-Garcia, Spain, M. Inomistova, Ukraine, R. Kumari, U.K., J. Mayrhofer, Austria, Y. Timasheva, Russia, M. Xylaki, Greece
20:30	farewell dinner

Wednesday June 1st

Departure

LECTURERS' ABSTRACTS

SLIDING DOORS AND TURNING POINTS: EVENTS THAT MAY SHAPE THE FUTURE OF A YOUNG SCIENTIST

Angelo Azzi

Tufts University, Boston, USA, angelo.azzi@tufts.edu

In a movie of 1998 a London woman's love life and career both hinge on whether or not she catches a train before the sliding doors close. We see in this film both very different destinies, in parallel. To what extent the future of a young scientist depends on essential decisions early in their life - on some sliding doors? Based on the biographies of important scientists, can we identify the turning points at the basis of their success? Many other questions need to be discussed, such as “is scientific recognition a component of success?”; or “is success itself an important element to be pursued by a young researcher?”. Many answers to these questions have been provided by the contact with scientists that have made great discoveries, crucial for the progress of human beings. Many more questions are posed by a world that does not offer equal sliding doors and turning points to everyone.

ATAXIA WITH VITAMIN E DEFICIENCY: A DISEASE STILL IN SEARCH FOR A MECHANISM BUT ALREADY WITH A STRAIGHTFORWARD THERAPY

Angelo Azzi

Tufts University, Boston, USA, angelo.azzi@tufts.edu

Ataxia with vitamin E deficiency is also called AVED, Ataxia with Isolated Vitamin E Deficiency, Familial Isolated Vitamin E Deficiency or Friedreich-Like Ataxia. The disorder was first reported in 1981. AVED is inherited as autosomal recessive disease. The TTPA gene encodes the αTTP protein, responsible for retaining and using dietary vitamin E. Consequent to TTPA gene mutations, the function of αTTP as well as the plasma and tissue vitamin E levels are greatly reduced. Patients with AVED show at puberty common characteristics of the disease: they include progressive ataxia, clumsiness of the hands, loss of proprioception and areflexia. Disease manifestations can be handled with oral vitamin E supplementation at high doses, a treatment that, early after disease onset may partially reverse ataxia and mental decline. Vitamin E deficiency is associated with atrophy and reduced dendritic branching of Purkinje neurons. However the molecular basis of vitamin E protection is not completely understood. The radical scavenging properties of vitamin E, traditionally described as an antioxidant, do not explain why its vital protection is predominantly expressed at the level of Purkinje neurons. More recently, vitamin E has revealed to possess specific cellular functions that are independent of its radical scavenging properties. It inhibits protein kinase C and PI3 Kinase, as well as activates protein phosphatase 2A and diacylglycerol kinase. Furthermore, at transcriptional level, several genes are modulated by α-tocopherol, the natural form of vitamin E. α-Tocopheryl phosphate is synthesized and hydrolysed in animal cells and tissues; it modulates also several cell functions. While it is similar to α-tocopherol, α-tocopheryl phosphate appears to be much more potent than α-tocopherol, possibly representing an activated form of the vitamin. It is in these novel functions that vitamin E activity as survival factor for Purkinje neurons is being searched.

THE BACTERIAL PATHOGEN *LISTERIA MONOCYTOGENES*: A MULTIFACETED MODEL IN BIOLOGY

Pascale Cossart

Unité des Interactions Bactéries Cellules, Inserm U604, INRA USC2020,Institut Pasteur, Paris, France
pcossart@pasteur.fr

Listeria monocytogenes is a pathogen which has emerged as a model system in several areas of biology including Infection biology, cell biology, fundamental microbiology and also RNA biology. *Listeria* is an environmental bacterium which can contaminate food products resulting in foodborne infections which can be dramatic for pregnant wormen leading to abortions or immunocompromised individuals leading to meningitis and/or deaths in 30% of the cases. This microorganism is able to adapt its transcriptional and translational programmes to a variety of life conditions in both the environment and the infected host. Many of these adaptations are RNA-mediated, and involve novel riboswitches which will be discussed during the talk.

References :

- Mellin, J.R., T. Tiensuud, C. Bécavin, E. Gouin, J. Johansson and P. Cossart (2013). A riboswitch-regulated antisense RNA in *Listeria monocytogenes*. Proc Natl Acad Sci U S A, 110:13132-7
- Mellin, J.R., M. Koutero, D. Dar, M.-A. Nahori, R. Sorek and P. Cossart (2014). Sequestration of a two-component response regulator by a riboswitch-regulated non-coding RNA. Science, 345:940-3
- Mellin, J.R., and P. Cossart (2015). Unexpected versatility in bacterial riboswitches. Trends Genet., 31:150-156
- Dar, D., M. Shamir, J. R. Mellin, M. Koutero, N. Stern-Ginossar, P. Cossart, and R. Sorek (2016). Term-seq reveals abundant ribo-regulation of antibiotics resistance in bacteria. Science, In Press

THYROID HORMONE DISRUPTION AND THE INCREASED INCIDENCE OF AUTISM SPECTRUM DISORDERS

J. B. Fini, B. Mughal, M. Leemans & B. A. Demeneix

UMR CNRS MNHN 7221, Evolution of Endocrine Regulations, 75231 Paris, France.

During vertebrate evolution thyroid hormone (TH) acquired multiple roles in development, especially brain development. Examples of thyroid action on nervous system development include division, differentiation, migration, myelination and synaptogenesis. In the last 15 years we have acquired new knowledge on cell specific control of thyroid hormone signaling, the roles of TH in neuronal lineage decisions[1] and the unexpected requirement of TH in early gestation and neurogenesis. It is also in these last 15 years that we have witnessed an unprecedented and dramatic increase in Autism Spectrum Disorders (ASD) incidence. Although, changes in diagnosis and awareness could contribute to some of the increase, many authors consider that environmental factors, possibly exacerbating genetic susceptibilities, are involved[2]. Our hypothesis is that disruption of thyroid signaling could be implicated. Four arguments support this hypothesis. First, increasing numbers of chemicals are found routinely in human amniotic fluid. These include, pesticides, plasticizers (such as phthalates, BPA), nitrates, perchlorate, antimicrobials (such as Triclosan), flame-retardants, surfactants and mercury (produced by fossil fuel burning). Second, many of these chemical categories can interfere with TH signaling. Third, prenatal exposure to some of these chemicals, such as organophosphate pesticides are already documented as being associated with increased ASD risk. Fourth, production of many of these chemicals has risen exponentially in the last few decades, continually increasing exposure. I will present and discuss data showing that cocktails of these chemicals found in amniotic fluid can interfere with TH signaling and brain development, affecting genes and developmental pathways that are regularly associated with ASD.

1. Lopez-Juarez, A., et al., *Thyroid hormone signaling acts as a neurogenic switch by repressing Sox2 in the adult neural stem cell niche.* Cell Stem Cell, 2012. **10**(5): p. 531-43.
2. Demeneix, B., 2014 *Losing our minds: how environmental pollution impairs human intelligence and mental health*. Oxford University Press

1966-2016: A BRIEF HISTORY OF SPETSES SUMMER SCHOOLS

Horst Feldmann

University of Munich, Germany, Horst.feldmann@med.uni-muenchen.de

In my presentation I want to outline the beginning and the development of these famous Lecture Courses on Molecular and Cellular Biology during the past 50 years.

It was the initiative of Marianne Grunberg-Manago to establish the first of these venues in 1966 with generous support from NATO. With the aid of Thanos Evangelopoulos she found the island of Spetses, an ideal place, where renowned Lecturers could scientifically interact with young researchers in a relaxed atmosphere. Due to the 'putsch of the colonels', the School could not be organized in 1967 and 1968, but Francis Crick, Brian Clark and Mark Bretscher in 1969 succeeded to revive the enterprise with great success. They suggested that the Germans – Hans Zachau and I – should be involved as a third party. However several of the lecturers decided not to go back to Spetses. The most vigorous protest against the unsafe political situation in Greece was pronounced by the students in a letter to Nature. So the Germans preferred to organize the Schools in 1971 and 1974 in an exile at Erice (Sicily). But in 1972 and 1973, the French and British organizers went back to Spetses, and from 1975 onwards the series of successful Summer Schools in Spetses could be continued.

I will then describe some of Spetses geographic peculiarities, the role of Spetses during the Greek War of Independence, and the founding of Anargyrios & Korgialenios School.

Interesting aspects – not only for future organizers - will be discussed in two sections to follow: 'How financing changed over the years' and 'How Spetses Summer Schools were run'.

After 50 years tradition, we now have to think about the future of the Spetses Summer Schools. Finally, it is most appropriate to remember all those people who substantially contributed to the success of these Schools and to heartily thank them for their untired commitment.

ROLE OF TYPE III SECRETION SYSTEM EFFECTORS OF *AEROMONAS* IN UNDERMINING THE HOST'S IMMUNE RESPONSE

Joachim Frey and Francesco Origgi

Institute of Veterinary Bacteriology, University of Bern, Switzerland. joachim.frey@vetsuisse.unibe.ch

Infections by the waterborne *Aeromonas* species cause significant multi-systemic disease in human and many animal species. Differently from most *Aeromonas* species, *Aeromonas salmonicida* is an obligate pathogen of salmonid fish causing the severe disease furunculosis and provides an excellent homogeneous model for human disease. The type-three secretion system (T3SS) is the major virulence attribute in *Aeromonas* sp. It is used by the bacterium to secrete and translocate a large number of toxins and effector proteins into the host cell. Some of these factors such as the bi-functional ADP ribosylating – GTPase activating protein AexT have been shown to have a detrimental impact on the integrity of the cell cytoskeleton, impairing phagocytosis. Other effector proteins that are injected into the host cell such as ApoP act by inhibiting the NF-κB signalling pathway blocking the nuclear translocation of NF-κB (p65) into the nucleus, thus influencing the host's inflammatory response. Several additional effectors that are secreted and translocated via the T3SS including Ati2, AopN and ExsE have been suggested to modulate the host's immune response in particular by downregulating the inflammatory reaction. The analysis of the immune response in rainbow trout infected with either fully virulent or T3SS defective *A. salmonicida* revealed that a functional T3SS in *A. salmonicida* leads to a rapid, strong and persistent downregulation of several immune-relevant markers associated with Th-1, Th-2, and an upregulation of T-regulatory markers, in contrast to an T3SS defective mutant that only showed a weak and transient downregulation of Th-1, Th-2, and a relatively poor induction of the T-regulatory markers. T3SS-delivered effector molecules and toxins of *Aeromonas salmonicida* do not only impair the host's cytoskeleton thus damaging cell physiology and phagocytosis, but also directly affect the transcription of critical immune markers including the shut-down of important warning signals to recognize infection and induce immune defense.

GENETIC AND ENVIRONMENTAL FACTORS OF HUMAN AGING AND LONGEVITY

Efstathios S. Gonos

National Hellenic Research Foundation, Institute of Biology, Medicinal Chemistry & Biotechnology, Athens, Greece, e-mail: sgonos@eie.gr

Aging and longevity are two multifactorial biological phenomena whose knowledge at molecular level is still limited. We have studied proteasome function in replicative senescence and cell survival (Mol Aspects Med 35, 1-71; Ageing Res Rev 23, 37-55). We have observed reduced levels of proteasome content and activities in senescent cells due to the down-regulation of the catalytic subunits of the 20S complex (J Biol Chem 278, 28026-28037). In support, partial inhibition of proteasomes in young cells by specific inhibitors 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 increased cell survival following treatments with various oxidants. Importantly, the developed "proteasome activated" human fibroblasts cell lines exhibit a delay of senescence by approximately 15% (J Biol Chem 280, 11840-11850; J Biol Chem 284, 30076-30086). Moreover, additional findings indicate that the recorded proteasome activation by many inducers is Nrf2-dependent (J Biol Chem 285, 8171-8184). Finally, we provide evidence that proteasome activation is an evolutionary conserved mechanism, as it can delay aging in vivo and, importantly, it also confers deceleration of aggregation-related pathologies, such as Alzheimer's or Huntington's diseases (FASEB J 29, 611-622). Given these findings, recent work has identified a proteasome activator that decelerates aging and Alzheimer's disease (Antiox Redox Signal, in press).

We have also developed biobanks of donors of different ages, including healthy centenarians and long-lived siblings. Using these biobanks we have cloned several novel longevity genes (Biogerontology 5, 401-409) and we have 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 healthy aging and longevity. Finally, we determine the rate of aging and the efficacy of anti-aging protocols using molecular biomarkers in PBMCs of healthy individuals. To this end we measure the levels of 4 established biomarkers of ageing, namely telomeres length, Clu/ApoJ, proteasome content and levels of oxidized proteins in volunteers every 3-6 months.

REGULATION OF NEURONAL GLYCOGEN METABOLISM IN HEALTH AND DISEASE

Joan J. Guinovart

Institute for Research in Biomedicine (IRB Barcelona) and University of Barcelona, Spain
guinovart@irbbarcelona.org

Glycogen is present in the brain, where it has been observed mainly in glial cells. Therefore, all physiological roles of brain glycogen have been attributed exclusively to astrocytic glycogen. Working with primary cultured neurons, as well as with genetically modified mice and flies, we have found that—against general belief—neurons contain a low but measurable amount of glycogen. Moreover, these cells express the brain isoform of glycogen phosphorylase, allowing glycogen to be fully metabolized. Most importantly, we show that an active glycogen metabolism protects cultured neurons from hypoxia-induced death and flies from hypoxia-induced stupor.

Lafora disease is a fatal neurodegenerative condition characterized by the accumulation of abnormal glycogen inclusions known as Lafora bodies. It is an autosomal recessive disorder caused by mutations in either the laforin or malin gene. We generated a malin knockout mouse model with impaired (totally or partially) glycogen synthesis. These animals did not show the increase in markers of neurodegeneration seen in the malin knockout model. Interestingly, the autophagy impairment that has been described in the latter was also rescued in this double knockout model. Conversely, two other mouse models in which glycogen is over-accumulated in the brain, independently of the lack of malin, showed altered autophagy.

These findings change the current view of the role of glycogen in the brain and demonstrate that neuronal glycogen metabolism participates in tolerance to hypoxia. They also reveal that glycogen accumulation accounts for the neurodegeneration, as well as the impaired autophagy, seen in the malin knockout model.

HIGH CHOLESTEROL INDUCED CARDIAC FAILURE AND UNFOLDED PROTEIN RESPONSE

Erdi Sözen, Burak Yazgan, Betul Karademir, Nesrin Kartal Ozer

Department of Biochemistry, Faculty of Medicine, Marmara University, 34854, Maltepe, Istanbul, Turkey, nkozer@marmara.edu.tr

Most important characteristics of cardiovascular diseases are cardiac hypertrophy and heart failure. Among many other factors, high cholesterol takes an important place for the progress of these diseases results in atherosclerosis. Cholesterol is involved in the disruption of several molecular pathways related to cardiac failure. Endoplasmic reticulum (ER) play crucial role in the multifunctional organel support of cardiomyocytes. Proper synthesis and correct folding of proteins in ER is extremely important for the normal function of heart and ER-associated functions are shown to be key regulators for cardiac physiology and pathology. IRE1, PERK, ATF6 pathways are involved in unfolded protein response (UPR) of cardiac ischemia. When unfolded proteins increased, and proteosomal system impairs, autophagy provides a possible alternate pathway for removing aggregated proteins. Incidentally, the ratio of the protein expression of membrane associated LC3-II to cytosolic LC3-I is often used to assess autophagic activity. Beclin-1 is negatively regulated by its interaction with the anti-apoptotic protein Bcl-2 under normal conditions. However, increased oxidative stress and ER stress activates the ubiquitin-proteosome system, which functions to degrade Bcl-2. This allows for beclin-1 activation subsequently resulting in autophagic cell death.

In this study, the effects of enhanced oxidative stress and ER stress in heart of hypercholesterolemic rabbits have been investigated. The results of molecular components of ER stress, autophagy and apoptosis (such as ATF6, BiP, Grp94, LC3 II/I, beclin-1, Bcl-2, Bax, Aif, Caspase-9 and Caspase-3) indicate that hypercholesterolemia increases unfolded protein response via increasing oxidative stress and ER stress and this leads autophagic cell death and contributes to progression of cardiac failure.

Supported by Marmara University Research Fund SAG-A-130612-0202, SAG-C-DRP-130515-0164 and SAG-C-DRP-130515-0165.

TOWARD SINGLE CELL PROTEOME ANALYSIS USING RECOMBINANT ANTIBODIES

Peter Kristensen

Aarhus University, Department of Engineering, Denmark. pk@eng.au.dk

In recent years the importance of cellular heterogeneity has become increasingly clear. In developing therapies for important diseases, such as cancer, the ability to isolate and characterize rare cell populations will be important, allowing targeting of minor tumor cell populations such as the tumor stem cells or circulating tumor cells. Monitoring normal human health statues, in the future, also could take advantage of a characterization of rare cell populations in the circulation, such as the Endothelial Progenitor Cell.

We have advanced the phage display technology, thus allowing the isolation of specific antibodies binding to one identified cell in a heterogeneous mixture of cells. In this presentation the technology for single cell analysis using phage antibody technology will be described, building on examples from model systems, endothelial progenitor cells and other pathological situations.

In addition novel antibody libraries and formats will be discussed.

MITOCHONDRIAL PROTEASES IN HEALTH AND DISEASE

Thomas Langer

Institute for Genetics and CECAD Research Center, University of Cologne, Cologne, Germany. *Thomas.Langer@uni-koeln.de*

Mitochondrial proteases ensure protein quality control within the organelle and mediate regulated proteolytic reactions important for mitochondrial function, integrity and homeostasis, such as protein synthesis, phospholipid trafficking, mitochondrial dynamics, mitophagy and apoptosis. Impaired or dysregulated function of mitochondrial proteases is associated with ageing and with many pathological conditions, such as neurodegenerative disorders, metabolic syndromes and cancer.

Recent experiments established an essential function of mitochondrial proteases in the regulation of mitochondrial morphology, which is maintained by balanced fusion and fission events and proteolytic cleavage of the dynamin-like GTPase OPA1 by two peptidases, the *i*-AAA protease YME1L and OMA1. Uncleaved, long forms of OPA1 mediate mitochondrial fusion, whereas cleaved, short OPA1 forms are associated with mitochondrial fission. Various stress conditions activate OMA1 and induce OPA1 cleavage limiting fusion and triggering mitochondrial fragmentation, a hallmark of pathologic conditions that are associated with a dysfunction of mitochondria. Inhibition of this pathway upon ablation of *Oma1* protects against heart failure and neurodegeneration in mouse models for tissue-specific mitochondrial dysfunctions in the heart and the brain, respectively, highlighting the physiological significance of OMA1 and stress-induced mitochondrial fragmentation for cell survival.

