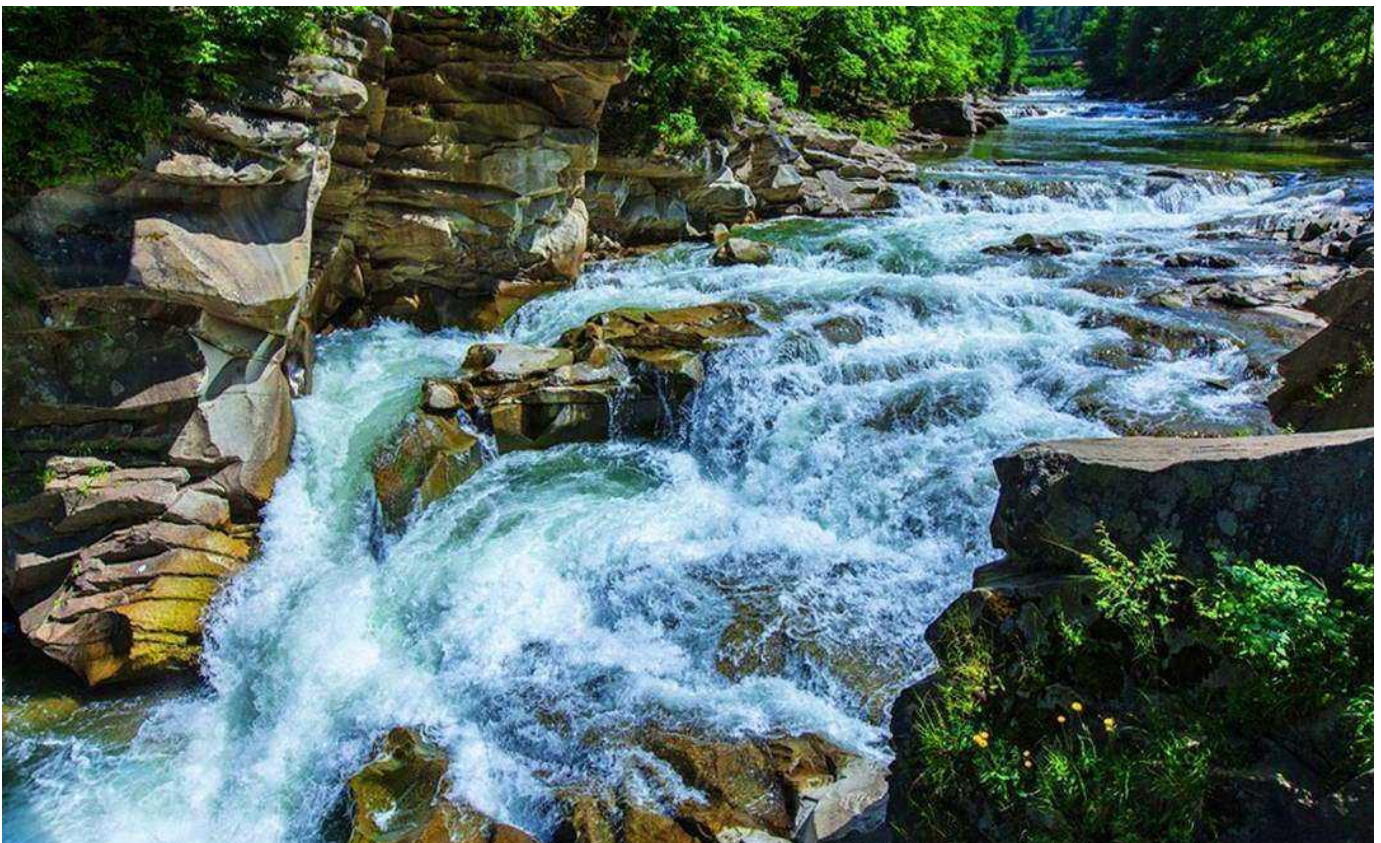


**Ukrainian Society of Cell Biology
Institute of Cell Biology NAS of Ukraine
Ivano-Frankivsk National Medical University**

6th Ukrainian Congress for Cell Biology with international representation

PROCEEDINGS



18-21 June 2019, Yaremche, Ukraine

6th Ukrainian Congress for Cell Biology with international representation

Proceedings. – Yaremche, 2019. – 180 p.

Proceedings contain the materials of *6th Ukrainian Congress for Cell Biology with international representation*, which was focused on novel insights in cell biology and biotechnology in Ukraine and abroad. The authors are solely responsible for the content of the abstracts.

Edited by:

Prof. Sibirny A.A.
Dr. Sci. Panchuk R.R.
Dr. M. Semkiv

Desktop publishing, cover design by: **Rostyslav Panchuk**

CONTENTS

Conference Program.....	1
Sessions	
Plenary lectures.....	5
Apoptosis, autophagy, cell signaling.....	10
Cell response on stress.....	25
Cellular, genetic and metabolic engineering.....	62
Tumor cell biology.....	88
Plant cell biology.....	125
Biology of stem cells and specialized cells and tissues.....	156
Index of authors.....	176

6th Ukrainian Congress for Cell Biology with international representation

*June 18-21, 2019,
Yaremche*

6th Ukrainian Congress for Cell Biology with international representation

CONFERENCE PROGRAM

*June 18-21, 2019,
Yaremche*

6th Ukrainian Congress for Cell Biology with international representation

*June 18-21, 2019,
Yaremche*

CONFERENCE PROGRAM

June 18 (TUESDAY)

- 13:00 – 15:00 Registration of participants of the conference, recreation center “Arnika”, central building, I. Petrasha Str. 30, Yaremche
- 15:00 – 15:30 Conference opening, press release for the media**
- 15:30 – 17:30 **Plenary session**
Chair, Andriy Sibirny (Lviv)
- 15:30 – 16:10 Maryna Skok. "Mesenchymal stem cells as a therapeutic tool to cure cognitive impairment caused by neuroinflammation or alpha7 nicotinic acetylcholine receptor deficiency "
- 16:10 – 16:50 Grzegorz Wegrzyn. "Changes in transcriptomes of cells derived from patients suffering from different types of mucopolysaccharidosis"
- 16:50 – 17:30 Patrick Fickers. "The quest for the best cell factory for the production of recombinant proteins: *Pichia pastoris* vs *Yarrowia lipolytica*"
- 17:30 – 19:00 **Get together party**

June 19 (WEDNESDAY)

- 9:00 – 11:00 Session "**Cell signaling, apoptosis, autophagy**"
Chair, Oleh Stasyk (Lviv)
(recreation center “Arnika”, central building, I. Petrasha Str. 30, Yaremche)
- 9:00 – 9:20 Lecture 1. Andriy Sibirny. "Autophagy is the primary mechanism involved in degradation of the cytosolic enzymes of methanol metabolism in the methylotrophic yeast *Komagataella pastoris*"
- 9:20 – 9:40 Lecture 2. Lyudmila Drobot. "The epithelial-mesenchymal plasticity is a driving force of tumor progression"
- 9:40 – 10:00 Lecture 3. Olexandr Korchynskyy. "Overexpression of direct bone morphogenetic protein/Smad target gene inhibitory kBa upon BMP7 treatment is insufficient to prevent and treat rheumatoid arthritis"
- 10:00 – 10:20 Lecture 4. Kostyantyn Dmytruk. "The role of peroxisomes in xylose alcoholic fermentation in the engineered *Saccharomyces cerevisiae*"
- 10:20 – 10:40 Lecture 5. Yuriy Kit. "Identification of cortactin-like proteins in human urine at norm and pathology: diagnostic and prognostic potentials"
- 10:40 – 11:00 *Presentation of "Bionix Lab" company*
- 11:00 – 11:30 **Coffee break**
- 11:30 – 13:30 Session "**Tumor cell biology**"
Chair, Rostyslav Stoika (Lviv) (recreation center “Arnika”, central building, I. Petrasha Str. 30, Yaremche)

6th Ukrainian Congress for Cell Biology with international representation

- 11:30 – 11:50 Lecture 1. Rimantas Daugelavicius. "Conditions and factors leading to multidrug resistance in bacteria and eukaryotic cells"
- 11:50 – 12:10 Lecture 2. Rostyslav Stoika. "Potentials of nanocarriers in circumventing tumor cell resistance to anticancer drugs and diminishing negative side effects of their action in the treated organism"
- 12:10 – 12:30 Lecture 3. Oleh Stasyk. "Metabolic anticancer enzymotherapy based on arginine deprivation and new combinational approaches"
- 12:30 – 12:50 Lecture 4. Rostyslav Panchuk. "Circumvention of tumor drug resistance by quinone-containing compounds: role of extramitochondrial ROS"
- 12:50 – 13:10 Lecture 5. Nataliya Hudz. "Leaning points around biocompatibility of PD solutions measured as *in vitro* proliferation of HepG2 and Vero cells"
- 13:10 – 13:30 Lecture 6. Nelia Kyshynets. "Biological drugs obtained by means of high-tech processes: quality assurance in accordance with the requirements of the State Pharmacopoeia of Ukraine"
- 13:30 – 14:30 **Lunch** (in cafeteria at central building of recreation center "Arnika", I. Petrasha Str. 30, Yaremche)
- 14:30 – 16:50 Session "**Cell response on stress**"
Chair, Rostyslav Panchuk (Lviv)
(recreation center "Arnika", central building, I. Petrasha Str. 30, Yaremche)
- 14:30 – 14:50 Lecture 1. Alexander Rapoport. "Anhydrobiosis: Survival without water"
- 14:50 – 15:10 Lecture 2. Sergiy Beschasnyi. "Effects of the carbon monoxide releasing molecule-2 on human erythrocyte membranes"
- 15:10 – 15:30 Lecture 3. Kostyantyn Kuznetsov. "Cell membrane permeability changes under exposure to neutron radiation"
- 15:30 – 15:50 Lecture 4. Oksana Stolyar. "Application of the novel integrative index of oxidative stress basing on the experience of aquatic mollusks study"
- 15:50 – 16:10 Lecture 5. Raisa Piskun. "Compensatory-adaptive potential of cardiocytes under conditions of experimental cholesterol loading the animal models"
- 16:10 – 16:30 Lecture 6. Anastasiia Rozhyna. "The influence of low power microwave radiation of 2,4 GHz frequency on human cells"
- 16:30 – 16:50 Lecture 7. Oksana Novikova. "Viability and proliferative activity of cryopreserved cultures of dermal papillas of rabbits"
- 16:50 – 17:10 **Coffee break**
- 17:10 – 18:30 Poster session №1 (sections 1, 2, 4, 6)

6th Ukrainian Congress for Cell Biology with international representation

18:30 – 19:30 **Dinner** (in cafeteria at central building of recreation center “Arnika”, I. Petrasha Str. 30, Yaremche)

June 20 (THURSDAY)

9:00 – 11:50 Session "**Cellular, genetic and metabolic engineering**"

Chairs, Yaroslav Blume (Kyiv)

(recreation center “Arnika”, central building, I. Petrasha Str. 30, Yaremche)

- 9:00 – 9:20 Lecture 1. Mykhailo Gonchar. "Recombinant enzymes, human arginase i and microbial arginine deiminase, as the biorecognition elements for arginine-selective amperometric biosensors and enzymatic kits"
- 9:20 – 9:40 Lecture 2. Justyna Ruchala. "Importance of peroxisomes and peroxisomal enzymes of pentose phosphate pathway for xylose alcoholic fermentation in the thermotolerant methylotrophic yeast *Ogataea polymorpha*"
- 9:40 – 10:00 Lecture 3. Daria Fedorovych. "New approaches to improve riboflavin production in the yeast *Candida famata*"
- 10:00 – 10:20 Lecture 4. Olena Kurylenko. "Evaluation of transcriptional factors involved in regulation of xylose metabolism and fermentation in the thermotolerant yeast *Ogataea polymorpha*"
- 10:20 – 10:40 Lecture 5. Marta Semkiv. “Crude glycerol bioconversion to fuel ethanol by methylotrophic yeasts”
- 10:40 – 11:00 Lecture 6. Nataliya Finiuk. "Application of poly-DMAEMA-containing carriers for delivery of plasmid DNA to mammalian cells"
- 11:00 – 11:20 Lecture 7. Oleh Smutok. “The recombinant yeast *Ogataea polymorpha*, overproducing flavocytochrome b2, as a tool for L-lactate and chromate analysis, as well as for chromate bioremediation”
- 11:20 – 11:40 Lecture 8. Volodymyr Granovski. "Expression of heterologous proteins in *E.coli* and *H. polymorpha* using recombinant DNA technologies for vaccine development and diagnostics applications"
- 11:40 – 11:50 *Presentation of Xema Company*
- 11:50 – 12:10 **Coffee break**
- 12:10 – 13:00 Poster session №2 (sections 3, 5)
- 13:00 – 14:00 **Lunch** (in cafeteria at central building of recreation center “Arnika”, I. Petrasha Str. 30, Yaremche)
- 14:00 – 18:00 Yaremche tour
- 18:00 – 19:00 **Dinner** (in cafeteria at central building of recreation center “Arnika”, I. Petrasha Str. 30, Yaremche)

Or

19:00 – 22:00 **Banquet**

CONFERENCE PROGRAM

June 21 (FRIDAY)

- 9:00 – 10:40 Session "**Plant cell biology**"
Chair, Alla Yemets (Kyiv)
(recreation center “Arnika”, central building, I. Petrasha Str. 30, Yaremche)
- 9:00 – 9:20 Lecture 1. Alla Yemets. "Lactoferrin expression as a tool for the enhancement of non-specific plant pathogen resistance"
- 9:20 – 9:40 Lecture 2. Yuriy Kolupayev. "Nitric oxide, synthesized by nitrate reductase, as participant of transduction of hydrogen sulphide signal at induction of heat resistance of wheat plantlets"
- 9:40 – 10:00 Lecture 3. Galyna Shevchenko. "Adaptative changes of proteome in arabidopsis seedlings from chernobyl zone"
- 10:00 – 10:20 Lecture 4. Olena Kravets. “Role of cytomixis in a mechanism of microsporocyte kariotype rearrangement”
- 10:20 – 10:40 Lecture 5. Volodymyr Shkarupa. ”Optimized system of analysis of mutagenic modifiers based on the model plant of *Allium cepa L.*”
- 10:40 – 11:00 Coffee break
- 11:00 – 12:20 **Plenary session**
Chair, Andriy Sibirny (Lviv)
- 11:00 – 11:40 Yaroslav Blume. "New inhibitors of FtsZ proteins: the way from high-throughput molecular screening up to verification *in vitro*"
- 11:40 – 12:20 Alicja Jozkowicz. “Nrf2 and Keap1: a quintessential duet moonlights in endothelium”
- 12:20 – 12:40 Report of Audit Commission on the activities of the Ukrainian Society of Cell Biology for the 2016-2019.
- 12:40 – 13:20 Short oral presentations of 5 best posters of young scientists (under 35 years). 5 min presentation+2 min questions.
- 13:20 – 13:40 Conference closing ceremony. Young Scientists Award for the best poster presentation.
- 13:40 – 14:40 **Lunch** (in cafeteria at central building of recreation center “Arnika”, I. Petrasha Str. 30, Yaremche)

6th Ukrainian Congress for Cell Biology with international representation

PLENARY LECTURES

*June 18-21, 2019,
Yaremche*

6th Ukrainian Congress for Cell Biology with international representation

*June 18-21, 2019,
Yaremche*

Plenary lectures

**MESENCHYMAL STEM CELLS AS A THERAPEUTIC TOOL TO CURE COGNITIVE
IMPAIRMENT CAUSED BY NEUROINFLAMMATION OR ALPHA7 NICOTINIC
ACETYLCHOLINE RECEPTOR DEFICIENCY**

Skok Maryna

Plenary Lecture

Skok M.V.

*Palladin Institute of Biochemistry, National Academy of Science of Ukraine, 9, Leontovycha str, 01030
Kyiv, Ukraine*

E-mail: skok@biochem.kiev.ua

Mesenchymal stem cells (MSCs) are self-renewing multipotent cells able to differentiate into multiple cell types including neurons. In addition, MSCs produce numerous trophic and growth factors affecting neurogenesis, synaptogenesis and cell survival. The efficiency of regenerative MSC therapy has been proved in many experimental models. Neuroinflammation caused by injections of bacterial lipopolysaccharide (LPS) results in memory impairment in mice accompanied by elevated amounts of amyloid beta peptide A β (1-42) and decreased levels of α 7 nicotinic acetylcholine receptors (nAChRs) in the brain. Immunization of mice with α 7 nAChR extracellular domain (1-208) also results in inflammatory effect and memory impairment. Finally, mice lacking α 7 nAChRs (α 7^{-/-}) possess a pro-inflammatory phenotype and demonstrate impaired episodic memory. Taken together, these data illustrate a key role of α 7 nAChRs in neuroinflammation and memory. Our experiments were undertaken to find out if MSCs can prevent and cure the pathogenic symptoms caused by either inflammation or α 7 nAChR deficiency. We used MSCs isolated from either human umbilical cord (hMSCs) or mouse placenta (mMSCs) and the conditioned medium obtained after 2 days of MSCs culturing in serum-free medium. MSCs injected intravenously penetrated the brain of LPS-pre-treated mice and prevented episodic memory impairment, mitochondria damage, A β (1-42) accumulation and nAChR decrease in the brain caused by LPS. Moreover, MSCs could reverse the pathogenic symptoms developed 3 weeks after LPS injection. MSCs also penetrated the brains of α 7^{-/-} mice and transiently improved their episodic memory. Similar, although weaker and shorter, effects were observed after intraperitoneal injections of MSCs conditioned medium suggesting that memory improvement was caused by soluble factors produced by MSCs. Cultured MSCs produced IL-6 in response to LPS and stimulated an IL-6 increase in the brain, which coincided with the improvement of episodic memory. Injections of recombinant IL-6 also improved episodic memory of α 7^{-/-} mice accompanied by the up-regulation of α 3, α 4, β 2 and β 4 nAChR subunits in the brain. In whole, the data obtained indicate that MSCs, injected intravenously, are able to cross the blood-brain of mice loosened by inflammation and persist there for at least two weeks. They improve episodic memory of mice and make their mitochondria more resistant to apoptogenic influence. One of the soluble factors responsible for the memory improvement is IL-6.

CHANGES IN TRANSCRIPTOMES OF CELLS DERIVED FROM PATIENTS SUFFERING FROM DIFFERENT TYPES OF MUCOPOLYSACCHARIDOSIS

Wegrzyn Grzegorz

Plenary Lecture

Lidia Gaffke*, Karolina Pierzynowska*, Magdalena Podlacha, Joanna Brokowska,
Grzegorz Wegrzyn

Department of Molecular Biology, University of Gdansk, Wita Stwosza 59, 80-308 Gdansk, Poland

E-mail: grzegorz.wegrzyn@biol.ug.edu.pl

* These authors contributed equally to this work.

Mucopolysaccharidoses (MPS) are a group of genetic disorders belonging to lysosomal storage diseases (LSD). They are caused by genetic defects leading to either a lack or severe deficiency of activity of one of lysosomal enzymes involved in degradation of glycosaminoglycans (GAGs). Partially degraded GAGs accumulate in lysosomes which results in dysfunctions of cells, tissues and organs. Depending on the kind of the deficient enzyme, 11 types and subtypes of MPS are distinguished. Until recently, it was assumed that GAG accumulation in cells is the major, if not the only, mechanism of pathogenesis in MPS, as GAGs may be a physical ballast for lysosomes causing inefficiency of cells due to a large amount of a stored material. However, recent reports (including articles published by our team) suggested that in MPS cells there are changes in many different processes which might be even more important for pathogenesis than lysosomal accumulation of GAGs *per se*. Therefore, the aim of this work was to analyze changes in transcriptomes in fibroblasts derived from patients suffering from all known types and subtypes of MPS. The RNAseq method was used to determine genes which expression is disturbed in particular MPS types. We found that each MPS type and subtype is characterized with specific changes in expression of a various genes relative to cells derived from a healthy person. Nevertheless, some changes in the gene expression patterns were common for various MPS types. Generally, one can conclude that several cellular processes are affected in MPS cells due to disturbed regulation of expression of specific genes, coding for proteins involved in particular cellular functions. The putative changes include following processes: cytoskeleton dynamics, vacuolar transport, cell cycle, DNA replication, functions of lysosomes, mitochondria and ribosomes, apoptosis and autophagy. Further studies will lead to detailed determination of biochemical pathways which are disturbed in cells of different MPS types. Nevertheless, information gained from the transcriptomic studies suggests hypothetical molecular mechanisms responsible for the changes in particular cellular processes.

THE QUEST FOR AN EFFICIENT CELL FACTORY FOR THE PRODUCTION OF RECOMBINANT PROTEINS: *PICHA PASTORIS* VS *YARROWIA LIPOLYTICA*.

Fickers Patrick

Plenary Lecture

Marie Vandermies, Chrispian Theron, Patrick Fickers

Microbial Processes and Interactions, TERRA Teaching and Research Centre, University of Liège - Gembloux AgroBio Tech, Av de la Faculté, 2B. B-5030 Gembloux, Belgium

E-mail: pfickers@uliege.be

The non-conventional yeasts *Y. lipolytica* and *P. pastoris* are both well-established cell factories for the synthesis of recombinant proteins (rProt) in industry. Surprisingly, no direct comparison of their performances for rProt synthesis has been reported so far. Here, we report such a comparison using the CalB lipase from *Candida antarctica* as a model protein. The corresponding codon optimized gene sequence was cloned under the control of strong inducible promoters, namely *pEYKA3B* and *pAOX1*, and expressed in recipient strains *Y. lipolytica* *EYK1ko* and *P. pastoris* MutS, respectively. With the resulting strains, cell growth, gene expression, carbon uptake rates and extracellular lipase activity were monitored over time during cultures in bioreactor in optimized conditions. *Y. lipolytica* performances were by far superior in terms of cell growth rate and extracellular lipase activity, although *P. pastoris* showed a significantly higher level of CalB gene expression. This lower protein productivity in *P. pastoris* was found related to the intracellular protein degradation by the proteasome complex. From this study, *Y. lipolytica* appears a more efficient cell factory that is in addition not related to the utilization of flammable methanol as inducer.

Plenary lectures

NEW INHIBITORS OF FTSZ PROTEINS: THE WAY FROM HIGH-THROUGHPUT MOLECULAR SCREENING UP TO VERIFICATION *IN VITRO*

Blume Yaroslav

Plenary Lecture

Yaroslav Blume, Alex Rayevsky, Svitlana Spivak, Serhii Ozheredov, Oleg Demchuk, Alla Yemets, Pavlo Karpov

*Institute of Food Biotechnology and Genomics, Natl. Acad. of Sci. of Ukraine
Osypovskoho str., 2A, Kyiv, Ukraine 04123*

E-mail: blume.yaroslav@nas.gov.ua

Due to the emergence of multidrug-resistance tuberculosis (MDR-TB) and TB's role as a major opportunistic pathogen in patients with HIV/AIDS, the need for new chemotherapeutics that will be effective against both sensitive and MDR-TB has become prominent. The current area of investigation for the development of new novel drugs against TB is bacterial cell division.

The purpose of our project was selection of novel anti-TB compounds targeted the main cell division protein of *Mycobacteria*, based on results of high-throughput screening, molecular dynamics simulations, *ab initio* fragment molecular orbital calculations, as well as the *in vitro* analysis of ligand-protein interactions based on the inhibition of GTP hydrolysis of FtsZ-protein, inhibition of its polymerization *in vitro*.

Within the framework of virtual organization CSLabGrid, high-throughput molecular screening has been performed for new anti-tuberculosis compounds. Using the FlexX program installed on the Institute of Food Biotechnology and Genomics (IFBG) Cluster and models of four promising ligand binding sites on the surface of FtsZ protein from *Mycobacterium tuberculosis*, virtual screening has been done for the database containing 2886 compounds synthesized in the Institute of Organic Chemistry of the NAS of Ukraine. Based on the LE and ΔG scores, the docking scores of CCDC Gold, and the results of molecular dynamics, a group of Mycobacterial FtsZ inhibitors has been selected. *In vitro* validation have revealed 6 compounds with the highest inhibition of GTPase activity of FtsZ. Also, based on *in vitro* experiment, three of selected compounds demonstrate strong inhibition of FtsZ polymerization together with inhibition of its GTPase activity.

Respectively we can propose a short list of most promising leading compounds of new perspective agents for TB-treatment for testing on living bacteria culture.

NRF2 AND KEAP1: A QUINTESSENTIAL DUET MOONLIGHTS IN ENDOTHELIUM

Józkowicz Alicja

Plenary Lecture

Anna Grochot-Przeczek¹, Aleksandra Kopacz, Damian Kloska¹, Dominik Cysewski², Nicolas Personnic¹, Aleksandra Piechota-Polanczyk¹, Jozef Dulak¹, [Alicja Jozkowicz](mailto:alicja.jozkowicz@uj.edu.pl)¹

1 – Department of Medical Biotechnology, Faculty of Biochemistry Biophysics and Biotechnology, Jagiellonian University, 30-387 Krakow, Poland

2 – Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics, Polish Academy of Science, 02-106 Warsaw, Poland

E-mail: alicja.jozkowicz@uj.edu.pl

Oxidative stress and premature senescence is conducive to aging and cardiovascular diseases. Nrf2 transcription factor, the master orchestrator of adoptive response to cellular stress, has been implicated in regulation of premature senescence in fibroblasts, neural and mesenchymal stromal cells by transactivation of antioxidant gene expression. However, a molecular switch between normal, senescent and apoptotic fate remains unknown.

Recently we have shown that GDF-15- and SDF-1-induced angiogenesis strongly depends on the presence of Nrf2 protein in endothelial cells (EC) but does not rely on its transcriptional activity. Instead, Nrf2 serves as a protein tethering Keap1, its known transcriptional repressor, to allow podosome assembly and angiogenesis. We have also demonstrated that human primary ECs devoid of Nrf2, and murine Nrf2 transcriptional knockout (tKO) aortas are senescent. Surprisingly, they do not encounter oxidative stress and damage, and senescence is not a primary cause of the decrease in their angiogenic potential.

We were looking to elucidate the mechanism of Nrf2-related premature senescence of vascular system, to understand why Nrf2 deregulation does not cause oxidative stress in ECs, and to indicate a molecular switch determining EC fate. We evidenced that ECs deficient in Nrf2 protein, or with limited Nrf2 activity in shear stress conditions, exhibit excessive S-nitrosylation of proteins. It is also a characteristic feature of Nrf2 tKO murine aortas, as determined by biotin switch assay *in situ*. Mass spectrometry analysis reveals that NOX4 is S-nitrosylated exclusively in ECs devoid of Nrf2. We suppose that a functional role of S-nitrosylation is protection of ECs from death by inhibition of NOX4-mediated oxidative damage. As a result, the Nrf2-deficient ECs preserve oxidative balance but are redirected to premature senescence. The same phenotype is seen in Nrf2 tKO aortas. These effects are mediated by Keap1 repressor, a direct binding partner of Nrf2, remaining in cytoplasm unrestrained by Nrf2. S-nitrosylation, followed by senescence, can also be triggered in smooth muscle cells by EC-derived paracrine induction of iNOS.

To sum up, disturbed Nrf2 signaling in endothelial cells leads to the Keap1-dependent S-nitrosylation of NOX4, what hampers oxidative detriment and may provide defense in the adjacent aortic cells. Overabundance of unrestrained Keap1 in the cytoplasm determines the fate of endothelial cells.

6th Ukrainian Congress for Cell Biology with international representation

*June 18-21, 2019,
Yaremche*

Session 1

Apoptosis, autophagy, cell signaling

AUTOPHAGY IS THE PRIMARY MECHANISM INVOLVED IN DEGRADATION OF THE CYTOSOLIC ENZYMES OF METHANOL METABOLISM IN THE METHYLOTROPHIC YEAST *KOMAGATAELLA PASTORIS*

Andriy Sibirny

Lecture 1

Andriy A. Sibirny^{1,2}, Olena V. Dmytruk¹, Marta V. Semkiv¹

1 – Department of Molecular Genetics and Biotechnology, Institute of Cell Biology, NAS of Ukraine, Drahomanov Street, 14/16, Lviv 79005 Ukraine

2 – Department of Biotechnology and Microbiology, University of Rzeszow, Zelwerowicza 4, Rzeszow 35-601 Poland

E-mail: sibirny@yahoo.com

Methylotrophic yeasts metabolize methanol using peroxisomal (alcohol oxidase, catalase, dihydroxyacetone phosphate synthase) and cytosolic (fructose-1,6-bisphosphatase, formaldehyde dehydrogenase, S-formylglutathione hydrolase, formate dehydrogenase) enzymes. After shift of methanol-grown cells to glucose medium, rapid drop in the activities of peroxisomal and cytosolic enzymes of methanol metabolism occur and such drops show similar kinetics. It is known for many years that the drop in activities of peroxisomal enzymes after shift of methanol-grown cells to glucose medium occurs due to autophagic degradation of peroxisome (pexophagy). Mechanisms of rapid decrease in activities of cytosolic enzymes of methanol metabolism after such shift remained unknown for long time. We have found that inactivation of fructose-1,6-bisphosphatase in methanol-grown cell transferred into glucose occurs due to protein degradation. We also observed that inactivation of fructose-1,6-bisphosphatase, formaldehyde dehydrogenase and formate dehydrogenase under mentioned condition in the yeast *Komagataella pastoris* is slowed down in the mutants with defected vacuolar proteinases whereas inhibitor of the located in cytosol proteasomal protein degradation MG-132 did not affect this process. These data unequivocally indicates that inactivation of cytosolic enzymes of methanol metabolism occurs due to their degradation and this degradation occurs in vacuole in autophagic process. Our main goal is to isolate the collection of the mutants defective in specific autophagic degradation of the cytosolic proteins. However, there are not convenient procedures for selection of the mutants defective in degradation of fructose-1,6-bisphosphatase, formaldehyde dehydrogenase or formate dehydrogenase. Therefore we decided to construct *K. pastoris* strains which express heterologous β -galactosidase coding by LAC4 gene of *Kluyveromyces lactis* under control of *K. pastoris* FLD1 promoter of formaldehyde dehydrogenase. Resulted *K. pastoris* transformants indeed synthesized β -galactosidase in during cultivation in methanol medium and such activity was dropped after shift the cells into medium with glucose. Inactivation was impaired in the strain defective in vacuolar proteinases. Strains of *K. pastoris* which express Lac4-GFP hybrid protein have been constructed. Localization of the hybrid protein during inactivation caused by glucose is under study. Possible mechanisms of autophagic degradation of cytosolic enzymes in *K. pastoris* will be discussed.

THE EPITHELIAL-MESENCHYMAL PLASTICITY AS A DRIVING FORCE OF CANCER PROGRESSION

Liudmyla Drobot

Lecture 2

Liudmyla Drobot

Palladin Institute of Biochemistry, National Academy of Science of Ukraine, 9 Leontovycha str., Kyiv, 01030, Ukraine

E-mail: drobot@biochem.kiev.ua

The ability of tumor cells of epithelial origin to reciprocal reprogramming/trans-differentiation in the course of epithelial-to-mesenchymal/amoeboid transitions (EMT/MAT) is associated with acquisition of a more aggressive tumor phenotype tightly interrelated with conversion of noninvasive tumor cells into stem-like states, increase of therapy resistance and metastatic potential, and is currently referred as epithelial-mesenchymal plasticity (EMP). In many cases, to undergo EMP, cancer cells do not require additional genetic changes. Signaling strategies that orchestrate EMP involve context-dependent dynamic changes in extracellular milieu, consequent modulation of receptor-mediated signaling networks providing a fine control of epigenetic events, gene and miRNA expression patterns, translation and post-translational modifications, cell morphology and behavior. Adaptor/scaffold proteins belong to the critical components of intracellular signaling networks that determine formation and localization of signaling complexes in dynamic and selective fashion, and can support or inhibit signal transduction depending on their stoichiometry in a particular compartment thus regulating signal specificity, efficiency and the amplitude of signal propagation. In addition, the ability of some adaptor/scaffold proteins to act as ultra-sensitive switches that determine alternative cell fates has been currently recognized. Data that demonstrate the key role of adaptor protein Ruk/CIN85 in the control of EMP will be presented.

Acknowledgments. This work was partially supported by SCOPES grant № IZ73ZO from Swiss National Science Foundation (SNSF).

OVEREXPRESSION OF DIRECT BONE MORPHOGENETIC PROTEIN/SMAD TARGET GENE INHIBITORY κ B α UPON BMP7 TREATMENT IS INSUFFICIENT TO PREVENT AND TREAT RHEUMATOID ARTHRITIS

Olexandr Korchynskyi

Lecture 3

Olexandr Korchynskyi^{1,2,3,4}, Frank P. Luyten⁵, Rik Lories⁵, Peter ten Dijke⁴

1 – Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology NAS of Ukraine, Drahomanov Street, 14/16, Lviv 79005 Ukraine

2 – Medical Faculty, Rzeszów University, Poland

3 – Thurston Arthritis Research Centre, University of North Carolina at Chapel Hill, NC, U.S.A.

4 – Department Cell and Chemical Biology, Leiden University Medical Centre, Leiden, The Netherlands

5 – Skeletal Biology & Engineering Research Centre, KU Leuven, Belgium

E-mail: olexkor@hotmail.com

Introduction: Loss of articular cartilage in the joints associated with bone erosions is a common problem in patients with chronic inflammatory arthritis. Several reports suggest a role for BMP7 in preventing joint damage and in promoting regeneration in part by mitigating the catabolic effects of TNF α and IL-1 β . The precise molecular mechanism mediating the preventive and regenerative effects of BMP7 is unknown. Our transcriptional profiling studies revealed that the activation of BMP signaling leads to increased expression of *Inhibitory κ B α* (IkB α), which is a key negative regulator of a major proinflammatory NF- κ B pathway. We therefore explored whether anti-catabolic effects of BMPs based on BMP-induced IkB α expression in cartilage and bone could be used to prevent joint damage in an animal model of inflammatory arthritis.

Methods: cDNA microarrays-based gene expression profiling was used to discover novel BMP target genes. Positive hits were confirmed using Northern and Western blotting and Real-Time PCR. Luciferase reporter assays and ChIP assay were used to characterize the BMP-responsive region in IkB α promoter. Anti-catabolic effects of BMPs and their effect on osteoblast differentiation were validated by genetic shRNAs-mediated depleting studies using pre-osteoblastic cell lines and primary human mesenchymal stem cells (hMSC). To interrogate the *in vivo* relevance of our findings we used a model of experimentally-induced arthritis in DBA/1 mice upon inoculation of BMP7-expressing adenovirus into a knee. All experiments were approved by the Ethics Committee for Animal Research (KU Leuven, Belgium; P198/2012).

Results: Real-Time PCR showed that activation of IkB α mRNA by BMPs does not require *de novo* protein synthesis, thus suggesting IkB α is a direct BMP target gene. Using ChIP assays we demonstrated BMP intracellular effectors, i.e. Smad1/5 and Smad4, bound to the highly conserved proximal region of IkB α promoter. A proximal fragment of IkB α promoter was found to be activated by BMP2 and BMP7. EMSA assay showed that BMP7-induced IkB α expression blocks formation of TNF α -induced NF- κ B transcriptional complex. In addition, BMP treatment was found to inhibit TNF α and LPS-induced NF- κ B transcriptional response in mouse preosteoblasts and hMSC and rescued the osteogenic differentiation of MSC from proinflammatory inhibition. shRNA-mediated knockdown of IkB α expression confirmed an essential role of IkB α in mediating of anti-catabolic effects of BMPs. However, anti-catabolic effects of BMPs *in vivo* appeared to be insufficient for effective prevention and treatment of experimental arthritis in DBA/1 mice.

Conclusion: IkB α is a direct BMP/Smad target gene and its expression could have a potential to be a basis for preventive and BMP7 regenerative effects on degrading cartilage and bone. Unfortunately, our *in vivo* studies showed effects of BMP7 as insufficient to affect cartilage and bone regeneration in inflamed joints.

**THE ROLE OF PEROXISOMES IN XYLOSE ALCOHOLIC FERMENTATION IN THE
ENGINEERED *SACCHAROMYCES CEREVISIAE***

Kostyantyn Dmytruk

Lecture 4

Kostyantyn Dmytruk¹, Liubov Dzanaieva¹, Justyna Ruchala², Barbara Kruk², Jens Nielsen³, Andriy Sibirny^{1,2}

1 – Institute of Cell Biology National Academy of Science of Ukraine,
Drahomanov Street 14/16, 79005 Lviv, Ukraine

2 – Department of Microbiology and Biotechnology, University of Rzeszow,
Zelwerowicza 4, 35-601 Rzeszow, Poland

3 – Chalmers University of Technology, Department of Biology and Biological Engineering,
Kemigården 1, SE-412 96 Göteborg, Sweden

E-mail: dmytruk77@gmail.com

Peroxisomes are membrane-enclosed organelles occurring in the cytoplasm of most *eukaryotic cells*. Peroxisomes contain more than 50 different enzymes, which are involved in a variety of biochemical pathways. In yeasts, peroxisomes are involved in fatty acid beta-oxidation, glyoxylic acid cycle, catabolism of unusual carbon sources like purines, methanol etc. Peroxisomes reside many oxidases producing hydrogen peroxide and catalase, which decomposes this toxic compound. Very few are known on the role of peroxisomes on the metabolism of carbohydrates.

Recently we have found that peroxisomes are indispensable for xylose (but not glucose) alcoholic fermentation in the methylotrophic yeast *Ogataea polymorpha*. Peroxisome-deficient *pex3Δ* mutant completely lost ability to produce ethanol from xylose (Kurylenko et al., 2018). This phenomenon is most probably associated with mislocalization of peroxisomal enzymes transketolase (another name, dihydroxyacetone synthase) and transaldolase involved in xylose catabolism, since overexpression of both enzymes resulted in significant increase of ethanol production from xylose.

Wild type strains of Saccharomyces cerevisiae are not able to grow on xylose or ferment this pentose to ethanol. In this work, we have studied role of peroxisomes in xylose alcoholic fermentation in the engineered xylose-utilizing strain of *S. cerevisiae* (Scalcinati et al., 2012). Peroxisome-deficient *pex3Δ* mutant was constructed on the background xylose-utilizing strain of *S. cerevisiae*. The *pex3Δ* revealed slight decrease in ethanol production from xylose, while glucose alcoholic fermentation remained unimpaired. The Pex34 belongs to the peroxisome integral membrane proteins, which are responsible for constitutive peroxisome division (Zhou et al., 2016). The *PEX34* gene was overexpressed under control of strong constitutive *TEF1* promotor aiming to increase peroxisome proliferation. Constructed strain possessed slight increase of ethanol production from xylose relative to that of parental strain. Obtained results let us to suppose that peroxisomes somehow involved in xylose alcoholic fermentation in xylose-utilizing strain of *S. cerevisiae*.

Kurylenko O. et al. *Biotechnol Biofuels*. 2018, 11:197.

Scalcinati G. et al., *FEMS Yeast Res*. 2012, 12(5):582-97.

Zhou Y.J. et al., *J Am Chem Soc*. 2016, 138(47):15368-15377.

IDENTIFICATION OF CORTACTIN-LIKE PROTEINS IN HUMAN URINE AT NORM AND PATHOLOGY: DIAGNOSTIC AND PROGNOSTIC POTENTIALS

Yuriy Kit

Lecture 5

Marina Starykovych¹, Serhiy Souchelnytskyi², Orest Abrahamovych³, Mar' yana Abrahamovych⁴, Oksana Fayura³, Nazar Lukavetsky⁵, Rostyslav Stoika¹, Yuriy Kit¹

1 – Institute of Cell Biology, NAS of Ukraine, Drahomanov Str. 14/16, Lviv, 79005, Ukraine.

2 – College of Medicine, Qatar University, Doha, Qatar

3 – Department of Internal Medicine № 1, Danylo Halytsky Lviv National Medical University, Pekarska Str. 69, Lviv, 79010, Ukraine

4 – Department of Family Medicine, Danylo Halytsky Lviv National Medical University, Pekarska Str. 69, Lviv, 79010, Ukraine

5 – Lviv State Oncology Regional Treatment and Diagnostic Center, J. Hasheka Str., 2A 79000, Lviv, Ukraine

E-mail: kit@cellbiol.lviv.ua

The protein compositions of human urine reflect the changes of the biochemical state in a human organism, thus, possessing an essential diagnostic value. We used precipitation/extraction approach (1) toward samples of human urine and isolated a Mr ~100 kDa protein. MALDI TOF/TOF mass-spectrometry demonstrated similarity of structure of this protein with cortactin (UniProtKB/Swiss-Prot: Q14247) that is known to be a substrate for human Src protein kinase. Screening of urine samples using Western-blot analysis with specific anti-human cortactin antibodies suggests an existence of different cortactin isoforms in healthy donors and patients with liver cirrhosis and oncological diseases, the presence of which in urine could be an important diagnostic criterion for detection of the mentioned diseases. To evaluate their diagnostic importance further investigations are needed.

1. Myronovskij S., et al, Biochem. Biophys. Rep. 2016, 5:175-179.

CALIX[4]ARENE C-956 AS A PROMISING SUPRAMOLECULAR COMPOUND TO REGULATE THE ACTIVITY OF SARCOPLASMIC RETICULUM Ca^{2+} , Mg^{2+} -ATPase OF SMOOTH MUSCLE CELLS

Tetyana Veklich

Poster 1

Tetyana Veklich, Olexander Shkrabak, Sergiy Kosterin

*Palladin Institute of Biochemistry, National Academy of Science of Ukraine, 9,
Leontovycha str, 01030 Kyiv, Ukraine*

E-mail: veklich@biochem.kiev.ua

Calcium transporters, which include, for example, the calcium pumps of plasma membrane (PM) and sarcoplasmic reticulum (SR), play an essential role in controlling Ca ion concentration in the smooth muscle (SM) cytoplasm. The SR Ca^{2+} , Mg^{2+} -ATPase reduces intracellular Ca^{2+} concentration owing to cation accumulation in reticular pool. Recent literature data have indicated that SR is one of the largest cellular calcium depots. Therefore, it is promising to search for a compound that would allow the activity of these pumps to change. In previous studies, it was shown that the synthetic compound calix[4]arene C-956 (5,11,17,23-tetra(trifluoro)methyl(phenylsulfonylimino)methylamino-25,27-dioctyloxy-26,28-dipropoxycalix[4]arene) in concentration 100 μM effectively (by 75% relative to control) and specifically inhibited the activity of PM Ca^{2+} , Mg^{2+} -ATPase of the uterine myocytes, without affecting the activity of other PM ATPases.

Thus, the aim of this work was to study the patterns of the inhibitory effect of calix[4]arene C-956 on the SR Ca^{2+} , Mg^{2+} -ATPase activity of the uterine SM cells. Calix[4]arene were synthesized and characterized using NMR and infrared spectroscopy in the Phosphoranes Chemistry Department of the Institute of Organic Chemistry, NASU (head of the department - Academician of NASU, prof. V.I. Kalchenko). All enzymatic activities were assayed in cell suspension perforated with 0.1 % digitonin. In 100 μM concentration, the highest inhibition of SR Ca^{2+} , Mg^{2+} -ATPase activity was in the presence of calix[4]arene C-956, which inhibited enzyme activity to 36.5 ± 0.2 % relative to control. The calculated inhibition coefficient $I_{0.5}$ was 42.73 ± 1.03 μM . The Hill coefficient was 0.54 ± 0.03 (n=5).

It was also shown that application of 20 μM calix[4]arene C-956 into uterine myocytes caused a temporary increase of intracellular Ca^{2+} concentration. Interestingly, during 2.5 minutes $[\text{Ca}^{2+}]_i$ decreased that could be explained by involvement of compensatory mechanisms which regulate calcium homeostasis. In addition, 50 μM calix[4]arene C-956 induced a decrease in myocyte's hydrodynamic diameter to 45 % that could be explained by contraction of myocytes. Such change in the hydrodynamic diameter could be interpreted as a combination of events that accompany the SMC contraction/relaxation processes, namely changes in osmotic-water balance, rearrangement of the cytoskeleton elements. Since it has been previously shown that the decrease in the SMC hydrodynamic diameter upon the action in the presence of contractile agents correlates with the state of SM contraction, these results suggest a promising use of calix[4]arene C-90 as a regulator of the contractile activity of the uterus SM.

It is assumed that the obtained data on the effect of calix[4]arene C-956 might be valuable for further of ascertaining the ionic, molecular and membrane mechanisms of calcium metabolism in SM. The discovered phenomenon of increase in the SMC intracellular Ca^{2+} concentration in the presence of calix[4]arene may also be promising for development new medicinal drugs on the basis of indicated supramolecular compound, namely a stimulator of uterine basal tone.

We are thankful to the academician of NASU V.I. Kalchenko for scientific cooperation.

**MITOCHONDRIAL NICOTINIC ACETYLCHOLINE RECEPTORS INTERACT WITH
BCL-2-FAMILY PROTEINS IN U373 CELL LINE**

Olena Kalashnyk

Poster 2

Kalashnyk O.M., Uspenska K.R., Lykhmus O.Y., Skok M.V.

Palladin Institute of Biochemistry, National Academy of Science of Ukraine, 9, Leontovycha str, 01030 Kyiv, Ukraine

E-mail: o.kalashnyk56@gmail.com

Nicotinic acetylcholine receptors (nAChRs) expressed in the outer membrane of mitochondria regulate the early events of mitochondria-dependent apoptosis like cytochrome c (cyt c) release by affecting the activity of intramitochondrial kinases. However, the mechanisms of functioning, as well as potential partners of nAChR in mitochondria are far from being understood. The aim of the present study was to reveal potential connection of mitochondrial nAChRs with the pro- and anti-apoptotic mitochondrial proteins BAX and Bcl-XL. Bioinformatic analysis performed using the pyDockWEB software demonstrated the high probability of both BAX and Bcl-XL binding with the transmembrane portions of nAChR molecule. Experiments were performed in cultured human astrocytoma cell line U373, mitochondria of which were shown to express $\alpha 3$, $\alpha 4$, $\alpha 7$, $\alpha 9$, $\beta 2$ and $\beta 4$ nAChR subunits. The cells were cultured in the presence of 1 mM H₂O₂ shown to cause cyt c release from both isolated and cell-included mitochondria. Sandwich ELISA using the pairs of nAChR subunit-specific and BAX- or Bcl-XL-specific antibodies demonstrated the presence of complexes of both Bcl-XL and BAX with $\alpha 7$, $\alpha 3$ and $\beta 2$ nAChR subunits in both mitochondrial and cytosole cell fractions. Incubation with hydrogen peroxide resulted in redistribution of such complexes: $\alpha 7$ -BAX, $\alpha 7$ -Bcl-XL and $\beta 2$ -Bcl-XL left mitochondria for the cytosole, while $\alpha 7$ -Bcl-XL and $\beta 2$ -BAX moved from the cytosole to mitochondria. Incubation of U373 cells with H₂O₂ in the presence of $\alpha 7$ nAChR agonist PNU282987 prevented cyt c release from mitochondria and decreased the connection of $\alpha 7$ nAChRs with both BAX and Bcl-XL. The level of free BAX and Bcl-XL was quite low. This data indicate that mitochondrial nAChRs are connected to Bcl-2-family proteins and can affect their involvement in mitochondria pore formation, which is the starting point of mitochondrial pathway of apoptosis. Moreover, it looks like the nAChRs are transported to mitochondria in complexes with BAX or Bcl-XL, which can serve like chaperones providing mitochondria-specific targeting signals for the nAChR molecules.

**EFFECT OF RESVERATROL TREATMENT ON MEIOTIC MATURATION OF OOCYTES,
VIABILITY AND DNA INTEGRITY OF FOLLICULAR CELLS**

Taras Blashkiv

Poster 3

Valentina Sribna¹, Oksana Kaleynykova¹, Igor Karvatskiy², Victoria Savchuk², Maria Stupchuk¹,
Taras Blashkiv¹, Tatyana Voznesenskaya¹

1 – Bogomoletz Institute of Physiology NAS of Ukraine, Kyiv, Ukraine

2 – Bogomolets National Medical University, Kyiv, Ukraine

E-mail: voz@biph.kiev.ua

Premature ovarian failure (POF), which is an ovarian function disorder affecting women under 40 years of age, is actively studied. This disease is becoming widespread due to the delay in maternity and is currently a medical and social problem. In accordance with contemporary concepts about the development of POF, the leading role is given to the deletion of autoimmune pathology.

Until now, it is unclear whether the development of the autoimmune process is a primary cause of this disease or it is the result of chronic pathology. Glomerulonephritis, in particular glomerulonephritis of immune etiology, represents a serious problem for reproductive health of women. The reproductive function may be affected by both, the glomerular disease itself and glucocorticoid and cytostatic therapy. Among the known antioxidants, Resveratrol (RES) has received numerous approvals when used in various disease patterns, including oocytes. According to recent studies, the effect of RES on reproductive function in women under conditions of experimental glomerulonephritis has not been studied yet, which makes this research relevant today.

The aim of the given study was to estimate under conditions of experimental glomerulonephritis the effect of Resveratrol treatment on oocyte passage of meiotic maturation stages - metaphase I and metaphase II, on the viability and integrity of DNA of cells of the follicular environment of oocytes as well as pre- and post-implantational embryonic mortality in mice.

Experiments (two series) have been conducted on CBA/lac mice (coloring wool – agouti; genotype - +, H-2k): 64 females (10 weeks, 20-22 g) and 12 males (25 weeks, 25-27 g).

Experimental glomerulonephritis in mice was achieved by immunization of white laboratory mice of the first generation with a kidney antigen suspension derived from a parent. Animal immunization was carried out at the rate of 10 mL of suspension per 10 g of body weight according to the following scheme: 3 times intra-abdominal 1 time per day; re-immunization was carried out after 3 weeks with a single intra-abdominal treatment of the same dose.

Animals were treated with intraperitoneal injections of Resveratrol (R5010, Sigma-Aldrich, USA) 4 times: 1 time per day for 1 hour before immunization of animals with suspension of kidney antigen; as well as in 3 weeks once with the same dose (50 mg/kg, 0.3 mL).

In this work we have found that under conditions of experimental glomerulonephritis the treatment of Resveratrol results in an increase in the percentage of oocytes that successfully undergo meiotic maturation, decrease in the percentage of apoptotic and necrotic cells of the follicular environment of oocytes and a decrease of the post-implantation mortality rate of embryos in mice.

CHANGES IN THE VACUOLAR TRANSPORT IN MUCOPOLYSACCHARIDOSIS

Lidia Gaffke

Poster 4

Lidia Gaffke, Karolina Pierzynowska, Zuzanna Cyske, Magdalena Podlacha, Grzegorz Wegrzyn

Department of Molecular Biology, University of Gdansk, Wita Stwosza 59, 80-308 Gdansk, Poland

E-mail: lidia.gaffke@phdstud.ug.edu.pl

Glycosaminoglycans (GAGs) are complex polysaccharides which play various roles in cells and tissues, particularly in cell-to-cell communication, facilitating binding of ligands to cell membrane receptors and maintaining flexibility of the connective tissue. In normal cells, synthesis and degradation of GAGs is in balance, due to activities in GAG synthetases and lysosomal GAG hydrolases. However, mutations in genes coding for the latter enzymes result in impaired GAG degradation and progressive storage of these compounds in cells. This is the cause of inherited metabolic diseases, mucopolysaccharidoses (MPS). There are 11 types and subtypes of MPS, distinguished on the basis of the kind of lacking or insufficient enzyme. MPS is a group of severe disorders, causing disability and significantly shortened life span. It was proposed previously that GAG storage is the sole reason for cellular dysfunctions in MPS. However, according to recent suggestions (including the proposal of our team), secondary cellular defects might play significant role in the development of MPS phenotypes, and they might be responsible for failures of full correction of the disease symptoms by using various therapeutic options. Therefore, we aimed to determine molecular mechanisms which may disturb particular cellular processes. In this work, we investigated vacuolar transport as an important cellular process, without which the cell does not have a chance to work properly. We have performed confocal microscopy analysis of clathrin vacuoles (COP), both COP I and COP II. Moreover, levels of marker proteins of the vacuolar transport, including their phosphorylated forms, were determined in all MPS types and control cells. The tested proteins were as follows: caleolin, clathrin, APPL, APPL1, EEA1, syntaxin, GOPC, and Rab5. We found various changes in the vacuolar transport in different MPS types and subtypes. These results indicate newly identified pathomechanism of MPS which might influence our understanding of this disease and further efforts to develop efficient therapy.

DISTURBANCES IN THE COURSE OF AUTOPHAGY PROCESS IN LYSOSOMAL STORAGE DISEASES FROM THE GROUP OF MUCOPOLYSACCHARIDOSIS

Karolina Pierzynowska

Poster 5

Karolina Pierzynowska, Lidia Gaffke, Zuzanna Cyske, Magdalena Podlacha, Grzegorz Wegrzyn

Department of Molecular Biology, University of Gdansk, Wita Stwosza 59, 80-308 Gdansk, Poland

E-mail: karolina.pierzynowska@biol.ug.edu.pl

Mucopolysaccharidoses (MPS) is a group of 11 enzymatic defects which are expressed as deficiency in degradation of glycosaminoglycans (GAGs). They are caused by mutations in genes coding for lysosomal enzymes involved in sequential reactions in the pathways of GAG catabolism. Deficiency of one of these enzymes results in inhibition of subsequent steps, and accumulation of partially degraded complex polysaccharides. Depending on the kind of mutated gene, various GAGs are accumulated, for example dermatan sulfate, heparan sulfate, keratan sulfate, chondroitin sulfate or combinations of them. It was assumed for a long time that GAG storage is not only the primary but also the only cause of cellular defects which are then reflected at the tissue and organ levels and appear as specific clinical symptoms. However, results of recent studies suggest that secondary effects of MPS-causing mutations may be as important in pathomechanism of the disease as primary GAG accumulation. Thus, impairment in various cellular processes may significantly contribute to the disease phenotype. In this light, the efficiency of autophagy process was examined as of one of the most important cell processes for homeostasis. The research was conducted on cell lines taken from patients with all types/subtypes of MPS, as well as on the HDFa line (human dermal fibroblasts, adult) as a healthy control. The results indicated differences in both the initial and final stages of the autophagy process. Levels of protein complexes involved in initiation (Ulk, Atg13, FIP200 and/or their phosphorylated forms), elongation (Atg12, Atg5, Atg16L and Atg3, Atg4A, Atg4B, Atg7, LC3-II) and nucleation (PI3K, Beclin-1, Atg14, UVRAG, Rubicon, Bif-1, Atg9A) were found to be altered in various types/subtypes of MPS. In addition, the autophagy flux itself is inhibited for particular types of this disease, as evidenced by the lack of increase in the level of LC3-II protein after inhibition of lysosomal hydrolases. There were also large changes in the proportions between individual vesicles involved in the autophagy process: lysosomes, autophagosomes and autophagolysosomes. The autophagy process plays an extremely important role in the cell to maintain its homeostasis, as well as to adapt the cell to new, changing environmental conditions. Dysfunction of this process in MPS may point to the unexplored pathogenesis mechanism of this disease.

ADAPTOR PROTEIN RUK/CIN85 IS A COMPONENT OF SIGNALING NETWORKS THAT REGULATES BREAST CANCER CHEMORESISTANCE IN CONCENTRATION-DEPENDENT MANNER

Iryna Horak, Olga Gudkova, Nely Latyshko, Olga Khudiakova, Serhiy Shandrenko, Liudmyla Drobot

Palladin Institute of Biochemistry, National Academy of Science of Ukraine, 9 Leontovycha str., Kyiv, 01601, Ukraine

E-mail: iryna.horak@gmail.com

Metastatic dissemination and resistance to chemotherapy are main causes of breast cancer mortality. Tumor cells chemoresistance is strongly associated with features of cancer stem cells (CSCs) – subpopulation of cells with the potential for self-renewal, tumorigenicity, and dedifferentiation. There are several mechanisms providing drug resistance: drug efflux by membrane ABC-transporters, enzymatic drug inactivation, resisting apoptosis, increased DNA repair, quiescence etc. Adaptor proteins play important roles in maintenance, regulation and directing cellular signaling in normal and malignant cells. The aim of present study was to investigate the effect of adaptor protein Ruk/CIN85 on drug resistance of murine breast adenocarcinoma 4T1 cells.

As a model we used murine breast adenocarcinoma 4T1 sublines with stable overexpression (RukUp-1 cells) and knockdown (RukDown cells) of Ruk/CIN85, and corresponding control sublines Mock and Scr. Cell survival rate and doxorubicin (Dox) IC₅₀ were examined by MTT-assay. Transforming potential *in vitro* was estimated by soft agar growth assay. Aldehyde dehydrogenase (ALDH) activity was measured by NADH production.

