Session II: Biology of stress response

Lectures

L2.1

Cross-talk between redox regulation and protein homeostasis

Ohad Suss[1], Oded Rimon[1], Ohad Yogev[1], Meytal Radzinski[1], Rosi Gillin[1], Dana Reichmann[1,✉]

[1]Department of Biological Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel
✉danare@mail.huji.ac.il

Keywords: redox regulation; redox switch proteins; protein homeostasis

Why do we age? How do we cope with environmental and physical changes? What makes pathogens smarter than their host? One of the biological processes linking all these questions relates to the amazing ability of cells to respond to changes in the environment, such as changes in oxidant levels, and protect its proteome against damage.

Cellular defense depends heavily on redox-regulated proteins, redox switch proteins, which play fundamental roles not only during cellular defense but also in signaling, and protein biogenesis. Here, I will cover different mechanisms and functions redox-regulated proteins play in stress-response mechanisms, including maintaining protein homeostasis during oxidative stress and aging. I will show two examples of redox regulated proteins, the bacterial ATP-independent chaperone, Hsp33, and an eukaryotic ATPase protein, CDC48/VCP/p57, members of protein homeostasis system, that use site-specific oxidation to protect cells against oxidative stress or/and regulate the aging process. I will describe a mass spectrometry-based method that we use to identify novel redox-regulated proteins, and to quantify redox status of cellular proteins during different physiological processes.

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L2.2

The role played by chromatin modifications and chromatin modifying enzymes in DNA repair mechanisms

Deborah Toiber[1,✉]

[1]Ben-Gurion University of the Negev, Beer Sheva, Israel
✉toiber@bgu.ac.il

Keywords: DNA repair; chromatin remodeling

The accurate maintenance of nuclear DNA is critical to cellular and organismal function. Defects in DNA integrity associate with tumorigenesis, immunodeficiencies and aging. Therefore, numerous DNA repair systems have evolved in mammalian cells to cope with daily damage. The DNA and the histones are arranged in the nucleus in a highly condensed structure known as chromatin. Cellular processes that require unwinding of the double helix, such as transcription, replication and DNA repair, must overcome this natural barrier to gain DNA accessibility. In my talk I would explain how the deacetylase sirtuin 6 (SIRT6) is one of the earliest factors recruited to double-strand breaks (DSBs). SIRT6 recruits the chromatin remodeler SNF2H to DSBs and focally deacetylates histone H3K56. Lack of SIRT6 and SNF2H impairs chromatin remodeling, increasing sensitivity to genotoxic damage and recruitment of downstream factors such as 53BP1 and breast cancer 1 (BRCA1). Remarkably, SIRT6-deficient mice exhibit lower levels of chromatin-associated SNF2H in specific tissues, a phenotype accompanied by DNA damage. SIRT6 is critical for recruitment of a chromatin remodeler as an early step in the DNA damage response, indicating that proper unfolding of chromatin plays a rate-limiting role. We present a unique crosstalk between a histone modifier and a chromatin remodeler, regulating a coordinated response to prevent DNA damage.

L2.3

Using 500 species to study the human diseasome

Yuval Tabach[1,✉]

[1]Hebrew University, Jerusalem, Israel
✉YuvalTab@ekmd.huji.ac.il

Keywords: comparative genomics; gene function

Different eukaryotes have developed extraordinary traits such as genetic resistance to cancer and hypoxia, increased life span, ability to hibernate, regeneration of lost tissue, and adaptation to severe environments. Comparing the genomes of these and other species can reveal the genetic – phenotype – environmental crosstalk and tackle fundamental bio-medical challenges.

In the recent years we have been analyzing hundreds eukaryotes genomes, evaluating simultaneously the evolution of each protein across the tree of life. By mapping all human genes into about 1000 clusters of genes, with distinct patterns of conservation across eukaryotic phylogeny, we demonstrated that sets of genes associated with cancer, metabolic diseases and diseases phenotypes in addition to most gene networks have similar evolutionary pattern (phylogenetic profiling).

In my talk I would demonstrate how we use comparative genomics to predict gene function, identify novel disease and pathway genes, reveal novel tumor suppressors and develop drug repositioning platform.

L2.4

Depolymerization of the desmin cytoskeleton by the ubiquitin ligase Trim32 precedes and accelerates myofibril breakdown and muscle wasting

Alexandra Volodin[1], Eitan Shimko[1], Shenhav Cohen[1,✉]

[1]Technion Institute of Technology, Haifa, Israel
✉shenhavc@technion.ac.il

Keywords: ubiquitin proteasome system; muscle atrophy

Loss of skeletal muscle innervation (i.e. denervation), as occur during aging, spinal cord injuries and motor-neuron diseases, leads to accelerated proteolysis and ultimately to muscle atrophy. During atrophy, there is a major loss of the fundamental contractile machinery in muscle, the myofibrils, whose destruction results in reduced muscle mass and strength, fatigue and disability. The mechanism for myofibril breakdown during atrophy has long been uncertain, although the ubiquitin-proteasome system seems to play a major role. In this study we show that myofibril destruction in denervated atrophying muscle is preceded and accelerated by the depolymerization of the desmin cytoskeleton. The solubilization of desmin requires phosphorylation and ubiquitination by the ubiquitin ligase Trim32. *In vivo* electroporation of a specific shRNA into an adult mouse muscle to downregulate Trim32 prevented desmin disassembly and attenuated the loss of myofibrils. The muscles expressing shRNA to Trim32 also
exhibited a major preservation of mass. Interestingly, desmin depolymerization preceded myofibril breakdown because it was evident 10 days after denervation, before there was any loss of actin or myosin from the myofibril. Furthermore, at 7 days after denervation, when desmin filaments and myofibrils are intact, the overexpression of an inhibitor of desmin assembly induced disassembly of desmin filaments and facilitated myofibril destruction. Thus, depolymerization of the desmin cytoskeleton during muscle atrophy is coupled to the loss of the myofibrillar apparatus, and Trim32 is critical in the destruction of both structures.