THE MOLECULAR BASIS OF NEURODEGENERATIVE DISEASES: HOW PARKINSON'S DISEASE STARTS – AND HOW IT MIGHT BE STOPPED

Gregory A. Petsko, Iva Perovic, Rui Wu, Wassilios Meissner, Erwan Bezard, Michael Foley, Dagmar Ringe and Quyen Hoang

Appel Alzheimer's Disease Research Institute, Weill Cornell Medical College, New York, NY USA, gpetsko@med.cornell.edu

α-Synuclein is a presynaptic protein whose aggregation in so-called Lewy Bodies is the defining pathological hallmark of Parkinson's disease. Mutations in the α-synuclein gene also cause familial PD, leading to the conclusion that aberrant α-synuclein folding may be a sufficient, as well as necessary, condition for pathogenesis. α-Synuclein is widely believed to be an intrinsically disordered protein in free solution; however, we have been able to show that recombinant α-synuclein can be prepared as a stable, largely helical, soluble, folded protein that is predominantly homotetrameric in common physiological buffers. Disease-associated mutations perturb this "native" protein conformation. This oligomeric folded protein is resistant to aggregation and amyloid formation, does not compromise membrane integrity, and is not toxic to cells, which raises the question: if a key physiological form of α-synuclein does not aggregate readily, how does Parkinson's disease get started? It has long been known that a C-terminally truncated fragment of α-synuclein is found in Lewy Bodies. We have shown that *in vitro* this fragment aggregates more readily than the full-length protein and can recruit the full-length protein into aggregates. We speculated that aberrant α-synuclein proteolysis might be an initiating event in Parkinson's disease, and set out to find the protease responsible. By a combination of yeast genetics and experiments in a neuroblastoma model of PD, we have tentatively identified the enzyme and have begun to develop specific inhibitors for testing in animal models. These inhibitors prevent synuclein fragmentation and toxicity in cultured human neurons, and the mouse knockout of the protease is resistant to Parkinson's-inducing toxins. Inhibition of synuclein proteolytic truncation is a novel strategy for the prevention and/or treatment of synucleinopathies such as Parkinson's disease and Lewy Body dementia.

OXIDATION OF DNA AND RNA IN CHRONIC DISEASES

Poulsen HE, Weimann A, Henriksen T, Cejvanovic V, Kjaer LK, Brandslund I, Christensen C, Simonsen AH, Jorgensen A, Jørgensen MB, Munkholm K..

Department of Clinical Pharmacology, Bispebjerg Frederiksberg Hospitals, University Hospital Copenhagen, Rigshospitalet, 9 Blewgdamsvej, DK 2100 Copenhagen, Denmark, hepo@rh.dk

Oxidative damage to nucleic acids have long been implication in chronic diseases, particularly cancer, type 2 diabetes, haemochromatosis, psychiatric diseases and degenerative brain diseases. Epidemiological studies have suggested a role for oxidative damage to DNA as a risk factor in lung cancer and in breast cancer. The effects, as measured from the urinary excretion of guanine oxidation, 8-oxodG, are rather low, indicating a low to moderate contribution in lung cancer and breast carcinogenesis. Still, although a minor individual risk factor, from a public health point of view, it could represent a large preventable factor. In haemochromatosis oxidation of DNA (urinary 8oxodG) apparently is not elevated, however, the oxidation of guanine in RNA, measured as the urinary excretion of 8oxoGuanosine, is considerably increased and returns to control values with treatment as does the risk of cancer and diabetes. In diabetes, DNA oxidation is not elevated, but increased RNA oxidation is prognostic for death from overall causes and from arteriosclerotic events. In neurodegenerative diseases preliminary data indicated the RNA oxidation in the brain, measured as 8oxoGuanosine concentration in cerebrospinal fluid, is increased.

Taken together these observations clearly indicate the involvement of oxidative damage to nucleic acids in human disease. Mechanistically, the molecular basis for cancer development from oxidation of guanine in DNA is well understood. With regard to RNA oxidation, the mechanisms are poorly understood. Base lesion in RNA show a differential effect on translation, some lesions produce only truncated products and some lesions produce a mixture of full-length and truncated products (Nucleic Acid Research 2015;43:4713), and furthermore oxidation of mRNA seems to produce ribosomal stalling.

The major challenge in the further studies on oxidation of nucleic acids is to identify a treatment or factor that can reduce the oxidations, and reduce or retard the development of chronic diseases.

TELOMERES IN AGEING AND CANCER

Daniela Rhodes

NTU Institute of Structural Biology, Nanyang Technological University, 59 Nanyang Drive, Singapore

The telomerase-based mechanism for telomere maintenance of linear chromosomes is conserved in most eukaryotes. Telomeres are the protein/DNA complexes that cap the ends of eukaryotic chromosomes and maintenance of their length is essential for genomic stability and cell viability. Each time a cell divides the telomeres get shorter. Telomere shortening correlates with cellular aging and in the majority cancer cells depend on the activation of the telomerase enzyme to gain proliferative immortality.

I will give an overview of telomere biology and then present our structural analysis of the human telomerase enzyme using single particle cryo-EM as well as our structural analysis of telomeric protein/DNA complexes.

Combinatorial Readout of Histone H3 Modifications Specifies Localisation of ATRX to Heterochromatin.
Eustermann, S., Yang, J-C., Law, M. J., Amos, S., Chapman, L. M., Jelinska, C., Garrick, D., Gibbons, R. J., **Rhodes, D.**, Higgs, D. R. and Neuhaus, D.
(2011) Nat. Struct. Mol. Biol., 18, 777-82

Structure of active, dimeric human telomerase.
Sauerwald, A., Sandin, S., Christofari, G., Scheres, S. H. W., Lingner, J., and **Rhodes, D.**
(2013) Nat. Struct. Mol. Biol, 20, 454-60.

Telomerase activated thymidine analogue pro-drug is a new molecule targeting hepatocellular carcinoma.
Tarocchi M, Polvani S, Peired AJ, Marroncini G, Calamante M, Ceni E, Rhodes D, Mello T, Pieraccini G, Quattrone A, Luchinat C, Galli A.
(2014) J. Hepatol. Nov; 61(5):1064-72

STOCHASTIC AND DETERMINISTIC MECHANISMS OF CELLULAR REPROGRAMMING

Dimitris Thanos

Biomedical Research Foundation Academy of Athens, 4 Soranou Efesiou Street, Athens 11527, Greece
thanos@bioacademy.gr

Cellular reprogramming of somatic cells to ground state pluripotency by the co-expression of the transcription factors *Oct4*, *Sox2*, *Klf4* and c-Myc (OSKM factors) is a prolonged gradual, asynchronous and inefficient process generating a small subpopulation of induced Pluripotent Stem Cells (iPSCs). Here, we found that reprogramming involves two waves of transcriptional changes. The first occurs at the first day of the process involving the rapid transcriptional activation of genes required for the establishment and maintenance of the pluripotent phenotype, whereas the second wave is dominated by prolonged and complex patterns of gene repression targeting the mesenchyme-specific genes. Monitoring the OSKM distribution by ChIP-seq at different times of reprogramming we were able to show an unprecedented dynamic redistribution of OSKMs, where early Oct4 binding marks Sox2, KLF4 and MYC binding at the early time points. By contrast, most KLF4 and MYC act as late markers for OCT4 and SOX2 binding events. These and other data suggest that Oct4 works as nucleation factor for the subsequent cooperative binding of additional reprogramming factors to pluripotency genes. Furthermore, by combining RNA-seq, chip-seq and mathematic modelling we identified a novel gene regulatory network composed of 9 transcriptional regulators that it is directly activated by the expression of the OSKM factors. This network assembles and operates stochastically in a small subset of reprogrammable cells early in the process. Thus, our data support a combination of stochastic and deterministic models to explain cellular reprogramming.

POSTER ABSTRACTS

1 INTERACTION OF NORMAL ENDOTHELIAL CELLS DERIVED FROM MESENCHYMAL STEM CELLS WITH TUMOR VASCULAR NETWORK IS ASSOCIATED WITH DOWN REGULATION OF VEGFR2 IN MOUSE BREAST TUMOR

Maryam Adelipour[a], Abdolamir Allameh, Zuhair M.Hassan, Masoud Soleimani, Seyed Mohammad Tavangar, Shahnaz Esmaeli

a Department of Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran. E-mail: m.adelipour@modares.ac.ir

A large body of research has shown the beneficial effects of stem cell therapy in tissue vascularization after ischemic injury. However, the interaction of endothelial cells derived from mesenchymal stem cells (MSCs) with capillary network within the tumor is not well understood. There are controversies over the endothelial cells therapy of tumor because the endothelial cells injected may contribute to tumor associated angiogenesis. However there are no reports showing that normal endothelial cells may inhibit abnormal vascularization in tumors. In present study, normal endothelial cells at early stage of differentiation from MSCs were injected intra tumor (I.T) into breast tumors in a mouse model. For this purpose, MSCs isolated from bone marrow of Balb/c mice were induced to endothelial cells in presence of VEGF (Vascular Endothelial Growth Factor) for 5 days and confirmed by expression of endothelial surface markers such as VEGFR2, CD34 and VCAM1. Besides, a syngeneic mouse model of breast cancer was developed and treated with either MSCs or endothelial cells at early stage of differentiation by I.T route. One month after cell therapy the expression of VEGFR2 gene in tumor tissues was measured by real time PCR. Mice treated with either undifferentiated MSCs or endothelial cells showed that tumor growth was significantly inhibited compared to untreated mice (42% and 59% inhibition respectively, $p<0.05$). VEGFR2 gene expression in tumor tissues of mice treated with endothelial cells at early stage of differentiation was markedly down regulated (3.6 fold, $p<0.05$), however VEGFR2 gene expression in mice treated with MSCs was within the range of untreated group. In conclusion, It was demonstrated that tumor inhibition in breast was associated with down regulation of VEGFR2 in animals received the endothelial cells. These data may suggest that differentiated endothelial cells compared to undifferentiated MSCs are more efficient in targeting abnormal vascularization in tumors.

2 NU-AGE: NEW DIETARY STRATEGIES ADDRESSING THE SPECIFIC NEEDS OF ELDERLY POPULATION - EVALUATION OF INFLAMMATORY AND IMMUNOLOGICAL STATUS, FUNCTION AND REGULATION

Sophia Athanasopoulou[1], Konstantina Asimaki[1], Vasiliki Delitsikou[1], Konstantinos Voutetakis[1], Niki Chondrogianni[1], Efstathios S. Gonos[1,2]

[1] *Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, 48 Vassileos Constantinou Ave., Athens 11635, Greece*

[2] *Örebro University, Medical School, Örebro 701 82, Sweden*

Cellular aging is characterised by the accumulation of oxidized proteins, either due to increased protein damage or decreased removal of oxidized proteins, as well as by the establishment of a chronic inflammatory environment, known as inflammaging. This process is directly associated with functional and structural modifications of a key cellular component, the proteasome. In this study, levels of oxidised proteins, along with proteasome composition and activity on a selected subgroup of 120 volunteers from Italy and Poland were analysed before and after the implication of specific dietary protocols. In order to elicit subjects' immunological status from another perspective, a modified proteasome complex, so called the immunoproteasome, was also detected and quantified.

In general terms, our findings have confirmed a significant negative correlation between levels of oxidised/carbonylated proteins and proteasomal function both before and after intervention. Furthermore, regarding specifically the proteasome content and activity, it has been proven that subgroups of non-frail subjects and women seem to receive a greater benefit after the intervention. Higher levels of proteasome content and activity were also found in subjects with specific dietary and sleep habits, combined with generally positive attitude and sensibly drink alcoholic beverages within the frames of a healthy diet. Lower levels were found in subjects with higher Body Mass Index (BMI). As for immunoproteasome, high levels of β1 constitutive subunit (β1i) were observed in subjects characterised as pre frail, as well as in obese subjects (BMI (Kg/ m^2) > 30) before the dietary intervention.

Regarding the pharmacological profile of the 120 volunteers, higher levels of oxidised/ carbonylated proteins were found in subjects of both Italian and Polish subgroups under medication for high cholesterol before intervention. Lower levels of oxidised/carbonylated proteins were detected after the intervention in subjects of the Polish subgroup who take aspirin as cardiovascular prevention. Lower levels of β1i before the intervention were observed in subjects of the Italian subgroup that use prescribed medicine regularly.

3 PROFILING OF GENE EXPRESSION OF BLOOD PLASMA USING DNA MICROARRAY ANALYSIS FOR BREAST CANCER BIOMARKERS DISCOVERY

Bakakina Y.S., Pozniak T.A., Dubovskaya L.V., Volotovski I.D.

Institute of Biophysics and Cell Engineering of National Academy of Sciences of Belarus, Minsk, Belarus

Breast cancer remains a major health concern of women in the world. Advances in early diagnosis have resulted in an effective disease screening, monitoring and significantly reduced breast cancer related mortality. Microarray expression analysis has become one of the most widely used functional genomics tools. DNA microarray analysis of blood plasma of women with breast cancer was performed. Microarray analysis was performed using commercial Breast Cancer Pathways™ Focused Human Genome Microarrays (Arrayit, USA) that contain oligonucleotide sequences for 89 genes involved in the human breast cancer pathway. To find unique biomarkers for early diagnosis of breast cancer we studied gene expression in blood plasma from women with triple negative breast cancer, fibroadenoma and healthy donors as a control. It was found that the relative expression levels of 31 genes were higher in blood plasma of women with triple negative breast cancer as compared to patients with fibroadenoma and donors. These genes are known to be potential markers of malignant breast tumors. We also identified some genes that encode Jun-oncogene and cyclin A1, D1 and E1 with relative expression levels being higher in blood plasma of women with fibroadenoma as compared to donors. These genes are suggested to be potential markers of predisposition to the development of non-malignant breast tumors in women of Belarusian population. We suppose that obtained results give the reason for breast cancer diagnosis and choice of the therapy.

This research is supported by the Belarusian Republican Foundation for Fundamental Research (grant M15CO-025).

4 NUCLEIC ACID OXIDATION RELATION TO IRON STATUS IN A GENERAL POPULATION

Vanja Cejvanovic, Christina Ellervik, Laura K Kjaer, Allan Weimann, Henrik E. Poulsen

Laboratory of Clinical Pharmacology, Rigshospitalet, Department of Clinical Pharmacology, Bispebjerg Hospital, Faculty of Health Sciences, University of Copenhagen
vanja.cejvanovic@regionh.dk

Background: Oxidation of nucleic acids has been linked to development of major human diseases. DNA oxidation can be measured as urinary excretion of 8-oxo-2´-deoxyGuanosine (8-oxodG) and RNA oxidation as 8-oxo-2´-guanosine (8-oxoGuo). In epidemiological studies high excretion of 8-oxodG is linked to development of lung and breast cancer, and high urinary excretion of 8-oxoGuo is linked to poor survival in type 2 diabetes patients. It has been shown that 8-oxoGuo is increased in patients with the iron accumulating disease hereditary hemochromatosis, and it has also been shown been shown that individuals in general populations with iron overload have increased risk of mortality. Iron is a metal with redox properties and can via the Fenton reaction produce reactive oxygen species (ROS). ROS can via secondary reactions cause oxidative damage to cellular macromolecules such as DNA and/or RNA.
Methods: We investigated a mixed suburban cohort population sample of 3567 individuals (males=1456) for urinary excretion of 8-oxodG and 8-oxoGuo. Iron levels were measured as ferritin, transferrin saturation and as the genotypes for hemochromatosis.
Results: We found a significant increased urinary excretion of 8-oxodG and 8-oxoGuo with increasing levels of ferritin. Only 8-oxoGuo was associated with transferrin saturation and hemochromatosis genotype.
Conclusions: We conclude that body iron stores are a determinant of oxidation of nucleic acids, in particular oxidation of RNA.

5 CHARACTERIZATION OF SKAP/KINASTRIN ISOFORMS

Anita Cindric Vranesic, Andreas Wohlmann, Jens Bratsch, Karlheinz Friedrich, Cagatay Günes and Otmar Huber

Institute of Biochemistry II, University Hospital Jena, Germany; anita.cindric@med.uni-jena.de

SKAP (Small Kinetochore-Associated Protein/Kinastrin) is an essential component of vertebrate mitotic spindle and kinetochores. It is required for proper and timely alignment and segregation of sister chromatids, as well as for maintenance of spindle pole architecture. Depletion of SKAP causes severe chromosome segregation defects, which probably represent an important pathway to generate aneuploidy during tumorigenesis. Besides its mainly studied role in regulation of mitosis, SKAP was recently associated with promotion of apoptosis, directional cell migration and is speculated to be an oncogene. Even though SKAP is obviously an important multifunctional protein, mechanisms underlying its roles in different processes are not yet fully understood. SKAP is predicted to have different isoforms, however, studies reported in the literature did not differentiate between them. As distinct molecular architectures of isoforms often affect their localization and functions, in order to better understand the roles of SKAP, we studied the expression profile and functional differences between SKAP isoforms in human. With this aim, we generated new monoclonal antibodies against SKAP, which enabled us to differentiate between the predicted protein isoforms. We analyzed human cell lines of different origin, as well as different human tissues and found for the first time that SKAP in human is expressed in two isoforms: ubiquitously present SKAP16 and testis/sperm-restricted SKAP1. This finding is critical for all the forthcoming studies, since most studies of SKAP in somatic cells were performed with the isoforms we could not detect in any of the human cells we analyzed. Additionally, we describe a novel interaction between SKAP1 and Pontin, a member of AAA+ ATPases (ATPases Associated with diverse cellular Actvities). The specific interaction of SKAP1 and Pontin in sperm cells suggests a sperm-specialized function. We reason SKAP1-Pontin interaction might be essential for normal human male fertility.

6 ALTERATIONS IN PERIPHERAL BLOOD MONONUCLEAR CELLS PHOSPHOLIPID COMPOSITION IN BREAST CANCER

Hasmik H.Davtyan, G.V.Hakobyan, K.A. Alexanyan[1], M.P.Lazyan, Yu.V.Tadevosyan

Institute of Molecular Biology, NAS RA, Yerevan, Armenia

[1] National Center of Oncology after V.Fanarjyan, MH RA, Yerevan, Armenia

The involvement of cell plasma membrane (PM) lipids in the regulatory mechanisms of various important membrane-associated processes at norm and malignancy is well documented. It is known that compared to normal individuals, patients with several types of cancer have an increased prevalence of regulatory T cells in the peripheral blood. Recent studies suggest also a potential impact of changes in different subpopulation of immune cells on solid tumors and hematological malignancies resulted in the alteration of balance between pro-and anti-tumor immunity in the peripheral blood. It was hypothesized by us earlier that alterations in lipid homeostasis of peripheral blood crude mononuclear cells (MNC) in human tumors may possibly represent information useful for early detection and evaluation of diverse cancers.

The aim of this study was to investigate the quantitative changes in the phospholipids (PL) content of MNC PMs in breast cancer (BC) compared to norm. Data obtained indicate that eight different PL fractions, in the PM of blood MNC, were reliably altered in all BC patients compared to healthy individuals. Particularly, it was shown significant increase in the content of lysophosphatidylcholine fraction in BC compared to norm. Importantly, regular and distinctly individual for each patient disturbance in the contents of different PL fractions reveled in BC were identical with those observed earlier in leukemia and some other forms of solid tumor.