It was demonstrated that survival of 4T1 cells in the presence of 0-10 μM Dox depends on Ruk/CIN85 expression level. As well, Dox IC₅₀ for RukUp-1 cells was higher by 23% in comparison to Mock control, while Ruk/CIN85 down-regulation in 4T1 cells led to IC₅₀ decrease by 20% compared to Scr control. In order to analyse anchorage-independent growth potential, required for cancer cells survival and spreading, we used soft agar colonies assay. It was shown that Ruk/CIN85 overexpression resulted in significantly increased number of colonies under control condition (untreated cells) as well as in the presence of 0,01-0,1 μM Dox. In contrast, RukDown cells revealed attenuated transforming potential. ALDH is a CSCs' marker enzyme, involved in chemo- and radioresistance, dedifferentiation and free radicals protection. We found that ALDH activity was 1,5 times higher in RukUp-1 cells, and decreased by 30% in RukDown cells in comparison to corresponding controls.

Our results indicate the essential role of adaptor protein Ruk/CIN85 in breast cancer chemoresistance, potentially reflecting enhanced CSCs properties of Ruk/CIN85-overexpressing cancer cells.

THE EFFECT OF RESVERATROL AND AN INHIBITOR OF NF- κ B ON THE FUNCTIONAL STATUS OF OVARIAN CELLS UNDER CONDITIONS OF OXIDATIVE STRESS *IN VITRO*

Mariia Stupchuk¹, Tatyana Voznesenskaya¹

*Department of Immunophysiology, Bogomoletz Institute of Physiology, NAS of Ukraine,
Bogomoletz street, 4, Kyiv, Ukraine, 01024*

E-mail: mariastupchuk@yahoo.com

Sirtuins (silent information regulator (SIRT) proteins), NAD⁺ dependent enzymes with deacetylase and/or mono-ADP-ribosyltransferase activity, are emerging as key antiaging molecules. Moreover, strong experimental evidence supports the notion that SIRT1 plays a crucial role in sensing and modulating the cellular redox status thus providing protective effects in cells and tissues exposed to oxidative stressors *in vitro* and *in vivo* [Voznesenskaya, Stupchuk, et al. 2018].

The effect of sirtuins activator - resveratrol and an inhibitor of NF- κ B (BAY11-7082) on the functional status of ovarian cells (oocytes and cells of follicular environment of oocytes (FEO)) under conditions of oxidative stress *in vitro* was assessed. An oxidative stress was reconstructed *in vitro* by adding hydrogen peroxide (H₂O₂) at a concentration of 100 μ M in the cultural medium where oocytes and FEO cells have been cultivated.

It is shown that in the conditions of oxidative stress, the inhibition of meiotic maturation of oocytes is observed. The influence of resveratrol (20 μ M concentration) under the conditions of oxidative stress *in vitro* was found to decrease the inhibition of meiotic maturation of oocytes both at the stage of dissolution of the germinal vesicle (metaphase I), and at the stage of the first polar body formation (metaphase II) on respectively 9% and 17% in comparison with such values under conditions of oxidative stress without resveratrol influence. Moreover, it has been established that under the conditions of oxidative stress *in vitro* and the influence of resveratrol there is an improvement in the indicators of viability of FEO cells - the percentage of living FEO cells increased, and the percentage of apoptotic and necrotic cells decreased. NF- κ B inhibitor was also found to decrease the harmful effect of oxidative stress on the functional status of ovarian cells. Therefore, we postulate that in the conditions of oxidative stress *in vitro*, the NF- κ B transcription factor is involved in the mechanism of resveratrols' (activator of sirtuin 1) action of both on the process of meiotic maturation of oocytes and on the viability of FEO cells.

Voznesenskaya T. Y., Stupchuk M. S., et al. Bulletin Probl Biol Med. 2018, Is 1, 1 (142): 20-25.

Ca²⁺-SIGNALS IN RESPONSE TO ATP IN SPERM CELLS OF INFERTILE MEN WITH DIFFERENT FORMS OF PATHOSPERMIA

Fafula R.V., Meskalo O.I., Vorobets Z.D.

Department of Medical Biology, Department of Biophysics, Danylo Halytsky Lviv National Medical University, Pekarska Street, 69, Lviv, Ukraine, 79010

E-mail: roman_fafula@ukr.net; kaf_medicalbiology@meduniv.lviv.ua

Calcium ions play a vital role in regulating of physiological processes in spermatozoa, in particular in motility and viability of ejaculated spermatozoa. Intracellular calcium level ($[Ca^{2+}]_i$) is important in the initiating process of sperm hyperactivation, capacitation, acrosome reaction and gamete interaction. Decreased fertility potential of spermatozoa is closely associated with the disturbances of Ca²⁺-homeostasis. Metabolic active form of ATP (strongly charged anion ATP⁴⁻) is present in the female reproductive tract and may play important role the fertilization process. It is critically important for sperm cells since it is the main energy source and substrate for the second messenger cAMP in spermatozoa. Extracellular ATP (ATP_e) plays a key role as a signaling molecule.

Since $[Ca^{2+}]_i$ is main determinant of many physiological processes occurring in sperm, we set out to describe the Ca²⁺-signals in response to ATP_e in spermatozoa of fertile (normozoospermia) and infertility men (oligo- and asthenozoospermia).

ATP_e-induced changes in $[Ca^{2+}]_i$ in spermatozoa were studied using 2 μM fluorescent probe Fluo-4. ATP_e caused a rapid transient elevation in $[Ca^{2+}]_i$. We found that kinetics and magnitude of the $[Ca^{2+}]_i$ changes induced by ATP_e were different in normo and pathospermic cells. Specifically, the average value of peak amplitudes of $[Ca^{2+}]_i$ rise induced by 5 mM ATP_e in oligozoospermic samples was not significantly different from normozoospermic samples. In asthenozoospermic samples the ATP_e-induced peak amplitude of $[Ca^{2+}]_i$ changes was in 1.5 fold lower (P<0.05) compared to that in normozoospermic samples. ATP_e-induced increase in $[Ca^{2+}]_i$ in sperm cell has a concentration-dependent manner in both normozoospermic and pathozoospermic samples. In oligozoospermic samples the $[Ca^{2+}]_i$ transient response was 2.5 fold (P<0.05) slower than in normozoospermic samples. Differences in ATP_e-induced $[Ca^{2+}]_i$ transients between astheno- and normozoospermic samples were also significant (P<0.05) although less pronounced.

Thus, the data presented here reveal that ATP_e-induced increase in $[Ca^{2+}]_i$ in asthenozoospermic samples was inhibited. In oligozoospermic samples ATP_e-induced increase in $[Ca^{2+}]_i$ was not significantly different from normozoospermic samples, but kinetics of $[Ca^{2+}]_i$ transients was significantly slower compared to normozoospermic samples.

Obtained results clearly demonstrates ATP_e-induced increase in $[Ca^{2+}]_i$ transients are disturbed in pathozoospermic samples which may be detrimental to sperm activation and may result in fertilization failure or abnormality. Taken into account the importance reproductive techniques, specifically for in vitro fertilisation and intrauterine insemination present study suggest that modulation of $[Ca^{2+}]_i$ signals and sperm function by ATP_e may be beneficial for artificial reproductive techniques used in reproductive biology and medicine.

УЧАСТЬ СІРТУЇНІВ 1 ТА 3 У НЕЙРОПРОТЕКЦІЇ ПРИ ХРОНІЧНІЙ ЦЕРЕБРАЛЬНІЙ ГІПОПЕРФУЗІЇ У МИШЕЙ

Гарматіна О.Ю., Портниченко А.Г.

Інститут фізіології ім. О.О. Богомольця НАН України, вул. Богомольця, 4, Київ, 01024, Україна

E-mail: harmatina@ukr.net

Критичні стенози та оклюзії брахіоцефальних артерій призводять до розвитку гіпоперфузії головного мозку та є причиною розвитку інсультів та хронічної ішемії. В основі механізмів розвитку цих патологічних процесів лежить гіпоксія, яка визначає реакцію мозкової тканини на стрес та ступінь пошкодження. Показано, що сіртуїни (Sirt) епігенетично регулюють відповідь на клітинний стрес та можуть бути чинником процесів транскрипції генів, посттрансляційної модифікації білків, регуляторами апоптозу, клітинного старіння тощо. Метою нашого дослідження було вивчення експресії та функції Sirt1 та Sirt3 в тканині мозку при моделюванні хронічної гіпоперфузії головного мозку (ХГГМ) у мишей. Експерименти проведені на 40 мишах лінії C57Bl/6j (6 тижнів, вага 18-20 г). Хірургічні маніпуляції проводились на анестезованих кетаміном (60 мг/кг, внутрішньоочеревинно) тваринах. ХГГМ моделювали перев'язкою лівої загальної сонної артерії. В якості блокатора Sirt1 вводили нікотинамід (NAM, 200 мг/кг, 10 діб, внутрішньоочеревинно), у якості активатора Sirt1 – ресвератрол (RV, 10 мг/кг, 10 діб, внутрішньоочеревинно). Через 8 тижнів проводили дослідження пошкодження ДНК нейронів (методом ДНК-комет) та експресію Sirt1 та Sirt3 (методами real-time PCR та імуногістохімії) в обох гемісферах головного мозку. Виявлено, що ХГГМ за результатами дослідження ДНК-комет супроводжувалась збільшенням пошкодження ДНК нейронів в обох півкулях головного мозку, унілатерально – майже у 7 разів ($P < 0.001$). При цьому експресія генів Sirt1 та Sirt3 знижувалася у 9 та 20 разів, відповідно, у порівнянні з контрольною групою ($P < 0.05$). Тривала модифікація функції сіртуїнів за допомогою NAM або RV призводила до зростання рівнів експресії Sirt1 та Sirt3 у порівнянні з контрольною групою ($P < 0.05$) та зменшення ступеня пошкодження ДНК унілатерально майже у 2 рази ($P < 0.001$). Таким чином, сіртуїни Sirt1 та 3 типів приймають участь у нейропротективних механізмах при хронічних захворюваннях головного мозку, пов'язаних з нестачею кисню та метаболічних субстратів. При негативній регуляції експресія сіртуїнів підтримується за рахунок потужних компенсаторних механізмів, що вказує на важливість цього епігенетичного механізму в цитопротекції.

ТЕРАПЕВТИЧНІ ЕФЕКТИ ГЕНЕТИЧНО МОДИФІКОВАНИХ МЕЗЕНХІМАЛЬНИХ СТОВБУРОВИХ КЛІТИН З ПОСИЛЕНОЮ ЕКСПРЕСІЄЮ ГЕНА ІНТЕРЛЕЙКІНУ-10.

Олена Топорова^{1,3}, Олександр Боцул², Олена Дерябіна³, Володимир Фіщенко², Яніна Похолєнко^{1,3}, Лачин Маммадов², Станіслав Яремін², Олександр Фіщенко², Марина Шульга⁴, Альберт Точиловський⁵, Віталій Кордюм^{1,3}.

1 – Інститут молекулярної біології і генетики НАН України, вул. Заболотного 150, 03143 Київ, Україна

2 – Вінницький національний медичний університет ім. М. І. Пирогова, вул. Пирогова 56, 21018 Вінниця, Україна

3 – ДУ «Інститут генетичної та регенеративної медицини НАМН України», вул. Вишгородська 67, 04114 Київ, Україна

4 – Київський національний університет ім. Тараса Шевченка, вул. Володимирська 60, 01601 Київ, Україна

5 – Біотехсом, вул. Отто Шмідта 2/6, 01004 Київ, Україна

E-mail: topp@meta.ua

Частота захворювань і травматичних ушкоджень колінного суглоба з подальшим розвитком його дисфункції настільки велика, що є не тільки медичною, а й соціально-економічною проблемою. Створено багато технологій як консервативного, так і оперативного лікування. Однак ключова проблема – пошук методу ефективного відновлення суглобового хряща – остаточно не вирішена. В останні роки проводяться численні клінічні випробування терапії стовбуровими клітинами. Мезенхімальні стовбурові клітини (МСК) є основним типом клітин, які використовуються лікарями в ортопедії. Оскільки показано, що застосування стовбурових клітин значно прискорює процес відновлення і помітно зменшує запалення, метою роботи було посилити протизапальну терапевтичну дію МСК людини за рахунок переносу гена протизапального інтерлейкіну-10.

МСК виділяли з пуповин людини методом експлантів. FACS-аналіз експресії поверхневих маркерів МСК з використанням моноклональних антитіл проти CD105, CD90, CD73 виконано на сортері *BD FACSAria* із застосуванням програмного забезпечення *BD FACSDiva*. Клітини 2-го пасажу трансфікували комплексами ДНК плазмідного вектора та реагента TurboFect. Створена плазміда містить біцистронну експресійну касету, що регулюється конститутивним промотором і забезпечує одночасну експресію цільового (il-10) та маркерного (egfp) генів. Показано, що через 48 год після трансфекції рівень синтезу трансгенного цитокіну на два порядки перевищує вихідний рівень синтезу власного інтерлейкіну-10.

Щурам з механічним ураженням суглобового хряща проведено внутрішньосуглобові ін'єкції 1×10^5 нативних МСК та генетично модифікованих МСК/IL-10. Гістологічний аналіз пошкоджених тканин після трансплантації клітин показав, що утворення зрілих хондроцитів, виражений ендостальний і периостальний хондрогенез, утворення зрілої ендостальної і периостальної хрящової тканини у щурів, які отримали МСК з посиленням синтезом антизапального інтерлейкіну-10 людини, відбувається в більш короткий термін. В обох експериментальних групах на відміну від контрольних тварин, яким не трансплантували МСК, не відмічалось надмірної продукції осифікуючого матриксу, не спостерігалась активація остеокластів ще до початку формування повноцінної кісткової тканини і були відсутні дистрофічні зміни хондроцитів і хондробластів.

Session 2

Cell response on stress

6th Ukrainian Congress for Cell Biology with international representation

***June 18-21, 2019,
Yaremche***

ANHYDROBIOSIS: SURVIVAL WITHOUT WATER

Alexander Rapoport

Lecture 1

Alexander Rapoport

*Laboratory of Cell Biology, Institute of Microbiology and Biotechnology, University of Latvia
Jelgavas Str., 1-537, Riga LV-1004, Latvia*

E-mail: rapoport@mail.eunet.lv

Anhydrobiosis is unique state of live organisms when their metabolism is temporary reversibly suspended as the result of strong cells' desiccation. In spite the discovery of this state has been made by great Dutch naturalist Anthony van Leeuwenhoek more than 300 years ago in 1701 its mechanisms still are "an unsolved problem" (Crowe, 2015). The development of modern methodological approaches gave the possibility to re-start the studies of this interesting and important phenomenon about 50 years ago practically simultaneously in 3 laboratories – in Davis (USA), Wageningen (The Netherlands) and Riga (Latvia). One of big groups of live organisms able to transfer themselves into the state of anhydrobiosis are microorganisms which in the nature rather regularly are affected by high temperatures of the environment and a severe drought. Therefore, with the goal to survive in such conditions they had to work out during long evolution period special protective mechanisms and anhydrobiosis is one of them. Studies of anhydrobiosis in yeasts which are perfect models of any eukaryotic cell showed that during transition into anhydrobiosis all intracellular structures are subjected to definite changes. A lot of them may be supposed as specific intracellular protective reactions. Such conclusions have been made, for example, regarding strong folding of plasma membrane, condensation of chromatin in nucleus and DNA in mitochondria. A lot of attention is devoted to the changes of plasma membrane and especially of its lipid component. It is supposed that the state of plasma membrane lipids may be one of the main factors which determine the maintenance of yeast cells viability during its dehydration, stay in the dehydrated state and subsequent rehydration/reactivation. At the same time recently it was shown that protein component of plasma membrane also is very essential for the survival of the cells. Mitochondria were supposed to be the most resistant to dehydration between intracellular organelles and as the result not too much attention was devoted to their studies linked with anhydrobiosis. Recently it appeared that also their membranes chemical content is very important for the resistance of yeasts at their transfer into anhydrobiosis. The studies of yeast cell walls led to the conclusion that mannoproteins of this structure may be rather critical factor for the survival of yeast cells. Similar results have been obtained in the studies of importance of endoplasmic reticulum. The main attention in the studies of intracellular protective compounds was devoted to trehalose. Last years showed that there are a lot of other protective compounds which are extremely important for the resistance of the cells at desiccation stress and which may function together with trehalose or substitute it in the case of its absence. Between these protective compounds there are various polyols, proline, glutathione, ergosterol, heat shock proteins and some other compounds. Summarizing information obtained during last 50 years of studies of anhydrobiosis it is clear now that there is necessary big complex of coordinated by the cell reactions and changes of various intracellular structures with the synthesis of a number of protective compounds to maintain the survival of organisms and their successful transfer into the state of anhydrobiosis.

Reference: Crowe J.H. Subcell. Biochem. 2015, 71:263-280.

EFFECTS OF THE CARBON MONOXIDE RELEASING MOLECULE-2 ON HUMAN ERYTHROCYTE MEMBRANES

Serhii Beschasnyi

Lecture 2

Serhii Beschasnyi, Olena Hasiuk, Anastasia Shkuropat
Kherson State University, Universitetska Street 27, 73000, Kherson, Ukraine

E-mail: beschasnyis@gmail.com

Carbon monoxide (CO) is the third most common agent that causes poisoning. Nevertheless, it is known that CO produced endogenously and can have a beneficial effect, especially at lower concentrations. The first reports have shown that CO is actively involved in the regulation of key intracellular functions. At physiological concentrations, CO can affect processes of signal transmission in various organs and in various cells, also including endothelial cells. For example, CO may regulate aspects of the cardiovascular system, such as platelet activation, inflammation or blood pressure, demonstrate neuroprotective and neurotherapeutic properties.

CORMs (Carbon monoxide releasing molecule-2) or molecules that release carbon monoxide make up a recently classified group of chemicals. These are compounds capable of releasing a controlled amount of CO into cells and tissues to induce biological activity. CORM molecules consist of carbonyl groups linked to metals, such as ruthenium (CORM-2 and CORM-3), and can act as drugs. The potential of a CORM lies in its ability to release a CO molecule bound to a metal when it reaches its destination.

We used washed and “packed” red blood cells of donors 28-30 years old. In 3.150 ml of medium (with different osmotic strength), 0.35 ml of erythrocytes was added and light scattering was measured. After this, the tricarbonyldichlororuthenium (II)-dimer carbon monoxide donor (CORM-2 was dissolved in DMSO (<1%)) was added and the light scattering was measured again. After the measurement, was adding the blocker of calcium-dependent K⁺ channels of the membrane clotrimazole.

In environments with different osmotic strengths after adding CORM-2, decrease in the intensity of light transmission was observed. In the environment of 220 μmol (hypoosmotic), the light scattering index changed from 2.898 ± 0.144 to 2.759 ± 0.138 , in the medium of 320 μmol (isoosmotic) from 2.984 ± 0.142 to 2.887 ± 0.144 . After adding erythrocytes to the environment of 420 μmol (hyperosmotic), the index changed from $3,000 \pm 0,15$ to $2,887 \pm 0,144$, and in medium 520 μmol from $3,000 \pm 0,14$ to $2,912 \pm 0,146$ (4.8, 3.2, 3.2 and 2.9%, respectively). In this case, the addition of clotrimazole did not significantly affect the light transmittance.

Thus, CORM-2 at a dose of 200 μM caused an increase in the volume of erythrocytes in hypertonic, isotonic and hyposmotic solutions. After melting the spectrin after the calcium-dependent potassium channel blocker was added, clotrimazole and CORM-2 erythrocytes swelled. The exception was the compression of erythrocytes in isoosmotic solution.

Thus, CORM-2 affects on the properties of the erythrocyte membrane, their ion channels. This is due to the erythrocyte cytoskeleton protein – spectrin. Thus, this should influence the micro-rheological properties of erythrocytes *in vivo* after consuming CORM-2 medication.

CELL MEMBRANE PERMEABILITY CHANGES UNDER EXPOSURE TO NEUTRON RADIATION

Kostyantyn Kuznetsov

Lecture 3

Kuznetsov K.A.¹, Kyzym P.S.², Berezhnoy A.Yu.², Shchus A.F.²,
Onyshchenko G.M.², Shckorbatov Y.G.³

1 – Medical Biology Department, Kharkiv National Medical University, 4 Nauky Av., 61022, Kharkiv, Ukraine

2 – Nuclear and Medical Physics Department, V.N. Karazin Kharkiv National University, 31 Kurchatov Av., 61108, Kharkiv, Ukraine

3 – Molecular Biology and Biotechnology Department, V.N. Karazin Kharkiv National University, 4 Svobody Sq., 61022, Kharkiv, Ukraine

E-mail: Konst.Kuznets@gmail.com

Exposure of living tissues to neutrons induces the hazardous biological effects at high doses as well as for X-ray and γ -radiation; however, the effects of low doses of neutron radiation may inflict delayed damage to membrane proteins even after several days after irradiation (A. Saeed et al., 2015).

The aim of present work was the detection of possible short-time effects of exposure to fast neutrons and description of dose-effect relations using sensitive index of cell membrane permeability (CMP).

Buccal epithelium cells of 3 non-smoking male donors (A – 21 years old, B – 25 years old, and C – 27 years old) were exposed to neutron radiation with equivalent dose range 2.3 – 146 mSv from Pu-Be source, corresponding to the time of exposure 1 – 64 min. Cell membrane permeability (CMP) was evaluated through staining with 5 mM indigocarmine. Student's test ($p < 0.05$) has been used for statistical processing. In each experiment, values of CMP control levels at the beginning and at the end of experiment was almost the same so they were averaged.

The doses of 18.3 and 36.5 mSv led to the increase of cell membrane permeability in the cells of all three donors. The donor's A cells had the highest reactivity to irradiation. Dose 36.5 mSv induced peak effect, CMP values decreased at higher and less doses of irradiation. The impact of radiation on CMP was donor-specific, but it generally reflected the main direction of changes in cell response.

The maximum of radiation induced damage to the membranes correlate with the state of chromatin in the nuclei as non-specific cell stress reaction which was investigated in previous work (Kuznetsov K.A., et al, 2018). The peak of chromatin condensation rate and CMP was at the same dose with further decrease for both of indexes. Thus, it seems likely that higher doses inflicted the cell response and activation of reparative mechanisms, which involves both decondensation of chromatin and restoration of the membrane.

1. Saeed, A., Raouf, G. A., Nafee, S. S. Effects of Very Low Dose Fast Neutrons on Cell Membrane and Secondary Protein Structure in Rat Erythrocytes. *PLoS One*, 2015, 10(10), <https://doi.org/10.1371/journal.pone.0139854>.
2. Kuznetsov, K. A., Kyzym, P. S., Onishchenko, G. M., Berezhnoy, A. Y., Shckorbatov, Yu. G. Human buccal epithelium cell response to low intensive neutron radiation, *Biophysical Bulletin of V.N. Karazin Kharkiv National University*, 2018, 40:17-25.

**APPLICATION OF THE NOVEL INTEGRATIVE INDEX OF OXIDATIVE STRESS
BASING ON THE EXPERIENCE OF AQUATIC MOLLUSKS STUDY**

Oksana Stoliar

Lecture 4

Oksana Stoliar¹, Lesya Gnatyshyna^{1,2}, Vira Khoma¹, Gunta Sprinģe³

*1 – Volodymyr Hnatyuk Ternopil National Pedagogical University, M. Kryvonosa Str., 2, 46027
Ternopil, Ukraine*

*2 – I.Ya. Horbachevsky Ternopil State Medical University, m. Voli, 1, 46001,
Ternopil, Ukraine*

3 – University of Latvia, Miera Str. 3, Salaspils, LV, 2169, Riga, Latvia

E-mail: Oksana.Stolyar@tnpu.edu.ua

The oxidative stress response is the common manifestation of the adverse environmental impact on the organism. However, depending on the severity and duration of impact, this response can be highly different. Consequently, the successfulness of the oxidative stress response is frequently unclear due to the variability of the applied set of the indices for its assessment and difficulties in the evaluation of the state of the equilibrium between the antioxidant activities and oxidative injury manifestations. Bivalve mollusks, due to their suspension-feeding and sedentary lifestyle, are on the first line of impact of the pollution. The aim of this study was the application of the elaborated novel Integrative index 'Preparation to the oxidative stress' (POS) (Moreira et al., 2016) to the available results of the antioxidant activities in the sentinel aquatic organism, bivalve mollusk.

The results of the assessment of the field exposures of three populations of bivalve mollusks during three seasons, and in their ability to withstand heating (25° C and 30° C during 14 days) and ionizing radiation (14 days after the acute exposure to 2 mGy) were analyzed. The parameters for the calculation of POS included the activities of superoxide dismutase, catalase and glutathione S-transferase, and the concentrations of the glutathione and metallothionein (from its thiol groups) in the digestive gland and gills. The values were calculated as the magnitude of change (as % change) in comparison to the corresponding control (less disturbed field group or non-exposed group). Only statistically significant differences, as stated by the authors, were considered. Based on the magnitude of change compare to the corresponded control, we classified each group as POS-positive, POS-negative or POS-neutral, considering the three different criteria. The first criterion was the occurrence of at least one statistically significant up-regulation event of antioxidant defense. The second criterion based on the definition of thresholds for up- and down-regulation events: the occurrence of at least one up-regulation above a 50% threshold or the down-regulation by 25% or more. The third criterion was the occurrence of more cases of up-regulation in comparison to down-regulation within a tissue. The analysis had shown that the POS responses were in the limits of adaptive ability in all studied cases. However, the depressive common direction in the POS response was estimated in the cases of the impact of extreme temperatures, irradiation and, mainly for the mollusks from the highly polluted sites. Summarizing, the key importance of POS as a survival strategy of the mussels exposed to adverse impact depending on the life history is evident. As far as we know, there is no analysis available of the prevalence of POS among mollusks depending on their history of population.

This work has been granted by the Ministries of Education and Science of Ukraine and Latvia (Projects 132B and M/35 for O. Stoliar and LV-UA/2017/5 for G. Sprinģe).

Moreira D. C. et al. *Comp. Biochem. Phys.* 2016. 200A: 64–78..

COMPENSATORY-ADAPTIVE POTENTIAL OF CARDIOCYTES UNDER CONDITIONS OF EXPERIMENTAL CHOLESTEROL LOADING THE ANIMAL MODELS

Raisa Piskun

Lecture 5

Piskun R.P., Shevchuk T.I., Shkarupa V.M., Hrynychak N.M.

Pyrohov Memorial Vinnytsia National Medical University, 56, vul. Pyrohova, Vinnytsya, Ukraine, 21018

E-mail: piskyn2006@gmail.com

Human cardiocytes demonstrate very low self-healing ability; they almost do not divide, therefore, restoration of a heart muscle and replacement of defected areas in patients with myocardial infarction occurs mainly with the participation of stromal cells. However, according to recent studies, it has been established that muscle cells of the myocardium are capable of proliferation, yet they cannot completely restore the damaged areas. Since cardiocytes are highly specialized cells, organs adapt to damage due to their regenerative hypertrophy by enlargement of certain organelles at ultrastructural level, namely, in mitochondria and myofibrils. In this case, organelles become hypertrophic, and cells increase in size. When rabbit models are loaded with cholesterol, pathological changes occur first in the coronary vessels in the form of narrowing their lumen, thickening the wall, the formation of atherosclerotic plaques, which lead to the phenomena of ischemia and hypoxia, and, as a consequence, the myocardial damage is developed. In this case, we can observe changes related to all stages of the adaptive syndrome: the beginning (tension), the development of adaptive reactions characterized by remodeling of basic cardiac structures, and the completion of the process. Once compensatory and adaptive potential of the myocardium exhausts, the compensation process ends with decompensation. The objective of our work was to identify the compensatory and adaptive potential of cardiocytes under conditions of experimental dislipoproteinemia, which was modeled by oral administration of cholesterol in animal test models. For study purposes, hearts were sampled, histological, micromorphometric and electron microscopic studies were performed.

The studies of the cardiac muscle presented with almost 3 times decrease in parenchyma-stromal ratio, indicating growing of interstitial connective tissue, that is, an increase in the number and size of collagen fibers. The cross-section area and the diameter of heart muscle cells declined by 12.04%, while their nuclei decreased by 14.06%. The nuclear-cytoplasmic ratio increased by almost 4%. The observed cell damage was associated with the phenomena of destruction, necrobiosis, and dissection of muscle fibers caused by accumulation of serous fluid, and the phenomenon of cariolysis. The proportion of damaged cardiocytes increased by 7.76 times against the norm. Structural heterogeneity of cardiocytes significantly strengthened against an increase in the number of atrophic and hypertrophic cells – by 2.82 and 5.17 times, accordingly, and against almost twice decrease in the proportion of normal cells compared to animals from the intact group.

At the ultrastructural level, pathological changes were mainly associated with myofibrils and mitochondria of cardiocytes. Thus, we observed an enlightenment of cytoplasmic matrix, an enlargement of the perinuclear space, an accumulation of the lipid drops in the cytoplasm, and a decrease in the content of ribosomes. Myofibrils were laminated, with the foci of lysis and rupture. The mitochondrial matrix was clear, with a disturbance of crista density and their destruction observed. Therefore, one of the manifestations of adaptive reaction of cardiocytes to ischemia and hypoxia in cholesterol loading model is their structural remodeling presented as an increase in the proportion of hypertrophied cells with activation of the protein synthesis system associated with an increase in the number and size of mitochondria and myofibrils, while the presence of foci of necrobiosis, destruction, and lysis of cardiocytes and their organelles indicates the phenomena of decompensation.

**THE INFLUENCE OF LOW POWER MICROWAVE RADIATION OF 2,4 GHz
FREQUENCY ON HUMAN CELLS**

Anastasiia Rozhyna

Lecture 6

Rozhyna A.A.¹, Kanuka A.S.¹, Kovalenko I.F.², Shckorbatov Y.G.¹

1 – V.N. Karazin Kharkiv National University, 4 Svobody Sq., Kharkiv, 61022, Ukraine

2 – Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, 23, Pereyaslavskaya str., Kharkov, 61015, Ukraine

E-mail: yuriy.shckorbatov@gmail.com

The purpose of the work was to study impact of low energy microwave irradiation on in the state of isolated human cells.

Methods. Studies were performed on exfoliated cells of human buccal epithelium of three donors (one man and two women of age 19-22). Three parameters of cell state were analyzed: structure of chromatin in cell nuclei after orcein staining, permeability of cell membranes to trypan blue, and free calcium content in cells estimated by Fluo-3 calcium probe applying Confocal Laser Scanning Microscope LSM 510 META (Carl Zeiss). The source microwave radiation was generator of microwaves elaborated at Department of Theoretical Radiophysics of the V. N. Karazin Kharkiv National University. The generator produced microwaves of 2,4 GHz frequency and flow of energy 2,3 $\mu\text{W}/\text{cm}^2$ at the level of cell sample subjected to microwaves. The exposure time was 1, 5, 10, 30, and 60 min. The microwaves of frequency 2,4 GHZ are used in wireless Wi-Fi communication systems, the energy level of microwaves used in the present study is far less than level ($10 \mu\text{W}/\text{cm}^2$) regarded as safe in Ukraine.

Results and Discussion. It was shown that the number of heterochromatin granules was significantly higher in comparison to control after 5 min exposure and gradually increased with exposure time. This phenomenon occurred in cells of all three donors. These data are in good agreement with our previous data and demonstrate cell stress reaction to microwave radiation (Shckorbatov Y., et al., 2011). The results obtained using trypan blue staining of cells from three donors show a general picture of the decrease in the percentage of stained cells after the exposure to microwave radiation. Our previous data demonstrated increase of membrane permeability after microwave irradiation at 36,64 GHz frequency, $10 \mu\text{W}/\text{cm}^2$ intensity, and 10 sec exposure of cells (Shckorbatov Y., et al., 2011). Differences in the response of cells to microwave irradiation appear to be related to differences in experimental conditions. The electromagnetic radiation at low intensities induced slight changes in the calcium content.

Conclusion. To sum up, cell exposure to microwave electromagnetic radiation increases the content of heterochromatin granules in cells as compared with the control sample – the chromatin condensation. Since the condensation of chromatin (heterochromatinization) is connected with the decrease of chromatin functional activity, microwaves induce a decrease in the functional activity of the cell nucleus as a whole.

ЖИТТЄЗДАТНІСТЬ ТА ПРОЛІФЕРАТИВНА АКТИВНІСТЬ КРІОКОНСЕРВОВАНОЇ КУЛЬТУРИ КЛІТИН ДЕРМАЛЬНОЇ ПАПІЛИ КРОЛИКІВ

Oksana Novikova

Lecture 7

Новікова О. Ю., Бондаренко Т.П., Божок Г.А.

Інститут проблем кріобіології і кріомедицини НАН України, вул. Переяславська, 23, 61016, м. Харків, Україна

E-mail: ksuhanew7@gmail.com

Клітини дермальної папіли (КДП) волосяного фолікула (ВФ) є популяцією плюрипотентних клітин – похідних нервового гребеня. Була показана можливість культивування й спрямованої індукції даного типу клітин *in vitro* (Driskell et al., 2011). У зв'язку з широкими перспективами застосування КДП у регенеративній медицині на сьогодні є актуальною розробка способів їх довготривалого зберігання. На даний момент існують одиничні роботи, в яких кріоконсервуванню піддавали експланти ВФ або мультиклітинні сфероїди, що утворюються при культивуванні КДП (Kajiura et al., 2015). Проте досі не було зроблено спроб кріоконсервування моношарової культури КДП.

Мета дослідження – вивчення впливу складу кріопротекторного середовища на основі ДМСО на життєздатність та проліферативну активність КДП кроликів при кріоконсервуванні з повільною швидкістю охолодження.

Культуру КДП отримували з вібрис новонароджених кроликів за методом (Sieber-Blum, 2004). Культивування клітин здійснювалось на середовищі ДМЕМ/F12 з 10% фетальної телячої сироватки (ФТС). Були отримані моношарові культури клітин 1 пасажу, одна половина з яких була піддана кріоконсервуванню, а друга слугувала в якості нативного контролю. Використовувались такі комбінації кріозахисних середовищ, приготовані на основі середовища ДМЕМ/F12: 1) 5% ФТС+5% ДМСО; 2) 7% ФТС+7% ДМСО, 3) 7% ДМСО; 4) 10% ДМСО. Швидкість охолодження складала 1°C/ хв, до – 80°C, потім зразки занурювали в рідкий азот. Через тиждень зберігання зразки були деконсервовані і поміщені в середовище ДМЕМ/F12 з додаванням 2% B27, а нативна культура в цей же час була пересіяна в аналогічне середовище.

Життєздатність оцінювали методом фарбування з трипановим синім. На третю добу росту здійснювався підрахунок концентрації клітин. Показник проліферативної активності КДП вираховувався як відношення отриманої концентрації клітин до вихідної.

Встановлена тенденція збільшення життєздатності КДП при кріоконсервуванні в середовищі з додаванням ФТС, що дало змогу знизити концентрацію ДМСО. Показник життєздатності клітин для середовища 1 склав 96%, для середовища 2 – 92%, для середовища 3 – 79% і для середовища 4 – 84%. Індекс проліферативної активності нативної культури становив 8,97. Кріоконсервовані культури мали деяке зниження даного показника, який перебував у межах від 7,60 (середовище 1) до 8,10 (середовище 4).

Таким чином, незначне зменшення проліферативної активності свідчить про відсутність серйозних летальних пошкоджень, здатних вплинути на швидкість ділення КДП після кріоконсервування. У той же час збільшення життєздатності клітин після заморожування-відігріву в середовищі 1 є перспективним чинником для подальшої розробки режимів кріоконсервування, які ґрунтуються на використанні кріозахисних середовищ зі зменшеною концентрацією ДМСО.

1. Driskell RR et al. J Cell Sci. 2011; 124(Pt 8):1179–1182.
2. Kajiura S, Mii S. Tissue Eng Part C Methods. 2015; 21(8):825–831.
3. Sieber-Blum M, Grim M, Hu YF, Szeder V. Dev Dyn. 2004 Oct;231(2):258-69.

DEMETALATION OF METALLOTHIONEINS AS A CONSTITUENT OF STRESS RESPONSE IN BIVALVE MOLLUSK CAUSED BY CO-EXPOSURE TO DICLOFENAC, NIFEDIPINE, GLYPHOSATE AND HEATING

Vira Khoma

Poster 1

Vira Khoma¹, Lesya Gnatyshyna^{1,2}, Yulia Rarok¹, Oksana Horyn¹, Oksana Stoliar¹

1 – Volodymyr Hnatiuk Ternopil National Pedagogical University, M. Kryvonosa Str., 2, 46027 Ternopil, Ukraine

2 – I.Ya. Horbachevsky Ternopil State Medical University, m.Voli, 1, 46001, Ternopil, Ukraine

E-mail: Oksana.Stolyar@tnpu.edu.ua

The pharmaceutical and agrochemical substances continuously release into the surface waters, mainly with the discharge of wastewater effluents. Bivalve mollusks are characterized by low activity of the CYP1A-dependent biotransformation and aromatase activity (Grosvik et al. 2006; Cubero-Leona et al. 2010). Therefore, we hypothesize that the biotransformation of these substances in these organisms can be slow, causing their enhanced toxicity. The aim of this study was to evaluate the ability of the mollusks to generate the response of stress under the effect of most distributed in the environment pharmaceutical and pesticide, acting in the environmentally realistic conditions of co-exposure and the heating. Freshwater mussels *Unio tumidus* were treated with non-steroidal anti-inflammatory drug diclofenac (600 ng L⁻¹), Ca-channel blocker nifedipine (700 ng L⁻¹), or phosphonate glyphosate (33.8 µg L⁻¹) separately at the 18° C and jointly at the 18° C and 25° C during 14 days. The indices of oxidative stress and toxicity were evaluated in the digestive gland.

The most common response was the down-regulation of glutathione *S*-transferase activity. It decreased in all exposures (especially, by four times, in the co-exposure under heating). However, the concentration of the glutathione (GSH) even increased in the co-exposures, and the concentration of oxidized glutathione (GSSG) and GSH/GSSG ratio did not differ from the control value in any exposure. The level of metal-keeping and stress-related protein metallothionein determined from the concentration of its sulfhydryl groups, was enhanced in all exposures except of nifedipine. Particularly, the glyphosate increased the level of metallothionein by the 47.7%. At that, the concentration of metalated metallothioneins did not differ from the control value in all exposures except exposure to glyphosate alone. In the last case, the level of the metalated metallothioneins dropped by two times. Consequently, the level of the non-metalated metallothioneins (apo-form) increased dramatically. This manifestation witnesses the highly oxidative impact of glyphosate. On the other hand, the level of the essential for the metallothioneins metals (Zn, Cu, Cd) in the unbound to the metallothioneins form increased, and maximally, in the exposures contained glyphosate. The lack in their buffering by metallothioneins and appearance in the non-bound form could be the additional reason for the oxidative damage. The inducing of the oxidative damage of lipids (detected as level of TBARS) and proteins (protein carbonyls) was detected in these exposures. The typical toxicity of this phosphonate was confirmed by the depletion of the cholinesterase activity. However, the heating diminished the response of cholinesterase. To summarize, the depletion of the biotransformation activity and metallothioneins demetalation were the most consecutive responses to the environmentally realistic effect of xenobiotics.

This work has been granted by the awards of Ministry of Education and Science of Ukraine to O. Stoliar (Projects M/35-2018 and 132B).

Grosvik B. E. et al. *Aquat. Toxicol.* 2006, 79: 334-340

Cubero-Leona C. M. et al. *Aquat. Toxicol.* 2010, 98:178-187

**TOXIC ENVIRONMENT DIMINISHES THE OXIDATIVE STRESS RESPONSE
IN THE BIVALVE MOLLUSKS**

Lesya Gnatyshyna

Poster 2

Lesya Gnatyshyna^{1,2}, Vira Khoma¹, Natalia Mishchuk¹, Oksana Horyn¹, Victoria Martynyuk¹,
Lubomir Tsaryk¹, Gunta Sprinģe³, Oksana Stoliar¹

1 – Volodymyr Hnatyuk Ternopil National Pedagogical University, M. Kryvonosa Str., 2, 46027
Ternopil, Ukraine

2 – I.Ya. Horbachevsky Ternopil State Medical University, m. Voli, 1, 46001, Ternopil, Ukraine

3 – University of Latvia, Miera Str. 3, Salaspils, LV, 2169, Riga, Latvia

E-mail: Oksana.Stolyar@tnpu.edu.ua

The aquatic inhabitants are chronically exposed to complex impact of the toxic effluents and climate abnormalities. When the native populations are examined, it can be expected either the specific responses of detoxification of the local impacts, or the tolerance to them, or the exhausting of the responses depending on the time and the severity of impact (Pain-Devin et al., 2014). In the current study, we focused on the stress and toxicity responses of two species of bivalve mollusks, *Unio tumidus* and *Dreissena polymorpha* depending on their settlement. The mollusks were sampled at the artificial river sites associated with the hydropower plants (HPPs). In Ukraine, the samples of *Unio tumidus* (Unionidae) were collected in the Dniester River basin from the reservoir of Kasperivtsi small HPP and before the dam of Krasnostavtcy micro HPP and from the sites after the dam of both HPPs. In Latvia, the specimens of *Dreissena polymorpha* were sampled from the reservoir of Riga HPP at Daugava River and from the pristine native lake. Several indices of antioxidant activities and oxidative damage were assayed, and the successfulness of the detoxification of the certain pollutants was evaluated. Integrated Biomarker Index (IBR) elaborated by Beliaeff and Burgeot (2002) was calculated including biochemical and cellular markers (totally 11).

In both species, the mussels sampled in the reservoirs of HPP, have shown the typical responses to the pollution by pesticides and personal care products. Particularly the mussels from the Kasperivtsi demonstrated the depletion of the cholinesterase activity typical for the effect of thiocarbamate pesticides. The concentration of metal-buffering protein metallothionein induced by toxic metals was higher in this group. The level of vitellogenin, determined as alkali labile phosphates, was highest in both groups from the reservoirs. This manifestation of the elevated vitellogenesis is usually caused by the presence in the water of endocrine disruptors. The most known endocrine disruptors in the surface waters are the pharmaceuticals and personal care products. However, in the mollusks from the Latvian sites, their level was similar. Hence, the specimens from the reservoirs indicated the pollution by the typical effluents, particularly in the Kasperivtsi. In the mollusks from both reservoirs, the depletion of GSH/GSSG was found. Unexpectedly, the mollusks from the reservoirs demonstrated lower level of lipid peroxidation products than the groups of comparison and greatest superoxide dismutase (Cu, Zn-SOD and/or Mn-SOD) activities. The standardized data for each marker (IBR) confirm that the manifestations of toxic impact were greatest in the mollusks from the large reservoirs, whereas the oxidative stress responses were intrinsic the specimens from the micro HPP and pristine sites.

Overall, the current study represents the first evaluation of the biochemical indices of bivalve mollusks depending on the water flow regime. They indicate the disagreement of the responses of stress and toxicity in the polluted areas.

This work has been granted by the Ministries of Education and Science of Ukraine and Latvia (Projects 132B and M/35 for O. Stoliar and LV-UA/2017/5 for G. Sprinģe).

Beliaeff B., Burgeot T. Environ. Toxicol. Chem. 2002, 21:1316–1322.

Pain-Devin S. et al. Aquat. Toxicol. 2014, 155:52-61.

REACTION OF RAT'S HEPATOCYTES AFTER THE INFLUENCE OF METHIONINE

Olena Chaka

Poster 3

Yanko R.V., Chaka O.G., Levashov M.I.
*O.O. Bogomoletz Institute of Physiology of NAS of Ukraine,
4, Bogomoletz St., Kiev 01024, Ukraine*

E-mail: biolag@ukr.net

Literary data on the effect of methionine on the functional activity and morphological changes in hepatocytes are rare and ambiguous, despite the fact that its role in the body is well-studied. It may be due to use in experiments the animals of different species and age, differences in the dosage of methionine, the duration of experiments, etc. Most of the available studies are devoted to studying the effect of methionine deficiency in food on the synthetic activity of the liver parenchyma. A question is unexplored as activity of hepatocytes will change after additional introduction of methionine to the standard diet. The purpose of our work is to investigate the effect of sulfuric amino acid methionine on morpho-functional changes in hepatocytes in adult rats.

The study was conducted on 24 Wistar male rats, aged 15 months. The animals, both the control and the experimental group, were in compatible terms with a standard diet. Rats of the experimental group in addition to the standard diet received methionine every day perorally during a 21 day in a dose of 250 mg/kg of body weight. Such dose of methionine may be considered prophylactic, because it does not lead to a substantial increase of methionine content in organism and the emergence of homocysteinemia. Histological preparations from the liver tissue were prepared according to the standard method. Morphometry of the liver was carried out on digital images using the computer program "Image J". The activity of succinate dehydrogenase in the suspension of hepatocyte mitochondria was determined by the Krivchenkova method. The concentration of the protein in the hepatocyte mitochondria was determined by the Lowry method.

The cross-sectional area of hepatocytes and their cytoplasm had a slight tendency to decline after completion of the methionine introduction to the experimental animals. The area of the nucleus, on the contrary, increased by 18 %. It led to an increase in the nucleus-cytoplasmic ratio by 27 % ($P < 0.05$) compared to the control values. The increase of this index indicates an increase of the cell functional activity, and may indicate the preparation of the cell to mitosis. The nucleolus of hepatocytes in the experimental animals are well visualized, mostly of medium size, with a rounded shape and clear borders. It was noted an increase the number of nucleolus in hepatocyte nucleus at 28 % ($P < 0.05$) and a nucleolus-nucleus ratio of 9 % compared to control. The nucleolus hyperplasia may indicate the activation of hepatocytes physiological regeneration at the intracellular level and the increase of their protein synthetic activity. The number of binucleus hepatocytes in the liver of experimental animals increased significantly by 16 % compared with control. Most authors believe that an increase the number of binucleus hepatocytes indicates an increase the intensity of the liver parenchyma regeneration at the intracellular level.

The activity of succinate dehydrogenase in the suspension of hepatocytes mitochondria tended to increase after methionine introduction. The concentration of protein in the suspension of hepatocyte mitochondria in the experimental rats was significantly higher by 73 % than in the control. It may indicate on the increase of protein-synthetic function of mitochondria cells.

Thus, according to most of the obtained indicators, we can conclude that 21-day introduction of methionine increases the functional and regenerative activity of hepatocytes.

ANTIFUNGAL ACTIVITY OF NEW STYRYLPYRIDINIUM DERIVATIVES AGAINST
CANDIDA ALBICANS

Simona Vaitkienė

Poster 4

Simona Vaitkienė¹, Neringa Kuliešienė¹, Egils Bisenieks², Laura Bekere², Laura Krasnova², Zenta Kalme², Gunars Duburs² and Rimantas Daugelavičius¹

1 – Department of Biochemistry, Vytautas Magnus University, Vileikos str. 8, Kaunas 44404, Lithuania

2 – Institute of Organic Synthesis, Aizkraukles str. 21, Riga 1006, Latvia

E-mail: simona.vaitkiene@vdu.lt

Yeast of *Candida* genus, especially *Candida albicans*, are the most common causes of fungal infections, which can lead to significant morbidity and mortality among immunosuppressed patients. The choice of drugs to treat fungal infections is limited due to the emerging yeast resistance to antifungal compounds. The main reason for *C. albicans* drug resistance is the presence of ATP-binding cassette (ABC) and Major facilitator superfamily (MFS) transporters in the plasma membrane, which decrease azoles binding to the yeast cells. Reduced susceptibility of *C. albicans* cells to antifungals force to search for new agents that could affect different cellular targets.

Aim. To evaluate antifungal efficiency of the synthesized styrylpyridinium derivatives on *C. albicans* wild type (WT) and clinical isolate (CI) cells, select the least toxic compounds to Chinese hamster ovary cells (CHO-K1), and test the selected compounds as inhibitors on *C. albicans* efflux transporters.

Material and methods. *C. albicans* WT strain ATCC 10231 and fluconazole resistant clinical isolate *C. albicans* CI strain 11017 were used in this study. Effects of 13 newly synthesized styrylpyridinium derivatives (compounds I-XIII, concentrations varying from 0.5 to 128 µg/ml) on the susceptibility of yeast cells were studied using standard broth microdilution method and minimal inhibitory concentrations preventing 50% of cells growth (MIC₅₀) were determined. Cytotoxicity of the compounds to CHO-K1 cells was determined using 2,3-bis [2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide (XTT) assay. This assay is based on the ability of metabolically active cells to reduce XTT tetrazolium salt and produce formazan compounds of orange colour. Rhodamine 6G, a fluorescent substrate of ABC transporters, was used to determine the transport activity of efflux pumps. *C. albicans* cells were de-energized using 5 mM 2-deoxyglucose and sodium azide and preloaded with R6G. Efflux of this indicator was monitored measuring the changes in fluorescence after addition of glucose to cell suspension.

Results. Three tested compounds effectively inhibited *C. albicans* growth and at the same time showed only slight to moderate toxicity to CHO-K1 cells: MIC₅₀ of **Comp IV** on WT cells was 2.8 µl/ml and 4 µl/ml were required for CI growth suppression. The MIC₅₀ of **Comp V** and **VI** on *C. albicans* WT varied from 1 to 4 µl/ml, at the same time the MIC₅₀ of the mentioned compounds on *C. albicans* CI varied from 4 µl/ml and higher than 6 µl/ml, respectively. The cytotoxicity results indicated that after 24 h of CHO-K1 cells treatment with MIC₅₀ of **Comp IV**, metabolic activity of CHO-K1 cells was reduced only up to 77.5 ± 5.6 %. It was demonstrated, that MIC₅₀ of **Comp V** and **VI** reduced CHO-K1 cells metabolic activity up to 62.1 ± 4.5 % and 79.1 ± 3.8 %, respectively. Moreover, WT and CI cells treated with **Comp V** showed suppressed R6G efflux, determined by decreased fluorescence intensity compared to the control cells with no compounds added.

Conclusions. Data of our experiments showed that several newly synthesized styrylpyridinium derivatives are active against *C. albicans*: effectively reduce growth of *C. albicans* cells and inhibit efflux pump activity. Therefore, activities of novel compounds should be further investigated analyzing structure-function properties of these potential antifungal agents.

The study was supported by the Research Council of Lithuania (project number 09.3.-LMT-K-712-02-0200)

COMPARATIVE ANALYSIS OF INHIBITORS PHE-ARG- β -NAPHTHYLAMIDE AND 1-(1 NAPHTHYLMETHYL) PIPERAZINE ON EFFLUX IN SALMONELLA ENTERICA CELLS

Sandra Sakalauskaitė

Poster 5

Sandra Sakalauskaitė and Rimantas Daugelavičius

Department of Biochemistry, Vytautas Magnus University, Kaunas, Lithuania

E-mail: sandra.sakalauskaite@vdu.lt

Antimicrobial resistance is a steadily growing worldwide problem. Efflux pumps play a key role in bacteria resistance to antibiotics. It is not easy to develop new antimicrobial compounds which would not be substrates of the efflux pump. Beside this, analysis of sides effects of new antimicrobials slow down their introduction into medical practice. Knowledge of ways how to increase efficiency of usual antibiotics is of crucial importance. Therefore, it is very important to understand the regulation of efflux in bacteria.

We used tetraphenylphosphonium and ethidium ions, these lipophilic cations selective electrodes and fluorescence measurements to assay the efflux activity in *Salmonella enterica* cells. The aim of our study was to explore effects of two efflux inhibitors – Phe-Arg- β -naphthylamide (PA β N) and 1-(1 Naphthylmethyl) piperazine (NMP) – on accumulation of indicator ions in bacteria. Also, ability of these inhibitors to increase efficiency of Chloramphenicol, Tetracycline and Ampicillin was studied. We used wild-type cells and efflux pump mutants to determine, how the mutations in pump genes affect efficiency of the inhibitors and sensitivity of the cells to antibiotics.

In experiments with wild-type and efflux mutant *S. enterica* cells we determined that PA β N and NMP enhanced accumulation of efflux pumps substrates and increased permeability of the outer membrane. We observed dependence of efflux inhibiting activity of the inhibitors on Mg⁺⁺ concentration in the medium: 0.2 mM and higher concentrations of MgCl₂ inhibited action of PA β N increasing ethidium fluorescence to the control level, and action of NMP was blocked at 0.8 mM and higher concentrations of MgCl₂.

EFFECTS OF PREINCUBATION WITH NON-FERMENTABLE CARBON SOURCES ON THE DESICCATION STRESS TOLERANCE OF SACCHAROMYCES CEREVISIAE CELLS

Deimantė Galalytė

Poster 6

Deimantė Galalytė, Neringa Kuliešienė, Rimantas Daugelavičius
Department of Biochemistry, Vytautas Magnus University, Kaunas, Lithuania

E-mail: deimante.galalyte@gmail.com

Saccharomyces cerevisiae is a unicellular eukaryotic organism that often undergoes transition into state of anhydrobiosis. During desiccation these cells lose intracellular water, but still protect their structures from damages for successful recovery during the rehydration. This study was performed to explore effects of cell preincubation in concentrated solutions of non-metabolizable carbon sources on the resistance of *S. cerevisiae* to dehydration. Two *S. cerevisiae* strains were studied: #14 – semi-resistant, and #77 – very resistant to dehydration. The cells were incubated in 1 M solutions of xylitol, lactose or glycerol for 3 h and desiccated at 30 °C for 21 h. During rehydration the metabolic activity of cells was assayed by oxygen consumption test, following intracellular accumulation of K⁺ and synthetic lipophilic anion phenyldicarbaundecaborane (PCB⁻), also by fluorescence microscopy.

This study revealed that incubation in various carbon source solutions differently affected cells. Respiration of #14 strain cells preincubated with glycerol and lactose was more intensive (consuming 90% and 70% of medium dissolved oxygen, respectively) than of #77 strain cells whose oxygen consumption was 2 fold slower. Meanwhile, xylitol pretreated cells of both strains were consuming the same amount of oxygen (45%). The PCB⁻ test showed that after preincubated in glycerol or lactose, plasma membrane of #14 cells was more permeable than of #77 ones. Another observed difference was that lactose-preincubated #14 cells recovered membrane barrier more slowly than the same cells preincubated in glycerol. When the cells were preincubated with xylitol, plasma membrane permeability of both strains was very similar. Results on the role of preincubation on accumulation of intracellular K⁺ showed that during rehydration #14 strain cells preincubated in hypertonic solutions of glycerol and xylitol accumulated more K⁺ ions compared to #77 strain cells.

Our results indicate that preincubation of yeast cells in hypertonic solutions of glycerol, lactose or xylitol highly increase their resistance to desiccation. The most intriguing result of this study is that after preincubation in concentrated solutions of lactose and glycerol the cells “switched” their phenotypes: #14 strain showed higher metabolic activity than #77 one. On the other hand, metabolic activity of both cells did not change after preincubation in 1 M xylitol. This effect should not be metabolism-driven, because all three carbon sources are non-fermentable in *S. cerevisiae* cells.

Acknowledgments: This study was supported by Research Council of Lithuania, funding grant No TAP-LLT-3/2016 and Ministry of Science and Technology, Taiwan. We thank prof. A. Rapoport (University of Latvia) for *S. cerevisiae* cells.

PHYSICOCHEMICAL QUALITY PARAMETERS, ANTIBACTERIAL PROPERTIES AND CELLULAR ANTIOXIDANT ACTIVITY OF POLISH BUCKWHEAT HONEY

Dorota Grabek-Lejko

Poster 7

Małgorzata Dżugan¹, Dorota Grabek-Lejko², Sylwia Swacha¹, Ireneusz Kapusta³, Monika Tomczyk¹, Sabina Bednarska⁴

1 – Department of Chemistry and Food Toxicology, Faculty of Biology and Agriculture, University of Rzeszów, Rzeszów, Poland

2 – Department of Biotechnology and Microbiology, Faculty of Biology and Agriculture, University of Rzeszów, Rzeszów, Poland

3 – Department of Food Technology and Human Nutrition, Faculty of Biology and Agriculture, University of Rzeszów, Rzeszów, Poland

4 – Department of Biochemistry and Cell Biology, Faculty of Biology and Agriculture, University of Rzeszów, Rzeszów, Poland

E-mail: dorobek@o2.pl

Buckwheat honey is the darkest Polish honey and has the strongest antibacterial and antioxidant activity; however, the mechanism of this bioactivity remains unknown. To determine the factors responsible for the bioactivity of buckwheat honey, the quality, antioxidant power, radical scavenging activity, and total phenolic and flavonoid contents of 20 buckwheat honey samples from southeastern Poland were measured. The antibacterial activity of the honey was tested against four bacterial strains. The effect of catalase on the antibacterial action of the honey was determined. Five buckwheat honey samples with different antioxidant and antibacterial activities were selected, and their phenolic profiles were characterized in detail with the UPLC-PDA-MS/MS method. *In vivo* experiment demonstrated that these samples protected cells of the yeast *Saccharomyces cerevisiae* exposed to hydrogen peroxide, which was used as a hydroxyl radical generator. The antibacterial activity of the tested honey was significantly correlated with antioxidant activity and phenolic compound content ($p < 0.05$). The removal of H_2O_2 by catalase partially eliminated (30-50%) the bacteriostatic activity of the tested honeys. The results indicated that among the 13 phenolic compounds identified in the selected buckwheat honey samples, only quercetin, rutin, chlorogenic acid and caffeic acid were correlated with the antioxidative and antibacterial activity of the honey, which was shown by the Principal Component Analysis (PCA) statistical analysis. For the first time the protective effect of buckwheat honey resulting from its polyphenols content was confirmed ($p < 0.05$) against *in situ*-generated hydroxyl radicals using the *S. cerevisiae* yeast cells as a biological model.