L2.5

Malaria parasites regulate secretion of exosomes carrying distinct cargo

Xavier Sisquella[1,2], Yifat Ofir-Birin[6], Matthew A. Pimentel[1,2], Lesley Cheng[3,4], Natália G. Sampaio[1,2], Thusitha Rupasinghe[4], Jocelyn Sietsma Penington[2,5], Thomas Nebl[1], Jennifer C. Volz[1,2], Benjamin James Scicluna[3,4], Eugene A. Kapp[1], Robyn A. Sharples[3,4], Andreea Waltmann[1,2], Louis Schofield[1,2], Anthony T. Papenfuss[1,2], Malcolm McConville[4], Emily M. Eriksson[1,2], Andrew F. Hill[3,4] Andrew Bowie[5], Neta Regev-Rudzki[6,✉]

[1]The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia; [2]Department of Medical Biology, The University of Melbourne; [3]Department of Biochemistry and Molecular Biology, The University of Melbourne; [4]Bio21 Molecular Science and Biotechnology Institute The University of Melbourne, Melbourne, Victoria 3010, Australia; [5]Trinity Collage Dublin, Department of immunology and biochemistry, Ireland; [6]Department of Biomolecular Sciences, Weizmann Institute of Science, Rehovot 76100, Israel
✉ neta.regev-rudzki@weizmann.ac.il

Keywords: malaria parasites; exosomes

Cells use extracellular vesicles to communicate, coordinate social activities and, in the case of pathogens, to export virulence effectors into target host cells. The lethal malaria parasite, *Plasmodium falciparum*, was recently shown to transfer episomal genes via secreted vesicles. However, a comprehensive characterization of the physiological cargo delivered by these vesicles and its function is still lacking. Here, we identify these nanovesicles as exosomes, and determine their molecular composition: nucleic acids, proteins and lipids. By establishing advanced nano-resolution techniques for analyzing exosome content, we found that malaria-derived exosomes deliver a large group of non-coding RNAs and parasite nuclear, apicoplast and mitochondrial genes, in a time dependent manner. Further, we demonstrate activation of pro-inflammatory responses of the human host upon exosome uptake.

This work identifies previously unknown molecular players in signalling pathway of malaria parasites, essential for its survival in the host, and provides a new insight into our understanding of how malaria parasites can manipulate their host environment.

Posters

P2.1

The role of beta-catenin in cardiomyocyte hypertrophy development

Oksana Palchevska[1,✉]

[1]Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine
✉ o.l.palchevska@imbg.org.ua

Keywords: beta-catenin; hypertrophy; cardiomyocytes

The canonical WNT signaling is one of the most crucial pathways that regulate cell fate. The main mediator of canonical WNT signaling is beta-catenin. Though the canonical WNT signaling has basal level of activity in postnatal heart, it can be reactivated during heart regeneration, remodeling, stress adaptation etc.

Our group is focused on heart hypertrophy development and role of canonical WNT signaling in this process. The data concerning the involvement of this signaling to postnatal are controversial. Also the precise terms of such activation are not clearly understood yet.

The aim of present work is to clarify the role of canonical signaling activation under beta-catenin haploinsufficiency condition.

In our work we used primary cultures of mouse cardiomyocytes treated with LiCl and H_2O_2 (known inductors of cell hypertrophy). The primary cultures were isolated from new-born transgenic animals bearing only one allele of beta-catenin in cardiomyocytes (Piven, 2011) and their wild-type sibs as control. The metabolic activity of primary cultures after 3 days of treatment was estimated using MTT-test. The cell width and cell length were measured for wild-type and beta-catenin haploinsufficient cells.

Some interesting peculiarities explaining our previous results of animal experiments have been revealed.

P2.2

Oxidative processes, structural and functional state of cord blood nucleated cells upon cryopreservation

Olga Mykhailova[1,✉], Olena Makashova[1], Pavel Zubov[1], Oksana Zubova[1], Lubov Babijchuk[1]

[1]Institute for Problems of Cryobiology and Cryomedicine, NAS of Ukraine, Kharkiv, Ukraine
✉mixolya@mail.ru

Keywords: cord blood; cryopreservation; ROS overproduct

BACKGROUND: Successful transplantation of cryopreserved cord blood (CB) largely depends on the total cell amount and CB quality. The aim of this study was to assess the oxidative processes and the structural and functional states of nucleated cells (NCs, $CD45^+$) in CB units after cryopreservation.
experimental approach: Dextran-isolated CBNCs were cryopreserved with 5% DMSO. Assessment of the intracellular content of reactive oxygen species (ROS) ($DCFH_2$-DA), cell viability (7AAD), phospholipid asymmetry (AnnexinV), and analysis of apoptosis/necrosis stages (7AAD/AnnexinV) were accomplished via flow cytometry. Prevention of ROS overproduction was achieved by adding 10 mM N-acetyl-L-cysteine (AC) into the cryoprotectant medium.
RESULTS: Cryopreserved CBNCs manifested a high viability level (82.73±1.41%) and low levels of loss (80.26±2.31%). Phosphatidylserine externalization was observed in 7.35±0.56% of NCs. The analysis of the apoptosis/necrosis stages of CBNCs revealed that 79.28±1.24% of CBNCs ($AnnexinV^-7AAD^-$) remained alive and cell injuries occurred mainly as part of the necrosis pathway (13.37±1.30% $AnnexinV^-7AAD^+$). The ROS content of cryopreserved CBNCs achieved 20.5±1.27% (DCF^+CD45^+). Supplementing the cryoprotective medium with AC decreased the amount of DCF^+CD45^+-cells up to 8.5±0.85% and followed with a rise in the quality and quantity of cells in CB, up to 5-8%.
CONCLUSIONS: Overproduction of ROS in cryopreserved CBNCs is responsible in part for decreases in cell viability, disturbances of phospholipid asymmetry, and induction of the necrosis pathway. Application of AC in the cryoprotective medium improves qualitative and quantitative features of CBNCs.

