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National Hellenic Research Foundation (NHRF),

Institute of Biology, Medicinal Chemistry & Biotechnology

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

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

National Hellenic Research Foundation

Scientific Programme

Monday May 23rd

19:30-20:00	Welcome & history of Schools
20:00-21:00	A. Goldberg, USA, “Regulation of Proteasome Function in Normal and Disease States”
21:00	welcome reception

Tuesday May 24th

9:00-10:00	W. Baumeister, Germany, “Structural studies of the 26S Proteasome ex situ and in situ”
10:00-11:00	S. Gonos, Greece, “Proteasome regulation in Aging and Longevity”
11:00-11:30	coffee break
11:30-12:30	A. Goldberg, USA, “Mechanisms for the activation of the ubiquitin-proteasome ` pathway that cause muscle atrophy and cachexia”
12:30-14:00	lunch
14:00-16:00	free time
16:00-17:30	Poster session 1
17:30-18:30	Tutorial 1 (A. Azzi, W. Baumeister, A. Goldberg, S. Gonos, W. Thomas)
18:30-19:00	coffee break
19:00-20:00	M. Walsh, Canada, “Signaling Pathways Mediating Excitation-contraction Coupling in the Vasculature”
20:30	dinner

Wednesday May 25th

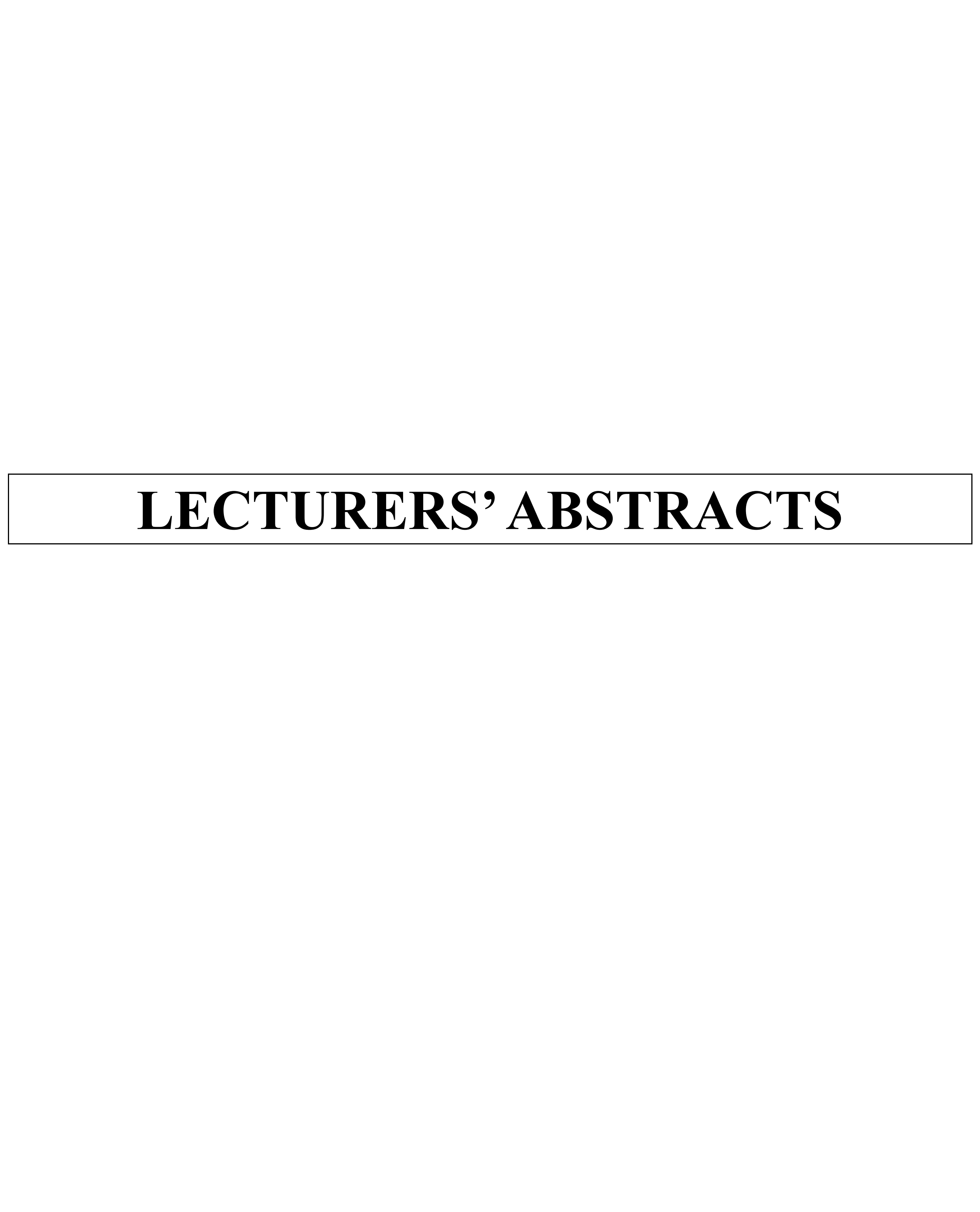
9:00-10:00	M. Brown, USA, “Membrane-mediated Signaling by G-protein-coupled Receptors”
10:00-11:00	A. Chattopadhyay, India, “Membrane Cholesterol and GPCRs: An Intimate Association”
11:00-11:30	coffee break
11:30-12:30	R. Sunahara, USA, “Probing G protein-coupled receptors as allosteric sensors linking hormone binding to G protein activation.”
12:30-14:00	lunch
14:00-16:00	free time
16:00-17:30	Poster session 2
17:30-18:30	Tutorial 2 (M. Brown, A. Chattopadhyay, D. Rhodes, R. Sunahara, M. Walsh)
18:30-19:00	coffee break
19:00-20:00	D. Rhodes, Singapore, “Telomeres in Ageing and Cancer”
20:30	dinner

Thursday May 26th

9:00-10:00	G. Petsko, USA, "The Molecular Basis of Neurodegenerative Diseases: Targeting protein trafficking for the treatment of Alzheimer's disease"
10:00-11:00	W. Thomas, Australia, "Bitter Taste Receptors in Heart: Identification and Potential Function"
11:00-11:30	coffee break
11:30-12:30	C. Glaubitz, Germany, "NMR Spectroscopy on GPCRs and Transporters"
12:30-14:00	lunch
14:00-16:00	free time
16:00-17:30	Young scientists' presentations: C. Bussi, Argentina, B. Fonseca, Portugal, N. Ismail, Malaysia, A. Peluso, Denmark, S. Radovic, Serbia, Y. Yang, China
17:30-18:00	coffee break
18:00-19:00	A. Azzi, USA, "Sliding doors and turning points: events that may shape the future of a young scientist"
20:30	farewell dinner

Friday May 27th

Departure



LECTURERS' ABSTRACTS

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

Angelo Azzi

Tufts University, Boston, USA

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.

STRUCTURAL STUDIES OF THE 26S PROTEASOME *EX SITU* AND *IN SITU*

Wolfgang Baumeister

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The 26S proteasome operates at the executive end of the ubiquitin-proteasome pathway for the controlled degradation of intracellular proteins. The 2.5 MDa complex is built of 34 different subunits and comprises two subcomplexes: the 20S core where proteolysis takes place and one or two regulating particles which prepare substrates for degradation. Whereas the structure of its 20S core particle has been determined by X-ray crystallography two decades ago, the structure of the 19S regulatory particle, which recruits substrates, unfolds them, and translocates them to the core particle for degradation, has remained elusive. Recently, the molecular architecture of the 26S holocomplex was determined by an integrative approach based on data from single particle cryoelectron microscopy, X-ray crystallography, residue-specific chemical cross-linking, and several proteomics techniques. Based on a 6Å structure of the *Saccharomyces cerevisiae* proteasome, and molecular dynamics-based flexible fitting we were able to generate a near-atomic model of the 26 holocomplex. Furthermore, by applying deep image classification to a very large data set, we defined its conformational landscape.

Electron cryotomography allows to perform structural studies of macromolecular and supramolecular structures *in situ*, i.e. in their functional cellular environments. We applied electron cryotomography with a new type of a phase plate, the Volta phase plate, to hippocampal neurons grown on EM grids and were able to locate 26S proteasomes with high fidelity and nanometer precision in these cells. Subtomogram, classification and averaging revealed the existence of different states of assembly and conformational states. From this we can infer the activity status of individual 26S complexes.