Dżugan M, Grabek-Lejko D, Swacha S, Kapusta I, Tomczyk M, Bednarska S. Food Bioscience (accepted)

ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF SELECTED MONOFLORAL HONEYS FROM UNUSUAL PLANT SOURCES

Dorota Grabek-Lejko

Poster 8

Edyta Rudny¹, Maciej Kluz², Aleksandra Wrona¹, Dorota Grabek-Lejko¹

1 – Laboratory of Biotechnology and Microbiology, Department of Bioenergetics and Food Analysis University of Rzeszow, Zelwerowicza 4, 35-601 Rzeszow, Poland

2 – University of Rzeszow, Zelwerowicza 4, 35-601 Rzeszow, Poland

E-mail: dorobek@o2.pl

Honey is a natural product of animal origin, produced by honey bees. It has been used for centuries because of its taste, nutritional and health benefits. The antimicrobial and antioxidant properties of atypical, selected honey types – cornflower, bean, goldenrod, raspberry, dandelion and sunflower – have been studied in this paper. It has been shown that all analyzed honeys, inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus saprophyticus* and *Salmonella enterica*. Cornflower and bean honey possessed the best antibacterial properties, which inhibit the growth of all bacteria at a concentration of 25%. The weakest antimicrobial properties were observed for sunflower honey. The highest antioxidant potential was observed for raspberry (978.26 $\mu\text{mol Trolox/kg}$), cornflower and bean honey. Lower values of antioxidant properties were observed for goldenrod, sunflower and dandelion honey (381.27 - 429.77 $\mu\text{mol Trolox/kg honey}$). Similarly, the polyphenol concentration decreased in the following order: raspberry > cornflower > bean > sunflower > dandelion > goldenrod honeys. There was a strong correlation between antioxidant properties and polyphenol content ($r = 0.99$), but antimicrobial properties of honey were not correlated with polyphenols content.

EFFECT OF HONEYBEE CHITOSAN-MELANIN FRAGMENTS UPON VIABILITY
IN VITRO OF BACTERIAL, FUNGI, AND MAMMALIAN CELLS

Nazar Manko

Poster 9

Nazar Manko¹, Maxim Lootsik¹, Stepan Tistechok², Olexander Gromyko², Rostyslav Stoika¹
1 – Institute of Cell Biology, National Academy of Science of Ukraine, Drahomanov Street 14/16,
79005, Lviv, Ukraine

2 – Department of Genetics and Biotechnology, Ivan Franko National University of Lviv, Hrushevsky
Street, 4, 79005, Lviv, Ukraine

E-mail: mankonazarcb@gmail.com

Antimicrobial effect of marine crustacean's chitosan and its derivatives is well known [1] and further investigations are developing on their biomedical application. Chitosan from insects, in particular honeybee, is less investigated, though it might be also of practical interest. Recently, we have isolated chitosan-melanin complex (CMC) from honeybees, and performed its cleavage by limited acid hydrolysis. It was found that obtained fragments exhibit the antimicrobial effect, especially towards *Candida albicans*.

The aim of this study was to investigate the influence of purified fragments of CMC on a survival of cells of various taxonomic origin – bacteria, fungi (*Candida sp.*) and mammalian, and compare their antifungal activity with that of nystatin and clotrimazol, a known antifungal medicines.

Material and methods. Fragments of CMC were obtained by limited hydrolysis with HCl: acetic acid mixture, chromatographic fractionation on a column of Toyopearl HW-60, as described in [2]. Water solution of fragments (2 mg/ml, pH 5.6) was used as a stock for introduction into culture medium. The test-objects included: *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa*, *Candida albicans* C88 (conventional), *C. albicans* N12 (MDR), *C. tropicalis*, as well as the mammalian cell lines – human breast carcinoma cells (MCF-7), murine macrophages J 774.2, NIH3T3 murine fibroblasts, human embryonic kidney cells (HEK 293), PHA-activated lymphocytes of human peripheral blood. The microbial cells were cultured in Saburo medium at pH 5.6 for *Candida sp.*, and at pH 7.2 - for bacteria. Viability of bacterial and mammalian cells was determined by MTT-test and expressed as decrease in color intensity of thiazolyl blue in percents relative to values in control at the end of incubation. Cytotoxic effect towards *Candida sp.* was measured with the method of colony-forming units (CFU) and expressed as changes in number of colonies in percents relative to start value of control. The apoptotic changes in cells were determined by fluorescent microscopy after staining with Hoechst 33342, DAPI and Ethidium bromide dyes.

Results. Fragments of CMC were eluted from the column of Toyo-60 as long-drawn peak which was conventionally divided into three parts: leading (15% of material), middle (75% of material) and the last (10% of material). They differed by molecular mass and melanin content: over 250 kDa and 16.2 % resp. (1st part), 250-70 kDa and 5.7 % (2nd), 80-15 kDa and 1.3 % (3rd). The leading part of fragments showed the highest antimicrobial activity, providing at concentration 1 mg/ml 99% growth inhibition of *C. albicans*, less inhibition of bacteria, i.e. *E. coli* (78%), *P. aeruginosa* (25%), *St. aureus* (13%). Damaging effect of mentioned CMC fragment towards *C. albicans* was compared with: that of nystatin and clotrimazole. 50% of growth inhibition was achieved at 50 µg/ml of CMC fragment, 12 µg/ml of clotrimazole, 9 µg/ml of nystatin, thus fragment being of a lower specific activity. However, CMC fragment equally well inhibited clotrimazole-sensitive (12 µg/ml) and clotrimazole-resistant (70 µg/ml) *C. albicans* strains. The high molecular mass CMC fragment in doses up to 200 µg/ml. did not exhibited significant pro-apoptotic effect towards pseudonormal and tumor mammalian cells

Conclusion. High molecular fragments of honeybee chitosan-melanin complex possess a distinct antifungal activity and are non-toxic towards mammalian cells. It can be used *per se* or as carrier of antimicrobial drugs for enhancement and prolongation of their effect.

1. Muzzarelli R. et al. Antimicrob. Agents Chemother. 1990, 34(10):2019-2023.

2. Lootsik M. et al. In.: Advances in Microbiology and Biotechnology. Abstract Book. Lviv, 2018, Oct 29-31, P.24.

ВПЛИВ ТКАНИННОГО ГІДРОЛІЗАТУ «ГЛОБУТРИН» НА ПЕРВИННУ КУЛЬТУРУ ФІБРОБЛАСТІВ ШКІРИ ПІСЛЯ УФ ОПРОМІНЕННЯ

Olena Novikova

Poster 10

Новікова О.Ю.

ПАТ «ФАРМСТАНДАРТ-БІОЛІК», Помірки, м. Харків, 61070, Україна

E-mail: ksuhanew7@gmail.com

Ультрафіолетове випромінювання (УФ) є екологічним чинником, що постійно впливає на людину. При тривалому впливі УФ-випромінювання викликає дегенеративні зміни клітин шкіри, фіброзної тканини і кровеносних судин, пригнічує імунну систему, особливо небезпечним є короткохвильове УФ. Основними молекулярними мішенями УФ є ДНК і білки, пошкодження може бути прямим, чи опосередкованим фотосенсибілізацією, що зрештою призводить до ланцюгових реакцій (Pattison DI, Davies, 2006). Нами було досліджено протекторні властивості субстанції «Глобутрин» на первинній культурі клітин фібробластів свині, опромінений УФ. Субстанція представляє собою низькомолекулярну фракцію продуктів глибокого гідролізу білків риби. Вивчення фармакологічної дії пептидів риби виявило їх здатність проявляти антиоксидантну, імуномодельную, протипухлинну та антимікробну активність, проте інформації щодо впливу на шкіру недостатньо (Najafian, Babji, 2012).

Культура первинних фібробластів шкіри свині була отримана ферментативним методом, культивування здійснювалося на стандартному середовищі - ДМЕМ, з додаванням 10% ФТС (Biowest, Франція). Фотопшкодження здійснювалося шляхом розташування культурального планшета з моношаром 30% конfluентності під УФ-випромінювачем з довжиною хвилі 254 нм. Час експозиції становив 20 і 30 хв. Після впливу середовища замінювалося на свіже, що містило досліджуваний препарат або без нього (контрольні варіанти). Через 48 годин культивування проводився підрахунок концентрації клітин і обчислення коефіцієнта проліферативної активності, а також приготування фіксованих препаратів для підрахунку патологічних форм поділів.

Встановлено, що вплив УФ 254 нм протягом 20 і 30 хвилин призводить до значного підвищення проліферативної активності – 3,60 і 3,74, що значно вище ніж в неопроміненому контролі – 2,50. При внесенні препарату до культурального середовища відразу після опромінення і подальшому культивуванні, відбувалося відновлення швидкості поділу до значень, наближеним до таких у неопромінених контролі – 2,50 та 2,66. У той же час, додавання препарату до культури неопромінених клітин не призводить до будь-яких істотних змін коефіцієнта проліферації – 2,60.

Підрахунок числа патологічних мітозів продемонстрував, що число патологій поділу, індукованих впливом УФ, достовірно знижувалося на $10 \pm 1,2\%$ ($p = 0,05$). Вочевидь, енергія УФ в даному випадку відіграє роль активатора, неспецифічно підвищуючи швидкість обмінних процесів, наслідком чого стає прискорення поділу клітин. Збільшення швидкості росту може призводити до накопичення помилок поділу, і як наслідок - до злоякісної трансформації клітин, через порушення проходження контрольних точок клітинного циклу. Таким чином, можна припустити, що препарат здатний активувати репаративні системи клітини, відновлюючи нормальну швидкість проходження всіх етапів клітинного циклу, а також бути донором непошкоджених структурних компонентів – амінокислот і низькомолекулярних пептидів. Ймовірно, подібна композиція може слугувати профілактикою променевого ураження клітин шкіри *in vivo*.

1. Pattison DI, Davies MJ. EXS. 2006;(96):131-57.
2. Najafian L, Babji AS. Peptides. 2012 Jan; 33(1):178-85.

ANTIHEMOLITIC ACTIVITY OF AMPHIPHILIC COMPOUNDS UNDER CONDITIONS OF POSTHYPERTONIC SHOCK OF HUMAN RED BLOOD CELLS

Olena Chabanenko, Natalia Yershova, Natalia Orlova, Natalia Shpakova

Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, 23, Pereyaslavskaya str., Kharkiv, 61016, Ukraine

E-mail: chabanenkoolena@gmail.com

Long-term storage of red blood cells (RBC) is currently a valuable approach for the donors with rare blood groups and combatants. Accumulation of the cryopreserved RBC can be useful in emergency or clinical situations, where the demand exceeds the supply (Henkelman S. et al., 2010). At the stage of thawing and removal of penetrating cryoprotectants, the cells are damaged as a result of the changes in medium temperature and osmolarity. In this research a posthypertonic shock was used as a model to investigate the effects of cryodamage factors on cells. Some amphiphilic compounds are known to prevent a cell damage under stress factors conditions (Semionova E.A. et al., 2017).

The aim of this work was to study the efficiency of amphiphilic compounds belonging to different classes on sensitivity of human red blood cells to the posthypertonic shock action.

Posthypertonic shock (PHS) of RBC carried out by transferring the cells from the dehydration medium (1.65 mol/L NaCl) into rehydration one (0.15 mol/L NaCl) at 0 and 37°C. Amphiphilic compounds were added to the rehydration medium before the transfer of RBC. The level of RBC hemolysis was determined spectrophotometrically at a 543 nm wavelength. Amphiphilic compounds, which belong to different classes of surfactants, were used in this work. Anionic surfactants are represented by sodium decyl sulfate (C10), cationic with chlorpromazine (CPR), nonionic with decyl β -D-glucopyranoside (DGP). The antihemolytic activity of amphiphilic compounds was expressed as a percentage reduction of hemolysis in the presence of compounds in relation to hemolysis an amphiphile-free sample.

The level of posthypertonic lysis of RBC was $70 \pm 5\%$. At 0°C all amphiphilic compounds were found to significantly reduce the hemolytic damage of cells. So the use of C10 and DGP led to a 3-time decrease in the level of human RBC hemolysis, and the supplement of CPR in 4 times. At 37°C, the protective effect of amphiphilic compounds under PHS action was not revealed. Effective concentrations, with which there was observed a minimal lysis of cells, for C10 and CPR, are close and make 400 and 600 $\mu\text{mol/L}$, respectively. Effective concentration for DGP is equal to 1,000 $\mu\text{mol/L}$. The antihemolytic activity of anionic C10 and nonionic DGP was 66 ± 5 and $65 \pm 5\%$, respectively, and cationic CPR was slightly higher, i.e. $77 \pm 3\%$.

Thus, amphiphilic compounds belonging to different classes demonstrated a high antihemolytic activity under PHS. The mechanism of posthypertonic lysis of human RBC is based on the processes associated with the formation of microdefects in plasma membrane at dehydration stage and their growth at rehydration stage. Apparently, amphiphilic compounds, incorporating into plasma membrane, prevent the evolution of microdefects up to the hemolytic pore size.

Henkelman S. et al. Transfusion. 2010, 50(11): 2393-2401.

Semionova E.A. et al. Probl Cryobiol Cryomed 2017; 27(1): 51–60

ASSESSMENT OF INTERCELLULAR RELATIONSHIPS IN YEAST COLONY

Liubov Zelena¹, Olena Kravets², Nataliia Tkachuk³

1 – Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Zabolotnogo Street 154, 03143 Kyiv, Ukraine

2 – Institute of Food Biotechnology and Genomics, National Academy of Science of Ukraine, Osipovskogo Street 2A, 04123 Kyiv, Ukraine

3 – Department of Biology, National University “Chernihiv Collegium” named after Taras Shevchenko, Getman Polubotko Street 53, 14013 Chernihiv, Ukraine

E-mail: zelenalyubov@gmail.com

Yeast population is characterized by the cellular heterogeneity with the formation of multicellular structures such as colonies, biofilms, filaments and others. It is considered as well-organized community of several cell groups with different molecular-biological properties. Growing on the solid surface yeast cells undergo specialization and form colonies. Differentiation of cells in the yeast colony begins on 7th day of development and leads to the formation of cell groups with a different specialization: some of them produce nutrients, while others consume those (Váchová L. et al., 2009a). Besides the heterogeneity of phenotypes, cells in the colony are differed by physiological, metabolic and biochemical heterogeneity. Relationships between cell groups in the colony are aimed at maintaining of multicellular structure, adapting to various environments and comprised of cooperation, interaction and reciprocity between cells of different localization. It is suggested that Ato proteins have influence on various aspects of colony biology and metabolic reprogramming (Váchová L. et al., 2009b) and Flo1 protein drives cooperation between yeast cells (Smukalla S. et al., 2008).

The aim of the present study was to analyze some cytological and genetic characteristics of cells with different localization in *Saccharomyces cerevisiae* colony at the 2nd acidic phase of development. For this purpose, cytological and gene expression analysis were carried out.

Results of cytological analysis using fluorescent dyes showed well-defined differentiation between central and outer cell layers of yeast colony. The central zone consisted of morphologically heterogeneous cells and up to 40% were apoptotic ones while cells of outer zone were more homogeneous and percent of apoptotic cells was lower, about 4-8%. Comparative analysis of *ato1* gene expression in cells of central and margin layers revealed decreasing of *ato1* gene activity in central zone by 2 times. There were no changes in *flo1* expression between cells of different localization in *S. cerevisiae* colony at the 2nd acidic phase of development although small alterations were detected at the 2nd alkali phase. Thus, results obtained in the study suppose that *flo1* expression might maintain the multicellular structure of yeast colony whereas cell differentiation could be defined cytologically and by *ato1* gene activity.

Váchová L. et al. Environ. Microbiol. 2009a, 11:494-504.

Váchová L. et al. Environ. Microbiol. 2009b, 11:1866-1877

Smukalla S. et al. Cell, 2008, 135(4):726-737.

BIOLOGICAL ROLE OF GSTs AGAINST OXIDATIVE STRESS IN SPERMATOZOA OF INFERTILE MEN

Fafula R.V., Onufrovych O.K., Iefremova U.P., Vorobets Z.D.

Department of Medical Biology, Department of Biophysics, Danylo Halytsky Lviv National Medical University, Pekarska Street, 69, Lviv, Ukraine, 79010

E-mail: roman_fafula@ukr.net; kaf_medicalbiology@meduniv.lviv.ua

Oxidative damage to the sperm membranes leads to reduced ejaculate quality and has been considered as one of cause of male subfertility or infertility. Since spermatozoa contain a large amount of polyunsaturated fatty acids in spermal membranes which might easily be oxidized by ROS, they are particularly vulnerable to oxidative stress. The appropriate balance between ROS generation and their neutralization is crucially important for protection of spermatozoa against oxidative damage and is maintained by antioxidant systems. Among antioxidant enzymes crucial role belong to glutathione S-transferases (GSTs). It is known that GSTs catalyze the conjugation of GSH to a variety of electrophilic compounds and have crucial role as cell housekeepers involved in the detoxification of both endogenous and exogenous substances. Regarding the GSTs that have been studied in sperm cells, little is known about their role in pathozoospermic patients.

In order to demonstrate the functional role of GSTs in sperm cells, we used a H₂O₂-induced stress on human ejaculated spermatozoa obtained from both normo- and pathospermic patients.

We report here the effect of GSTs inhibitor ethacrynic acid on sperm motility and viability. Pharmacological inhibition of sperm GSTs activity (using its potent inhibitor ethacrynic acid) leads to spermal membrane damage, rejected in the loss of motility and decrease of viability. The fact that GSTs were primarily responsible for protection of spermatozoa from H₂O₂-induced oxidative stress became evident when in the presence of ethacrynic acid, sperm motility and viability was drastically decreased in incubation medium containing H₂O₂ in both normo- and pathozoospermic samples. Presence of GSH in incubation medium attenuated this inhibitory effect only in normozoospermic samples, but not in asthenozoospermic samples.

For similar treatment conditions TBARS levels increased significantly leading to decrease in sperm motility and viability. The strong positive correlation between sperm motility ($r = 0.72$; $p < 0.05$) / sperm viability ($r = 0.64$; $p < 0.05$) and TBARS accumulation confirms that lipid peroxide-induced membrane damages are involved in disturbances of sperm function. It is suggested that these functional impairments are related to the intensification of lipid peroxidation as expressed by TBARS levels in spermal membranes after GSTs inhibitor treatment.

Finally, we checked the inhibition profiles of GSTs by ethacrynic acid in sperm cells obtained from normo- and pathozoospermic samples. It was shown that ethacrynic acid in the concentration range of 0.01 - 10 mM suppresses GSTs activity of spermatozoa in dose-dependent manner. The inhibition curves in spermatozoa obtained from astheno-, oligo- and oligoasthenozoospermic samples were not significantly different from normozoospermic patients. The inhibition constant and Hill's coefficient were not significantly different in sperm cells obtained from normo- and pathozoospermic samples.

This study provides evidence that sperm GSTs are important in the defense mechanism against oxidative stress. Evaluation of GSTs activity in sperm cells of infertile men can be helpful in fertility assessment and for the evaluation of treatment by antioxidants.

COMPARATIVE EVALUATION OF THE EFFECT OF MICRO AND NANO-PARTICLES OF ALUMINUM AND SILVER ON THE ENDOCRINE SYSTEM OF ANIMALS

Andrushyshyna I.N., Golub I.O., Melnik N.A.

SI «Kundiiev Institute of Occupational Health of the NAMS of Ukraine, Saksaganskogo str.,75 Kyiv

E-mail: andrusyshyna.in@gmail.com

It is well known that endocrine system is a frontline hand in maintaining the homeostasis during the development of long-term body adaptation including adaptation to heavy metals. The excess or lack of certain metals results in the metabolic imbalance in the human body and brings in various changes in endocrine, immune, reproductive and other systems, and may reduce the lifespan. The purpose of this research was to reveal the profile of specific impact of metals as low-dose factors on endocrine system and endocrine cells. In order to better understand and interpret the pathogenic essence of the above mentioned disturbances associated with endocrine system, we carried out the series of toxicological experiments. These experiments showed that the disruption of endocrine system depends on sex of animals, route of toxicant administration and chemical and physical properties of test toxicants. It was determined that upon subacute intraperitoneal administration and acute oral administration of metal doses of 0.05 and 0.5 mg/kg, Al, Ag, were accumulated in internal organs of test animals as well as in their endocrine organs in particular.

The biochemical markers of Ag and Al effect on adaptation process in animals (imbalance in oxidant and antioxidant processes, disruption of hormone metabolic process), which characterize the adaptation process at molecular and cellular level, are as follows. At "*training*" stage: in case of AgNO₃ exposure, increase in CP levels up to 2.87 μmol/l and C-peptide up to 0.15 ng/ml (p<0.05); in case of exposure to Ag₂O NPs, increase in albumin levels up to 83.77 g/l, in erythrocyte ATP up to 157.30 μmol/l and in MT up to 0.40 μmol/l (p<0.05); in case of Al(NO₃)₃ exposure, increase in albumin up to 72.0 g/l, MT up to 0.40 μmol/l and free T₄ up to 2.56 ng/ml (p<0.05); in case of exposure to Al₂O₃ NPs, increase in CP up to 2.32 μmol/l (p<0.05). At "*tension*" stage: in case AgNO₃ exposure, decrease in erythrocyte ATP down to 26.33 g/l, in serum glucose down to 0.68 g/l and in ThG iodine down to 35.10 μg/g and in serum TTH (thyrotropic hormone) to 0.18 ng/ml (p<0.05); in case of exposure to Ag₂O NPs, decrease in serum CP down to 1.42 μmol/l and in ThG iodine down to 56.36 μg/g (p<0.05); in case of Al(NO₃)₃ exposure, decrease in ThG iodine content down to 46.06 μg/g (p<0.05); in case of exposure to Al₂O₃ NPs, decrease in serum albumin down to 37.18 g/l and in serum free T₄ down to 1.14 ng/ml (p<0.05). At "*exhaustion*" stage: in case of AgNO₃ exposure, decrease in ThG iodine down to 24.37 μg/g and in serum TTH down to 0.18 ng/ml (p<0.05); in case of Al(NO₃)₃ exposure, increase in erythrocyte ATP up to 230.48 μmol/l and in whole blood MT up to 0.1 μmol/l (p<0.05).

The experimental evaluation of histological changes observed in endocrine organs demonstrated that the effects of salts of metals on ThG tissue are non-specific. When metal salts are administered intraperitoneally, the morphological transformation of ThG follicular cells is characterized by dystrophic changes in thyroid epithelium; follicles around incorporated parathyroid gland (PThG) tend to enlarge, and more deep aggregation is observed in PThG occasionally. When silver and aluminium were individually orally administered, the disruptions were limited to changes in the functional state of thyrocytes and apoptosis observed in their small fraction. Morphological transformations of PG were characterized by functional changes; and it is namely in case of aluminium exposure, when the reduced number of islets of Langerhans was registered.

In vitro studies elucidated the mechanisms of interaction between peptide hormones and metals in the form of micro- and nano-size particles. It was found that molecular mass changes in human serum albumin and insulin were caused by binding with individual atoms contained in metal NPs (in case of silver, nanoparticles were found in the form of oxides). This interaction brought in the conformation changes in peptide hormones, and the disruptive effect increased with the increase in metal concentration in the solution. The comparison of metal micro- and nanoparticles effects demonstrated, that the metal nanoparticle (NP) accumulation produces the functional effect predominantly, while metal microparticles cause the physical accumulation of the material. Metal microparticles are absorbed faster and their concentration increases with time. At the same time, the translocation rate of metal nanoparticles in endocrine organs of test animals is higher thus indicating their greater toxicity.

CYTOMORPHOLOGICAL DIFFERENCES OF LEUKOCYTE POOLS AS ADDITIONAL CRITERIA FOR DIAGNOSTICS OF COMMUNITY-ACQUIRED PNEUMONIA

Raksha-Slusareva O.A.¹, Trykhlіb V.I.², Slusarev O.A.¹, Tarasova I.A.³, Boyeva S.S.¹, Shundel T.O.¹

1 – Donetsk National Medical University, Pryvokzalna Str., 27, Lyman, Ukraine

2 – Ukrainian Military Medical Academy, Moscowska Str., 33, Kyiv, Ukraine

3 – SE “L.V. Hromashevskyi Institute of Epidemiology and Infectious Diseases NAMS of Ukraine”, M. Amosova Str. 5, Kyiv, Ukraine

E-mail: slusarev.alex@gmail.com

Topicality. Nowadays the differential diagnosis of acute community-acquired pneumonia and acute respiratory diseases is inadequate. In this regard, the development of new criteria for the diagnosis of community-acquired pneumonia both in the military and in the civilian population is timely and relevant.

Purpose. The purpose of this work was to study the cytomorphological features of peripheral blood leukocytes in patients with community-acquired pneumonia and acute respiratory diseases in order to develop additional criteria for differential diagnosis.

Methods. Under the research there were 84 patients with acute respiratory diseases (ARD) and 65 patients of military personnel with community-acquired pneumonia (CAP) who were treated at clinics of the National Military Medical Clinical Center “MMCH”. Clinical, hematological, immunological and radiological methods were used in the studies.

Results and discussion. It has been established that in the first days of the disease there are cytomorphological differences of peripheral blood leukocytes in patients with CAP and ARD. On the background of almost double increase in cellular neutrophils in patients with CAP the increase in the number of segmental neutrophils was recorded, while in patients with ARD the content of segmental cells remained up to the standard or even decreased on the background of the increase in the content of rodenuclear neutrophils. In CAP compared with ARD a more pronounced toxicosis-inflammatory process was registered which was reflected by a more significant increase in cellular decay, neutrophil content with the fragmented nucleus, destruction of the nuclear membrane, cells with toxogenous granularity of the cytoplasm, as well as increased adhesion of neutrophils and there was registered a more pronounced mutagenic process that was reflected in the increase in the content of neutrophils with chromatin vili, the bodies of Bar, hypersegmentation and hyposegmentation of the nucleus. From the side of the cells of the lymphocyte lineage of hematopoiesis with CAP the number of disintegration of Botkin-Gumprecht cells was registered much less and was three times less than in ARD. In some cases extracted and aberrant lymphocytes indicating the tension of the immune system at the level of its breakdown were recorded in CAP.

Conclusions. The obtained results of cytomorphological research give new opportunities as for the development of additional criteria for differential diagnosis of community-acquired pneumonia and acute respiratory diseases in the first days of the disease of CAP and ARD.

EFFECT OF THE ADENO-HERPETIC MIXED INFECTION ON STRUCTURAL AND FUNCTIONAL PROPERTIES OF CELLS

Biliavska L., Pankivska Y., Povnitsa O., Zagorodnya S.

Department of Virus Reproduction, Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Acad. Zabolotny str., 154, Kyiv, 03143, Ukraine

E-mail: bilyavskal@ukr.net

Mixed viral infection is one of the topical and unexplored issues of human infectious diseases (Da Palma et al., 2010). A special place in the development of these pathologies is occupied by adeno- and herpes viruses that are able to persist for a long time in a latent condition in the body. To understand the biological nature of mixed infections the model of adeno-herpetic infection in cells was developed. The features of the development of viral infections in this system were studied (Biliavska et al, 2016). However, the role of the associated viruses in the regulation functioning of the cell population is not fully understood. Therefore, the aim of this work was to investigate the viruses-cell interactions under condition of mixed infection.

The study of mitochondrial, proliferative and lysosomal activity of co-infected cells was conducted. It was found suppression of vitality and mitochondrial activity of MDBK cells co-infected with herpes simplex virus type 1 and human adenovirus type 5 up to 32% compared to uninfected cells and its increasing by 100% in comparison with herpetic mono infection.

The subversion of cell cycle pathways is a well-established mechanism by which viruses create the most suitable environment for their replication (Trapp-Fragnet et al, 2016). Notably, the induction of S-phase is either mandatory or at least advantageous for lytic replication of a number of viruses. MDBK cell cycle analysis under condition of the mono and mixed infections was carried out. It was determined the reduction of the cell population in G1 phases and its increasing in S phases under condition of both mono and mixed infections that demonstrated the suppression of the transition of cells through the mitotic phase. As infected cells enter S phase and G2/M phase is blocked, cells produce viral DNA, late viral proteins and virions.

The ultrastructure of MDBK cells and the features of viral morphogenesis under the condition of mono- and mixed infection have been investigated. The sharp changes in the morphology of MDBK cells under reproduction of adeno- or herpesviruses were detected, namely, the increase in the nucleus, the marginality of chromatin, the formation of crystalline intranuclear clusters of complete and incomplete viral nucleocapsids, hypertrophy of the nuclear membrane in conjunction with the expansion of perinuclear space and the disappearance of the nucleoli, the vacuolation of the cytoplasm and the formation of paracrystalline structures. All these morphological changes resulted in complete destruction of cells for 72-96 hours after infection. The co-infected cells were characterized by a lower level of pathomorphological changes and the presence of small amount of viral capsids.

Thus, significantly less change of the structural and functional properties of cells under condition of mixed infection were detected in comparison with adenoviral and herpetic mono infections.

1. Da Palma T.A et al. *Virus Research*. 2010, 149(1):1–9.
2. Biliavska L.O. et al. *Pharmaceuticals*. 2016, 9(14):21–23.
3. Trapp-Fragnet L. et al. *PLOS ONE*. 2016, 9:1–14.

PHYSIOLOGICAL ACTIVITY AND SYMBIOTIC PROPERTIES OF BRADYRHIZOBIUM JAPONICUM UNDER FUNGICIDAL INFLUENCE CONDITIONS

Vozniuk S.V., Tytova L.V., Iutynska G.O.

D.K. Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Ukraine, 03143, Kyiv, Zabolotny str., 154

E-mail: vozsvet@gmail.com

The ecological and phytosanitary situation in modern agrarian systems requires the widespread introduction of biotechnological products, such as microbial preparations with polyvector action. This is due to an increase in the pesticide load that acts on the soil microbiota as a stress factor and also leads to soil and food agrochemical's contamination and the emergence of pathogens and pest-resistant forms. In agricultural practice for the productive potential of cultivated plants sustainable management biological products are introduced based on the associations of useful soil microorganisms of local breeding. However, their basic properties under extreme conditions of fungicidal stress are not sufficiently investigated.

Ecovital is the complex bioformulation based on highly effective strains of *Bradyrhizobium japonicum* UCM B-6035, UCM B-6018, UCM B-6023 and phosphate-mobilizing bacteria *Bacillus megaterium* UCM B-5724. The influence of Vitavaks 200FF, Maxim Star 025 FS and Kinto duo fungicides on physiological activity and symbiotic properties on bioagents of bioformulation Ecovital has been studied. It was found that the bacteria that are included in the bioformulation Ecovital proved to be resistant to Maxim Star 025 FS and Kinto duo in recommended by the manufacturers of the fungicides working concentrations. Vitavaks 200FF inhibited the growth of *B. japonicum* UCM B-6035, UCM B-6018 and *B. megaterium* UCM B-5724 in laboratory conditions. Under the Vitavaks 200FF action the diameter of the growth inhibited zones of these strains was 14.5, 25.7 and 30.1 mm, respectively. In the plots experiments it was shown the application of Maxim Star 025 FS or Kinto duo with following inoculation resulted in an nodulation increase in 1,2 and 1,4 times, respectively, in comparison with the inoculated variant. Vitavaks 200FF inhibited the nodulation process by 1.5 times. The nitrogenase activity of symbiotic systems increased by a combined effect of the Kinto Duo and Ecovital in 2.2 times compared to the variant with inoculation. Seeds treatments by Maxim Star 025 FS or Vitavaks 200FF with subsequent Ecovital inoculation resulted in a decrease of nitrogenase activity by 1.5 and 2.8 times, respectively, in comparison with bacterized variant. In the field conditions, the nodulation activity in variants with application of fungicides and bioformulation increased by 3-4 times, but nitrogenase activity of symbiotic systems decreased by almost 32-44% in comparison with the inoculated variant.

Thus, the investigated strains of *B. japonicum* and *B. megaterium* exhibited resistance to fungicides Maxim Star 025 FS and Kinto duo and retained the nodulation activity under the conditions of these fungicides, but the formed symbiotic apparatus nitrogenase activity was reduced in almost all experimental variants.

REGULATORY ROLE OF MOLECULAR FORMS OF SUPEROXIDE DISMUTASE OF HEPATOCYTES AND MYOCARDIUM OF THE RAT UNDER ACUTE HYPOXIA-HYPERCAPNIA

Svitlana Khyzhnyak, Mariya Ribak, Svitlana Midyk, Volodimir Voitsitskiy
*National University of Life and Environmental Sciences of Ukraine,
Heroiv Oborony Street, 15, 03041 Kyiv, Ukraine
E-mail: khs2014@ukr.net*

The regulatory role in the adaptation of the organism of animals to hypoxia-hypercapnia is played by ROS that participate in cascades of the intracellular redox signals and are controlled by protective components, including the antioxidant defense system, which improves the organism resistance to exogenous effects. The purpose of the work is to investigate the activity of multiple forms of superoxide dismutase (SOD) in hepatocytes and myocardium of the rat under acute hypoxia-hypercapnia.

SOD is one of the key ROS scavengers whose functioning in eukaryotic cells is associated with the existence of cytosolic Cu, Zn-SOD and mitochondrial Mn-SOD enzyme isoforms. Electrophoretic analysis of multiple forms of SOD was carried out in a polyacrylamide gel after protein separation according to the molecular weight in native conditions. The overall activity of SOD was determined in hepatocytes and myocardial cells of rats. The model of acute hypoxia-hypercapnia with reduced body temperature for rats was created as described earlier (Melnychuk S.D. et al., 2013).

It is established that in hepatocytes the activity of multiple forms of cytosolic Cu, Zn-SOD is dominant, and in myocardium – high activity of both mitochondrial and cytosolic isoforms of enzyme is detected. In acute hypoxia-hypercapnia total activity of SOD in hepatocytes decreases by 24.2% ($P < 0.05$), in particular, of the dimeric and monomeric forms of Cu, Zn-SOD, however, the activity of Mn-SOD does not change. In myocardial cells under these conditions the total activity of SOD increases by 62.7% ($P < 0.05$), with Mn-SOD activity increasing by 24.6% ($P < 0.05$), while the dimeric form of Cu, Zn-SOD – by 27.6% ($P < 0.05$). At the same time, the activity of other subunits of Cu, Zn-SOD decreases. The revealed features of SOD isoform reaction to hypoxia-hypercapnia may be attributed to differences in the mitochondrial activity under these conditions. It is known that the main supplier of ROS in the organism is the mitochondrial respiratory chain. The ROS, in turn, can affect the functioning of mitochondria, and, on the other hand, modify the activity of the complexes of the respiratory chain, and thus the processes of free radical oxidation. The inhibition of the activity of Complexes II, III and especially the terminal part of the chain – Complex IV of mitochondria in hepatocytes under hypoxia-hypercapnia is observed (Melnychuk S.D. et al., 2013). At the same time, in the mitochondria of the myocardium, a partial uncoupling of the oxidative phosphorylation processes along with the increase of the activity of Complexes III and IV (Melnychuk S.D. et al., 2016) was revealed, indicating a decrease in the ROS production by the electron transport chain, along with the increase of Mn-SOD activity. In addition, the regulatory role of hypercapnia is discussed, which is manifested in the ability of carbon dioxide to act as an ROS scavenger, as well as to modulate the activity of enzymes of the antioxidant defense system, in particular SOD. The obtained results testify to the complex processes of free radical oxidation with the involvement of multiple forms of superoxide dismutase, which leads to a change in the intensity and course of the metabolic pathways, directed to adapting the organism to the action of external factors.

Melnychuk S.D., Khyzhnyak S.V. et al. Ukr. Biochem. J. 2013; 85(4):75-81.

Melnychuk S.D., Khyzhnyak S.V. et al. Inter. Journal of Physiol. and Pathophysiol. 2016; 7(2):127-36.

SIGNAL WAYS OF HYPOXIA-INDUCED CYTOPROTECTION

Babicheva V., Portnychenko A., Vasylenko M., Lapikova-Bryhinska T., Portnichenko V.

*Bogomoletz Institute of Physiology, NAS of Ukraine; ICAMER, NAS of Ukraine,
4 Bogomoletz str, 01024 Kyiv, Ukraine*

E-mail: port@biph.kiev.ua

Purpose. Insulin like growth factor IGF-1 through activation of kinases PI3K/Akt is known to exert cardioprotective effects and regulate myocardial metabolism, as well as mediates heart hypertrophy, promotes cell survival by reduction of apoptosis and oxidative stress. However, the cardioprotective mechanisms in the hypertrophied myocardium during hypoxic preconditioning is not fully elucidated. The aim was to characterize IGF-1/PI3K/Akt-mediated cardioprotection in normal and hypertrophied heart after hypoxic preconditioning in response to severe whole body hypoxia or isolated heart ischemia/reperfusion.

Methods. Experiments were performed in male adult Wistar rats with or without heart hypertrophy and spontaneously hypertensive rats (SHR) with pressure induced heart hypertrophy. In Wistar rats, short-term hypertrophy of left heart ventricle was induced by isoproterenol (5 mg/kg, 3 days). The animals were exposed to hypoxic preconditioning using mild hypobaric hypoxia séances in barochamber (5600 m, 3 h). In 24 h, hearts were isolated with urethane narcosis and subjected to ischemia/reperfusion in a Langendorff mode, infarct size was detected with TTC staining. The other group of Wistar rats after preconditioning were subjected to severe hypoxia (5600 m, 3 h). Inhibitor of PI3K wortmannin or blocker of IGF-1 receptors picropodophyllin (PPP) were used for testing of cytoprotective signalling. Dynamic changes in mRNA and protein expression in heart ventricles were examined using real-time PCR, and Western blotting.

Results. Isoproterenol-induced heart hypertrophy was accompanied in Wistar with IGF-1 and Akt protein induction by 79% or 50%, respectively, but reduction of IGF-1 mRNA expression. In SHR, the expression of IGF-1 mRNA and Akt protein was lower by 42% or 63%, respectively, compared to Wistar control. Hypoxic preconditioning intensified IGF-1/PI3K/Akt-mediated cardioprotection by induction of IGF-1 expression, Akt expression and phosphorylation, more pronounced in non-hypertrophied hearts. Blockade of PI3-kinase or IGF-1 receptors decreased Akt phosphorylation, and abolished cytoprotective effects of preconditioning in view of mortality caused severe hypoxia, or of infarct size and cardiac function recovery in postischemic reperfusion. In SHR, hypoxia-induced cardioprotective effects and influences of the blockers were significantly reduced, in hypertrophied Wistar hearts – partly decreased.

Conclusions. The left ventricular hypertrophy in Wistar rats is regulated by IGF-1-dependent prohypertrophic signalling, but after hypoxic preconditioning IGF-1-receptor/PI3K/Akt-mediated cardioprotective response is only partly maintained. The prolonged left ventricular remodeling in SHR is characterized with IGF-1 mRNA and protein suppression, and with limited cardioprotection. Hypoxic preconditioning mediated by IGF-1/PI3K/Akt-signalling loses its effectiveness depending on the degree and duration of cardiac hypertrophy.

**THE EFFECT OF MECHANICAL STRESS-STRAIN ON ACTIN ISOFORMS
REORGANIZATION IN FIBROBLASTS IN VITRO**

Kot Yu., Persky Ye., Kot K., Polonska A., Lukan R., Yehemberdinov Ye.
*V. N. Karazin Kharkiv National University, Biochemistry department,
4 Svobody Sq., Kharkiv, 61022, Ukraine*

E-mail: kot.juriy@gmail.com

The changes in the intensity of the Actb, Actg1 and Acta1 genes expression and the content and localization of their products – marker cytoskeleton proteins – β -, γ -, and α -actin in lung fibroblasts, cultured on a deformable elastic substrate, were studied.

The fibroblasts were isolated from 3 months' age Wistar rats skin. The cells of 2nd passage were cultivated during 16 hours on transparent latex substrate with adhesive cover made of collagen type I, type III and hyaluronic acid. Then the substrate was single-time monoaxially deformed on 10% of substrate length. The rate of substrate relaxation was 160 $\mu\text{m}/\text{h}$. The total time of cells deformation was 6 hours.

The localization of actin filaments was analyzed by fluorescence confocal microscopy after eGFP-actin transfection (eGFP-Effectene Reagent, QIAGEN (USA)). The determination of the ratio of actin isoforms was performed using isoelectric focusing (10% PAAG, pH 10.0 – 3.0, Bio-Rad ampholytes) after total actin purification by affinity chromatography on DNase-I-agarose (Pharmacia).

The gene expression analysis was performed on DNA-microchips (Arrayit) on Affymetrix 428 Scanner. The total RNA from the cells was isolated on spin columns with the RNeasy Mini Kit (Qiagen). Synthesis of cDNA by reverse transcription was performed using QIAGEN OneStep RT-PCR Kit (Qiagen). The final amount of the produced protein product was measured immunochemically on antibody-conjugated ELISA-microchips using the Antibody Array Assay Kit (Full Moon BioSystems, Inc.).

It has been shown that the mechanical deformation of fibroblasts leads to the intensification of cytoplasmic actin synthesis. This process is accompanied by a two-step reorganization of actin filaments: reducing the number of longer structures and increasing the pool of shorter ones with subsequent formation of long stress fibers directed along the long axis of the cell. The mechanical stress enhances the expression and the content of β -, γ -, and α -actin within the deformed lung fibroblasts. α SMA isoform is not specific for fibroblasts but is typical for myofibroblasts.

As it is known, the manner in which wound fibroblasts respond to strain influences the wound repair. The compaction of granulation tissue pulls on the surrounding dermis, generating tension. The fibroblasts transform into myofibroblasts, characterized by prominent cytoplasmic stress fibers with the alpha smooth muscle actin (α SMA) isoform of actin. More intensive stress fibers formation influences on the level of strain-dependent responses of cells and increases the production of structural extracellular matrix proteins, which leads to faster wound healing.

THE EFFECT OF LOW CONCENTRATION OF CADMIUM ON OSTEOGENIC DIFFERENTIATION OF RATS MESENCHYMAL STROMAL CELLS *IN VITRO*

Kot Yu., Wu Si, Persky Ye., Kot K., Kharchenko T., Lukan R.
*V. N. Karazin Kharkiv National University, Biochemistry department,
4 Svobody Sq., Kharkiv, 61022, Ukraine*

E-mail: kot.juriy@gmail.com

In our early studies, conducted on the effects of long-term cadmium low concentration intake with drinking water on laboratory rats' metabolism, it has been found that such an application leads to decrease in the concentration of total collagen and glycosaminoglycans in bone tissue. It is known that these components of bone tissue are produced by osteoblasts - cells derived from mesenchymal stromal cells. We assumed that perhaps cadmium interferes with the formation of new metabolically active osteoblasts from mesenchymal stem cells.

The experimental work is devoted to the evaluation of the influence of low cadmium concentrations on the differentiation of mesenchymal stromal cells (MSC) of the rats' bone marrow in the osteogenic direction. The studies were conducted on 3 months old rats' mesenchymal cells which were cultivated during 17 days in the presence of low concentrations of cadmium (1,0 $\mu\text{M/L}$). The bone marrow cells were isolated from diaphysis of os femoris. The cells viability (87%) was determined by flow cytometry (Millipore Guava PCA). CD90 positive and CD45 negative MSC were isolated from primary suspension by the immunomagnetic separation method (Anti-CD90 antibody, Abcam; Dynabeads™ Antibody Coupling Kit, Life Technologies). MSC were cultivated in 24-well plate (Nunclon Delta) in α -MEM medium (Gibco).

The cells of the second passage were used for the study. The osteogenic differentiation was induced by substitution of media to osteogenic media with 0.1 μM dexamethasone, 0.05 μM ascorbic acid, 10 μM glycerol-2-phosphate and 20% FBS (Gibco). After 14 days the cells had bipolar morphology and were characterized by CD34 surface marker expression, alkaline phosphatase activity, collagen type 1, osteopontin, vimentin and calcium accumulation in culture. These surface and metabolic markers were determined by Abcam and Merck-Millipore kits, analyzed by confocal microscopy or spectrophotometry methods and used for analysis of effect of cadmium ions.

It was shown that the exposure to cadmium in media for 17 days at a concentration of 1,0 $\mu\text{M/L}$ leads to decrease of alkaline phosphatase, collagen type 1, osteopontin, vimentin and CD34 protein expression in MSC and calcium accumulation in MSC culture. The results indicate that cadmium ions inhibit osteogenic differentiation of mesenchymal stromal cells of the bone marrow *in vitro*.

If extrapolated the results obtained to *in vivo* processes, cadmium induced inhibition of osteogenic differentiation of MSC can slow down the recovery of a pool of metabolically active osteoblasts, renewal of the biopolymer and mineral composition of bones and, as a consequence, lead to the development of osteoporosis.

THE ANTIOXIDANT EFFECT OF CERIUM DIOXIDE NANOPARTICLES IN GAMMA-IRRADIATED SKIN FIBROBLASTS

Kot Yu.¹, Persky Ye.¹, Kot K.¹, Filonenko S.¹, Shishkina N.¹, Klochkov V.², Kavok N.², Malyukin Yu.²

1 – V. N. Karazin Kharkiv National University, Biochemistry department, 4 Svobody Sq., Kharkiv, Ukraine

2 – Institute of Scintillation Materials, Nanocrystal Materials Department, 60 Nauky av., Kharkiv, Ukraine

E-mail: kot.juriy@gmail.com

The work was carried out on 3D-culture of skin fibroblasts of 3 months old male Wistar rats. The isolated and subcultured until passage 3 cells with viability of 89% were seeded on a three-dimensional matrix (Alvetex Scaffold, 96 well) and cultured for 8 days until the culture density of 90%. 1 hour prior to irradiation, a suspension of nanoparticles was added to the culture medium to a final concentration of 1.72 µg/L, 3.44 µg/L, 6.88 µg/L, and incubated for 1 hour. After replacing the medium with nanoparticles by the free one, the cells were irradiated on the gamma complex Rokus AM (Co⁶⁰, 0.75 Gy, 104kV, 9mA, 0.2 Gy/min, duration of irradiation – 225 sec). Two and six hours after irradiation, the determination of the concentration of reactive oxygen species (ROS, DCFDA), lipid hydroperoxides (LP, MitoPeDPP), the degree of DNA fragmentation by fluorometric assay and spectrophotometric determination of the enzymatic activity of superoxide dismutase (SOD) and catalase were carried out in the fibroblasts culture.

It was shown that in the absence of irradiation, nanoparticles at concentrations of 1.72 and 3.44 µg/L do not cause changes in the studied parameters at all investigation times. The concentration of the nanoparticles 6.88 µg/L caused a decrease in cell viability by 4% after 6 hours of cultivation on a background of absence of changes in other parameters. This concentration also proved to be ineffective for maintaining the vitality of cells after irradiation - the number of living cells decreased both in the presence and absence of nanoparticles after 6 hours of cultivation by 31%. Pre-incubation with nanoparticles at a concentrations of 1.72 and 3.44 µg/L resulted in an increase in the number of living cells in culture 6 hours after irradiation by 14% and 18%, respectively, compared with irradiated cells. In 2 and 6 hours after irradiation, the concentration of ROS and LP increased by 5.3 and 7.7 times, respectively, in the culture, and the degree of fragmentation of DNA - by 5.0 times. In this case, the activity of SOD and catalase decreased by 6 hours of observation by 2.8 and 4.7 times, respectively. Pre-incubation with CeO₂ at all used concentrations resulted in a significant decrease in prooxidant indices in irradiated culture. Herewith, the most corrective effect was provided by the concentration of 3.44 µg/L – for 6th hour of cultivation after irradiation, the concentration of ROS and LP were respectively 40% and 27% less, and the activities of SOD and catalase were 64% and 123% higher than in irradiated culture without nanoparticles. At the same time, regardless of the concentration of nanoparticles, the degree of DNA fragmentation remained at the level of irradiated cells, which apparently is either a consequence of long-term effects of ROS overproduction or the inability to fix defects by cell repair systems over the past observation time. Thus, spherical nanoparticles CeO₂ (D=1-2 nm) at a concentration of 3.44 µg/L can be used in cell therapy of skin lesions to normalize the prooxidant-antioxidant balance.

Acknowledgements. The authors express their gratitude to the chief doctor Vasiliev L. Ya., to the doctor-radiologist Vasilyev L. L., and to the doctor-roentgenologist Trofimov A. V. for the consultations and for the provided technical possibility of irradiation on the basis of Grigoriev Institute for medical Radiology NAMS of Ukraine.

ВПЛИВ БІОЛОГІЧНО АКТИВНИХ ПРОДУКТІВ КУЛЬТУРИ МАНТІЙНИХ ГЛІОЦИТІВ НА МОРФОЛОГІЧНІ ПОКАЗНИКИ СЕЧОВОГО МІХУРА ЩУРІВ З ІНФРАВЕЗІКАЛЬНОЮ ОБСТРУКЦІЄЮ

Глоба В.Ю., Бондаренко Т.П., Легач Є.І.

Інститут проблем кріобіології і кріомедицини НАН України,

вул. Переяславська, 23, 61016, м. Харків, Україна

E-mail: globa.1978@gmail.com

Інфравезікальна обструкція (ІВО) викликає стійке перевантаження уродинаміки, що в кінцевому підсумку призводить до розвитку гіпертрофії сечового міхура (СМ) і його дисфункції [Aikawa K. et al., 2012]. Сьогодні розробляються новітні методи регенеративної медицини для лікування незворотних ушкоджень нижніх сечових шляхів [Imamura T. et al., 2013]. Нейротрофічні фактори, які виробляють похідні глії, можуть сприяти процесам диференціювання нейронів, виживання і поширення аксонів нервових клітин [Hansebout C. et al., 2012]. Метою представленої роботи було вивчення морфологічних показників СМ щурів з ІВО після введення кондиціонованого середовища, отриманого від нативної та кріоконсервованої культур мантійних гліоцитів (МГ) і базового середовища культивування.

Культуру МГ отримували з спінальних гангліїв неонатальних поросят. Клітини висівали в концентрації 5×10^5 кл / мл на чашки Петрі (Orange Scientific, Бельгія) і культивували протягом 20 діб при 37°C , 5% CO_2 . Частину культури МГ кріоконсервували з додаванням 10% ДМСО зі швидкістю охолодження 1°C за хвилину до -40°C з подальшим зануренням в рідкий азот. Після відігріву і відмивання від ДМСО, клітини культивували по вищеописаній методиці. Базове середовище культивування містило α -МЕМ, 10% ФТС (BioSera, Франція) і антибіотики. Середовище від 15-20 діб культивування збирали в стерильні пробірки і аліквотували по 0,2 мл. ІВО моделювали на білих безпородних щурах-самицях (6 міс.) лігатурним методом. Після зняття лігатури через 1,5 міс тваринам внутрішньоочередивно вводили по 0,2 мл біологічно активних речовин на протязі 10 днів. Потім тварин виводили з експерименту. При морфометричному аналізі оцінювали товщину перехідного епітелію, товщина м'язового шару, загальну товщину стінки СМ, індекс співвідношення м'язовий шар/стінка на гістологічних препаратах. Експериментальні групи: 1-введення базового середовища культивування, 2-введення кондиціонованого середовища від нативних МГ, 3- введення кондиціонованого середовища від кріоконсервованих МГ, 4-без лікування, 5-інтактний контроль.

В 1-й, 2-й, 3-й групах вимірювання перехідного епітелію СМ показали його збільшення на 41,3%, 94% та 48,6%, відповідно, в порівнянні з інтактним контролем і 4-ю групою. При морфометрії товщина м'язового шару СМ збільшилася у всіх групах на 65,8%, 61,2%, 52,8% і 83,2%, відповідно, в порівнянні з контрольною групою. Цікаво, що в 2-й і 3-й групах спостерігалось найбільш наближені значення цього показнику у відношенні до контролю.

Результати експерименту виявили, що біологічно активні продукти культури МГ здатні чинити вплив на морфометричні показники, особливо на проліферацію перехідного епітелію та вираженість гіпертрофії СМ на тлі ІВО.

Aikawa K., et al. LUTS: Lower Urinary Tract Symptoms 2012, 4: 81–86.

Imamura T., et al. In book: Regenerative medicine and tissue engineering, 2013, 411-412.

Hansebout CR., et al. Neural Regen Res. 2012; 7(28):2165-217.

**ФУНКЦІОНУВАННЯ СИСТЕМИ ОКСИДУ АЗОТУ
В ЛІМФОЦИТАХ КРОВІ ПРИ АЗОСПЕРМІЇ ЧОЛОВІКІВ**

Воробець М.З., Луцик М.М., Борис Ю.Б.

*Львівський національний медичний університет ім. Данила Галицького,
м. Львів, Україна*

E-mail: vorobetsm@i.ua

Патологічні процеси, що відбуваються у сім'яниках і придаткових залозах, призводять до порушення сперматогенезу, зміни структури та форми сперматозоїдів, що знижує їх рухливість і запліднювальну здатність, чи, навіть, відсутності сперматогенезу внаслідок деструктивних змін у структурі сперматогенного епітелію сім'яних каналців. Азооспермія – форма чоловічого непліддя, обумовлена відсутністю сперматозоїдів в еякуляті. На її частку припадає 10-20 % всіх форм непліддя чоловіків. Необструктивна форма азооспермії полягає в порушенні процесу сперматогенезу в сім'яних каналцях яєчок. Оскільки вивчати регуляторні та сигнальні системи в клітинах біоптатів яєчок вкрай складно із-за відсутності достатньої кількості матеріалу, метою даної роботи було з'ясувати стан аргіназо-NO-синтазної системи в лімфоцитах крові чоловіків із необструктивною формою азооспермії. Відомо, що лімфоцити крові є «метаболічним дзеркалом» організму і швидко реагують на всі патологічні впливи.

У дослідженнях використовували лімфоцити крові чоловіків віком 20–44 роки, які проходили обстеження на кафедрі урології Львівського національного медичного університету ім. Данила Галицького, і у яких був верифікований діагноз «необструктивна азооспермія». Аналіз гістологічних змін у біоптатах яєчок показав набряк або фіброз строми яєчка, деструктивні зміни тестостерон-продукуючих клітин, порушення структури синцитіальних комплексів сперматогенного епітелію та повну відсутність процесу сперматогенезу в окремих звивистих сім'яних каналцях, витончення стінки звивистих сім'яних каналців, відсутність контактів між суспендоцитами (порушення структури гемато-тестикулярного бар'єру), у просвітах судин еритроцитарні складжі (порушення мікроциркуляції крові), інфільтрацію лімфоцитами строми яєчка та, навіть, наявність у деяких пацієнтів спор дріжджового грибка у стромі та просвітах каналців.

Встановлено зниження активності аргінази у лімфоцитах неплідних чоловіків з азооспермією у 1,6 раза, щодо її активності у клінічно здорових чоловіків із нормозооспермією. З'ясовано, що у лімфоцитах крові неплідних чоловіків з азооспермією активність ендотеліальної ізоформи NO-синтази нижча, ніж у чоловіків із нормозооспермією в 1,8 раза. Виявлено підвищення активності індукцйбельної ізоформи NO-синтази у лімфоцитах неплідних чоловіків у 12,6 рази щодо чоловіків із нормозооспермією.

Таким чином, отримані результати перш за все засвідчують порушення структури сперматогенного епітелію сім'яних каналців яєчок та, опосередковано свідчать про важливу роль системи оксиду азоту в порушенні процесу сперматогенезу при необструктивній формі азооспермії.

ВПЛИВ ВІКОВОГО СТАТУСУ ЕРИТРОЦИТІВ ЩУРІВ НА ЇХ ЧУТЛИВІСТЬ ДО ГІПЕРТОНІЧНОГО СТРЕСУ

Лілія Коба¹, Олена Ніпот², Ольга Шапкіна², Катерина Семіонова², Ася Жуйкова¹

1 – Харківський національний університет ім. В.Н. Каразіна, площа Свободи 4, м. Харків, 61022, Україна

2 – Інститут проблем кріобіології і кріомедицини НАН України, вул. Переяславська 23, м. Харків, 61016, Україна

E-mail: nipotel71@gmail.com

Відомо, що фізіологічні зміни під час старіння зумовлюють поступове порушення фізико-хімічних властивостей і метаболізму еритроцитів. Це зменшує здатність еритроцитів до деформації, що впливає на постачання кисню до всіх тканин та органів. Але ці зміни можуть носити неявний характер і не виявляються звичайними рутинними методами. У цьому випадку дія екстремального фактора на еритроцити *in vitro* дозволить оцінити їх адаптивний потенціал і виявити ймовірні структурно-функціональні зміни. Стійкість еритроцитів людини та ссавців до нефізіологічних осмотичних та температурних умов середовища залежить від стану структурно-функціонального комплексу цитоскелету та плазматичної мембрани. Суттєву роль у цьому відіграють білки, які визначають взаємозв'язки між компонентами цієї структури. Модифікація цитоскелет-мембранного комплексу може виявити компоненти, які відповідальні за вікові зміни на клітинному рівні.