P2.3

Inhibition of IRE1 signaling affects expression of proliferation-related transcription factors in U87 glioma cells

Dariia Tsymbal[1,✉], Oleksandr Minchenko[1]

[1]Palladin Institute of Biochemistry, NAS of Ukraine, Kyiv, Ukraine
✉dariiabova@gmail.com

Keywords: ER stress; glioma cells; IRE1 inhibition; mRNA expression; transcription factors

IRE1 (inositol-requiring enzyme 1) is a key effector of ER stress. Downstream IRE1 signaling is mediated by its kinase and endoribonuclease domains. Inhibition of IRE1 strongly reduces the proliferation of glioma cells. In U87 glioma cells with stable knockdown of IRE1, we observed altered mRNA expression in a subset of proliferation-related transcription factors. For instance, inhibition of IRE1 led to down-regulation of mRNA levels of *E2F8* (E2F transcription factor 8), *EPAS1* (endothelial PAS domain 1), *HOXC6* (homeobox C6), *ATF3* (activating transcription factor 3), *FOXF1* (forkhead box F1), *TBX2* (T-box 2), *IFRD1* (interferon-related developmental regulator 1), *GTF2F2* (general transcription factor 2F, polypeptide 2), and *GTF2B* (general transcription factor 2B). However, the mRNA levels of *TBX3* (T-box 3), *MYBL1* (V-Myb avian myeloblastosis viral oncogene homolog-like 1), *MYBL2* (V-Myb avian myeloblastosis viral oncogene homolog-like 2), and *MAZ* (MYC-associated zinc finger protein) were up-regulated under identical conditions. Inhibition of endoribonuclease activity alone had a more profound effect on the expression of *E2F8, HOXC6,* and *TBX3* genes, than a complete knockdown, whereas for *FOXF1* it even resulted in an opposite effect: a 23-fold increase in mRNA levels versus 2-fold decrease. Endoplasmic reticulum stress, glycolysis, glutaminolysis, and hypoxia are important factors of tumor progression; the effects of these conditions on the expression of most studied genes depend on IRE1 activity in glioma cells. In conclusion, the inhibition of IRE1 complexly regulates the expression of genes implicated in malignant growth, as a rule decreasing the expression levels of pro-proliferative genes and increasing the expression of anti-proliferative genes.

P2.4

*At*FTSH4 regulates mitochondrial proteostasis by controlling turnover of the essential protein import component

Magdalena Opalińska[1,✉], Katarzyna Parys[1], Monika Murcha[2], Hanna Jańska[1]

[1]Department of Cellular Molecular Biology, Faculty of Biotechnology, University of Wroclaw, Wroclaw, Poland; [2]ARC Centre of Excellence in Plant Energy Biology, The University of Western Australia, Crawley, Western Australia

✉ magdalena.opalinska@uwr.edu.pl

Keywords: i-AAA protease; mitochondria; protein import

Mitochondria are double-membrane organelles that play a central role in energy metabolism. The bulk of mitochondrial proteins are involved in diverse metabolic pathways, such as the biosynthesis of cofactors like iron-sulphur clusters, and the metabolism of sugars, amino acids, and lipids. Due to the essential functions of these organelles, mitochondrial content, quality, and dynamics are tightly controlled. Highly conserved ATP-dependent proteases are the machinery critical for maintaining proper mitochondrial functioning. This study focuses on FTSH4, an *i*-AAA protease from *Arabidopsis thaliana*. Lack of FTSH4 causes severe morphological and developmental abnormalities in plants grown under stress conditions (short photoperiod and increased temperature). This is accompanied by decreased levels and activities of specific respiratory complexes, anomalies in mitochondrial morphology, and increased oxidative stress. However, the molecular mechanism that directly links the observed phenotypes of *ftsh4* plants with the FTSH4 protease is missing. The goal of this work is to identify *in vivo* substrates and binding partners of FTSH4. Interestingly, using distinct approaches we found that the essential component of the TIM23 translocase, Tim17-2, constitutes an *in vivo* target of FTSH4. Our data suggests that FTSH4 might play a role in the removal of excess Tim17-2 subunits from TIM23. Accurate regulation of the level and composition of TIM23 is vital for mitochondrial functioning.

This work is supported by grant FUGA 2015/16/S/NZ3/00364 from National Science Center awarded to Magdalena Opalińska.

P2.5

An inhibitory action of N-stearoylethanolamine on aggregation of human platelets

Iegor Hudz[1,✉], Volodymyr Chernyshenko[1], Ludmila Kasatkina[1], Lesia Urvant[1], Eduard Lugovskoy[1], Nadia Hula[1]

[1]Palladin Institute of Biochemistry, NAS of Ukraine, Kyiv, Ukraine

✉ goudz@mail.ru

Keywords: lipids; aggregation; platelets; activation; ristocetin; N-stearoylethanolamine

Introduction: N-stearoylethanolamine (NSE), a lipid mediator that belongs to the N-acylethanolamines family, has anti-inflammatory action. The aim of present work was to evaluate the effect of NSE on activation and aggregation of human platelets *in vitro*.

Methods: In aggregation measurements human platelet rich plasma (PRP) in the presence/absence of NSE (10^{-6}–10^{-10} M) was activated by ADP, adrenaline, PAF, and ristocetin. Binding of fibrinogen to GPIIbIIIa receptor was estimated by indirect ELISA using anti-fibrinogen monoclonal antibody. Spectrofluorimetry was used for detection of the platelet activation by ADP or ristocetin in the presence of 10^{-7} M NSE. The changes of the granularity and shape of resting platelets and platelets stimulated with ADP in the presence of NSE were monitored using flow cytometry.

Results: In concentration of 10^{-7} M NSE did not inhibit platelet aggregation induced by thrombin, slightly inhibited ADP- or PAF-induced aggregation of platelets and substantially decreased the platelet aggregation induced by ristocetin. Spectrofluorimetry showed that NSE did not change the rate of platelet activation induced by ADP but inhibited ristocetin-induced degranulation. It was shown that NSE did not affect the shape or granularity of resting or ADP-induced platelets but decreased the amount of fibrinogen that binds to the GPIIbIIIa receptor of adhered platelets in ELISA.

Conclusions: NSE was shown to be efficient inhibitor of aggregation of human platelets. NSE was the most effective in the case of ristocetin-induced aggregation and affected the activation stage of aggregation process preventing induced platelet degranulation and the activation of GPIIbIIIa receptors.

P2.6

BCG Immunization Influence on Bone Metabolism in Rats With Alimentary Osteoporosis

Olga Zaitseva[1,✉], Serhiy Shandrenko[1], Mykola Veliky[1]

[1]Palladin Institute of Biochemistry, NAS of Ukraine, Kyiv, Ukraine
✉Zaitseva_OV@ukr.net

Keywords: osteoporosis; vitamin D_3; nitrous oxide; BCG

Osteoporosis remains a serious clinical problem. One of the possible causes of osteoporosis is vitamin D_3 insufficiency, which results in impaired bone mineralization and immune system function. Because the BCG vaccine stimulates the immune system and leads to nitrous oxide (NO) synthesis activation, we decided to investigate the effect of BCG vaccination on bone metabolism in rats with alimentary osteoporosis.