MEMBRANE-MEDIATED SIGNALING BY G-PROTEIN-COUPLED RECEPTORS

Michael F. Brown, Udeep Chawla, Suchithranga M. D. C. Perera, Michael C. Pitman, Andrey V. Struts, and Xiaolin Xu

Departments of Chemistry and Physics, University of Arizona, Tucson, Arizona 5721, USA

The *Rhodopsin* family of G-protein–coupled receptors (GPCRs) comprises the targets of the majority of pharmaceuticals used worldwide. Here we address the question: what is the role of the lipids and water of cellular membranes in GPCR signaling [1]? Together with theoretical molecular dynamics (MD) simulations [2], solid-state NMR data [3,4] reveal increased local mobility of retinal upon light activation. Changes in local dynamics of the retinal cofactor initiate large-scale fluctuations of transmembrane helices that expose recognition sites for the signal-transducing G-protein. Effects of membrane lipids on GPCRs are revealed by UV-visible and FTIR spectroscopic studies of the conformational energetics of rhodopsin in recombinant membranes [5]. A new flexible surface model (FSM) describes how the curvature stress field of the membrane governs the energetics of active rhodopsin, due to the spontaneous monolayer curvature of the lipids [4]. The new biomembrane model challenges the standard fluid mosaic model. Rhodopsin becomes a sensor of the spontaneous (intrinsic) curvature of the lipids upon light activation [1]. Additional influences of osmotic pressure reveal that a large number of bulk water molecules are implicated in rhodopsin activation. Rhodopsin signaling is directly coupled to changes in internal hydration ("the flood") within the lipid bilayer. We propose that bulk water underlies the remarkable signaling efficacy of rhodopsin through wet-dry cycling (sponge-like mechanism) of the recognition cleft for its cognate G-protein transducin bound to the lipid membrane. Besides GPCRs, ion channels, transporters, and membrane-bound peptides are all affected by the lipid bilayer thus giving a new paradigm in structural biology. [1] M. F. Brown (2012) *Biochemistry* **51**, 9782. [2] N. Leioatts et al. (2014) *Biochemistry* **53**, 376. [3] X. Xu et al. (2014) *eMagRes* **3**, 275. [4] A. V. Struts et al. (2011) *PNAS* **108**, 8263. [5] U. Chawla et al. (2016) *Angew. Chem. Int. Ed.* **128**, 598.

MEMBRANE CHOLESTEROL AND GPCRS: AN INTIMATE ASSOCIATION

Amitabha Chattopadhyay

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G protein-coupled receptors (GPCRs) are the largest class of molecules involved in signal transduction across membranes, and represent major drug targets in all clinical areas. The serotonin$_{1A}$ receptor is an important neurotransmitter receptor of the GPCR superfamily and is implicated in the generation and modulation of various cognitive, behavioral and developmental functions. We previously demonstrated that membrane cholesterol is necessary for ligand binding, and G-protein coupling of serotonin$_{1A}$ receptors. Interestingly, recently reported crystal structures of GPCRs have shown structural evidence of cholesterol binding site(s). In this context, we reported the presence of cholesterol recognition/interaction amino acid consensus (CRAC) motifs in the serotonin$_{1A}$ receptor. We also showed that the receptor is more stable and compact in the presence of membrane cholesterol. Our recent results utilizing coarse-grain molecular dynamics simulations to analyze the molecular nature of receptor-cholesterol interaction offer interesting insight in cholesterol binding site(s) in the receptor and oligomerization of the receptor. We showed utilizing homo-FRET that the serotonin$_{1A}$ receptor is constitutively oligomerized in live cells, with the possibility of higher order oligomers of the receptor. Progress in deciphering molecular details of the nature of GPCR-cholesterol interaction in the membrane would lead to better insight into our overall understanding of GPCR function in health and disease.

NMR SPECTROSCOPY ON GPCRS AND TRANSPORTERS

Clemens Glaubitz

Institute for Biophysical Chemistry and Centre for Biomolecular Magnetic Resonance, Goethe University Frankfurt, Germany

The tremendous progress in crystallography-based structural biology of membrane proteins and in particular of GPCRs during the last decade has created an urgent need for further spectroscopic analysis of these proteins. In particular NMR spectroscopy offers the opportunity to bridge the gap between structural and functional data. This lecture will provide an introduction and overview about NMR spectroscopy on GPCRs. Experimental requirements and boundary conditions for solution-state NMR on detergent-solubilized and solid-state NMR on membrane embedded proteins will be discussed. Special emphasize will be given to latest technical development such as dynamic nuclear polarisation (DNP). Examples will involve NMR studies on dynamic and kinetic events of receptor activation and the analysis of peptide ligands bound to human GPCRs. These examples will be complemented by application of lipid and drug transporters.

REGULATION OF PROTEASOME FUNCTION IN NORMAL AND DISEASE STATES

Alfred Goldberg, Zhe Sha, Nikolay Kukushkin, and Sudarsanareddy Lokireddy

Dept Cell Biology, Harvard Medical School, Boston, MA 02115, USA

It has generally been assumed that rates of protein degradation by the ubiquitin-proteasome pathway are controlled only through regulation of ubiquitin conjugation. However, we recently uncovered several surprising mechanisms that regulate 26S proteasome function and protein degradation in mammalian cells. Inhibitors of its peptidase activities are valuable research tools (e.g. MG132) and have greatly advanced the treatment of multiple myeloma (e.g. bortezomib). When proteasomes are inhibited or become stalled, the ubiquitin-receptor subunit, Rpn13, becomes polyubiquitinated by a 26S-associated ubiquitin ligase Ube3c. This modification prevents the binding of ubiquitinated substrates and presumably evolved to redirect Ub conjugates from stalled to functional proteasomes. In addition, when proteasomes are inhibited, cells increase the expression of new subunits of the 26S proteasomes, PA200, and p97/VCP complex. This response is triggered by activation of the NRF1 transcription factor. Instead of completely degrading the NRF1 precursor, partially inhibited proteasomes release the active NRF1 fragment, which enters the nucleus and promotes proteasome expression.

Proteasome function in mammalian cells is tightly regulated. The binding of an ubiquitinated protein enhances the proteasome's capacity for proteolysis by stimulating realignment of the ATPases, opening of the 20S's gated entry channel, and ATP hydrolysis. This activation occurs when the ubiquitin chain binds to the 2bS-associated deubiquitinating enzymes, Usp14/Ubp6 or Uch37, and an unfolded domain binds to the ATPases. Substrate deubiquitination, unfolding, and translocation depend on ATP hydrolysis, and the amount of ATP consumed in degrading a ubiquitinated substrate and the time required depend on the tightness of its folding.

There is growing evidence that in the neurodegenerative diseases, the functioning of 26S proteasomes in impaired. In Mad Cow Disease, in mice overexpressing mutant tau in a model of Frontotemporal Dementia, and in mouse models of Amyotrophic Lateral Sclerosis, the proteasome's capacity to degrade ubiquitinated proteins is inhibited. The proteasome's capacity to degrade such ubiquitinated proteins, peptides, and ATP can be activated by phosphorylation of the 19S subunit Rpn6 by Protein Kinase A (PKA) both in cells and in vitro. Agents that activate PKA in cells and cause proteasome phosphorylation promote the degradation of short-lived proteins, but not the bulk of cell proteins. These treatments also stimulate the clearance of several types of aggregation-prone mutant proteins that cause neurodegenerative diseases in cell culture and in vivo. Thus, pharmacological agents that raise cAMP and activate proteasomes may have therapeutic benefits in treating proteotoxic diseases.

MECHANISMS FOR THE ACTIVATION OF THE UBIQUITIN-PROTEASOME PATHWAY THAT CAUSE MUSCLE ATROPHY AND CACHEXIA

Alfred Goldberg, Jinghui Zhao, Jeffrey Brault, Rosanna Piccirillo, Shenhav Orit Cohen

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In recent years there has been dramatic progress in our understanding of the cellular mechanisms responsible for the atrophy of skeletal muscle that occurs with fasting, disuse, nerve injury, and many systemic diseases (e.g. cancer cachexia). In these diverse conditions, the rapid muscle wasting results largely from excessive protein degradation by the ubiquitin proteasome pathway. In these rapidly atrophying muscles, there is a common program of transcriptional changes, in which a set of atrophy-related genes ("atrogenes") are induced or repressed coordinately. Among the most induced proteins are components of the ubiquitin-proteasome pathway, especially the muscle-specific ubiquitin ligases, atrogin-1 and MuRF1.

These atrogenes are induced by the FoxO family of transcription factors, and overproduction of FoxO3 by itself causes dramatic muscle atrophy. MuRF1 is critical in the ordered disassembly and degradation of the myofibrillar apparatus, specifically components of the filaments. However, a distinct E3, Trim32, catalyzes loss of thin (actin) filaments, as well as associated components. Downregulation of Trim32 blocks muscle atrophy and surprisingly can cause rapid growth of normal muscle. The p97/VCP ATPase appears essential for the accelerated breakdown of muscle proteins during atrophy. p97 probably extracts ubiquitinated components from the myofibril since p97DN mutants also block atrophy and become tightly associated with myofibrils. In addition, FoxO3 stimulates the cell's other main proteolytic system, the autophagic/lysosomal pathway, and in atrophying muscles, mRNAs for many autophagy-related genes are induced by FoxO3. Thus, during various types of atrophy, the cell's two main proteolytic systems are activated coordinately to cause the breakdown of different cellular components; the loss of contractile proteins via the ubiquitin-proteasome pathway and of organelles (e.g. mitochondria) via autophagy.