We conclude that pathological alterations in PLs content of crude MNC PMs have been similarly involved in the onset and evolution of diverse forms of cancer and can be used for early detection and definition of cancer as well as for discovery of new personalized treatment modes.

7 SERUM METAL CONCENTRATIONS AND PROTEOMICS OF ALS PATIENTS ORIGINATING FROM A SMALL GEOGRAPHICAL AREA

Stefano De Benedetti, Giorgio Lucchini, Alessandro Marocchi, Silvana Penco, Christian Lunetta, Stefania Iametti, Elisabetta Gianazza, Francesco Bonomi

Department of Food, Environmental and Nutritional Sciences – University of Milan - Italy
e-mail: stefano.debenedetti@unimi.it,

Amyotrophic Lateral Sclerosis (ALS) is a rare neurodegenerative disorder characterized by selective degeneration of both upper and lower motor neurons in the brain, brainstem, and spinal cord. This results in paralysis due to muscle weakness and atrophy, leading to death in 3-5 years[1]. Genetic and environmental factors are involved in the pathogenesis of the disease and metals metabolism have been linked to ALS[2].
This study enrolled seven patients and five controls (age matched, living in the same geographical area). For metal quantitation, samples of serumwere analyzed by ICP-MS. For proteomic analyses, immobilized pH gradient covered the 4-10and 3-7pH range.Statistical analyses were carried out with Student's t-test and Artificial Neural Networks.
Among the 20 metals analyzed, As concentration resulted significantly lower in patients than in controls ($p=0.007$); Hg too was found in lower concentration in patients, but with a lower statistical significance ($p=0.13$). Higher concentration of Al in patients was detected ($p=0.08$). In this study, we were not able to confirm the higher concentrations of Ni and Pb in patients previously described in a smaller cohort[3]. Our proteomics data show that APOA2 is decreased by 30% in patients with respect to controls. Furthermore, AHSG, and SAP showed a significant decrease in patients with a story of more than 10 years of disease.
Impaired metal homeostasis, attributable to environmental exposure, could lead to mineral overload. Besides promoting oxidative stress, metals can compete for the binding sites of metal-containingproteins, such as those containing iron-sulfur clusters[3]. Currently, there are no literature data linking APOA2 to ALS, but the fact that its mRNA is processed by TDP43, provides a possible connection with the disease. The proteins differentially expressed belong to the group of Acute Phase Reaction proteins, suggesting a possible link between ALS and a chronic inflammation status.

References.

1Chiò et al. (2013) Global epidemiology of amyotrophiclateral sclerosis: a systematic review of the published literature. *Neuroepidemiology*
2Hadzhieva et al. (2014) Review: iron metabolism and the role of iron in neurodegenerative disorders. *Neuropathol Appl Neurobiol*
3De Benedetti et al. (2016) Serum metal evaluation in a small cohort of Amyotrophic Lateral Sclerosis patients reveals high levels of thiophylic species. *Peptidomics*

8 MECHANISM OF THE CROCETIN-INDUCED APOPTOSIS IN THE PRIMARY EPITHELIAL BREAST CANCER CELLS ISOLATED FROM IRANIAN BREAST TUMORS

Nassim Faridi[1], Seyedeh Zhara Bathaie[1], Saeed Abroun[2], Maryam Kadivar[3]

[1]Department of Clinical Biochemistry, Tarbiat Modares University, Tehran, Iran.
Email: Nassim_Faridi@yahoo.com
[1]Department of Clinical Biochemistry, Tarbiat Modares University, Tehran, Iran
[2]Department of Hematology, Tarbiat Modares University, Tehran, Iran,
[3]Iran University of Medical Sciences, Tehran, Iran.

Saffron as an important natural product was used to prevent and treat various diseases, especially cancer, and might be a good candidate for the development of anticancer drugs. Saffron carotenoids and monoterpene aldehydes are two potent ingredients of the saffron stigma. Breast cancer is the first leading cause of cancer death among Iranian women. Our previous studies showed that saffron carotenoids, crocin and crocetin induce apoptosis and cell cycle arrest in animal model of breast cancer, as well as the breast cancer cell lines. Apoptosis occurs through two main pathways; extrinsic (cytoplasmic) and intrinsic (mitochondrial) pathways. The intrinsic pathway ariseswhen stimulated leads to the release of cytochrome C from the mitochondria, activation of caspase-9 and induction of the death signal. PI3K is up-regulated in many cancers. Akt kinase activation leads to phosphorylation of caspase-9 that blocks the induction of apoptosis. In this study, the normal and cancerous human epithelial cells were isolated from the mammoplasty and human breast tumors, respectively; and characterized by flow cytometry using ESA, CD44, CD24 and CD49f antibodies. Then, they were exposed to different concentrations of crocetin at different time intervals. The IC50 of crocetin at 48 h was obtained 1 mg/ml, as determined by MTT assay. Flow cytometric analysis using annexin/PI and increasing the expression of caspase-9 (determined by western blot) indicated that crocetin induces apoptosis in these cancerous epithelial cells, while it was safe for normal cells, even at higher concentrations. These results are compatible with our previous data obtained on therapeutic effect of crocetin on NMU-induces breast cancer in rat. Other mechanisms involved in this process are investigating. In conclusion, crocetin could be used as a chemopreventive, as well as a chemotherapeutic agent for breast cancer in human.

9 NOVEL CANDIDATE GENES IN PRIMARY CILIARY DYSKINESIA

Mahmoud Fassad[1, 2], Jane Hayward[1], Mitali Patel[1], Christopher Boustred[3], UK National PCD Service, Lucy Jenkins[3], Anne- Marie Tassin[4], Thomas Cullup[3], Hannah M. Mitchison[1]

[1]Genetics and Genomic Medicine, UCL Institute of Child Health, London, UK. [2]Human Genetics Department, Medical Research Institute, Alexandria University, Egypt. [3]NE Thames Regional Genetics Service, Great Ormond Street Hospital for Children, London, UK.[4]Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Univ. Paris Sud, Université Paris-Saclay,

Primary ciliary dyskinesia (PCD) is a debilitating autosomal recessive inherited condition caused by structural defects and dysmotility of cilia. The estimated prevalence is about one per 10 000 births, but it is more prevalent in populations where consanguinity is common.

Genetic testing has great potential to enhance early diagnosis and improve patient lives, especially in difficult cases without laterality defects or clear-cut ciliary defects. Accurate genetic diagnosis also allows appropriate genetic counselling in affected families. Disease-causing mutations have been identified in over 30 genes; however, it is thought that these genes still only account for up to 65% of cases, with many additional genes still to be identified.

We are trying to identify novel causative genes through next-generation sequencing, currently using an Agilent SureSelect-design 651-gene motile Ciliome research panel. We collate putative disease-causing sequence variants in affected individuals using standard QC and variant calling pipeline based on a recessive disease model.

This study analyzed 90 unrelated patients with a diagnosis of PCD confirmed through the UK National PCD Service. Biallelic disease-causing mutations in novel candidate PCD genes were identified in 16 patients (18%). All mutations were confirmed by Sanger sequencing and segregation analysis was done whenever the family members were available.

We have begun functional studies to elucidate the role of the novel candidate genes in ciliated cells, including immunofluorescence studies in patient respiratory cilia. We have identified the orthologs of our genes of interest in *Paramecium*, a unicellular multiciliated organism that represents a new model of PCD, being easy to cultivate allowing molecular and biochemical analyses of the role of candidate proteins in cilia motility functions. Here, I present the results of RNAi knockdown experiments to study the effect of PCD candidate gene silencing on cilia formation and beating after depletion of the endogenous orthologs in *Paramecium*.

10 COMBINATIONAL TREATMENT OF NATURAL EXTRACTS WITH ADRIAMYCIN IN PC-3 PROSTATE CANCER CELLS

Katerina Gioti, Georgios Papaefstathiou, Nektarios Aligiannis, Alexios-Leandros Skaltsounis, Roxane Tenta

*Harokopio University, Athens, Greece, Department of Dietetics and Nutritional Science, Kallithea, kategio@hua.gr*PC-3 is an androgen-independent human prostate cancer cell line, widely used for the study of prostate cancer. Two natural extracts were administrated alone or in combination with Adriamycin in PC-3 cells. Silymarin extract was isolated from the plant *Silybum marianum (Asteraceae)* whereas glycyrrhiza extract was isolated from the plant *Glycyrrhiza glabra (Fabaceae)*. The dominant substances of silymarin extract are silibinin and silicristine. Adriamycin is a widely used chemotherapeutical drug, yet with severe cardiotoxic side effects. The goal of the present study was to evaluate the cytotoxic effect of the natural extracts silymarin and glycyrrhiza when they are administrated alone or in combination with Adriamycin in PC-3 cells, to identify their bioactive substance, and also to determine the implicated mechanism of action. PC-3 cells were treated with silymarin (6-200 μg/mL) or glycyrrhiza (6-200 μg/mL) extracts alone, with silibinin (1-120 μg/mL) or silicristine (1-120 μg/mL) alone and in combination with Adriamycin (ADR) and the inhibition of cell proliferation was evaluated by the MTT assay. In order to identify the implicated mechanism of action, cell cycle alterations and apoptosis rate were measured through flow cytometry. In parallel, expression levels of autophagy related proteins were measured with specific ELISA kits, western blotting and Real Time PCR. Both plant extracts tested alone, inhibited PC-3 cell proliferation in a dose-dependent manner, however only the co-treatment of ADR-silymarin extract significantly enhanced the cytotoxic effect of ADR. Cell cycle alterations were observed in addition to changed expression levels of autophagy related proteins. In conclusion, the combinational treatment of ADR-silymarin extract significantly enhances the cytotoxic effect of ADR in PC-3 cells, which is attributed to the bioactive substance silibinin while the mechanism of autophagy seems to be implicated. The results of the present study are very promising, regarding the application of silymarin extract in prostate cancer therapy.

11 PROPHYLACTIC INFLUENCE OF NANOCRYSTALLINE CERIUM DIOXIDE ON THE GASTRIC MUCOSA INJURIES

Golyshkin Dmytro, Virchenko Oleksandr, Falalyeyeva Tetyana, Beregova Tetyana, Spivak Mykola

ESC "Institute of biology", Taras Shevchenko National University of Kyiv
dgolyshkin@gmail.com

According to the world statistics, ulcer disease is diagnosed in 10% of Europeans. However, disease relapses are observed in 60% patients after conservative therapy. Innovations in nanoscience and nanomedicine lead to the construction of new materials. They are applicable in molecular diagnostics, nanodiagnostics and improvements in the discovery, design and delivery of drugs, including nanopharmaceuticals. The aim of this study was to investigate nanocrystalline cerium dioxide ($nCeO_2$) influence on gastric mucosa (GM) injuries of rats induced by Selye's restraint stress.

24 male albino nonlinear rats were enrolled into this study (weigh: 150-200 g). We established that $nCeO_2$ prophylactic administration (*per os*) significantly reduced by 58.3% ($p<0.05$) lesion areas induced by Selye's restraint stress. The development of erosive and ulcerative lesions in the GM was accompanied with elevated degradation of collagen fibers (by free hydroxyproline level) and mucous epithelial barrier protective proteins (by free fucose and hexuronic acids level). Prophylactic introduction of $nCeO_2$ decreased free hydroxyproline by 16 % ($p<0.05$), free fucose by 14 % ($p<0.05$) and hexuronic acid by 28.7% ($p<0.05$)) compared with the stress-control group. The attenuation of inflammation and decrease of lipid peroxidation in the conditions of gastric injuries and prophylactic administration of $nCeO_2$ were detected. That was confirmed by the decrease of pro-inflammatory cytokines (interleukin 1β, 12B p40) and increase of anti-inflammatory cytokines (interleukin 10 and transforming growth factor β).Measurement of lipid peroxidation products proved the antioxidant properties of $nCeO_2$. Prophylactic administration of nanocompounds decreased the level of conjugated dienes and thiobarbituric acid active products in the conditions of gastric ulceration induced by stress.

In this study we had shown protective properties of $nCeO_2$ on GM injuries under stressful conditions.

12 Curcumin improves high glucose induced lipogenesis via autophagy induction in HepG2 cells

Sattar Gorgani-Firuzjaee [1,2] **and Reza Meshkani** [1]

1- *Department of Biochemistry, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, I.R Iran*
2- *Department of Medical Laboratory Sciences, School of Allied Health Medicine, AJA University of Medical Sciences, Tehran, Iran*
Presenting author email: Gorgani59@gmail.com

Hepatic de-novo lipogenesis is the trigger of non-alcoholic fatty liver disease (NAFLD) progression. The beneficial effect of herbal remedies such as curcumin in managment of fatty liver and NAFLD was reported but the molecular mechanisms are not be fully understood. In this study we investigated the role of autophagy in lipid-lowering effect of curcumin in HepG2 cells.. The results showed that curcumin significantly reduces high glucose-induced total lipid content, intra and extra cellular triglyceride levels. Our data demonstrated that curcumin enhances p-AMPK, p-FOXO1, p-ACC levels and reduces SREBP1c and FAS expression but did not significantly affect MTP mRNA expression and apoB level. High glucose reduced autophagic key protein expression (ATG7, ATG5 and LC3II) and co-treatment of high glucose with curcumin reversed these effects. To confirm the effect of curcumin on autopahgy pathway, co-treatment of choloroquine (inhibitor of autophgy pathway) with high glucose led to a reduction in autophagy signaling pathway. Taken together, these findings suggest that curcumin could attenuate high glucose-induced lipogensis by a mechanism involving the activation of autophagy pathway.

Key words: Curcumin, Fatty liver, NAFLD and high glucose

13 Fibrinogen-erythrocyte binding as prognosis biomarker in heart failure patients

Ana Filipa Guedes[1], Filomena A. Carvalho[1], Inês Malho[1], Nuno Lousada[2], Luís Sargento[2], Nuno C. Santos[1]

[1]Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal; [2]Heart Failure Unit, Cardiology Department, Hospital Pulido Valente, Centro Hospitalar Lisboa Norte, Lisbon, Portugal.

Introduction and Aims: High fibrinogen levels are a relevant cardiovascular risk factor, but the biological mechanisms associated with pathologic alterations are not totally clear. Fibrinogen-erythrocyte binding in chronic heart failure (CHF) patients and its prognostic value were evaluated.
Methods: 15 Ischemic and 15 non-ischemic CHF patients, as well as 15 healthy donors were enrolled in the study. Fibrinogen-erythrocyte interactions were evaluated, at the single-molecule level, by atomic force microscopy (AFM)-based force spectroscopy. These measurements were performed in buffer, with the protein covalently attached to the AFM tip, and the erythrocytes on a poly-L-lysine coated-glass slide. Clinical outcome was assessed during a 12-months follow-up.
Results: As presented in Figure 1A-B, force spectroscopy data showed that CHF patients presented stronger specific fibrinogen-erythrocyte binding forces than the control group (60.6 ± 6.6 pN *vs.* 40.4 ± 3.0 pN, p=0.038), despite a lower binding frequency (13.0 ± 2.38 pN *vs.* 27.6 ± 4.2 pN; p=0.003). According to etiology, ischemic patients had higher binding forces than donors (74.9 ± 10.7 pN *vs.* 40.4 ± 3.0 pN; p=0.004) and lower binding frequency (11.7 ± 2.1 % *vs.* 27.3 ± 4.2 %, p=0.002). Ischemic patients presented increased fibrinogen-erythrocyte binding forces relative to non-ischemic (74.9 ± 10.7 pN *vs.* 45.4 ± 5.6 pN; p=0.021). Non-ischemic patients also had a lower binding frequency than donors (14.3 ± 4.3 % *vs.* 27.3 ± 4.2 %, p=0.040). Their cell stiffness is also altered. These variations were only statistically significant for the median, in which we observed an increased cell stiffness (or decreased elasticity) on both groups of CHF patients, with the larger variation being observed for the non-ischemic patients (390 Pa for control group *vs.* 743 Pa for non-ischemic *vs.* 568 Pa for ischemic patients). Follow-up data demonstrated that patients presenting higher fibrinogen-erythrocyte binding forces at the beginning of the study had a higher probability of being hospitalized due to cardiovascular complications on the subsequent year (Figure 1C).
Conclusions: As fibrinogen-erythrocyte interactions, evaluated by AFM, are modified in CHF patients and associated with short-term clinical outcome, here we demonstrate the power of this nanotechnology-based evaluation as potential biomarker for cardiovascular risk and patients' clinical prognosis evaluation [Guedes *et al.* (2016) *Nature Nanotech.*, in press].

Keywords: erythrocyte; fibrinogen; heart failure; atomic force microscopy; clinical prognosis.

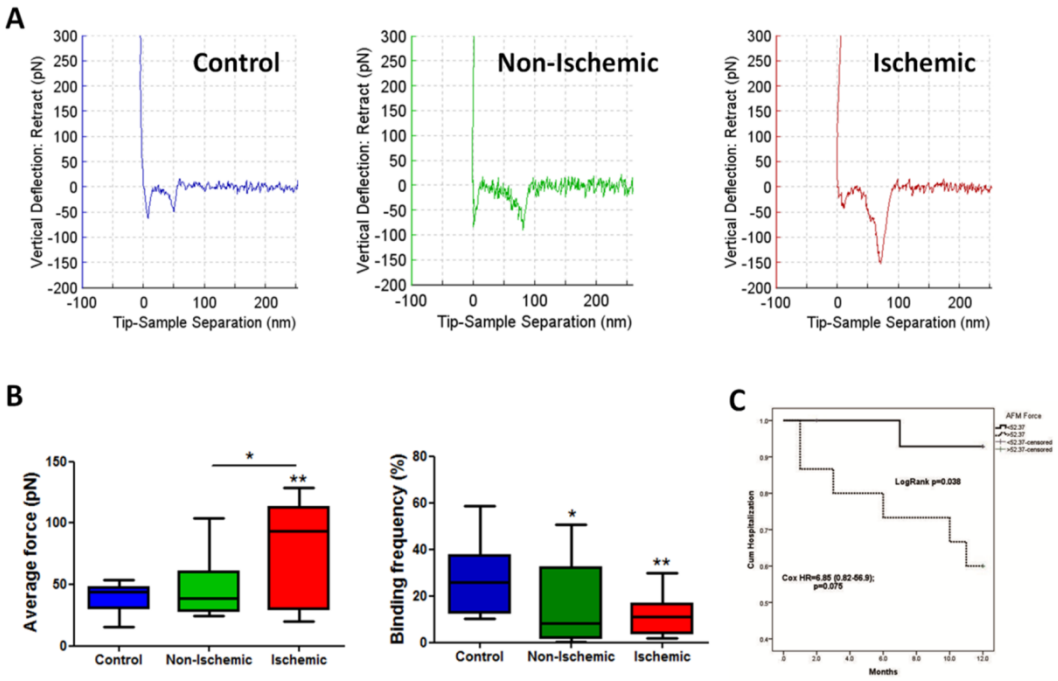

Figure 1 – **A.** Typical force-distance curves obtained for the interaction of fibrinogen with erythrocytes from the control healthy donors (blue curve), non-ischemic CHF patients (green) and ischemic CHF patients (red). **B.** Average (un)binding forces and binding frequencies for patients divided according to etiology *vs.* control subjects. **C.** Fibrinogen-erythrocyte (un)binding forces above the median (52.37 pN) were associated with the hospitalization curve and tendency to higher risk of hospitalization.