Alimentary osteoporosis was characterized by hypocalcaemia, hypophosphatemia, decreased 25-hydroxyvitamin D_3 content, and increased alkaline phosphatase activity in blood serum. There was also severe bone tissue demineralization (reduced ash content, mineral and organic components) and typical osteoporosis seen with X-ray imaging. Immune system dysfunction manifested as decreased granulocyte phagocytic activity, which impaired the ability to produce bactericidal oxidants. Intraperitoneal BCG vaccine injection (1 mg/animal) caused increased granulocyte phagocytic activity, which led to increased mineral content and decreased total alkaline phosphatase activity by 40% compared to osteoporosis in blood plasma. Femur bone ash content and mineral levels were markedly elevated. We also observed decreased citric soluble collagen content in bones by 48% compared to osteoporosis, which is related to the normalization of mineral levels in blood plasma and bones.

Therefore, we suggest that immune system stimulation and high NO concentrations, because of BCG vaccination, may affect bone cell formation/functioning and bone collagen structure by increasing its stability.

P2.7

Immune response of *Galleria mellonella* after natural infection and injection of blastospores of the fungus *Beauveria bassiana*

Lidiia Vertyporokh[1,✉], Paulina Taszłow[1], Iwona Wojda[1]

[1]Maria Curie-Sklodowska University, Faculty of Biology and Biotechnology, Institute of Biology and Biochemistry, Department of Immunobiology, Lublin, Poland
✉lydia.vertyporokh@gmail.com

Keywords: *Galleria mellonella*; *Beauveria bassiana*; biotic stress; infection; immune response; defense peptides

Beauveria bassiana is an entomopathogenic fungus that infects insects through adhesion of the aerial conidia to the body surface and subsequent penetration through the cuticle. Inside an animal, the fungus grows in the form of spherical cells – blastospores and kills the insect through the synthesis of toxic secondary metabolites and mechanical damage to animal tissues.

We intended to explore the biotic stress caused by natural *B. bassiana* infection (i.e. occurring as a result of contact with aerial conidia) and by infection caused by injection of *B. bassiana* blastospores. We chose *Galleria mellonella* larvae, a widely used object of host-pathogen interaction studies, as a model organism.

We found that the hemolymph of naturally infected larvae lacked antimicrobial activity and demonstrated antifungal activity on the fifth day of infection, whereas the hemolymph of larvae infected with blastospores had both antimicrobial and antifungal dose-dependent activities. We chose three antimicrobial peptides (AMPs) to track their expression in the fat body (the main site of AMPs synthesis): antifungal peptides galiomicin and gallerimycin, and an antimicrobial peptide cecropin. Both natural infection and injection of blastospores induced the expression of galiomicin and gallerimycin, which was dose-dependent in the case of the injection. The expression of cecropin was higher after the blastospore injection and declined with time.

The data obtained allows us to conclude that natural infection activates a more specific defense response, but blastospore injection might initiate a stronger response and faster development of infection.

P2.8

Paracetamol hepatotoxicity: Is there any chance for protection?

Adriana Piwoni[1,✉], Daria Terepka[1], Nina Filipczak[1], Maria Stasiuk[1], Jerzy Gubernator[1]

[1]Faculty of Biotechnology, University of Wroclaw, Wroclaw, Poland
✉adriana.piwoni@gmail.com

Keywords: paracetamol; resorcinolic lipids; alkylresorcinols; HepG2 cell line

The aim of this project is to investigate if 5-n-alk(en)ylresorcinols (ARs) can protect HepG2 cells (a human liver model cell line) against the effects of paracetamol – a well-known hepatotoxic agent.

ARs are phenolic lipids that are synthesized mainly by graminaceous plants, such as cereals, and they have relatively high concentrations in the bran fraction of wheat and rye. Because ARs occur only in the outer layer of the grain, it has been proposed that the AR content can be recognized and used as a biomarker for whole grain product intake. ARs present in daily diet are absorbed and circulated in the blood stream and finally metabolized in the liver. ARs have been shown to be promising compounds that have antioxidant, antigenotoxic and antibacterial activity.

Our recent, unpublished data showed that when ARs are added to the culture medium, the number of surviving cells increased in an AR incubation time- and concentration-dependent manner, and they also eliminated the toxic effect of paracetamol. We also examined the physiological and morphological state of cells after treatment with paracetamol and ARs (alone and together). Indicators of the cells' physiological state included the lactate dehydrogenase leakage assay and measurements of cellular ATP levels. Morphological changes of the cells' general condition (nuclei and cytoskeleton staining) and apoptosis (Annexin V-FITC staining) were visualized using fluorescence microscopy.

Our results suggest that a whole grain diet can protect liver cells from paracetamol hepatotoxicity.

P2.9

Oligoribonucleotide effects on expression of *nos2*, *arg2*, *xdh*, *nfkbia*, *nfkb1* genes under influenza virus infection *in vivo*

Nataliya Melnichuk[1,✉], Alina Rybenchuk[1], Ganna Gerashchenko[1], Volodymyr Kashuba[1], Larysa Semernikova[1], Tetiana Yakovenko[1], Zenovii Tkachuk[1]

[1]Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine
✉natalia.melnichuk8@gmail.com

Keywords: oligoribonucleotides; influenza; *nos2*; *xdh*; *nfkbia*; *nfkb1*

Infection by influenza (flu) virus causes activation of NOS2, Arg2, XOD enzymes involved in the production of free radicals, such as $NO^{\cdot}$, $O_2^{\cdot-}$, $ONOO^{-}$. Free radicals involved in signal transduction pathways activate transcription factors, such as NFκB, and may lead to lung tissue damage. It was shown that NFκB takes part in flu virus life cycle. Natural and synthetic oligoribonucleotides (ORNs) have a wide range of biological effects, playing a key role in antiviral activity. However, the mechanisms underlying ORNs effects are not clear for today. So the aim of the research was to study ORNs effects on the expression of *nos2, arg2*, *xdh, nfkbia*, *nfkb1* genes under influenza virus infection *in vivo*.

Methods: The ORNs were modified by D-mannitol. The gene expression in mouse lung cells was investigated with RT-PCR.

Results: The overexpression of all investigated genes in virus-infected vs. healthy mice was shown. The decreased mRNA expression level of *arg2, nfkbia* and *nfkb1* genes was detected after prevention and treatment with ORNs compared to the virus-infected mice. ORN injection for prevention and treatment reduced the mRNA level of *arg2* expression by 50% and 22%, respectively, vs. virus-infected mice, whereas the mRNA expression of *xdh* remained unchanged. The mRNA expression level of the *nos2* gene decreased by 36% and increased by 73% when the ORNs were injected for prevention and treatment, respectively.