У роботі були досліджені вікові особливості чутливості еритроцитів 1- та 12-місячних щурів до гіпертонічних умов середовища після їх температурної модифікації при +49 °С та/чи витримці у розчинах сахарози. Отримані дані показали, що температурна модифікація клітин підвищує їх чутливість до дії гіпертонічного стресу. При цьому більш чутливими є еритроцити 1-місячних щурів. Попередня інкубація в гіпертонічній сахарозі від 0,4 до 0,8 М значно збільшує чутливість до гіпертонічного шоку як 1-місячних так і 12-місячних щурів. Збільшення часу інкубації в сахарозі також підвищує чутливість еритроцитів обох вікових груп до дії гіпертонічного розчину, у цьому випадку більш чутливими також виявилися клітини 1-місячних тварин. Припускається, що денатурація анкірину при температурі 49 °С призводить до часткового відкріплення спектринів від мембрани і підвищує чутливість еритроцитів 1-місячних щурів до гіпертонічного стресу. Показано, що для нативних клітин ступінь сенсibilізації еритроцитів щурів різного віку до гіпертонічного впливу практично не залежить попередньої інкубації в розчинах сахарози, в той час як після попередньої температурної модифікації еритроцитів при 49 °С, еритроцити 1-місячних щурів стають чутливішими. Отже, низька іонна сила однаково впливає на клітини щурів різного віку, що демонструє повну сформованість комплексу спектрин-актин у 1-місячних щурів. Усунення шляхом термоденатурації зв'язку спектрин-анкірін призводить до підвищення чутливості тільки 1 місячних тварин. Це вказує на наявність додаткових зв'язків в еритроцитах дорослих тварин які з'являються як заміна частини зв'язків спектрин-анкірін в процесі подорослішання організму. Таким чином, осмотична стійкість клітин еритроцитарної популяції щурів на ранніх етапах онтогенезу визначається станом структурно-функціонального комплексу спектрин-анкірін, а зрілі клітини відрізняються більш різноманітним комплексом зв'язків плазматична мембрана-цитоскелет, що зумовлює їх стійкість до обраних модифікацій.

РЕАКЦІЯ АРГІНАЗО-НО-СИНТАЗНОЇ СИСТЕМИ ЛІМФОЦИТІВ КРОВІ НА ДІЮ АНТИБІОТИКІВ ФТОРХІНОЛОНОВОГО РЯДУ

Коваленко І.В., Онуфрович О.К., Корчинська О.С., Корнійчук О.П.
Львівський національний медичний університет ім. Данила Галицького,
Львів, Україна
E-mail: iryana0012@gmail.com

Для лікування важких хворих із гнійно-запальними захворюваннями, зокрема спричинених золотистим стафілококом, широко використовують такі фторхінолони як ципрофлоксацин (II покоління), левофлоксацин (III покоління), моксіфлоксацин (IV покоління) та інші. Вони мають специфічний механізм дії на бактеріальні клітини – інгібують ДНК-гіразу переважно грамнегативних і топоізомеразу IV грампозитивних бактеріальних клітин, що призводить до зниження активності цих ензимів, порушення біосинтезу ДНК та РНК, та унеможливлення суперспіралізації хромомом. Цей механізм дії принципово відрізняється від такого антибіотиків інших класів, що зумовлює відсутність перехресної резистентності між фторхінолонами та іншими протибактеріальними засобами. Оскільки фторхінолони володіють гідрофільними та ліпофільними властивостями, при будь-якому їх застосуванні вони попадають у кров де спричиняють різноманітні біохімічні ефекти. У цьому плані біологічна дія фторхінолонів дуже мало досліджена.

Метою даної роботи було дослідження впливу ряду фторхінолонів на активність ензимів системи аргіназа/NO-синтаза в лімфоцитах периферичної крові. Показано, що у всьому діапазоні досліджуваних концентрацій *L*-аргініну активність аргінази лімфоцитів крові при дії фторхінолонів була підвищена у порівнянні з такою величиною в контрольній групі. Активність зростала в ряді: контроль → ципрофлоксацин → левофлоксацин → моксіфлоксацин. Найвища активність спостерігається при дії моксіфлоксацину, який належить до IV покоління. Розрахунок кінетичних параметрів активності аргінази свідчить про те, що максимальна швидкість (V_{max}) метаболізму *L*-аргініну сапонін-пермеабілізованими лімфоцитами крові при дії ципрофлоксацину у 1,4, левофлоксацину в 1,6, а моксіфлоксацину в 1,9 рази вищі щодо контрольних значень. Оскільки *L*-аргінін є субстратом не тільки для аргінази, але й для всіх ізоформ NO-синтази, наступним етапом роботи було вивчення активності окремих ізоформ NO-синтази та їх кінетичних особливостей при дії фторхінолонів. В результаті проведених досліджень встановлено, що активність cNOS лімфоцитів крові практично здорових жінок становить $(71,4 \pm 6,9)$ нмоль NADPH(H^+)/хв на 1 мг протеїну. При дії досліджуваних фторхінолонів у концентрації 10^{-5} М, ципрофлоксацин зумовлює зниження активності cNOS в 1,9, левофлоксацин – у 3,0 та моксіфлоксацин – у 5,4 рази щодо групи контролю. Можна припустити, що таке суттєве зниження активності cNOS в лімфоцитах крові може бути маркером на дію фторхінолонів. При вивченні впливу фторхінолонів на активність iNOS лімфоцитів крові практично здорових жінок, її активації ми не спостерігали, а інгібуючий ефект неможливо було визначити через низьку активність. Для індукування активності iNOS в лімфоцитах крові використовували оксидативний стрес, преінкубуючи лімоцити з H_2O_2 . Преінкубація лімфоцитів із 0,2 мМ H_2O_2 призводить до зростання активності iNOS в 31,3 рази. На фоні активації iNOS гідроген пероксидом, ципрофлоксацин призводить до інгібування активності ензиму в 1,2 рази, левофлоксацин – у 1,4, а моксіфлоксацин – у 2,3 рази. Отже, за умов впливу фторхінолонів на лімфоцити крові порушується співвідношення NO-синтазного та аргіназного шляхів метаболізму *L*-аргініну, що свідчить про дисметаболичні зміни в системі синтезу NO.

ПОРІВНЯННЯ МЕТОДІВ RFFIT ТА MNT ПРИ КОНТРОЛІ ТИТРІВ АНТИРАБІЧНИХ АНТИТІЛ В ПРЕПАРАТІ АНТИРАБІЧНОГО ІМУНОГЛОБУЛІНУ

Варяниця В.В.^{1,2}, Новікова О.Ю.^{1,2}

1 – ПАТ «ФАРМСТАНДАРТ-БІОЛІК», Помірки, м. Харків, Україна, 61070

2 – Інститут проблем кріобіології і кріомедицини НАН України, вул. Переяславська, 23, м. Харків, Україна, 61016

E-mail: ksuhanew7@gmail.com

За даними ВООЗ, сказ на сьогодні залишається однією з найнебезпечніших хвороб у світі, спільних для людини і тварин, що наносять найбільший соціально-економічний збиток. Ця хвороба має 100% летальність після прояву клінічних ознак. За даними ВООЗ за 2018 рік, щорічно від сказу гине 59 000 людей. Активні осередки сказу, рівень захворюваності в яких зріс за останній рік, існують в ряді областей України, щорічно з приводу укусів тварин звертається близько 110 000 чоловік. Наприкінці 2018 року були проведені профілактичні заходи щодо пероральній імунізації диких тварин.

Основними методами боротьби зі сказом є профілактична та лікувально-профілактична імунізація людей та домашніх тварин за допомогою антирабічних вакцин та імуноглобулінів, а також пероральна імунізація диких тварин. У зв'язку з цим нагальною є проблема розробки та контролю якості специфічних засобів антирабічного захисту (антирабічна вакцина та імуноглобулін). На сьогоднішній день для контролю активності антирабічних препаратів окрім тестів *in vivo* з використанням білих лабораторних мишей Європейською Фармакопеею рекомендовано застосування серологічного методу спільно з використанням у якості моделі культури клітин (*in vitro*) – RFFIT (rabies fast focus inhibition test).

ПАТ «Фармстандарт-Біолік» – виробник гетерологічного препарату «Імуноглобулін антирабічний (кінський)», який є єдиним вітчизняним препаратом антитіл, що використовується для постекспозиційного антирабічного захисту. З метою валідації та модернізації контролю сировини та продукту впроваджуються нові методи визначення титрів антирабічних антитіл у крові коней-продуцентів та в готовому препараті антирабічного імуноглобуліну. Метою нашого дослідження було порівняння традиційного тесту *in vivo* – реакції нейтралізації антитіл на моделі білих лабораторних мишей (MNT – mouse neutralization test), що застосовувався раніше, з тестом RFFIT. За допомогою цих тестів були досліджені титри антирабічних антитіл 3-х серій антирабічного імуноглобуліну. Результати були подані в міжнародних одиницях на мл (МО/мл), для перерахунку щоразу послуговувались порівнянням ED₅₀ досліджуваних зразків з ED₅₀ Європейського Фармакопейного Стандарту антирабічного імуноглобуліну людини (EDQM). Для реакції нейтралізації використовувався вірус штаму CVS (Challenge standard virus), культивованій на перешеплюваній культурі клітин ВНК-21 (клітини нирки новонародженого сирійського хом'яка).

В ході дослідження 3-х серій імуноглобуліну антирабічного у 3-х повторах була підрахована внутрішньогрупова варіативність даних у кожному з тестів, для MNT цей показник склав 21,24%, для RFFIT – 17,98%. Було встановлено наявність прямого позитивного кореляційного зв'язку між даними в титрах антитіл, отриманими за двома методами. Фактичне значення коефіцієнту кореляції становило 0,90, дані є статистично значущими (p=0,01).

Таким чином, тест на культурі клітин має ряд переваг перед дослідженням *in vivo*. Методичні переваги полягають в тому, що час отримання результатів за тестом RFFIT становить 48 годин, тоді як при використанні MNT – 2 тижні. Різниця у варіативності даних свідчить про більшу відтворюваність тесту *in vitro*. Та найвагомішою перевагою методу RFFIT є відмова від використання лабораторних тварин, що відповідає умовам Європейської конвенції про захист хребетних тварин, що використовуються для дослідних та інших наукових цілей (Страсбург, 1986). Тому реакція RFFIT може бути використана в якості альтернативи MNT для контролю специфічної активності препарату «Імуноглобулін антирабічний (кінський)».

ВИВЧЕННЯ ВПЛИВУ ПРОПІЛТІОУРАЦИЛУ НА МОРФОЛОГІЧНІ ХАРАКТЕРИСТИКИ ФОЛІКУЛЯРНОГО ЕПІТЕЛІЮ ЩИТОВИДНОЇ ЗАЛОЗИ ЩУРІВ ЛІНІЇ SHR

Побеленський К.О.¹, Легач Є.І.¹, Побеленський О.М.², Побеленська Л.А.², Бондаренко Т.П.¹

1 – Інститут проблем кріобіології і кріомедицини НАН України, вул. Переяславська, 23, м. Харків, Україна, 61016

2 – Харківський національний університет імені В. Н. Каразіна, площа Свободи, 4, м. Харків, Україна, 61022

E-mail: pobelensky@gmail.com

Згідно статистики поширеність вузлових утворень щитовидної залози (ЩЗ) різної етіології, які виявляються методом ультразвукової діагностики, перевищує 50%. Поряд з цим, зростає група пацієнтів, які мають патології ЩЗ на фоні серцево-судинних захворювань. Враховуючи це, актуальним є вивчення патоморфологічних показників формування вузлового зобу на моделях, які відтворюють найбільш поширені серцево-судинні патології. Таку можливість надає використання спеціальних ліній лабораторних тварин, зокрема спонтанно-гіпертензивних щурів лінії SHR. Вважається, що за динамікою підвищення артеріального тиску, наявністю характерних морфологічних змін в серці та кровоносних судинах перебіг артеріальної гіпертензії у цих тварин відповідає ознакам гіпертонічної хвороби у людини (Conrad et al., 1995). З іншого боку, загальноприйнятим є моделювання гіперплазії та вузлових утворень ЩЗ у щурів шляхом введення пропілтіоурацилу (Polychronakos et al., 1989), однак досі не було вивчено особливостей розвитку цього процесу у щурів лінії SHR. Мета роботи – вивчення впливу пропілтіоурацилу (ПТУ) на морфологічні характеристики фолікулярного епітелію ЩЗ щурів лінії SHR на різних термінах.

Експеримент проводили на самках щурів лінії SHR віком 6 місяців та вагою 250–280 г. Тваринам давали розчин 0,1% ПТУ у питній воді на протязі всього експерименту, при цьому спонтанна загибель тварин склала 45%. В якості контролю використовували інтактних щурів. Тварин забивали на 17, 25, 31, 39 та 47 добу. Тканину ЩЗ піддавали фіксації у формаліні, гістологічній проводці та забарвленню гематоксиліном/еозином за стандартною методикою. Мікрофотозйомку здійснювали за допомогою світлооптичного мікроскопу AmScope XYL–403 з камерою. Вимірювання висоти епітелію фолікулів ЩЗ на мікрофотографіях серійних зрізів проводили з використанням програми AxioVision Rel. 4.8.

Мікрофотографії зрізів тканини ЩЗ у контролі демонстрували фолікули різних розмірів, які були вистелені кубічним епітелієм та містили вакуолізований ацидофільний колоїд. Середня висота епітелію фолікулів дорівнювала $5,5 \pm 2,1$ мкм. Фолікули ЩЗ тварин експериментальної групи були вистелені кубічними або циліндричним епітелієм, деякі з них мали більш ніж один шар клітин, завдяки чому ці ділянки тканини характеризувалися ущільненим фолікулярним малюнком. Висота епітелію в цій групі значуще зростала в порівнянні з контролем. На 17 добу вона дорівнювала $12,36 \pm 2,4$ мкм, на 25 – $12,5 \pm 2,9$ мкм, на 31 – $18,38 \pm 2,2$, на 39 – $14,9 \pm 1,8$, на 47 – $13,9 \pm 3,1$ мкм. Таким чином, при введенні 0,1% розчину ПТУ у раціон щурів лінії SHR морфологічні ознаки розвитку гіперплазії ЩЗ спостерігаються вже починаючи з третього тижня.

1. Conrad C.H. et al., Circulation. 1995, 91 (1): 161–170.
2. Polychronakos C. et al., Endocrinology. 1989, 124 (1):505–510.

**КОНТРОЛЬ ЕФЕКТИВНОСТІ ЛІКУВАННЯ ГОСТРИХ РЕСПІРАТОРНИХ
ЗАХВОРЮВАНЬ, УСКЛАДНЕНИХ ОБСТРУКТИВНИМ СИНДРОМОМ З
ДОПОМОГОЮ ЦИТОМОРФОЛОГІЧНИХ ДОСЛІДЖЕНЬ**

Ракша-Слюсарєва О.А., Тарасова І.А., Слюсарєв О.А.

*Донецький національний медичний університет, вул. Привокзальна, 27, м. Лиман, Україна
ДУ «Інститут епідеміології та інфекційних хвороб ім. Л.В. Громашевського НАМН України»
вул. М. Амосова 5, м. Київ, Україна*

E-mail: rakshaslusareva@gmail.com

Гострі респіраторні захворювання (ГРЗ), що ускладнюються обструктивним синдромом (ОС), є все більш частою патологією дітей раннього віку. Лікування цих захворювань не є досконалою, так як не попереджає часті повторні епізоди ОС. Проведені нами попередні дослідження дали можливість уточнити деякі особливості патогенезу й розробити нову схему лікування ГРЗ ОС. Метою роботи була оцінка ефективності лікування ГРЗ ОС за різними схемами за критерієм цитоморфологічних змін лейкоцитів периферичної крові.

Дослідження проводили у дітей з діагнозом ГРЗ ОС. 23 особи дітей контрольної групи (КГ) отримували лікування за традиційною схемою: небулайзерну терапію, індивідуально підібрані муколітичні, бронхолітичні, спазмолітичні, протиалергічні лікарські речовини, а 27 дітей основної групи (ОГ) додатково до базової схеми отримувала курс індивідуально підбраного антибіотика і комплекс вітамінів А, Е, С. До та після курсу терапії у дітей досліджували цитоморфологічні особливості пулів лейкоцитів периферичної крові.

До лікування у 100% КГ та ОГ реєструвались такі показники токсико-запалювального процесу й підвищеної пероксидації, як: збільшення клітинних розпадів (КР), підвищення вмісту нейтрофілів з фрагментацією ядра (ФЯ), набуханням хроматином ядра (НХЯ), клітин з токсогенною зернистістю цитоплазми (ТЗН), а також реєструвались клітини з ворсинчатістю хроматину, що віддзеркалюють, з одного боку наявність мутагенного процесу, а з іншого свідчать про зниження контролю генетичного гомеостазу організму з боку системи імунітету.

Після проведення терапії в ОГ знизилась кількість КР, вміст ФЯ, НХЯ та ТЗЦ, відповідно з: $34,8 \pm 6,8\%$, $21,6 \pm 4,7\%$, $51,2 \pm 4,5\%$ і $70,2 \pm 6,3\%$ до, відповідно: $14,6 \pm 4,6\%$, $11,5 \pm 3,1\%$, $43,0 \pm 1,7\%$ і $35,9 \pm 5,4\%$. На противагу цьому, в КГ підвищились кількість КР, вміст ФЯ, НХЯ та ТЗЦ, відповідно: з $30,1 \pm 2,4\%$, $41,4 \pm 4,9\%$, $39,9 \pm 2,8\%$ і $27,2 \pm 4,4\%$ до відповідно: $46,7 \pm 3,7\%$, $46,2 \pm 11,1\%$, $67,6 \pm 8,0\%$ і $67,6 \pm 8,0\%$.

Таким чином, за проведеними дослідженнями цитоморфологічних змін нейтрофілів і лімфоцитів периферичної крові, найбільш ефективною схемою лікування гострих респіраторних захворювань, ускладнених обструктивним синдромом є нова, яка включає, крім традиційної терапії, курс індивідуально підібраних антибіотиків і комплексного препарату водорозчинних вітамінів А, Е, С – «V- каротин».

РОЛЬ ГЛІЦЕРИНУ В РОЗВИТКУ ПОСТГІПЕРТОНІЧНОГО ЛІЗИСУ ЕРИТРОЦИТІВ ЛЮДИНИ

*Олена Чабаненко, Наталя Єршова, Наталія Орлова, Наталія Шпакова
Інститут проблем кріобіології і кріомедицини Національної академії наук України,
вул. Переяславська, 23, Харків, Україна, 61016*

E-mail: chabanenkoolena@gmail.com

Метод низькотемпературного консервування еритроцитів під захистом гліцерину дозволяє зберігати клітини протягом тривалого часу. При розморожуванні біологічного матеріалу ряд факторів кріопшкоджень діє на клітини. Серед них особлива роль приділяється зміні концентрації солі та гліцерину. Для дослідження впливу зміни концентрації солі на еритроцити використовують модель постгіпертонічного шоку, при якій клітини після інкубування в середовищі дегідратації переносять в середовище регідратації.

Мета – дослідити постгіпертонічний лізис еритроцитів людини при варіюванні концентрацій солі (NaCl) і кріопротектора (гліцерин) при незмінній осмоляльності середовища дегідратації при 37°C.

Вихідну суспензію еритроцитів отримували шляхом додавання осаду еритроцитів до фізіологічного розчину (0,15 моль/л NaCl) в співвідношенні 1:1. Постгіпертонічний шок еритроцитів здійснювали наступним чином. Аліквоту суспензії клітин додавали в середовище, що містить 1,2 моль/л NaCl і інкубували 20 хв (етап дегідратації). Після чого клітини переносили до фізіологічного розчину (етап регідратації) на 5 хв. Рівень гемолізу еритроцитів визначали методом спектрофотометрії при довжині хвилі 543 нм.

В роботі варіювали умови проведення етапу дегідратації. У першій постановці використовували комбіновані середовища, в яких змінювали зміст гліцерину (2-15%) і NaCl так, щоб зберігалася сумарна осмоляльність розчину близько 2370 мосм/л (що відповідає осмоляльності 1,2 моль/л NaCl). У другій постановці фізіологічний розчин містив гліцерин в різних концентраціях (2-15%). У вищеописаних розчинах (етап дегідратації) інкубували клітини, після чого їх переносили в фізіологічний розчин (етап регідратації).

Рівень постгіпертонічного лізису (ПГЛ) еритроцитів, перенесених з 1,2 моль/л в 0,15 моль/л NaCl, становив близько 10%. Присутність гліцерину в діапазоні концентрацій 2 -10% (при відповідній зміні вмісту NaCl 1,06 - 0,50 моль/л) практично не викликає зміни рівня ПГЛ еритроцитів. Підвищення концентрації гліцерину до 11-15% (при відповідному зменшенні концентрації солі) призводило до різкого збільшення рівня ПГЛ еритроцитів від 80 до 100%.

У разі, коли клітини переносили в фізіологічний розчин з середовища, що містить 0,15 моль/л NaCl і різні концентрації гліцерину, спостерігалось різке збільшення рівня гемолізу клітин при концентраціях кріопротектора, що перевищують 2%, і практично повне їх пошкодження при використанні гліцерину в концентрації 7 %.

Отримані дані свідчать про те, що рівень ПГЛ еритроцитів не залежить від загальної осмоляльності середовища дегідратації, а визначається концентрацією гліцерину. Таким чином, виходячи з того, що рівень ПГЛ еритроцитів значно нижче в комбінованих середовищах, можна припустити, що присутність в середовищі сольового компонента (вище фізіологічного рівня) впливає на проникність еритроцитарних мембран для гліцерину.

Session 2: Cell response on stress / Відповідь клітини на стрес

ЦИТОТОКСИЧНА ДІЯ НАНОЧАСТИНОК В КУЛЬТУРІ КЛІТИН СНЕВ

Дерев'яно С.В., Решотько Л.М.

Інститут сільськогосподарської мікробіології та агропромислового виробництва НААН
вул. Шевченка, 97, Чернігів, 14027, Україна

E-mail: biopreparat@i.ua

Наночастинки (НЧ) та нанотехнології все частіше використовують у різних галузях народного господарства. Сучасні наукові досягнення у галузі нанотехнологій відкривають широкі перспективи для виробництва та використання НЧ, що можуть існувати у формах оксидів, гідроксидів, колоїдних сполук, гідратованих чи цитратованих формах (наноаквахелатів). Зокрема, на їх основі розробляють медичні та ветеринарні антивірусні препарати.

Відомо, що деякі НЧ володіють антивірусними властивостями, здатні блокувати активні центри клітин і тим самим запобігати проникненню в них вірусів. З іншої сторони, НЧ здатні зв'язувати антиген, що призводить до направленої поглинання його макрофагами. Окрім антивірусних властивостей НЧ можуть впливати на антиоксидантний стан, репарацію генів клітин господаря та мають цитотоксичний ефект, що обмежує їх використання в якості противірусних препаратів. Тому, метою наших досліджень було вивчити цитотоксичну дію НЧ в перещеплюваній культурі клітин нирки ембріону свині (СНЕВ) та встановити максимально допустиму концентрацію (МДК) НЧ для подальших вірусологічних досліджень.

В дослідях використовували культуру клітин СНЕВ, цитрати наночастинок Ti, Ce, Zn, Al, Co, V, Ni, CeO₂, композицій НЧ Se та I, S та I, НЧ Al₂O₃, SiO₂ і бентоніту. Пітримання культури клітин СНЕВ проводили за використання загальноприйнятих методів. У сформований моношар клітин вносили поживне середовище, що містить досліджувану речовину в різних концентраціях. Ми використовували двократні розведення НЧ у таких концентраціях: 400, 200, 100, 50, 25 мкг/см³ і так далі. На кожену концентрацію використовували по 4 пробірки з культурою клітин. У контролях також проводили заміну поживного середовища але без досліджуваних речовини. Облік результатів проводили щодоби впродовж 7 діб. Моношар клітин досліджували в оптичному мікроскопі на наявність цитотоксичної дії речовини.

У результаті проведених дослідів встановлено МДК наночастинок для перещеплюваної культури клітин СНЕВ. Так, МДК для бентоніту становить 500 мкг/см³, Al₂O₃, та CeO₂ – 100 мкг/см³, Ni – 50 мкг/см³, V – 25 мкг/см³, Al – 20 мкг/см³, Ti – 12,5 мкг/см³, I+S, Zn та Co – 5 мкг/см³, Se+I – 0,5 мкг/см³. За більш високої концентрації НЧ спостерігали порушення цілісності моношару, округлення та зморщування клітин, появу вогнищ дегенерованих клітин, вакуолізацію, зернистість, підвищення, розпластанність клітин (рис. 1). Для подальшого вивчення антивірусної активності в культурі клітин СНЕВ НЧ будуть використані у максимально допустимій концентрації.

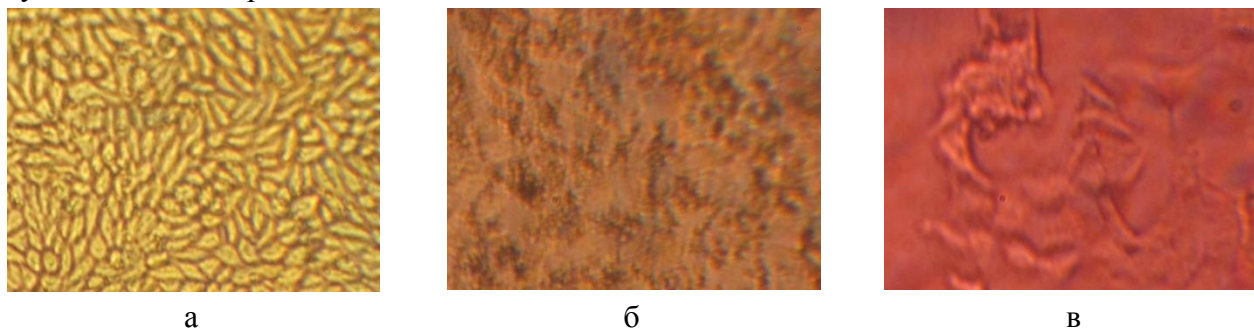


Рис. 1. Цитотоксична дія наночастинок в культурі клітин СНЕВ: а – культура клітин СНЕВ не інокульована НЧ; б та в – дегенеративні зміни в культурі клітин СНЕВ за дії НЧ.

Session 3

Cellular, genetic and metabolic engineering

*June 18-21, 2019,
Yaremche*

RECOMBINANT ENZYMES, HUMAN ARGINASE I AND MICROBIAL ARGININE DEIMINASE, AS THE BIORECOGNITION ELEMENTS FOR ARGININE-SELECTIVE AMPEROMETRIC BIOSENSORS AND ENZYMATIC

Mykhailo Gonchar

Lecture 1

Mykhailo Gonchar

Department of Analytical Biotechnology, Institute of Cell Biology, National Academy of Sciences of Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine

E-mail: gonchar@cellbiol.lviv.ua; mykhailo1952@gmail.com

L-Arginine, a semi-essential amino acid, plays an important role in cellular metabolism. It is dispensable in healthy individuals as it may be formed from other amino acids. However, for ill or injured people or for individuals in specific physiological state (pregnancy, intensive work at extreme conditions, sport activity), higher quantities of L-arginine are required. On the other hand, certain cancers may be auxotrophic for a particular amino acid, and amino acid deprivation is one of the approaches used to fight with these tumours. It has been suggested that arginine-degrading enzymes may be effective in the control of arginine-dependent cancers. Therefore, it is a demand to develop fast and valid methods for monitoring the level of arginine in biological liquids, drug formulations and nutrition.

We developed two amperometric biosensors for sensitive and selective detection of L-Arg using as the biorecognition elements different arginine-hydrolyzing recombinant enzymes, human arginase I (coupled with commercial urease) and arginine deiminase from *Mycoplasma hominis*. The NH_4^+ ions, generated during the enzymatic transformation of L-Arg, were monitored amperometrically using ammonium-sensing Nafion/Polyaniline composite placed at a working electrode. The bioanalytical parameters of the constructed biosensors were studied and the created devices were tested for assay of L-Arg in biological samples and some commercial pharmaceuticals.

A novel enzymatic method of manganese (II) and cobalt (II) ions assay, based on using apo-enzyme of Mn^{2+} -dependent recombinant human arginase I and 2,3-butanedione monoxime (DMO) as a chemical reagent, has been proposed. The principle of the method is the evaluation of the activity of L-arginine-hydrolyzing arginase holoenzyme after the specific binding of Mn^{2+} or Co^{2+} with apo-arginase.

The work was supported by NAS of Ukraine in the frame of the Scientific-Technical Program “Smart Sensor Devices of a New Generation Based on Modern Materials and Technologies”.

References:

1. Stasyuk N., Gayda G., Gonchar M. Novel arginine deiminase-based method to assay L-Arginine in beverages // *Food Chemistry*. – 2016. – V. 201. – P. 320-326.
2. Zhybak M.T., Fayura L.Y., Boretsky Y.R., Gonchar M.V., Sibirny A.A., Dempsey E., Turner A.P.F. Amperometric L-arginine biosensor based on a novel recombinant arginine deiminase // *Microchim. Acta*. – 2017. – V. 184, Nr 8. – P. 2679–2686.
3. Stasyuk N., Gayda G., Zakalskiy A., Zakalska O., Errachid A., Gonchar M. Highly selective apo-arginase based method for sensitive enzymatic assay of manganese (II) and cobalt (II) ions // *Spectrochim. Acta Part A: Molecular and Biomolecular Spectroscopy*. – 2018. – V. 193. – P. 349-356.

IMPORTANCE OF PEROXISOMES AND PEROXISOMAL ENZYMES OF PENTOSE PHOSPHATE PATHWAY FOR XYLOSE ALCOHOLIC FERMENTATION IN THE THERMOTOLERANT METHYLOTROPHIC YEAST *OGATAEA POLYMORPHA*

Justyna Ruchala

Lecture 2

Justyna Ruchala¹, Olena Kurylenko², Kostyantyn Dmytruk², Andriy Sibirny^{1,2}

1 – Department of Microbiology and Biotechnology, University of Rzeszow, Zelwerowicza 4, 35-601 Rzeszow, Poland

2 – Institute of Cell Biology, National Academy of Science of Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine

E-mail: jruchala@ur.edu.pl

Peroxisomes are ubiquitous organelles responsible for many reactions of oxidative metabolism, fatty acid β -oxidation, glyoxylic acid cycle, catabolism of unusual carbon sources and some biosynthetic reactions. The role of peroxisomes and peroxisomally-located enzymes in xylose metabolism and alcoholic fermentation remains unknown. We have found that peroxisomal transketolase (also known as dihydroxyacetone synthase, gene *DASI*) and transaldolase (gene *TAL2*) are important for xylose alcoholic fermentation but not growth on this substrate as deletion strains *das1 Δ* and *tal2 Δ* are well on xylose but did not produce ethanol from this pentose. In contrast to that, the mutants with deletions of genes coding for cytosolic transketolase *TKL1* and transaldolase *TAL1* did not grow on xylose though effectively fermented this sugar to ethanol. The strains of *O. polymorpha* with overexpression of all four mentioned enzymes overproduced ethanol from xylose. Deletion and overexpression of peroxisomal transketolase and transaldolase did not have an influence on glucose utilization and fermentation. Obtained results suggested on the role of peroxisomal enzymes in xylose fermentation. Therefore we decided to study if peroxisomes as organelles are important for xylose fermentation. For this, the mutants of *O. polymorpha* defective in peroxisome biogenesis *pex3 Δ* and *pex6 Δ* have been used which are known to contain only peroxisomal remnants. It was found that these mutants, similarly to *das1 Δ* and *tal2 Δ* mutants, normally grew on xylose though did not produce ethanol from this sugar at all. We also constructed for the first time the *O. polymorpha* strains with overexpression of *RKII* gene coding for ribulose-5-phosphate isomerase. The transformant with a 14-fold increase in *RKII* expression was characterized by a two-folds increase in ethanol production from xylose while glucose fermentation was unchanged. We also found that the mutants of *O. polymorpha* with overexpression of *AOX1* gene coding for alcohol oxidase, are characterized by significantly activated xylose alcoholic fermentation. As *AOX1* gene expression could control the cellular volume of peroxisomes, we suggest that peroxisomes are important organelle for active xylose alcoholic fermentation. Further studies of the role of peroxisome in xylose metabolism and fermentation are needed.

This work was supported by the grant of National Science Centre, Poland (NCN) Opus 2016/21/B/NZ1/00280

NEW APPROACHES TO IMPROVE RIBOFLAVIN PRODUCTION IN THE YEAST CANDIDA FAMATA

Daria Fedorovych

Lecture 3

Fedorovych D.V.¹, Dmytruk K.V.¹, Tsyurulnyk A.O.¹, Ruchala J.², Pavliukh K.V.¹, Sibirny A.A.^{1,2}

1 – Institute of Cell Biology, National Academy of Science of Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine

2 – Department of Microbiology and Biotechnology, University of Rzeszow, Zelwerowicza 4, 35-601 Rzeszow, Poland

E-mail: fedorovych.d@gmail.com

Flavins are manufactured to use as vitamins in human and animal nutrition, as pharmaceuticals and as a food colorant. Currently, riboflavin (RF) is produced on a large scale by microbial synthesis. Yeast *Candida famata* represents the organism with high flavinogenic potential. The mutant strains of this yeast were used in industry for RF production. However, used before microbial RF producers require increasing productivity and genetic stability. The RF overproducing strain of *C. famata* was constructed via simultaneous overexpression of the genes *SEF1*, which codes for a transcriptional activator of RF synthesis, as well as of the *RIB1* and *RIB7* genes, which encode the first and the last structural enzymes of RF synthesis, GTP-cyclohydrolase II and RF synthase, respectively. This strain accumulated a 45% increased RF titer in the flask when compared to the stable parental RF producer of *C. famata* AF-4 isolated by classical mutagenesis and selection for antimetabolite resistance. The activation of metabolic flux toward purine nucleotide biosynthesis is promising approach to improve RF production since GTP is the immediate precursor of RF synthesis. The phosphoribosyl pyrophosphate synthetase and phosphoribosyl pyrophosphate amidotransferase are the rate limiting enzymes in purine biosynthesis. Corresponding genes *PRS3* and *ADE4* from yeast *Debaryomyces hansenii* were modified to avoid feedback inhibition and co-overexpressed on the background of a previously constructed a RF overproducing strain of *C. famata*. The strain expressing both genes *PRS3m* and *ADE4m* revealed 2-fold increase in RF production when compared to the best RF producer described in our previous work.

Particular attention deserves the search for ways to increase the excretion of flavins by natural and cosntructed RF overproducing yeasts. Flavinogenic yeasts *C. famata*, *Meyerozyma guilliermondii*, *Debaryomyces hansenii* contain genes homologous to the mammal *BCRP* gene coding the protein responsible for secretion of RF from mammal breast to the milk. *BCRP* gene homolog was isolated from genome of *D. hansenii* and expressed under control of own promoter in the heterologous host, the best available flavinogenic strain of the yeast *C. famata*. Resulted transformants revealed 1.3-1.5 fold increase in RF production relative to that of parental strain.

In addition to construction of the productive strains, the design of a fermentation medium is of critical importance because medium compositions can significantly affect production, yield and volumetric productivity. The cultivation conditions for constructed strains directed to increased production of RF were optimized. We screened the different media components (in particular carbon and nitrogen sources) for the best RF production. We are also focused on extension of the substrate range to produce the vitamin B₂ from cheaper carbon sources from beer wort, hydrolysate of bagasse, **cheese** whey or lignocellulosic substrates.

This study was supported by Polish National Science Center, grant Opus UMO-2018/29/B/NZ1/01-497 and by National Academy of Sciences of Ukraine (Grant 36-19).

EVALUATION OF TRANSCRIPTIONAL FACTORS INVOLVED IN REGULATION OF XYLOSE METABOLISM AND FERMENTATION IN THE THERMOTOLERANT YEAST *OGATAEA POLYMORPHA*

Olena Kurylenko

Lecture 4

Olena Kurylenko¹, Justyna Ruchala², Roksolana Vasylyshyn¹, Kostyantyn Dmytruk¹, Andriy Sibirny^{1,2}
1 – Institute of Cell Biology, National Academy of Science of Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine
2 – Department of Biotechnology and Microbiology, University of Rzeszow, Zelwerowicza 4, 35-601 Rzeszow, Poland

E-mail: olenkakurylenko@gmail.com

Lignocellulosic biomass contains multiple fermentable sugars and represents a potentially valuable feedstock for fuels and chemicals. The pentose sugar xylose, which is the second-most abundant monosaccharide in nature following glucose, is present in significant amounts in lignocellulosic biomass hydrolysates. *Ogataea polymorpha* belongs to the most thermotolerant xylose-fermenting yeast species. As the efficiency of xylose fermentation in the *O. polymorpha* wild-type strains is very low, several targeted metabolic engineering approaches have been successfully applied for improvement of ethanol production. However, regulation of xylose metabolism in the natural xylose-assimilating yeasts is poorly understood. Therefore, identification of specific regulatory genes involved in xylose-dependent regulation of gene expression and in xylose alcoholic fermentation are of great importance.

In this work the effect of deletion or overexpression of transcriptional factors Hap4A, Hap4B, Tup1 on metabolism and fermentation of xylose and glucose was studied. The transcriptional regulator Hap4 is known to be involved in the balance between fermentation and respiration in *Saccharomyces cerevisiae*. Two putative orthologs of Hap4 protein were identified in the genome of *O. polymorpha*, named Hap4A and Hap4B. The activation of xylose alcoholic fermentation was observed in *hap4Δ* mutant whereas overexpression of *HAP4A* gene led to decreased ethanol production from xylose. The deletion or overexpression of *HAP4B* gene did not result in significant change in the amount of accumulated ethanol from xylose as compared to the wild type strain. The Tup1-Cyc8 (Ssn6) corepressor complex is required for repression of transcription in several regulatory pathways in yeast cells, including glucose repression. The knock out of *TUP1* gene in *O. polymorpha* resulted in immediate increase of ethanol production during xylose alcoholic fermentation and total inability to ferment glucose. The overexpression of *TUP1* gene under control of strong constitutive promoter or native promoter in the frame of the multicopy plasmid decreased ethanol production from xylose. The putative targets of the Tup1 in the genome of *O. polymorpha* are under investigation.

This work is supported by grant of Polish National Scientific Center (NCN) Opus UMO-2016/21/B/NZ1/00280

CRUDE GLYCEROL BIOCONVERSION TO FUEL ETHANOL BY METHYLOTROPHIC YEASTS

Marta Semkiv

Lecture 5

Marta Semkiv¹, Iwona Kata², Anastasya Zazulya¹, Kostyantyn Dmytruk¹, Andriy Sibirny^{1,2}

1 – Institute of Cell Biology, National Academy of Science of Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine

2 – Department of Biotechnology and Microbiology, University of Rzeszow, Zelwerowicza 4, 35-601 Rzeszow, Poland

E-mail: smarta0309@gmail.com

Biodiesel production is a fast-growing industry. Biodiesel is obtained through transesterification of different kinds of oils with methanol. This process results in a formation of substantial amounts (up to 10% of a total product mass) of the by-product fraction that mainly contains glycerol, but also some toxic contaminations (spent catalyst, salts after neutralization, residual methanol, methyl esters, and free fatty acids), and that is therefore called crude glycerol. Efficient utilization of this fraction is imperative to the sustainability of the biodiesel industry. Crude glycerol could be converted by yeasts to organic acids, polyols, ethanol, microbial oil, carotenoids, γ -decalactone, sophorolipids, heterologous proteins, yeast biomass etc. Our aim was to produce fuel ethanol from crude glycerol with methylotrophic yeasts *Ogataea polymorpha* or *Komagataella phaffii* (formerly *Pichia pastoris*). Methylotrophic yeast is a good candidate for crude glycerol utilization due to its ability to tolerate high methanol concentrations.

The initial steps of glycerol catabolism in yeast cells are catalyzed by glycerol dehydrogenase (Gcy1) and dihydroxyacetone kinase (Dak1) or glycerol kinase (Gut1) and glycerol-3-phosphate dehydrogenase (Gpd1). Obtained dihydroxyacetone phosphate is converted to phosphoenolpyruvate, which is then either used in TCA cycle or converted to ethanol through the action of pyruvate decarboxylase (Pdc1) and alcohol dehydrogenase (Adh1). The mechanism of glycerol transport into *O. polymorpha* cells is unclear, whereas in *K. phaffii* it is known to be imported through the channel formed by Fps1 protein.

The *O. polymorpha* strain NCYC495 was subjected to the following genetic modifications: overexpression of the genes *ADH1*, *PDC1*, pairs of genes *GCY1-DAK1* or *GPD1-GUT1* and heterologous gene *FPS1* *K. phaffii*. Obtained recombinant strains produced up to 10.2 g of ethanol/L from pure glycerol or 3.1 g of ethanol/L from crude glycerol (Semkiv et al., 2019).

Homologous or heterologous (from *Saccharomyces cerevisiae*) genes *ADH1* and *PDC1* were overexpressed in the *K. phaffii* strain GS200. Obtained recombinant strains produced up to 5 g of ethanol/L from crude glycerol. These strains will be further modified similar to *O. polymorpha* recombinant strains described above.

Semkiv M.V. et al. Yeast 2019, Mar 23. doi: 10.1002/yea.3387.

APPLICATION OF POLY-DMAEMA-CONTAINING CARRIERS FOR DELIVERY OF PLASMID DNA TO MAMMALIAN CELLS

Nataliya Finiuk

Lecture 6

Nataliya Finiuk¹, Olena Bahniuk², Olga Klyuchivska¹, Olena Paiuk³, Natalia Mitina³, Alexander Zaichenko³, Rostyslav Stoika^{1,2}

1 – Institute of Cell Biology, National Academy of Sciences of Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine

2 – Ivan Franko National University of Lviv, Hrushevskyy Street 4, 79005 Lviv, Ukraine

3 – Lviv Polytechnic National University, Bandera Street 12, 79013 Lviv, Ukraine

E-mail: nataliyafiniuk@gmail.com

Gene delivery to targeted mammalian cells is an attractive technique for treatment of hereditary diseases via changing the expression of the endogenous gene. The non-viral gene delivery systems are promising alternative to viral vectors. Cationic materials possess low immunogenicity, are not limited to a type or size of carried genetic cargo, and have a controlled chemical diversity for further functionalization.

The aim of this study was to evaluate the gene delivery potential of poly(2-dimethylaminoethyl methacrylate) {poly-DMAEMA} carriers for various tumor and non-tumor adherent cell lines.

The block-copolymers, namely poly(2,2,3,3,4,4,5,5-octafluoropentyl methacrylate (FMA)-block-poly(DMAEMA) {F8DM1; 2}, poly(butyl acrylate)-block-poly((DMAEMA) {BADM1; 2}, and poly(lauryl acrylate)-block-poly(DMAEMA) {LADM1; 2} carriers with different length of poly-DMAEMA block were used for delivery of plasmid DNA to human breast adenocarcinoma MCF-7 cells, colon carcinoma HCT116 cells, glioblastoma U-251 MG cells, hepatocellular carcinoma HepG2 cells, non-small cell lung cancer A549 cells, and embryonic kidney HEK293 cells.

Poly-DMAEMA cationic polymers electrostatically interact with negatively charged plasmid DNA. Gel retardation of plasmid DNA bands was detected when the F8DM1 and F8DM2 polymers were used at 0.01%, and BADM1, BADM 2, LADM1, LADM2 – at 0.03%. Studied polymers do not intercalate the DNA. Transfection efficiency of the polymer/pEGFP c-1 complexes measured at 48 h was 14-56% and depended on cell line. The gene transfer efficiency in HEK293 and MCF-7 cell lines was the highest. The gene delivery efficiency of poly-DMAEMA/pDNA complexes was not affected by the presence of 10% of blood serum at complex formation in the transfection medium. The detected transfection efficiency was comparable with efficiency found at using a commercial reagent, linear polyethyleneimine (PEI), however, a cytotoxic effect of the studied carriers was lower compared with the PEI. The gene delivery efficiency was increased with an elongation of poly-DMAEMA block in the carrier. DNA comet assay at alkaline conditions and diphenylamine measuring of DNA fragmentation did not reveal DNA damaging by the polymeric carriers in HEK293 and MCF-7 cells.

Thus, poly-DMAEMA cationic polymers are of interest as potential gene delivery vectors. The optimization of poly-DMAEMA carriers for improving gene transfection efficiency is in progress.

THE RECOMBINANT YEAST OGATAEA POLYMORPHA, OVERPRODUCING FLAVOCYTOCHROME B₂, AS A TOOL FOR L-LACTATE AND CHROMATE ANALYSIS, AS WELL AS FOR CHROMATE BIOREMEDIATION

Oleh Smutok

Lecture 7

Oleh Smutok¹, Taras Kavetsky^{2,3}, Mykhailo Gonchar¹

1 – Institute of Cell Biology NAS of Ukraine, 79005, Lviv, Ukraine

2 – Drohobych Ivan Franko State Pedagogical University, 82100 Drohobych, Ukraine

3 – The John Paul II Catholic University of Lublin, 20-950 Lublin, Poland

E-mail: smutok@cellbiol.lviv.ua

Lactic acid (L-lactate) is among the most important analytes, since it is a universal metabolite of nearly all living organisms. The indication of the L-lactate level is used in clinical diagnostics of hypoxia, lactic acidosis, some acute heart diseases and in drug toxicity tests, as well as for monitoring the athletic performance to evaluate the best training equipment and regimes during the training of the sportsmen. Therefore, reliable determination of L-lactate is important for many fields of human activity. Hexavalent chromium Cr(VI) is widely used in different branches of industry, so, soluble Cr(VI)-pollutants are distributed in wastewater and natural water streams. Cr(VI) is a strong oxidizing species with very toxic and carcinogenic influences on living organisms, including human.

The yeast L-lactate:cytochrome *c* oxidoreductase (flavocytochrome *b*₂, FC *b*₂) has absolute specificity for L-lactate, although is non-selective with respect to the nature of electron acceptors. Such properties allow consider this enzyme as a potential candidate for L-lactate and chromate analysis, as well as for reduction of chromate.

For construction of new microbial amperometric biosensors for L-lactate analysis, an increasing the target enzyme (FC *b*₂) in the cells is required. Two approaches for this we have used: 1) enrichment of the yeast cells by the target enzyme on genetic level – by over-expression of the corresponding *HpCYB2* gene, in the recombinant strain of *Ogataea polymorpha* «tr1» (*gcr1 catX CYB2*) which possesses a 6-fold increased FC *b*₂ enzymatic activity; 2) using nanotechnological approach – by the transfer of FC *b*₂-bound nanocarriers into the recombinant yeast cells. The yeast cells with both types of enzyme enrichment have been used for construction of highly sensitive and selective microbial biosensors for L-lactate. The Cr(VI)-selective biosensor is based on ability to catch electrons from the reduced FC *b*₂ on the surface of platinum electrode. It was clearly shown that with increasing concentration of Cr(VI) the peak of enzyme-mediated L-lactate oxidation is decreased, indicating Cr(VI)-dependent competition between reaction of chromate with reduced FC *b*₂ and direct electron transfer from the enzyme to the electrode surface.

For investigation of bioremediation ability of the recombinant *O. polymorpha* «tr1» cells, they were tested in respect of chromate reducing activity *in vitro* in the presence of L-lactate (as an electron donor for chromate reduction) and a low-molecular redox-active dye – dichlorophenolindophenol (as a final electron acceptor). The capacity of living cells for Cr(VI) bioremediation *via* the reductive pathway of recombinant yeast, over-producing FC *b*₂, was established. The tested recombinant yeast cells seem to be perspective for chromate detoxification, using as a source of cheap L-lactate dairy waters as a reductant.

The work was supported by the Ministry of Education and Science of Ukraine (project #0118U000297).

**METABOLIC ENGINEERING OF THE YEAST *SACCHAROMYCES CEREVISIAE* FOR
ENHANCEMENT OF GLYCEROL SYNTHESIS AND EXCRETION**

Volodymyr Granovski

Lecture 8

Volodymyr Granovski

*Xema, Bioprocess and Biotechnology department,
Akademica Efremova Street 23, 03179, Kyiv, Ukraine,*

E-mail: granovski.v@gmail.com

The use of recombinant DNA technologies for expression of foreign proteins in different expression hosts is not new in pharma industries and laboratories. However, still in many cases, these technologies represent a challenge, starting from basic plasmid engineering, passing through the correct target protein isolation and identification, stopping at a cost evaluation and finalizing with licensing. All these problems should be tackled in corrected ways, yet the highlight moments influencing all these barriers go back to the DNA engineering and correct plasmid development. Influencing the protein behavior, the plasmid construction must be done predicting several aspects of the host system. Some strains of *E.coli* do not process well some codons or the protein does not fit the purpose of the development. Some proteins are unfitted to be done in bacteria in the first place, making a place for yeast to perform that duty. However, yeasts plasmids are different from bacterial and must be engineered differently and yet are suited for bacteria, for amplification purposes. Here we present several different approaches to express heterologous proteins using recombinant DNA technologies for vaccine and diagnostics applications in bacteria and yeast.

YEASTS AND FUNGI AS THE TOOLS FOR GREEN SYNTHESIS OF NANOMATERIALS

Galina Gayda

Poster 1

Galina Gayda, Natalia Stasyuk, Olha Demkiv, Tetiana Prokopiv, Olga Klyuchivska, Roman Serkiz, Mariya Ivash, Mykhailo Gonchar
Institute of Cell Biology, NAS of Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine
E-mail: galina.gayda@gmail.com

Many plants, algae, microorganisms, as well as redox-imbalanced mammalian cells and systems are known to produce nanostructured mineral crystals and metallic nanoparticles (NPs) with properties similar to chemically-synthesized materials. Green synthesis processes (GSP) are rapid, eco-friendly and cost-effective. Metallic NPs obtained *via* green synthesis (gNPs) offer better manipulation, stabilization and control over crystal growth due to slower kinetics. gNPs of noble metals possess low cytotoxicity, enable easy modification of their surfaces, allow straightforward synthesis processes and have excellent biocompatibility. Such advantages make gNPs of noble and transitional metals prospective for applications in industry and medicine. gNPs are used as nanocarriers for drug delivery, biocatalyzators in biosensors and biofuel cells, as well as for bioimaging in clinical diagnostics and for bioremediation of toxic ions in the environment. A special role of gNPs is related to antimicrobial and antibiofilm agents.

The exact mechanism of gNP synthesis using biological agents has not been elucidated to date. However, it has been suggested that various biomolecules are involved in GSP. Biological agents secreted by cells are able to reduce metal ions to gain gNPs. Extracellular GSP are mediated through different enzymes, including hydrogenases, nitrate reductase, phenol-oxidizing enzymes (laccases, tyrosinases, and Mn-peroxidases), *etc.* Capping and stabilizing agents (polysaccharides, polypeptides and other bioorganic compounds) prevent the gNPs from further growth and agglomeration in resulted colloidal solution.

In our experiments, different types of NPs of noble metals were synthesized by chemical methods and via GSP. To obtain gNPs, cells of yeasts, fungi and their extracellular metabolites were applied. To study a possible toxic effect of the synthesized NPs on a living organism, the cells of the yeast *Ogataea polymorpha* were used. As a result of investigations, different variants of novel low-cost gNPs, containing Au, Ag, Pt, Pd, Cr₂O₃ etc. were obtained and characterized by UV-VIS spectroscopy, scanning electron microscopy, transmission electron microscopy, atomic force microscopy, fluorescence microscopy. On the model of yeast cell, the possibility of enzymes delivery, using biocompatible gNPs as carriers, were studied. The method of cell imaging with fluorescence gNPs was proposed. On the other hand, investigations of GSP are useful to elucidate the mechanisms of the eukaryotic cells output on stress, caused by toxic compounds, in particular, initial salts of metals and resulted NPs. It will bring new knowledge about the detailed mechanism of NPs interaction with cell: what proteins/enzymes or other biomolecules respond for the gNPs formation inside/outside the cells.

This work was financially supported by NAS of Ukraine (Project 10/3-/2019 of the Program “Smart sensor devices of a new generation based on modern materials and technologies”).

References:

- 1) Stasyuk N, Gayda G, Serkiz R, Gonchar M (2015) Cell Imaging with Fluorescent Bi-Metallic Nanoparticles. Journal of Advances in Chemistry 11: 3499-3511.
- 2) Stasyuk N, Gayda G, Serkiz R, Gonchar M (2016) The “green” synthesis of gold nanoparticles by the yeast *Hansenula polymorpha*. Visnyk of the Lviv University. Series Biology 73: 96–102.
- 3) Gayda GZ, Demkiv OM, Stasyuk NYe, et al (2019) Metallic nanoparticles obtained *via* “green” synthesis as a platform for biosensor construction. Appl Sci 9:720; doi:10.3390/app9040720.

**YEAST MELANIN AS A TEMPLATE FOR GOLD NANOPARTICLES GREEN SYNTHESIS
– PHYSICOCHEMICAL AND BIOLOGICAL CHARACTERIZATION OF AuNPs**

Małgorzata Kus-Liśkiewicz

Poster 2

Imen Ben Tahar¹, Dariusz Płoch², Andrzej Dziedzic², Patrick Fickers³, Małgorzata Kus-Liśkiewicz⁴

1 – Microbial Processes and Interactions, TERRA Teaching and Research Centre, University of Liège - Gembloux AgroBio Tech, Av de la Faculté, 2B. B-5030 Gembloux, Belgium

2 – Faculty of Mathematic and Natural Science, University of Rzeszow, Pigonia 1, 35-310 Rzeszow, Poland

3 – Faculty of Biotechnology, University of Rzeszow, Pigonia 1, 35-310 Rzeszow, Poland

E-mail: mkus@ur.edu.pl

Synthesis of nanoparticles (NPs) and their incorporation in materials are amongst the most studied topics in chemistry, physics and material science. Gold NPs have applications in medicine due to their antibacterial and anticancer activities, in biomedical imaging and diagnostic test. Despite chemical synthesis of NPs are well characterized and controlled, they rely on the utilization of harsh chemical conditions and organic solvent and generate toxic residues. Therefore, greener and more sustainable alternative methods for NPs synthesis have been developed recently. These methods use microorganisms, mainly yeast or yeast cell extract. NPs synthesis with culture supernatants are most of the time the preferred method since it facilitates the purification scheme for the recovery of the NPs. Extraction of NPs, formed within the cells or cell-wall, is laborious, time-consuming and are not cost effective. The bioactivities of NPs, namely antimicrobial and anticancer, are known to be related to NPs shape, size and size distribution.

Here, we proposed the method for a green synthesis of gold nanoparticles by purified yeast metabolite; melanin. We evaluate the potential to manipulate key parameters to control the monodispersity, size and shape of NPs. Therefore, the effect of pH, temperature, substrate concentration, time and addition of stabilizing agent on the synthesis of gold nanoparticles were investigated. The physico-chemical characterizations of nanoparticles, with size distribution (DLS), UV-Vis spectroscopy, Transmission and Scanning Electron Microscopy (TEM, SEM) and measurement of the NPs stabilization, were employed. A profile of the bioactivity of these nanoparticles compared to purified melanin, was assessed with cell lines and microbiological strains.

MICROBIAL PRODUCERS OF ANALYTICALLY IMPORTANT OXIDO-REDUCTASES: SCREENING AND OPTIMIZATION OF CULTIVATION CONDITIONS

Olha Demkiv

Poster 3

Olha Demkiv, Galina Gayda, Andriy Zakalskiy, Oxana Zakalska, Vasyl Kutsyaba,
Mykhailo Gonchar

*Department of Analytical Biotechnology, Institute of Cell Biology, National Academy of Sciences of
Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine*

E-mail: demkiv@yahoo.com

Enzymatic processes are the basis of many productions, and application of microbial enzymes has been widely distributed since the early 20th century. Microbial oxido-reductases are used in many different spheres of human activity, and this use is increasing rapidly due to reduced processing time, low energy input, cost effectiveness, nontoxic and eco-friendly characteristics. Enzymes are necessary in genetic engineering and biotechnology, in various industries, including food, agriculture, chemicals, and pharmaceuticals, as well as in medicine. Oxido-reductases of microbial origin are capable of degrading toxic chemicals from industrial and domestic wastes (phenolic compounds, nitriles, amines *etc.*) either *via* degradation or conversion to non-toxic products. However, the greatest role of enzymes is in analytical practice. Thus, obtaining of a wide range of enzymes in industrial scale from the different sources, including recombinant microorganisms, is an urgent problem of biotechnology and enzymology.

Global market for industrial enzymes, including recombinant, was valued at USD 4.61 billion in 2016, and is projected to grow at a compound annual growth rate of 5.8 % from 2017, to reach USD 6.30 billion by 2022. This branch of science and business requires rationally chosen cell factories (hosts) and cost-efficient protocols of proteins isolation. To obtain highly purified microbial enzyme the general approaches may be marked out: screening or gene-engineering of the effective strains as producers of the target enzyme(s); optimization of cultivation conditions for the most effective hosts to achieve both the highest yield and specific activity of a target enzyme in producing cells or in extra-cellular medium; development of effective method for enzyme isolation, purification; concentration and stabilization.