14 NEW HISTONE DEACETYLASE INHIBITORS AS SENSITIZERS OF TUMOR CELLS TO CHEMOTHERAPY

Hernández, P.1; De Negri, M.1,2; Lavaggi, M.L.2; Martínez, W.1

1Laboratorio de Epigenética e InestabilidadGenómica, Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay; 2Grupo de Química Medicinal, Facultad de Ciencias, Montevideo, Uruguay.

Regarded as chemosensitizers, the addition of well-tolerated doses of histone deacetylase inhibitors (iHDACs) to platinum-based chemotherapeuticdrugshave shown synergistic improvement in cytotoxicity to many cancer types and stages.
In this sense, we developed phenazine 5,10-dioxide derivatives designed as hybrid compounds that combine a bio-reductive group and a possible HIF-1 (*hypoxia inducible factor)* or HDAC inhibitor moiety. The HIF-1 activity is regulated by HDAC7 acetylation and its inhibition results in a decrease of gene transcription.
In the present study, we developed new compounds using conventional synthesis methods. We first determined the citotoxicity *in vitro*in normal and in tumor cells. The HDAC inhibitory activity of the synthesized compounds was then assayed at a subtoxicdose in osteosarcoma and bladder tumor cells by western blot. This study demonstrated that some molecules had a good HDAC inhibitory activity in tumoral cell lines. These molecules were able to almost duplicate the acetylation level of histone H4 respect to the negative control.The chromosomal aberrationsand micronucleus test indicated that at least two compounds have sensitizer activity to cisplatin.
At the present we are currently evaluating the level of VEGF expression in cells treated with these active compounds under normoxia or hypoxia conditions, to determine its potential use as a hypoxic tumor cell sensitizer to other chemotherapeutic agents.
In conclusion, through this work we identified new lead compounds sensitizers to chemotherapy that will allow usthe re-design of more activeiHDACsmolecules.

15 RECOGNITION OF DOMINANT BIOENERGETIC PROCESSES BY COMPARING SUBSTRATE UTILIZATION AND ITS REGULATORS IN TUMOUR CELLS

Zoltán Hujber[1,2], András Jeney[1], Gábor Petővári[1], Noémi Nagy[1], Norbert Szoboszlai[2], Júlia Oláh[1], Ágnes Márk[1], Titanilla Dankó[1], Anna Sebestyén[1,3]

[1]1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest
[2] Laboratory of Environmental Chemistry and Bioanalytics, Department of Analytical Chemistry, Institute of Chemistry, Eötvös Loránd University, Budapest
[3] Tumour Progression Research Group of Joint Research Organization of Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary

zoltan.hujber56@gmail.com

The alterations in cellular metabolism are considered as a core hallmark of cancers, however, to recognize its variable forms, moreover to understand their mechanisms appropriate techniques are required. The purpose of this study was to test the hypothesis whether bioenergetic signature may be estimated by comparing the utilization of glucose and acetate by human tumour cells. To define the dominant bioenergetic pathway different metabolic tests were used. CO_2 production from [1-^{14}C]-glucose and [1-^{14}C]-acetate and the effect of glucose and acetate on adenylate energy charge, moreover [U-^{13}C]-glucose or [2-^{13}C]-acetate labelled glycolytic and TCA cycle metabolites were analysed. These tests provided data to indicate the higher glycolytic phenotype in HT-1080 relative to ZR-75.1 tumour cells. Using [2-^{13}C]-acetate tracer the low number of ^{13}C atoms incorporated into citrate during the labelling period suggested the impairment of TCA cycle of HT-1080 tumour cells. Regulatory factors which may maintain the high glycolytic phenotype of HT-1080 tumour cells and mRNA expressions of some metabolic enzymes furthermore protein levels of mTOR complexes and their targets examined. We found that the dominant glycolytic phenotype involving impaired TCA cycle were associated by high expression of IL-1, IL-6, HO-1 and mTORC1 activity with negligible protein level of mTORC2 in HT-1080 cells.
The applied methods of energy substrate utilisation and other measurements represent simple assay system using ^{13}C-acetate and glucose to recognize dominant bioenergetic pathways in tumour cells.
In summary we demonstrated these assays are suitable to define the bioenergetic signature with the help of using acetate and glucose utilization in cancer cell lines. The studied experimental setting provided further evidence for the relationship between altered phenotype and impaired TCA cycle.

Supported by OTKA K84262 and Bolyai Grant Foundation of Hungarian Academy of Sciences

16 SEARCH OF POTENTIALLY ANTICANCER PROTEIN TYROSINE KINASE LIGANDS

V.V. Hurmach[1], A.A. Varnek[2], O. M. Platonov[3], Yu.I. Prylutskyy[1]

[1]*Taras Shevchenko National University of Kyiv, Volodymyrska Str., 64, 01601 Kyiv, Ukraine*
[2]*University of Strasbourg, 1, rue B. Pascal, Strasbourg, France*
[3]*Institute of Molecular Biology and Genetics, NAS of Ukraine 150, Akademika Zabolotnoho Str., 03680 Kyiv, Ukraine*
gyrmach@gmail.com

SH2 domain is a compact domain involved in intracellular signaling pathways (e.g. MAPK/ERK pathway). They are the largest class of pTyr-binding domains. Their dysfunction is commonly related with different cancer diseases (Basal cell carcinoma, T cell acute lymphoblastic leukemia, B cell lymphoma). The aim of this work is to investigate the properties of SH2 domain binding, and to search new potential active compounds for the whole SH2 domains class, which, in turn, could be used as starting points for drugs development.

219 SH2 domain structures were retrieved from Protein Data Bank and divided to six groups which display high/average level of conservation (group 1 – 91.25%, group 2 – 78%, group 3 – 47%, group 4 – 32%, group 5 – 39%, group 6 – 45%) and not significant Rmsd difference (0.8 Å, 1.16 Å, 1.6 Å, 1.9 Å, 2 Å, 1.4 Å). We found that the binding pocket is more conserved (91.25%, 90%, 65%, 55%, 56%, 51%, respectively) and comprise 20-24 main amino acids: FLVRESETT (pTyr binding part), βB-sheet; KHYKIR (central part), βD-sheet; ITSR and ADGLC (hydrophobic part of pocket), βG-sheet and αB-helix.

Seven representative binding pockets were used for molecular docking of the entire *Enamine Ltd* database (> 1 M compounds). This resulted in selection of 10463 compounds which potentially bind the SH2 domain.

Finally, based on compound with known activity and “SH2 domain – ligand” complexes, eight ligand-based and one structure-based pharmacophore models were build. They involve the following pharmacophoric features: (1) H-bond acceptor (pTyr binding part), (2) aromatic/hydrophobic part, (3) H-bond donor/acceptor, (4) hydrophobic part. Overall performance of these models in virtual screening is rather high: the area under ROC curve (AUC) varies between 75% and 100%. Virtual screening with these models of the molecules selected at the docking stage resulted in 1816 hits representing potentially active compounds.

17 MicroRNA-34 family role in neuroblastoma progression

Inomistova M., Khranovska N., Skachkova O., Klymniuk G., Svergun N., Demydov S.

National Cancer Institute, Kiev, Ukraine
m.inomistova@gmail.com

Background. Neuroblastoma is the most frequent childhood solid tumor. Despite the rare *TP53* mutations, p53 pathway alterations often occur in neuroblastoma. MDM2 is a direct p53antagonist, leading to its suppressor activity decrease. MYCN gene amplification (MNA) is a marker of aggressive neuroblastoma course. MiR-34 family members are the most prevalent p53-induced miRNAs and important mediators of tumor suppression implicated in the regulation of proliferation, apoptosis, epithelial to mesenchymal transition, migration, invasion, and metastasis. The aim of this study was to determine the significance of miR-34a,b,c expression deviations in neuroblastoma progression and association with clinical features of the disease.
Material and methods. We examined miR-34a,b,c expression levels in tumor samples without p53 deletion from 64 neuroblastoma patients (mean age: 39.5 ± 4.8 months, recurrence tumor – 5 % metastatic foci – 9 %) using real-time PCR TaqMan MicroRNA Assay.
Results. The lowest miR-34a expression levels were detected in samples with unfavorable clinical neuroblastoma characteristics – relapsed, metastatic, advanced stage and with MNA. Strong correlation (R=0,95) was detected between miR-34b and -34c, their reduced expression was associated with deterioration of clinical course at trend ($p=0.06$). We found reliable inverse dependence of miR-34 family expression with MDM2 and GSTP1 expression which are negative regulators of p53($p<0.05$). Downregulation of miR-34 family associates with significant reduction of 3-year overall survival in patients with MDM2 overexpression regardless MNA. In not-MNA neuroblastoma with low MDM2 expression 3-year overall survival was on 44 % lower ($p<0.05$) in downregulated miR-34a cases compared to miR-34a high expressed neuroblastoma which excludes their impact in p53 regulation.
Conclusion. The dependence of miR-34 family and p53 inhibitors expression indicates on indirect regulation links in neuroblastoma. Decrease of miR-34 family may be a useful marker of unfavorable prognosis of neuroblastoma.

18 THE ROLE OF PVR, A RECEPTOR TYROSINE KINASE RELATED TO MAMMALIAN PDGF AND VEGF RECEPTORS, IN *DROSOPHILA* AIRWAY REMODELING

Constantina Ioannou

constantina_ioannou_t@hotmail.com

The *Drosophila* tracheal system is a tubular network of interconnected epithelial tubes responsible for gas exchange. It is an excellent model system to study developmental and physiological processes that govern branching morphogenesis of various mammalian tubular organs such as the lung, the kidney and the vascular system. Importantly, the trachea is extensively remodeled during development, a process that requires the participation of multiple signaling pathways. Nevertheless, the role of many key players controlling tracheal development is currently unknown. The aim of my master thesis is to identify how specific components of the PVR (PDGF/VEGF-receptor related) signaling pathway affect airway remodeling in the *Drosophila melanogaster* larva. The overexpression and down-regulation of key molecules that function in the PVR pathway, using the Gal4-UAS system, in a very dynamic part of the *Drosophila* trachea, the spiracular branch (SB), sheds light into their potential role during remodeling. PVR is a receptor tyrosine kinase related to the mammalian PDGF (Platelet Derived Growth Factor) and VEGF (Vascular Endothelial Growth Factor) receptors. In *Drosophila*, it controls hemocyte development and cell migration in ovaries, but its potential role in tracheal remodeling remains unknown. My results indicate that the PVR signaling pathway is sufficient for SB cell proliferation, since the expression of a constitutive active form of the receptor resulted in increased proliferation rates of SB tracheoblasts. In addition, PVR is necessary for SB remodeling, since PVR inhibition, either via the expression of a dominant negative form of PVR or via RNAi, compromises SB development. Using, RNAi, we show that among the three PVF (PDGF/VEGF related) ligands (PVF1, PVF2 and PVF3) encoded by the *Drosophila* genome, PVF1 is the PVR ligand which acts redundantly with PVF3 to activate PVR in the SB. Because, the PDGF/VEGF cascade is evolutionarily conserved, our work in *Drosophila* will provide insights for its role in development of organisms and might also allow the understanding of human diseases including cancer.

19 THE APPLICATION OF MODELS TO **THE MICRORNAS MEDIATED VIRAL ONCOGENESIS.**

Devaraj.J, AlfredSelleshteen.A and Sujesh.M

Center for Molecular Biology, C-21, Taramani CSIR Road, Chennai – 600 113, India.

Email:dr_cmurugan@yahoo.co.in

MicroRNAs are a recently discovered class of small noncoding functional RNAs. These molecules mediate post-transcriptional regulation of gene expression in a sequence specific manner. MicroRNAs are now known to be key players in a variety of biological processes and have been shown to be deregulated in a number of cancers. The discovery of viral encoded microRNAs, especially from a family of oncogenic viruses, has attracted immense attention towards the possibility of microRNAs as critical modulators of viral oncogenesis. The host-virus crosstalk mediated by microRNAs, messenger RNAs and proteins, is complex and involves the different cellular regulatory layers. In this commentary, we describe models of microRNA mediated viral oncogenesis.

20 CONDITIONAL TRANSGENIC EXPRESSION OF PIM1/PIM2 KINASES IN HORMONE-DEPENDENT TISSUES INDUCES MAMMARY GLAND TUMORS

Manuel P Jiménez-García[1,*], Maja Narlik-Grassow[2], Carmen Blanco-Aparicio[2] and Amancio Carnero[1].

[1]Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío (HUVR), Consejo Superior de Investigaciones Científicas (CSIC), Universidad de Sevilla (US). Avenida Manuel Siurot s/n, 41013, Sevilla, Spain.
[2]Experimental Therapeutics Programme, Spanish National Cancer Centre (CNIO), C/Melchor Fernández Almagro 3, 28029, Madrid, Spain.
**E-mail: mpjimenez-ibis@us.es*

PIM proteins (Proviral Insertion site in Moloney murine leukemia virus), are a highly evolutionarily conserved family of serine/threonine kinases composed of three different isoforms (PIM1, PIM2 and PIM3). These proteins are regulated by transcription and stability through pathways that are controlled by JAK/STAT transcription factors. PIM family proteins have been implicated in regulation of apoptosis, metabolism, cell cycle, homing and migration processes, and they have been found to be overexpressed in hematological malignancies and solid tumors, which it has led to the postulation of these proteins as interesting targets for anticancer drug therapy. Although PIM kinases have been identified as oncogenes in transgenic models, they have weak transforming abilities on their own. However, they have been shown to greatly enhance the capacity of other genes or chemical carcinogens to induce tumors. The effects vary depending on affected tissues and pathways activated. Proto-oncogene PIM1 is a novel estrogen receptor target associated with high grade breast tumors. Furthermore, PIM1 expression levels were elevated in most mammary carcinoma cell lines and it was stated that progesterone both increased PIM1 protein expression to some extent in non-tumorigenic mammary epithelia, and matched its expression pattern with PIM activity in mouse mammary glands. To characterize the proto-oncogenic role of PIM1/PIM2 in mammary gland tissues, we generated two conditional murine models: PIM1 and PIM2, with confined expression in hormone-dependent tissues, due to MMTV promoter which expression is induced by steroid hormones. MMTV-Cre/PIM1 and MMTV/Cre-PIM2 models showed a reduced survival and a higher percentage of tumors at clinical endpoint. Both models generated tumors in sexual organs, and only MMTV-Cre/PIM1 generated mammary gland tumors, which indicate PIM1 have a stronger proto-oncogene activity. As a conclusion, PIM1/PIM2 over-expression induces a neoplastic phenotype in mammary gland and female reproductive system, indicating a role in oncogenic induction in these tissues.

21 RAISING ANTIBODIES AGAINST CIRCULATING TUMOUR CELLS BY RECOMBINANT ANTIBODY PHAGE DISPLAY TECHNOLOGY

Mathias Lindh Jørgensen, Peter Kristensen

Department of Engineering Aarhus University Denmark, mlindh@eng.au.dk

Circulating Tumour Cells (CTCs) may be a source of immense importance to our understanding of the dissemination of cancer from primary tumour to distant organs. Being rare in patient blood, the isolation and characterisation of CTCs can pose a challenge. A challenge that to some extent has been overcome by delicate isolation techniques together with DNA and RNA based technologies. Most CTC capture technologies currently rely on the expression of EpCAM on the surface of CTCs. This expression is, however, not always the case for CTCs partly or fully undergoing the epithelial-to-mesenchymal transition. Accordingly, there is a need for identification of additional markers that enable the capture of different subsets of CTCs. We describe the selection of a tumour cell-specific antibody by applying recombinant antibody phage display technology in combination with micromanipulation. The antibody has been selected against an $EpCAM^{+}/CD45^{-}$ cell identified in peripheral blood from a patient with metastatic colon cancer. This antibody has been tested against peripheral blood mononuclear cells, patient samples, SW480, MCF-7, SK-BR3, a breast cancer cell line of disseminated tumour cells, and two different CTC cell lines of breast and colon cancer, respectively. The presented technology is a possible avenue for the direct identification of CTC surface molecules from a proteomic approach. Furthermore, this work may contribute to the development of CTC specific antibodies readily implemented in the already existing CTC capture platforms.

22 PROTEASOME ACTIVATION ENHANCES STEMNESS AND LIFESPAN OF HUMAN MESENCHYMAL STEM CELLS.

Marianna Kapetanou *[1,2], Niki Chondrogianni [1] and Stathis Gonos [1].

1: National Hellenic Research Foundation, Institute of Biological Research & Biotechnology, 48 Vas. Constantinou Ave., Athens, 116 35, Greece
2: Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Athens, Panepistimiopolis, 15701 Athens, Greece
Email: mkapetanou@eie.gr

Adult stem cells are critical for rejuvenating tissues and persist throughout the lifespan of the organism. However, the age-associated demise of their function contributes to the physiological decline of homeostasis during ageing, leading to tissue degeneration and age-related diseases. The proteasome, being the main cellular proteolytic system, plays a key role in the maintenance of protein homeostasis and its failure is associated with various biological phenomena including cellular senescence and ageing. Even though stem cell biology has attracted intense attention during the recent years, the role of proteasome in stemness and in the age-dependent deterioration of stem cell function remains largely unclear. In order to shed light on this process, we employed both Wharton-jelly and Adipose derived adult mesenchymal stem cells (hMSCs). Our results indicate a significant age-related decline in proteasome content and peptidase activities, accompanied by alterations of proteasomal complexes. This impairment of the proteasome-mediated protein degradation might account for the observed accumulation of oxidatively modified proteins in senescent stem cells. Interestingly, we show that ageing and the concomitant failure of proteostasis negatively impacts on their stemness. Remarkably, insights suggested that the progressive deterioration of proteostasis can be counteracted through proteasomal activation and compensate for the loss of proliferative capacity and stemness of hMSCs. A firm understanding of the mechanisms regulating the proteostasis network in stem cells will pave the way to innovative stem cell-based interventions to improve healthspan and lifespan.

23 C5L2 RECEPTOR, A NEW PLAYER IN ARRHYTHMOGENIC CARDIOMYOPATHY

N. Kappa1*, I. Kostavasili1, A. Varela2, E. Lekka1, C. Tzimas2, I. Kloukina1, D. Sanoudou2, C. Davos2, Y. Capetanaki1, and M. Mavroidis1

1 Basic Research , Biomedical Research Foundation, Academy of Athens, Athens Greece.
2 Clinical, Experimental Surgery, & Translational Research, Biomedical Research Foundation, Academy of Athens, Athens Greece
**Master student, National and Kapodistrian University of Athens e-mail: nikikappa@hotmail.com*

Background/Introduction: Arrhythmogenic cardiomyopathy (ACM), is a progressive and primarily heritable heart disease usually caused by mutations in desmosomal genes. Recently a severe cardiac phenotype with right ventricular predominance and arrhythmias fulfilling the criteria for ACM has been identified in patients with point mutations in the desmin gene. We previously demonstrated (1) that the desmin-null mouse (Des-/-) recapitulates most of the pathognomonic features of human ACM, and inhibition of the complement receptor C5aR could ameliorate disease progression.