Conclusions: Our results show that the modification of ORNs by D-mannitol modulates the expression of *nos2*, *arg2*, *xdh*, *nfkbia*, *nfkb1* genes under the flu virus infection *in vivo*.

P2.10

Hyperosmotic stress response of erythritol-producing yeasts *Yarrowia lipolytica*

Dorota A. Rzechonek[1,✉], Waldemar Rymowicz[1], Aleksandra M. Mirończuk[1]

[1]Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland
✉dorota.rzechonek@up.wroc.pl

Keywords: hyperosmotic stress; *Yarrowia lipolytica*; erythritol

Hyperosmotic stress is one of the main factors that inhibits the growth of microorganisms. Yeasts such as *Saccharomyces cerevisiae* have developed a comprehensive response known as the high osmolality glycerol (HOG) pathway to counteract hyperosmotic stress. The HOG pathway results in the production and accumulation of glycerol in cells. However, there is a group of non-conventional yeasts, including *Yarrowia lipolytica*, which produce erythritol instead of glycerol. Erythritol is a non-toxic polyol that is used as low-caloric sugar substitute. The connection between the HOG pathway and erythritol production is still unclear. In order to determine the significance of this osmotic response in *Y. lipolytica*, the gene homologous for *Hog1* (YALI0E251359) was deleted. This study showed the impact of *Hog1* deletion on various vital functions and the production of erythritol when cells are cultured in hyperosmotic stress conditions.

This work was financed by the Polish National Centre for Research and Development under project LIDER/010/207/L-5/13/NCBR/2014

P2.11

Modeling homocysteine effects on folate-related metabolism in the human placenta

Ruslan Rodriguez[1,✉], Andrew Kostiuk[2], Serhii Ralchenko[1], Alexandra Martynovska[2], Victoria Kosach[1], Ihor Konovets[3], Larysa Porubleva[1], Maria Obolenska[1]

[1]Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine; [2]Education and Scientific Center 'Institute of Biology', Kyiv, Ukraine; [3]Institute of Hydrobiology, NAS of Ukraine, Kyiv, Ukraine
✉r.r.rodriguez@imbg.org.ua

Keywords: homocysteine; placenta; metabolism; modeling

Homocysteine (Hcy) is known to adversely affect human placental trophoblasts, impairing proliferation, DNA methylation, and inducing apoptosis, but the exact mechanism is still debated. This research provides insights in these processes using the computational model of folate-related metabolism along with experimental verification. We modeled the behavior of over 60 reactions (methionine, folate cycles, *de novo* purine, glutathione and taurine syntheses) under a 20 mkM Hcy load. The main compounds of the methionine cycle, S-adenosylmethionine and S-adenosylhomocysteine, had increased concentrations by 40-60% under high Hcy, which was verified from placental explants using LC/MS analysis. However, *de novo* purine synthesis remained untouched by Hcy. This was verified using LC/MS tracking of newly synthesized purines from the MCF-7 cell line and placental explants supplemented with heavy-labeled glycine. We hypothesize that Hcy influences DNA methylation through disturbance of the methionine cycle, which is responsible for all methylation processes. In addition, impairment of Hcy on proliferation is not due to *de novo* purine synthesis inhibition. The computational model used in this study is a useful tool and can predict metabolic system behavior rather accurately.

P2.12

Effect of different carbon sources and the level of ergosterol in plasma membrane on the Cdr1 transporter of *Candida albicans*

Jakub Suchodolski[1,✉], Magda Siwek[1], Anna Krasowska[1]

[1]Faculty of Biotechnology, University of Wroclaw, Wroclaw, Poland
✉jakub.suchodolski@uwr.edu.pl

Keywords: *Candida albicans*; ABC transporters

Candida albicans is a pathogenic yeast-like fungus that causes exogenous and endogenous infections in humans. *C. albicans* strains exhibit multidrug resistance to commonly used antifungal agents, such as azoles and amphotericin B, which correlates with overexpression of Cdr and Mdr transporters located in the plasma membrane (PM) [1]. Recent literature reports that the level of ergosterol in the PM determines the susceptibility of *C. albicans* to azoles. For our study, we deleted *C. albicans*' genes encoding the ABC transporters, Cdr1 and Cdr2, as well as genes involved in ergosterol production (Erg11 and Erg3) using the SAT1-FLIP SAP2 cassette. We found that fungal growth varied depending on the environmental carbon source and type of deletion. Depending on the growth phase, we observed mislocalization of the Cdr1 transporter from the PM into the interior of cells. Intensification of this process was also dependent on the carbon source. Using standard HPLC methods and a filipin dye-based fluorescent method, we measured ergosterol in the PM. We examined altered sterol levels in the PM of different *C. albicans* mutants cultured in the presence of various carbon sources. Activity of the ABC transporters was measured using a DiSC3(3) dye-based fluorescent method. We hypothesized that the mislocalization and activity of Cdr1 may occur because of altered ergosterol level and plasma membrane fluidity.

[1] Szczepaniak J. et al., *Frontiers in Microbiology*, 2015, 6:176

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P2.13

Dinucleoside polyphosphates affect phenylpropanoid pathway in *Vitis vinifera* cv. Monastrell

Małgorzata Pietrowska-Borek[1,✉], Jakub Dąbrowski[2], Joanna Kowalska[2], Jacek Jemielity[2], Andrzej Guranowski[1]

[1]Poznan University of Life Sciences, Poznan, Poland; [2]Centre of New Technologies, University of Warsaw, Warsaw, Poland
✉gospi@up.poznan.pl

Keywords: dinucleoside polyphosphates; signalling molecules; phenylpropanoid pathway; *Vitis vinifera* cv. Monastrell

Dinucleoside polyphosphates (Np_nN's) are naturally occurring compounds in cells. Some of these accumulate in cells under stresses such as elevated temperature, ethanol and cadmium. For this reason, Np_nN's have been named alarmones. Previously we demonstrated that exogenous Ap_3A and Ap_4A acted as signalling molecules in *Arabidopsis thaliana* seedlings and *Vitis vinifera* cv. Monastrell cells [1, 2]. These nucleotides increased the expression of genes, enzyme activities and accumulation of phenylpropanoid pathway products. In plants, these are the phenylpropanoids that protect them against various types of stress. A small group of these phenolic compounds are stilbenes, which exhibit phytoalexin activity. In *Vitis vinifera* they are called viniferins, which are derivatives of the *trans*-resveratrol (*t*-R) monomeric units.