In this work, the screening of microorganisms (bacteria, yeasts and fungi) on a capability to synthesize oxidases, sensitive to D-xylitol (X), glycerol (Gl), galactose (Gal), L-arginine (Arg), cholesterol (Ch), phenolic compounds (PhC) was carried. These analytes are important biomarkers in medicine, food industry, pharmaceuticals and environmental control services. The best producers of xylitol oxidase (XO), glycerol oxidase (GIO), galactose oxidase (GalO), arginine oxidase (ArgO), cholesterol oxidase (ChO) and laccase were selected and the optimal conditions for cells cultivation to achieve the highest specific activities of these enzymes were estimated. The listed enzymes have a high analytical value: XO, GIO, GalO and ArgO are essential tools for enzymatic analysis of X, G, Gal and Arg in different branches of industry (pharmaceutical, cosmetics, food, dental *et al.*). The listed enzymes and ChO are promising biocatalysts in enzymatic diagnostic kits for clinical diagnostics. Laccase-based monitoring of PhC is necessary for food and environmental safety.

This work was financially supported by NAS of Ukraine (Project 10/3-/2019 of the Program “Smart sensor devices of a new generation based on modern materials and technologies”) and by the Ministry of Education and Science of Ukraine (projects Nos. 0118U000297 and 0119U100671).

**CHARACTERIZATION OF THE NEW FLAVIN PRODUCT OF THE HETEROLOGOUS
ROS B GENE OF STREPTOMYCES DAVAWENSIS EXPRESSION IN THE YEAST
KOMAGATAELLA PHAFFII**

Lyubov Fayura

Poster 4

Fayura L.R.¹, Dmytruk K.V.¹, Ruchala J.², Tsyurulnyk A.O.¹, Svyshch I.V.³, Motyka O.I.⁴, Fedorovych D.V.¹, Sibirny A.A.^{1,2}

1 – Institute of Cell Biology, National Academy of Science of Ukraine, Drahomanov Street 14/16, 79005, Lviv, Ukraine

2 – Department of Biotechnology and Microbiology, University of Rzeszow, Zelwerowicza 4, 35-601, Rzeszow, Poland

3 – Ivan Franko Lviv National University, Hrushevsky Street, 4, 79005, Lviv, Ukraine

4 – Research Institute of Epidemiology and Hygiene Danylo Halytsky Lviv National Medical University, Zelena Str. 12, 79005, Lviv, Ukraine

E-mail: fayural@gmail.com

Roseoflavin and its biosynthetic precursor aminoriboflavin (ARF) are produced by gram-positive bacteria *Streptomyces davawensis* and *Streptomyces cinnabarinus*. ARF shows the strong inhibitory effect against gram-positive bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus* and *Micrococcus luteus* and *Listeria monocytogenes* being non-toxic to mammalian cells. ARF is synthesized from flavin mononucleotide (FMN), which in turn is formed from riboflavin.

The aim of this work was to obtain the preparations of new product of the heterologous expression of the key gene of ARF biosynthesis *rosB* *S. davawensis*, encoding 8-dimethyl-8-aminoryboflavin-5'-phosphate synthetase along with homologous *FMN1* gene encoding riboflavin kinase on the background in of *Komagataella phaffii* riboflavin overproducing strain with the introduced native *FMN1* gene. The transformed strain *K. phaffii* with prTEF1-FMN1-prGAP-rosB expression cassette resulted in accumulation of new yellow fluorescent compound present in relatively large amounts in the culture medium. Flavins from the culture media were analyzed by paper chromatography in 0.5% Na₂HPO₄ (pH = 8.0) according to retardation value (Rf: FMN– 0.50; RF (riboflavin) – 0.37; ARF – 0.10). The cultivation conditions for constructed strain favored for increased production of the new flavin (Rf =0.10) were optimized. Two absorbance peaks near 445 and 478 nm corresponding to riboflavin and ARF were detected. The flavins of culture supernatants were adsorbed on Florisil (60-100 mach) (column 15 x 30 mm) and eluted with gradient of 50% acetone:H₂O (1:1). Fractions containing flavin with Rf 0.10 were pooled and applied to cellulose column (100 x 25 mm) previously washed with H₂O. Non-adsorbed flavins were eluted with H₂O. Obtained fractions were analyzed by paper chromatography in 0.5% Na₂HPO₄. Fractions containing only the new flavin with Rf = 0.1 were pooled and analyzed. Spectra analysis of obtained fractions flavins was performed. Spectra of flavins were recorded employing Hach Lange DR 6000 UV-VIS spectrophotometer. Absorbance peak at 478 nm corresponding to ARF was detected for researched preparation.

To obtain the solid samples of new flavin, freeze dryer was used. The optimal lyophilization conditions, namely, the composition of mixture of stabilizing additives as well as the parameters of freeze-drying procedure have been found. As a result, the solid preparations of ARF were obtained and characterized. This preparation showed relatively strong growth inhibition of *Staphylococcus aureus* ATCC 25923 (minimum inhibitory concentration, 100 mkg/ml).

This study was supported by Polish National Science Center, grant Opus UMO-2018/29/B/NZ1/01-497 and by National Academy of Sciences of Ukraine (Grant 36-19).

**THE MECHANISMS OF FRUCTOSE-1,6-BISPHOSPHATASE DEGRADATION IN
*KOMAGATAELLA PHAFFII***

Olena Dmytruk

Poster 5

Olena Dmytruk¹, Nina Bulbotka¹, Kostyantyn Dmytruk¹, Andriy Sibirny^{1,2}

1 – Institute of Cell Biology, National Academy of Science of Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine

2 – Department of Biotechnology and Microbiology, University of Rzeszow, Zelwerowicza 4, 35-601 Rzeszow, Poland

E-mail: verbaolena@gmail.com

Many enzymes of methanol metabolism of methylotrophic yeasts are located in peroxisomes whereas some of them have the cytosolic localization. During shift of methanol grown cells of methylotrophic yeasts to glucose medium, a decrease in the activity of cytosolic (formaldehyde dehydrogenase (FIDH), formate dehydrogenase (FDH), fructose-1,6-bisphosphatase (FBP)) enzymes of methanol metabolism is observed. Inactivation of peroxisomal enzymes occurs due to the autophagic degradation (pexophagy) whereas mechanisms of inactivation of cytosolic enzymes remain unknown. We aimed to study of the mechanisms of FBP degradation in methylotrophic yeasts *Komagataella phaffii*.

The changes of the specific activity of FBP in the wild type strain GS200, the strain with the deletion of the *GSS1* hexose sensor gene and strain defected in autophagy pathway SMD1163 of *K. phaffii* in short-term and long-term induction with methanol, and with or without the addition of the MG132 (proteasome degradation inhibitor) was investigated. Degradation of fructose-1,6-bisphosphatase by the Western blot analysis in GS200, SMD1163 and $\Delta gss1$ strains was studied. It was shown that the duration of cell incubation on methanol has no particular effect on the inactivation of the enzyme. The effect of the proteasome inhibitor MG132 was insignificant. Catabolic inactivation of FBP is damaged in the $\Delta gss1$ mutant as glucose signaling is impaired. FBP degrades by a vacuolar pathway, regardless of the duration of methanol induction, which correlates with the activity data of this enzyme.

To confirm FBP degradation pathway the recombinant strains with GFP-labeled Fbp1 of *K. phaffii* (or *E. coli*) and RFP-labeled peroxisomes were constructed on the background of GS200, *pex3Δ* and SMD1163. The fluorescent microscopy analysis of the constructed strains, using the vacuolar membrane dye FM4-64, was performed. It was shown that Fbp1 in *K. phaffii* degrades by autophagy pathway.

IMPACT OF THE GENE *TMII* ON THE EFFICIENCY OF ETHANOL PRODUCTION FROM XYLOSE IN *SCHEFFERSOMYCES STIPITIS*

Marta Semkiv

Poster 6

Marta Semkiv¹, Mariia Borbuliak¹, Orest Hryniv¹, Krzysztof Berezka², Tomas Linder³, Kostyantyn Dmytruk¹, Volkmar Passoth³, Andriy Sibirny^{1,2}

1 – Institute of Cell Biology, National Academy of Science of Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine

2 – Department of Biotechnology and Microbiology, University of Rzeszow, Zelwerowicza 4, 35-601 Rzeszow, Poland

3 – Swedish University of Agricultural Sciences, Uppsala, Sweden

E-mail: smarta0309@gmail.com

Lignocellulosic biomass represents an abundant renewable energy source and it is considered an ideal substrate for fuel ethanol production. However, the feasible technology for the bioethanol production from lignocellulosic materials has not been developed yet. The main reason is the absence of a robust microorganism capable of efficient alcoholic fermentation of the main sugars of lignocellulose, most importantly, glucose and xylose. Yeast *Scheffersomyces stipitis* is able to ferment both glucose and xylose with a high ethanol yield.

We aimed to identify potential effectors of fermentation in *S. stipitis* by the insertional mutagenesis combined with the positive selection of ethanol overproducers and to investigate the role of identified gene. Inhibitor of key glycolytic enzymes 3-bromopyruvate was used as a selection agent for obtaining of ethanol overproducers.

Among the selected 3-bromopyruvate resistant mutants, strain #4.6 revealed reproducible increase of ethanol accumulation during xylose fermentation. In this strain, the insertion was found within the ORF of a gene homologous to *Saccharomyces cerevisiae* gene *YDL119C*, encoding mitochondrial glycine transporter. We designated identified gene *TMII*. Wild-type phenotype was restored via complementation of the insertional mutation by the wild type allele of *TMII* gene, and the deletion of *TMII* gene on the background of *Ku80* strain improved its ethanol production on xylose containing media. The respiration efficiency, heme content and key glycolytic enzymes activities were measured in all investigated strains. Obtained results revealed that the gene *TMII* is involved in the regulation of alcoholic fermentation of xylose in *S. stipitis*.

**A NEW APPROACH TO THE STUDY OF PROTEIN DEGRADATION IN YEAST
KOMAGATELLA PHAFFII BASED ON THE FUSION PROTEIN COMPRISING β -
GALACTOSIDASE AND GFP**

Anastasiya Zazulya

Poster 7

Anastasiya Zazulya¹, Marta Semkiv¹, Nina Bulbotka¹, Olena Dmytruk¹, Kostyantyn Dmytruk¹, Andriy Sibirny^{1,2}

1 – Institute of Cell Biology, National Academy of Science of Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine

2 – Department of Biotechnology and Microbiology, University of Rzeszow, Zelwerowicza 4, 35-601 Rzeszow, Poland

E-mail: zazulya.n.z@gmail.com

The methylotrophic yeast *Komagataella phaffii* (formerly *Pichia pastoris*) as well as *Escherichia coli* and *Saccharomyces cerevisiae* has become an important host organism for recombinant protein production. *K. phaffii* has very strong and tightly regulated promoters of the genes of the methanol utilization pathway such as the alcohol oxidase, formaldehyde dehydrogenase gene etc., which are very convenient for directed expression of heterologous proteins. Proteolytic degradation has been a perpetual problem when yeasts are employed to express recombinant proteins. The maximum reduction in the level of degradation of the target recombinant protein in the cytosol is one of the prerequisites for successful overproduction of the proteins of industrial significance. To reduce the level of degradation of the protein in cytosol, it is necessary to understand the mechanism by which it occurs.

There are no convenient methods for the selection of *K. phaffii* mutants with damaged pathways of proteins degradation. The new model has been developed to detect mutants and analyze the way in which they degrade the proteins. This model involves constructing a vector for expression of β -galactosidase gene *LAC4* from *Kluyveromyces lactis* fused with fluorescent tag green fluorescent protein (GFP). This fusion protein was cloned under the control of the methanol-regulated promoter of the gene *FLD1* (encoding formaldehyde dehydrogenase). Obtained vector was used for the transformation of *K.phaffii* wild type strain GS200, as well as the strain SMD1163 with damaged autophagy. Methanol-induced gene expression will result in the formation of a chimeric protein that will retain β -galactosidase activity and, at the same time, will be noticeable in the cells under a fluorescence microscope due to the GFP presence.

Two types of transformants have been obtained and tested for the presence of integrated vector at the moment. Subsequent stages of the study involve cultivation on methanol to activate β -galactosidase and GFP gene expression. It is planned to observe the cellular localization of β -galactosidase-GFP fusion after it has been produced and during degradation. Obtained recombinant strains will be further used for the identification of insertional mutants with impaired protein degradation, which will possibly allow as to identify genes involved in protein degradation in *K. phaffii*.

USE OF LOCAL AGRICULTURAL WASTE MATERIAL FOR PRODUCTION OF BIOETHANOL

Linda Rozenfelde

Poster 8

Rozenfelde L.¹, Vedernikov N.², Puke M.², Khroustalyova G.¹, Saulite L.¹, Zala D.³, Rapoport A.¹

1 – Laboratory of Cell Biology, Institute of Microbiology and Biotechnology, University of Latvia, 1 Jelgavas Str, LV-1004, Riga, Latvia

2 – Laboratory of Polysaccharides, Latvian State Institute of Wood Chemistry, 27 Dzerbenes Str, LV-1006, Riga, Latvia

3 – Department of Microbiology and Biotechnology, Faculty of Biology, University of Latvia, 1 Jelgavas Str, LV-1004, Riga, Latvia

E-mail: linda.rozenfelde@lu.lv

Research for alternative forms of energy is becoming more and more essential nowadays. Bioethanol considered to serve not only as agent of environmental pollution reduction but also as a means to secure an energy supply that is local, renewable and independent of a financially volatile and potentially unreliable oil market. Different types of resource materials have a potential for bioethanol production. Biomass and biomass derived materials have been pointed out to be one of the most promising. It has been considered to be the best sustainable source of organic carbon on earth and the perfect equivalent to petroleum for the production of fuels. In this context, lignocellulosic biomass, which is the most abundant and bio-renewable biomass on earth, has a critical importance. In this work colza straw was tested as the resource material for the production of bioethanol. The new waste-less technology is used where pretreated colza straw is subjected to special enzymatic treatment with commercial enzymes which leads to the formation of glucose. Production of bioethanol from glucose is realized by the yeast *Saccharomyces cerevisiae*. In our work native and dehydrated yeast *Saccharomyces cerevisiae* is used for the fermentation. The last step of new technology is linked with the use of lignin for the cultivation of medical mushrooms.

Acknowledgements

This work has been financed by European Regional Development Fund project Nr. 1.1.1.1/16/A/113

**NEW APPROACHES FOR CONSTRUCTION OF THE STRAINS OF THE *OGATAEA*
POLYMORPHA YEAST WITH ACTIVATED XYLOSE UTILIZATION AND
FERMENTATION**

Małgorzata Bednarzak

Poster 9

Małgorzata Bednarzak¹, Justyna Ruchala¹, Andriy A. Sibirny^{1,2}

1 – Department of Microbiology and Biotechnology, University of Rzeszow, Zelwerowicza 4, 35-601 Rzeszow, Poland

2 – Institute of Cell Biology, National Academy of Science of Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine

E-mail: mbednarzak@ur.edu.pl

The methylotrophic yeast *Ogataea polymorpha* is apparently the most thermotolerant yeast with maximal border growth temperature above 50°C. This yeast well grows on glucose and cellobiose and efficiently ferments these sugars to ethanol under elevated temperatures whereas galactose and L-arabinose do not support yeast growth at all. At the same time, xylose supports relatively active growth; however, its conversion to ethanol is negligible. Active xylose fermentation is prerequisite for the development of yeast strains capable of efficient production of bioethanol from lignocellulosic hydrolyzates. Based on our findings, we decided to develop the simple system of positive selection of the mutant strains which show improved xylose growth and fermentation. The rationale of selection is as follows. Galactose and L-arabinose themselves do not support yeast growth but they could penetrate the cell. If so, they could inhibit xylose transport and possibly some intracellular reactions of xylose metabolism so on the mixtures of xylose with galactose or L-arabinose growth will be inhibited or totally absent. Our assumption was right as indeed growth on the mixture of xylose and galactose was absent. We plan to isolate spontaneous or UV-induced mutants which could metabolize xylose in spite of galactose (or/and L-arabinose) presence. Characteristics of xylose growth and alcoholic fermentation of the isolated strains of *O. polymorpha* will be analyzed. In the case some of the mutants will show improved xylose alcoholic fermentation, insertion mutagenesis will be subsequently used to tag the corresponding genes involved in regulation of xylose alcoholic fermentation.

THE ROLE OF TRANSCRIPTIONAL FACTORS MIG1, ACE1 AND CAT8 ON XYLOSE ALCOHOLIC FERMENTATION IN THE RECOMBINANT STRAINS OF SACCHAROMYCES CEREVISIAE AND OGATAEA POLYMORPHA

Barbara Kruk

Poster 10

Barbara Kruk¹, Justyna Ruchala¹, Olena Kurylenko², Kostyantyn Dmytruk², Andriy Sibirny^{1,2}

1 – Department of Microbiology and Biotechnology, University of Rzeszow, Zelwerowicza 4, 35-601 Rzeszow, Poland

2 – Institute of Cell Biology, National Academy of Science of Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine

E-mail: kruk.golab@gmail.com

Development of microorganisms capable of efficient fermentation of lignocellulosic materials is one of the most important goals of contemporary biotechnology. Xylose is the second most abundant monosaccharide after glucose found in lignocelluloses. Significant efforts have been focused on the metabolic engineering of *Saccharomyces cerevisiae* and *Ogataea polymorpha* for effective xylose utilization. However, the role of transcription factors in xylose alcoholic fermentation is poorly understood. In this work, we paid attention to function of genes that encode several transcriptional factors and their possible role in xylose alcoholic fermentation.

Transcription repressors Mig1, and its close functional homolog, Mig2, are involved in regulating the filamentous growth pathway in response to glucose limitation. A copper-binding regulatory protein Ace1 is transcriptional activator which we suggest could influence fermentation. We hypothesized that *O. polymorpha* cells that overproduce Mig1 and Ace1 will change glucose and xylose metabolism and fermentation. To examine this assumption we constructed strains of *O. polymorpha* that overexpressed *MIG1* and *ACE1* genes and studied their properties. The fermentation assaying showed insignificant effects of the derepression of the mentioned genes on glucose and xylose fermentation.

Transcription activator Cat8 is involved in expression of genes involved in gluconeogenesis, respiration and alternative carbon source utilization. We hypothesized that *S. cerevisiae* cells that lack of Cat8 transcription activator will have disturbed xylose alcoholic fermentation. To investigate this hypothesis, we performed series of experiments on *S. cerevisiae* GS010 strain capable of xylose utilization and *cat8Δ* mutants isolated from GS010 strain. We have found that the growth of the wild-type strain as well as *cat8Δ* mutant cells of *S. cerevisiae* on xylose was a little bit retarded whereas xylose fermentation was significantly reduced in the mutant. In contrast, both wild-type and *cat8Δ* strains showed similar growth rate and ethanol production. The drop in xylose conversion to ethanol by *cat8Δ* cells suggests that Cat8 transcription activator is required for xylose metabolism and conversion to ethanol in *S. cerevisiae*.

The research was carried out from the project funds 2016/21/B/NZ1/00280 financed from the funds of the National Science Center, Poland.

**CONSTRUCTION OF THE RIBOFLAVIN OVERPRODUCING YEAST STRAINS OF
CANADIDA FAMATA AND *KOMAGATAELLA PHAFFII* WITH HETEROLOGOUS
EXPRESSION OF *STREPTOMYCES DAVAWENSIS ROSB* GENE**

Andriy Tsyurulnyk

Poster 11

Tsyurulnyk A.O.¹, Ruchala J.², Fayura L.R.¹, Dmytruk K.V.¹, Fedorovych D.V.¹, Marx H.³, Mattanovich D.³, Sibirny A.A.^{1,2}

1 – Institute of Cell Biology, National Academy of Science of Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine

2 – Department of Biotechnology and Microbiology, University of Rzeszow, Zelwerowicza 4, 35-601 Rzeszow, Poland

3 – University of Natural Resources and Life Sciences Vienna, Muthgasse 18, 1190 Vienna, Austria

E-mail: andriy_tsyurulnyk@ukr.net

Aminoriboflavin (AF), the biosynthetic precursor of antibiotic roseoflavin, is produced by gram-positive bacteria *Streptomyces davawensis* and *Streptomyces cinnabarinus*. AF shows a strong antibiotic effect on Gram-positive bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus* and *Micrococcus luteus* being non-toxic to mammalian cells. Since AF is synthesized from flavin mononucleotide (FMN), which is formed from riboflavin, it is promising to apply FMN or riboflavin overproducers as basic strains for the construction of AF producers.

The aim of this study was to express the key gene of AF biosynthesis *rosB* *S. davawensis*, encoding 8-dimethyl-8-aminoryboflavin-5'-phosphate synthetase in the riboflavin overproducing strains of the yeasts *Candida famata* and *Komagataella phaffii* to achieve AF production. The *FMN1* and *rosB* genes were cloned into the genomes of the recipients under the control of number strong constitutive and maltose/methanol-inducible promoters. The transformed strain *K. phaffii* with *prTEF1-FMN1-prGAP-rosB* expression cassette resulted in accumulation of new yellow fluorescent compound present in relatively large amounts in the cultural medium. *K. phaffii* transformants with *prGAP1-FMN1-prTEF1-rosB*, *prDAS2-FMN1-prAOX1-rosB* and *prGAP1-rosB* expression cassettes produced lower amounts of this new product. The absorbance spectra analysis of accumulated flavins in of *prTEF1-FMN1-prGAP-rosB* transformant was performed. Two absorbance peaks near 445 and 478 nm corresponding to RF and AF were detected. Also new flavin compounds in *C. famata* cultural medium strains transformed by *prTEF1-rosB* and *prMAL2-rosB* were detected.

THE ROLE OF TRANSCRIPTION FACTORS IN XYLOSE FERMENTATION OF ENGINEERED YEAST *SACCHAROMYCES CEREVISIAE*

Liubov Dzanaieva

Poster 12

Liubov Dzanaieva¹, Kostyantyn Dmytruk¹, Andriy Sibirny^{1,2}

1 – Institute of Cell Biology, National Academy of Science of Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine

2 – Department of Biotechnology and Microbiology, University of Rzeszow, Zelwerowicza 4, 35-601 Rzeszow, Poland

E-mail: liubadzanaieva@gmail.com

Today biofuel industry primarily produces ethanol from a corn or sugarcane. However, this so-called first generation ethanol produced from starch and sugar, is in competition with a food and animal feed industry. In contrast, lignocellulosic biomass (crop wastes, agricultural and forestry residues, and municipal waste) offers a high potential as feedstock for biofuels, because it is the most abundant sustainable raw material worldwide and occurs as byproduct without competing uses. Studies on conversion of lignocellulosics to ethanol focused on the searching for natural microbial strains and construction of recombinants able to ferment efficiently all sugars of lignocellulosic hydrolysates. Effective alcoholic fermentation of xylose, the second abundant after glucose sugar of lignocellulose hydrolysates (consists approx. 30% of hydrolyzate sugars) is one of the main unresolved problems.

Although there are many bacterial and yeast strains capable of naturally utilizing xylose, *Saccharomyces cerevisiae* has advantages over the innate xylose-utilizing microorganisms regarding robustness against various stresses in industrial environments, such as low pH, high osmotic pressure, high alcohol concentration, and phage contamination. *S. cerevisiae* cannot naturally utilize xylose. Metabolic engineering approaches for introducing heterologous xylose utilization pathways and optimizing internal metabolisms have been undertaken to develop efficient xylose-fermenting *S. cerevisiae* strains. Despite of intensive engineering of *S. cerevisiae* strains, xylose fermentation rate still remains lower than for glucose. Study the regulation of xylose catabolism in engineered *S. cerevisiae* can facilitate xylose fermentation performance.

Effect of deletion and overexpression of corresponding genes encoding transcription factors Adr1, Asg1, Cat8, Hap4, Sip4, Tup1 and Znf1 on xylose alcoholic fermentation of engineered *S. cerevisiae* was studied. In this study, we report the isolation of *adr1Δ*, *asg1Δ*, *cat8Δ*, *hap4Δ*, *sip4Δ*, *tup1Δ* and *znf1Δ* mutants on the background of xylose-utilizing *S. cerevisiae* strain. In addition, recombinant strains overexpressing *ZNF1*, *SIP4*, *HAP4*, *ASG1* and *CAT8* genes under control of strong constitutive promoter were constructed. Ethanol production during xylose and glucose fermentation were significantly reduced in *hap4Δ* and *znf1Δ* as compared to that of parental strain.

ENGINEERING OF THE HEXOSE TRANSPORTERS IN THE YEAST *OGATAEA POLYMORPHA* FOR IMPROVED UTILIZATION OF XYLOSE DURING HIGH-TEMPERATURE ALCOHOLIC FERMENTATION

Roksolana Vasylyshyn

Poster 13

Roksolana Vasylyshyn¹, Olena Kurylenko¹, Rimantas Daugelavičius², Kostyantyn Dmytruk¹, Andriy Sibirny^{1,3}

1 – Institute of Cell Biology, National Academy of Science of Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine

2 – Department of Biochemistry, Vytautas Magnus University, Vileikos 8, LT-44404, Kaunas, Lithuania

3 – Department of Biotechnology and Microbiology, University of Rzeszow, Zelwerowicza 4, 35-601 Rzeszow, Poland

E-mail: vasylyshyn.r.v@gmail.com

Xylose transport is one of the bottlenecks in the conversion of lignocellulosic biomass to ethanol. To improve the xylose uptake activity in yeasts it is necessary to identify specific transporters mediating the uptake of xylose. *Saccharomyces cerevisiae* strain lacking the key transporters Hxt1-17 and Gal2 was found to be unable to grow on xylose and glucose. In this strain, growth on xylose could be restored by the reintroduction of Hxt1, Hxt2, Hxt4, Hxt5, Hxt7, or Gal2. *Ogataea polymorpha* is known as one of the thermotolerant xylose-fermenting yeast species.

To increase the specific xylose uptake rate the modified *O. polymorpha* Hxt1 transporter was engineered by substitution of asparagine to alanine residues at position 358. Furthermore, N-terminal lysine residues of Hxt1 predicted to be the target for ubiquitination were replaced for arginine residues. The *S. cerevisiae* Gal2 was modified with the substitution of asparagine residue to serine at position 376 and the Hxt7 with the substitution of the corresponding asparagine residue to phenylalanine at position 370. The modified versions of Gal2 and Hxt7 as well as Hxt1 transporters were introduced into genome of *O. polymorpha* wild-type strain and advanced ethanol producer. The utilization of glucose and xylose as well as ethanol production during high temperature co-fermentation of both sugars were studied in obtained recombinant strains.

AN ESTIMATION OF THE IMPACT OF *VMA1* GENE-DELETION ON THE RIBOFLAVIN PRODUCTION IN THE FLAVINOGENIC YEAST *CANDIDA FAMATA*

Yuliia Andreieva

Poster 14

Yuliia Andreieva¹, Kostyantyn Dmytruk¹, Andriy Sibirny^{1,2}

1 – Institute of Cell Biology National Academy of Sciences of Ukraine, Drahomanov St, 14/16, Lviv, 79005, Ukraine

2 – Department of Biotechnology and Microbiology, University of Rzeszow, Zelwerowicza 4, 35-601 Rzeszow, Poland

E-mail: yuliiaandreievapeng@gmail.com

Yeast *Candida famata* belongs to so-called flavinogenic yeast able to riboflavin (vitamin B2) oversynthesis under iron starvation. It is known that iron ions repress the synthesis of enzymes involved in conversion of GTP and ribulose-5-phosphate to riboflavin. Earlier studies defined that the deletion of *VMA1* gene coding for vacuolar ATPase in the filamentous fungus *Ashbya gossypii* and the flavinogenic yeast *Pichia guilliermondii* resulted in increase of riboflavin production. Riboflavin oversynthesis in *A. gossypii* is regulated by vacuolar ATPase due to extrusion of excess of riboflavin, which normally accumulates in vacuoles. In contrast, *P. guilliermondii* does not accumulate the synthesized riboflavin in vacuoles. Therefore, regulatory impact of *Vma1* in flavinogenic yeasts remains elusive.

It was decided to isolate *vma1Δ* strain of *C. famata* and study its properties. For that reason deletion cassette, containing *selectable* marker gene *ble* conferring resistance to phleomycin flanked with noncoding regions of *VMA1* gene was constructed. Deletion was obtained by gene replacement. Among 46 analyzed transformants selected on phleomycin containing medium, one was found with the deletion of the *VMA1* gene. Proper deletion of target gene was verified by PCR. It was shown that the *vma1Δ* mutant possessed 16-fold increase in riboflavin accumulation, as compared to that of the parental wild-type strain on the medium supplemented with iron. Growth of the mutant in iron-deficient medium, as well as in the higher temperature conditions (34 °C) was retarded. Isolation of the mutants with deletion of *VMA1* gene in riboflavin overproducing strains *C. famata* AF-4 and #91 is under way.

EFFECTS OF GENE *SFU1* DELETION ON RIBOFLAVIN SYNTHESIS IN THE YEAST *CANDIDA FAMATA*

Yana Petrovska

Poster 15

Yana Petrovska¹, Kostyantyn Dmytruk¹, Andriy Sibirny^{1,2}

1 – Institute of Cell Biology, National Academy of Science of Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine

2 – Department of Biotechnology and Microbiology, University of Rzeszow, Zelwerowicza 4, 35-601 Rzeszow, Poland

E-mail: yanapetrovska1@gmail.com

Riboflavin (RF) or vitamin B2 is an important vitamin for all living organisms. Riboflavin serves as a precursor of flavin coenzymes FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide) involved in numerous biochemical processes. Riboflavin biosynthesis pathway is well studied in the yeast *Candida famata*, however, its regulation is poorly understood. Several regulatory genes involved in this process, particularly, the gene *SEF1* coding for transcription activator, have been identified. It has been shown that in the pathogenic yeast *C. albicans*, Sfu1 (GATA-type transcription factor) represses *SEF1* (Chen C et al. 2011).

The aim of this work was to study the influence of *SFU1* gene on the suppression of the riboflavin synthesis in *C. famata*. It was decided knock out this gene by the constructed deletion cassette. Strains L20105 (wild type), AF4 and #91 (flavinogenic mutants) were transformed with plasmid, which contains the *SFU1* gene deletion cassette and selective marker *ble* gene conferring resistance to antibiotic phleomycin. The deletion of *SFU1* was confirmed in *C. famata* L20105. The four fold increase in the level of riboflavin production was found in *sfu1Δ* mutant.

The effect of deletion in the *SFU1* gene on the riboflavin synthesis in overproducing strains of flavinogenic yeast *C. famata* is currently under investigation.

Chen C, Pande K, French SD, Tuch BB, Noble SM. An iron homeostasis regulatory circuit with reciprocal roles in *Candida albicans* commensalism and pathogenesis. *Cell Host Microbe*. 2011;10(2):118–135. doi:10.1016/j.chom.2011.07.005

INVESTIGATION OF THE ROLE OF PEROXISOMAL ENZYMES IN XYLOSE METABOLISM AND ALCOHOLIC FERMENTATION IN THE YEAST *OGATAEA POLYMORPHA*

Dmytro Bratiichuk

Poster 16

Dmytro Bratiichuk^{1,2}, Olena Kurylenko², Kostyantyn Dmytruk² and Andriy Sibirny^{2,3}

1 – Ivan Franko National University of Lviv Hrushevskogo St. 4, 79005, Lviv, Ukraine

2 – Institute of Cell Biology National Academy of Sciences of Ukraine, Drahomanov St. 14/16, 79005, Lviv, Ukraine

3 – University of Rzeszow, Zelwerowicza 4, Rzeszow 35-601 Poland

E-mail: dmytrobrat1998@gmail.com

Ogataea (Hansenula) polymorpha is one of the most thermotolerant methylotrophic yeast species with ability to utilize xylose and ferment this sugar to ethanol. Methylotrophic yeasts possess well-developed peroxisomes which could occupy up to 80% of cellular volume during growth on methanol. The genes coding for peroxisomal enzymes are strongly induced by methanol as sole carbon source, repressed during growth on glucose and partly derepressed in the presence of xylose.

The key peroxisomal enzyme is alcohol oxidase. Overexpression of *AOX1* gene coding for alcohol oxidase in *O. polymorpha* resulted in increased ethanol production from xylose relative to the wild type strain. Moreover, overexpression of *AOX1* gene led to increase in the expression of *DAS1* and *TAL2* coding for peroxisomal transketolase (known also as dihydroxyacetone synthase) and transaldolase, respectively. It was shown that peroxisomal transketolase and transaldolase in *O. polymorpha* are required for xylose alcoholic fermentation but not for growth on this pentose. Mutants with knock out of *DAS1* and *TAL2* normally grew on xylose but were defective in its conversion to ethanol. Separate overexpression or co-overexpression of *DAS1* and *TAL2* in the wild type strain increased ethanol production from xylose 2-4 times with no effect on glucose alcoholic fermentation. For comparison, we also overexpressed the genes, *TKL1* and *TAL1*, coding for cytosolic transketolase and transaldolase, respectively. We found that overexpression of these genes also stimulated ethanol production from xylose during fermentation.

In the current study, genes coding for peroxisomal enzymes (*AOX1*, *DAS1*, *TAL2*) were overexpressed together with genes for cytosolic enzymes (*TKL1* and *TAL1*) in the genome of the previously isolated *O. polymorpha* advanced ethanol producer from xylose and obtained recombinant strains were studied regarding their ethanol production during high-temperature xylose alcoholic fermentation.

НОВІТНІ ВИМОГИ ДО КОНТРОЛЮ ЯКОСТІ (CD34/CD45+)-ВМІСНИХ ЛІКАРСЬКИХ ЗАСОБІВ

Юлія Меркулова

Poster 17

Кишинець Н.В., Меркулова Ю.В., Тимченко О.В.

Державне підприємство «Український науковий фармакопейний центр якості лікарських засобів», 61085, м. Харків, вул. Астрономічна, 33

E-mail: nelkish@gmail.com

Трансплантація гемопоетичних стовбурових клітин – висока медична технологія, яка в останні роки набуває швидкого розвитку завдяки позитивним результатам лікування злоякісних та інших, вкрай тяжких, гематологічних захворювань та потенційній можливості медикаментозної терапії солідних пухлин. Цитометрична оцінка кількості клітин попередників за умови трансплантації мобілізованих периферичних стовбурових клітин, пуповинної крові або кісткового мозку є обов'язковою процедурою фармакопейного аналізу (CD34/CD45+)-вмісних лікарських засобів, бо саме від кількості CD34-клітин залежить успішне відновлення основних показників крові під час аутологічної та алогенної трансплантації кровотворної тканини. Найчастіше для цього використовується поєднання маркера стовбурових клітин CD34 та загальнолейкоцитарного антигену CD45.

Відповідно до вимог Державної Фармакопеї України (ДФУ), кількісне визначення клітин CD34/CD45+, які містяться в продуктах гемопоезу проводять за допомогою імунологічного мічення з подальшим визначенням методом проточної цитометрії за одноплатформною технологією із використанням калібрувальних флуоросфер, за необхідності після лізису еритроцитів проби (ДФУ, стаття 2.7.23 «Підрахунок гемопоетичних клітин CD34/CD45+» та стаття 2.7.24 «Проточна цитометрія»).

Метод, що включено до ДФУ, специфічний щодо всіх типів гемопоетичних препаратів та цільної крові. Результати випробування за даним методом наводяться у вигляді відсотка клітин CD34/CD45+ та абсолютного числа на мікролітр або на кілограм маси тіла реципієнта. Доведено, що запропонований метод характеризується високою чутливістю і, закономірно, дає можливість контролювати лікарські засоби з дуже малим відсотковим вмістом клітин CD34/CD45+. У валідаційних дослідженнях підтверджена точність та внутрішньо лабораторна прецизійність цитометричного методу. Правильність, та прецизійність методу, його чутливість та специфічність забезпечує клінічно значущі та достовірні результати, а швидкість виконання дозволяє проводити аналіз та отримувати результати у реальному часі. Таку валідаційну характеристику, як міжлабораторна відтворюваність, що має гарантувати надійність результатів фармакопейного випробування (CD34/CD45+)-вмісних лікарських засобів, відповідно до вимог ДФУ, рекомендується перевіряти у межах програми з професійного тестування.

НАНОКОМПЛЕКСИ ЯК ПЕРСПЕКТИВНИЙ НАПРЯМОК РОЗВИТКУ У ФАРМАКОЛОГІЇ ТА МЕДИЦИНІ

Левашова В.М.

Національний фармацевтичний університет, кафедра біології

м. Харків, Україна

E-mail: vika55510@meta.ua

У роботі окреслено можливості використання наноконкомплексів у вигляді ліпідних везикул або ліпосом, що являють собою сферичні двошарові мембрани, які можуть містити лікарські засоби та транспортувати їх до клітини. Історія ліпосом починається з 60-х років ХХ століття, коли англійський учений Алек Бенгхем (Bangham A.D.) разом з колегами, проводячи дослідження фосфоліпідів у водних середовищах, на електронних мікрофотографіях побачив шароподібні частки, схожі на мембранні структури клітини. Подальші дослідження показали, що неорганічні іони, присутні в розчині, транспортуються усередину цих частинок та утримуються там тривалий час. Так вперше було встановлено, що фосфоліпіди, які є основними компонентами клітинних мембран, здатні утворювати у воді замкнуті мембранні оболонки, які захоплюють частину навколишнього водного розчину, а фосфоліпідна мембрана, що їх оточує, має властивості напівпроникного бар'єру.

Фармакотерапевтичні переваги ліпосом обумовлені низкою факторів: природною біосумісністю матеріалу ліпосом, вибірковістю депонування щодо клітин, які перебувають у стані гіпоксії, можливістю регулювати ліпідний склад ліпосом і тим самим змінювати їх фармакокінетику і фармакодинаміку. Найпоширеніші серед них - це сімейство інтегринів, тобто трансмембранних глікопротеїнів - рецепторів, що складаються з альфа- і бета-субодиниць, різні поєднання яких визначають специфічність зв'язування позаклітинного рецептора з тим чи іншим лігандом. Лігандами для інтегринів найчастіше є різні білки позаклітинного матриксу: колаген, ламінін, фібронектин, значна частина яких розташована на ендотелії, що вистилає внутрішню поверхню судин. Завдяки інтегринам циркулюючі клітини «дізнаються», де їм потрібно зупинитися і прикріпитися, а також, якщо є необхідність, то й увійти із судини до тканини.

Через те, що мембрана ліпосом складається з природних фосфоліпідів, ліпосоми нетоксичні, мають здатність до біодеградації, а їх мембрана може зливатися з клітинною мембраною, що призводить до внутрішньоклітинного транспорту їх вмісту та його поглинання клітиною. Крім того, речовина, що міститься у ліпосомі, захищена від гідролітичного впливу ферментів, що збільшує ефективність препаратів, схильних до біодеструкції в біологічних рідинах. Ліпосомальні системи або наноконкомплекси слід розглядати не тільки як носії лікарських засобів, але і як самостійні чинники фармакокорекції патологічних станів.

На основі ліпосом В.М. Стефановим, був створений антигіпоксичний антиоксидантний препарат «Ліпін» – уперше в світі промислово освоєний ліпосомальний лікарський засіб. До ліпосомальних препаратів розроблених Стефановим В.М., відносяться також «Ліюлів» та «Ліпофлавіон». Полегшена дифузія з адсорбованих ліпосом, механізм дії ліпосом, імовірно, полягає в модифікації фосфоліпідного оточення іонних каналів, мембранних рецепторів і ферментів: якщо оточення змінюється, то відповідно змінюється і їх активність. Змінюючи ліпідний склад ліпосом, можна цілеспрямовано змінювати їх фармакологічний ефект. Таким чином, ліки у формі фосфоліпідних наночасток мають високу біодоступність та ефективність.

6th Ukrainian Congress for Cell Biology with international representation

***June 18-21, 2019,
Yaremche***

Session 4

Tumor cell biology

**CONDITIONS AND FACTORS LEADING TO MULTIDRUG RESISTANCE IN BACTERIA
AND EUKARYOTIC CELLS**

Rimantas Daugelavičius

Lecture 1

Rimantas Daugelavičius

Department of Biochemistry, Vytautas Magnus University, Vileikos str. 8, Kaunas 44404, Lithuania

E-mail: rimantas.daugelavicius@vdu.lt

Multidrug resistance (MDR) caused by multidrug transporters (MT) is a complex phenotype when cells acquire resistance to wide variety of structurally unrelated drugs. MDR is one of the most common reasons of bacterial resistance to antibiotics and information on the efflux transporters is of vital importance for effective usage of available antibacterials and discovery of the new ones. At the same time resistance to anticancer drugs is the main limitation of effective chemotherapy. It is of crucial importance to understand conditions and factors determining the increased efficiency of efflux and the overexpression of MT genes.

MTs need energy to transport compounds against their electrical and/or chemical gradients. Members of clinically the most important resistance-nodulation-division family of MTs in gram-negative bacteria obtain energy from the proton motive force generated as a result of energy metabolism. Efflux inhibitors are considered as attractive means for the prevention of antibiotic efflux from clinically relevant bacterial pathogens. Possibilities of the usage of efflux inhibitors will be discussed.

One of the best known MTs in human cells is P-glycoprotein, the product of *ABCB1* gene, able to extrude from cells a variety of lipophilic drugs and widely contributing to chemoresistance. Cells resistant to high concentrations of chemotherapy drugs can be developed exposing the cell cultures to stepwise increase of lipophilic compound, i.e., tetraphenylphosphonium (TPP⁺) concentrations. TPP⁺-induced resistance is accompanied by a high efficiency of doxorubicin efflux and more than 1 million-fold upregulation of *ABCB1* expression. Compounds inducing the overexpression of MT genes in bacteria and eukaryotic cells will be discussed.

POTENTIALS OF NANOCARRIERS IN CIRCUMVENTING TUMOR CELL RESISTANCE TO ANTICANCER DRUGS AND DIMINISHING NEGATIVE SIDE EFFECTS OF THEIR ACTION IN THE TREATED ORGANISM

Rostyslav Stoika

Lecture 2

Rostyslav Stoika

*Institute of Cell Biology, National Academy of Sciences of Ukraine,
Drahomanov Street 14/16, 79005 Lviv, Ukraine*

E-mail: stoika.rostyslav@gmail.com

There are two principal problems at using the anticancer chemotherapy: 1) only 0.01% of drugs applied intravenously reach their biological targets in the organism, while 99.99% can cause negative side effects; 2) during a year term, over 50% of cancer patients gain resistance to applied chemotherapeutic drugs. Both these problems can be solved through using nanomaterials (<100 nm) for drug delivery, since such delivery: a) enhances an effectiveness of drug action *in vitro* and *in vivo*; b) accelerates drug delivery to target cells; c) permits circumventing natural biological barriers, particularly, the multi-drug resistance in tumor cells; d) reduces the adverse side effects (cardio-, hepato-, nephro-, neuro-, immuno-toxicities) caused by drugs in the treated organism; e) prolongs a stability of anticancer drugs and their treatment effects; f) provides water solubility to water insoluble drug substances that improves their application.

At a design of nanoparticles for biological and medical application, one should take into account the following characteristics of the particles: 1) size (important for particles' clearance); 2) core (defines bio-degradability); 3) coating (affects biocompatibility); 4) labeling (important for detection at bio-imaging); 5) activation (necessary for bio-functionalization); 6) bio-functionalization (provides a possibility of particles' recognition by specific cells).

Here we demonstrated that immobilization of anticancer drug by the nanocarriers of the organic (polymer) or mineral (fullerene C₆₀) nature decreased the acting concentration of the drug, thus, enhancing its cytotoxic (pro-apoptotic) action. Such enhancement also included circumvention of the multidrug resistance barriers, probably due to "hiding" the drug from special membrane transporters protecting cells from the action of low molecular weight toxic agents, particularly drug substances. Besides, such "masking" of highly toxic anticancer drugs prevented their direct interaction with cells of normal tissues and organs at their non-addressed action in the treated organism. In such way, the negative side effects of the anticancer drug, namely its cardio-, hepato-, and nephro-toxicities were significantly decreased. Perspectives of using specific nanocarriers for delivery of anticancer drugs to tumor cells *in vitro* and in tumor bearing animals (mice) are considered.

Acknowledgements for: R. Panchuk, N. Finiuk, Yu. Senkiv, N. Boiko, N. Skorokhyd, N. Kashchak, Yu. Kozak, O. Klyuchivska (Institute of Cell Biology, NAS of Ukraine), N. Mitina, A. Ryabtseva, O. Zaichenko (National University "Lviv Polytechnica"), L. Kobylinska, R. Lesyk (Danylo Halytsky Lviv National Medical University), Yu. Prylutsky (Taras Shevchenko Kyiv National University).

METABOLIC ANTICANCER ENZYMOTHERAPY BASED ON ARGININE DEPRIVATION AND NEW COMBINATIONAL APPROACHES

Oleh Stasyk

Lecture 3

Oleh Stasyk

*Institute of Cell Biology, National Academy of Sciences of Ukraine,
Drahomanov Street 14/16, 79005 Lviv, Ukraine*

E-mail: stasyk@cellbiol.lviv.ua

Metabolic anticancer therapies based on a single amino acid (such as asparagine, methionine, and arginine) deprivation was developed as potentially more selective and less toxic alternatives to the existing classical therapies. Several recombinant arginine-degrading enzymes that can be applicable in humans have been elaborated to support such enzymotherapy.

However, despite of the recent progress in laboratory, several problems still have to be addressed before it is approved for the practical use. For instance, clinical trials demonstrated that this monotherapy is quite efficient in controlling growth of many highly aggressive tumors which are auxotrophic for arginine, but is less efficient than had been originally expected as a curative approach. Therefore, to increase therapeutic efficacy of arginine deprivation, new more efficient rationally designed combinatory procedures are sought.

We established on diverse cancer cell models that such auxilliary therapeutics as inhibitors of autophagic protein degradation, arginine proteomimetic analogues such as canavanine, and nitric oxide donors specifically enhance antitumor effects of arginine deprivation. We have recently described that one of the critical cellular responses to single amino acid starvation is mediated by endoplasmic reticulum stress and resulting unfolded protein response (Bobak et al., 2016). In collaboration with Prof. Jolanta Redowicz (Nencki institute of experimental biology PAN, Warsaw, Poland) we observed for the first time that arginine deprivation specifically leads to transient actin cytoskeleton remodeling and profound impairment of cells metastatic properties (Pavlyk et al., 2015). In addition, in collaboration with Prof. Leoni Kunz-Schughart (Oncoray, Technical University Dresden, Germany) it was demonstrated that arginine deprivation, especially in combination with antimetabolite canavanine, leads to profound radiosensitization of tumor cells (Kurlishchuk et al., 2016). Future research and animal studies should reveal whether potential of the mentioned combinatory approaches is translated to *in vivo*.

Bobak Y. et al. The International Journal of Biochemistry & Cell Biology. 2016, 70:29-38.

Pavlyk Y. et al. Amino Acids. 2015, 47:199–212.

Kurlishchuk et al. Oncotarget. 2016, 7(45):73292-73308.

CIRCUMVENTION OF TUMOR DRUG RESISTANCE BY QUINONE-CONTAINING COMPOUNDS: ROLE OF EXTRAMITOCHONDRIAL ROS

Rostyslav Panchuk

Lecture 4

Rostyslav Panchuk¹, Nataliya Kashchak¹, Nadya Skorokhyd¹, Jurgen Rohr², Vasyl Hurmach⁴, Yuriy Prylutsky⁴, Petra Heffeter³, Walter Berger⁴, Rostyslav Stoika¹

1 – Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine

2 – University of Kentucky, College of Pharmacy, Lexington, USA

3 – Institute of Cancer Research, Vienna Medical University, Vienna, Austria

4 – Taras Shevchenko National University of Kyiv, 64 Volodymyrska Str., 01601 Kyiv, Ukraine

E-mail: rpanchuk@ukr.net

Cancer drug resistance is considered one of the main reason for the failure of conventional chemotherapy, and is mainly caused by overexpression of ABC-transporter proteins (P-glycoprotein, MRP-1, bcrp) in plasma membrane of tumor cells. However, these processes are tightly dependent on ATP production, thus lowering cellular ATP pool by pro-oxidants should inhibit efficiency of drug efflux pumps and circumvent cancer drug resistance. Recently we have shown that quinone-containing angucycline antibiotic landomycin E leads to massive extramitochondrial hydrogen peroxide production in tumor cells (Panchuk et al, *Free Rad Biol Med*, 2017), which allows him to easily circumvent multi-drug resistance, caused by overexpression of P-gp, MRP-1, bcrp. However, the molecular mechanisms of this phenomenon remained poorly understood. The main aim of this study was to dissect structure-activity relationships underlying ROS-producing and MDR-circumventing potential of various quinone-containing compounds.

It was revealed that presence of 1,4-benzoquinone motif in molecule of menadione (vitamin K₃) was sufficient for successful circumvention of cancer drug resistance (caused by P-gp and MRP-1 overexpression) in human leukemia cells by this compound. However, the overall cytotoxic action of menadione was low (LC₅₀=50 μM), but subsequent addition of extra benzene rings (landomycinone, LC₅₀=10 μM) and 6 dideoxysugars (landomycin A, LA, LC₅₀=1 μM) to menadione scaffold resulted in 50-fold increase of its anticancer activity with full preservation of its MDR-circumventing potential. Increased complexity of quinone molecules also significantly changed their mode of action. In particular, menadione led to early burst both of H₂O₂ (2,5-fold) and O₂⁻ production (1,5-fold) already at 1h after addition to cell culture, reaching its peak at 3h timepoint. No signs of depolarization of mitochondria were detected at 1-6h indicating that both of these ROS were produced by cytosolic enzymes. However, at late timepoints (24h) menadione led to massive (96%) depolarization of mitochondria, accompanied by 12-fold increase in superoxides, indicating on time-dependent involvement of both cytosolic enzymes and mitochondria in cell death, induced by this drug.

On contrast to it, LA led only to strong (5-fold) increase of H₂O₂ levels at 1h without any visible impact on superoxide production. Weak depolarization (30%) of mitochondria under LA action was observed only at 24h, indicating that cytosolic enzymes play crucial role in H₂O₂ burst and hence MDR circumvention by this antibiotic. *In silico* studies have revealed two potential targets of quinone-containing compounds – NADPH dehydrogenases NQO1 and NQO2, which are involved in H₂O₂ and O₂⁻ production, correspondingly. It was revealed that menadione demonstrated high affinity both to NQO1 and NQO2, which positively correlated with increased H₂O₂ and O₂⁻ levels under its action *in vitro*. However, LA was able to form a tight complex only with NQO1 due to presence of extra binding pocket in this enzyme for LA's oligosaccharide chain, which was closed in NQO2. These data explain specific H₂O₂ production under the action of LA, which may be of key importance in circumvention of MDR by this experimental drug.

**LEARNING POINTS AROUND BIOCOMPATIBILITY OF PD SOLUTIONS MEASURED AS
IN VITRO PROLIFERATION OF HEPG2 AND VERO CELLS**

Natalia Hudz

Lecture 5

Hudz N.¹, Lagutina O.²

1 – Department of Drug Technology and Biopharmaceutics, Danylo Halatsky Lviv National Medical University, Lviv 79010, Ukraine

2 – Institute for Occupational Health of National Academy of Medical Science of Ukraine, Kyiv

E-mail: nataligudz03021972@gmail.com

Introduction. Issues of biocompatibility studies of solutions for peritoneal dialysis (PD) is very important from a point of view of saving peritoneum functions and finding factors influencing biocompatibility.

Methods. The following research methods were used: analytical methods for determination of pH, glucose degradation products (GDPs) contents and cell viability using neutral red (NR), MTT and sulforhodamin B (SRB).

Results. Assessing all the laboratory-made PD solutions with the HepG2 and Vero cells, there were non-significant (weak) predicted positive relationships (0.35 and 0.32) between viability and pH in the MTT-test (the more was pH, the higher was viability) and negative unpredicted relationships in the NR- (-0.88 and -0.50) and SRB-test (-0.72 and -0.38), respectively. There were non-significant unpredicted relationships (-0.12 and -0.20) between viability and GDPs concentration in the MTT-test and a strong and middle positive (the more was concentration, the higher was viability) unpredicted relationships in the NR- (0.96 and 0.50) and a middle and weak unpredicted relationships (0.66 and 0.18) in the SRB-test, respectively, with the HepG2 and Vero line. There were positive unpredicted relationships between viability of the HepG2 and Vero line and glucose concentration: 0.12 and 0.20 in the MTT-test, 0.37 and 0.14 in the NR-test and 0.37 in the two SRB-tests, respectively.

Discussion. The study results established influence of numerous factors on the bioavailability of PD solutions: type of cells, test of the viability determination, pH, etc. However, only MTT-test gives a non-significant but predicted relationship between increasing both cell types viability and increasing solutions pH.

Conclusion. Our study has several important learning points around biocompatibility. The first is that cytotoxicity is related as much to pH and other unknown mechanisms as it is to GDPs and glucose. The second point is that GDPs or glucose did not exert an exceptionally strong effect upon PD solutions cytotoxicity.

Acknowledgement:

Co-author Nataliia Hudz is grateful to the International Visegrad Fund (contract No. 51700107) for providing scholarship for studies related to solutions for dialysis therapy.

**БІОЛОГІЧНІ ЛІКАРСЬКІ ЗАСОБИ: ХАРАКТЕРИСТИКА, ЗАСТОСУВАННЯ,
ОБ'ЄМ СВІТОВОГО РИНКУ, ФАРМАКОПЕЙНІ ВИМОГИ ДО ЯКОСТІ**

Nelia Kyshynets

Lecture 6

Кишинець Н.В.

ДП «Фармакопейний центр», 61085, м. Харків, вул. Астрономічна, 33

E-mail: nelkish@gmail.com

Застосування біологічних лікарських засобів (БЛЗ), виготовлених за допомогою високотехнологічних процесів, відкрили нові можливості в терапії захворювань, які важко піддаються лікуванню: аутоімунних, інфекційних, онкологічної патології, цукрового діабету, розсіяного склерозу, артриту та ін.

Біологічний лікарський засіб (БЛЗ) — лікарський засіб, що містить як активний інгредієнт біологічну речовину. До великої групи БЛЗ належать алергени, антигени, вакцини, імунні сироватки, цитокіни, інтерферони, альбуміни, фактори згортання крові, імуноглобуліни, імуномодулятори бактеріального походження, моноклональні антитіла, живі біотерапевтичні лікарські засоби (пробіотики) та інші у вигляді фармацевтичних препаратів, активних фармацевтичних інгредієнтів, нерозфасованих лікарських засобів та препаратів *in bulk*. Під час їх виготовлення, в основному, використовують такі високобіотехнологічні процеси, як застосування методів генної інженерії; перенос генів біомолекулами та/або біологічно модифікованими клітинами, що є діючими речовинами або частинами діючих речовин; технологію рекомбінантної ДНК; контрольовану експресію генів, що кодують біологічно активні білки у прокаріотів та еукаріотів (зокрема трансформовані клітини ссавців; гібридомну технологію та моноклональні антитіла); репродукцію живих агентів у культурах клітин, ембріонах чи тваринах; екстракцію речовин з біологічних тканин (включаючи тканини людини, тварин і рослин); культивування штамів мікроорганізмів і клітин еукаріотів.

На даний час на світовому ринку фармацевтичними компаніями представлено близько 220 БЛЗ і більше 250 знаходяться в стадії розробки. Обсяг світового ринку біотехнологій на сьогоднішній день оцінюється в 270 млрд. дол., а до 2020 р. складуть близько 600 млрд. дол. (Джерело: IMS Consulting Group).

Характеристика та визначення якості БЛЗ, одержаних за допомогою високотехнологічних процесів, є складним завданням та потребує комбінації фізико-хімічних і біологічних випробувань, а також оцінки технологічного процесу та його контроль.

Контроль якості БЛЗ відповідно до фармакопейних стандартів є невід'ємною частиною забезпечення вітчизняних споживачів лікарськими засобами, які відповідають вимогам, встановленим законодавством України. Якість європейського рівня, що є безперечною умовою безпеки та ефективності застосування лікарських засобів, забезпечує дотримання вимог Державної Фармакопеї України, правового акту, який містить загальні вимоги до лікарських засобів, вимоги до упаковки, умов і терміну зберігання та методів контролю їх якості, та повністю гармонізована з Європейською Фармакопеєю.

L-CANAVANINE INFLUENCE ON HUMAN GLIOBLASTOMA CELLS UNDER ARGININE DEPRIVATION

Olena Karatsai

Poster 1

Olena Karatsai¹, Oleh Stasyk², Maria Jolanta Rędownicz¹

1 – Laboratory of Molecular Basis of Cell Motility, Nencki Institute of Experimental Biology PAS, 3 Pasteur Street, 02-093 Warsaw, Poland

2 – Institute of Cell Biology, National Academy of Science of Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine

E-mail: o.karatsai@nencki.gov.pl

It is known that numerous cancers are defective in arginine biosynthesis and therefore become hypersensitive to deprivation of this amino acid. Auxotrophy of several tumors for arginine, including glioblastomas, is mainly related to impairment of activity of argininosuccinate synthetase, one of the key enzymes of arginine biosynthesis. However, arginine deprivation affects only tumor cell growth and does not induce cell death. One of the drugs that can be used in combination with arginine deprivation is arginine analogue, L-canavanine.