In normal muscle, these two proteolytic systems are coordinately inhibited, and atrophy is prevented by IGF-1 and insulin, which activate the PI3K-mTOR-Akt pathway, and by contractile activity, which causes production of the transcriptional coactivator, PGC-1α. In addition to causing mitochondrial production, PGC-1α inhibits FoxO3 and thus reduces muscle protein degradation by both autophagic and proteasomal systems. Overproduction of PGC-1α in culture or mouse muscles inhibits ubiquitin ligase induction and retards atrophy. This program for muscle wasting in disease states is also activated by myostatin (another TGF family member). Antagonists of the myostatin/activin pathway inhibit FoxO-induced proteolysis and are an attractive therapeutic approach. These agents not only dramatically block cancer cachexia and muscle wasting, but also can prolong the viability of tumor-bearing animals.

PROTEASOME REGULATION IN AGING AND LONGEVITY

Efstathios S. Gonos

National Hellenic Research Foundation, Institute of Biology, Medicinal Chemistry & Biotechnology, Athens, Greece

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.

THE MOLECULAR BASIS OF NEURODEGENERATIVE DISEASES: TARGETING PROTEIN TRAFFICKING FOR THE TREATMENT OF ALZHEIMER'S DISEASE

Vincent J. Mecozzi, Diego E. Berman, Chris Vetanovetz, Sabrina Simoes, Mehraj R. Awal, Remy T. Schneider, Scott A. Small, Rebecca Cox, Burce Ergel, Dagmar Ringe, and Gregory A. Petsko

Appel Alzheimer's Disease Research Institute, Weill Cornell Medical College, New York, NY USA

Retromer is a multi-protein complex that traffics endosomal cargo back to the Golgi and to the plasma membrane. It also plays a vital role in lysosomal maturation and homeostasis. Implicated by human genetics in both sporadic and familial Alzheimer's disease (AD), and more recently in familial Parkinson's disease (PD), retromer has been shown to traffic the Amyloid Precursor Protein (APP) away from the endosome, where the beta-secretase is optimally active, thereby regulating Aβ peptide accumulation. The complex has also been implicated in the trafficking of alpha-synuclein. We have identified pharmacological chaperones that enhance retromer stability and function, with the goal of redirecting APP away from those compartments in which it is proteolyzed. First, we relied on biophysical measurements to identify the 'weak link' of the complex, and to complete an in silico screen of small molecules predicted to enhance retromer stability (the Figure below shows the docked complex). Among the hits, an in vitro assay identified one molecule that stabilized retromer against thermal denaturation by more than 10 degrees C. Second, we turned to cultured hippocampal neurons, showing that the small molecule increases the levels of retromer proteins, shifts APP away from the endosome, and decreases Aβ accumulation dramatically, in a dose-dependent manner. Similar studies have also shown that increase in retromer function rescues several different models of Parkinson's disease. Together, these findings clarify mechanisms of retromer stability, and identify a strategy that has therapeutic potential for AD and possibly also for other neurodegenerative disorders.

TELOMERES IN AGEING AND CANCER

Daniela Rhodes

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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

PROBING G PROTEIN-COUPLED RECEPTORS AS ALLOSTERIC SENSORS LINKING HORMONE BINDING TO G PROTEIN ACTIVATION.

Roger K. Sunahara

Department of Pharmacology, University of California San Diego, 4062 Biomedical Sciences Building, 9500 Gilman Drive, La Jolla, CA 92093, USA.

G protein-coupled receptors (GPCR) are vital communication systems that sense extracellular stimuli and transduce signals across the plasma membrane to intracellular compartments. They serve as sensors for environmental stimuli such as light, odors and tastes, as well as serving the receptors for hormones, chemokines and even pathogens. GPCRs couple to effectors systems that regulate second messengers such as cAMP, cations, and inositol phosphates, through interacting with the heterotrimeric family of G proteins. Recent advances in the structural biology of G protein-coupled receptors by themselves or more recently bound to G proteins have helped to unravel the intricacies of ligand binding. Similarly, structural and biochemical analyses of heterotrimeric G proteins have affirmed our understanding of the mechanism underlying effector interactions and GTPase activity. Our recent crystal structure of the β_2-adrenergic receptor (β_2AR) in a complex with the stimulatory G protein, Gs, trapped in its nucleotide-free state, in particular, has now provided insight into the physical relationship between G proteins and GPCRs. Analysis of the structural data, along with pharmacological and biochemical data, have helped to delineate how hormone binding to GPCRs leads to GDP release on G proteins, the principle step that precedes GTP binding and G protein activation. Here we describe the functional relationship between the receptor and G protein leading to G protein activation and the initiation of cell signaling.

BITTER TASTE RECEPTORS IN HEART: IDENTIFICATION AND POTENTIAL FUNCTION.

Walter G Thomas

The University of Queensland, Australia

G protein-coupled receptors (GPCRs) are seven transmembrane-spanning proteins that represent the largest receptor superfamily in the human genome. GPCRs recognise and bind an array of sensory inputs and ligands, transducing extracellular stimuli into intracellular signals, thereby mediating diverse cellular and physiological responses, as well as the senses of smell, taste, and vision.

GPCRs are critical for cardiovascular function and are targeted by frontline therapies (e.g., β-blockers and angiotensin receptor antagonists) for the treatment of hypertension and heart failure. Current therapies target only a small fraction of the known cardiac GPCR repertoire, indicating that there are many more potential receptor targets and that numerous aspects of heart physiology remain poorly defined.

Although taste receptors are generally considered as exclusive mediators of taste, recent evidence suggests that taste GPCRs have specific functions in tissues beyond the oral cavity, including in the brain, gastrointestinal tract and respiratory system, where they mediate gut hormone release and nutrient absorption, bronchodilation, and innate defence, respectively. Using a combination of RT-qPCR, in situ hybridisation, and gene-targeted reporter mice, we have demonstrated the widespread expression of individual taste receptors (specifically the bitter taste T2R family of GPCRs) in both rodent and human heart. In rodents, these receptors are expressed throughout the myocardium (in cardiomyocytes and fibroblasts) and ligands (that we identified) for these receptors have profound effects on ex vivo heart function. T2R transcripts are readily detected in all four chambers of human heart with comparable abundance to the classically-targeted angiotensin (AT1R) and β1-adrenergic receptors. We now have preliminary data that T2R ligands exert profound decreases in cardiac contractility in human myocardium, which predicts important, new cardiac physiology, controlled by currently unknown systems and ligands.

SIGNALING PATHWAYS MEDIATING EXCITATION-CONTRACTION COUPLING IN THE VASCULATURE

Michael P. Walsh

Department of Biochemistry & Molecular Biology, Cumming School of Medicine, University of Calgary, 3330 Hospital Drive N.W., Calgary, Alberta T2N 4N1, Canada

Vascular smooth muscle contraction regulates blood flow to tissues and organs and protects delicate structures, e.g. glomerular capillaries, from damage due to sudden increases in systemic blood pressure. Contraction is triggered by circulating or locally released molecules, such as angiotensin II, serotonin, endothelin-1 and norepinephrine and, in some blood vessels, by an increase in intraluminal pressure via the myogenic response. Such stimuli elicit an increase in cytosolic free Ca^{2+} concentration by activating Ca^{2+} entry from the extracellular space via voltage-gated or receptor-operated Ca^{2+} channels, and/or by triggering Ca^{2+} release from the sarcoplasmic reticulum via inositol 1,4,5-trisphosphate receptors (mediated by G protein-coupled receptors (GPCRs) coupled to the $G_{q/11}$ family of heterotrimeric G proteins) or ryanodine receptors (via Ca^{2+}-induced Ca^{2+} release). Ca^{2+} binds to calmodulin and the $(Ca^{2+})_4$-calmodulin complex activates myosin light chain kinase (MLCK), which phosphorylates the 20-kDa regulatory light chains of myosin II (LC_{20}) at Ser19. This simple phosphorylation reaction induces a conformational change in myosin II, enabling actin interaction, which enhances the MgATPase activity of the myosin II, thereby providing the energy necessary for cross-bridge cycling and contraction. Following removal of the stimulus, Ca^{2+} is removed from the cytosol, MLCK is inactivated, LC_{20} is dephosphorylated by myosin light chain phosphatase (MLCP) and the muscle relaxes. Some contractile stimuli also induce Ca^{2+} sensitization via a pathway involving the small GTPase RhoA and Rho-associated kinase (ROCK), which phosphorylates the myosin targeting subunit (MYPT1) of MLCP to inhibit its activity, thereby increasing the MLCK: MLCP activity ratio, LC_{20} phosphorylation and contraction. Finally, acting via pathways involving ROCK and protein kinase C, contractile stimuli lead to the phosphorylation of cofilin and HSP27, respectively, to induce polymerization of a cortical pool of actin, which increases the formation of adhesome complexes that distribute and transmit force across the plasma membrane and to the extracellular matrix.