The question is whether dinucleotide polyphosphates other than Ap_3A or Ap_4A also evoke the induction of *t*-R synthesis. We studied the effects exerted by 5 μM Ap_3U, Up_3U, Up_4U and Gp_3G on the phenylpropanoid pathway in Monastrell cells. Each of these compounds increased accumulation of *t*-R in an extracellular medium and in cells during experiments lasting up to 72 h. In addition, gene expression of phenylalanine ammonia lyase, cinnamate-4-hydroksylase, 4: coumarate-CoA ligase and stilbene synthase was modified by the dinucleotides. The accumulation of *t*-R and gene expression depended on the kind of applied dinucleotide and the time point.

[1] Pietrowska-Borek M. et al. (2011). *FEBS Open Bio* 1: 1-6
[2] Pietrowska-Borek M. et al. (2014). *Plant Physiol. Biochem.* 84: 271-276

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P2.14

Genome variability of *Deschampsia antarctica* Desv. plants with different chromosome numbers in tissue culture

Daria Navrotska[1,✉], Igor Andreev[1], Maryana Twardowska[1], Viktor Kunakh[1]

[1]Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine
✉navrotska.daria@gmail.com

Keywords: *Deschampsia antarctica* Desv.; genome variability; aneuploidy; chromosomal variation *in vitro*; tissue culture

Stressful environmental factors are known to influence plant genome stability, causing structural changes (i.e., alterations in chromosome number and structure) and nucleotide sequences changes.

Previously, cytogenetic analysis of *Deschampsia antarctica* Desv. (*Poaceae*) from the Antarctic region revealed new forms of chromosome polymorphism associated with aneuploidy, mixoploidy, polyploidy, and the occurrence of supernumerary B-chromosomes. These karyotype forms have not been found earlier in the central part of the range, and their occurrence may be considered signs of genome instability caused by stress due to harsh environments at the limits of the species' range.

In this study, genetic variation in tissue cultures of *D. antarctica* was investigated with the aim to elucidate the genome stability of *D. antarctica* plants with normal and abnormal karyotypes under stress caused by *in vitro* culture.

Analysis of *D. antarctica* tissue cultures demonstrated a wide variation in chromosome number, which ranged from 15 to 63 (mean 26.4 chromosomes per metaphase). The percentage of aneuploid cells reached 60.6%. In cell cultures derived from diploid plants (2n=26) and plants with supernumerary B-chromosome (2n=26+1B) diploid cells predominated. In contrast, in tissue culture derived from plant with near-triploid modal chromosome number (2n=36, 38), a shift to a predominance of cells with diploid (2n=26) and near-diploid chromosome numbers was observed.

Results indicate a higher genomic instability of *D. antarctica* plants with abnormal chromosomal number compared to diploid plants in tissue cultures, thus suggesting that alterations in karyotype may result in destabilization of the genome.

P2.15

Identification of putative protein substrates for the molecular chaperone ClpB in the pathogenic bacterium *Leptospira interrogans*

Joanna Krajewska[1,✉], Zbigniew Arent[2], Daniel Więckowski[1], Sabina Kędzierska-Mieszkowska[1]

[1]Department of General and Medical Biochemistry, University of Gdansk, Faculty of Biology, Gdansk, Poland; [2]University Centre of Veterinary Medicine JU-UAK, University of Agriculture in Cracow, Cracow, Poland
✉joanna.krajewska@biotech.ug.edu.pl

Keywords: molecular chaperone; *Leptospira interrogans*; virulence

Bacterial ClpB is a molecular chaperone that reactivates aggregated proteins in cooperation with the DnaK chaperone system. It is known that ClpB is essential for survival of bacteria under stress conditions. There are also a number of data, which indicate the involvement of ClpB function in bacterial virulence. However, the specific role of ClpB in bacterial pathogens during infection of a host organism is unknown. It is assumed that ClpB, due to its role as the molecular chaperone, mediates refolding of some bacterial proteins including known virulence factors.

The aim of this study was to identify putative protein substrates of ClpB from *Leptospira interrogans* (ClpBLi), a pathogenic spirochaete responsible for leptospirosis in both humans and animals. Using his-tagged ClpBLi affinity chromatography and mass spectrometry, 59 proteins were preliminarily identified in cell lysates from *L. interrogans* submitted to thermal and oxidative stresses. Among them, for instance, there are TrkA, a potassium transporter, ferritin, proteins involved in transcriptional regulation (ArsR family transcriptional regulator), translational process (30S ribosomal protein S15), chemotaxis protein, enzymes involved in various metabolic pathways (e.g., succinate dehydrogenase, malate dehydrogenase, citrate synthase, fructose-bisphosphate aldolase, crotonyl-CoA reductase, branched-chain amino acid aminotransferase) and in biosynthesis of the oligosaccharide core of LPS (3-deoxy-8-phosphooctulonate synthase). Identification of such proteins supports the ClpB's impotance in virulence of *L. interrogans*. This finding may help to reveal the underlying mechanism by which ClpB influences virulence traits in *L. interrogans*.

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P2.16

E. coli AlkA and AlkB proteins repair 1,N6-α-hydroxypropanoadenine and 3,N4-α-hydroxypropanocytosine

Małgorzata Dylewska[1,✉], Beata Sokołowska[2], Karolina Korzeniowska[1], Jarosław T. Kuśmierek[1], Agnieszka M. Maciejewska[1]

[1]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland; [2]Medical Research Center, Polish Academy of Sciences, Warsaw, Poland
✉ mdylewska@ibb.waw.pl

Keywords: AlkA; AlkB; HPA; HPC; DNA repair

1,N6-α-hydroxypropanoadenine (HPA) and 3,N4-α-hydroxypropanocytosine (HPC) are formed when adenine and cytosine react with acrolein, respectively. Acrolein is an environmental mutagenic agent. It is also generated endogenously during oxidative stress as a by-product of lipid peroxidation. AlkA (3-methyladenine DNA glycosylase II) is DNA repair protein with a broad substrate specificity that removes lesions via the Base Excision Repair pathway. As part of the superfamily of 2-oxoglutarate and Fe(II) dependent dioxygenases, AlkB removes alkyl lesions from bases via an oxidative mechanism, thus restoring native DNA structure. Both AlkA and AlkB are induced in *E. coli* as part of the adaptive response to alkylating agents. Our experimental system uses pIF101-106 plasmids that bear the lactose operon from *E. coli* CC101-106 which allows us to monitor Lac+ revertants that arise by particular base substitutions. Acrolein-modified plasmids were introduced into *wt*, *alkA*, and *alkB* cells and mutants were selected. To differentiate between strains with induced or not adaptive response in their repair ability, algorithms of pattern recognition were used. We found that both lesions are mutagenic. HPA causes mainly A→T and A→G substitutions, whereas HPC treatment results in C→T transitions followed by C→A transversions. For the first time, we have shown that AlkA glycosylase, together with AlkB dioxygenase, efficiently repairs HPA and HPC *in vivo*. Because AlkB and AlkA act on ssDNA and dsDNA, respectively, together they constitute a complete defense against those new substrates.