Purpose: Following these results, we analyzed the role of the second C5a receptor (C5L2) which has been linked to energy metabolism and inflammation, by generating C5L2-/-Des+/- mice.

Methods: Cardiac histology, echocardiography, and electrocardiography were performed in C5L2-/-Des+/-, Des+/- and WT mice. In order to identify the molecular pathways implicated in C5L2 receptor mediated pathogenesis, we analyzed the global gene expression in the cardiac tissue of the above animal models. Western blot analysis was performed for proapoptotic, antiapoptotic, inflammatory markers, metabolic components and ionic channels. Electron microscopy of cardiac tissue sections from atrium and left-right ventricles was performed.

Results: Our results indicate the progressive development of severe cardiac dysfunction in C5L2-/-Des+/- mice, where 56% decrease of left ventricle fractional shortening (FS) is observed at the age of 12 months, compared to wild-type mice (FS=20.6±1.58, versus 47.15±0.89). The FS of Des+/- mice is 39±0.94, and by histological analysis these animals are similar to wild-type ones. Interstitial and perivascular fibrosis is observed in the ventricles of C5L2-/-Des+/- mice with dilatation of both ventricles. In addition, enlargement and extended fibrosis is detected in the atria of these mice, which are reminiscent of the histological findings in mouse models with atrial fibrillation. The C5L2-/-Des+/- mice also developed severe arrhythmias (ventricular tachycardia, bidirectional ventricular tachycardia, AV-block). RNAseq analysis of the hearts from the different mice models revealed altered expression of several genes involved in metabolic pathways (fatty acid beta-oxidation, glycolytic pathway), as well as in the ionic equilibrium and mitochondrial structure, function. Moreover, electron microscopy revealed mitochondrial abnormalities in the C5L2-/-Des+/- mice.

Conclusions: Our hypotheses is that the C5L2 receptor exerts a crucial role in the metabolic support of the cardiac tissue which is under stress (Des+/- mice) by regulating lipid metabolism and glucose uptake and it's ablation has detrimental consequences.

Mavroidis M, et al., (2015) Complement system modulation as a target for treatment of arrhythmogenic cardiomyopathy. Basic Res Cardiol. May;110:27.

24 A NOVEL TOPOLOGICAL CENTRALITY MEASURE CAPTURING AUTOIMMUNE DISEASE RELATED PROTEINS IN HUMAN INTERACTOME

Muhammed Erkan Karabekmez and Betul Kirdar

Bogazici University, Department of Chemical Engineering, Istanbul, Turkey
erkan.karabekmez@boun.edu.tr

Topological centrality in protein interaction networks and its biological implications have widely been investigated in the past. In the present study, a novel metric of centrality—weighted sum of loads eigenvector centrality (WSL-EC)—based on graph spectra is defined and its performance in identifying topologically and biologically important nodes is comparatively investigated with common metrics of centrality in a human protein–protein interaction network. The metric can capture nodes from peripherals of the network differently from conventional eigenvector centrality. WSL-EC outperforms other metrics of centrality in detecting biologically central nodes such as pathogen-interacting, cancer, ageing, HIV-1 or disease-related proteins and proteins involved in immune system processes and autoimmune diseases in the human interactome. Out of the top five most central hubs identified by the novel metric the four nodes, *VCAM1, ITGA4, HSPA8* and *HSPA5* are related to stress response and/or the immune system and closely related to the diseases like Mooren's ulcer, vasculitis, breast sarcoma, chronic venous insufficiency, autoimmune inner ear disease, borna disease and wolfram syndrome.

25 HIGH CHOLESTEROL INDUCED CARDIAC FAILURE AND UNFOLDED PROTEIN RESPONSE

Erdi Sözen, Burak Yazgan, Betul Karademir, Nesrin Kartal Ozer

Department of Biochemistry, Faculty of Medicine, Genetic and Metabolic Diseases Research Center (GEMHAM), Marmara University, 34854, Maltepe, Istanbul, TURKEY
betulkarademir@marmara.edu.tr, nkozer@marmara.edu.tr

Most important characteristics of cardiovascular diseases are cardiac hypertrophy and heart failure. Among many other factors, high cholesterol takes an important place for the progress of these diseases results in atherosclerosis. Cholesterol is involved in the disruption of several molecular pathways related to cardiac failure. Endoplasmic reticulum (ER) play crucial role in the multifunctional organel support of cardiomyocytes. Proper synthesis and correct folding of proteins in ER is extremely important for the normal function of heart and ER-associated functions are shown to be key regulators for cardiac physiology and pathology. IRE1, PERK, ATF6 pathways are involved in unfolded protein response (UPR) of cardiac ischemia. When unfolded proteins increased, and proteosomal system impairs, autophagy provides a possible alternate pathway for removing aggregated proteins. Incidentally, the ratio of the protein expression of membrane associated LC3-II to cytosolic LC3-I is often used to assess autophagic activity. Beclin-1 is negatively regulated by its interaction with the anti-apoptotic protein Bcl-2 under normal conditions. However, increased oxidative stress and ER stress activates the ubiquitin-proteosome system, which functions to degrade Bcl-2. This allows for beclin-1 activation subsequently resulting in autophagic cell death.

In this study, the effects of enhanced oxidative stress and ER stress in heart of hypercholesterolemic rabbits have been investigated. The results of molecular components of ER stress, autophagy and apoptosis (such as ATF6, BiP, Grp94, LC3 II/I, beclin-1, Bcl-2, Bax, Aif, Caspase-9 and Caspase-3) indicate that hypercholesterolemia increases unfolded protein response via increasing oxidative stress and ER stress and this leads autophagic cell death and contributes to progression of cardiac failure.

Supported by Marmara University Research Fund SAG-A-130612-0202, SAG-C-DRP-130515-0164 and SAG-C-DRP-130515-0165.

26 NEUROPROTECTIVE IMPACT OF MACROVIPERA LEBETINA OBTUSA VENOM ON HIPPOCAMPAL NEURONS IN THE MODEL OF ALZHEIMER'S DISEASE

Kishmiryan A.V., Ghazaryan N.A., Danielyan M.H., Chavushyan V. A., Ayvazyan N.M.

L.A. Orbeli Institute of Physiology, Orbeli bros. 22, 0028 Yerevan, Armenia

The commotion of cholinergic neurotransmission, the activation of astrocytes and microglia as well as the accumulation of β-amyloid (Aβ) represent the pathological symptoms of Alzheimer's disease (AD). The experimental structure of the research consists of injecting rats with $A\beta_{25–35}$ amyloid intracerebroventricular (ICV) $A\beta_{25–35}$ together with intramuscular injection of MLO venom (5 % solution of LD 50 dose, 50 ml, per animal seven times at intervals of 1 day). The phosphatase activity has extremely dropped in the hippocampus of Aβ -induced rats. The most susceptible neurons have been in the field of the CA1 and CA3. According to results of the study application of small amounts of viper's MLO venom has had constructive alterations in the structural properties of neurons, enhanced metabolism, improved Ca^{2+} -dependent phosphorylation processes, also the density of neurons improved in the CA1 and CA2 fields which controls cell survival. MLO venom increases the rate of TD-PTP responses after high frequency tetanic stimulation of ipsilateral entorhinal cortex. Overall increase in firing rate of hippocampal neurons can contribute to recovery processes after Aβ -induced neurodegeneration in hippocampus. Thus, MLO venom could reduce neuronal cell death and afford neuroprotection to rat brain.

27 URINARY RNA OXIDATION MARKER 8-OXOGUO PREDICTS LONG-TERM MORTALITY IN WELL-ESTABLISHED TYPE 2 DIABETICS

Laura Kofoed Kjaer, Vanja Cejvanovic, Trine Henriksen, Cramer Christensen, Ivan Brandslund, Henrik Enghusen Poulsen

Laura Kofoed Kjær, Q7642, Laboratory of Clinical Pharmacology, Rigshospitalet, Department of Bispebjerg University Hospital, Blegdamsvej 9, 2100 Copenhagen, Denmark
E-mail: laura.kofoed.kjaer@regionh.dk

ABSTRACT

Objective: In type 2 diabetes we have suggested that high urinary excretion of 8-oxo-guanosine (8-oxoGuo), as a measure of global RNA oxidation, is associated with poor survival independent of classical risk factors (Diabetes Care 2011;34:2594). As a proof-of-concept and extrapolation to the established diabetic state we investigated 8-oxoGuo in a new larger cohort of type 2 diabetes patients.

Research design and methods: We obtained a urine sample from a cohort of 2778 type 2 diabetics and followed them for a median of 6.3 years and the association with long-term mortality was assessed by Cox proportional hazards regression.

Results: Cox regression survival analysis showed that the decile with the highest decile of 8-oxoGuo urinary excretion had a 7.84 hazard ratio for death ($P<0.0001$) compared with the decile with the lowest 8-oxoGuo urinary excretion after multivariate adjustment for age, sex, BMI, smoker status, year of diagnosis, insulin treatment, s-HbA1c, s-triglycerides, s-cholesterol, microalbuminuria, proteinuria, retinopathy, neuropathy, AMI and stroke. Like our previous finding, DNA oxidation urinary marker 8-oxodG did not show any prognostic value.

Conclusions: We conclude that it is now established that RNA oxidation marker, 8-oxoGuo, is an independent risk factor for death in type 2 diabetes.

Perspectives: RNA oxidation represents oxidative stress intracellularly, hypothesized to result from mitochondrial dysfunction with increased production of hydrogen peroxide in diabetes due to the diabetic state. We suggest that the intracellular RNA oxidation is compartmentalized from the traditional biomarkers from the extracellular compartment, and therefore provides independent prognostic value. RNA modification can result in formation of truncated and/or modified proteins, thus RNA oxidation could be a novel disease mechanism.

Taken together RNA oxidation may be 1) a valid biomarker in the clinical setting, 2) a novel explanatory mechanism for development of late complications in type 2 diabetes.

28 DISSECTING THE MOLECULAR BASIS AND SIGNALLING PATHWAYS UNDERLYING CELLULAR SENESCENCE AND AEGING

Ruchi Kumari[1], Parmjit Jat[2]

Department of Neurodegenerative Disease, UCL, London, United Kingdom r.kumari@prion.ucl.ac.uk

Cellular senescence is a stable cell cycle arrest that normal cells undergo in response to a variety of intrinsic and extrinsic stimuli. Being implicated in ageing and age related diseases including cancer it is of great importance to elucidate the signalling pathways involved in regulating the senescent state. The p53-p21 and p16-pRB tumour suppressor pathways have clearly been implicated in senescence but the critical downstream targets of these pathways are unclear.

My primary goal is to identify and characterize the transcription factors (TFs) that act downstream of the p16-pRB and p53-p21 pathways to regulate senescence growth arrest. Previous research in the group has compared mitotic and senescent cells by microarray analysis, and identified genes that are differentially expressed upon senescence. TFs regulate gene expression at different stages of embryonic development and are key to the establishment and maintenance of specific cell fates. To identify the TFs that act downstream of the p16-pRB and p53-p21 pathways to regulate senescence growth arrest, we overlaid the list of differentially expressed transcripts with the 1391 manually curated sequence-specific DNA binding factors, identified by Luscombe and colleagues. This list was further refined by examining what happened to their expression, when senescence was bypassed upon inactivation of the p16-pRB and/or p53-p21 pathways.

To determine if silencing expression of up-regulated TFs individually bypasses senescence, lentiviral pGIPZ shRNAmir constructs, corresponding to the up regulated TFs are being tested for their ability to bypass senescence. To determine if ectopic expression of down-regulated TFs individually bypasses senescence we have prepared, full length lentiviral expression constructs for all down-regulated TFs. These are now being systematically tested for their ability to bypass senescence in a conditionally immortal human fibroblast cells as well as other senescence assays.

This will enable us identify key downstream TFs that have a causal role in senescence and may thus serve as molecular targets for therapeutic intervention against cancer.

29 TOWARDS THE STRUCTURE OF A LIPID FLIPPASE USING ELECTRON MICROSCOPY

Milena Laban, Jakob J. Ulstrup, Joseph Lyons, Miriam-Rose Ash, Thomas Boesen, Arne Möller, Guillaume Lenoir, Poul Nissen

The Danish Research Institute of Translational Neuroscience – DANDRITE, Aarhus University, Denmark, Department of Molecular Biology and Genetics, Aarhus University, Denmark
milena@mbg.au.dk

One of the least understood and characterized members of the P-type ATPase family, are the P4 ATPases or “lipid flippases”. These transporters are involved in the flipping of phospholipids across the membrane bilayer, thus maintaining an asymmetry in the distribution of lipids, which is important in for example cell-to-cell signaling and initiation of endocytosis. A central question in the study of P4 ATPases is, how they are able to accommodate a much larger substrate than other P-type ATPases, which usually transport small cations.
Humans have fourteen genes coding for P4 ATPases, of which mutations in ATP8B1 cause cholestasis, while ATP8B4 has been linked to Alzheimer's disease.
The Drs2/Cdc50 complex is situated in the Trans-Golgi Network of *Saccharomyces cerevisiae* where it transports mainly phosphatidylserine (PS). It requires phosphatidylinositol 4-phosphate (PI4P) for relief of autoinhibition from the C-terminal.
With the aim of studying the structure and dynamics of lipid flippases, the Drs2/Cdc50 heterodimer was recombinantly expressed in *S.cerevisiae*, and has successfully been reconstituted into amphipol A8-35 – an artificial environment, where the complex is found to retain its activity.
Initial negative stain Electron Microscopy (EM) studies of amphipol-reconstituded samples of Drs2/Cdc50 with inhibitors as well as PS and PI4P provide a promising start to the structural characterization of P4 ATPases.

30 Dysferlin ablation leads to cardiac function improvement in a cardiomyopathy animal model

Evangelia Lekka[1], Ioanna Kostavasili[1], Aimilia Varela[2], Niki Kappa[1], Constantinos Davos[2], Yassemi Capetanaki[1] and Manolis Mavroidis[1]*

1 Basic Research, Biomedical Research Foundation, Academy of Athens, Athens Greece.
2 Clinical, Experimental Surgery, & Translational Research, Biomedical Research Foundation, Academy of Athens, Athens Greece

Mutations in the muscle specific intermediated filament protein desmin have been identified in patients suffering from dilated cardiomyopathy (DC) and recently from arrhythmogenic cardiomyopathy (AC). Mice null for desmin develop DC and also fulfil the major criteria as an AC mice model. Desmin null mice, also develop mild skeletal myopathy.
On the other hand dysferlinopathies are characterized by the absence of dysferlin in striated muscle and so far, three main phenotypes have been reported: Miyoshi myopathy, limb girdle muscular dystrophy type 2B, and distal myopathy. Patients with dysferlin mutations usually do not have overt cardiac involvement as is also the case in dysferlin null (dysf-/-) mice where the cardiomyocyte injury is sublethal and causes only mild cardiomyopathy even at advanced ages.
Unexpectedly, in desmin-dysferlin double knock out (d.k.o) mice by histological and echocardiography analysis we observed 60% reduced cardiac injury compared to des-/- mice (injury index 1.1±0.31 versus 2.7±0.11) and an improved (54%) cardiac function (left ventricle fractional shortening 38.19±1.43 versus 24.66±1.73). Also the d.k.o animals have preserved diastolic dimension with no difference to wild type animals, where the des-/- animals have severe left ventricle dilatation. In contrast, the skeletal muscle of the d.k.o. mice develop a severe form of muscular dystrophy compared to des-/- or dysf-/- skeletal muscle (muscular dystrophy index: 2.3±0.3 compared to 0.8±0.15 and 1.1±0.12 accordingly), indicating a differential role of dysferlin in cardiac and skeletal muscle function under stress.
Dysferlin plays an important role in fusion of vesicles with the plasma membrane and therefore has been proposed as a significant molecular player in the process of cytokine secretion. Thus containment of the inflammatory reaction in the injured myocardium with consequently reduced myocardial remodeling could explain the alleviated cardiac phenotype in d.k.o. mice compared to des-/- mice. Apart from the "cytokine hypothesis", the existence of a remote protective mechanism from the injured skeletal muscle through the release, or transport of unknown "protective factors" to the myocardium in a mechanism analogous to "remote conditioning" could also be present in the d.k.o mice.
Data supporting both hypothesis will be presented, indicating that dysferlin downregulation could potentially be a new therapeutic target for dilated cardiomyopathy, caused by mutations in desmin and/or other genes of cytoskeleton related proteins.

31 ROLE OF LIPID DROPLET ASSOCIATED PROTEIN (PLIN1) IN HEPATOCELLULAR CARCINOMA

Elisavet Lioulia*, Alexandros Karagiannopoulos, Georgios Sioutas, Evangelia Parlapani, Ioanna Vallianou, Margarita Hadzopoulou-Cladaras, Dimitra Dafou

**Laboratory of Developmental Biology, Department of Genetics, Development and Molecular Biology, School of Biology, Faculty of Sciences, Aristotle University of Thessaloniki, 54 124 Thessaloniki, Greece*
e-mail: lioulia@bio.auth.gr

Hepatocellular carcinoma (HCC) represents the 3rd most common cancer, leading to 600,000 deaths annually. Hepatocyte nuclear factor 4α (HNF4α) is the most abundant liver transcription factor (TF), with important roles during organ development and metabolic functions. Aiming to understand the role of HNF4α in HCC lipid metabolism, RNA-interference, RNASequencing and subsequent bioinformatics analysis identified PLIN1, a lipid droplet (LD) associated protein and master regulator of lipid metabolism in adipocytes, as a potential target. Firstly, we investigated PLIN1 promoter regulatory sites. TRANSFAC analysis revealed binding sites for liver TFs: HNF4α, HNF3α and C/EBPα. Next, a pGL3-luciferase reporter vector was constructed containing upstream sequences -2100/+60 of PLIN1 promoter, followed by co-transfection experiments in HEK293T cells, which do not express liver TFs. PLIN1 promoter activity was increased in HNF4α, HNF3α and C/EBPα- transfected cells by 8.6, 3.2 and 26.9-fold, respectively. PLIN1 promoter activation was confirmed by co-transfection experiments in HCC cell lines Huh7 and HepG2. In accordance, we observed increased PLIN1 promoter transactivation in HNF4α, HNF3α and C/EBPα transfected cells by 81.4, 3.1, 5.4-fold, and 52.7, 9.6, 6.4-fold, in Huh7 and HepG2. Significant increase of PLIN1 mRNA expression in HNF4α-transfected Huh7 and HepG2 cells, by 8.4 and 1.9-fold, was also observed. We are investigating the role of PLIN1 during *de novo* long-term steatosis by free fatty acids in Huh7 and HepG2. Initial experiments with PPARα and PPARγ antagonists indicate the requirement of these TFs for PLIN1 and HNF4α transcriptional regulation during chronic steatotic conditions. Reduced PPARα expression led to an increase of PLIN1 and HNF4α, whereas reduced PPARγ resulted in a decrease of PLIN1 and HNF4α protein levels. Ongoing experiments are currently being performed to identify the post-transcriptional/translational regulation of HNF4α and its target PLIN1 in liver exacerbated steatosis for elucidation of the mechanisms involving activation of lipogenic genes and *de novo* lipogenesis.