POSTER ABSTRACTS

1 NEOADJUVANT CHEMOTHERAPY CONTRIBUTES TO THE LONGER SURVIVAL OF THE PATIENTS DIAGNOSED WITH NONSMALL-CELL LUNG CARCINOMA WITH SPECIFIC MOLECULAR ALTERATIONS

[a]Jasna Bankovic, [b]Jelena Stojsic, [a]Tijana Stankovic, [a]Sonja Stojkovic, [a]Jelena Dinic, [a]Zorica Milosevic, [b]Zorka Milovanovic, [a]Nikola Tanic

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Accounting for approximately 80% of all lung carcinomas, the non-small cell lung carcinoma (NSCLC) is the most common clinical form of lung cancer with its two predominant histological types, adenocarcinoma (ADC) and squamous cell carcinoma (SCC). Neoadjuvant chemotherapy (NAC) is an accepted treatment modality since despite surgical resection relapse is still high. In this study we examined whether some of the key molecules associated with the RAS/RAF/MEK/ERK and PI3K/AKT/mTOR signaling pathways could have predictive and prognostic value for the NAC application. The expression status of PTEN, pAKT, pERK and loss of heterozygosity (LOH) of PTEN were analyzed in two groups of NSCLC patients, those who received and those who did not receive NAC. LOH PTEN and low pERK expression was shown to be correlated with the longest survival of patients with SCC and ADC, respectively, who received NAC. These results point that the application of NAC is beneficial in the NSCLC patients with specific molecular alterations which could further help to improve constant search for the druggable molecular targets used in personalized therapy.

2 INDUCTION OF AUTOPHAGY IN BV2 MICROGLIAL CELLS MODULATES PRO-INFLAMMATORY MEDIATORS LEVELS AND RESCUES BOTH LPS AND ALPHA-SYNUCLEIN-INDUCED NEURONAL CELL DEATH.

Bussi C., Peralta Ramos J., Gaviglio EA., Arroyo DS., Gallea JI, Celej MS, Iribarren P.
Center for Research in Clinical Biochemistry and Immunology (CIBICI-CONICET), Argentina.
cbussi@fcq.unc.edu.ar

Autophagy is a fundamental cellular homeostatic mechanism, whereby cells autodigest parts of their cytoplasm for removal or turnover. Paradoxically, although autophagy is primarily a protective process for the cell, it can also play a role in cell death. Microglial cells (MC) are resident macrophages in the central nervous system (CNS) and have multiple functions, such as phagocytosis, production of growth factors and cytokines, and antigen presentation.

The aim of this study was to evaluate the effects of autophagy on the production of pro-inflammatory mediators by BV2 MC, and on neuronal viability in a co-culture model.

Autophagy was induced in MC before or after TLR or alpha-synuclein (α-syn) stimulation by rapamycin or trehalose and blocked by using 3-Methyladenine. Supernatants were isolated and analyzed by ELISA and Griess assay to determine cytokines and nitric oxide (NO) levels, respectively. Autophagic activity was followed by confocal microscopy and WB. Cell death was evaluated using AnnexinV/propidium iodide staining and subsequent flow cytometric analysis.

Autophagy induction in BV2 cells before LPS or α-syn stimulation downregulated IL1β, IL-6, TNFα and NO production ($p<0.01$).

In addition, we observed in BV2/N2A co-cultures stimulated with LPS or α-syn fibers that induction of autophagy in microglial cells rescued LPS and α-syn-induced neuronal cell death ($p<0.05$).

Moreover, we wanted to determine if autophagy activity could regulate the phosphorylation status (p-) of p38 and ERK1/2, two MAPKs involved in cellular programs such as inflammation and cell death. We observed a reduction in both p-p38 and p-ERK1/2 levels after inducing autophagy in MC stimulated with α-syn fibers or LPS ($p<0.05$).

Despite the increasing reports studying the effects of autophagy in the CNS, slightly emphasis is placed on MC. These results suggest that modulation of microglial cells by autophagy could be an important strategy in the context of neurodegenerative diseases.

3 TRANSCRIPTOME PROFILING IN THE AGED RAT BRAIN FOR THE PREDICTION OF AGE-ASSOCIATED COGNITIVE DECLINE

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Background: Cognitive decline is a key threat to successful ageing and presence in continuum with advancing of age. To date, it is still uncertain the indicator between physiological and pathological changes. Alterations in pathways related to the ageing process include oxidative stress, lipid metabolism, and inflammation. However, the exact mechanism involved in age-associated cognitive decline is still unclear. This study is sought to determine the differentially expressed genes in rat's brain regions at different ages and correlate the findings with the cognitive functions that may predispose to the brain ageing process.

Methods: Cognitive functions tests including open field test, Morris water maze and object recognition test were determined at the age of 14, 18, 23 and 27 months old and were compared with young group (3 months old). Upon the completion of the cognitive assessment at each age, brain regions associated with cognitive functions hippocampus (HC), striatum (ST) and medial prefrontal cortex (mPFC) were carefully dissected and total RNA was extracted and proceed with microarray analysis (Affymetrix GeneChip® Rat Gene 2.0 ST Array). Raw signal intensity data was subjected to background subtraction and quantile normalization by Affymetrix® Expression Console™ Software. Analysis of variance (ANOVA), Gene Set Enrichment Analysis (GSEA) and pathway enrichment analysis of normalized probe intensities values were performed using Partek® Genomic Suite® (v. 6.6).

Results: Across different ages, our behavioral data indicated that the exploratory behaviors were negatively correlated with age. We also found that the levels of anxiety in the older rats were higher compared to the younger rats. In respect to the microarray data, a total of 4,619 genes were differentially expressed ($p<0.05$) in 14, 18, 23, and 27 months old HC when compared with young 3 months old groups. Among these genes, 2,384 genes were downregulated and 2,235 genes were upregulated. These genes were enriched, showing that the genes were involved in 22 KEGG pathways with enrichment score more than 3.0 ($p<0.05$), which include ErbB signaling pathway, synthesis and degradation of ketone bodies, antigen processing and presentation, metabolic pathways and oxidative phosphorylation. Besides, a total of 3,045 genes were differentially expressed ($p<0.05$) in ST when compared with young 3 months old groups where 1,460 genes were downregulated and 1,585 genes were upregulated. These genes were enriched in 16 KEGG pathways with enrichment score more than 3.0 ($p<0.05$), which include oxidative phosphorylation, metabolic pathways, glutathione metabolism, and synthesis and degradation of ketone bodies. On the other hand, a total of 656 genes were differentially expressed ($p<0.05$) in mPFC when compared with 3 months old group. Among these genes, 348 genes were downregulated and 308 were upregulated. These genes were enriched in 6 KEGG pathways with enrichment score more than 3.0 ($p<0.05$). Pathways were ECM-receptor interaction, complement and coagulation cascade, RNA degradation and protein digestion and absorption.

Conclusion: Our studies suggest that the analysis of transcriptome in specific brain region could be utilized to gain more comprehensive understanding of brain ageing process and identify potential biomarkers for age-associated cognitive decline.
Keywords: Cognitive decline, normal ageing, rat, brain, gene expression

4 EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF VOLATILE CONSTITUENTS ISOLATED FROM *TRICHODERMA VIRIDE* AND *CHAETOMIUM GLOBOSUM*

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Abstract:

The present study aimed to evaluate the antioxidant and antimicrobial activities of the volatile constituents isolated from two fungal species. The volatile constituents have been isolated from the fresh mycelia of *Trichoderma viride* (0.19%) and *Chaetomium globosum* (0.2%) then subjected to GC/MS analysis. Cyclooctanol, Caryophyllene oxide and α-Bisabolol (8.48%, 5.12 % and 5.04%, respectively) are the major volatile constituents in *T. viride* mycelia. While Limonene, 1,8-Cineole, *ρ*-Allylanisole and α-Myrcene (15.96%, 10.99%, 10.07% and 6.66%, respectively) are the major constituents in the volatiles of *C. globosum* mycelia.

The volatiles isolated from *T. viride* and *C. globosum* are subjected to evaluation of the antioxidant activities using DPPH- free radical, and the antimicrobial activities using the disc diffusion method, the volatiles isolated from the two fungi record high antioxidant effects by 29.62%,63.12%, 70.37% for *T. viride* and 33.33%, 55.55%, 66.60% for *C. globosum* at concentrations of 10, 50 and 100µg, respectively. Also, evaluation of the antifungal activities was performed against the yeast *Candida albicans,* and the phytopathogenic fungi (*Fusarium solani*, and *Rhizoctonia solani*). While evaluation of the antibacterial activities was performed against *Bacillus subtilis* (Gram-positive bacteria), and *Escherichia coli* (Gram-negative bacteria).

The volatile constituents of both fungi show significant antifungal and antibacterial effects. On the other hand, the volatile constituents isolated from *T. viride* show higher antibacterial effect against both *B.subtilis* and *E. coli* and antifungal effect against both *F. solani*, and *R. solani* than that isolated from *C.globosum.* Moreover, the volatiles isolated from *C. globosum* record higher antifungal effect against *Candida albicans* than that isolated from *T. viride.*

Keyword: *Trichoderma viride*, *Chaetomium globosum,* Volatile Constituents, Antioxidant*,* Antifungal, Antibacterial.