We decided to analyse the effect of L-canavanine under arginine deprivation on human U251MG and U87MG glioblastoma cells. We demonstrated that L-canavanine under the limitation of arginine increases the amino acid response signal transduction pathways. In particular, we observed changes in eIF2 α phosphorylation, activation of PI3K/Akt/mTOR pathway, and expression of ATF4, a master regulator controlling the transcription of key genes essential for adaptative functions. Also, we examined the effects of L-canavanine on MAPK/ERK signaling pathway in glioblastoma cells. We observed that under the cotreatment phosphorylation of ERK1/2 was decreased only in U251MG cell line. We also noted that the level of p38 phosphorylated protein was significantly increased in both cell lines thus suggesting suppression of cell proliferation. Moreover, we demonstrated that L-canavanine under arginine deprivation induced apoptotic cell death in human glioblastoma cells. These observations indicate that this combinational treatment might be potentially used for the development of an effective anticancer therapy against these treatment-resistant and highly malignant tumors.

This work was supported by European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No.665735 granted to the Nencki Institute.

THE COMPLEX INFLUENCE OF NEWCASTLE DISEASE VIRUS LASOTA STRAIN AND TESTOSTERONE ON HUMAN PROSTATE CANCER CELLS WITH DIFFERENT SENSITIVITY TO HORMONE THERAPY *IN VITRO*

Tamara Kozak

Poster 2

Tamara Kozak, Oleksandra Lykhova, Natalia Bezdieniezhykh, Nazar Vydasov
RE Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of NASU, Kyiv, Ukraine
E-mail: kozaktoma@ukr.net

Prostate cancer (PC) therapy is severely hampered by lack of response and development of resistance to conventional chemotherapeutic drugs in patients, and some types of PC are insensitive to the hormone therapy which is obstacle for anti-androgen treatment. Furthermore, according to the experimental data (Song W., 2014), physiological normal levels of androgen inhibit proliferation of PC cells *in vitro*, while lower concentrations than the optimal androgen level promoted the proliferation of PC cells. Considering this data we have a necessity to search new ways for PC treatment. Newcastle disease virus (NDV) has a low-level pathogenicity for the human organism and is used for the complex treatment of cancer because the virus is cytotoxic for tumor cells and can stimulate producing of some cytokines with anti-tumor activity (Schirmacher V., 2017).

The aim of this work was to study the influence of NDV of avirulent vaccine strain LaSota in complex with testosterone (T) on androgen-dependent LNCaP and androgen-independent PC-3 cell lines of human prostate cancer. In this work were used virology and cell culture methods. To the cells, infected by NDV, were added various concentration of testosterone and cultured during 48 hours.

The results of the experiment showed that a number of alive LNCaP cells for concentration at NDV=208333.33 EID₅₀/ml in mono mode was 57.31±1.13%, while for T=0.16 ng/ml this number was 82.41±9.41%. In conditions of complex using these agents at such concentrations the number of alive cells was 40.72±3.73%, and it's may point to additive/synergetic effect of NDV (208333.33 EID₅₀/ml) and testosterone (0.16 ng/ml) on androgen-dependent LNCaP cells *in vitro*. Also similar effects for other few combination of NDV/Testosterone were observed. For example, for NDV=416666.67 EID₅₀/ml in monomode was 30.09±5.28% alive cells, while for T=0.04 ng/ml this number was 101.79±2.24%, and for complex of these concentrations was 14.87±2.82% alive cells. For androgen-independent PC-3 cells a number of alive cells for concentrations of NDV=208333.33 EID₅₀/ml and T=0.16 ng/ml in monomode were 67.42±2.3% and 92.77±5.13% respectively. The complex using of these concentrations gave 58.45±4.16% of PC-3 alive cells, and it's also additive/synergetic effect. For such type of effect for androgen-independent PC-3 cells were also observed other combinations of NDV/T. Also, should be noted that LNCaP cells were more sensitive to the NDV in compare to PC-3 cells, because for LNCaP IC₅₀=232989.97 EID₅₀/ml, while for PC-3 IC₅₀=559812.25 EID₅₀/ml. These results can become a base for further investigations of features of complex therapy by NDV LaSota and testosterone on human prostate cancer and for creating other alternative ways for cancer biotherapy.

Song W., Khera M., Physiological normal levels of androgen inhibit proliferation of prostate cancer *in vitro*, Asian J. Androl. 2014, 16(6):864-868.

Schirmacher V., Immunobiology of Newcastle Disease Virus and Its Use for Prophylactic Vaccination in Poultry and as Adjuvant for Therapeutic Vaccination in Cancer Patients, Int. J. Mol. Sci. 2017, 18:1–20.

ОСОБЛИВОСТІ ПОРУШЕНЬ ВУГЛЕВОДНЕВОГО ОБМІНУ В ГОРМОН-ЧУТЛИВИХ І РЕЗИСТЕНТНИХ КЛІТИНАХ РАКУ ПЕРЕДМІХУРОВОЇ ЗАЛОЗИ ЛЮДИНИ *IN VITRO*

Олександра Лихова

Poster 3

Лихова О.¹, Безденежних Н.¹, Лупан В.¹, Козак Т.¹, Видасов Н.¹, Чехун В.¹

Інститут експериментальної патології, онкології та радіобіології

ім. Р.С. Кавецького НАН України, Васильківська 45, 03022, Київ, Україна

E-mail: AlexxDNA@gmail.com

Рак передміхурової залози (РПЗ) та цукровий діабет – одні з найбільш поширених захворювань, що вражають чоловіків старшого віку. Дослідження показали, що саме РПЗ серед пацієнтів із цукровим діабетом зустрічається рідше. Разом з тим, в поєднанні з цукровим діабетом РПЗ протікає більш агресивно і частіше супроводжується метастазуванням в регіонарні лімфатичні вузли. Особливості такого перебігу онкологічного захворювання пов'язані з різними порушеннями біосинтезу гормонів або надмірною експресією рецепторів до них в клітинах РПЗ, що супроводжується пригніченням апоптозу та підвищенням проліферації пухлинних клітин. З огляду на це, актуальним є дослідження впливу інсуліну на проліферацію та метаболізм глюкози гормон-чутливими і резистентними клітинами РПЗ *in vitro*. Об'єкти дослідження: клітини РПЗ людини лінії LNCaP – гормон-чутливі та Du-145 – резистентні. Методи: культури клітин, біохімічні, молекулярної біології та статистичні. Аналіз кількості FITC-інсулін⁺ клітин та рівня експресії рецептора інсуліну в популяції досліджуваних клітин показав, що на рівні тенденції в гормон-чутливих клітинах LNCaP кількість FITC-інсулін⁺ клітин та рівень експресії рецептора інсуліну на клітинах вищий, ніж в гормон-рефрактерних клітинах Du-145. Показано, що за умов культивування клітин LNCaP у поживному середовищі з високим вмістом глюкози (4,5 г/л) інсулін у всіх досліджених концентраціях (2000-30 нг/мл) стимулював проліферацію цих клітин на 20-30%, в порівнянні з контролем. Культивування LNCaP в присутності таких же концентрацій інсуліну, але в поживному середовищі з низькою концентрацією глюкози (1 г/л) суттєво не впливало на їх проліферацію. Аналіз проліферативної активності клітин Du-145 після їх обробки інсуліном показав, що лише високі концентрації гормону (2000-1000 нг/мл) спричиняли пригнічення їх проліферації на 28-20% в поживному середовищі з високим вмістом глюкози і на 18-12% в середовищі з нижчою концентрацією глюкози, в порівнянні з контролем. Дослідження особливостей поглинання глюкози клітинами LNCaP в присутності інсуліну показало, що LNCaP в присутності 2000-125 нг/мл інсуліну метаболізують глюкозу на 32,5-17,3% інтенсивніше ніж інтактні клітини ($p < 0,05$). Аналіз кількості метаболізованої глюкози в культуральному середовищі з клітин Du-145 показав, що лише високі концентрації інсуліну (2000-1000 нг/мл) статистично достовірно підвищують кількість поглинутої клітинами глюкози на 34-32%, в порівнянні з контролем. Культивування Du-145 в присутності 30 нг/мл інсуліну призводило до статистично достовірного зменшення кількості поглинутої глюкози на 23%, в порівнянні з контролем.

Висновки: В гормон-чутливих клітинах LNCaP на рівні тенденції була відмічена більша кількість FITC-інсулін⁺ клітин та вищий рівень експресії рецепторів інсуліну, ніж в гормон-рефрактерних клітинах Du-145. Інсулін в усіх досліджених концентраціях підвищував проліферативну активність клітин LNCaP, але в клітинах Du-145 високі концентрації гормону призводили до пригнічення проліферації клітин. На відміну від клітин LNCaP, лише високі концентрації інсуліну суттєво підвищували кількість поглинутої глюкози клітинами Du-145.

**ОСОБЛИВОСТІ ЕКСПРЕСІЇ РЕЦЕПТОРА ІНСУЛІНУ В КЛІТИНАХ РАКУ
МОЛОЧНОЇ ЗАЛОЗИ ЛЮДИНИ З РІЗНИМ СТУПЕНЕМ МЕДИКАМЕНТОЗНОЇ
РЕЗИСТЕНТНОСТІ ДО ДОКСОРУБІЦИНУ**

Назар Видасов

Poster 4

Видасов Н.В., Лихова О.О., Безденежних Н.О., Козак Т.П., Чехун В.Ф.

*Інститут експериментальної патології, онкології та радіобіології
ім. Р.С. Кавецького НАН України, Васильківська 45, 03022, Київ, Україна*

E-mail: vydasov.nazar@gmail.com

Проблема медикаментозної резистентності пухлинних клітин до дії цитостатиків (ЦС) досі залишається не вирішеною. Особливість метаболізму глюкози пухлинними клітинами є однією з основних ознак, які впливають на чутливість пухлинних клітин до дії ЦС. Однак, наскільки змінюється метаболізм пухлинної клітини з різним рівнем медикаментозної резистентності до дії ЦС є до кінця не вивченим. Найважливішим гормоном, що відповідає за засвоєння глюкози клітиною є інсулін. Інсуліновий рецептор (PI) відіграє ключову роль в регуляції гомеостазу глюкози, функціонального процесу, який при дегенеративних умовах може призвести до ряду клінічних проявів, в тому числі діабету і раку. З огляду на це, важливо дослідити особливості експресії PI в пухлинних клітинах з різним ступенем медикаментозної резистентності до ЦС та встановити як діє інсулін на експресію PI та проліферативну активність таких клітин. У роботі використовували клітини аденокарциноми молочної залози людини вихідної лінії MCF-7/S та клітини з різним ступенем медикаментозної резистентності до доксорубіцину: MCF-7/Dox(2) – рівень резистентності 2 та MCF-7/Dox(4) – рівень резистентності 4. Для досліджень застосовували молекулярні методи та методи культури клітин. Всі дослідження проводили за умов культивування клітин в поживному середовищі з низьким вмістом (0,5%) фетальної сироватки. Інсулін людини кон'югований з FITC в комплексі з проточною цитофлуориметрією використовували для дослідження експресії PI в пухлинних клітинах. Не було виявлено статистично достовірної різниці між кількістю FITC-інсулін⁺ клітин в популяції чутливих клітин лінії MCF-7/S та клітин з фенотипом медикаментозної резистентності до доксорубіцину. Культивування досліджених клітин (MCF-7/S, MCF-7/Dox(2) та MCF-7/Dox(4)) в присутності інсуліну (2000 нг/мл та 20 нг/мл) також суттєво не впливало на кількість FITC-інсулін⁺ клітин в популяції. Однак, оцінка рівня експресії PI в досліджених клітинах показала, що за умов їх культивування в середовищі з низьким вмістом ростових факторів джерелом яких є фетальна сироватка, в клітинах MCF-7/Dox(4) рівень експресії PI статистично достовірно нижчий в 3,2-3,7 рази, ніж в клітинах ліній MCF-7/S та MCF-7/Dox(2). Було показано також, що культивування клітин MCF-7/S та MCF-7/Dox(2) в присутності 2000 нг/мл інсуліну призводило до статистично достовірного підвищення рівня експресії PI на 50,3% та 27,6%, відповідно, в порівнянні з контрольними інтактними клітинами цих ліній. В клітинах MCF-7/Dox(4) інсулін не впливав на рівень експресії PI. Відомо, що чутливість клітин до інсуліну залежить перш за все від кількості PI та їх активності. Представлені вище дані корелюють з результатами аналізу проліферативної активності досліджених клітин. Було показано, що інсулін у високих концентраціях (2000 нг/мл) пригнічує проліферативну активність клітин вихідної лінії MCF-7/S на 55%, клітин MCF-7/Dox(2) на 35% і суттєво не впливає на проліферацію клітин MCF-7/Dox(4). Отже, саме в клітинах з вищим ступенем резистентності до ЦС (MCF-7/Dox(4)), за умов їх культивування в середовищі з низьким вмістом ростових факторів, спостерігали значно нижчий рівень експресії PI ніж в клітинах MCF-7/S, і відповідно, меншу чутливість резистентних клітин до інсуліну.

IMPACT OF SELENOMETHIONINE AND D-PANTETHINE ON THE FUNCTIONAL STATUS OF THE GLUTATHIONE SYSTEM IN HUMAN PSEUDONORMAL CELLS TREATED WITH DOXORUBICIN *IN VITRO*

Yuliya Kozak

Poster 5

Yuliya Kozak¹, Rostyslav Panchuk¹, Nadia Skorokhyd¹, Dmytro Semenovych², Andriy Moiseenok², Rostyslav Stoika¹

1 – Institute of Cell Biology, National Academy of Sciences of Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine

2 – Institute of Biochemistry of Biologically Active Compounds, NAS of Belarus, Leninskogo Komsomola Boulevard 50, Grodno, 230030, Republic of Belarus

E-mail: juliana.kozzak@gmail.com

One of the main problem of modern chemotherapy is rapid development of multiple drug resistance (MDR) of tumor cells leading to ineffective treatment. In our previous studies we have shown that antioxidants selenomethionine (SeMet) and D-Pantethine (D-Pt) enhanced cytotoxic effect of doxorubicin (Dx) by 20%-30% toward drug-resistant malignant cells ($P \leq 0.05$). This can be explained by the ability of these antioxidant compounds to reduce the level of reduced (GSH) glutathione and glutathione-S-transferase activity (GST) which are necessary for work of ATP-binding cassette transporters (ABC-transporters). Also SeMet and D-Pt inhibited cytotoxic action of Dx towards pseudonormal cells, sensitive to chemotherapy treatment, by 15%-30% ($P \leq 0.05$). However, the molecular mechanisms underlying such features of SeMet and D-Pt remain poorly understood.

The aim of our work was to investigate the impact of selenomethionine and D-pantethine on the functional status of the glutathione system in human keratinocytes of HaCat line under doxorubicin treatment.

It was found that the basal GSH level in human pseudonormal cells was 9.4 nmol/mg protein. At the same time the basal level of oxidized glutathione (GSSG) was lower by 3.1-fold. Thus, typical GSH/GSSG ratio in human keratinocytes is 3.1:1. Treatment of cells with Dx led to statistically significant 1.7-fold decrease of the GSH level ($P \leq 0.01$) and 2.4-fold increase of GSSG level ($P \leq 0.001$) compared to these parameters in untreated pseudonormal cells. This leads to imbalance in GSH/GSSG ratio and the development of oxidative stress in studied cells. We have shown that a combined effect of SeMet or D-Pt on the background of the cytotoxic action of doxorubicin on HaCat cells is accompanied by 1.4-fold ($P \leq 0.05$) and 1.6-fold increase of GSH level ($P \leq 0.01$), correspondingly with a simultaneous 1.5-fold and 2.5-fold decrease of GSSG level ($P \leq 0.001$); correspondingly, in human keratinocytes compared to these parameters in Dx-treated cells.

We didn't observed significant changes in the activity of glutathione-S-transferase (GST) in this study. But the activity of glutathione peroxidase (GP) was increased 1.3-fold ($P \leq 0.001$) under Dx action in human keratinocytes, and it was recovered under the action of this drug in a combination with SeMet or D-Pt thus leading to decrease in GSSG level. Dx treatment was accompanied by a 1.8-fold decrease ($P \leq 0.001$) in the activity of glutathione reductase (GR) in cells of HaCat line. Both SeMet or D-Pt effectively normalized GR activity to a basal level, thus leading to increase in GSH level.

Thus, the studied antioxidants reduce Dx-induced oxidative stress in pseudonormal cells of HaCat line by normalizing the activity of GPx and GR and stabilizing the GSH/GSSG ratio.

**IMMUNOPHENOTYPIC CHARACTERISTICS OF HUMAN B LYMPHOCYTIC
CELL LINES**

Alena Duzh

Poster 6

Alena V. Duzh, Andrei Y. Hancharou

*Institute of Biophysics and Cell Engineering of National Academy of Sciences of Belarus,
Belarus, Minsk, Akademicheskaya st., 27*

E-mail: lenaduzh@gmail.com

Background: Hematopoietic B-cell lines are widely used in virology, immunology, oncology and immunopharmacology research. The application of immortalized b-cell lines is one of the more promising approaches to conducting experimental preclinical studies of new immunomodulatory drugs. This will allow to exclude the use of laboratory animals along with the opportunity to standardize the method and significantly increase its reproducibility and productivity.

Methods: 5 immortalized B-lymphocyte cell lines were used: Daudi (human, Burkitt's lymphoma), IM-9 (human, myeloma), Raji (human, Burkitt's lymphoma), RPMI-1788 (human, peripheral blood leukocytes of a healthy donor) and CCRF-SB (human, acute lymphoblastic leukemia). Cell lines obtained from cryobanks were cultured in the RPMI-1640 medium supplied with 10 % FCS, L-glutamine, sodium pyruvate, hepes and gentamycin. The expression of the following molecules was determined: CD1c, CD5, CD10, CD11b, CD11c, CD14, CD15, CD19, CD23, CD27, CD33, CD40, CD43, CD45, CD54, CD62L, CD69, CD79a, CD80, CD86, CD273, CD274, HLA-DR, HLA-ABC, kappa-, lambda- chains.

Results: An extended analysis of expression of surface molecules in human hematopoietic B-cell lines was conducted. B-lymphoid line, which was the most suitable for screening and testing substances with immunodulating properties, was determined. The results show that cell line Daudi express the most comprehensive set of surface markers to assess the immunobiological properties of drugs. This line was selected for future studies.

Conclusion: Daudi cell lines were selected as a B-cell model for further research in the field of testing of immunomodulatory drugs.

ЕКСПРЕСІЯ КО-ІНГІБІТОРНИХ МОЛЕКУЛ СІМЕЙСТВА В7 ДЕНДРИТНИМИ
КЛІТИНАМИ ХВОРИХ НА РАК ПІДШЛУНКОВОЇ ЗАЛОЗИ

Оксана Тимохіна

Poster 7

Гончаров А.Є.¹, Тимохіна О.В.¹, Антоневіч Н.Г.¹, Прохоров О.В.^{1,2}, Романовська С.Є.²

1 – Інститут біофізики і клітинної інженерії Національної академії наук Білорусі; Білорусь, Мінськ, вул. Академічна, 27

2 – Білоруський державний медичний університет; Білорусь, Мінськ, пр. Дзержинського, 83

E-mail: andrei.hancharou@gmail.com

Обґрунтування. Експресія як ко-інгібіторних, так і коstimуляторних молекул є ключовою особливістю антигенпрезентуючих клітин, які дозволяють ініціювати або пригнічувати активацію Т-клітин. Серед 9 членів родини В7 є як коstimуляторні, так і інгібіторні молекули. Канонічні коstimуляторні молекули включають В7.1 (CD80) і В7.2 (CD86), тоді як серед молекул, які зазвичай вважаються ко-інгібіторами, є 7 білків, які в першу чергу пов'язані з придушенням імунної відповіді: В7-DC (CD273), В7-Н1 (CD274), В7-Н2 (CD275), В7-Н3 (CD276), В7-Н4, В7-Н5, В7-Н6 і В7-Н7. В той же час результируючий ефект взаємодії молекул В7 залежить від зв'язуючих лігандів (наприклад, CD80 з CD28 проти CD152). Надмірна експресія ко-інгібіторних молекул може бути пов'язана з посиленими толерогенними властивостями дендритних клітин (ДК). Метою дослідження було оцінити експресію ко-інгібіторних молекул ДК родини В7 від хворих на рак підшлункової залози (РПЗ).

Методи. Досліджено зразки крові 14 хворих на РПЗ II-III стадії і 9 здорових добровольців (К). Культивуванням моноцитів з ГМ-КСФ і ІЛ-4 отримані ДК. Експресію CD80 (клон МЕМ-233), CD86 (клон FUN-1), В7-DC (CD273) (клон МІН18), В7-Н1 (CD274) (клон МІН1), В7-Н2 (CD275) (клон 2D3 / В7-Н2), В7-Н3 (CD276) (клон FM276), В7-Н4 (клон МІН43), В7-Н5 (клон МІН65), В7-Н6 аналізували за допомогою методу проточної цитометрії. Визначали як відсоток позитивних клітин (%), так і відносну інтенсивність флуоресценції (RFI). Лінію клітин Сасо-2 (ATCC® HTB-37™) використовували в якості позитивного контролю для експресії CD275, CD276, В7-Н4 і В7-Н5. Для аналізу результатів застосовували методи непараметричної статистики.

Результати. Експресія коstimуляторних молекул – CD80 і CD86 на зрілих ДК, генерованих від хворих на РПЗ, не мала суттєвих відмінностей від К. У пацієнтів з РПЗ 23,6 (20,6-38,3)% ДК експресували молекулу CD273, що в 3 рази вище, ніж на ДК від К ($p = 0,03$). У той же час інтенсивність експресії В7-DC не мала статистично достовірних відмінностей від К. Інтенсивність експресії CD274 на зрілих ДК від пацієнтів з РПЗ була в 2 рази більшою, ніж у К, а кількість CD274⁺ клітин в 3,5 рази вище, ніж на ДК, генерованих з крові донорів ($p = 0,02$). Експресія молекули CD276 становила 100% на ДК обох груп. Не було виявлено достовірних відмінностей в експресії регуляторної молекули CD275 на ДК обох груп. Експресія В7-Н4, В7-Н5 і В7-Н6 була відсутня в більшості культур ДК. Лише 2 зразки ДК від хворих на РПЗ експресували В7-Н4 (2,6 % і 3,1 %) і 2 інші зразки ДК мали помітну експресію В7-Н5 (3,0 % і 4,2 %). В7-Н6 виявлено тільки в одному зразку ДК (2,6 %).

Висновки. CD80, CD86 і CD276 є конститутивними для зрілих ДК, тоді як експресія CD273, CD274 і CD275 була присутня у відносно низьких кількостях. Експресія В7-Н4, В7-Н5 і В7-Н6 також була присутня в деяких зразках ДК від хворих на РПЗ. Експресія цих молекул ДК у пацієнтів з РПЗ може знизити імуногенний потенціал клітин. Незважаючи на те, що кардинальних відмінностей в експресії коінгібіторних молекул сімейства В7 на ДК пацієнтів з РПЗ і донорів не було виявлено, патерн експресії коінгібіторних молекул передбачає посилений толерогенний потенціал ДК хворих на РПЗ. У подальших дослідженнях планується встановити залежність імунофенотипу ДК і їх ефективності в лікуванні РПЗ.

**THE IMPACT OF ARGININE LIMITATION ON CELL VIABILITY AND
PI3K/AKT/MTORC1 SIGNALING PATHWAY IN COLON CARCINOMA CELLS WITH
KNOCK OUT OF TSC2 GENE**

Serhii Chorny, Yaroslav Bobak, Oleh Stasyk

*Department of Cell Signaling, Institute of Cell Biology, National Academy of Science of Ukraine,
Drahomanov Street 14/16, 79005 Lviv, Ukraine*

E-mail: chorny.serhii95@gmail.com

Many aggressive cancer types in course of malignant transformation become auxotrophic for arginine. Subsequently, deprivation of arginine with arginine-degrading enzymes has been adopted as a promising anticancer therapy. Reduction of arginine availability alone is not sufficient to eradicate malignant cells in an organism but increases their sensitivity to conventional anticancer drugs with various cytotoxic mechanisms. Alternative approach is to target signalling pathways, which are involved in cancer cell response to arginine limitation and determine cellular sensitivity to the lack of arginine. The goal of the project was to investigate how direct impact on mTORC1 signalling pathways will change the behaviour of cancer cells under arginine starvation condition to gain new knowledge on how mTORC1 status in colorectal carcinoma cells affects cells response to arginine deprivation therapy and whether mTOR or TSC2 (inhibitor of mTORC1) may be utilized as predictive markers of sensitivity to this metabolic therapy.

For this project we generated a colorectal carcinoma cell model with hyperactivated mTORC1. It's known that TSC2 protein is one of the most important inhibitors of mTOR. The technology of gene knocking out (CRISP/Cas9) was used to remove the TSC2 gene. Cells were cultured in the arginine-supplied (complete) or in the arginine-deplete medium. The sensitivity of these cell lines to the arginine withdrawal was analyzed by Western blot and WST cell viability assay.

As was expected, TSC2 knock out cell lines cannot deactivate mTORC1 under serum withdrawal. Unfortunately, no significant changes in sensitivity to arginine deprivation therapy in the generated cell line compared to the wild-type cell line were detected. Also, we found interesting specific changes in Akt signalling under arginine deprivation (48-72h) in TSC2 knock out cell lines but not in the wild-type cell line. Additional canavanine treatment led to an even stronger decrease in Akt signalling.

KNOCKDOWN OF ADAPTOR PROTEIN RUK/CIN85 IN LEWIS LUNG CARCINOMA CELLS IS FOLLOWED BY MESENCHYMAL-EPITHELIAL TRANSITION ASSOCIATED WITH ATTENUATION OF TUMOR GROWTH AND BLOCKAGE OF PULMONARY METASTASIS *IN VIVO*

Tetyana Skaterna, Denys Gerashchenko, Dmytro Shytikov, Olga Khudiakova, Iryna Horak,
Liudmyla Drobot

*Palladin Institute of Biochemistry, National Academy of Science of Ukraine,
9 Leontovycha str., Kyiv, 01601, Ukraine*

E-mail: scaterna.t@ukr.net

Lung cancer is a common cause of cancer mortality associated with distant metastases. Progress in metastatic research, however, is constrained by the lack of tumor-bearing animal models that would allow to comprehensively understanding the complex network of signaling pathways that drives the multistep process of metastatic cascade. Adaptor/scaffold proteins are key regulators able to effectively process the information through signaling networks. It was shown previously that up/down regulation of adaptor protein Ruk/CIN85 in murine breast adenocarcinoma cells is followed by epithelial-to-mesenchymal/mesenchymal-to-epithelial transition (EMT/MET) respectively associated with reversible control of their malignancy features. In addition, we found high amounts of this adaptor in aggressive Lewis lung carcinoma cells (LLC cells). In the current study, we set a goal to determine interplay between Ruk/CIN85 down-regulation in LLC cells and their MET state as well as metastatic potential using experimental and spontaneous metastasis models in syngeneic C57BL/6 mice.

To down-regulate Ruk/CIN85, LLC cells were stably infected with lentivirus encoding Ruk/CIN85-specific shRNA as well as irrelevant virus to obtain control cells. The expression levels of Ruk/CIN85 in LLC cells and primary tumors were assessed by Western-blotting and qRT-PCR. The influence of Ruk/CIN85 down-regulation on the morphology of LLC cells was studied by confocal microscopy. The expression levels of specific EMT markers were evaluated by qRT-PCR. To estimate efficiency of experimental and spontaneous metastasis, C57BL/6 mice were inoculated intravenously or subcutaneously into right hind leg with control and Ruk/CIN85 knockdown LLC cells. Primary tumors and lungs were processed for histological evaluation according to standard protocol. Statistical analysis was carried out using ANOVA with Newman-Keuls correction.

It was demonstrated that Ruk/CIN85 knockdown LLC cells acquired a more epithelial phenotype associated with gene expression patterns characteristic of MET. Especially, the results of qRT-PCR analysis showed that Ruk/CIN85 down-regulation in LLC cells led to decrease in expression levels of EMT markers such as transcription factors Zeb1, Zeb2 and Snail1, decrease of mesenchymal marker vimentin and simultaneous increase of epithelial marker E-cadherin. In addition, the pulmonary metastasis was almost completely eradicated for Ruk/CIN85 down-regulated LLC cells both in experimental and spontaneous metastasis models *in vivo*.

According to changes in cell morphology and qRT-PCR data, we suggest that the suppression of aggressiveness of Ruk/CIN85 knockdown LLC cells was associated with mesenchymal-to-epithelial transition.

**CARDIOMYOPATHY IN RATS WITH WALKER 256 CARCINOSARCOMA:
GENERATION OF REACTIVE OXYGEN SPECIES INDUCES DAMAGE OF
CARDIOMYOCYTES**

Hudenko N.V.¹, Sarnatskaya V.V.¹, Paziuk L.M.², Yusko L.O.¹, Maslenny V.N.¹, Nikolaev V.G.¹

1 – RE Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the NAS of Ukraine, Vasylkivska 45, Kyiv 03022, Ukraine

2 – Department of Cytology, Histology and Developmental Biology, Educational and Scientific Centre “Institute of Biology & Medicine”, Taras Shevchenko National University of Kyiv, Kyiv 03608, Ukraine

E-mail: hudenkonataliia@gmail.com

Malignant tumors exert systemic influence on an organism and therefore could cause the development of paraneoplastic syndrome, which could be represented by complex changes of structure and functions of some organs, anemia and endogenous intoxication. The latter could be significantly affected by oxidative stress via activation of free radicals and imbalanced rate of generation of compounds with anti- or pro-oxidant properties. In the majority of cells defense enzymes of antioxidant system (superoxide dismutase, catalase and glutathione peroxidase) reduce to minimum the damaging action of these factors, but the pool of endogenous antioxidants of cardiomyocytes is very limited and couldn't provide adequate reaction to induced oxidative stress what finally leads to degeneration and descent of cardiac muscle contractility. In this study, we have analyzed the myocardium damage caused by enhanced production of reactive oxygen species (ROS) due to the W256 progression at the background of gradual weakening of antioxidant defense of the body. The studies were performed on female Wistar rats with an average weight of 185.0 ± 13.8 g and the age of 2.5 months. After W256 transplantation, rats were distributed in 6 groups (5 rats per group) corresponding to the day after W256 transplantation, when they have been examined (1st day, 2nd day, 3rd day, 5th day, 7th day, 9th day). As intact control, healthy animals were used, the age, gender and weight of which fully corresponded to the experimental rats at the start of the study (n = 5). We detected the two-fold increase of ROS generation in blood plasma of experimental animals starting from the second day after W256 transplantation. At the same time, a gradual decrease of catalase activity, the index of body's antioxidant defense, was also noted - from 10.0 mmol/ml/min on the 1st day up to 7.3, 6.1 and 4.3 mmol/ml/min on the 3rd, 5th and 9th days, respectively. Dynamics of changes in morphological structure of myocardium in rats showed that on the 1st day after W256 transplantation it was equal to the control group of intact animals. At the 3rd and 5th days after transplantation the syncytial structure of the myocardium is preserved, places of dystrophic changes are detected, while the amount of fibrous connective tissue of stroma increases with the development of full-blooded vessels. On the 5th day after transplantation, the flattening and reducing of the nuclei of cardiomyocytes were noted, and the majority of nucleus had sharpened contours. Also, there was observed violation of microcirculation in a form of a sludge phenomenon and permeation of muscle fibers with erythrocytes. At the 7th and 9th days, the syncytial structure was broken and the areas of myocytolysis were detected. Polymorphism and anionic nucleosis of cardiomyocytes were intensified. We noted the presence of small vacuoles, which were diffusely located in the sarcoplasm of cardiomyocytes.

Conclusion: W256 progression induce the myocardial disorganization and degenerative changes in cardiomyocytes since the 3rd day of tumor transplantation.

A COMPARISON OF THE DIELECTRIC PROPERTIES OF HEMOGLOBIN BEFORE AND AFTER RADIOTHERAPY

Liliya Batyuk¹, Volodymyr Berest²

1 – Department of Medical and Biological Physics and Medical Information Science, Kharkiv National Medical University, 4 Nauky Avenue, Kharkiv, Ukraine, 61022

2 – Department of Molecular and Medical Biophysics, V.N. Karazin Kharkiv National University

E-mail: liliya-batyuk@ukr.net

As known, the hemoglobin performs the vital function of carrying oxygen from the lungs to the tissues and facilitates the transport of carbon dioxide from the tissues to the lungs. The dielectrically permittivity of red blood cells has been found to depend on the hemoglobin and water content, temperature and the frequency relaxation of molecules of water in cells (Farsaci F., Ficarra S., Galtieri A., et al., 2017). In general each cell contains an intracellular fluid surrounded by a membrane. The packed blood cells have therefore been known to behave as a heterogeneous medium. In the radio frequency range the dispersion exhibited by such a medium has often been explained by the Maxwell-Wagner model and it has been suggested that it originates mainly from the polarization effects in which the cellular membranes are charged through the electrolytes (Batyuk L., Kizilova N., 2018).

In this paper the dielectric properties of aqueous hemoglobin solutions of donors and oncological patients are investigated under a variety of parameters, and the results are discussed in terms of the most probable orientation polarization. The study used the blood of 10 donors and 10 cancer patients with breast cancer. The group received postoperative radiation therapy in the mode of classical fractionation, a single focal dose per tumor was 6 Gy, and a total focal dose was 45 Gy. Blood sampling was performed before and after irradiation. The human hemoglobin was prepared by method (Casimir W. V., Kaiser N., Keilman F., et al., 1968). All measurements were done using UHF-dielectrometry method the temperature dependencies of the dielectric permittivity for water molecules and the relation of free and bound water. The complex dielectric constant of aqueous hemoglobin solution was measured at frequency 9.2 GHz. The temperature was varied from 20 to 37 °C. For all samples linear dependence of both real and imaginary part of dielectric constant of hemoglobin solution on concentration was found. The shift in relaxation wavelength and hydration ratios was calculated. We found that the frequency of relaxation time the molecule is determined mainly by the type of procedure of radiotherapy and by the viscosity of the solution, which is also responsible for the temperature and concentration dependence.

1. Farsaci F., Ficarra S., Galtieri A., et al. *Fluids*. 2017, 2:59
2. Batyuk L., Kizilova N. *Development trends in medical science and practice: the experience of countries of Eastern Europe and prospects of Ukraine: monograph / edited by authors*. 2018, 18-37.
3. Casimir W. V., Kaiser N., Keilman F., et al. *Biopolimer*. 1968, 6:1705-1715.

MATHEMATICAL APPROACHES TO THE STUDY OF THE DISTRIBUTION OF OXYGEN IN HETEROGENEOUS TUMOR TISSUES

Liliya Batyuk¹, Natalya Kizilova², Hanna Chovpan³

1 – Department of Medical and Biological Physics and Medical Information Science, Kharkiv National Medical University, 4 Nauky Avenue, Kharkiv, Ukraine, 61022

2 – Warsaw University of Technology, Warsaw, Poland

3 – Department of Medical and Biological Physics and Medical Information Science, Kharkiv National Medical University, 4 Nauky Avenue, Kharkiv, Ukraine, 61022

E-mail: liliya-batyuk@ukr.net

Cancer is a complex process that involving many different cell types. Traditionally, mathematical models of cancer growth fall into two broad camps: descriptive and mechanistic (Araujo R.P. et al., 2004), (Kozusko F. et al., 2007). Descriptive models tend to focus on reproducing the gross characteristics of tumours, such as size and cell number. The mechanistic models focus on specific aspects of tumour progression (Batyuk L., Kizilova N., 2018), (Ponomarenko N., Batyuk L., et al. 2014). The mathematical model of cancer growth includes three steps. The first is the modeling of vasculature formation by sprouting angiogenesis. Second is fluid flow in interstitial space, and third is blood flow through vasculature and solute transport in interstitium. As known, tissue is heterogeneous, consists of cells and extracellular spaces. These heterogeneities affect the distribution of oxygen in the tissue. A model of tumor tissue composed of plane layers with different diffusion characteristics was proposed (Batyuk L.V., Kizilova N.N. et al., 2017). The Krogh tissue cylinder model of oxygen transport between blood capillaries and tissue has served as the foundation and starting point for many theoretical studies (Kreuzer F. et al. 1983). It has also been broadly used in physiological studies for estimating oxygen distribution in tissue. Tumors especially dangerous after they induce blood vessel growth. The new vessels of blood can not only carry oxygen and nutrition that further facilitate tumor growth but help also spreading for tumor cells to spread to other it's of the body. Thus tumor growth can be divided into two phases: avascular and vascular growth. We investigate how growth of tumor is related to the oxygen concentrations in the environment that tumor lives in. Oxygen concentration distribution is governed by the diffusion-consumption equation.

The above analysis is the preliminary step of the role of the parameters of the model upon the asymptotic behavior of the solutions. Using such a model, we tested two saturation hypotheses for avascular tumor growth separately. *In vivo*, it should be a combination of the mechanisms that all together contribute to the stop of growth, and we are on the way of putting them together.

1. Araujo R.P. et al. Bull. Math. Biol. 2004, 66:1039-1091.
2. Kozusko F. et al. Cell Prolif. 2007, 40:824-834.
3. Batyuk L., Kizilova N. AS Cancer Biology. 2018, 2(10):55-60.
4. Ponomarenko N., Batyuk L. et al. Visnyk of the Lviv University. Series Biology. 2014, 68:263-268.
5. Batyuk L.V., Kizilova N.N. et al. International research and practice conference «Innovative technology in medicine: Experience of Poland and Ukraine». 2017:158-161.
6. Kreuzer F. et al. Adv. Exp. Med. Biol. 1983, 3:159.

**ARGININE ANALOGUES AS COMPONENTS OF ANTICANCER THERAPY BASED ON
ARGININE DEFICIENCY**

Galyna Shuvayeva, Yaroslav Bobak, Olena Vovk, Yuliya Kurlishchuk, Oleh Stasyk

*Institute of Cell Biology, National Academy of Sciences of Ukraine, Drahomanov Street 14/16, 79005
Lviv, Ukraine*

E-mail: shuvayeva77@gmail.com.ua

Deprivation for the single amino acid arginine is a rapidly developing metabolic anticancer therapy, which allows growth control in a number of highly malignant tumors. The combinative therapy approach may increase effectiveness of the treatment. The aim of the work was to study cancer cells response to impact of natural and synthetic arginine analogues combined with arginine deprivation.

In this study we first analyzed effects of non-toxic concentrations of canavanine or thioarginine, known natural and synthetic arginine analogues, respectively, as well as indospicine-containing extract of the plant *Indigofera spicata* for different tumor cell models. It was shown that arginine starved cells of human colon HCT-116 and human ovarian SKOV3 adenocarcinomas treated with the mentioned arginine analogues are characterized by rapid loss of proliferative potential and cell death. It is known that viability and cell proliferation are largely determined by the regulation of MAPK and AKT/mTOR signaling pathways. Impairment of these signaling cascades in cancer cells under arginine deficiency combined with selectively toxic amino acid analogues may affect the cell vitality. We have found that incubation of cells in this experimental condition for 72 hours is accompanied by MAPK signaling disregulation, namely inactivation of proliferative ERK and activation of proapoptotic p38 MAPK. There was also a significant decrease in the level of AKT phosphorylation, whereas mTOR activity remained unaffected. This mTOR activity provides the maintenance of general translation machinery and the incorporation of arginine analogues into synthesized *de novo* proteins rendering them abnormal and triggering induction of endoplasmic reticulum stress. As a result, caspase-dependent apoptosis is induced.

Our data suggest that the abnormal regulation of the key pro-survival signaling pathways such as MAPK, AKT/mTOR cascades and the induction of ER stress cause a selective decrease in cell viability in malignant cells under combined treatment of arginine deprivation and low doses of arginine analogues. The revealed features form a basis for further development of this metabolic anticancer therapy.

This work was supported in part by the grant №F76/51-2018 of State Fund for Fundamental Research of Ukraine.

THE ROLE OF ER STRESS RESPONSE IN HNSCC CELLS UPON ARGININE DEPRIVATION

Chen O.^{1,2}, Manig F.², Lehmann L.², Sorour N.², Dubrovskaya A.², Kunz-Schughart L.², Stasyk O.¹

1 – Institute of Cell Biology, National Academy of Sciences of Ukraine, Drahomanov Street 14/16, 79005 Lviv, Ukraine

2 – OncoRay – National Center for Radiation Research in Oncology, Faculty of Medicine and University Hospital Carl Gustav Carus, TU Dresden, Fetscherstr. 74, PF 41, 01307 Dresden, Germany

E-mail: oleh.chen@gmail.com

Arginine deprivation therapy (ADT) is a relatively new metabolic type of treatment for numerous solid cancers with high therapeutic potential. Previously, it has been shown that low concentrations of the natural proteomimetic arginine (Arg) analog – canavanine (Cav) strongly intensify the cytotoxic effect of ADT in a broad spectrum of cancer cells. In the present study, we examined the mechanistic role of endoplasmic reticulum (ER) stress response in head and neck squamous cell carcinoma (HNSCC) cells upon ADT alone or combined with Cav. We showed for the first time that ADT alone triggers massive and long-term ER stress response mainly via IRE1-sXBP1 and eIF2 α -ATF4 signaling pathways much more pronounced in sensitive to ADT cancer cells. However, Cav induced catastrophic ER stress in all tested HNSCC upon ADT, which was associated with cancer cell apoptosis. Our gene expression analysis revealed that the levels of ER stress marker genes were dramatically up-regulated. It was shown that ER stress is accompanied by strong up-regulation of *IRE1*, *GADD34*, *ATF4* and *CHOP* genes, which are critical for ER stress-induced apoptosis in human HNSCC cells. siRNAs-mediated knockdowns of these genes blocked Cav-induced apoptosis upon ADT in human HNSCC cells. Moreover, the inhibitor of ER stress-induced apoptosis – salubrinal strongly protected HNSCC cells against Cav-dependent cell death upon ADT. We confirmed that IRE1-sXBP1 pathway was not involved in apoptosis induction upon ADT with Cav. Apparently, activated IRE1-sXBP1 pathway rather mediates transcription of chaperone-encoding genes, whose products have a role in ER protein folding machinery to eliminate abnormal proteins from the ER lumen. Altogether, our findings suggest that Cav induces ER stress-mediated cancer cell death upon ADT via eIF2 α -ATF4(GADD34)-CHOP signaling pathway. We propose that combination of ADT with low doses of Cav and possibly other ER stress modulators is a feasible treatment approach for solid cancer cells.

Keywords: ADT, ER stress, arginine, canavanine, HNSCC

Bobak et al. *Int J Biochem Cell Biol*, 2016, 70: 29-38.

Chen et al. *Curr Med Chem*, 2018, 25(21):2465-2502.

**THE MATHEMATICAL MODELING OF THE QUANTITATIVE RELATIONSHIP
BETWEEN INCREASING THE SURVIVAL RATE OF ONCOLOGICAL PATIENTS AND
THE GROWTH OF CANCER MORBIDITY**

Vladimir Knigavko, Liliya Batyuk, Olga Zaytseva, Marina Bondarenko

*Department of Medical and Biological Physics and Medical Information Science,
Kharkiv National Medical University, 4 Nauky Avenue, Kharkiv, Ukraine, 61022*

E-mail: vkni@mail.ru

The issue of assessing the dependence of oncological morbidity on the probability of cure of such a disease is considered in this paper. It is considered that malignancy of cells requires damage to a certain number of certain genes (Lopez-Lazaro M., 2010), (Knigavko V. et al., 2014). Since the damage to one or another gene is accidental, cell malignancy for the same initial (from birth) gene numbers in the genotype should occur at different times of time with varying probabilities. Obviously, due to the randomness of the mutation process, the initial number of these genes in the genotypes of different people is different. Thus, the less unharmed such genes are in the genotype of the individual, the greater is the likelihood of his oncological disease occurring in him. To solve such a question, the method of mathematical modeling was used. Let α is the probability of damage to some a-gene in one cycle of division, n is the number of cycles of division from the moment of formation of this tissue, k is the initial number of a-genes in the cell, $t = n \cdot T$, where T is the duration of the cell cycle. Let also m is the initial number of a-genes in gametes, $\varphi(m)$ is the probability that m a-genes are contained in the gamet in the beginning of the reproductive cycle in gametes, $\psi(m)$ is the probability that at the end of the reproductive cycle in gametes contains m a-genes, $s(t)$ is the function of distribution of the life expectancy of those individuals who do not have cancer; $v(\tau)$ is the density of the probability of reproduction time, $\Phi(m,t)$ and $F(k,t)$ - the function of distribution of the time of the formation of a malignant tumor in the initial presence in gametes m a-genes and time the formation of a malignant tumor with an initial presence in the genotype of individuals and k a-gene, respectively, L - probability cure cancer. At first, the probability that gamete, originally having 4 genes subject to malignancy, was preserved during the reproductive cycle was funded. Let's denote this probability $P_{4 \rightarrow 4}$. To preserve such a gamete, it is necessary that the mutations do not damage any of these genes, so that the life expectancy of the individual containing this gamete is greater than the duration of the reproductive period. Necessary also that the individual with such a gamete has not acquired or acquired an oncological illness during the reproductive period. The probability that the reproduction will occur at the time t (more precisely, in the interval of time from t to $t + dt$) is equal to $v(t)dt$. The probability that an individual is still alive by this moment is equal to $1 - s(t)$. The probability that by the time t , none of the a-genes in the gamete considered is not damaged is equal to $\beta^{\frac{4t}{T}}$. The probability that an individual with a gamete to be considered will not acquire at the time of the oncological illness is equal to $1 - \Phi(4,t)$. The probability that this individual will acquire an oncological illness before that time, but then cured, is equal to $\Phi(4,t) \cdot L$.

In the experiment, a formula is obtained that allows us to calculate the mean value of the time (\bar{t}_k) of occurrence in an individual oncologic disease in the initial presence in the genotype of the individual k -genes. The solution of this system of equations for the determination of probabilities $\varphi(m)$ allows, in turn, to calculate the probability values ($\xi(k)$) of the presence of k a-genes in the individual in the genotype. Thus, with known values of $\xi(k)$, the average time (\bar{t}) of appearance in an individual oncologic disease in the general case is calculated by the following formula:
$$\bar{t} = \sum_{k=1}^8 \bar{t}_k \cdot \xi(k)$$

In terms of assessing the dependence of oncological morbidity on the probability of cure of such a disease the probability of occurrence of cancer to a certain age of a person should be considered as major indicator. Obviously, the probability of preserving gametes depends on the time of reproduction and on the length of life of the individual. Factors that affect life expectancy can be divided into those that are related to cancer patients and those that are not associated with such diseases. In turn, the life expectancy of a cancer patient and the possibility of reproduction depend on the effectiveness of treatment.

**SERUM MIR-155, -320A, AND -205 LEVELS AS PROGNOSTIC MARKERS OF BREAST
CANCER COURSE**

Borikun T.V., Yalovenko T.M., Lukianova N.Y., Chekhun V.F.

*R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of
Science of Ukraine, Department of Monitoring of Tumor Process and Therapy Design, Vasylykivska
Street 45, 03022, Kyiv, Ukraine*

E-mail: yalovenkotm@gmail.com

Introduction: The exosomal miRNAs are considered to be perspective markers of cancer course. For now, there is a lot of evidence about the role of circulating miRNAs, derived from a tumor, in breast cancer (BC) diagnostic and prognosis. But before clinical use, there is a need to validate their prognostic value in the local population and estimate their expression in different groups of patients.

Aim: To identify the features of circulating of miR-155, -320a, and -205 levels in patients with BC and validate their usage as diagnostic and prognostic markers of this tumor localization.

Materials and methods: The levels of miR-155, -320a, and -205 in serum samples from 89 BC patients and 14 healthy individuals were estimated using RT-PCR in real time. The clinical-pathological characteristics were obtained using standard clinical methods.

Results: The analysis of circulating miR-155, -320a, and -205 showed no correlation of the studied miRNAs levels in the serum of BC patients with the stage of the tumor process and tumor grade. The expression of miR-155 and miR-320a differ in patients with metastases in regional lymph nodes. Level of miR-320a was lower than 0.5 fold and expression of miR-155 was higher than 3.5 fold in patients with more than 3 lymph nodes with metastases. The expression of miR-205 increased in accordance with this parameter but we did not find significant differences with patients without metastases.

It is remarkable, that the levels of serum miR-155, -320a, and -205 in patients with triple-negative BC differed from the patients with other BC subtypes, especially luminal A. Patients with basal BC were characterized with the lowest miR-320a levels and highest miR-155 and -205 levels.

Conclusions: These data demonstrate the potential role of serum miR-155, -320a, and -205 as the additional non-invasive markers for BC course. But it should be considered that these miRNAs separately have low specificity for basal BC diagnosis.

The study was supported by scientific grant of the NAS of Ukraine 2015–2019 “Molecular and Cell Biotechnologies for the needs of medicine, industry and agriculture”: Estimation of cancer-associated microRNAs as extratumoral markers of breast cancer course (2.2.5.395, 0115U001378).

COPY NUMBER VARIATIONS AND EXPRESSION OF HER-2/NEU, C-MYC AND CYCLIN E1 PROTEINS IN ENDOMETRIAL CANCER PATIENTS WITH FAMILY HISTORY OF CANCER

Buchynska L.G., Brieieva O.V., Iurchenko N.P., Glushchenko N.M., Nesina I.P.

R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Science of Ukraine, Vasylkivska Street 45, 03022, Kyiv, Ukraine

E-mail: olha.brie@gmail.com

Endometrial cancer (EC) is a multifactorial disease, the pathogenesis of which may be associated with hereditary predisposition related to the development of specific molecular profile of tumors. Such changes can be accompanied by activation of oncogenes and affect the aggressiveness of the disease.

Aim: To investigate the copy number variations of oncogenes *HER-2/neu*, *c-MYC* and *CCNE1* and protein expression in endometrial tumors, taking into account the clinical and morphological features of EC and the family history of cancer of patients.

Materials and methods. 68 EC patients with I-II stage by FIGO were included in the study (mean age 60.3 ± 2.7 years). The analysis of *HER-2/neu*, *c-MYC* and *CCNE1* gene copy number changes was performed by qPCR. Expression of the proteins was determined using immunohistochemical method by counting the number of positively stained cells (labeling index, LI). The proliferative activity was evaluated by the expression of the Ki-67 marker. To analyze family history of patients, a special clinical-genealogical card was used, which included information about relatives' diseases, patients' living conditions and concomitant diseases.

Results. Genetic and mathematical analysis showed that the contribution of the genetic component in the predisposition to the EC was $45.4 \pm 9.2\%$. Significant heterogeneity in copy number changes and expression of *HER-2/neu*, *c-Myc* and *Cyclin E1* proteins was identified in the group of EC patients with family history of cancer as well as in the group of patients with sporadic tumors. It was shown that 16.7% of tumors of patients with family history of cancer were characterized by amplification of *HER-2/neu* gene, and 14.3% of these samples showed high expression of its protein product. Among patients with sporadic tumors, the similar indicators were 19.2 and 15.4% respectively. The tendency to increase the percentage of cases with *c-MYC* amplification in the group of EC patients with family history of cancer was determined in comparison with the group of sporadic tumors (42.9 and 19.0% respectively). Among patients with a family history of cancer, a significantly higher number of persons with high expression of *c-Myc* protein (43.8%) was found than among women with sporadic forms of EC (17.3%) ($p < 0.05$). It was found that in EC patients with family history of cancer and high expression of *c-Myc*, tumors were characterized by low degree of differentiation and deep myometrial invasion. In these tumors, a significantly higher Ki-67 expression (LI 42.1 ± 3.3 and $32.5 \pm 2.3\%$ respectively) was observed compared to a well differentiated carcinoma without invasion in myometrium (12.9 ± 2.3 and $18, 9 \pm 2.4\%$, respectively). The analysis of copy number variations of *CCNE1* gene in endometrial carcinomas has shown that the amplification of this marker was observed in 19.0% of the sporadic endometrial carcinoma, whereas it was not found in tumors of patients with family history of cancer. The high expression of this protein was more often found in a group of patients with sporadic tumors than with family history of cancer (72.0% and 42.9% respectively).

Conclusion. The high expression of *c-Myc* protein in tumors of EC patients with family history of cancer is associated with a low degree of tumor differentiation, high invasive and proliferative potential, that can become the basis for the selection of women with an aggressive form of the disease among such patients.

MIR-155, -320A, AND -205 EXPRESSION DIFFERENCES IN TUMOR FROM PATIENTS WITH BENING AND MALIGNANT BREAST DISEASE

Chekhun V.F., Borikun T.V., Lukianova N.Y.

R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Science of Ukraine, Department of Monitoring of Tumor Process and Therapy Design, Vasylykivska Street 45, 03022, Kyiv, Ukraine

E-mail: tborikun@gmail.com

Introduction: It is well-known that microenvironment is essential for cancer progression. At last decades there were a lot of successful attempts to create the molecular profile of tumor microenvironment. But there is still a need to clarify the role of miRNAs in tumor-microenvironment interaction, especially their role in the process of malignancy formation during breast disease.

Aim: To identify the features of miRNA expression derived from the tumor microenvironment, in patients with breast cancer (BC) and benign breast disease (BBD).

Materials and methods: The levels of miR-155, -320a, and -205 in tissue samples from BC and BBD patients were estimated using RT-PCR in real time. The ER, PR, Her2/neu expression was analyzed using standard immunohistochemical analysis. The study was conducted on 156 subjects, 89 of which were those who suffer from BC, and 53 had BBD.

Results: In the total group of BC samples, we have found a significant increase in miR-155 (≈ 2 times) expression and decrease in miR-205 ($\approx 2,6$ times) levels compared to adjacent normal tissue. While levels of miR-320a in most samples were lower than in normal tissue (almost twice), there were no significant differences between all studied groups. The analysis of BBD samples showed no differences both from normal and cancerous tissue, the meanings were in intermedia range. Probably, on the larger sample collection, the expression of miR-155, -320a, and -205 can be proven as a distinguishing marker of BBD.

Conclusion: As far as the expression of miR-155 and -205 in BC samples differs from one in normal adjacent tissue, it can be used in further investigation of their prognostic value for BC course.

The work was carried out with the support of the Research Program of the Scientific Research Program of the National Academy of Sciences of Ukraine "Molecular Biological Factors of the Heterogeneity of the Malignant Cells and the Variability of the Clinical Course of Hormone Dependent Tumors "(2.2.5.411, 0117U002034).

PROGNOSTIC VALUE OF NANOG LEVEL IN PATIENTS WITH PROSTATE CANCER

Cekhun V.F., Zadvornyi T.V., Lukianova N.Y.

*R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology,
National Academy of Science of Ukraine, Vasylykivska Street 45, 03022, Kyiv, Ukraine*

E-mail: tito132007@ukr.net

Introduction. Prostate cancer (PCa) take over second place of the incidence among cancer and is the third cause mortality rate among men in Ukraine. In view of these, the choice of optimal treatment tactics and monitoring of disease course are important tasks. Today, NANOG, a transcription factor that is involved in maintaining pluripotency and is considered one of the markers of tumor stem cells, is esteemed promising marker in this direction.

Aim: to investigate the level of NANOG expression in the tumor tissue of patients with PCa and to evaluate the possibility of its use as a prognostic marker for the disease.

Materials and methods. The research is based on the results of the examination and treatment of 50 patients with II-III PCa stage who were treated in the National Cancer Institute in 2015-2017. The average age of the patients was 57.6 ± 5.4 years. Expression of the NANOG mRNA in tumor tissue was determined using RT-PCR in real time. STATISTICA 6.0 software was used to process the results.

Results. It was found that the level of NANOG mRNA in tumor tissue was 3.21 ± 0.23 fold change with individual oscillations from 0.91 ± 0.04 to 8.33 ± 1.2 fold change. We proved the correlation between the level of NANOG expression in the tumor tissue of patients and the clinical and pathological characteristics of PCa patients ($r = 0.53$, $p \leq 0.05$), as well as with the presence of metastases in regional lymph nodes ($r = 0.65$, $p \leq 0.05$), and the Gleason score ($r = 0.48$, $p \leq 0.05$) and the PSA level ($r = 0.68$, $p \leq 0.05$).

Conclusions. The obtained results testify the association of levels of expression of NANOG in tumor tissue with the main clinical and pathological characteristics of PCa and indicate the promising use of it as a marker for prediction of the disease course.

The work was carried out with the support of the Research Program of the Scientific Research Program of the National Academy of Sciences of Ukraine "Molecular Biological Factors of the Heterogeneity of the Malignant Cells and the Variability of the Clinical Course of Hormone Dependent Tumors "(2.2.5.411, 0117U002034)

A TUMOR AND A RADIATION STRESS

Domina E.A., Druzhina M.O., Makovetska L.I., Glavin O.A., Mikhailenko V.M.