32 ACCUMULATION OF ACYLCARNITINES IS RELATED TO INSULIN RESISTANCE IN DIABETIC DB/DB AND HIGH FAT DIET-FED MICE

Elina Makarova, Marina Makrecka-Kuka, Solveiga Grinberga, Maija Dambrova, Edgars Liepinsh

Latvian Institute of Organic Synthesis, Riga, Latvia
elina.makarova@farm.osi.lv

An increasing number of studies indicate that the important pathological consequences of diabetes result from the detrimental effects induced by long-chain acylcarnitines. The aim of this study was to assess the role of long-chain acylcarnitines in the development of insulin resistance and to test potential application of acylcarnitine measurements in the diagnosis of insulin resistance and diabetes.

Concentrations of acylcarnitines were measured in plasma and muscles of diabetic db/db and control db/L mice, as well as high fat diet-fed mice in fasted and fed states. To induce insulin resistance C57bl/6 mice were treated with high fat diet for 8 weeks. Plasma biochemical parameters were measured and glucose tolerance test (GTT) was performed. In addition, we tested whether the decreased content of acylcarnitines improves insulin sensitivity.

Db/db mice developed severe hyperglycemia, hyperinsulinaemia and insulin resistance. High fat diet induced an increase in fasted state glucose levels, hyperinsulinemia and disturbed glucose tolerance. Long-chain acylcarnitine levels were significantly increased in the fed state of diabetic db/db and high fat diet-fed mice compared to respective control animals, while in fasted state no substantial differences were observed. In control animals 2h after glucose administration the plasma concentrations of long-chain acylcarnitines decreased by 30%, but the levels of long-chain acylcarnitines in animals with insulin resistance decreased only by 16%. Decrease in acylcarnitine levels improved glucose and insulin tolerance, and significantly reduced blood glucose levels in db/db and high fat diet-fed mice.

Our results provide evidence that plasma concentrations of long-chain acylcarnitines are increased in fed state of experimental model of type 2 diabetes. The reduction of long-chain acylcarnitine levels is an effective strategy to improve insulin sensitivity. Measurements of long-chain acylcarnitines after glucose tolerance test could be a useful method to diagnose insulin resistance.

33 SCAFFOLDING FUNCTIONS OF GPCR'S

Mayrhofer JE*, Bachmann VA*, Ilouz R, Röck R, Tschaikner PM, Raffeiner P, Aanstad P, Taylor SS, Stefan E

Institute of Biochemistry and Center for Molecular Biosciences, University of Innsbruck, Austria
johanna.mayrhofer@uibk.ac.at

Signal transmission emanating from the G protein-coupled receptor (GPCR) family to intracellular effector cascades is organized by pleiotropic scaffolding proteins. Signaling scaffolds such as A-kinase anchoring proteins (AKAPs) compartmentalize kinase activities and ensure substrate specificity [1]. In previous studies we have identified dynamic protein:protein interactions (PPI) of compartmentalized protein kinase A (PKA) [2] with distinct molecular switches downstream of receptor cascades [3,4]. In order to gain a more comprehensive and mechanistic understanding of cAMP-controlled macromolecular PKA complexes we chose a phospho-proteomics approach to map dynamic PPIs. We affinity-purified endogenous PKA complexes from osteosarcoma cells and generated a dynamic PPI network. Besides well-known connections to AKAPs, we identified possible links of PKA to metabolic pathways, protein transport, RNA binding, GTP binding, calcium signaling, and nuclear signaling. We selected and investigated a novel PPI between the cAMP/PKA cascade and an orphan GPCR, the GPR161, which has been shown to be involved in Hedgehog signaling [5]. We show that the orphan GPCR GPR161 contains the structural features to function as selective high-affinity type I AKAP. Binary complex formation of GPR161 exclusively with type I PKA regulatory subunits affect plasma membrane targeting in cells and provokes GPR161-mediated PKA recruitment into the primary cilium of zebra-fish embryos. We illustrate that receptor-anchored PKA complexes enhance cAMP-mediated GPR161 phosphorylation, which is regarded as one general principle for regulating GPCR desensitization. In addition we revealed that distinct 'rare disease' mutations of PKA regulatory subunits differentially contribute to spatially restricted interactions of GPR161-anchored PKA type I holoenzyme complexes. We propose that the ciliary GPR161-PKA signalosome is a compartmentalized signaling hub that directly integrates receptor-sensed input signals with spatiotemporal cAMP dynamics.

Acknowledgement: This work is supported by the Austrian Science fund FWF (P22608, P27606, SFB-F44).

[1] Langeberg LK & Scott JD. Nat Rev Mol Cell Biol 16, 232-244 (2015).
[2] Taylor SS, Ilouz R, Zhang P & Kornev AP. Nat Rev Mol Cell Biol 13, 646-58 (2012).
[3] Bachmann V et al. *Proc. Natl. Acad. Sci. USA* 110, 8531-8536 (2013).
[4] Stefan E et al. Nat.Commun. 2, 598 (2011).
[5] Mukhopadhyay S. et al. Cell 152, 210-223 (2013).

34 LONG-TERM DIETARY RESTRICTION MODULATES BRAIN INSULIN AND NEUROPEPTIDE Y EXPRESSION AND EXPLORATORY BEHAVIOUR IN MIDDLE-AGED RATS

Mladenović-Đorđević A., Todorović S., Smiljanić K., Pešić V., Ruždijić S., Kanazir S.

Department of Neurobiology, Institute for Biological Research "Sinisa Stankovic", University of Belgrade, Belgrade, Serbia

Dietary restriction (DR) extends lifespan and delays age-related disorders, including neurodegenerative diseases. Emerging data indicate that insulin and neuropeptide Y (NPY) play a crucial role in the maintenance of nutrient homeostasis in the hypothalamus, while in the hippocampus they affect learning and memory and can be neuroprotective. Moreover, NPY is one of the key players involved in explorative behaviour and decisive for beneficial effects of DR on aged animals. On that account, our aim is to assess the influence of long-term DR on behavioural parameters and expression levels of insulin and NPY in rat hippocampus and hypothalamus.

Experiments were performed on male middle-aged (18-months-old) Wistar rats fed ad libitum (AL) or dietary restricted (DR group fed 60% of AL intake starting from 6-months of age). The habituation of exploratory movement was examined in an open field box. The expression levels of insulin and NPY protein were determined by Western blot analysis.

Animals on DR demonstrated significantly higher exploratory behaviour compared to AL group. These changes were followed with a significant increase of NPY protein level in the hippocampus of DR animals, while no changes of NPY levels in hypothalamus were observed. Insulin protein level did not vary in any of examined brain regions.

This study demonstrated that long-term DR is capable to increase exploratory behaviour in middle-aged animals which was accompanied by the increased level of NPY in a region-specific manner. Our data point to NPY role in beneficial effects of DR, but mechanism underlying these effects need to be further clarified.

35 SEARCH AND CHARACTERIZATION OF THE MTORδ SPLICING ISOFORM IN MAMMALIAN CELL LINES

N. Nosachuk, O. Malanchuk, S. Palchevskyy

Department of Cell Signaling
Institute of Molecular Biology and Genetics, 150 Zabolotnogo st., 03143 Kyiv, Ukraine
e-mail: natali_vov@ukr.net

The mammalian target of rapamycin (mTOR) is a phosphoinositide 3-kinase-related protein kinase, which controls cell growth in response to nutrients and growth factors and is frequently deregulated in some pathologies, including cancer. Researches of previous years, indicate the possible presence of additional splicing isoforms along with the existence of classical mTORα. Recently, mTORβ isoform was identified. It acts as protooncogen. The aim of this work was to attempt to identify the hypothetical splicing isoform of mTOR kinase, exactly mTORδ in a number of mammalian cell lines for further study of the functioning of this isoform in the cell. In our experiments we used eight cell lines of different etiology to identify transcripts, which encode mTORδ isoform. At the first stage the total RNA was isolated from mammalian cell lines with the following performance of cDNA synthesis. For the search of potential splicing variants we were using two pairs of primers. Primers of the first forward pair were identical. At the same time primers from the second reverse pair were different.
Amplification of fragments was made in two rounds using some of PCR mixture of the first round as matrix for PCR of the second round. In fact, the presence of mTORδ isoform was confirmed by the appearance of the predicted size DNA fragment. This DNA fragment contained the expected site connection of the nucleotide sequence from the twenty seventh exon and the sequence of the 3'-untranslated mTORα area. The advanced sequence-analysis of the received PCR fragments showed the presence of a group of splicing variants – which are similar by principle of the formation to the mTORδ isoform.
Further research of structural and functional features of the mTORδ isoform will help to make better understanding of the functioning of mTOR kinase isoforms in general.

36 ANALYSIS OF miRNA TRANSCRIPTOME PROFILE OF HUMAN ENDOTHELIAL CELLS EXPOSED TO ESTRADIOL.

Daniel Pérez-Cremades, Xavier Vidal-Gómez, Ana Mompeón, Ana Paula Dantas, Susana Novella and Carlos Hermenegildo.

Dep. Physiology, Univ. Valencia and INCLIVA Biomedical Research Institute. daniel.perez@uv.es

Introduction.
Epidemiological studies reveal sex differences in cardiovascular diseases. These discrepancies may be a consequence of the action of the sexual hormones on vascular tissues. Endothelium, as target of estrogens, has a key role in the modulation of vascular physiology. MicroRNAs (miRNAs) are a small non-coding RNAs that modulate post-transcriptional expression of numerous genes implicates in a wide range of biological processes. The objective of this study was to determine important cardiovascular pathways regulated by estradiol-sensitive miRNAs in cultured human endothelial cells.

Methods.
Human umbilical vein endothelial cells (HUVEC) were isolated and cultured in Medium 199 with endothelial cell growth supplement (Sigma). HUVEC from passages 3-5 were exposed to 1 nM estradiol for 24 hours and miRNAs were isolated by miRNeasy Mini Kit (Qiagen). miRNAs expression was performed with GeneChip miRNA 4.0 Array (Affymetrix). Global interrelationships between samples were measured by Principal Components Analysis (PCA) and changes in the expression profile were analyzed using Partek Genomic Suite v6.6 software (Partek Inc.). High predicted and experimentally observed mRNA targets of differentially expressed miRNAs were used to canonical pathway analysis using Ingenuity Pathway Analysis software.

Results.
Global differences among samples studied by PCA analysis shown differences between samples from estradiol-treated and non-treated cells. We identified 120 miRNAs with significant differential expression compared between estradiol-treated and non-treated cells: 47 up-regulated and 73 down-regulated. Bioinformatic analysis of predicted mRNA targets revealed significant canonical pathways important for endothelial function, including ERK/MAPK signalling, integrin signalling and actin cytoskeleton signalling, among other pathways.

Conclusions.
Estradiol regulates miRNA expression profile of human endothelial cells. Analysis of mRNA targets reveal the role of estradiol in different biological processes implicated in vascular function.

Supported by the Spanish MINECO, ISCIII-FEDER-ERDF (grants FIS PI13/00091, PI13/00617, and RD12/0042/0052). DPC is an "Atracció de Talent" fellow. A.M. is a "Formación de Profesorado Universitario" fellow.

37 METABOLIC ADAPTATION AFTER MTOR INHIBITOR TREATMENTS IN CANCER CELL LINES

Gábor Petővári[1], Zoltán Hujber[1], Titanilla Dankó[1], Norbert Szoboszlai[2], Noémi Nagy[1], Júlia Oláh[1], László Kopper[1], András Jeney[1], Anna Sebestyén[1,3]

[1]First Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary
[2]Laboratory of Environmental Chemistry and Bioanalytics, Department of Analytical Chemistry, Institute of Chemistry, Eötvös Loránd University, Budapest, Hungary
[3]Tumor Progression Research Group Joint Research Organization of Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary
gaborpetovari@gmail.com

The dysregulation of the activity of mTOR kinase was described in different tumors. The tumor type dependent changes of expression and activity of mTOR complexes (C1, C2) could correlate to the different sensitivity againts currently available mTOR inhibitors. However, the mTOR inhibitor treatments related effects in therapeutic drug sensitivity and in the metabolic alterations of tumor cells even less revealed.

We studied two cell lines with different metabolic capacity and mTOR related protein expression profile. The mTOR inhibitor (rapamycin, BEZ235, PP242) sensitivity, the alterations in their mTOR activity related proteins expresssion pattern and the metabolic profile and glucose, acetate, glutamine consumptions were analysed.

We found different metabolic activities and parallel diverse mTORC1, mTORC2 complex activity in HT-1080 and ZR-75.1 cells. HT-1080 cells have characteristic high glycolytic activity accompanied with proteins related to mTORC1 complex activity. However, ZR-75.1 expresses low level of p-S6 (related to mTORC1 complex), and high level of Rictor (related to mTORC2) at the same level of mTOR kinase activity and these cells were characterised with high activity of Krebs cycle. Surprisingly, the mTOR inhibitor sensitivity of these cells did not show any differencies. Rapamycin and BEZ235 have almost similar anti-proliferative effects (~40%) and PP242 (mTORC1 and mTORC2 inhibitor) was the most effective. Based on energy substrate consumption of the cell lines we assume that mTOR inhibitors influence the metabolic adaptations of the cells and alter the metabolite ratios of gycolysis and Krebs cycle.

These results emphasize that mTOR complex activities influence the adaptation in cellular microenvironment at metabolic level, as well. To understand the regulation and the mTOR activity of this metabolic adaptation and the role of mTOR complexes in these need further studies especially the metabolic characterization of different tumors and the related therapy resistance..

Supported by OTKBA T84262 and MTA Bolyai Grant (A.S.).

38 ASSOCIATION OF IL -1A C(-889)T AND IL -1B C(+3953/4)T GENETIC POLYMORPHISM WITH CHRONIC PERIODONTITIS IN A PERUVIAN POPULATION

Ivonne F. Reyes-Mandujano[1,2,a], José E. Olivera-García[1, 2, 3b], Manuel Taboada-Vega[1,4c], Patricia Galvan-Sanchez [1,2d], Amilcar Azcarza-Acuña[1e], Aldo M.Yactayo-Flores[1, 2f], Luis A. Zuñiga-Ccoicca[1,2g], Evelyn V. Leiva-Pachas[1,2h], Efraín Paccori-García[1,5i]

1 Centro de Investigación de Bioquímica y Nutrición "Alberto Guzmán Barrón". Universidad Nacional de San Marcos. Lima, Perú.
2 Facultad de Ciencias Biológicas. Universidad Nacional de San Marcos. Lima, Perú
3 Instituto de Investigación Estomatológica de la Facultad de Odontología. Universidad Nacional de San Marcos. Lima, Perú.
4 Departamento Académico de Ciencias Básicas de la Facultad de Odontología. Universidad Nacional Mayor de San Marcos, Lima, Perú.
5 Facultad de Odontología. Universidad San Martín de Porras. Lima, Perú.

a Biólogo con mención en Biología Celular y Genética, Magíster en Biología Molecular. ivonnefanny@gmail.com
b Biólogo con mención en Genética , Magíster en Bioquímica. joliverag@unmsm.edu.pe
c Cirujano Dentista, Doctor en Odontología. mtaboadav@unmsm.edu.pe
d Biólogo con mención en Biología Celular y Genética. patriciagalvans@gmail.com
e Cirujano Dentista, Maestría en Periodoncia. amilcar_aa@hotmail.com
f Biólogo con mención en Zoología. aldoyf@gmail.com
g Bachiller en Genética y Biotecnología. luisz_207@hotmail.com
h Bachiller en Genética y Biotecnología. evelynleiva1423@gmail.com
i Cirujano Dentista, Magister en Docencia e Investigación en Estomatología. paccori@hotmail.com

Chronic periodontitis is the most common cause of tooth loss in the world and many risk factors such as infections, inflammatory response, oral hygiene, age, smoking habits and individual genetic characteristics are involved in the development of this disease. The identification of genetic factors controlling oral inflammation may help us understand the genetic predisposition to developing periodontitis ***Objective***. To determine the association of polymorphisms of the IL-1A C(-889)T, IL-1B C(+3953/4)T with chronic periodontitis in a Peruvian population. ***Design***. Observational study of cases (94) and controls (96). ***Materials and methods***. Genotyping IL-1A and IL-1B, it was performed with the PCR-RFLP. Data were analyzed with the test $\chi 2$, $\chi 2$de linear trend, logistic regression and odds ratio (OR) with 95% confidence interval. ***Results***. The IL-1B, meets the Hardy Weinberg ($\square\square 2 = 0.6894$, p = 0.4063); genotype (IL-1B) and the composite genotype (IL-1A / IL-1B) are associated with chronic periodontitis, OR: 2.065, 95% CI: 1.012-4.210, p = 0.0144 and OR: 3.023, 95% CI: 1.487-6.145, p = 0.002, respectively. ***Conclusion.*** There is a statistically significant positive association between IL-1B genotype positive and composite genotype with chronic periodontitis.

Key words: Interleukins, cytokines, Chronic Periodontitis, Genetic Polymorphism, PCR-RFLP.

39 A PROTEASOME ACTIVATOR DECELERATES AGING AND ALZHEIMER'S DISEASE PROGRESSION

Nikoletta Papaevgeniou[1,2*], Marianthi Sakellari[1,3*§], Sweta Jha[4], Nektarios Tavernarakis[5,6], Carina I. Holmberg[4], Efstathios S. Gonos[1,3] and Niki Chondrogianni[1]

[1] Institute of Biology, Medicinal Chemistry and Biotechnology, National Hellenic Research Foundation, 48 Vassileos Constantinou Ave., Athens 11635, Greece
[2]Institute of Nutrition, Faculty of Biology and Pharmacy, Friedrich Schiller University of Jena, 07743 Jena, Germany
[3] Örebro University, Medical School, Örebro 701 82, Sweden
[4] Research Programs Unit, Translational Cancer Biology Program, University of Helsinki, FI-00290 Helsinki, Finland.
[5] Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion 70013, Crete, Greece
[6] Department of Basic Sciences, Faculty of Medicine, University of Crete, Heraklion 71110, Crete, Greece

** These authors contributed equally in this study*
§Email address: mirella_sak@hotmail.com

Proteasomes are constituents of cellular proteolytic networks and responsible for the degradation of a pleiad of both normal and abnormal (in any way) proteins. Genetically-mediated proteasome activation in multicellular organisms has been shown to promote longevity and to exert protein anti-aggregation activity. Here we sought to investigate whether compound-mediated proteasome activation is feasible in a multicellular model system using *Caenorhabditis elegans* and we dissect the effects of such approach in aging and Alzheimer's disease (AD) progression. We provide evidence that treatment of wild type *C. elegans* with 18α-glycyrrhetinic acid (18α-GA; a previously shown proteasome activator in cell culture) induces proteasome enhancement leading to a SKN-1- and proteasome activation-dependent lifespan extension. We also demonstrate that 18α-GA treatment confers a positive effect against AD progression in a proteasome activation-dependent manner in various AD nematode models and in cells of nervous origin. In total, our results show the potential use of 18α-GA as pro-longevity and anti-aggregation compound in the context of a multicellular organism.