5 CANNABINOIDS INDUCE ENDOMETRIAL CANCER CELL DEATH THROUGH DIFFERENT SIGNALLING PATHWAYS: INVOLVEMENT OF TRANSIENT RECEPTOR POTENTIAL VANILLOID 1 IN APOPTOSIS OF ER^{+} CELLS

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The endogenous cannabinoids-endocannabinoids (eCBs) have a range of physiological activities that are, like in the case of Δ9-tetrahydrocannabinol (Δ9-THC), mediated by cannabinoid receptors. Anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are considered the major eCBs. Endometrial cancer (EC) is the most common gynecological cancer in developed world and may occur in two forms: type 1, which is classified as being estrogen-dependent and type 2, which is estrogen independent. Several different types of cancer have abnormal regulation of the endocannabinoid system that contributes to cancer progression and correlates to clinical outcomes. Here, besides the transient receptor potential vanilloid 1 (TRPV1), we report the biochemical characterization of the endocannabinoid system in Ishikawa and Hec50co cell lines, which models for the two relevant types of EC, type 1 and type 2, respectively. Using the MTT assay, we observed that at concentrations higher than 5 µM, eCBs induced a significant reduction in cell viability in both Ishikawa and Hec50co cells, whereas THC treatment did not show any significant differences in cell viability. We further demonstrate that AEA-induced cell death in Ishikawa cells resulted in chromatin condensation, as revealed by Giemsa and Hoescht staining, and a significant increase in caspase -3/-7 activities, through TRPV1 mediated action indicating an apoptotic process. Moreover, these effects were accompanied by a decrease in mitochondrial membrane potential, an increase in ROS release and intracellular Ca^{2+} levels in response to TRPV1 activation. However, in Hec50co cells, eCBs induced cell death with an increase in LDH release, suggesting a cytotoxic effect. Understanding the exact mechanism by which eCBs regulate different signaling pathways will eventually lead to a targeted clinical approach. Altogether, the present data indicates that eCBs modulate EC cell death and, thus, eCBs machinery may constitute a new target for the development of chemotherapeutic agents to be used against EC.

6 SURFACE ACOUSTIC WAVES-BASED MOLECULAR RECOGNITION OF A COLLAGEN RECEPTOR ON HUMAN ERYTHROCYTES

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The integrin - mediated binding is important for the metastatic dissemination of different types of cancer cells. Snake venom disintegrins obtustatin and echistatin are potent, irreversible and selective inhibitors of $\alpha1\beta1$ and $\alpha v\beta3$ integrins respectively. Aiming to describe the structural requirements of disintegrins for membrane-target recognition, the affinity of these specific binding have to be elucidated, being this topic an issue of extremely importance for the human health. Obtustatin is the shortest disintegrin yet described, containing only 41 amino acids. It contains a similar pattern of cysteines to the short disintegrin echistatin but contains the sequence KTS rather than RGD in its active site loop. To confirm molecular recognition of disintegrins by their substrates, a surface acoustic wave-biosensor was applied. The human erythrocyte ghost cells were immobilized at the sensors to allow for detection of kinetic binding constants of disintegrins compared to GUVs surface. Obtustatin binds to erythrocyte ghost membrane with affinity in mid-nanomolar range (2.32×10^{-7} M), and of echistatin in the low micromolar range, which clearly indicates specific molecular recognition for both disintegrins, but the higher response for obtustatin. The data provide evidence for a direct confirmation of disintegrin binding to erythrocyte ghost membrane and thus, contribute to prove the presence of integtrins in the red cell membranes earlier neglected.

7 CURCUMIN IMPROVES HIGH GLUCOSE INDUCED LIPOGENESIS VIA AUTOPHAGY INDUCTION IN HEPG2 CELLS

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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

8 EFFECTS OF ACE GENE POLYMORPHISM AND KIDNEY MALFUNCTION IN THE OCCURRENCE OF MYOCARDIAL INFARCTION IN BANGLADESHI POPULATION

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Abstract

Myocardial infarction (MI), which is the most important manifestation of coronary artery disease, is the leading cause of morbidity and mortality in the world. The aim of the present study was to find out the effects of ACE gene polymorphism and kidney malfunction in the risk of Myocardial Infarction in Bangladeshi population. A case-control study on 100 cardiac patients who has experienced MI one or more times and 120 control subjects was conducted. The serum troponin I level and kidney functional tests were was measured using Demension Xpand Plus Biochemistry analyzer. The genotyping of ACE insertion/deletion (I/D) was done using polymerase chain reaction. Over all we found significantly ($p < 0.01$) higher level of troponin I in cardiac patients when compared to the control subjects. The serum creatinine, blood urea nitrogen (BUN) and uric acid levels were significantly ($p < 0.001$, respectively) higher in patients when compared to controls. A positive correlation of troponin I was found with creatinine, blood urea nitrogen and uric acid. The percentage of II allele was significantly ($p < 0.05$) higher in control subjects. On the other hand, the DD genotype frequency was significantly ($p < 0.01$) higher in patients. The individual with DD allele was at 3.2 fold risk (odds ratio = 3.28; 95 % confidence interval =1.6 to 6.7; $p < 0.01$) of experiencing MI while individual with ID genotype was at lower risk. The cigarette smokers with DD genotypes were found to have a 4.1-fold increased risk to develop cardiac disease (OR=4.1; 95 % CI=1.7 to 10.5; $p < 0.01$). The patients who have DD genotypes of ACE gene have significantly ($p < 0.05$) higher level of troponin I when compared to II genotypes. Thus our recent study suggested that ACE (I/D) gene and kidney malfunction may have a strong association with the occurrence of myocardial infarction in Bangladeshi population.

Keywords: ACE, Kidney malfunction, Myocardial infarction, Polymorphisms.

9 PROTEOMICS ANALYSIS OF THE EPITHELIAL SODIUM CHANNEL (ENAC) AND S100A10 INTERACTION

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Abstract

S100A10 is a calcium-binding protein that binds to the light chain of annexin A2 to form a heterotetramer prior trafficking of many ion protein channels to the plasma membrane. This study proposed the interaction of S100A10 and epithelial sodium (Na^+) channel (ENaC) as a potential candidate in ENaC regulation. Proteomics analysis revealed the overexpression of S100A10 in HEK293 cells interacts with ENaC subunits and many of other regulatory proteins. We scrutinized many of trial and errors in S100A10 overexpression and further clarified its expression with spectrometry analysis. Trafficking of ENaC to the cell surface is poorly understood and we anticipate this approach would confirm that a complex of ENaC and S100A10 forms in cells. The identity of other proteins closely associated with the ENaC-S100A10 complex has shed some light to understand ENaC trafficking to the plasma membrane. Identification of novel cellular molecules and mechanisms that alter the delivery of ENaC to the cell surface may lead to new gene candidates for high and low blood pressure conditions.

10 MODULATION OF MEMBRANE CHOLESTEROL AND SPHINGOLIPIDS IN LIVE CELLS ENHANCES LIGAND BINDING AND G-PROTEIN COUPLING OF HUMAN SEROTONIN$_{1A}$ RECEPTORS

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The serotonin$_{1A}$ receptor is an important neurotransmitter receptor that belongs to G protein-coupled receptor (GPCR) family. It is involved in the generation and modulation of a variety of cognitive and behavioral functions and serves as an important drug target.[1,2] The emergence of GPCRs in general as major clinical drug targets has led to increased focus of research in exploring important aspects of their function, such as ligand binding, G-protein coupling and downstream signaling.[3] Earlier work from our laboratory has demonstrated the sensitivity of the function of the serotonin$_{1A}$ receptor to membrane cholesterol and sphingolipids.[4-6] In our previous work, we highlighted the structural features of cholesterol required for the function of the serotonin$_{1A}$ receptor.[7] In the present study, in order to explore the structural stringency of cholesterol required for the receptor function, we oxidized hydroxyl group of membrane cholesterol in live cells, stably expressing the receptor, utilizing cholesterol oxidase. In parallel, to understand the role played by membrane sphingolipids in the function of the serotonin$_{1A}$ receptor, we stably expressed the receptor in CHO conditional mutant cell lines defective in sphingolipid biosynthesis (termed LY-B cells). Importantly, our results show that the oxidation of hydroxyl group of membrane cholesterol and the modulation of sphingolipid content in live cells resulted in enhancement of agonist binding and G-protein coupling to the serotonin$_{1A}$ receptor. These results extend our understanding of the structural requirements of cholesterol and the role of sphingolipids in the function of the serotonin$_{1A}$ receptor and could provide better insight into receptor function in health and disease.

References:

1. Pucadyil, T.J., Kalipatnapu, S., Chattopadhyay, A. (2005) *Cell. Mol. Neurobiol.* 25, 553-580.
2. Chattopadhyay, A. (2014) *Adv. Biol.* 2014, 143023.
3. Katritch, V., Cherezov, V., Stevens, R.C. (2013) *Annu. Rev. Pharmacol. Toxicol.* 53, 531-556.
4. Pucadyil, T.J., Chattopadhyay, A. (2006) *Prog. Lipid Res.* 45, 295-333.
5. Jafurulla, M., Chattopadhyay, A. (2013) *Curr. Med. Chem.* 20, 47-55.
6. Jafurulla, M., Chattopadhyay, A. (2015) *Eur. J. Pharmacol.* 763: 241-246.
7. Chattopadhyay, A., Rao, B.D., Jafurulla, M. (2015) *Methods Enzymol.* 557: 117-134.