*R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology,
National Academy of Science of Ukraine, Vasylkivska Street 45, 03022, Kyiv, Ukraine*

E-mail: veterok61@ukr.net

The ionizing radiation is used in an oncology clinic as a powerful stress agent, causing the mass death of tumor cells through apoptosis and necrosis. However, the therapeutic irradiation of oncological patients does not always guarantee the successful treatment due to the following reasons: the micro metastases are often formed in the body before the therapy is initiated; during the therapy some of the tumor cells migrate through the circulatory and lymphatic pathways; a pool of radio-resistant cells may be stored in a radiation zone. Nevertheless, a radical and a palliative radiotherapy causes a powerful stress and an immunosuppressive effect on the body of the patients, which promotes the survival and the proliferation of viable tumor cells. The tumor cells that survived the course of radiation therapy are, at the same time, the most suited to survival and the most resistant to the repeated exposure. The endogenous stress caused by the oncological process has a profound depressive effect on the immune system of the patient, which, in combination with a radiation stress, can contribute to the progression of the disease. In this case, the lymphocytes reflect the individual radio sensitivity of the body of the patients (G2-radiating sensitivity assay). They die even when being exposed to the small doses. The radiation stress also leads to a profound inhibition of the functioning of neutrophils, which increases the sensitivity of the body of cancer patients to the infections and the development of inflammatory processes. These processes occur due to the development of the oxidative stress - the effect of the ionizing radiation. The attack of free radicals on biological structures causes a damage to the healthy cells that have fallen into the zone of irradiation.

Thus, the radiation stress has a dual effect: the devitalization of tumor cells and, at the same time, the radiation side effects in the healthy cells in the tumor environment. This represents the main problem of radiation oncology. The cellular DNA repair systems, which are aimed at preserving and stabilizing the integrity of the genome, counteract the death of tumor cells. This reduces the effectiveness of the radiation therapy. The suppression of the activity of DNA repair enzymes in tumor cells is considered as one of the modern promising areas of oncology. The whole family of poly (ADP-Ribose) polymerase (PARP-ase) is considered as an inhibitor of reparation. The (PARP-ase) family is involved in maintaining the integrity of the genome, its repair, gene expression, telomere homeostasis, differentiation and cell death. The repair of the DNA damage in tumor cells occurs more actively than in normal tissue cells. This is due to the increased expression of repair enzymes in tumor cells. We see the solution to this problem in the search for ways to inhibit repair systems in tumor cells and to preserve their activity in the normal cells of the patients. We are currently conducting research aimed at finding and examining such radio modifiers. One of the possible candidates of this diverse influence on the repair enzymes may be the drug metformin.

REDOX-DEPENDENT MECHANISMS OF FORMATION OF METASTATIC MICROENVIRONMENT OF DISTANT SITES OF METASTASATION OF PATIENTS WITH COLORECTAL CANCER

Anatoliy Burlaka¹, Iryna Ganusevich¹, Anton Burlaka², Anastasiya Vovk¹, Sergiy Virko³

1 – R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Science of Ukraine, Vasylkivska Street 45, 03022, Kyiv, Ukraine

2 – National Cancer Institute, Lomonosova str. 33/43, Kyiv, Ukraine

3 – B.E. Lashkarev institute of semiconductor physics NAS of Ukraine, Nauky av. 45, Kyiv, Ukraine

E-mail: apburlaka@gmail.com

Objective: to investigate the redox state of metastases (M), liver tissue (LT), blood and urine of metastatic colorectal cancer patients (mCRC) with liver metastases.

Object and methods: the research of M tissue was conducted; LT, which directly contacts with M; LT taken at a distance of 5 cm from M; urine and blood of 25 patients with mCRC with liver damage. The activity of the iron-sulfur N-2 protein (N-2 FeS proteins) of the electron transport chain (ETC) mitochondria, levels of lactoferrin (LF), "free iron" ("FI") was determined by the method of electron paramagnetic resonance (EPR) (77 K). The levels of superoxide radicals (SR) and nitric oxide (NO) were determined using Spin Traps technology. The activity of matrix metalloproteinases (MMP-2 and MMP-9) was recorded by zymography in a polyacrylamide gel.

Results: mCRC with M in the liver revealed a violation of the functioning of cytochrome P-450 in the system of detoxification of hepatocytes, defects in ETC mitochondria (NO complexes with FeS proteins – NOFeS-proteins), damage to the metabolism of oxygen and iron, changes in the degree of destruction of the intercellular matrix. The most pronounced these violations were in the LT adjacent to M (the site of the formation of metastatic microenvironment), which was manifested by a decrease in the levels of cytochrome P-450 (oxidized and low-spin forms of cytochrome P-450 and isoform CYP 1A2), the growth of levels of complexes of NO with FeS proteins, LF, "FI", MMP-2, -9 and speed of generation of SR and NO in comparison with LT at a distance of 5 cm from M. In the blood of patients with mCRC, high levels of superoxide and NO-producing activity of neutrophils, active forms of gelatinases were detected (compared to those without metastases).

Conclusions: the investigated liver redox states, operated on mCRC, can be used to evaluate the functional state of distal metastasis of organs and tissues, the risk of cancer recurrence and the improvement of therapeutic approaches to anticancer therapy.

Key words: metastatic colorectal cancer, liver, redox state of distant metastasis sites.

ARGINASE ACTIVITY AND EXPRESSION IN BLOOD CELLS IN DIFFERENT KINDS OF LEUKEMIA

Sergiy Gogol, Sophia Zaletok, Yuriy Yanish, Lilia Sklyarenko

*R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology,
National Academy of Science of Ukraine, Vasylkivska Street 45, 03022, Kyiv, Ukraine*

E-mail: tantattoo72@gmail.com

Introduction. Arginase (L-arginine amidinohydrolase, E.C.3.5.3.1) catalyses L-arginine hydrolysis into L-ornithine and urea. It was discovered in the mammalian liver as the end enzyme of urea cycle. Arginase activity (AA) was found also in other tissues not having complete urea cycle. Some researchers have found AA in malignant tissues to be higher than in normal ones. At the same time, there are only a few of the works dealing with AA in the malignant blood cells of the leukemia patients. Especially, AA in the leukemia cells of the chronic lymphoid leukemia patients was found to be twice lower than in the healthy donor lymphocytes; also was found enhanced AA in the patients with drug-resistant chronic myeloid leukemia.

Aim: To study AA and arginase protein expression (APE) in the peripheral blood lymphocytic fraction of patients with different kinds of leukemia.

Object and methods: AA measurement was done in the peripheral blood lymphocytic fraction of patients with chronic B-cell leukemia (B-CLL, 71 patient); acute myeloid leukemia (AML, 53 patients); acute B-cell lymphoblastic leukemia (B-ALL, 8 patients); non-Hodgkin's lymphomas (NHL, 30 patients) and of 10 donors. APE was investigated in the lymphocytes cellular extracts. AA was measured by the method of Corraliza I. et al., APE – using Western blotting analysis by the Lemmly's method.

Results: Both AA and APE level was highest in the B-CLL patients. The lowest AA level was found in the blastic cells of the B-ALL patients.

Conclusion: AA and APE measurement in the peripheral blood cells may be proposed as supplementary diagnostic criteria for certain types of leukemia.

**MRPS18-2 AND TP53 PROTEINS, CONJUGATED ON GOLD NANOPARTICLES,
ENHANCE ACTION OF VINCRISTINE AND DOXORUBICINE ON PROSTATE CANCER
CELLS**

Kovalevska L.M., [Kashuba O.V.](#)

*R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology,
National Academy of Science of Ukraine, Vasylykivska Street 45, 03022, Kyiv, Ukraine*

E-mail: Kashuba@nas.gov.ua

For the treatment of prostate tumors, the cytostatic drugs are often used, such as vincristine and doxorubicin. Vincristine is a synthetic alkaloid, which can be isolated from the rose periwinkle (*Catharanthus roseus*). Vincristine blocks separation of chromatids in the metaphase, thus causing apoptosis. Doxorubicin is an anthracycline antibiotic, that can be isolated from a mutated strain of bacteria *Streptomyces peucetius*. Doxorubicin blocks DNA replication, which results in the death of rapidly proliferating cells.

In the present work, PC3 prostate carcinoma cell line and immodalized PNT2 prostate cells were used, grown in 12-well plates.

Vincristine and doxorubicin were used at concentrations of 5-600 nM and 0.5-50 µg/ml, respectively. IC₅₀ for both cell lines was determined, which was 50-100 nM for PC3 and 30-50 nM for PNT2. For doxorubicin, these values were 10-30 µg/ml and 5-10 µg/ml, respectively.

The effect of cytostatic drugs was also studied in the presence of GST-fusion proteins MRPS18-2 and TR53 in the form of conjugates on a surface of golden nanoparticles. These conjugated were delivered to cells by pinocytosis. The number of apoptotic cells was counted and the percentage of such cells was calculated as compared to the total number of cells at 24, 48 and 72 hours after drug treatment.

The presence of GST-fusion proteins reduced the IC₅₀, in the case of doxorubicin, to 0.5-1 µg/ml. The effect was clearly expressed in both lines of prostate cells.

It should be noted that vincristine acted more slowly; only after 48 hours formation of multinucleated cells was observed. However, in the presence of protein conjugates, multicellular cells appeared much earlier (after 24 hours). In the culture of PNT2 cells, stress fibers were formed.

Thus, protein conjugates on the surface of gold nanoparticles reduce the IC₅₀ for vincristine and doxorubicin, which opens the prospect of using conjugated proteins to increase the sensitivity of tumor cells to chemotherapy.

PROTEINS OF THE MRPS18 FAMILY, CONJUGATED ON GOLD NANOPARTICLES, ENHANCE THE TP53-INDUCED APOPTOSIS IN PROSTATE CANCER CELL LINES

Kovalevska L.M., Kashuba O.V.

*R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology,
National Academy of Science of Ukraine, Vasylkivska Street 45, 03022, Kyiv, Ukraine*

E-mail: kreyl@yahoo.com

We have discovered the new mechanism of regulation of the cell life cycle, depending on the overexpression of the MRPS18-2 protein. The purpose of this work is to propose new methods of delivery of stabilized functional proteins to mammalian cells.

To deliver polypeptides, the conjugates of GST-fused proteins MRPS18-1-3 and TP53 were synthesized on the surface of gold nanoparticles via an NHS-ester with a PEG spacer. Approximately 17×10^{11} nanoparticles and 9×10^8 nanourchins in a complex with GST-fusion proteins were delivered to 30-50 000 of prostate cancer cells PC3 and immortalized prostate gland cells PNT2, grown in six-well plates. Nanoparticles were taken up by pinocytosis.

TR53 and mutated TR53 did not affected much the studied cell lines. However, a proportion of cells (up to 1%) showed fragmented large nuclei. An insignificant increase in the number of apoptotic cells was observed in the PC3 cell culture when GST-protein was administered. Basically, the same observations concerned PNT2 cells. However, the percentage of apoptotic cells in PNT2 was higher than in the tumor cell line PC3 (up to 8%).

PC3 cells do not express the endogenous TR53 protein, due to deletion of the chromosome region 17, where the TR53 gene is located. This explains differences in the level of induced apoptosis - in PNT2 cells, expressing wild-type TP53, apoptosis is higher than in tumor PC3 cells.

Subsequently, the conjugates of the GST-fused proteins of the S18 family were added together with the wild type and mutated TP53 proteins. The number of apoptotic cells (indicating abnormality of nuclei or fragmentation of the nucleus) was calculated. The percentage of apoptotic cells was calculated, in comparison with the total number of cells on a glass slide surface.

Unexpectedly, the administration of TR53 (wild type and mutated), together with any of the S18 family proteins led to the appearance of abnormal cells at much higher rate, compared with a single protein (up to 20% in PNT2 cells).

It should also be noted that the synergy level is more pronounced in PNT2 cells. Thus, the number of apoptotic cells is 3-4 fold higher upon delivery of two proteins, while in PC3 cells – only 2-3 fold.

The synergy of apoptosis is more pronounced when the spherical nanoparticles were used, and not nanourchins. This might be due to a more stable three-dimensional structure of proteins on nanospheres.

The observed phenomenon indicates the possible usage of conjugates of GST-fused proteins (the S18 family and TP53) to increase the effectiveness of chemotherapy, namely, increasing the sensitivity of tumor cells to the action of cytostatic drugs.

MORPHOLOGICAL AND FUNCTIONAL ALTERATIONS IN CARCINOSARCOMA WALKER-256 CELLS OVER THE COURSE OF RESISTANT PHENOTYPE FORMATION

Lozovska Yu.V.¹, Lukianova N.Yu.¹, Andrusishina I.M.², Naleskina L.A.¹, Todor I.N.¹, Kunska L.N.¹, Chekhun V.F.¹

1 – R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv, 03022, 45, Vasylykivska st., Ukraine

2 – SI “Yu.I. Kundiev Institute of Occupational Health”, NAMS of Ukraine, Kyiv, 01033, 75, Saksaganskogo st., Ukraine

E-mail: Lozovskaya.2012@ukr.net

Currently, the approaches to the results of fundamental research that can be used for the individualized treatment of cancer patients, especially resistant forms of breast cancer, are revised. The studies of the mechanisms underlying the morphological and functional alterations in tumor cells (TC) accompanying the formation of drug resistance are of high priority.

Aim: To determine the specific features of TC architectonics, cell cycle and content of the essential elements in sensitive and doxorubicin resistant Walker-256 carcinosarcoma.

Materials and methods. Sensitive and doxorubicin resistant Walker-256 carcinosarcoma strains were used in the study. Cytoarchitectonics of TC was studied in histological specimens with the aid of Primo Star microscope (Carl Zeiss, Germany). Cell cycle distribution was analyzed by flow cytometry (Beckman Coulter Epics XL). The content of essential elements (Fe, Cu, Zn, Mg and Ca) in tumor tissue (TT) was determined by atomic emission spectrometry on the Ortima 2100 DV device (Perkin-Elmer, USA).

Results. Doxorubicin-sensitive tumors were characterized by a rather compact arrangement of rounded cells with slightly pronounced signs of polymorphism in the form of large cells and alveolar structures separated by thin fibrous layers of connective tissue. The nuclei were characterized by a homogeneous nucleoplasm with a small amount of chromatin granules with increased number of aneuploid cells. The resistant TC were characterized by a more pronounced polymorphism, a denser arrangement, a slightly larger area and an intense coloration of nuclei, with increased cell ploidy. The morphological features were in line with the patterns of cell cycle distribution. In sensitive TC, percentage of cells in the G₂/M phase was higher than in resistant TC (25.51 ± 0.18% vs. 10.14 ± 0.45%) percentage of cells in the G₀/G₁ phase was respectively lower (42.64 ± 1.24% vs. 72.21 ± 0.19%). Such changes in the patterns of DNA status were accompanied by the redistribution of the content of the essential elements involved in cell cycle regulation. The corresponding values were in sensitive TC: Fe – 25.18 ± 4.79 µg/g, Cu – 1.56 ± 0.33 µg/g, Zn – 10.98 ± 1.83 µg/g, Ca – 131.16 ± 19.34 µg/g, Mg – 349.62 ± 34.64 µg/g; Ca/Mg – 0.37 ± 0.08, Cu/Zn – 0.14 ± 0.03, and in resistant TC: Fe – 34.51 ± 5.23 µg/g, Cu – 1.98 ± 0.21 µg/g, Zn – 9.17 ± 1.75 µg/g, Ca – 60.09 ± 16.21 µg/g, Mg – 181.41 ± 9.73 µg/g; Ca/Mg – 0.61 ± 0.04, Cu/Zn – 0.18 ± 0.01.

Conclusions: The morphological and functional characteristics of tumor cells have been shown to change over the course of resistant phenotype formation. We suggest that the new biological behavior of the resistant cells seems to be connected with reprogramming of their essential homeostasis.

The work was carried out with the support of the Research Program of the Scientific Research Program of the National Academy of Sciences of Ukraine "Molecular Biological Factors of the Heterogeneity of the Malignant Cells and the Variability of the Clinical Course of Hormone Dependent Tumors "(2.2.5.411, 0117U002034).

CONNECTION BETWEEN CELL SURFACE RECEPTOR STATUS OF CLL B CELLS AND THEIR SENSITIVITY TO CHEMOTHERAPY AGENTS *EX VIVO*

Valeriia Shcherbina, Inna Gordiienko, Tetiana Ivanivskaya and Larysa Shlapatska

*R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology NAS of Ukraine
Vasylkivska str. 45, 03022, Kyiv, Ukraine*

E-mail: L.knolodniuk@ukr.net

Chronic lymphocytic leukemia (CLL) is a disease with variable clinical outcome. Traditionally patients with CLL are divided into two groups based on disease symptomatic and biological features of malignant B cells. The first group consists of cases that do not require immediate treatment and for these patients' strategy "watch and wait" is used. Others are characterized by rapidly progression and typical clinical manifestations. For such cases, immediate application of treatment using polychemotherapy courses is applied. Basic scheme of first-line therapy for patients with aggressive CLL include combination of fludarabine, cyclophosphamide and rituximab (FCR) or bendamustine with rituximab (BR). The choice of treatment strategy is based mainly on age and genetic characteristics of the patient. In recent years medicine became more personalized and new markers for differentiation, prognosis and the exact selection of the course of therapy are required. In this aspect, attractive candidates are cell surface receptors. The aim of our study was to explore whether sensitivity of CLL B cells to chemotherapy agents depends on expression level of cell surface receptors. The study was performed on peripheral blood mononuclear cells isolated from previously untreated patients with CLL. Flow cytometry was used for immunophenotyping CLL B cells. Metabolic activity of the CLL B cells was determined after 48h of incubation with chemotherapeutic agents *ex vivo* by Alamar Blue test. For our research we chose several surface receptors which are expressed in CLL: CD5, CD20, CD22, CD37, CD38, CD40, CD150 and CD180. Based on flow cytometry results all CLL samples were divided into groups according to cell surface expression level of each receptor. It was shown that CD38⁻ cases were more sensitive for bendamustine than CD38⁺ and B cells characterized by CD40^{high} expression were more sensitive for cyclophosphamide than cells with middle CD40 expression. Response of CLL B cells to fludarabine alone or in combination with cyclophosphamide were enhanced in CD150⁺CD180⁺ B cells compare to CD150⁻CD180⁻ ones. Besides, sensitivity of CD180⁺ B cells to combine effect of fludarabine with cyclophosphamide was also better than in CD180⁻. For other receptors any differences in sensitivity of CLL B cell to chemotherapy agents between groups was not observed. So, expression levels of CD38, CD40, CD150 and CD180 on B cells could be used as predictive markers for the effectiveness of chemotherapy in choosing a treatment strategy for patients with CLL.

POLYAMINES IN THE PERIPHERAL BLOOD LYMPHOCYTES OF PATIENTS WITH DIFFERENT KINDS OF LEUKEMIA

Sophia Zaletok, Oleg Klenov, Sergiy Gogol, Veronika Bentrud, Oleksiy Orlovskiy, Lilia Sklyarenko

*R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology,
National Academy of Science of Ukraine, Vasylkivska Street 45, 03022, Kyiv, Ukraine*

E-mail: sophiazaletok@ukr.net

Introduction. Polyamines (PA) – spermine (spn), spermidine (spd), putrescine (put) — play an important role in normal and malignant cell proliferation, growth and differentiation. Existing knowledge about role of PA and enzymes of their metabolism in the leukemia cells is very incomplete, and that's why the data on the possible use of the PA metabolism parameters for leukemia differential diagnosis and prognosis are almost absent. There are only a few of publications in this direction. Especially, it was demonstrated that enhanced activity of spn/spd acetyltransferase (SSAT) correlate with clinical course in the myeloid kinds of leukemia but not in lymphoid ones. Patients with drug-resistant forms of chronic myeloid leukemia had enhanced arginase (the first enzyme of the PA synthesis) activity.

Aim: to investigate features of PA level and ODC (ornithine decarboxylase, the key enzyme of the PA biosynthesis) protein expression in the peripheral blood lymphocytes (PBL) of the patients with the different types of leukemia and elucidate their significance for the disease differential diagnosis and prognosis.

Object and methods: Clinical material of 202 patients with different types and cytological variants of leukemia (82 patients with chronic B-lymphocytic leukemia (B-CLL), 65 patients with acute myeloid leukemia (AML M1-M5), 25 patients with acute B-lymphoblastic lymphoid leukemia (B-ALL), 30 patients with non-Hodgkin's lymphomas (NHL)) and 10 donors was investigated. Each diagnosis was verified in the Department of oncohematology of IEPOR (head of the Department – prof. D.F. Glusman). PBL fraction was isolated by centrifugation in ficoll-urografin gradient. This fraction, in the case of leukemia patients, contained 75%-86% of leukemia cells. HPLC, PAAG-electrophoresis, Western blotting and statistical methods were used.

Results: PBL fraction of B-CLL patients was characterized by low ODC expression level, high spn level and low value of spd/spn ratio in comparison with acute leukemia patients. PA level and spd/spn value in the PBL fraction were essentially different in different groups of the NHL patients. PBL fraction of the Mantle-zone lymphoma without leukemization was characterized by low ODC expression, put content and spd/spn ratio and high spn content. If leukemization was present, ODC, put, spd and spd/spn ratio were 3-9 times higher. The highest values ODC expression, put content and spd/spn ratio were found in the patients with T-lymphoblastic leukemia/lymphoma and B-prolymphocytic leukemia.

Conclusion: ODC protein expression level, as well as spn and spd level and spd/spn ratio may be used as supplementary markers for refining of diagnosis and prognosis of different kinds of leukemia.

MATHEMATICAL MODELING OF DETERMINATION TIME CHARACTERISTICS OF THE FORMATION OF SECOND OTHER TUMORS

Liliya Batyuk, Vladimir Knigavko, Natalya Ponomarenko, Alexey Rukin,

Oksana Morozova, Tetiana Utytskykh

Department of Medical and Biological Physics and Medical Information Science,

Kharkiv National Medical University, 4 Nauky Avenue, Kharkiv, Ukraine, 61022

E-mail: liliya-batyuk@ukr.net

Causes of carcinogenesis are diverse (Aaronson S.A. et al., 1991), but one of the key factors of this phenomenon is damage of certain genes in the tumor cells. Presumably, these genes are responsible for reparation of DNA damage. Thus, the damage to all these genes in the cell is considered a necessary condition for the malignancy of this cell. Proceeding from such a concept, one can construct probabilistic mathematical models that allow calculating the numerical characteristics of the time of formation of other tumors (Adam J.A., 1986), (Batyuk L.V., et al. 2017). Let's proceed from the assumption that the formation of the first and second tumor is an independent random event. Let t be a random variable that is the time of the formation of one tumor, and $F_1(t)$ is the distribution function of this time. The formation of a second tumor for some time is a random event. The probability of formation at the time t of two tumors is a function of the distribution ($F_2(t)$) of the time of formation of these two tumors, and this function is defined by the expression: $F_2(t) = F_1^2(t)$. Expressions for the distribution functions of tumors are dependent on the number of genes that are initially (at birth) contained in the genotype of the individual. Therefore, these characteristics are amounts of this type: $F(t) = \sum_{k=1}^m (F_k(t)p_k)$ and

$f(t) = \sum_{k=1}^m (f_k(t)p_k)$, where m is the maximum value of k , and p_k is the probability of find encountering

an individual with an initial number of genes equal to k . The statistics of the time of the formation of the first and second tumors could use to evaluate the values of p_k . If there is sufficient data on the age of the individual at the time of the tumor formation, the above values can be estimated on the basis of such considerations. τ is a period of time equal to one year, i is the age (in years) in which the first tumor formed, j – the age (in years), in which the second tumor formed and the probability that one of the tumors will appear at age j , and the other at age i ($p(j\tau)$ and $i\tau$). Let φ_k is a probability to find a human with an initial number of genes equal to k . The value ($p(j\tau)$ and $i\tau$) is denoted as $p(i, j)$. Let $p(i, j)/k$ is the probability of the formation in the individual of two tumors in the ages i and j , provided that in the

genotype it originally was k number of genes. Then $p(i, j) = \sum_{k=1}^m (\varphi_k \cdot p(i, j) / k)$. If the values of $p(i, j)/k$

are calculated and the values of $p(i, j)$ are determined from the statistical data, then a redefined system of equations is formed, from which it is possible to determine the value of the quantities φ_k .

1. Aaronson S.A. et al. Science Wash. 1991, 254:11460-1153.
2. Adam J.A. Math. Biosci.1986, 81:229-244.
3. Batyuk L.V. et.al. Medical Review. 2017: 4: 46-47.

ІНДУКЦІЯ АПОПТОЗУ МОНОНУКЛЕАРІВ ПЕРИФЕРИЧНОЇ КРОВІ ХВОРИХ НА РАК ПЕРЕДМІХУРОВОЇ ЗАЛОЗИ ЗА ДІЇ ІОНІЗУВАЛЬНОГО ВИПРОМІНЮВАННЯ

Фільченков О.О., Завелевич М.П., Дьоміна Е.А.

Інститут експериментальної патології, онкології і радіобіології ім. Р.Є. Кавецького

НАН України, вул. Васильківська 45, Київ 03127, Україна

E-mail: a.philch@onconet.kiev.ua

Попри впровадження в онкологічну практику новітніх технологій терапевтичного опромінення, залишається загроза розвитку побічних реакцій з боку оточуючих пухлину тканин. Особливу актуальність у зв'язку з цим набувають радіобіологічні дослідження, які б сприяли зниженню частоти та тяжкості променевиx ускладнень. Мета роботи – порівняльний аналіз рівня “спонтанного” та радіаційно-індукованого апоптозу клітин периферичної крові хворих на рак передміхурової залози (РПЗ) до початку, після першої фракції та по закінченні курсу променевої терапії. Зразки периферичної крові отримували від 16 хворих на РПЗ (переважно II–III стадії захворювання, середній вік 65,9 року) та 4 умовно здорових донорів. Мононуклеари периферичної крові (МПК) виділяли з гепаринізованої крові в градієнті Histopaque®-1077 (“Sigma”, США). Одну з двох проб клітин від кожного з хворих піддавали дії рентгенівського опромінення в дозі 2,0 Гр на апараті “РУТ-250-15-2”. Після цього клітини культивували впродовж 48 год за стандартними методиками. Вміст гіподиплоїдних клітин визначали методом проточної цитометрії, використовуючи цитометр BD™ FACSCalibur (“Becton Dickinson”, США). Результати обробляли за допомогою програми CellQuest (“BD Bioscience Pharmingen”, США). Окремо визначали дозозалежність апоптотичної загибелі при тест-опроміненні зразків МПК хворих на РПЗ або здорових донорів в дозах 1,0, 2,0, 4,0 та 6,0 Гр з подальшим культивуванням в умовах, зазначених вище.

Встановлено лінійну залежність між рівнем апоптотичної загибелі та дозою тест-опромінення в діапазоні від 1,0 до 6,0 Гр для МПК донорів та хворих на РПЗ до початку курсу променевої терапії. У хворих на РПЗ лінійний характер залежності “доза-апоптоз” зберігається, але кут нахилу апроксимованої прямої зменшується після першої фракції опромінення (2,5 Гр) і значно зменшується по закінченні курсу променевої терапії (76,0 Гр). Це може свідчити про набуття радіорезистентності МПК хворих впродовж променевої терапії. Рівень “спонтанного” апоптозу в МПК здорових донорів був нижчим, ніж у хворих.

В рамках виконання пілотного проекту спостерігається тенденція до зниження радіаційно-індукованого апоптозу МПК хворих після проходження курсу променевої терапії. Широкий діапазон значень апоптотичного індексу опромінених МПК хворих на РПЗ свідчить про варіабельність індивідуальної радіочутливості. При проведенні кореляційного аналізу за Спірменом виявлено позитивну кореляцію між показниками “спонтанного” апоптозу й апоптозу, індукованого тест-опроміненням, в МПК хворих як до початку опромінення ($r = 0,88$), так і після першої фракції опромінення ($r = 0,77$). В то й же час такої кореляції між показниками “спонтанного” апоптозу й апоптозу, індукованого тест-опроміненням, після повного курсу променевої терапії виявлено не було ($r = 0,48$).

Визначення показників апоптозу в МПК хворих на РПЗ в поєднанні з іншими біохімічними, клінічними та цитогенетичними показниками може бути прогностичним маркером реакції здорових тканин, що потрапляють у зону терапевтичного опромінення. Робота виконана в рамках цільової програми наукових досліджень ВБФМБ НАН України “Молекулярно-біологічні фактори гетерогенності клінічного перебігу гормоно-залежних пухлин”.

СИРОВАТКОВИЙ МІОЗИН C1 У ПЕРЕБІГУ ХРОНІЧНОЇ ЛІМФОЦИТАРНОЇ ЛЕЙКЕМІЇ

Шалай О.¹, Кіт Ю.², Барілка В.¹, Мироновський С.², Стойка Р.², Зотова О.¹, Логінський В.¹

1 – ДУ «Інститут патології крові та трансфузійної медицини НАМН України, 79044, м. Львів, вул. Г. Чупринки, 45

2 – Інститут біології клітини НАН України, 79005, м. Львів, вул. Драгоманова 14/16

E-mail: Oliashalai@ukr.net

Хронічна лімфоцитарна лейкемія (ХЛЛ) – хронічне лімфопроліферативне захворювання, клінічні прояви, перебіг, відповідь на лікування і прогноз якого у окремих хворих значно відрізняються. У зв'язку з цим останнім часом широко обговорюється, та продовжується пошук нових діагностичних маркерів та прогностичних факторів цієї хвороби.

Раніше нами було встановлено, що в сироватці крові хворих на ХЛЛ на відміну від здорових донорів присутній міозин C1 (Myonovskij et al., 2018). Метою роботи було дослідити вміст цього білка в сироватці крові 15 хворих ХЛЛ в динаміці перебігу хвороби. Діагноз ХЛЛ встановлювали на підставі клініко-лабораторних досліджень відповідно до сучасних міжнародних критеріїв Національного інституту раку (NCI), включно з цитологічним та імунофенотиповим дослідженням клітин периферичної крові і/або кісткового мозку. Серед пацієнтів 10 чоловіків та 5 жінок віком 48-69 років (медіана віку становила 58 років), середній вік – 57,6±1,7 років, при цьому до 60 років було 75,0% осіб, а старших за 60 років – 25,0%. Пальпаторно та сонографічно констатовано ураження лімфатичних вузлів (у 60% пацієнтів), селезінки (33,3%), збільшення печінки (20,0%). Відповідно до загальноприйнятих класифікацій стадій ХЛЛ за Rai у 9 пацієнтів встановлено ранні (0-II) стадії та у 6 хворих пізні (III–IV) стадії хвороби. Кількість лейкоцитів периферичної крові складала $10,2 \times 10^9/\text{л}$ – $160,0 \times 10^9/\text{л}$. Середній показник у хворих становив $(69,7 \pm 12,8) \times 10^9/\text{л}$. Анемія (Hb < 110 г/л) виявлена у 4 (26,7%) осіб, тромбоцитопенія (< 100 Г/л) у 5 (33,3%) хворих. Лімфоцитоз периферичної крові складав понад 50% у всіх випадках. Абсолютна кількість лімфоцитів периферичної крові становила $(5,2-148,8) \text{Г/л}$. У пунктаті кісткового мозку спостерігався високий відсоток лімфоцитів, який у всіх хворих перевищував 40% (65%-90%). Рівень $\beta 2\text{-МГ}$, негативного прогностичного маркеру активності хвороби, коливався у межах 1,84 мг/л, – 12,67 мг/л. Середній вміст становив $(4,4 \pm 0,9)$ мг/л. Середня кількість лімфоцитів з ознаками активації, які несли на своїй поверхні антигени CD38 становила $(59,65 \pm 5,76)\%$. Визначення вмісту міозину C1 проводили перед початком лікування, через 3 та 6 місяців після лікування. Хворих розділено на дві групи. 1 група – 8 пацієнтів у яких в перебігу хвороби не виявлено міозин C1 та 2 група – 7 пацієнтів у яких в перебігу хвороби виявлений міозин C1. При статистичному аналізі вірогідної різниці у кількості лейкоцитів, тромбоцитів, рівня гемоглобіну та стадій хвороби у цих групах нами не виявлено. Звертає увагу вірогідна ($p < 0,05$) різниця рівня експресії CD38. У групі хворих у перебігу хвороби яких виявлений міозин C1, рівень CD38 значно вищий і становив $(70,5 \pm 6,1)\%$, порівняно з іншою групою $(48,8 \pm 7,8)\%$. Існує різниця між групами в показниках рівня $\beta 2\text{-МГ}$, $(3,2 \pm 0,8)$ мг/л у хворих з міозином C1 та $(5,28 \pm 1,41)$ мг/л без виявленого міозину C1, однак вона ще недостатньо доведена. Ми хотіли дізнатися чи існує зв'язок у групі №2 між рівнями прогностичних маркерів CD38, $\beta 2\text{-МГ}$ та вмістом міозину C1. Встановлено зворотній корелятивний зв'язок середньої сили ($r = -0,410$) між вмістом міозину C1 і рівнем експресії CD38, та прямий сильний ($r = 0,681$) корелятивний зв'язок з рівнем $\beta 2\text{-МГ}$. Результати проведених досліджень вказують на те, що наявність міозину C1 у крові хворих на ХЛЛ може бути пов'язана з прогресією хвороби та в майбутньому слугувати додатковим прогностичним критерієм перебігу цього захворювання.

**ВПЛИВ ІНСУЛІНУ НА ПРОЛІФЕРАТИВНУ АКТИВНІСТЬ
ТА ЧУТЛИВІСТЬ ДО ДОКСОРУБІЦИНУ КЛІТИН ЛІНІЇ MCF-7
РАКУ ГРУДНОЇ ЗАЛОЗИ ЛЮДИНИ *IN VITRO***

Завелевич М.П., Фільченков О.О., Лихова О.О., Лук'янова Н.Ю., Чехун В.Ф.

*Інститут експериментальної патології, онкології і радіобіології ім. Р.Є. Кавецького
НАН України, вул. Васильківська 45, Київ 03127, Україна*

E-mail: mzavelevych@yahoo.com

Останнім часом з'являється все більше даних про зв'язок метаболічного синдрому з ризиком розвитку ряду злоякісних новоутворень, включаючи рак грудної залози (РГЗ), та про зниження ефективності хіміотерапії при наявності метаболічного синдрому. Високі рівні глюкози й інсуліну можуть сприяти проліферації та інвазії клітин РГЗ. Однак дані про механізми лікарської стійкості, асоційовані з метаболічними змінами у хворих на РГЗ, обмежені. Мета дослідження полягала у порівнянні впливу інсуліну *in vitro* на проліферацію та чутливість до доксорубіцину клітин MCF-7 РГЗ та сублінії MCF-7/Dox, резистентної до доксорубіцину.

Клітини MCF-7 отримані з клітинного банку ліній з тканин людини та тварин ІЕПОР НАН України; клітини сублінії MCF-7/Dox отримані з вихідної лінії у відділі моніторингу пухлинного процесу та дизайну терапії ІЕПОР НАН України. Використовували препарат інсуліну з підшлункової залози бика ("Sigma", США) та доксорубіцин ("Ebewe Pharma", Австрія). Клітини культивували 48 год з інсуліном в повному поживному середовищі. Для визначення чутливості до доксорубіцину його вносили на 48 год після попередньої 24 год інкубації з інсуліном або без нього. Підрахунок клітин здійснювали після їх фарбування 0,1% розчином трипанового синього. Розподіл субпопуляцій клітин, пофарбованих пропідію йодидом, за фазами мітотичного циклу визначали на цитометрі BD™ FACSCalibur ("Becton Dickinson", США). Результати обробляли в програмі ModFit LT2.0 ("Verity Software House", США). Проліферативний індекс оцінювали за сумарним відсотком клітин у фазах S та G₂/M. Методом проточної цитометрії визначали також рівень рецептора інсуліну на поверхні клітин, використовуючи FITC-кон'югований інсулін. Життєздатність клітин визначали з використанням 3-(4,5-диметилтіазол-2-іл)-2,5-дифенілтетразоліум броміду (MTT, "Sigma").

Встановлено, що інсулін у концентраціях 5–25 нмоль/л незалежно від вихідної щільності культури не змінює проліферативний індекс клітин MCF-7/Dox та MCF-7. Однак на відміну від клітин MCF-7, в клітинах MCF-7/Dox при інкубації з інсуліном відбувається вірогідний перерозподіл (в межах біля 10%) між фазами S і G₂/M циклу на користь G₂/M фази. Крім того, попередня інкубація з інсуліном у концентрації 25 нмоль/л знижує чутливість клітин MCF-7 до цитотоксичної дії доксорубіцину. Рівень виживаності клітин MCF-7/Dox за комбінованої дії інсуліну та доксорубіцину залежить від вихідної щільності культури. В клітинах MCF-7/Dox виявлено вірогідне підвищення рівня рецепторів інсуліну в порівнянні з клітинами MCF-7 (27,4 ± 2,4 у.о. та 18,1 ± 1,4 у.о., відповідно). Разом з тим, різниця в ефектах інсуліну в клітинах з фенотипом лікарської резистентності ймовірно може бути пов'язана також і з відмінностями у швидкості інтерналізації ліганд-рецепторних комплексів, у функціонуванні аденілатциклазної та інозитолфосфатної сигнальних систем, тощо. Робота виконана в рамках цільової програми наукових досліджень ВБФМБ НАН України "Молекулярно-біологічні фактори гетерогенності клінічного перебігу гормонозалежних пухлин".

Session 5

Plant cell biology

*June 18-21, 2019,
Yaremche*

6th Ukrainian Congress for Cell Biology with international representation

*June 18-21, 2019,
Yaremche*

LACTOFERRIN EXPRESSION AS A TOOL FOR THE ENHANCEMENT OF NON-SPECIFIC PLANT PATHOGEN RESISTANCE

Yemets Alla

Lecture 1

Alla Yemets, Anastasiya Buziashvili

*Institute of Food Biotechnology and Genomics, Natl. Acad. of Sci. of Ukraine
Osypovskoho str., 2A, Kyiv, Ukraine 04123*

E-mail: yemets.alla@nas.gov.ua

Lactoferrin (Lf), one of the most important human milk proteins, occupies the expanding biotechnological market niche due to its important versatile properties. Lf exhibits antiviral, antimicrobial, antiprotozoal and antioxidant activities, modulates cell growth rate, binds glycosaminoglycans and lipopolysaccharides, and also inputs into the innate/specific immune responses. Development of highly efficient human recombinant Lf expression systems employing yeasts, filamentous fungi and higher plants as bioreactors for the large-scale Lf production is a biotechnological challenge.

Hereby, due to multiple protective Lf properties, its genes (*bLf*, *hLf*, *hLfN*) are desirable candidates for the introduction into the genomes of economically important higher plant species for the enhancement of their immune response. It has been demonstrated that the transformation of some higher plant species (tobacco, rice, pear, etc) by different Lf genes confers broad-spectrum resistance against viral, bacterial and fungal plant diseases and is the promising tool for the enhancement of plant biotic stress resistance crucial for food production safety.

In our study *hLf* gene was used for *Agrobacterium*-mediated transformation of different tomato and potato cultivars. Transgenic lines were selected *in vitro* on media supplemented with kanamycin as selective agent. Integration of *hLf* gene into their genomes was confirmed by PCR using specific primers to the gene of interest. Expression of *hLf* gene was confirmed by Western blotting hybridization with specific monoclonal antibodies against lactoferrin. Also, the resistance against bacterial pathogens *Clavibacter michiganensis* subsp. *michiganensis* and *Ralstonia solanacearum*, and fungal pathogene *Phytophthora infestans* of obtained lines was tested *in vitro*.

Obtained results show that genetic transformation of plants with human lactoferrin gene is one of the promising technology for increase their resistance to different phytopathogens.

**NITRIC OXIDE, SYNTHESIZED BY NITRATE REDUCTASE, AS PARTICIPANT OF
TRANSDUCTION OF HYDROGEN SULPHIDE SIGNAL AT INDUCTION OF HEAT
RESISTANCE OF WHEAT PLANTLETS**

Yuriy Kolupaev

Lecture 2

Kolupaev Yu.E., Karpets Yu.V., Shklyarevskiy M.A., Shvydenko M.V.

Dokuchaev Kharkiv National Agrarian University,

p/o Dokuchaevske-2, Kharkiv, 62483, Ukraine

E-mail: plant_biology@ukr.net

Signal functions of gasotransmitters are substantially defined by their interplay. NO and H₂S are most closely functionally bounded among themselves. Their crosstalk is the component of formation of adaptive responses to influence of stressors. It is known that hydrogen sulphide can induce synthesis of NO in plants. In experiments with lucerne plants the effect of removal of positive influence of hydrogen sulfide donor NaHS on the salt resistance of plants and the gene expression of antioxidant enzymes by treatment with nitric oxide scavenger PTIO (2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide) is established (Wang et al., 2012). The treatment of pea plantlets with sodium hydrosulfide raised their resistance to the toxic action of arsenic, at the same time the increase of content of nitric oxide was noted (Singh et al., 2015). However, the mechanisms of change of content of nitric oxide in plant cells, induced by hydrogen sulphide, remain not clear. Also the causal relationship between the change of homeostasis of reactive oxygen species (ROS) and reactive nitrogen species, and the development of plant resistance to stressors under the influence of hydrogen sulphide remain low-investigated. The purpose of studying was in clarification of mechanisms of functional interplay between exogenous hydrogen sulphide, ROS and nitric oxide at the induction of heat resistance of wheat (*Triticum aestivum* L.) plantlets.

The treatment of roots of wheat plantlets (var. Doskonala) with the solution of hydrogen sulfide donor NaHS in concentration of 100-300 μM caused the increase in their survival after the potentially lethal heat stress (heating at 45°C during 10 min). At the same time in 1-4 h after the influence of exogenous hydrogen sulphide on cells of roots the transitional intensifying of generation of hydrogen peroxide and nitric oxide happened, further their content in roots did not differ from quantities of control variant. Hydrogen peroxide scavenger dimethylthiourea (DMTU) and the inhibitor of NADPH oxidase imidazole completely leveled the effect of increase in NO content in roots, caused by the influence of donor of hydrogen sulphide. At the same time scavenger of nitric oxide PTIO, and also inhibitors of NO-synthase L-NAME (N^G-nitro-L-arginine methyl ester) and nitrate reductase sodium tungstate did not influence almost on the increase of content of hydrogen peroxide, induced by exogenous hydrogen sulphide in roots. Increase in NO content in roots, which is provoked by treatment with NaHS, was considerably suppressed with the nitrate reductase inhibitor tungstate and almost did not change at their treatment with NO-synthase inhibitor L-NAME. During the influence of hydrogen sulphide on roots the transitional increase of activity of nitrate reductase was registered with the maximum in 2 h after the beginning of treatment. This effect was eliminated by the treatment with DMTU or imidazole.

In general, the increase of heat resistance of plantlets, caused by exogenous hydrogen sulphide, was leveled by both antagonists of ROS (DMTU and imidazole), and antagonists of NO (PTIO, L-NAME and tungstate). Thus, the induction of heat resistance of plant cells by exogenous hydrogen sulphide is mediated by the intensifying of ROS generation, the subsequent activation of nitrate reductase and increasing of synthesis of nitric oxide, which depends on this enzyme.

**ADAPTATIVE CHANGES OF PROTEOME IN ARABIDOPSIS SEEDLINGS FROM
CHERNOBYL ZONE**

Galyna Shevchenko

Lecture 3

Shevchenko G.V., Brykov V.O., Klymenko O.

Institute of Botany, NAS Ukraine, Tereshchenkivska 2., 01004, Kyiv

E-mail: galli.shevchenko@gmail.com

As sessile plants possess high capacity for adaptation to contaminated environment, mechanisms of such processes are of significant interest to agriculture and biotechnology. To understand details of plant adaptation we consider it significant to investigate reaction to genotoxins in plants from Chernobyl which develops certain tolerance to chronic radiation. *A. thaliana* seeds were collected in highly contaminated areas of Chernobyl zone and subsequently screened for tolerance to heavy metals and radiomimetic which resulted in obtaining *Arabidopsis* line (Che_07) resistant to genotoxins. Che_07, Col and control plants were grown on 100uM CdCl₂ and checked for proteome changes by means of 2D electrophoresis, mass spectrometry and bioinformatics. Experiments revealed proteins involved in photosynthesis/chlorophyll biosynthesis, carbohydrate and energy metabolism, amino acid metabolism, protein transport/proteolysis, protein synthesis/folding, stress/detoxification and ascorbate-glutathione cycle. Our results showed high overlapping in altered functional categories among *Arabidopsis* accessions. At the same time, proteome of *Arabidopsis* descendant (accession Che_0,7) from radioactively contaminated place exhibits specific proteins which with highly probability are involved in promoting plant stress resistance. Thus, accumulation of enzymes involved in the biosynthesis of sulfur-containing amino acids, might be connected with phytochelatin biosynthesis and plant protection against heavy metals; and abundant energy related proteins could facilitate detoxification mechanisms. Distinctive feature of tolerant *Arabidopsis* accession versus not tolerant is increase in annexin D1 level which is known to protect photosynthetic pigments from degradation. We suggest that identified proteins in tolerant *Arabidopsis* could be implemented in biotechnology for crops stress resistance and used as markers for molecular breeding.

**ROLE OF CYTOMIXIS IN A MECHANISM OF MICROSPOROCTE KARIOTYPE
REARRANGEMENT**

Olena Kravets

Lecture 4

Olena Kravets, Svitlana Plokhovska, Inna Horiunova, Alla Yemets, Yaroslav Blume
*Institute of Food Biotechnology and Genomics, National Academy of Sciences of Ukraine,
Osypovskogo Street 2a, 04123 Kyiv, Ukraine*

E-mail: kravetshelen@gmail.com

Cytomixis is a type of nuclear and cytoplasmic cell-to-cell migration typical for plant and animal tissues. This process involves the interaction of dynamic cytoskeletal components with the nucleus through signalling systems and linker complexes (Kravets et al., 2017a, b; Mursalimov et al., 2019). The most numerous studies regarding cytomixis are researches on microsporogenesis in angiosperms. The wide occurrence of cytomixis in terrestrial plants testifies its being involved in the important genomic reorganization processes, which allow its carriers to be maintained due to natural selection process. However, despite significant progress in study of cytomixis its role as mechanism to increase genetic diversity and speciation in plants are still not clear. We have studied the assignment and actions of cytomictic chromatin in meiosis as well the mechanisms of rearrangement of microsporocyte and microspore genotype. Several monocotyledonous species with spontaneous cytomixis were investigated: Saffron lily (*Lilium croceum* Chaix.), Welsh onion (*Allium fistulosum* L.), Onion (*Allium cepa* L.). Light and fluorescent microscopy were used. Based on the cytogenetic analysis we have concluded that in microsporocytes of investigated species of lily are formed extra chromosomes presumably of a cytomictic nature. Aneuploidic microsporocytes are remained as functionally viable cells. Extra chromosomes are detected not only in hyperchromosomal but in euchromosomal microsporocytes. The markers of additional chromosome are the aberrations, weakened synapsis between homologues and synapsis with other chromosomes, including bivalents of the basic karyotype via formation of secondary chromosome associations. This allows extra chromosomes to be partially fixed in the meiotic division apparatus and participate in the restructuring of the karyotype. The bulk of the cytomictic chromatin after recombination is the cell "genetic ballast" which is gradually eliminated using chromosome rearrangements, chromatin diminution, differential chromosomal distribution, asymmetric division, cytomixis and PCD. Nevertheless, a part of extra chromosomes can adapt to the microsporocyte genome and participate in its reorganization. In this favor indicates the presence of extra chromosomes in eu- and hypochromosomal microsporocytes as well as polymorphism of pollen grains. It should be noted that the above events can be caused both by cytomixis and by the potential hybrid nature of these species.

Kravets E.A. et al. Cytol. Genetics 2017a, 51(3):192–201.

Kravets E.A. et al. Cell Biol Int 2017b. doi: 10.1002/cbin.10842.

Mursalimov S.R. et al. Biologia 2019. <https://doi.org/10.2478/s11756-019-00203-4>

ОПТИМІЗОВАНА СИСТЕМА АНАЛІЗУ МОДИФІКАТОРІВ МУТАГЕНЕЗУ НА ОСНОВІ
МОДЕЛЬНОГО РОСЛИННОГО ОБ'ЄКТА *ALLIUM CEPA L.*

Володимир Шкарупа

Lecture 5

Шкарупа В.М.¹, Клименко С.В.², Піскун Р.П.¹

1 – Вінницький національний університет ім. М.І. Пирогова, Вінниця, вул. Пирогова 56

2 – Національний науковий центр радіаційної медицини НАМНУ, Київ, вул. Мельникова 53

E-mail: Piskyn2006@gmail.com

Однією з сучасних проблем антимутагенезу є розрив між великою кількістю відомих речовин з антимутагенними властивостями та їх обмеженою кількістю, що успішно застосовуються в якості засобів профілактики/терапії наслідків індукованого мутагенезу. Це обумовлює необхідність виходу за рамки спрощеного визначення так/ні наявності антимутагенних властивостей, створення комплексних підходів виявлення і порівняльної оцінки антимутагенів; з'ясування умов, що визначають характер антимутагенезу в залежності від цілого ряду параметрів: шляхів та часу дії, дози мутагенів та модифікаторів; розробки математичних підходів оцінки ефективності антимутагенів та ін. У зв'язку з цим на перший план виступає завдання розробки методологічних основ та методичних підходів для якісної і кількісної оцінки модифікаторів мутагенезу. Вирішення цих завдань в повному об'ємі з використанням батареї тестів є довготривалою, трудомісткою, вартісною та важко виконуваною роботою. В цьому відношенні рослинні тест-системи з використанням цитогенетичних параметрів мають істотні переваги. Разом з тим, класична тест-система на основі кореневої меристеми цибулі (*Allium-test*), на нашу думку є недооціненою в плані розширення спектру критеріїв оцінки мутагенезу і його модифікації та можливостей для вирішення зазначених завдань. Метою роботи було створення та апробація комплексної системи якісних та кількісних критеріїв оцінки модифікаторів мутагенезу з використанням в якості модельної тест-системи *Allium cepa L.*

Розроблена система базується на використанні комплексу цитогенетичних критеріїв та параметрів оцінки модифікації мутагенезу, таких як частота аберантних клітин (та аберацій), пошкодженість аберантної клітини, частота мультиаберантних клітин, спектр аберацій хромосом, співвідношення «мостів» та фрагментів (як показник ефективності репаративних процесів), мітотичний індекс, співвідношення фаз мітозу. Параметри системи: використання в якості модельних – мутагенних чинників з різними механізмами дії та ефективністю (алкілюючі мітоміцин С та тіофосфамід, прооксидантний діоксидин, іонізуюче випромінення), роздільна та сумісна дія мутагена та модифікатора, їх вплив на різних стадіях мітотичного циклу (та впродовж всього мітотичного циклу), еквідозиметричний підхід (аналіз ефективності антимутагенів при дії мутагенів в дозах, що спричиняють однаковий рівень цитогенетичних пошкоджень), залежність антимутагенної ефективності від концентрації антимутагену, від рівня мутагенезу та ефективності мутагенів, діапазон антимутагенної ефективності. Додаткові математичні параметри оцінки: статистичний аналіз поклітинного розподілу аберацій (відповідність розподілам Пуасона, геометричному та компаунду цих розподілів), визначення інтегрального показника – коефіцієнта антимутагенної ефективності на основі теоретичних моделей залежностей доза мутагену-ефект, що дозволяє оцінити антимутагенну ефективність незалежно від рівня мутагенезу та прогнозувати ефект антимутагену при дії мутагенів в різних дозах.

Висновки. Розроблена оптимізована (в аспектах економічності, швидкості та інформативності аналізу) система якісних та кількісних критеріїв оцінки модифікаторів мутагенезу на прикладі дослідження антимутагенних властивостей гумінових речовин дозволяє значно збільшити інформативність комплексного аналізу модифікаторів мутагенезу.

SOIL SICKNESS AS A RESULT OF THE OCCURRENCE OF TOXINOGENIC FUNGI IN CULTIVATED SOILS

Wieslaw Barabasz

Poster 1

Wiesław Barabasz, Anna Pikulicka
East European State Higher School in Przemyśl

E-mail: rbaraba@cyf-kr.edu.pl

Soil sickness is a disadvantage occurring all over the world and more and more often in European arable soils, on which cereal cultivation begins to dominate. It is estimated that Europe in the structure of cereal sowing accounts for about 70%. This is unfavorable in many ways because it contributes to the degradation of arable soils, which in turn leads to a reduction in crop yields. Among the many factors that cause soil sickness and affect the reduction of crops attention should be paid to the biological factor, and especially the occurrence of an increased number of fungi including pathogenic fungi and toxinogenic fungi. It should be noted that plants grown in Europe are an excellent substrate for the development of various species of fungi, and climatic conditions including heavy rainfall during the harvest season favor the production of mycotoxins. The most well-known mycotoxins and particularly dangerous for all soil organisms and higher plants, animals and humans are species belonging to the genera *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria*. And it is mycotoxins that seem to be one of the main negative biological factors that significantly affect the microbiological activity of arable soils, contributing to a breakdown in the microbiocenotic systems of soils, which results in a decrease in crop yields. The harmful effect of mycotoxins on the soil microbiota manifests itself mainly in inhibiting the intensity of organic matter decomposition processes, weakening of biological nitrogen fixing processes, adverse effects on nutrient biogens in agroecosystems and weakening of symbiosis between microorganisms and crops, as well as elimination of bacteria recognized as PGPR (plant growth promoting rhizobacteria). It should be noted that soil sickness is an example of unfavorable changes in the natural environment caused by disturbances in the functioning of individual elements of soil biocenosis, and examples of soil sickness are familiar to farmers: clover, execution; tobacco, grinding; flax, eradication; beets, breakage etc.

ОДЕРЖАННЯ ХІТОЗАНУ ПРИ КОМПЛЕКСНОМУ ВИКОРИСТАННІ ХРЯЩА-МОЛОЧНИКА ПЕРГАМЕНТНОГО (*LACTARIUS PERGAMENUS* (FR.)FR): ТА ДОСЛІДЖЕННЯ ЙОГО ВЛАСТИВОСТЕЙ

Володимир Антонюк

Poster 2

Антонюк В.О.^{1,2}, Панчак Л.В.¹

1 – Львівський національний медичний університет імені Данила Галицького

2 – Інститут біології клітини НАН України, м. Львів, вул. Драгоманова 14/16

E-mail: antonjukvo@gmail.com

Метою даної роботи була розробка методу одержання хітину і хітозану з висушених вичавок хряща-молочника пергаментного в рамках їх комплексного використання та дослідження його фізико-хімічних властивостей.

Матеріали та методи. Плодові тіла хряща-молочника пергаментного збирали у мішаному лісі в липні під час їх масової появи у Сколівському районі Львівської області, та на протязі 6 годин доставляли до лабораторії, де їх подрібнювали на м'ясорубці і сік витискали на потужному пресі. Сік був використаний для одержання лектину, а вичавки висушували і екстрагували метанолом. Метанольний екстракт фракціонували органічними розчинниками і проводили хроматографію на колонці силікагелю і таким чином одержали фракції, що містили сесквітерпени, фталати, стеаринову кислоту [1-2]. Вичавки після екстракції метанолом повторно висушували і використовували для одержання хітину і хітозану.

Висушені вичавки порошокували в електроміксері і просівали через сито з розміром пор 0,5 мм. Для одержання хітозану ЛНМ заливали 50% розчином NaOH у співвідношенні 1:10 і поміщали на киплячу водяну баню на 60 хв. Після цього порошок хітину промивали дистильованою водою до тих пір, поки промивні води не обезбарвлювалися. Хітозан із вичавок, оброблених таким способом, екстрагували 2 % розчином оцтової кислоти при інтенсивному перемішуванні суміші протягом 30 хв, і потім процес повторювали ще раз. Надосадову рідину обох екстракцій об'єднували і хітозан осаджували 0,1 н. розчином NaOH при доведенні рН до 9,5-10,0. Осад хітозану промивали водою, далі 96° етанолом, ацетоном, диетиловим ефіром і висушували при кімнатній температурі. Молекулярна вага визначалась віскозиметричним методом. Ступінь деацетилювання зразків хітозану визначали шляхом титрування 0,1 н. розчином NaOH використовуючи як індикатор фенолфталеїн. Електрофоретичний аналіз хітозану проводили в пластині поліакриламідного гелю з поступовим градієнтом концентрації акриламідру 5, 10, 15 і 20% за допомогою кислої буферної системи (β -аланін-оцтова кислота, рН 4,5). Фарбування здійснювали 0,1 % р-ном кумасі G-250.