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P2.17

Lipid peroxidation affects cells from Fanconi anemia groups, FANCD2 and FANCJ

Ewelina M. Borsuk[1,✉], Agata Wilińska[2], Barbara Tudek[1], Elżbieta Speina[1]

[1]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland; [2]University of Warsaw, Warsaw, Poland
✉ e.szmajda@ibb.waw.pl

Keywords: Fanconi anemia; DNA repair

Fanconi anemia (FA) is a rare genetic disorder manifested mainly by progressive bone marrow failure and increased incidences of cancers. It arises through mutations in genes of the FA pathway, which is an important DNA repair pathway involved mainly in the repair of interstrand crosslinks (ICLs). On a cellular level patients exhibit defects in double strand breaks (DSBs) repair as well as in the restart of stalled replication fork. The status of endogenous factors, which can form ICL and block replication, such as lipid peroxidation (LPO) products, in etiology of FA has not yet been established. The aim of the study is to evaluate the influence of LPO on phenotype of FA. We found that FA patients' cells (*FANCD2*$^{-/-}$ and *FANCJ*$^{-/-}$) displayed hypersensitivity to major LPO products – acrolein (Acr), croton aldehyde (Cro) and 4-hydroxynonenal (HNE). In response to aldehydes treatment we observed accumulation of γH2A.X in FA cells, which is the marker of DSBs. Examination of replication also revealed increased fork stalling. DNA damage after HNE treatment was accompanied by increased phosphorylation of DNA damage response kinases, such as ATM, ATR and Chk2, and, surprisingly, by inhibited phosphorylation of Chk1 kinase. Moreover, damage induced by HNE and Acr caused increased cellular senescence. Cell cycle analysis of *FANCD2*$^{-/-}$ and *FANCJ*$^{-/-}$ lines revealed accumulation in G2 phase in response to HNE and Acr. These results support the hypothesis on the involvement of FA pathway in repair of DNA damage caused by LPO.

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P2.18

DNA epigenetic marks in wild-type and *Ercc1*$^{-/-}$ primary mouse embryonic fibroblasts

Jolanta Czerwińska[1,✉], Agnieszka Tupalska[2], Laura J. Niedernhofer[3], Marek Foksiński[4], Daniel Gackowski[4], Ewelina Zarakowska[4], Marta Starczak[4], Barbara Tudek[1,2], Ryszard Oliński[4]

[1]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland; [2]Institute of Genetics and Biotechnology, University of Warsaw, Warsaw, Poland; [3]The Scripps Research Institute, Jupiter, FL, USA; [4]Collegium Medicum, Nicolaus Copernicus University, Bydgoszcz, Poland

✉ jczerwinska@ibb.waw.pl

Keywords: 5-hydroxymethylcytosine; 8-oxodeoxyguanine; Ercc1; primary mouse embryonic fibroblasts

Epigenetic marks, like 5-hydroxymethylcytosine (5-hmC) and 5-formylcytosine (5-fC) are stable DNA base modifications generated from 5-methylcytosine (5-mC) by the ten-eleven translocation (TET) protein family. The quantity of these DNA modifications inversely correlates with the proliferation rate of mammalian cells and tissues. Five-hydroxymethyluracil (5-hmU) may also be generated from thymine by TET enzymes and has epigenetic functions. We assessed these epigenetic marks in the DNA of *Ercc1*-deficient primary mouse embryonic fibroblasts (MEFs), which are a model of premature aging, grown under low (3%) or high (21%) oxygen concentration. The amount of 5-hmC was significantly higher in knock-out cells when compared to congenic wild-type (WT) cells. The proliferation rate of *Ercc1*$^{-/-}$ cells was lower than that of WT cells cultured in 3% oxygen. Surprisingly, in 21% oxygen, both WT and *Ercc1*$^{-/-}$ MEFs showed decreased 5-hmC levels when compared to cells cultured in 3% oxygen, although the proliferation rate was much lower in 3% oxygen. Additionally, another DNA base modification, 8-oxodeoxyguanine (8-oxodG), a well-known marker of oxidative DNA damage, was measured. The level of 8-oxodG was significantly higher in *Ercc1*$^{-/-}$ cells than in WT cells at both oxygen concentrations; however, when cultured in 21% oxygen, both cell lines had more 8-oxodG than those cultured in 3% oxygen. These data support the hypothesis that active demethylation of genes in aging cells is regulated by oxygen concentration.

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P2.19

The influence of pectin extract from flax shives on the expression of genes involved in the extracellular matrix remodelling in NHDF cell line after induction of the inflammatory state with LPS

Wioleta Wojtasik[1,✉], Anna Kulma[1], Jan Szopa[1]

[1]Department of Genetic Biochemistry, University of Wroclaw, Wroclaw, Poland

✉ wioleta.wojtasik@uwr.edu.pl

Keywords: pectin extract; flax shives; inflammation; extracellular matrix remodelling

Pectin, a structurally complex polysaccharide, is an underutilized natural resource with multi-aspect applications in the food, pharmaceutical, and medical industries. New research has revealed that pectin possibly plays a role in extracellular matrix remodelling, suggesting its participation in the wound healing process. Therefore, a better understanding of the role of pectin in this mechanism is essential for the development of novel strategies for tissue engineering and regenerative medicine.

This study verified the influence of pectin isolated from flax shives on the extracellular matrix remodelling process after induction of the inflammatory state. Flax shives enriched in pectin were obtained from transgenic flax that overexpressed *β-glucanase*.