11 PROTEASOME ACTIVATION DELAYS SENESCENCE AND IMPROVES STEMNESS OF HUMAN MESENCHYMAL STEM CELLS.

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Adult stem cells are critical for tissue regeneration 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 degeneration of tissues and to age-related diseases. The proteasome, being the main cellular proteolytic system, plays a key role in the maintenance of protein homeostasis and loss of its function 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 indicated 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 detected accumulation of oxidatively modified proteins in senescent stem cells. Interestingly, we demonstrated that progression of senescence and the concomitant failure of proteostasis negatively impacts on hMSC stemness. Remarkably, insights suggested that the progressive deterioration of proteostasis can be countered through proteasomal activation and compensate for the loss of proliferation and differentiation capability of hMSCs. In support, we confirmed an interaction between factors of the pluripotency network and the proteasome system. A better understanding of the mechanisms regulating proteostasis in stem cells will pave the way to the development of advanced stem cell-based approaches to improve human healthspan and lifespan.

12 EMT AND MIGRATION IN OVARIAN CARCINOMA CELLS ARE REGULATED BY UTP AND ADENOSINE

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Nucleotides and nucleosides are signaling molecules that have a variety of roles mediating paracrine or autocrine activities by acting through specific membrane receptors. Their participation in cancer has been studied but it is still not clear. In this work, we studied the role played by UTP and adenosine in the migration of ovarian carcinoma-derived cells SKOV-3.
Stimulation of carcinoma-derived SKOV-3 cells with UTP (100 µM) increased migration (≈57%), while apyrase (10 U/mL), an ectonucleotidase that catalyzes dephosphorylation of purine and pirimidine nucleotides, decreased basal migration (≈47%). P2RY2 was found to be the receptor mediating these effects because the knock down of this receptor blocked the UTP-induced cell migration, and was dependent on EGFR transactivation. UTP effect on migration was also associated with epithelial to mesenchymal transition (EMT), since it was associated with an increase of *snail* and *twist* expression, known EMT inductors, as well as an increase of vimentin expression, a marker protein for mesenchymal phenotype. In turn, the inhibitory effect of apyrase over SKOV-3 basal migration was associated with an enrichment of E-cadherin in the cell contacts, suggesting the establishment of an epithelial phenotype. This observation strongly suggests the possible role of adenosine inhibiting the invasive ability in these cells.
To analyze the effect of extracellular adenosine over cell migration, we studied the effect of adenosine and a set of drugs that modify the adenosine activity, adenosine 5'-(α,β-methylene) diphosphate (APCP), an inhibitor of the enzyme that turns AMP into adenosine (NT5E); adenosine deaminase (ADA), the enzyme that catalyzes the desamination of adenosine to inosine; dipyridamole (DPR), an inhibitor of the adenosine uptake mediated by concentrative nucleoside transporters (CNT). By blocking NT5E enzyme with APCP and degrading adenosine with ADA, migration was unaltered even in the presence of apyrase. However incubation with DPR induced a reduction of basal migration (≈36%), that was even more accentuated with adenosine (100 µM) (≈64%), suggesting that extracellular adenosine could be acting upon ADORA receptors.
Our results suggest that released nucleotides acting upon P2RY2 receptors are inductors of mesenchymal phenotype, while adenosine acting over an ADORA receptor could have antagonistic effects by promoting an epithelial phenotype in ovarian carcinoma cells.
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13 PINEAL INVOLVEMENT IN CIRCADIAN REGULATION OF CGMP OSCILLATION IN RAT LEYDIG CELLS

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It is established that pineal is involved in circadian regulation of testosterone secretion from Leydig cells. However, the precise routes of this regulatory involvement are still unknown. As cGMP has been also regarded as modulator of steroidogenesis we sought to study the effects of pineal removal on the circadian pattern of cGMP variations and expression of the genes that encode elements of NO-cGMP signaling pathway in adult rat Leydig cells.

Data revealed circadian transcriptional pattern of *Nos2*, *Nos3* (genes encoded NO producers) and *Pde5a* (gene for cGMP remover) in Leydig cells from adult rats. Pinealectomy significantly increased expression of *Nos2* which lost rhythm and increased and delayed amplitude of *Nos3* expression. Further, pinealectomy initiated cyclic transcription of *Gucy1b3* and non-cyclic transcription of *Gucy1a3* (genes encoded cGMP producers) and increased mesor and amplitude of *Pde5* transcription. The transcription of *Prkg1*, the main effector in this signaling pathway was not affected with pineal abolition. Additionally, pinealectomy did not influence the circadian transcription profile of *Coxi2* or other investigated genes (*Coxi1*, *Nrf1*, *Nrf2a*, *Pgc1a*) related to mitochondrial function and biogenesis. Finally pinealectomy reversed phase of circadian cGMP oscillation in Leydig cells, increased amplitude and slightly advanced peak of serum testosterone oscillation.

Results suggested pineal influence on circadian rhythm of NO-cGMP signaling in Leydig cells. Further studies based on these data are needed to better understand the relationship between pineal and circadian rhythm of testosterone production

This work was supported by the MS173057 and APV0970.

14 EFFECTS OF MASTOPARAN ON HUMAN RED BLOOD CELLS: LIGHT AND ELECTRON MICROSCOPIC OBSERVATIONS IN CORRELATION WITH MEASUREMENTS OF WATER PERMEABILITY BY NMR

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Mastoparan (MP), an amphipathic peptide with 14 amino acid residues, the most studied toxin in wasp venom, has many effects on cell membranes (formation of pores, perturbation of transmembrane signalling by direct interaction with G proteins on the cytoplasmic face), depending on the type of cells and structures (liposomes, secretory vesicles, mitochondria). MP has been discussed as a potential antimicrobial, antiviral, antitumor (Moreno and Giralt, *Toxins* 2015). In case of red blood cells (RBCs) hemolytic effects have been described, as well as a possible regulation by GTP of the water channel in aquaporin 1 (AQP 1) (Abu-Hamdah *et al.*, *Cell Biol. Int.,* 2004).
We studied the effects of MP on the human RBC morphology by light microscopy (using an Olympus BX 43 with a CCD camera CX30) and by environmental scanning electron microscopy (ESEM, on a FEI Quanta 250), in correlation with the effects on the diffusional water permeability (P_d) using a NMR methodology previously described (see the review by Benga, *Mol Asp Med,* 2012a).
Exposure of RBCs to MP induced increases in diameter values and other morphological changes (fusion of swollen cells observed by ESEM analysis), dependent on the MP concentration and exposure to p-chloromercuribenzoic acid (PCMB). In addition, MP produced a small, but highly statistically significant increase, in the P_d. This effect of MP on P_d was very rapid, concentration dependent and partial reversible. The effect was abolished after inhibition of AQP1 by PCMB.
Taken together these results indicate that MP is activating the water channel of AQP1 from the human RBC membrane. This conclusion is very important, since it is for the first time that an activation of P_d in human RBCs has been observed. So far only inhibition of P_d by various compounds has been described (reviewed by Benga, *Eur Biophys J.,* 2013).

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

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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.

16 EPINEURIUM-MIMICKING CONDUITS ENRICHED WITH SIGNALING MOLECULES FOR PERIPHERAL NERVOUS TISSUE ENGINEERING

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An increasing need for improving the life quality of patients suffering from peripheral nerve injuries has inspired scientists to seek for alternative approaches to existing solutions. Nowadays, the standard clinical procedures for treating PNIs, which are based on end-to-end coaptations and autogenic or allogenic grafting, are being replaced by biofunctionalized polymeric implants called nerve guidance conduits. The newest concept is focused on creating a structure with properties mimicking key peripheral nervous system morphological features as well as developmental mechanisms, and thus enhancing regeneration of transected peripheral nerves.

An attractive alternative for treatment of peripheral nerve injuries seems to be a bioartifical conduit structurally mimicking the epineurium of nerves. This approach can be supported by the fact that native epineural allografts have been shown to be an attractive alternative for autografts. Unfortunately, the allografts evoke substantial immune response and the simultaneous administration of immunosuppressive agents is needed. Therefore, the use of allografts is limited only to extreme clinical situations.

Taking the above-mentioned advances and concerns into account, we focused our efforts on recreating the native physicochemical as well as biological performance of the epineurium in artificial conduits. For this purpose we used an electrodeposition method, which was developed in our laboratory and described earlier (Nawrotek et al., 2015). The method allows controlling both the thickness as well as the porosity of conduits. Moreover, the method shows off-the-shelf convenience as conduits can be produced with the desired inner dimension. In this study, we modified the structure of conduits by admixing both polymers constituting native epineural tissue and developmental signaling cues. The obtained conduits show good cell adhesion, proliferation, and viability when contacting with hippocampal cell lines. Moreover, their physicochemical as well as mechanical features are comparable to the ones of the native peripheral nervous tissue.