40 Validation of pyrosequencing and high resolution melting analysis in detecting mutations in EGFR gene related to exon 20 and 21 in non-small cell lung carcinomas

[1,2,]**Myla Santiago**, [1]Anne France Dekairelle,[1]Jerome Ambroise and [1]Jean-Luc Gala

[1] *Université catholique de Louvain- Bruxelles, Cliniques Universitaires Saint-Luc A.S.B.L., Centre de Genetique Humaine, Centre de Technologies Moleculaires Appliquees (CTMA), Clos Chapelle aux Champs, 30 boite 30.46, 1200 Woluwe Saint-Lambert, Bruxelles 1200 Belgium*

[2] *University of Santo Tomas, Department of Biochemistry, Faculty of Pharmacy, Espana Manila 1015 Philippines*
Email correspondence: myla.santiago@gmail.com

Abstract
Lung cancer is the leading cause of cancer deaths worldwide with non-small cell lung cancer (NSCLC) as most prevalent and majority of cases are diagnosed at advanced stage. Mutations related to exon 20 (T790M) and 21 (L828R) are most prevalent in NSCLCs and determines response to EGFR-tyrosine kinase inhibitors (TKI) therapy.

Two of the most frequently used methods in detecting EGFR mutations in both exons, namely high resolution melting analysis (HRM) and pyrosequencing were cross validated based on frequency and accuracy of mutation detected using genomic DNA of 174 formalin-fixed paraffin embedded (FFPE) tissue samples in CTMA laboratory Brussels, Belgium. HRM is a cheap-gel free method based on the formation of heteroduplexes between mutant and wild-type alleles that exhibits high sensitivity and specificity and detects mutant genes at levels of 2.5-10%. While, pyrosequencing is an alternative method to Sanger sequencing, its quick, gel-free method can detect mutations in at least 20% tumour cells.

Based on mutation detection, pyrosequencing can be detected in at least 1ng DNA compared to 5ng DNA in HRM. Based on accuracy, sensitivity of two methods were evaluated using different proportions of EGFR wild-type and mutant cell lines, NCI-H1650 and NCI-H1975. Overall, pyrosequencing can be used as first line assays to screen EGFR mutations in both exons. However, benchmarking of these detection methods are still needed to validate its reliability, efficiency and cost-effectiveness in routine clinical diagnosis of NSCLC.

Funding and Acknowledgement: This research work is supported by the Université Catholique de Louvain (UCL)- Bruxelles, Cliniques Universitaires Saint-Luc A.S.B.L., Centre de Genetique Humaine, Centre de Technologies Moleculaires Appliquees headed by Prof. Jean Luc Gala. This is part of the speaker's doctoral research training (2011-2013) under the Erasmus Mundus Action II-MAHEVA scholarship program. The speaker would also like to thank Prof. Annie Robert from Université Catholique de Louvain- Bruxelles, Pôle de recherche en épidémiologie et biostatistique for her excellent mentorship in parts where biostatistical analysis must be employed and continued trust and motivational support from Prof. Claire de Burbure as UCL-MAHEVA coordinator.

41 MOLECULAR DYNAMICS SIMULATIONS OF TYROSYL-tRNA SYNTHETASE MUTANT FORMS ASSOCIATED WITH CHARCOT-MARIE-TOOTH NEUROPATHY

Oleksandr V. Savytskyi and Alexander I. Kornelyuk

Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine
e-mail: savytskyi@moldyngrid.org

Certain mutations in aminoacyl-tRNA synthetases lead to Charcot-Marie-Tooth disease (CMT) – a group of heterogeneous inherited disorders that are characterized by degeneration of peripheral nerve fibers, loss of muscle tissue and touch sensation. There are some of possible mechanisms: loss of charging function, aggregation, mischarging, nucleolar dysfunction, dimerization, non-canonical functions, novel interactions resulting from mutations, mitochondrial toxicity or dysfunction, impaired axonal transport that leads to deficits in local translation (Motley, 2009). The common mechanism is still unknown. Two heterozygous missense mutations (G41R, E196K) and one *de novo* deletion (153-156delVKQV) in *Homo sapiens* tyrosyl-tRNA synthetase (*Hs*TyrRS) were identified in different families of patients with DI-CMTC (Jordanova, 2006).
All thee mutants of *Hs*TyrRS, structural complexes with cognate $tRNA_{Tyr}$ and translation elongation factor eEF1A2 were constructed *in silico* using Modeller 9.7 software. Molecular dynamics (MD) simulations were carried out for all *Hs*TyrRS mutants for 100 ns using GROMACS package. All MD simulations and trajectories analysis were performed using the grid services of MolDynGrid virtual laboratory (*http://moldyngrid.org*) (Savytskyi et al., 2011).

The melting of H9 helix (T141-A148) and subsequent partial melting of H11 helix were observed in 153-156delVKQV mutant of TyrRS. A novel β-sheet formation was observed in K147-E157 region in G41R and in 153-156delVKQV mutants for 20-100 ns time interval of MD simulation in CP1 region of Rossmann fold, which is a specific part for recognition with $tRNA_{Tyr}$. Calculation of hydrogen bonds for region K147-E157 with $tRNA_{Tyr}$ shows (lifetime more >10%): E151:C75 – 38.71%, Q155:A76 – 12.90%, K147:G72 – 12.90%, K147:G71 – 9.68%. MD simulations showed the attraction of the chloride ion instead of potassium ion for G41R mutant (active side localization), which has functional role replacing the second lysine of the catalytic KMSSS loop (KMSKS motif) that affects the catalytic properties of human TyrRS. Our results confirm the similar effect as for glycyl-tRNA synthetase mutation (G526R) and propose the idea of conformational changes in both enzymes in the active site after mutations.

42 TARGETING CELL APOPTOSIS AND NECROPTOSIS BY SHIKONIN, IRRESPECTIVE OF STEROID RECEPTOR STATUS IN THE BREAST CANCER CELL LINES

Zahra Shahsavari[1], Fatemeh Karami-Tehrani[2], Siamak Salami[3]

[1]Department of Laboratory Medicine, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

[3]Department of Clinical Biochemistry, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

E-mail: shahsavari.z@gmail.com

Responses of cells to death inducers or inhibitors are different. Therefore, recognition of a novel therapeutic agent may activate an alternative programmed cell death for the treatment of breast cancer, irrespective of steroid receptor status. Here, we tried to evaluate the effects of shikonin, a naphthoquinone derivative of *Lithospermum erythrorhizon*, on the induction of programmed cell deaths, apoptosis and necroptosis, mediating by RIP1K-RIP3K in the ER positive breast cancer cells lines, MCF-7 and T-47D. Cell death modalities, cell cycle pattern, RIP1K and RIP3K expressions, caspase-3 and caspase-8 activities, reactive oxygen species and mitochondrial membrane potential have been evaluated in the shikonin-treated cells. Programmed cell deaths, necroptosis and apoptosis, could be occurred by shikonin, with a significant increase in the RIP1K and RIP3K expressions in both cell lines, although necroptosis is the main rout. Under caspases inhibition, shikonin induces necroptosis which could be arrested by Nec-1. An increase in ROS levels and a decrease in mitochondrial membrane potential have been observed in the shikonin-treated cells. In the presence of Nec-1, caspase-3 mediating apoptosis has been occurred in the shikonin-treated T-47D cells but not in MCF-7 cells.

We found that shikonin induces necroptosis in both ER negative, MDA-MB-468 and ER positive, MCF-7 and T-47D breast cancer cell lines which suggest it as a drug more suitable for the treatment of breast cancer than tamoxifen, although further investigations are required.

43 MAGNETIC RESONANCE SPECTROSCOPY OF THE ISCHEMIC BRAIN UNDER LITHIUM TREATMENT INDICATES CHANGES IN METABOLITES

[1]Silachev D.N., [1]Zorova L.D., [1]Khailova L.S.,[2]Gulyaev M.V., [1]Plotnikov E.Y., [1]Zorov D.B.

[1]Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, 119992, Leninskye gory, house 1, building 40, Moscow, Russia; E-mail: silachevdn@genebee.msu.ru
[2]Faculty of Fundamental Medicine, Lomonosov Moscow State University, 117192, Lomonosovsky Prospekt, house 31-5, Moscow, Russia;

In the last two decades, a growing body of evidence has shown that lithium has several neuroprotective effects. Lithium is a classic drug for the treatment of bipolar disorder. The major mechanism underlying lithium-induced neuroprotection is inhibition of glycogen synthase kinase-3β, however, the certain metabolic changes mediated by lithium is still unclear. At the same time, many studies indicate mitochondrial dysfunction in stroke. Along these lines, *in vivo* analysis of metabolites associated with mitochondrial function may provide the key to understanding the mechanisms of brain damage and neuroprotection by lithium.

In this study, ^{1}H-MRS was employed to examine the metabolic changes in the cortex and thalamus during the acute phase of rat brain ischemia/reperfusion and those after lithium treatment.

Incubation of mitochondria isolated from normal rat brain at neutral and slightly acidic pH, mimicking the intracellular pH of normal and ischemic tissues correspondingly, revealed serious changes in mitochondrial bioenergetics, partially reflected in the magnitude of respiratory control and the basal and maximally stimulated respiration rates. Measurement of available metabolites by 1H-MR spectra of normal and ischemia-damaged brains showed a significant increase in lactate and myo-inositol and a moderate decrease in N-acetylaspartate 24 h after reperfusion. Remarkably, the administration of lithium chloride in the reperfusion phase normalized the levels of metabolites. Moreover, the introduction of lithium salts (chloride or succinate) in the bloodstream, restored after ischemia, reduced both the size of the ischemia-induced brain damage and the degree of brain swelling. Besides, post-ischemic introduction of lithium salts largely restored the neurological status of the animal.

Therefore using this approach one can estimate the severity of brain damage and effectiveness of applied therapy. In that way we were able to show lithium-mediated neuroprotection, associated with preserving mitochondrial function.

This study was supported by RFFI 15-34-20074.

44 DIFFERENTIAL INHIBITION EFFECTS OF BENZOTHIAZOLE SULFONAMIDE DERIVATIVES ON TUMOR-ASSOCIATED CARBONIC ANHYDRASE IX, XII ENZYMES

Emine Terzi, Anthi Petrou, Athina Geronikaki, Ozen Ozensoy Guler, Tuziano Tuccinardi, Claudiu Supuran

Department of Medical Biology , Faculty of Medicine, Yildirim Beyazit University, Bilkent Campus , Ankara , Turkey. E-mail address:emineterzi1990@hotmail.com

Carbonic anhydrases (CAs, EC 4.2.1.1) involved in respiration and transport of CO_2/bicarbonate between metabolizing tissues and lungs, pH and CO_2 homeostasis, electrolyte secretion in various tissues, biosynthetic reactions (gluconeogenesis, lipogenesis, ureagenesis etc.), bone resorption, calcification, tumorigenicity and many other physiologic or pathologic processes.

CA-I and CAII are two major cytosolic CA isoenzymes that found in mammalian red blood cells. CA-IX and CA-XII are tumor associated isoenzymes which have contribution to tumor development by stimulating extracellular acidification.

This study deals with inhibition effects of new synthesized benzothiazole sulfonamide derivatives on CA-I, CA-II, CA-IX and CA-XII. All inhibition studies were performed with SX.18MV-R Applied Photophysics Stopped-Flow Kinetic Instrument. According to the findings of the study, several of the investigated derivatives showed interesting inhibition activity and selectivities for inhibiting hCA-IX and hCA-XII over the off-target ones hCA-I and hCA-II.

45 MICROARRAY ANALYSIS OF DIFFERENTIALLY EXPRESSED GENES RELATED TO CYTOKINE SIGNALING IN PERIPHERAL BLOOD LEUKOCYTES OF PATIENTS WITH ESSENTIAL HYPERTENSION

Y.R. Timasheva, V.V. Erdman, V.A. Matveeva, T.R. Nasibullin, I.A. Tuktarova, O.E. Mustafina

Institute of Biochemistry and Genetics Ufa Science Centre Russian Academy of Sciences, Ufa, Russia
ianina_t@mail.ru

Vascular aging and hypertension are major risk factors for cardiovascular disease. Inflammation and endothelial dysfunction contribute to arterial wall remodeling and blood pressure elevation. Our aim was to explore the role of inflammatory mediators in the molecular regulation of essential hypertension, and to identify specific patterns of gene expression in the group of individuals with enhanced survival (beyond 80 years).

We performed the analysis of gene expression patterns in peripheral blood leukocytes of hypertensive individuals and healthy controls using RT² Profiler PCR Array (Qiagen), with subsequent validation of the obtained results by quantitative real-time RT-PCR. Study group consisted of 30 patients with essential hypertension and 32 control subjects aged between 30 and 60 years, and 12 individuals aged between 82 and 113 years (6 patients with hypertension, 6 normotensive controls).

In the group of middle-aged hypertensive patients, we found altered transcriptional activity of a number of genes: *CCL16, CCL17, CCL18, CCL19, CCL23, CCL8, CCR6, CCR8, CX3CR1, CXCL1, CXCL13, ICEBERG, IL17C, IL1F10, IL1F6, IL1F9, SPP1, CD40LG, XCR1, CCL2*. Further quantitative analysis confirmed significant differences of *CCL18, CX3CR1, CXCL1, CXCL13, IL10, IL13*, and *CCR2* expression level between cases and controls (P=0.001). Relative expression level changes in EH patients were more pronounced for *CX3CR1* gene (29.2-fold), *CXCL13* (13.8-fold), *IL1F6* (12.9-fold), *CD40LG* (8-fold), *CXCL1* (7.2-fold). In elderly hypertensive individuals compared to healthy controls, transcriptional activity of *NFKB1* and *IL18R1* genes was increased (FC, fold change, 3.21 and 2.41, respectively, P<0.05), while *CCL5, CFS1, CD4, RPLP0, BCL2, SRC, IL15, CD40LG, CFS3, CDKN1A, MYC, CFS2* genes were down-regulated. Functional analysis of has shown that the genes differentially expressed in hypertensive patients encode for molecules involved in immune response and inflammation, and their action is mediated via cytokine signaling pathway.

46 LOSS OF COREPRESSOR GPS2 SENSITIZES MACROPHAGE ACTIVATION UPON METABOLIC STRESS IN OBESITY AND TYPE-2 DIABETES

Rongrong Fan[1], Amine Toubal[2], Saioa Goñi[1], Zhiqiang Huang[1], Anastasios Damdimopoulous[1], Ning Liang[1], Nicolas Venteclef[2,3] and Eckardt Treuter[1,3]

[1]Karolinska Institutet, Department of Biosciences and Nutrition, S-14183 Stockholm, Sweden [2]Université Pierre et Marie-Curie, INSERM, UMR_S 1138 Cordeliers Research, Paris, France
[3]Co-senior authors, E-mail: eckardt.treuter@ki.se

Obesity and type-2 diabetes (T2D) are considered today as metabolically driven inflammatory diseases. Particularly, metabolic macrophage activation, adipose tissue dysfunction and the resulting systemic low-grade inflammation (metaflammation) have emerged as the closely linked central components and key drivers of insulin resistance and T2D. Our study aimed at identifying mechanisms that might explain why individual obese (but also non-obese) humans differ in their susceptibility to develop T2D. We reasoned that such individual differences could relate to epigenetic differences (despite the absence of, or in addition to, genetic differences), i.e. distinct epigenomes and specifying factors. Here we present a key role of GPS2 (G-Protein Pathway Suppressor 2, subunit of a fundamental corepressor complex containing HDAC3) in the epigenomic reprograming of metabolic macrophage activation. We found that GPS2 levels were reduced in adipose tissue macrophages of obese and diabetic humans. Correlation analysis suggested a causal relationship between macrophage GPS2, adipose tissue inflammation and T2D status. The study of macrophage-specific GPS2 knockout mice confirmed this causality and revealed some underlying mechanisms. These mice were characterized by the hallmarks of metaflammation: (i) chronic elevated low-level inflammation under basic/chow diet conditions, (ii) accelerated adipose tissue inflammation and macrophage infiltration, and (iii) development of systemic insulin resistance under diet-induced obesity conditions. The comparative analysis of macrophage cistromes, epigenomes and transcriptomes suggested that GPS2-dependent alterations of H3K27ac-marked enhancers establish an epigenomic memory that accelerates inflammatory responses to metabolic signals. Overall, our study highlights how cell type-specific epigenomes (e.g. in macrophages) due to alterations of a single regulatory component (i.e. a corepressor) can determine the individual pathophysiological responses (i.e. inflammation, insulin resistance) to a common disease environment (i.e. obesity). Since the underlying mechanisms appear conserved between mice and humans, therapeutic possibilities to target the GPS2-metaflammation-T2D axis may emerge, for example via epigenomic drugs that restore GPS2 expression and function.

47 CIRCULATING NUCLEOPROTEIN COMPLEXES IN BLOOD: PROTEIN COMPOSITION AND BREAST CANCER DIAGNOSTIC POTENTIAL

Tutanov O.S., Tamkovich S.N., Duzhak T.G., Ryabchikova E.I., Grigoreva A.E., Laktionov P.P..

Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences
e-mail: ostutanov@gmail.com

Cell-free DNA (cfDNA) was found to circulate in blood by apoptotic bodies or nucleosomes. However only small number of serum proteins except from histones (Bulter P, 1990) were shown to be potentially involved in cfDNA binding and circulation although a lot of cellular and plasma proteins could bind DNA or histones. We have investigated DNA and protein content of deoxyribonucleoprotein complexes (DNPC) circulating in blood of healthy donors (HD) and breast cancer patients (BCP). DNPC were isolated from blood plasma of HD and primary BCP by affinity chromatography using immobilized polyclonal anti-histone antibodies. DNA isolated from NPC was analyzed by Agilent 2100 Bioanalyser TM using High Sensitivity DNA Kit, proteins were identified using MALDI-TOF mass- spectrometry after 10-20% SDS-PAGE. 170-180 b.p.

DNA was found to be main component of DNPC circulating in plasma of HD, whereas equal amount of 170-180 b.p. and more than 6 k.b.p. DNA was found in DNPC from blood of BCP, more than 200 additional proteins (excluding histones) were identified with a reliable score in DNPC of HD and BCP, morever considerable amount of identified proteins are DNA-binding or containing DNA-binding motifs. Seven proteins (G1/Sspecific cyclin-E2, Rho GTPaseactivating protein 30, NADP-dependent malic enzyme, SHC SH2 domain-binding protein 1-like protein, protein Mdm4, Rho guanine nucleotide exchange factor 9, Neuroplastin) previously described as tumor specific proteins were found in DNPC of BCP and none of them was found in HD. The data obtained demonstrate involvement of number of cellular and extracellular matrix proteins in cfDNA circulation. The meaning of the proteins in cfDNA circulation is unclear but their diagnostic potentialities and use for tumor-specific cfDNA isolation can be potentially explored and employed by system of eraly diagnosis of breast cancer.