The main fraction of pectin, the CDTA soluble fraction, from the control and transgenic plants and commercially available citrus pectin were analysed in order to determine how genetic modification affects the structure and composition of pectin. The uronic acid content, the monosaccharide composition, and the phenolic acid content associated with pectin were determined. The antioxidant potential was determined with the DPPH test. Changes in the mRNA level was determined using real time PCR of selected genes involved in the extracellular matrix remodelling in the NHDF cell line after induction of the inflammatory state by LPS and treatment with the pectin extract from the flax shives.

The obtained results, in particular the strong antioxidant properties of pectin from flax shives and its significant influence on the genes participating in the extracellular matrix remodelling process, suggest that pectin has a possible application as a wound healing activator.

P2.20

Aldehyde dehydrogenase is inactivated by S-sulfhydration *in vitro* and *in vivo*

Małgorzata Iciek[1,✉], Magdalena Górny[1], Anna Bilska-Wilkosz[1], Danuta Kowalczyk-Pachel[1]

[1]Chair of Medical Biochemistry, Jagiellonian University Medical College, Cracow, Poland
✉ miciek@cm-uj.krakow.pl

Keywords: aldehyde dehydrogenase; S-sulfhydration; reactive sulfur species; diallyl trisulfide; hydropersulfides

Protein S-sulfhydration is a kind of reversible oxidation of –SH groups to hydropersulfides (-SSH) under the influence of reactive sulfur species. In the last years, many proteins have been observed to be modified through this process. Aldehyde dehydrogenase (ALDH) catalyzes the oxidation of various aldehydes, including acetaldehyde formed in the metabolism of ethanol.

The aim of the present study was to examine whether the activity of aldehyde dehydrogenase can be regulated by the S-sulfhydration process. Studies were performed *in vitro* using yeast ALDH and *in vivo* in the rat liver.

In *in vitro* studies, ALDH was incubated with various S-sulfhydrating species: Na_2S, GSSH, diallyl trisulfide (DATS) and K_2S_x, and activity of the enzyme was measured. All tested compounds produced an inhibitory effect on the activity of ALDH, and polysulfides (DATS and K_2S_x) had the strongest influence. Dithiothreitol and dihydrolipoic acid restored the inhibited activity of the enzyme. The content of protein-bound sulfane sulfur significantly increased in samples of the enzyme treated with polysulfides. In *in vivo* studies, ALDH activity was estimated in the rat liver after *ip* administration of DATS and compared with control animals. ALDH activity was decreased vs. control after treatment with garlic-derived DATS.

The obtained results clearly demonstrated that ALDH activity could be regulated by S-sulfhydration and in this case, the addition of a sulfur atom to the key Cys residue led to the inhibition of the enzyme. This is the first study reporting the regulation of ALDH activity by S-sulfhydration.

P2.21

TOR/Insulin signaling in intestinal stem cells as a target for new anti-aging, anti-obese and anti-diabetic drugs

Oleh Lushchak[1,✉], Olha Stril'bytska[1]

[1]Biochemistry and Biotechnology Department, Precarpathian National University, Ivano-Frankivsk, Ukraine
✉ olushchak@yahoo.com

Keywords: stem cells; TOR; insulin; longevity; metabolism

Intestinal stem cells (ISCs) are involved in maintenance of gut integrity and its regeneration after damages. Their activity is regulated by signals from surrounding niche cells formed by enterocytes, enteroendocrine and muscle cells. We used temperature sensitive modification of GAL4/UAS system to manipulate TOR/IIS pathway specifically in ISCs. We found that lifespan was significantly affected when TOR/IIS was manipulated in ISCs. Unexpectedly, both inhibition and activation of pathway shorten the lifespan. The shortest lifespan of about two days was observed when insulin receptor was knocked-down by expression of InR-RNAi. Lifespan-shortening effects were partially rescued by malnutrition. Inhibition of TOR or activation of IIS increased reproduction but it was not accompanied by changes in feeding behaviour. Activation of TOR in ISCs (esg^{ts}>UAS-Rheb) induced the development of diabetic phenotype that was defined by increased concentration of glucose in hemolymph. Fat amount was significantly lower in flies with inhibited TOR and activated insulin pathway. This changes in fly physiology and metabolism may be in part explained by changes in endocrine regulation. We found that TOR (Tsc1/Tsc2) and insulin (InR-RNAi) pathway inhibition significantly increased the transcript levels of brain-derived insulin-like peptides (ILP). These results together with upregulation of tobi show an increased production of ILPs and thus increased insulin signalling. Finally, TOR/IIS activation increased transcript level for dilp6 that in flies is used for feedback regulatory signalling for fat body to insulin-producing cells. Taken together, these findings suggest the possibility to use TOR/IIS signalling in ISCs to extend the lifespan and treat metabolic disorders.

P2.22

Fibrinogen and soluble fibrin monomer complex blood content in patients with ischemic stroke and diabetes mellitus

Olha Kravchenko[1,✉], Tetiana Tsarenko[1], Oleksiy Savchuk[1]

[1]Taras Shevchenko National University of Kyiv, Kyiv, Ukraine
✉demidiko@gmail.com

Keywords: fibrinogen; soluble fibrin monomer complex; ischemic stroke; diabetes mellitus

Research has demonstrated that fibrinogen is a risk marker for cerebrovascular diseases in the general population, and it may also contribute to the increased atherosclerotic risk in patients with diabetes, especially type II diabetes mellitus (DM). The aim of the study was to test fibrinogen (F), soluble fibrin monomer complex (SFMC) levels and ancistron time test (AT) in blood of patients with ischemic stroke (IS) and stroke complicated by type II diabetes mellitus (IS+DM).

Eighty-five patients with computed tomography- and magnetic resonance imaging-confirmed IS were selected for the investigation, 25 of whom had type II DM. Isolation of SFMC was performed using the phosphate method, and F content was determined via thrombin-stimulated clot formation. AT and glucose levels were estimated using standard methods.

There were significant changes of fibrinogen and SFMC levels in patients with both investigated pathologies compared with controls. Fibrinogen indices were higher in patients with IS and DM, while SFMC was significantly different in patients with IS compared with control parameters in patients with IS alone. AT for plasma clotting was prolonged to 22.4 s in the patients with IS and to 19.9 s in patients with DM and stroke compared with control (18.5 s). This shows that IS and stroke complicated by diabetes are characterised by similar changes in hemostasis parameters, but that a higher quantity of blood SFMC and a more prolonged AT were established in the patients with stroke alone.