Reference:

1. Nawrotek, K., Tylman, M., Rudnicka, K., Balcerzak, J., Kamiński, K., 2016. Chitosan-based Hydrogel Implants Enriched with Calcium Ions Intended for Peripheral Nervous Tissue Regeneration. Carbohydr. Polym. 136, 764–771

17 THE ROLE OF ARF6 IN HUMAN EMBRYONIC STEM CELL SIGNALING

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ARF6 is a low molecular weight GTPase localizing to the plasma membrane and endosomal compartments where it regulates endocytic membrane trafficking and actin remodeling[1]. As ARF6 cycles through its active and inactive conformations, it regulates cell surface ligand internalization, post-internalization trafficking along the endocytic pathway and endosomal recycling and fusion of an endosomal membrane with the plasma membrane[2]. Through its effector proteins, Arf6 affects many cellular functions including cell motility, adhesion[3], abscission[4] and lipid homeostasis[5].
Human embryonic stem cells (hESCs) are typically isolated from the inner cell mass (ICM) of preimplantation blastocysts[6]. Their ability to differentiate into all germ layers and self-renew indefinitely *in vitro* has drawn significant scientific attention, as hESCs constitute a potential therapeutic tool for a plethora of different diseases. A vast number of excellent publications has underlined the importance of various signaling pathways that are implicated in the elaborate processes of pluripotency and differentiation. Key components of this finely balanced network are members of the TGF-β superfamily. The ActivinA/TGF-β family ligands, which are able to sustain the pluripotent profile of hESCs[7], [8], signal through heteromeric complexes of type I and type II transmembrane serine/threonine kinase receptors which phosphorylate SMAD2/3 proteins. The phosphorylated SMAD2/3 proteins oligomerize with SMAD4, translocate to the nucleus and regulate transcription using a large network of interactions with transcription factors, co-activators and co-repressors[9]. On the other hand, the BMP4 family ligand, which signals through SMAD1/5/8, promotes differentiation of hESCs through a similar mechanism[10].
Taking into account the significant role of ARF6 in all the aforementioned cellular processes along with their obvious implication in hESCs signaling, an involvement of the GTPase could be reasonably hypothesized.

References:

[1] C. D'Souza-Schorey and P. Chavrier, "ARF proteins: roles in membrane traffic and beyond.," *Nat. Rev. Mol. Cell Biol.*, vol. 7, no. 5, pp. 347–58, May 2006.

[2] C. D'Souza-Schorey, E. van Donselaar, V. W. Hsu, C. Yang, P. D. Stahl, and P. J. Peters, "ARF6 targets recycling vesicles to the plasma membrane: insights from an ultrastructural investigation.," *J. Cell Biol.*, vol. 140, no. 3, pp. 603–16, Feb. 1998.

[3] N. Balasubramanian, D. W. Scott, J. D. Castle, J. E. Casanova, and M. A. Schwartz, "Arf6 and microtubules in adhesion-dependent trafficking of lipid rafts.," *Nat. Cell Biol.*, vol. 9, no. 12, pp. 1381–91, Dec. 2007.

[4] J. K. Schweitzer and C. D'Souza-Schorey, "Localization and activation of the ARF6 GTPase during cleavage furrow ingression and cytokinesis.," *J. Biol. Chem.*, vol. 277, no. 30, pp. 27210–6, Jul. 2002.

[5] N. Naslavsky, R. Weigert, and J. G. Donaldson, "Characterization of a nonclathrin endocytic pathway: membrane cargo and lipid requirements.," *Mol. Biol. Cell*, vol. 15, no. 8, pp. 3542–52, Aug. 2004.

[6] J. a Thomson, J. Itskovitz-Eldor, S. S. Shapiro, M. a Waknitz, J. J. Swiergiel, V. S. Marshall, and J. M. Jones, "Embryonic stem cell lines derived from human blastocysts.," *Science*, vol. 282, no. 5391, pp. 1145–7, Nov. 1998.

[7] L. Vallier, M. Alexander, and R. a Pedersen, "Activin/Nodal and FGF pathways cooperate to maintain pluripotency of human embryonic stem cells.," *J. Cell Sci.*, vol. 118, no. Pt 19, pp. 4495–509, Oct. 2005.

[8] D. James, A. J. Levine, D. Besser, and A. Hemmati-Brivanlou, "TGFbeta/activin/nodal signaling is necessary for the maintenance of pluripotency in human embryonic stem cells.," *Development*, vol. 132, no. 6, pp. 1273–82, Mar. 2005.

[9] A. Nakao, T. Imamura, S. Souchelnytskyi, M. Kawabata, A. Ishisaki, E. Oeda, K. Tamaki, J. Hanai, C. H. Heldin, K. Miyazono, and P. ten Dijke, "TGF-beta receptor-mediated signalling through Smad2, Smad3 and Smad4.," *EMBO J.*, vol. 16, no. 17, pp. 5353–62, Sep. 1997.

[10] R.-H. Xu, X. Chen, D. S. Li, R. Li, G. C. Addicks, C. Glennon, T. P. Zwaka, and J. a Thomson, "BMP4 initiates human embryonic stem cell differentiation to trophoblast.," *Nat. Biotechnol.*, vol. 20, no. 12, pp. 1261–4, Dec. 2002.

18 AT2-RECEPTOR STIMULATION PROMOTES NO RELEASE THROUGH ENOS SERINE 1177 PHOSPHORYLATION AND ENOS TYROSINE 657 DEPHOSPHORYLATION

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Angiotensin AT2 receptor (AT2R) stimulation promotes vasodilation by increased nitric oxide (NO) release from endothelial cells. However, the mechanisms underlying AT2R-induced NO synthesis are still not completely characterized. We investigated whether AT2R-stimulation activates endothelial NO synthase (eNOS) by phosphorylation at Ser1177 or dephosphorylation at Tyr657 thus increasing the production of NO, and the participation of phosphatases in mediate these effects.
Human Aortic Endothelial Cells (HAEC) were stimulated with the AT2R-agonist C21 (1µM) in the presence or absence of PD123319 (10µM) (an AT2R antagonist), L-NAME (10µM) (an eNOS inhibitor), okadaic acid (10nM; serine/threonine phosphatase inhibitor) or sodium orthovanadate (10nM; tyrosine phosphatase inhibitor). NO release was estimated by quantifying (DAF-FM) fluorescence over a period of 10 minutes. HAEC cells were also stimulated with C21 and intracellular calcium transient analysis was performed using FURA-2 probe. Furthermore, HAEC cells were stimulated with C21 1µM for 5, 10, 15 minutes, 24 and 48 hours, and expression of phospho-Ser1177-eNOS (activation site), phospho-Tyr657-eNOS (inhibition site) and total eNOS determined by Western blotting. Best time response of eNOS activation using C21 was performed again in presence of PD123319.
Stimulation of HAEC by C21 resulted in a significant increase in NO release, which was blocked by PD123319 or L-NAME preincubation. Both phosphatase inhibitors were also able to block the increase in NO release promoted by AT2R stimulation. No intracellular calcium transient was observed after C21 stimulation. Moreover, a significant increase in Ser1177-eNOS phosphorylation which was blocked by PD123319, as well as a significant decrease in Tyr657-eNOS dephosphorylation was observed. AT2R stimulation did not alter expression of total eNOS. From these data, it was concluded that AT2R-stimulation increases NO synthesis by endothelial cells through modulation of eNOS phosphorylation resulting in increased eNOS activity, but not by modulation of total eNOS expression. This pathway is not mediated via intracellular calcium transient.

19 GENERATION OF NEW MITOCHONDRIA IS POSSIBLE PROTECTION MECHANISM OF BASAL STEROIDOGENESIS IN LEYDIG CELLS

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Mitochondria are the most important component of stress response in all cells and for steroid-hormones-producing cells they are the starting point for steroid biosynthesis. Here we investigated the parameters of mitochondrial biogenesis in Leydig cells from rats exposed to the psychophysical stress by immobilization (IMO).
IMO stress was applied for 2 h daily for one (1xIMO), two (2xIMO) or ten (10xIMO) days. Proof that IMO was effective stressor were elevating serum adrenaline and corticosterone as well as decreasing serum testosterone level in all stressed groups. Quantification of TMRE fluorescence in Leydig cells revealed reduced mitochondrial membrane potential ($\Delta\psi_m$) in 1x and 2xIMO groups, while $\Delta\psi_m$ was restored in 10xIMO rats. There was positive correlation between $\Delta\psi_m$ of Leydig cells and androgens production of Leydig cells also reduced in all stressed rats but partially recovered in 10xIMO group. The increased mitochondrial mass in Leydig cells from 10xIMO group was detected by quantitative analysis of MitoTracker-Green fluorescence as well as relative intensity of fluorescence. In line with this were results of transmission electron microscopy showing that acute and two times repeated stress altered architecture of mitochondrial cristae, while 10xIMO increased number of mitochondria and recovered mitochondrial architecture. Results of RQ-PCR and Western blot analyses revealed significant increase in the expression of the all markers of mitochondrial biogenesis in Leydig cells from 10xIMO rats. In the same cells, a similar pattern was observed for essential kinases related to regulation of steroidogenesis and PGC1 activation.
Our results support the conclusion that stress, a constant factor in life of humans, induces mitochondrial biogenesis in Leydig cells, probably to protect the basal steroid production in stress conditions.