48 A STRUCTURAL AND FUNCTIONAL STUDY ON THE MYCOPLASMA HOMINIS ADHESIN VAA

Jakob J. Ulstrup, Manuela Gorgel, Theis Sommer, Søren R. Midtgaard, Nykola Jones, Søren Vrønning Hoffmann, Andreas Bøggild, Poul Nissen and Thomas Boesen.

Department of Molecular Biology and Genetics, University of Aarhus, Denmark
jakobu@mbg.au.dk

The *Mycoplasma* species are one of the smallest known self-replicating organisms and are often associated with a variety of diseases. This includes *M. hominis*, which has been connected with inflammatory diseases of the kidneys and urogenital tract. The lack of a cell wall makes them unsusceptible to treatment with β-lactam based antibiotics, such as members of the penicillin family. The bacteria gain entry to the host cell by adhering to the cell surface via several known adhesins encoded by the *vaa* gene (variable adherence-associated). Of these, the *Vaa5* variant have been shown to bind HeLa cells *in vitro.*

Despite several known crystallization conditions for *Vaa5*, experimental phases have remained elusive for years. Recently, a construct introducing additional methionines has been engineered and expressed using seleno-methionine as an anomalous scatterer. From the resulting crystals several MAD/SAD data sets were collected, which gave rise to new problems, namely high anisotropic diffraction and low isomorphism. Crystal optimization resulted in successful phasing with electron-density maps extending to 3.4-3.7 Å. From these data, an initial model was built and used to phase a previously obtained native 2.65 Å dataset using MR. Refinement of the structure is currently on-going.

49 ALLOSTERIC SIGMA-1 RECEPTOR MODULATORS: A NOVEL APPROACH TO TREAT MEMORY DISORDERS

Edijs Vavers[1,2], Liga Zvejniece[1], Baiba Svalbe[1], Ilona Domracheva[1], Reinis Vilskersts[1,2], Marina Makrecka-Kuka[1], Grigory Veinberg[1], Maija Dambrova[1,2]

[1]*Latvian Institute of Organic Synthesis, Latvia*
[2]*Riga Stradins University, Latvia*

The Sigma-1 receptor plays an important role in the pathophysiology of neurological and psychiatric disorders where cognitive impairment is a common symptom. The Sigma-1 receptor serves as an inter-organelle signalling modulator, a unique ligand-regulated chaperone protein at the mitochondria-associated endoplasmic reticulum membrane. Sigma-1 receptor activity has been found for an array of known CNS drugs and newly synthesised ligands, but only few of them act as allosteric receptor modulators. Several lines of evidence suggest that the Sigma-1 receptor agonists are effective in treatment of cognitive impairments while antagonists might be used to treat neuropathic and other chronic pain conditions. The mechanism of action of allosteric modulators should be associated with the regulation of endogenous ligand activity. We have discovered novel 4,5-disubstituted derivatives of piracetam which act as positive allosteric modulators of Sigma-1 receptor and possess memory enhancing properties.

The mechanism of action of the novel piracetam derivatives were characterised in competitive radioligand binding assay, electrically stimulated isolated rat vas deferens model, bradykinin-induced intracellular Ca^{2+} concentration increase assay in NG-108 hybrid cells and rat brain tissue mitochondrial respiration assay using high-resolution respirometry. Results showed that pre-treatment with novel derivatives of piracetam can significantly enhance the activity of selective Sigma-1 receptor agonist PRE-084. Acute pre-treatment with compounds improved memory and alleviated the scopolamine-induced cognitive impairment in mice. The *in vitro* and *in vivo* effects of compounds were blocked by treatment with the selective Sigma-1 receptor antagonist NE-100.

Since the activity of novel 4,5-disubstituted derivatives of piracetam is mediated through Sigma-1 receptor, our future studies will address the role of Sigma-1 receptor as a novel drug target for neurological diseases. Allosteric modulation of Sigma-1 receptors provides a novel approach for discovering new drugs to treat cognitive impairments.

50 ROLE OF CYCLOOXYGENASE IN VASCULAR SUPEROXIDE ANION PRODUCTION IN RESPONSE TO THROMBOXANE A2 IN AN EXPERIMENTAL MODEL OF MENOPAUSE

X Vidal-Gómez[1*], A Mompeón[2], D Pérez-Cremades[2], G Segarra[1,2], C Hermenegildo[1,2], P Medina[1,2], S Novella[1,2]

[*] *e-mail: xavier.vidal@uv.es*

[1] *INCLIVA Biomedical Research Institute, Valencia (Spain)*
[2] *Physiology Dept., Faculty of Medicine and Dentistry, University of Valencia, Valencia (Spain)*

Ageing is currently associated with a rise in oxidative stress. Thromboxane A_2 (TXA_2) is an inflammatory and oxidant endogenous vasoconstrictor which action is increased by ageing and lack of estrogens, both conditions found in menopause. Our aim was to determine superoxide anion ($O_2\cdot^-$) levels in response to TXA_2 in aortic vascular tissue from senescent and ovariectomized mice and the role of cyclooxygenase as source of superoxide.

Senescence-accelerated mice (SAM) were used in this study. 5 months-old prone (SAMP8, n=15) and SAM resistant (SAMR1, n=15) were separated in three groups: Sham-operated, ovariectomized (OVX) and ovariectomized treated with estradiol (OVE, 10µg/Kg/day 17ß-estradiol). 28 days after surgery, mice were sacrificed and aorta was isolated and fixed in OCT. Aortic frozen sections (10 µm-thickness) were incubated with a stable analogue of TXA_2 (U46619, 10^{-8}M) in the absence or presence of TXA_2 receptor (TP) antagonist (GR-3219, 10^{-8}M) and cyclooxygenase inhibitor (indomethacin, 10^{-5}M). Vascular $O_2\cdot^-$ production was detected by dihydroethidium (DHE) staining. All values were relativized to SAMR1 Sham and represented with mean ± SEM.

TXA_2 increase vascular $O_2\cdot^-$ production in both strains, but was higher in senescent mice ($p<0.001$). In aorta from OVX, TXA_2 produced a further increase of $O_2\cdot^-$ production in senescent mice ($p<0.01$). This effect was estrogen dependent as $O_2\cdot^-$ levels were reversed in OVE mice ($p<0.001$). In all groups, the treatment with indomethacin and GR-3219 prevented the increase of $O_2\cdot^-$ levels in response to U46619, suggesting a specific role of cyclooxigenases after TP receptor activation.

TXA_2, through TP receptor, induces an increase of vascular $O_2\cdot^-$ production mediated by cyclooxygenase activation. This response is enhanced by senescence and by ovariectomy. Estrogen administration reduces partially the O_2^- production.

Supported by MINECO-ISCIII (FIS-PI13/00617 and RC12/0042/0052). A.M. is FPU-fellow (FPU13/02235, MECD). D.P-C is A.d.T-fellow (PREDOC13-110541, UV).

51 THE ROLE OF GEMININ IN COLORECTAL CANCER

Maria Villiou[1], Spyridon Champeris Tsaniras[1], Zoi Lygerou[2] & Stavros Taraviras[1]

[1]*Department of Physiology, Medical School, University of Patras, Rio, 26504 Patras, Greece*

[2]*Department of Biology, Medical School, University of Patras, Rio, 26504 Patras, Greece*

Introduction

Colorectal cancer is is a malignant tumor arising from the inner wall of the large intestine and is considered the third leading cause of cancer-related deaths in the Western world. Colorectal cancer can begin as a small adenomatous polyp. Over time some of these polyps become colon cancers. Genomic instability is a hallmark of cancer and occurs early in carcinogenesis.as a result of the loss of control mechanisms present in the normal cell cycle. In our study, we have focused on the role of Geminin, a small .protein that inhibits formation of the pre-replicative complex (pre-RC) at origins of DNA replication and, therefore, cell cycle progression. Geminin, by binding to and inhibiting Cdt1, prevents premature loading of the MCM2-7 helicases complex, thus regulating the fidelity of DNA replication, which occurs once per cell cycle.

Materials & Methods

In order to provide in vivo evidence for the role of Geminin on tumor initiation, we created an induction model of colorectal cancer, with conditionally inactivation of Geminin in the murine large intestine, using the Cre-LoxP system. $Geminin^{flox/flox}$ mice were crossed with $Gem^{KO/WT}$ Lgr5-EGFP-IRES-CreERT2 and $Gem^{KO/WT}$;Villin-Cre transgenic mice, to inactivate the gene in intestinal stem cells and epithelial cells, respectively. The experimental mice were administered azoxymethane (AOM) intraperitoneally and were exposed to the inflammatory agent dextran sodium sulphate (DSS) to develop efficient murine models for colorectal carcinogenesis. Study of macroscopic and histological analysis of the early stages of tumorigenesis was held.

Results

We show that we have a specific action of Cre- recombinase only in the murine intestine of mice, so we can conditionally inactivate Geminin in intestinal stem cells and epithelial cells. We develop an efficient animal model of colitis-related carcinogenesis and we show that mice lacking Geminin develop higher diameters of tumors and have a larger tumor burden compared to sham-treated controls.

Conclusion

We have demonstrated that deregulation of Geminin expression is associated with carcinogenesis. Our study will present the in vivo effect of loss of Geminin in the intestinal cells upon carcinogen exposure as well as the tumorigenic potential of the two mouse lines.

52 RATIONAL DISCOVERY OF APOPTOSIS SIGNAL-REGULATING KINASE 1 INHIBITORS

Galyna Volynets, Segiy Starosyla, Volodymyr Bdzhola, Sergiy Yarmoluk

Institute of Molecular Biology and Genetics, NAS of Ukraine, 150 Zabolotnogo St., 03680, Kyiv, Ukraine
e-mail: galina.volinetc@gmail.com

Increased activity of apoptosis signal-regulating kinase 1 (ASK1) is associated with the pathogenesis of Alzheimer disease, Parkinson's disease, several types of cancer, autoimmune disorders and diabetes suggesting that small compounds inhibiting ASK1 could be useful for the treatment of these pathologies.

We have identified novel chemical class of ASK1 inhibitors, namely benzothiazol-2-yl-3-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-one, using combination of computer modeling techniques and *in vitro* kinase assay. The system based on DOCK software was used to perform semi-flexible docking.

The virtual screening experiments were carried out targeting the ATP-binding pocket of ASK1 by browsing compound library of 270,000 organic compounds. After the docking followed by visual inspection of the best-scored ligand binding poses, the most promising 186 compounds from different chemical classes have been selected for the kinase assay study. In vitro experiments revealed that 5-(4-Chloro-phenyl)-4-(furan-2-carbonyl)-3-hydroxy-1-(6-methoxy-benzothiazol-2-yl)-1,5-dihydro-pyrrol-2-one inhibited ASK1 with an IC_{50} of 4.2 μM. The core structure of this compound was used for developing more potent and selective inhibitors of ASK1. A series of derivatives has been synthesized and evaluated *in vitro* towards human protein kinase ASK1. It was revealed that the most active compound inhibits ASK1 with IC_{50} of 0.52 μM. Structure-activity relationships of 34 compounds have been studied and binding mode of this chemical class has been proposed. Accordingly to the in silico modeling results benzothiazole in the structure of tested compounds is involved in hydrophobic interactions with adenine-binding region of ASK1 ATP-binding site and forms sulfur-p interaction with Val757 located in the hinge region. Also, it was shown, that the structure of substituent which interacts with hydrophobic pocket I is important for compound inhibitory activity toward ASK1.

53 INVESTIGATION OF THE PROTECTIVE POTENTIAL OF NOVEL PLA_2 INHIBITORS AGAINST α-SYNUCLEIN INDUCED INFLAMMATION

Mary Xylaki[1], Evangelia Emmanouilidou[1], Victoria Magrioti[2], George Kokotos[2], Kostas Vekrellis[1]

[1] Center for Basic Research, Biomedical Research Foundation of the Academy of Athens, Greece
[2] Laboratory of Organic Chemistry, Department of Chemistry, University of Athens, Greece
e-mail: mxylaki@bioacademy.gr

α-synuclein accumulation and oligomerization has a major role in the pathogenesis of PD and has been linked with neuronal toxicity and inflammation. Due to an increased significance of inflammation in the development of PD we focused in using novel experimental PLA_2 inhibitors as potential anti-inflammatory agents against α-synuclein toxicity. α-synuclein was shown to interact with fatty acids in a manner that promotes its aggregation and toxicity. Fatty acids such as arachidonic acid and DHA, are released from membrane phospholipids by PLA_2. Elevated activity of PLA_2 and loss of essential membrane glycerophospholipids have been observed in PD. Application of PLA_2 inhibitors in the treatment of Parkinson's disease might be valuable for prevention. We used an SHSY5Y neuroblastoma cell line with inducible α-synuclein overexpression by a TET-OFF system. Our aim was to investigate the effect of PLA_2 inhibitors in the viability of cells overexpressing α-synuclein vs control cells expressing basal levels of the protein. At the same time we investigated the inhibitors' effect in the intracellular levels of a-synuclein and the protein oligomerization. Our data so far indicate significant decrease in α-synuclein endogenous levels and its mediated toxicity. The most potent inhibitors were administered IP in mouse. Two inhibitors were found to cross the BBB using HR-MS. Qualitative analysis of the α-synuclein levels in different brain regions indicated significant changes in protein levels in the striatum. Understanding the role of PLA_2 inhibitors in PD may lead to the identification of a PLA_2inhibitor as a candidate drug for this disease.

LIST OF PARTICIPANTS

Name	Surname	Country	E-mail
Maryam	Adelipour	Iran	m.adelipour@Modares.ac.ir
Alfredselleshteen	Alfred James	India	dr_cmurugan@yahoo.co.in
Sofia	Athanasopoulou	Greece	sathan@eie.gr
Angelo	Azzi	USA	angelo.azzi@tufts.edu
Yulia	Bakakina	Belarus	bakakinay@mail.ru
Vanja	Cejvanovic	Denmark	Vanja.Cejvanovic@regionh.dk
Anita	Cindric Vranesic	Germany	anita.cindric4@gmail.com
Margaret	Clark	Denmark	margaret.clark603@gmail.com
Pascale	Cossart	France	pascale.cossart@pasteur.fr
Hasmik	Davtyan	Armenia	dav.hasmik@gmail.com
Stefano	De Benedetti	Italy	stefano.debenedetti@unimi.it
Barbara	Demeneix	France	bdem@mnhn.fr
Nassim	Faridi	Iran	nassim_faridi@yahoo.com
Mahmoud	Fassad	UK	mahmoud.fassad.14@ucl.ac.uk
Horst	Feldmann	Germany	horst.feldmann@med.uni-muenchen.de
Joachim	Frey	Switzerland	joachim.frey@vetsuisse.unibe.ch
Aikaterini	Gioti	Greece	kategio@hua.gr
Dmytro	Golyshkin	Ukraine	dgolyshkin@gmail.com
Efstathios	Gonos	Greece	sgonos@eie.gr
Sattar	Gorgani-Firuzjaee	Iran	gorgani59@gmail.com
Ana Filipa	Guedes	Portugal	anaguedes@medicina.ulisboa.pt
Joan	Guinovart	Spain	guinovart@irbbarcelona.org
Lisbeth	Heilesen	Denmark	lh@mbg.au.dk
Paola	Hernandez	Uruguay	phernandez@iibce.edu.uy
Zoltan	Hujber	Hungary	zoltan.hujber56@gmail.com
Vasyl	Hurmach	Ukraine	gyrmach@gmail.com
Mariia	Inomistova	Ukraine	m.inomistova@gmail.com
Constantina	Ioannou	Cyprus	constantina_ioannou_t@hotmail.com

Manuel Pedro	Jimenez-Garcia	Spain	manubiomed42@gmail.com
Mathias	Jorgensen	Denmark	mlindh@eng.au.dk
Marianna	Kapetanou	Greece	mkapetanou@eie.gr
Ippolyti	Karvouni	Greece	ipkarvouni@eie.gr
Niki	Kappa	Greece	nikikappa@hotmail.com
Muhammed Erkan	Karabekmez	Turkey	karabekmez@gmail.com
Betul	Karademir	Turkey	betulkarademir@marmara.edu.tr
Nesrin	Kartal Ozer	Turkey	nkozer@marmara.edu.tr
Arsen	Kishmiryan	Armenia	akishmiryan@gmail.com
Laura Kofoed	Kjaer	Denmark	laura.kofoed.kjaer@regionh.dk
Dimitrios	Kletsas	Greece	dkletsas@bio.demokritos.gr
Peter	Kristensen	Denmark	pk@eng.au.dk
Ruchi	Kumari	UK	r.kumari@prion.ucl.ac.uk
Milena	Laban	Denmark	milena@mbg.au.dk
Thomas	Langer	Germany	Thomas.Langer@uni-koeln.de
Evangelia	Lekka	Greece	e.lekka@yahoo.gr
Elisavet	Lioulia	Greece	lioulia@bio.auth.gr
Elina	Makarova	Latvia	elina.makarova@farm.osi.lv
Johanna Elisabeth	Mayrhofer	Austria	Johanna.Mayrhofer@uibk.ac.at
Aleksandra	Mladenovic Djordjevic	Serbia	anamikos@ibiss.bg.ac.rs
Natalia	Nosachuk	Ukraine	natali_vov@ukr.net
Daniel	Perez Cremades	Spain	daniel.perez@uv.es
Gabor	Petovari	Hungary	gaborpetovari@gmail.com
Greg	Petsko	USA	gpetsko@med.cornell.edu
Henrik	Poulsen	Denmark	Henrik.Enghusen.Poulsen@regionh.dk
Ivonne Fanny	Reyes Mandujano	Peru	ivonnefanny@gmail.com
Daniela	Rhodes	Singapore	DRhodes@ntu.edu.sg
Marianthi	Sakellari	Greece	mirella_sak@hotmail.com
Myla	Santiago	Philippines	myla.santiago@gmail.com

Oleksandr	Savytskyi	Ukraine	savytskyi@moldyngrid.org
Zahra	Shahsavari	Iran	shahsavari.z@gmail.com
Denis	Silachev	Russia	silachevdn@genebee.msu.ru
Emine	Terzi	Turkey	emineterzi1990@hotmail.com
Dimitris	Thanos	Greece	thanos@bioacademy.gr
Yanina	Timasheva	Russia	ianina_t@mail.ru
Eckardt	Treuter	Sweden	Eckardt.Treuter@ki.se
Oleg	Tutanov	Russia	ostutanov@gmail.com
Jakob	Ulstrup	Denmark	jakobu@mbg.au.dk
Edijs	Vavers	Latvia	edijs.vavers@farm.osi.lv
Xavier	Vidal Gomez	Spain	xavier.vidal@uv.es
Maria	Villiou	Greece	mavilliou@gmail.com
Galyna	Volynets	Ukraine	galina.volinetc@gmail.com
Mary	Xylaki	Greece	m.xylaki@gmail.com