20 NICOTINE ALTERS CARDIAC MITOCHONDRIAL CALCIUM HANDLING VIA UP-REGULATED SUPEROXIDE GENERATION

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Activation of nicotinic cholinergic receptors (nAChR) is protective against myocardial ischemia-reperfusion (I/R) injury. These effects however are abolished in settings of chronic nicotine administration, the mechanisms of which are not resolved. Mitochondrial Ca2+ handling maintains intracellular Ca2+ content and protect cardiomyocytes from necrosis during I/R. This study sought to characterize mitochondrial Ca2+ handling in freshly-isolated rat heart mitochondria following chronic nicotine administration. Male Sprague-Dawley rats (n=6-8, 180-230g) were administered with nicotine (0.6 mg/kg i.p.) or saline alone (control) for 28 days. Plasma cotinine levels in nicotine-administered rats were 109±12ng/mL. These levels are comparable to our previous study demonstrating nicotine aggravation of myocardial I/R injury. In this study, freshly-isolated cardiac mitochondria from nicotine-treated rats exhibited lower Ca2+ retention capacity (241±31µM vs. 835±26µM), suggestive of enhanced permeability transition, further supported by increased sodium-induced Ca2+ release (by 56±9%, $p<0.05$). Nicotine treatment also increased mitochondrial superoxide generation, by 63±5% ($p<0.05$), accompanied by lower mitochondrial superoxide dismutase (SOD) and glutathione (both $p<0.05$). Furthermore, pre-treatment of mitochondria with either mitochondrial-targeted Tempo (100 nmol/L) or bovine SOD (50 U/mL) significantly attenuated the nicotine-induced impairment in mitochondrial Ca2+ retention capacity. Taken together, these results suggest that nicotine alters mitochondria Ca2+ handling, at least in part via upregulating superoxide generation, which may contribute to the aggravated myocardial I/R injury evident after chronic nicotine administration. These effects might be responsible for impaired cardiac function in chronic smokers post myocardial infarction. Further studies are thus warranted to identify therapeutic benefits of mitochondrial-targeted drugs in chronic smokers post myocardial infarction.

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

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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.

22 THE ROLE OF ADENOSINE DEAMINASES IN CONTROLLING THE INFLAMMATORY RESPONSES TO INFECTIONS.

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Homology gene search revealed that ADA2 (CECR1) belongs to a new family of adenosine deaminase growth factors (ADGF). Our studies show that ADA2 is secreted by the cells of myeloid lineage and thus the activity of ADA2 could be a selective marker in diseases where monocytes, macrophages and dendritic cells get activated or their function is deregulated. Adenosine deaminase activity was found to be elevated during some cancers and immunological disorders such as tuberculosis and HIV.

Recently, we showed that monocytes release cytokines in response to ADA2, and those cytokines induce T helper cell proliferation and differentiation into IL-17 secreting Th17 T cell subtype. This finding may explain the mechanism of Th17 cells proliferation and cross-talk with activated monocytes, dendritic cells and macrophages. In the same time we demonstrated that secretion of cytokines by activated monocytes and survival of the cells is strictly controlled by the type of adenosine receptors expressed on the activated monocytes, although ADA2 does not directly bind adenosine receptors and ADA2 cell receptor is still enigmatic.

23 STRESS CAUSES DIFFERENT EXPRESSION OF MITOCHONDRIAL BIOGENESIS MARKERS IN RAT STEROID-PRODUCING CELLS OF ADRENAL GLAND AND TESTES

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Functional mitochondria of steroid producing cells of adrenal cortex and Leydig cells of testes are essential for steroid hormones biosynthesis and regulation. The aim of this study was to determine transcriptional profile of mitochondrial biogenesis markers in adrenal cortex and Leydig cells by applying *in vivo* and *in vitro* studies. Immobilization stress (IMO), was performed for 2 hours daily for one (1xIMO), two (2xIMO) or ten (10xIMO) consecutive days. In *in vitro* studies, primary cultures of purified Leydig cells from undisturbed rats were stimulated with stress hormone adrenaline, propranolol (nonselective β-ADRs-blocker) and prazosin (the selective α1-ADRs antagonist). RQ-PCR results showed that the transcription of the main regulator of mitochondrial biogenesis, *Ppargc1a* and *Ppargc1b,* significantly decreased in adrenal cortex of 10xIMO rats. Oppositely, the significant increase of the same transcript was registered in Leydig cells from the same rats. In parallel, transcription of *Ucp1*, the mediator of regulated proton leak, decreased in adrenal cortex, but increased in Leydig cells of the same group of rats. Incubation of Leydig cells with adrenaline, increased transcription of the main markers of mitochondrial biogenesis (*Ppargc1a*, *Ppargc1b*, *Nrf1* and *Nrf2a*). Nonselective β-ADRs-blocker attenuated this effect. The selective α1-ADRs antagonist did not change adrenaline-induced stimulation of *Ppargc1a, Ppargc1b, Nrf1* and *Nrf2a* transcription in Leydig cells, indicating that the most of the effects are probably mediated by β-adrenergic receptors, not by α1-ADRs of Leydig cells. In summary, the results suggest that reduction of transcription of mitochondrial biogenesis markers could be a possible mechanism that protects body from excessive glucocorticoid production from adrenal glands in stress conditions, while at the same time stimulation of mitochondrial biogenesis markers transcription in Leydig cells could serve as mechanism to preserve testosterone production.

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24 GONADOTROPIN RELEASING HORMONE RECEPTOR PATHWAY IN B CELLS AFFECTS IMMUNOSENESCENCE PROCESS

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Aging is associated with a progressive decline in immune function, leading to the increased vulnerability to infection and increased frequency of autoimmune diseases and cancers. To slow down aging of the immune system, it is necessary to understand and investigate the signalling pathways which modulate its function, including the function of B lymphocytes. Recently, the expression of GnRH and its receptor (GnRHR) known from their role in brain, has been detected in many extra-hypothalamic (GnRH) and extra-pituitary (GnRHR) tissues, including B lymphocytes. We found that the impaired GnRHR signalling in B lymphocytes, at least in part, is responsible for aging-related decline in the function of these cells. Such a role for GnRHR was suggested by the results of our extensive in silico analysis of 1,355 genes involved in the aging process, identified by the means of large-scale studies of longevity, as well as by information from databases such as Aging Atlas and Longevity Maps. Ontological analysis of the pathways in which these genes are involved, demonstrated that they were most significantly (p corr=$4.36E^{-21}$) involved in the GnRHR signaling pathway. The Cytoscape bioinformatics tool enabled us to position GnRHR in a complex network of neuroimmunoendocrine interactions. Furthermore, we observed a significant difference in the expression of GnRHR in the peripheral blood mononuclear cells (PBMC) originating from 30 young and 30 long-lived individuals. Accordingly, using qPCR and immunoblot techniques we found that in pre-immortal EBV-transformed B lymphocyte cell lines derived from young and long-lived donors, there is an age-related downregulation of GnRH1 and upregulation of GnRHRv2 expression. MTT assays revealed that activation (Buserelin) or inhibition (WAY 207024 dihydrochloride) of GnRHR significantly influence B lymphocyte mitochondrial activity in a donor age-dependent manner. These results support our hypothesis that GnRHR signalling pathway plays role in B lymphocyte aging process.

25 TRANSLATIONAL REPRESSOR PUM1 IS ASSOCIATED WITH A KEY NMD REGULATOR RENT1 IN MOUSE EMBRYONIC STEM CELLS

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It is well established that stem cells employ the master transcriptional factors to maintain self-renew and proliferation. However, relatively little is known about post-transcriptional regulation of stem cells. Pumilio, a RNA binding protein, is important for translational regulation in stem cell maintenance by degrading or repressing the translation of or its target RNA. In the mouse genome there are two Pumilio genes, Pumilio1 (Pum1) and Pumilio2 (Pum2). They are expressed at stages of ESCs and early embryos. We previously generated knock-out mouse models for these two genes and found that Pum1 knock-out mice are developmental delayed, while Pum2 mutant has no significantly change. Our lab showed that double knock-out mice are embryonic lethal. We performed immune-precipitation and found that RENT1 was associated with Pum1. RENT1 expressed in mESCs is a key regulator of nonsense-mediated mRNA decay (NMD), plays a critical role in normal mRNA decay, and regulates more than 200 mRNAs. Immuno-fluorescence staining showed that both Pum1 and RENT1 localized in cytoplasm. Pum1 knock-out mESCs have smaller clones and a durative apoptosis, and similar as RENT1 knock-down mESCs. And double knock-down mESCs have aggravated phenotype. Co-IP assay of truncated Pum1 and RENT1 shows that Pum1 can interact with RENT1 by N-terminal fragment, and the N-terminal and C-terminal of RENT1 are important for Pum1 interaction. We proposed a cooperative model for the interaction between Pum1 and RENT1: that both Pum1 and RENT1 can reduce protein level by repressing translation and degrading mRNA respectively. Pum1 represses target mRNA by interacting with RENT1. When the NMD pathway is activated for a Pum1 target mRNA, both RENT1 and Pum1 are recruited to the RNA, and the translation was quickly shut off.

Key words: Pum1, RENT1, translational repression, NMD, mESC

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