Результати і обговорення. При комплексному використанні плодкових тіл хряща-молочника із 100,0 г свіжих сирих плодкових тіл можна одержати 7,54 г сухих вичавок, звільнених від водо- і метанолорозчинних речовин. Нами було встановлено, що розчинення хітину з сухих вичавок можна здійснити 36 % соляною кислотою, але для максимального зменшення гідролізу розчинення необхідно здійснювати дотримуючись температури льодяної бані. Вихід хітину з вичавок плодкових тіл після екстракції водо- і метанолорозчинних речовин становив $13,6\% \pm 3,5\%$. Вихід хітозану з сухих вичавок – $6,27\%$. Середня молекулярна маса хітозану плодкових тіл *Lactarius pergamenus*, визначена віскозиметрично, становила 112 ± 3 кДа. Ступінь деацетилювання – $87,1\%$. Результати диск-електрофорезу вказують на сильну гетерогенність одержаних зразків хітозану. Кислий гідроліз одержаних зразків хітозану до моносахаридів показав наявність у ньому наявність D-глюкози та відсутність D-фруктози, крім N-ацетил-D-глюкозаміну та D-глюкозаміну. Очевидно, що на відміну від хітозану креветок, хітозан хряща молочника пергаментного формує хітозан-глюкановий комплекс, залишки глюкози у якому міцно зв'язані ковалентними зв'язками.

1. Панчак Л. В. та ін. Біотехнологія, 2011. 4 (5): 90 - 96.

2. Tsivinska M. V. et al. // J. Adv. Biol., 2015. 6(3): 1023- 1035

3. Mati-Baouche N et al.// European Polymer J. 2014. 60: 198–213

**NITRIC OXIDE AS A SIGNALLING MOLECULE IN HEAT-INDUCED RESPONSE
INVOLVING MICROTUBULES IN *ARABIDOPSIS THALIANA***

Svitlana Plokhovska

Poster 3

Svitlana Plokhovska, Alla Yemets, Yaroslav Blume

*Institute of Food Biotechnology and Genomics, National Academy of Science of Ukraine,
Osipovskogo Street 2a, 04123, Kyiv, Ukraine*

E-mail: svetaplohovska@gmail.com

With the global temperature raised, heat stress is an important environmental factor limiting plant growth, development and productivity. Heat stress has also been shown to disturb coordination of plant organelles, damage to the cytoskeleton, thereby altering cell differentiation and elongation. Many previous studies have shown that the plant cytoskeleton participates in the response to heat stress (Malerba et al., 2010, Parrotta et al., 2015). NO is signaling molecule in mediating various plant responses such as photosynthesis, oxidative defense, osmolyte accumulation, gene expression, and protein modifications under heat stress. The literature describes an increase in NO accumulation in various plant species in response to high temperature treatments (Yu et al., 2014). Nevertheless, deep insights into the functional intermediaries or signal transduction components associated with NO-mediated heat stress signaling are important establish the role of NO in plant heat tolerance.

The aim this study was to investigate the influence of high temperatures (+38°C, +41°C, +45°C) and these temperature in combination with NO donor or scavenger on microtubule organization in living plant cells using *A. thaliana* (GFP-MAP4) line. It has been found that 100 µM SNP (NO donor) stimulates formation of root hairs and their active growth, whereas 100 µM cPTIO (NO scavenger) treatment leads to cell swelling in transition and elongation zones of primary roots and induction of primordial formation of root hairs. Exposure of *Arabidopsis* roots at +38°C did not cause visible changes in microtubule organization of s, they only changed their orientation. Reorganization of microtubules occurred after exposure to temperatures +41°C and +45°C: from singly disorganized microtubules to partially and completely depolymerization microtubules in some cells. We found that the most sensitive to heat stress were cortical microtubules in the epidermal cells of meristematic and elongation root as well as in the root hairs. SNP causes to microtubules network reorganization, while both high temperatures and NO scavenger (cPTIO) increase its randomization and fragmentation. The obtained results testify the existence of a functional relationship between changes in the intracellular NO content and the organization of microtubules after high temperature treatment of plant cells. The obtained results allows us to conclude that microtubules are important intermediates in the realization of high temperature effects in plant cells, and NO is involved in cell response to heat stress by signaling through these cytoskeletal structures.

Malerba M. et al. Protoplasma. 2010, 239:23-30.

Parrotta L. et al. Planta. 2015, 243(1):43-63.

Yu M. et al. New Phytol. 2014, 202:1142-1156.

**BIOCHEMICAL CHARACTERIZATION OF MICROALGAE ISOLATED FROM
PODKARPACIE REGION IN POLAND**

Daniel Broda

Poster 4

Daniel Broda¹, Magdalena Podbielska², Ewa Szpyrka², Grzegorz Chrzanowski¹

1 – Laboratory of Molecular Biotechnology, Faculty of Biotechnology, University of Rzeszow, Pigoia 1, 35-310 Rzeszow, Poland

2 – Department Chemistry of Analytical Chemistry, Faculty of Biotechnology, University of Rzeszow, Pigoia 1, 35-310 Rzeszow, Poland

E-mail: danielbroda@wp.pl

Phytochemicals are bioactive compounds which is found in vegetables, fruits, or in different plants, They are not considered as an essential nutrients, however they are provide a lot of health benefits. Moreover, they are not plant-specific, they were found in microalgae and cyanobacteria. Some of the microalgae species were identified as a valuable source of the biocompounds; they are rich in polysaccharides, proteins or lipids. Epidemiological and animal studies have previously demonstrated therapeutic effects of phytochemicals extracted from microalgae, and particularly their antioxidant, anti-inflammatory, and anti-hypertensive properties, as well as their ability to protect damage of DNA and cardiovascular disease. The commercial cultivation of microalgae has begun five decades ago and its application of algae products was first introduced in Japan. Microalgae are generally obtained from a saltwater environment and commercial producers are located primarily in the Asia-Pacific region (China, Taiwan, and India) with wide access to the seas. Closed systems (photobioreactors, PBR) and open ponds (raceways) are used for microalgae cultivation and both have advantages and disadvantages. Thus growth characteristics and cell composition of microalgae are significantly dependent on the environmental source of microalgae and their cultivation conditions.

For this purpose new freshwater algae was obtained from an environment of Podkarpacie region in Poland, and characterized. Lipid content, fatty acid profile, glycan content, including β -glycani, carotenoids, phenols, vit. C and antioxidant capacity was determined.

INFLUENCE OF LEAD ON THE ORGANIZATION OF MICROTUBULES IN *ARABIDOPSIS THALIANA* ROOT CELLS

Inna Horiunova

Poster 5

Inna Horiunova, Alla Yemets

Institute of Food Biotechnology and Genomics, National Academy of Science of Ukraine, Department of Cell Biology and Biotechnology, Osipovskogo Street 2a, 04123, Kyiv, Ukraine

E-mail: inna.horiunova.ukr@gmail.com

Plant cytoskeleton orchestrates such fundamental processes in a cell as division, growth and development, cell inner and outer motility, vesicle transport, polymer cross-linking, membrane anchorage, etc. The dynamic instability of some of the cytoskeletal components allows it to be adaptively rearranged in response to different environmental stimuli including metal content-pollutants. Lead (Pb^{3+}) is one of the most toxic non-essential heavy metals and a major environmental pollutant. Microtubules and actin filament are important targets of lead and other heavy metals action. The inhibitory effects of toxic metals on tubulin assembly, protein synthesis, and kinesin-related microtubule mortality have been extensively studied (Scortegagna et al., 1998).

Influence of lead on plant microtubules was investigated in our study. 4-day-old seedlings primary roots *A. thaliana* (GFP-MAP4) ecotype Landsberg erecta (Ler) expressing GFP-MAP4 (a green fluorescent protein (GFP)–microtubule-associated protein4 (MAP4) fusion protein) (Mathur and Chua, 2000) was used in this research. The primary roots of seedlings were treated with an aqueous solution of 1, 10 μ M (Lead (II) nitrate) for 1 (to study the effects on the microtubule), and for 24, 48, 72 h (to study the effects on root growth and morphology). It was established that high concentrations of lead induce inhibition of growth and changes in the morphology of *A. thaliana* seedlings primary roots after 24, 48, 72 h of lead (II) nitrate treatment. Root hair development, meristematic, epidermal cells of the differentiation zone, root apex and elongation zone, were extremely sensitive to all tested concentrations of lead (II) nitrate. The major reason of these changes is a disruption of the organization of microtubules which were visualized *in vivo* with a confocal laser scanning microscope LSM 510 META (Carl Zeiss, Germany). Minor reorientation in microtubule organization were observed after treatment with 1 μ M lead (II) nitrate. Whereas 10 μ M lead (II) nitrate provoked a dose-dependent microtubules reorganization ranging from randomization to strong depolymerization in epidermal as well as in cortex cells of all *A. thaliana* primary root zones immediately after treatment. Similar changes were observed in the meristematic cells, that is one of the reason of growth inhibition and morphology disruption of *A. thaliana* seedlings primary roots. Thus, it has been clearly demonstrated that plant microtubules is one of the target of lead (II) nitrate action.

Mathur J, Chua N-H. Plant Cell 2000, 12: 465-77.

Yemets A.I. et al Cell Biol Int. 2008, 32(6): 630-637.

Scortegagna, M. et al., Neurochem. Int. 1998, 32: 353-359.

CUSCUTA AND OROBANCHE GENERA AS THE MOST WIDESPREAD PARASITIC PLANTS IN UKRAINE

Inna Horiunova

Poster 6

Yulia Krasylenko^{1,2}, Inna Horiunova¹, Svitlana Plokhovska¹, Maria Borova¹, Nadia Pushkarova¹
1 – Institute of Food Biotechnology and Genomics, National Academy of Sciences of Ukraine,
Osipovskogo Street 2a, 04123, Kyiv, Ukraine

2 – Palacký University, Department of Cell Biology, Centre of the Region Haná for Biotechnological and
Agricultural Research, Šlechtitelů Street 27, 78371, Olomouc, Czech Republic

E-mail: inna.horiunova.ukr@gmail.com

Among the flowering plants, there are approximately 3,900 known parasitic plant species in more than 20 plant families (Westwood et al., 2010). Well-known and agriculturally important genera include *Striga* and *Orobanche* from the *Orobanchaceae* family and *Cuscuta* spp. from the *Convolvulaceae* family. Parasitic plants are widespread pathogens that infect numerous plant species and cause devastating agricultural losses. They efficiently withdraw water, nutrients and sugars from their hosts by fusing tissues and connecting their vasculature to the host vasculature. Despite multiple independent characteristics, a common feature to parasitism is the formation of an invasive organ termed the haustorium. Parasitic plants form haustoria in their stems or roots and use this structure to penetrate host tissues and form vascular connections, often with distantly related species (Spallek et al., 2013; Yoshida et al., 2016). Despite the extensive study of the dodder and broomrape parasitic lifestyle peculiarities and the differences in their morphology from non-parasitic species of higher plants, the issue of the distribution of these plants of parasites in Ukraine is learn insufficiently. The current paper addresses the distribution of parasitic plants from *Cuscuta* L. (*Convolvulaceae*) and *Orobanche* L. (*Orobanchaceae*) genera in Ukraine based on the analysis of the herbarium specimens from the National Herbarium of M.G. Kholodny Institute of Botany, NAS of Ukraine (KW) and literature reports. In order to study the places of growth of the dodder and broomrape the traditional method based on the literary sources and herbarium funds data. About 1600 samples from KW were analyzed and it was found that the distribution range of these parasitic plants is attributed mostly to the Steppe natural zone of Ukraine including the Crimean Peninsula. Creation of specialized databases and maps that would cover the diversity and distribution of these families in Ukrainian flora is useful for monitoring the changes in their range in order to control them. The results will be used in further research to develop an efficient strategy of parasitic species phytoquarantine control.

Westwood J.H. et al. Trends Plant Sci. 2010, 15:227-235.

Spallek T. et al. Mol Plant Pathol. 2013, 14:861-869.

Yoshida S. et al. Annu Rev.Plant Biol. 2016, 67:643-667.

**EXON-INTRON STRUCTURE OF ACTIN GENES AS AN EFFICIENT TOOL
FOR WHEAT AND BARLEY GENOTYPING**

Yaroslav Pirko

Poster 7

Yaroslav Pirko, Anastasiia Postovoitova, Anastasiia Rabokon, Yuliia Bilonozhko, Liubov Kalafat,
Yaroslav Blume

*Institute of Food Biotechnology and Genomics, National Academy of Sciences of Ukraine, Osypovskoho
Str., 2a, 04123 Kyiv, Ukraine*

E-mail: yarvp1@gmail.com

Introns as non-coding parts of genes which can be used powerful instrument of molecular genetic investigation. Introns as hypervariable sequences became the basis for the creation of widely distributed ILP (Intron Length Polymorphism) markers, which allows to evaluate genotypes and to explore plant diversity on different taxonomic levels. In addition, actin gene introns can be involved in the gene expression regulation and play the key role in cell division and differentiation. Actin genes are represented by a numerical conservative gene family in plants.

Wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), whose genomes are sequenced completely, were selected as the model plants for the actin gene search. However, for today, full reliable information about the quantity and exon-intron composition of actin genes of wheat and barley is absent. It served as a reason to make a search of actin genes sequences in wheat and barley genomes. This procedure was realised using the Phytozome v12 database (www.phytozome.net) and the on-line tool BLASTN version 2.2.26+.

As a result of bioinformatic search 15 actin genes were detected in wheat genome and 8 actin genes in barley. In general, most genes of wheat actin have 3-4 exons and 2-3 introns. Exception is the actin gene Traes_4DS_E47C518AD, which contains 5 exons and 4 introns. The exons of wheat actin genes have a length from 57 bp to 764 bp, and introns - 50 - 842 bp. Actin genes in barley have 3-4 exons and 2-3 introns. However, barley actin genes HORVU1Hr1G002840 and HORVU4Hr1G008310 don't contain IIIrd intron and IVth exon, while the IInd intron absents in HORVU3Hr1G083370 and HORVU3Hr1G083380 barley actin genes, and the IInd and IIIrd exons are combined in one exon (1008 bp). The barley actin exons have a length from 55 bp to 1008 bp and introns - 79-518 bp. In general, the analyzed data show systematic quantitative exon composition of actin genes in wheat and barley. In the vast majority actin exons contain same number of nucleic acid pairs: 60 (Ist exon), 394 (IInd exon), 614 (IIIrd exon) and 66 (IVth exon). There is no systematic and orderly in the intron structure.

The received data is subsequently used to study the intron functioning and their effects on actin gene expression, as well as for development of gene specific ILP-markers. Thus the consensus sequences inside of exons can be used for estimation of rate of gene expression, but for genotyping – the consensus sequences of neighboring exons.

RECONSTRUCTION OF SPATIAL STRUCTURE OF α - AND β -TUBULINS IN THE COMPLEX WITH CURCUMIN

Svitlana Spivak

Poster 8

Alex Rayevsky, Svitlana Spivak, Dariya Samofalova, Pavlo Karpov, Serhii Ozheredov, Oleg Demchuk, Alla Yemets, Yaroslav Blume

Institute of Food Biotechnology and Genomics, Natl. Acad. of Sci. of Ukraine

Osyповskoho str., 2A, Kyiv, Ukraine 04123

E-mail: SpivakS@nas.gov.ua

Curcuminoids are natural polyphenol compounds of diarylheptanoid group. They show antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory and antiproliferative effects (Mishra et al., 2008). Also, curcuminoids demonstrate positive effects in neurological diseases that directly associated with microtubule (MT) disorders (Sahu et al., 2009). Currently, the number of syntactic derivatives of curcumin show ability to interact with tubulin. Such direct binding was found to inhibit assembly of MT, what characterizes curcuminoids as bifunctional ligands (Srivastava et al., 2011). In turn, α - and β -tubulin molecules are involved in many biological processes, which are strongly associated with their vital functions in cell. As the key elements of MTs tubulins are main molecular targets for MT-binding agents. At the same time, many syntactic drugs with antimicrotubular activity are unsafe and cause structural and functional disorders in MT organization. The purpose of this study was to find out how tubulins can interact with curcumin.

To detect appropriate site on the tubulin surface and to estimate affinity against different candidate sites, we used blind screening and further performed precise docking of different curcuminoids on a whole surfaces of the tubulin molecules. Also, we used docking as molecular modeling to predict positions, orientations and energies of curcumin binding with α - and β -tubulin. The spatial structures of α - and β -tubulins from human and *Arabidopsis* were reconstructed using homology modeling method. The structures of native curcuminoids were obtained from the ChEMBL database and were used in site screening (based on molecular docking). Structural features of potential curcumin binding sites in different tubulin molecules were compared with spatial fitting method. To verify the stability of obtained complexes we used molecular dynamics computations in GROMACS (50 ns and temperature 310K).

Studying of α - and β -tubulin surfaces in monomer and dimer states revealed several potent binding sites for curcumin. The part of total pool of ligand poses occupied in colchicine binding site of α -tubulin. The other part was located in vinblastine binding site region. Also, much smaller number of conformations with different level of predicted affinity was observed in GTP- and taxol binding sites. In this way, our results on curcumin binding correspond to experimental data and confirms that their direct interaction with tubulin is possible.

The work was supported by grant NAS of Ukraine project N 0118U102391 «Investigation of the interaction of curcumin with cellular targets and the selection and creation of its functionally effective forms for the treatment of neurodegenerative diseases».

Mishra S. et al. Ann. Indian Acad. Neuro. 2008, 11(1):13-19.

Sahu R.P. et al. Br. J. Cancer. 2009, 100(9):1425-1433.

Srivastava S. et al. Biosci. Rep. 2016, 36(2):e00323.

THE INFLUENCE OF DROUGHT ON CHANGES IN CONTENT OF HYDROGEN PEROXIDE AND ACTIVITY OF CATALASE IN SOYBEAN, INOCULATED WITH STRAIN AND Tn-5 MUTANTS *BRADYRHIZOBIUM JAPONICUM*

Kots Sergey, Mamenko Tatyana, Pukhtajevich Peter, Zhemoyda Alla
*Institute of Plant Physiology and Genetics, National Academy of Sciences of Ukraine,
Vasylkivska Street 31/17, 03022, Kyiv, Ukraine*

E-mail: t_mamenko@ukr.net

The aim of the work was to investigate the effect of prolonged drought on changes in the content of hydrogen peroxide and the activity of catalase in the soybean roots and root nodules by inoculation *Bradyrhizobium japonicum* strains and Tn5 mutants with contrasting symbiotic properties. The objects of the study are chosen symbiotic systems, formed with the participation of soybean plants (*Glycine max* (L.) Merr.) and *B. japonicum* strains 646 (active, virulent) and 604k (inactive, highly-virulent) and Tn5 mutants B1-20 (active, virulent) and 107 (low-activity, virulent) from the museum collection of nitrogen-fixing microorganisms at the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine. The studies were conducted in strictly controlled conditions of the model experiment. Methods used were microbiological, physiological, biochemical, gas chromatography, spectrophotometry.

It was shown that the studied symbiotic systems realized their protective mechanisms in different ways and adapted to the conditions of drought. In particular, in the symbiotic system formed with the participation of soybeans and the active strain of rhizobium 646, an increase in hydrogen peroxide content was observed and a slight decrease in the activity of catalase in the root nodules during drought. It was recorded that in the symbiotic system formed with the participation of the active Tn5 mutant B1-20 there is the inclusion in the work of protective systems for the long-term effects of stress. This indicates an increase in catalase activity and an insignificant increase in hydrogen peroxide content. At the same time there was a rapid restoration of their level to optimal after the restoration of watering the plants. This underlines the ability of effective symbiosis to quickly mobilize their own defense systems and adapt to growing conditions. Investigated that the symbiotic system, formed with the participation of soybean plants and the low-activity Tn5 mutant 107, was characterized by high content of hydrogen peroxide and intensification of catalase activity in the root nodules during the period of drought. At the same time, in the symbiosis of plants with an inactive strain of rhizobium 604k, an increase in hydrogen peroxide content and a decrease in the activity of catalase in the root nodules for prolonged dehydration was observed. Both symbiotic systems had a low capability to restore the level of prooxidant and enzyme to control in the post-stress period. We noted that in all the studied symbiotic systems there was tendency to gradually increase the content of hydrogen peroxide in the roots with increasing drought and its partial restoration to the level of control in the post-stress period. It should be noted that under optimal conditions of plant growth, the total level of peroxide in soybean roots, inoculated with rhizobia, was lower compared to plants that were not inoculated. This indicates possible changes in the prooxidant system in the roots of plants by inoculation of rhizobia. It was proved that, regardless of the effectiveness of the symbiotic system, catalase activity in soybean roots, inoculated rhizobia increased with moderate drought. With the increase in stress, the level of catalase declined, especially in the roots of soybean, inoculated with an inactive strain of rhizobia 604k. In the post-stress period, the restoration of the catalase activity in the roots to the optimal level is noted only in effective symbiotic systems.

Thus, the efficiency of the functioning of the symbiotic systems *Glycine max* - *B. japonicum* under drought conditions is marked by adaptive changes in catalase activity in root nodules and roots, which helps regulate the content of hydrogen peroxide and maintain prooxidant-antioxidant balance during long-term exposure to drought.

**CYTOGENETIC STUDY OF *DESCHAMPSIA ANTARCTICA* E. DESV.
PLANTS REGENERATED FROM TISSUE CULTURE**

Daria Navrotska, Igor Andreev, Viktor Kunakh

*Institute of Molecular Biology and Genetics of the National Academy of Science of Ukraine,
Acad. Zabolotnogo Street 150, 03143 Kyiv, Ukraine*

E-mail: d.o.navrotska@imbg.org.ua

In vitro culture is often accompanied by the appearance of genetic or phenotypic changes. It is believed that main causes of the occurrence of somaclonal variation are the heterogeneity of the cells of the initial explant as well as genetic and epigenetic changes induced by culture. The earlier studies of somaclonal variation in Antarctic hairgrass *Deschampsia antarctica* E. Desv. demonstrated the absence of genetic and phenotypic differences between the regenerants and donor plants collected from natural populations (Cuba *et al.*, 2005; Osorio *et al.*, 2014). However, no cytogenetic study has been carried out up to now on *D. antarctica* cultured *in vitro*. Therefore, our aim was to investigate the variation in chromosome number of *D. antarctica* regenerated plants.

Root apical meristems of nine plants were analyzed. The plants were regenerated through indirect organogenesis from tissue cultures at 2-3 passages initiated from two diploid ($2n=26$) and one hypotriploid ($2n=36-39$) plants. Only one of two regenerants, which were originated from diploid donors, was diploid, while in the other 30.9 % of cells were aneuploid. Seven regenerated plants originated from hypotriploid donor were mixoploids, in which the cells with different chromosome numbers ($2n=26, 28, 33, 36$) were observed, with a modal class of diploid cells (33.3% - 85.7%).

The results of the study allow us to conclude that *D. antarctica* tissue cultures of both diploid and hypotriploid origins possess the ability to regenerate. The diploid cells prevailed in all of the regenerated plants regardless of the ploidy of the explant donor plant. Obtained results might indicate that the diploid cells in a heterogeneous cell population show enhanced ability to form regenerated plants.

Cuba *et al.* Antarctic Science 2005, 17(1):69. DOI: 10.1017/S0954102005002440

Osorio *et al.* Polar Biol 2014, 37(2). DOI: 10.1007/s00300-013-1425-2

COLD-INDUCED ULTRASTRUCTURAL CHANGES IN CHLOROPLASTS OF *GALANTHUS NIVALIS* L. LEAVES AND THE EFFECT OF EXOGENOUS SUCROSE

Fediuk O.M., Bilyavska N.O.

*M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine,
Tereshchenkivska, 2, 01004, Kyiv, Ukraine*

E-mail: olgamuronivna@ukr.net

The leaves of *Galanthus nivalis* L. develop during the early spring in zones with the temperate climate, where the low temperatures of the atmospheric air and of the soil surface layer predominate. The method for enriching the leaves with exogenous sucrose (Deryabin et al., 2011) was used to study the role of sucrose (Suc) in enhancing the resistance of *G. nivalis* chloroplasts to the influence of a low temperature.

The results of the study in conditions of experimental cultivation of *G. nivalis* at an average air temperature of +5 °C and the appearance of leaves over the soil surface showed that significant damages in the ultrastructure of granal thylakoids in leaf chloroplasts did not observe.

Following gradual cooling the leaves of control plants to a temperature of –5 °C, a sharp swelling of the granae was revealed, whereas this effect was much weaker in leaves treated with Suc solution. The treatment with the Suc led to an increase in the number of thylakoids per grana at 0.02 M Suc and 0.1 M Suc (by 17 and 119%), while the length of the thylakoids decreased by 33 and 22%, and the thickness of thylakoids - by 3 and 22%. When a temperature was lowered to –15 °C, the destruction of the chloroplasts in the control leaves did not observe, however, there were fragmentations and swelling the granae. In chloroplasts treated with a solution of 0.1 M Suc, significant positive changes occurred in compare to the corresponding control without Suc.

Compared to the data obtained at +5 °C, after processing 0.02 M Suc at –15 °C, the amount of thylakoids per grana was less by 10%, however, it increased by 88% with 0.1 M Suc, the thickness of the thylakoids was greater at 0.02 M and 0.1 M Suc, respectively, by 18% and 10%. The cross area of the grana at 0.02 M Suc was 31% higher as a result of an increase in the number of thylakoids (by 16%), as well as their thickness (by 18%); treatment with 0.1 M Suc induced the area reduction by 26%, mainly due to the decrease in the length and width of the thylakoids. The presence of high granae formed with tightly packed, relatively short thylakoids, and the absence of deformations in thylakoids indicates the cryoprotector properties of 0.1 M Suc.

Thus, sucrose is involved in the stabilization of the granal system in the chloroplasts of *G. nivalis* leaves at low temperatures. Pre-treatment with 0.1 M Suc helps to preserve the intactness of the granae, increase the number of thylakoids and the area of the granae at low temperatures (from –5 to –15 °C) that prevents damage in the structure of the photosynthetic apparatus and ensures its effective functioning at low temperatures.

Deryabin A.N., Sabelnikova E.P., Burakhanova E.A. The dependence of the cold resistance formation in plants *in vitro* on the sucrose concentration in environment of raising. Bulletin of Mordovia University. 2011, 4:200.

STRUCTURAL-ADAPTIVE RESPONSE OF PHRAGMITES AUSTRALIS LEAF CELLS TO REDUCED SOIL MOISTURE

Olena Nedukha, Elizabeth Kordyum

*M.G. Kholodny Institute of Botany of National Academy of Sciences of Ukraine,
Tereschenkivska str., 2, 01601, Kiev, Ukraine*

E-mail: o.nedukha@hotmail.com

The study of plant adaptation of plants to changes in soil moisture is the most important and decisive factor of the environment in modeling the impact of global changes on plants, is of fundamental and applied importance. We have compared the structural-functional signs in leaves common reed *Phragmites australis* (Cav.) Trin. ex Steud grown under different soil moisture, using light-, electron microscopic, laser confocal techniques and X-ray analysis. The soil moisture and leaf moisture was detected by the classic biochemical methods. *Ph. australis* leaves of water and terrestrial plants were harvested at the vegetative stage. Water plants grew in water along the left bank of the Dnipro River (in Kiev zone); terrestrial plants grew near 12-15 meter far bank in a sandy soil. Upon nature soil drought, the thickness of leaf blade and cell size of epidermis and mesophyll were significantly reduced compared to those signs of air-aquatic plants. The density of the stomata and trichome on adaxial and abaxial leaf surface terrestrial plants were almost twice less than that in leaves of air-aquatic plants. A decrease in the density of the stomata on the leaf surface of drought plants may indicate a decrease in transpiration in plants growing on soil with reduced humidity, and that this structural feature possible helps the plant to optimize water balance in conditions of natural drought. Changes in the ultrastructure of leaf epidermis cells of *Ph. australis* were accompanied by the deposition of silicon inclusions (in the form of different crystals) in epidermis cell walls of terrestrial plants. Whereas in leaves of aquatic plants silicon was mainly in amorphous form and its content was lower in all types of epidermal cells. It is assumed that like localization silicon and increased content of this chemical element in leaf epidemis can to decrease of cuticular transpiration, to optimize the water balance of terrestrial plants, and also to increase their resistance to soil drought. It is proposed to strengthen attention to study of silicon role in the adaptation of plants to adverse changes in abiotic environmental factors.

**ІНДУКЦІЯ КАЛЮСОУТВОРЕННЯ ТА ОДЕРЖАННЯ КУЛЬТУРИ ТКАНИН
DESCHAMPSIA ANTARCTICA E. DESV.**

Ірина Конвалюк, Людмила Можилевська, Віктор Кунах
Інститут молекулярної біології і генетики НАН України,
вул. Акад. Заболотного, 150, 03143, Київ, Україна

E-mail: konvalyuk.i.i.@gmail.com

Щучник антарктичний (*Deschampsia antarctica* E. Desv.) – злакова рослина-абориген Антарктики. Це унікальна модель для вивчення механізмів пристосування рослинного організму до екстремальних кліматичних умов. Дослідження мінливості *D. antarctica* мають особливе прикладне значення, а біотехнологічні маніпуляції *in vitro* є простим і ефективним методом швидкого отримання достатньої кількості матеріалу, цікавого як з генетичної, так і фізіологічної точки зору, без нанесення шкоди природним екосистемам та порушення домовленостей, прописаних в Протоколі про охорону навколишнього середовища до Договору про Антарктику (http://www.ats.aq/r/ep_faflo.htm).

Метою роботи було підібрати умови індукції калюсоутворення та одержання культури тканин *D. antarctica* з різних локалітетів Морської Антарктики. Вихідним матеріалом були рослини *D. antarctica*, раніше вивчені нами на цитогенетичному рівні (Navrotska et al., 2014): диплоїди (2n=26) з мису Расмусен (R35), острова Галіндез (G/D12-2a), о. Скуа (S22), диплоїд з додатковими 1-3 В-хромосомами з о. Дарбо (DAR12) та гіпотриплоїд (2n=36-39) з о. Ялур (Y66). Для індукції калюсоутворення використовували листові, кореневі експланти та точки росту, які висаджували на 5 типів живильних середовищ: Мурасіге-Скуга (МС), Гамборга-Евелей (В5) та 5С (Кунах и др., 1996) з різними концентраціями фітогормонів бензиламінопурина (БАП), кінетину (Кін), α -нафтилооцтової (НОК) та 2,4-дихлор-феноксоцтової (2,4Д) кислот: №1 – В5 +2 мг/л 2,4Д + 0,1 мг/л БАП; №2 – В5 +10 мг/л 2,4Д + 0,2 мг/л БАП; №3 – МС + 5 мг/л 2,4Д + 0,1 мг/л Кін; №4 – 5С + 2 мг/л 2,4Д + 0,1 мг/л БАП; №5 – 5С + 2 мг/л 2,4Д + 2 мг/л НОК + 1 мг/л Кін.

Встановлено, що інтенсивність калюсоутворення залежала від мінерального складу живильного середовища, співвідношення і концентрації регуляторів росту, типу експланта, вихідного генотипу рослини-донора. Оптимальними середовищами для індукції калюсогенезу виявилися варіанти № 1, 2, 3. Індукція калюсу на живильному середовищі 5С відбулась на корневих експлантах лише у двох випадках: DAR 12 – 7,1%, S22 – 6,7%.

Отримано пасивовану культуру тканин з листових, корневих експлантів та точок росту. Калюс характеризувався пухкою консистенцією та світло-жовтим забарвленням. Найбільша калюсогенна активність була у точок росту (наприклад, 100% у гіпотриплоїда Y66). Найвищий відсоток калюсоутворення з корневих експлантів був у генотипа DAR12 з В-хромосомами (23,1% та 20% на середовищі № 3 та № 1 відповідно), а також у диплоїда G/D12-2a (20% на середовищі № 2). Найвищу здатність до калюсогенезу з листових експлантів спостерігали у гіпотриплоїда Y66 (25%) та у диплоїда R35 (18,8%) на середовищі № 1. Загалом, калюсогенна активність із точок росту перевищувала таку з корневих та стеблових експлантів у 3,4–4,1 рази. Окрім калюсоутворення, зрідка спостерігали спонтанний органогенез – у рослин генотипів DAR12 з В-хромосомами та диплоїда G/D12-2a.

Отже, підібрано оптимальні умови для індукції калюсоутворення з листових, корневих експлантів та точок росту *D. antarctica* з різних локалітетів Морської Антарктики. Отримані калюси здатні до проліферації та можуть бути використані для одержання достатньої кількості біологічного матеріалу для подальших досліджень.

**PARTICIPATION OF CALCIUM IONS IN IMPLEMENTATION OF HYDROGEN SULFIDE
PHYSIOLOGICAL EFFECTS IN PLANT CELLS**

Kolupaev Yu.E., Yastreb T.O., Havva E.N., Lugova G.A.

*Dokuchaev Kharkiv National Agrarian University,
p/o Dokuchaevske-2, Kharkiv, 62483, Ukraine*

E-mail: plant_biology@ukr.net

Currently, hydrogen sulfide (H₂S) is considered to be one of the signal mediators in plant cells. It is established that H₂S participates in the control of photosynthesis intensity, seed germination, regulation of the flower lifespan, and resistance to the effect of many stressors (Yamasaki, Cohen, 2016). However, the role of other signal mediators in its physiological effects realization remains poorly understood. It is not much known about calcium as universal secondary messenger in transduction of hydrogen sulfide signal.

The goal of the work was the investigation of calcium ions participation in the induction by hydrogen sulfide donor of antioxidant system components, heat resistance, and stomatal reactions of plants. The study was performed using winter wheat (*Triticum aestivum* – var. Doskonala) coleoptiles' segments, and leaves of four-week-old green *Arabidopsis* (*Arabidopsis thaliana* – Col-0) plants.

The treatment of wheat coleoptiles with 100 μM hydrogen sulfide donor NaHS enhanced their survival after potentially lethal heat stress (heating at 43°C in a water ultra-thermostat for 10 min). Under the action of H₂S donor in wheat coleoptiles it was noted a transient increase in the superoxide anion radicals generation and hydrogen peroxide synthesis with a maximum of 2-4 hours after the start of treatment. Further (4-24 h) the rise in activity of antioxidant enzymes, namely superoxide dismutase, catalase, and guaiacol peroxidase, was observed. The specified effects were almost completely eliminated by pre-treatment of coleoptiles' segments with chelator of extracellular calcium EGTA or phospholipase C inhibitor neomycin. These calcium antagonists also leveled off the effect of enhancing heat resistance of coleoptiles' cells, caused by hydrogen sulfide donor.

Arabidopsis leaves treatment with NaHS in concentrations of 5-250 μM caused a decrease in the stomatal aperture. The maximum effect of stomatal closing was observed at 90 min after H₂S donor treatment starting, and after 180 min of exposition the stomatal aperture in NaHS variants, vice versa, became wider than in control. Caused by hydrogen sulfide donor the reduction in stomatal aperture and relative number of open stomata was almost leveled off by leaves pre-treatment with a nonspecific calcium channel blocker – lanthanum chloride, chelator of extracellular calcium – EGTA, phospholipase C inhibitor – neomycin, and antagonist of the formation of cyclic adenosine-5'-diphosphate ribose – nicotinamide.

Thus, it was shown on two model objects (wheat coleoptiles and *Arabidopsis* leaves) the importance of calcium ions entering the cytosol from extracellular space and intracellular compartments for the development of physiological reactions induced by hydrogen sulfide – enhancing heat resistance, activation of antioxidant system and stomatal closure.

SYNTHESIS OF AUXINS, ABCISIC ACID AND ETHYLENE BY SOME PECTOLITIC BACTERIA BELONG TO GENUS *PECTOBACTERIUM* AND *DICKEYA*.

Liudmyla A. Dankevych¹, Natalia O. Leonova²

1 – Department of phytopathogenic bacteria, Zabolotny Institute of Microbiology and Virology (IMV), National Academy of Sciences of Ukraine, 154 Acad. Zabolotny str., Kyiv 03143, Ukraine

2 – Department of general and soil microbiology, Zabolotny Institute of Microbiology and Virology (IMV), National Academy of Sciences of Ukraine, 154 Acad. Zabolotny str., Kyiv 03143, Ukraine

E-mail: ldankevich@ukr.net

It is known that the synthesis of some classes of phytohormones (auxins, cytokinins, abscisic acid (ABA) and ethylene) as a key factor in pathogenicity is quite common for phytopathogenic bacteria. The action of the above-mentioned phytohormones produced by tumor-inducing species has been the most studied among the agent of plant's bacterial diseases. On the other hand, the role of phytohormones synthesized by bacteria in the processes of development such diseases as wilting, chlorosis, rotting and spottiness has been not studied enough attracting the researcher's attention although. Thus the bacteria that belong to the genus *Pectobacterium* and *Dickeya* are necrotrophs and inducing rotting of the many plants, they are synthesize large numbers of specific pathogenic factors namely: pectinases, cellulases, hemicellulases, proteinases, protein-inducers of plant cell necrosis and etc. But despite its necrotrophic way of lives in the initial stages of interaction with the plant cell, bacteria that belong to these genera can subsist as biotrophs. It is very little known about the mechanisms that are using by these pectolytic bacteria allowing them to avoid the action of the host's protective responses during the asymptomatic stage of infection. One from such strategies is an ability of pathogenic bacteria to imbalance hormonal signaling in plants, through the production of plant's hormones or their analogues, or to influence cross-links between ways of their synthesis. Among the bacteria that cause rotting of some plants, only certain species of *Dickeya* genus revealed the ability to synthesize auxins. Particularly, it has been shown that the synthesis of auxins by these agents has a regulatory role in the expression of virulence genes, but they are not known about the effect on hormonal signaling in host plants. For representatives of the genus *Pectobacterium* data about phytohormones synthesis as well as information on the association of these compounds with specific factors of pathogenicity or hormonal signaling in plants is almost absent. That is why the aim of our research was to determine the ability to synthesize extracellular auxins, ABA and ethylene by representatives of some species of genera *Pectobacterium* and *Dickeya*. In our researches we used the isolated *Pectobacterium* sp. 10G and 10OG by us, collection *P. carotovorum* 8418 and typical *P. carotovorum* susp. *carotovorum* UCM B-1075^T, *P. atrosepticum* UCM B-1084^T, *D. chrysanthemi* UCM B-1087^T strains of pectolytic bacteria. The quantitative and qualitative composition of extracellular auxins and the content of ABA and ethylene were determined by gas and liquid chromatography. It is shown that all studied strains of phytopathogenic bacteria are capable of synthesizing extracellular auxins but not – abscisic acid. Among the wide range of indole compounds, we have determined the ability of these strains to synthesize such compounds as: indolyl-3-acetic acid and indole-3-carboxylic acid. The amount of these auxin nature compounds correlates with their pathogenic properties. Thus, the highest content of determined auxins was found in highly aggressive strains of *Pectobacterium* sp. and typical strains of genera *Pectobacterium* and *Dickeya*. It should also be noted that all researched strains are capable of producing ethylene. Like the case of indole compounds, the level of this phytohormones production correlates with their pathogenic properties.

INDUCTION OF “BYSTANDER EFFECT” IN SOYBEAN SEEDLINGS ROOT MERISTEM

Olena German, Olena Legostaeva

V.N. Karazin Kharkiv National University, 4 Svobody Sq., Kharkiv, 61022, Kharkiv, Ukraine.

E-mail: elenagerman2009@gmail.com

The “bystander effect” is the occurrence of radiation damages in the cells that have not been directly exposed to ionizing radiation behave as though they have been exposed. “Bystander effect” has been demonstrated on number of biological objects that formed the opinion that genetic changes, including induction of mutations, gene expression and apoptosis can occur in the cells that did not receive direct irradiation. The mechanism of the phenomenon is poorly understood and refers to the “non-target effects” of radiation.

The ability of cells to divide is extremely essential for normal organism functioning and the mitotic activity of the cells is often used as an indicator in the assessment of environmental pollution. One can judge the degree of mutagenic effects on the environment by the change in mitotic activity.

The aim of the study is to investigate the possibility of forming the “bystander effect” during the germination of irradiated and intact seeds of some soybean varieties in the general aquatic environment.

The soybean seeds of the Raiduga and Sprytyna varieties (obtained by selection) and the genetically modified Apollo variety had been exposed to γ -radiation at a dose of 40 Gy. The mitotic activity of the seedlings root meristem cells of irradiated (IR) and intact (IN) seeds, as well as intact seeds, which were germinated in the same aqueous medium together with irradiated (IN^{IR}), was analyzed.

The similar levels of mitotic activity were observed in seedlings of breeding varieties (3.4–3.7%) in “IN” variant, while cells in genetically modified variety divided more intensively (5.1%).

Radiation exposure in a dose of 40 Gy increased the level of mitotic activity in all varieties (6,4-7,2%). The values of the mitotic indices increased 2 times in the varieties of Raiduga and Sprytyna. The increase in Apollo variety was less significant, but this may be due to a high level of mitotic activity in the normal range. Irradiation also contributed to the appearance of chromosomal aberrations: fragments and bridges. The increase in mitotic activity in the meristem of the studied varieties to the mentioned levels may indicate the presence of a pool of meristem cells that can accelerate the passage of phases of the mitotic cycle under extreme conditions.

Mitotic activity increased in the “IN^{IR}” variant in all investigated varieties. The largest excess over the “IN” was in Sprytyna (37% of control), a little less - 23% - in the variety Raiduga. The proliferative activity in the Apollo variety meristem remained almost unchanged; the excess over the control in the “IN^{IR}” variant was only 9%.

Transmission of the “bystander effect” is possible both in the direct contact of irradiated and non-irradiated cells (through intercellular contacts), and through the mediators released in the culture medium (active forms of oxygen, hormones). Obviously, the concentration and spectrum of substances released in the aquatic environment during germination of irradiated in a dose of 40 Gy seeds, were insufficient to induce an intensive “bystander effect” in intact seeds.

Thus, the work shows the possibility of a “bystander effect” forming under condition of joint germination of irradiated and intact seeds in the same aquatic environment. The intensity of “bystander effect” formation depends on the genotype, starting mitotic potential and exposure dose.

**THE PHYTOCHEMICAL PROFIL OF *VACCINIUM CORYMBOSUM* (cv. ELLIOTT)
UPGROUND PART**

Yavorska N., Vorobets N.

*Department of Pharmacognosy and Botany, Danylo Halytsky Lviv National Medical University,
Pekarska Street 69, 79010, Lviv, Ukraine,*

E-mail: yavorska.natali@gmail.com

Vaccinium corymbosum L. (Ericaceae family), which is native of Northern America has become widespread in Ukraine for the last decade, and is interesting because its fruits, which in comparison with other fruit crops, are with high content of phenolic compounds, flavonoids, tannins, phenolic acids, proanthocyanidins etc. (McGhie, Walton, 2007). Phenolic compounds, ubiquitous in the plants of Ericaceae are an essential part of the human diet, and are of considerable interest due to their antioxidant properties. The research of the vegetative parts of *V. corymbosum*, namely of shoots, the content of biologically active substances such as phenolic has been paid little attention. It is known that the range of phenolic compounds is very varied, even within the same species. The purpose of this work was to investigate the total content of phenolic compounds, flavonoids and tannins in the shoots of *V. corymbosum* of the variety Elliott, depending on the extractive solvents and the period of vegetation of plant, and to evaluate the prospects of their usage as medicinal plant material.

Shoots of the plants were harvested manually, at the stages of flowering, fruiting, after ripening of fruits, during the winter rest in 2017-2018. Phytochemical screening for was done using standard chemical tests as described by Chew et al., 2011. The total phenolic contents of the aqueous and ethanolic (20%, 30%, 40%, 50%, 60%, 70%, 80%, 96%) extracts of shoots of the *V. corymbosum* of the variety Elliott were estimated using Folin-Ciocalteu reagent, total flavonoid content with aluminium chloride and calculated using standard calibration curves prepared from gallic acid ($y = 0.0025x + 1,5755$, $R^2 = 0.99825$); and quercetin ($y = 0,0004x - 0,0243$, $R^2 = 0,9871$), respectively. The total phenolic and total flavonoid content were expressed as g of gallic acid equivalent and quercetin equivalent per g of dry weight (DW), respectively.

Phytochemical screening of the shoot extracts of *V. corymbosum* revealed the presence of phenolics. Among them are flavonoids which are powerful antioxidants capable of scavenging free radicals by donating a hydrogen atom or electron to stabilize the radical species. Experimental results showed that the maximum amount of phenolic compounds 402.54 mg/g DW in gallic equivalent is observed in 70% water-alcohol extract, the minimum - 207.3 mg/g DW in gallic equivalent was noted in 40% water-alcohol extract, while their quantitative value in the remaining hydroalcoholic extracts was in the range of 285-340 mg/g DW, in the aqueous extract, the total amount of phenols was 319.95 mg/g DW in gallic equivalent. The total content of flavonoids and tannins in *V. corymbosum* shoots was 18.2% and 1, 6%, respectively, of the total amount of phenols.

The optimum extractant for the reception of phenolic compounds from *V. corymbosum* shoots of var. Elliott is a 70% aqueous-ethanol solution. So as phenolic-rich natural extracts have high antioxidant activities comparable to those of synthetic antioxidants, the practical aspects of their extraction and production of sufficient amounts from plant sources remain actual.

McGhie TK., Walton MC. Mol Nutr Food Res. 2007, 51(6):702-13

NEW VARIETIES OF LAVENDER OF UKRAINIAN BREEDING: CHEMICAL COMPOSITION AND PROSPECTS OF APPLICATION

Vorobets N.¹, Svydenko L.², Yavorska H.³

1 – Department of Pharmacognosy and Botany, Danylo Halytsky Lviv National Medical University, Pekarska Street 69, 79010 Lviv, Ukraine

2 – Institute of Rice of National Academy of Agrarian Sciences of Ukraine, with. Antonivka, Skadovsky area, Kherson region, 75705, Ukraine

3 – Department of Microbiology, Ivan Franko Lviv National University, Hrushevsky Street 4, 79004 Lviv, Ukraine

E-mail: vorobetsnatalia@gmail.com

Lavandins are group of natural and produced by the selection of interspecies hybrids of the genus *Lavandula*. Since ancient times they have been used in various fields, to a large extent due to the presence of essential oils in their composition. We have created lavandins Rabat and Inii, which need a comprehensive study for further use in medicine, pharmacy and cosmetology, and it was the aim of this investigation.

Flowering shoots of the plants Rabat and Inii lavandins were harvested manually, at the maximum flowering stage in 2017-2018. The mass fractions of essential oil have been determined by the method of hydrodistillation on the Klevenger apparatus from freshly harvested raw materials. The determination of the essential oil composition was carried out by high-performance gas-liquid chromatography on chromatograph Agilent Technology 6890N. Tannins, organic acids, hydrocynnamic and ascorbic acids content have been determined spectrophotometrically. Microorganisms as *Escherichia coli*, *Staphylococcus albus*, *Candida pseudotropicalis*, *Candida kefir*, *Candida parapsilosis*, *Candida tenuis*, *Candida curvata* and methods of glass cylinders and cup plate were used in determination of antimicrobial activity.

The mass fraction of essential oil of the above-ground mass of livandin Inii is 1.8%, and 205 kg of oil was obtained from hectare. The mass fraction of essential oil of the above-ground mass of livandin Rabat is 1.7%, and 114 kg of oil was obtained from hectare. The major component of essential oils of both lavandins is linalool, a slightly smaller proportion of linalil acetate: 57.7/11.1% and 49.5/8.7% in Inii and Rabat, respectively. The essential oils of both lavandins showed high antimicrobial activity to 15–30mm Zones of inhibitions diameter. Water-soluble groups of substances in lavandins are characterized by favorable concentrations of ascorbic acid, organic acids and tannins: 1.68%/DW, and 4.3-4.7 mg/g DW, respectively.

Due to large amounts of harvesting raw materials and high content of essential oils, organic acids and tannins, so as high antimicrobial activity, lavandins Inii and Rabat are medically, pharmaceutically and economically very promising.

KARYOTYPE INSTABILITY OF WHEAT REGENERATED PLANTS DERIVED BY *IN VITRO* SELECTION FOR TOLERANCE TO WATER DEFICIT

Serhii Pykalo, Tetiana Yurchenko, Nataliia Prokopik, Mykhailo Kharchenko

The V.M. Remeslo Myronivka Institute of Wheat, NAAS of Ukraine

Tsentralne, Myronivka district, Kyiv region, Ukraine

E-mail: pykserg@ukr.net

Wheat is the dominant crop in temperate countries being used for human food and livestock feed. In modern wheat breeding programs special attention is paid to combining high potential productivity of varieties and their ability to withstand biotic and abiotic factors. Water deficit is one of the major abiotic stresses that reduce plant growth and crop productivity worldwide. It is known that under action of salt agents a number of morphological and cytogenetic changes in cells cultured *in vitro* occurs. Specific *in vitro* tissue cultivation conditions increase the frequency of cells with karyological alterations, leading to the growth of regenerated plants with various genetic aberrations, including significant variations in the number of chromosomes in cells (Dubrovna, Bavol, 2011). The aim of the work is to analyze the ploidy level of regenerated plants of winter bread wheat derived by *in vitro* selection for tolerance to water deficit.

The material for study were regenerated plants of winter bread wheat line Erytrospermum 60068, derived by direct and gradual *in vitro* selection for tolerance to water deficit. Cytogenetic analysis of regenerated plants was carried out in the root meristem cells according to the standard method of crushed preparations. The material in a mixture of ethanol:glacial acetic acid (3:1) for 24 hours in a refrigerator at 4 °C was fixed and to 70 % ethanol was transferred. From the fixative the samples several times in distilled water were washed, transferred for maceration into 5N HCl solution at room temperature for 30 minutes. Then the samples in distilled water were washed and dyed with 2 % lactopropionim orcein for 24 hours at room temperature. Preparing temporary crushed preparations in 45 % acetic acid solution. 10-15 metaphases in each plant were analysed.

We have identified plants with different ploidy levels under the cytological analysis of regenerated plants derived by *in vitro* selection for tolerance to water deficit. The studied 38 plants derived from osmotolerant calluses included 35 hexaploids and 3 aneuploids. Aneuploid plants had 39–41 chromosomes. In the next cultivation of plants with a normal set of chromosomes, phenotypic differences from the initial morphotype were not found. The plants with aneuploid chromosome set were characterized by reduced viability and abnormal generative organs resulting they are not formed normal ears and not received seeds. Karp and Maddock conducted cytological studies of wheat regenerated plants derived from the culture of immature embryos and observed a high frequency of aneuploids (around 30 %). In most cases, aneuploidy was a consequence of a loss or the addition of one chromosome (sometimes, two chromosomes). It is known that the ploidy level of regenerated plants reflects the degree of heterogeneity of cell populations of callus cultures. In our investigations among the derived regenerated plants euploids were in most cases indicating a selective advantage of hexaploid cells in ability to morphogenesis.

Thus, the somaclonal variability of tolerant to water deficit regenerated plants of winter bread wheat was observed by ploidy level. The cytological instability of resistant's regenerated plants was revealed that was due in appearance of aneuploidy plants.

Dubrovna O.V., Bavol A.V. Cytol. Genet. 2011, 45(5):333-340.

Karp A., Maddock S. Theor. Appl. Genet. 1984, 67(2/3):249-255.

OBTAINING OF THE RESISTANT PLANTS LINES AGAINST PHYTOPATHOGENS AND NEMATODE INVASION BY THE ACTION OF NEW MICROBIAL BIOFORMULATION FHYTOVIT

Biliavska L.O.¹, Loboda M.I.¹, Tsygankova V.A.², Shysha E.N.³, Iutynska G.A.¹

1 – D.K. Zabolotny Institute of Microbiology and Virology, NAS of Ukraine

2 – Institute of Bioorganic Chemistry and Petrochemistry, NAS of Ukraine

3 – Institute of Food Biotechnology and Genomics, NAS of Ukraine

E-mail: marichka20loboda@gmail.com

The current task of the present is to develop the scientific base for the creation of the latest biotechnologies for organic farming and to improve the phytosanitary state of agrocenoses. One of the priority directions of its solution is the creation *in vitro* the new plants lines with genetically improved productivity and resistance to biotic and abiotic stresses by the using of microbial bioformulations [1-3].

The aim of the research was to study the effect of the action of metabolic microbial bioformulation on the production and stability of new tomato lines *Lycopersicon esculentum* Mill. variety Gentle to parasitic nematodes and pathogens. Fhytovit is the biological product received from the supernatant of the cell culture liquid and from the ethanolic extract of the producer's biomass *Streptomyces netropsis* IMV Ac-5025. Plant-regenerants were received in the process of callusogenesis *in vitro* on MSTI nutrient medium with the addition of the bioformulation Fhytovit in a concentration of 75 µl/L. Microbiological, biochemical, physiological, nematological and statistical methods were used in the research.

Morphological and physiological indices and the stability of tomato plant-regenerants *Lycopersicon esculentum* Mill to parasitic nematodes, including *Meloidogyne incognita* were analyzed in the conditions of the greenhouse. It was revealed an increase up to 33% the stem length in plant-regenerants, obtained with the addition of the bioformulation, in comparison with control plants without the addition of the bioformulation. It was shown the increase in 1.6-3.3 times the productivity by the index of fruit weight of tomato plant-regenerants, obtained with the addition of Phytovit, in comparison with similar indices of tomato plant-regenerants obtained without adding of the bioformulation, and the increase up to 15 - 100%, comparatively with similar indices of tomato plants grown from seeds.

By the application of Fhytovit, the resisrance of tomato plant-regenerants against phytohalmites was increased up to 96%. The degree of invasion of fruit by mixed bacterial-myco-nematodoses infections also was decreased. In the root zone of tomato plant-regenerants received on the nutrient medium with the addition of Fhytovit, the number of pedotropic and organotrophic microorganisms was in 2.2 - 8.6 times higher than in the rhizosphere of control plants. In the rhizosphere of plant-regenerants derived from the nutrient media containing the bioformulation the process of activating of phosphate-metabolizing microorganisms was ocured, the number of which was higher in 3.3 - 19.6 times in comparison with the control plant-regenerants.

Key-words: *Streptomyces netropsis*, tomato *Lycopersicon esculentum* Mill, bioformulation Phytovit, callusogenesis, *in vitro*, resistance, nematodes, phytopathogens/

References

1. Tsygankova V.A.et.al. Using of new microbial biostimulants for obtaining *in vitro* new lines of *Triticum aestivum* L. cells resistant to nematode *H. avenae*. European J. of Biotech.and Bioscience. 2016; 4(4): 39 - 53.
2. Tsygankova V.A. et.al. Impact of microbial biostimulants on induction of callusogenesis and organogenesis in the isolated tissue culture of *wheat in vitro*. J. Med. Plants. Stud. 2017; 5(3): 155 - 164.
3. Tsygankova V.A, et al. RNAi-mediated resistance against plant parasitic nematodes of wheat plants obtained in vitro using biostimulants of microbiological origin. Current Chemical Biology.2019; 13(1): 73–89.

МІКРОКЛОНУВАННЯ *IN VITRO* ДЛЯ ЗБЕРЕЖЕННЯ ТА РОЗМНОЖЕННЯ *IRIS PUMILA* L.

Мар'яна Твардовська, Ігор Андреев, Віктор Кунах
Інститут молекулярної біології і генетики НАН України,
вул. Акад. Заболотного, 150, 03143, Київ, Україна
E-mail: maryana.tvardovska@gmail.com

Дослідження дикорослих видів роду *Iris* L. та введення їх в культуру не тільки має значення для збагачення асортименту квітково-декоративних багаторічників, але й може сприяти охороні генофонду зникаючих рослин, зокрема, таких, що занесені до «Червоної книги України» та до охоронних регіональних списків. Щодо останніх актуальним та перспективним є поновлення природних популяцій шляхом реінтродукції в природне середовище посадкового матеріалу, отриманого *in vitro*. Метою роботи була розробка методики введення в культуру *in vitro* *I. pumila* для отримання проростків з наступною реінтродукцією в природне середовище, а також одержання культури тканин цього виду.

Вихідним матеріалом слугувало насіння *I. pumila* з природних популяцій зростання на території України. На динаміку проростання насіння значний вплив мали спосіб обробки насіння та тривалість його стратифікації. Найефективнішим способом подолання спокою насіння була холодова (-20°C) стратифікація протягом 1-2 місяців. Попередньо скарифіковане та стерилізоване насіння висаджували на агаризоване живильне середовище МС (Murashige, Skoog) з половинним вмістом макро- і мікросолей (МС/2) без фітогормонів. За таких умов перші сходи з'явилися на 11-14 день. Ефективність проростання насіння, зібраного з різних природних популяцій, була високою і варіювала в межах 77-95,6 %.

Культивування проростків *in vitro* показало, що формування повноцінної рослини, яка має розвинену кореневу систему і надземний пагін із 2-3 листків, відбувається протягом одного місяця.

Нами відмічено високі темпи росту асептичних проростків *I. pumila* на агаризованому живильному середовищі двох типів – без фітогормонів та з додаванням 0,1 мг/л НОК. При наступному перенесенні цих рослин у ґрунт, швидше приживалися рослини, вирощені на середовищі, доповненому НОК. Експерименти з адаптації рослин до умов закритого ґрунту виявили високий рівень (90-95%) приживання рослин. Тривалість адаптації рослин до зниженої вологості повітря та нестерильності субстрату складала в середньому 12-16 днів.

Для індукції калусоутворення використали експланти кореневого походження, які висаджували на середовище МС/2, доповнене різними концентраціями БАП та 2,4-Д. Відсоток калусогенезу був високим і складав 84%. Отриманий життєздатний та проліферативно активний калус мав жовте забарвлення, пухку консистенцію та був здатний до тривалого росту на живильному середовищі МС/2, доповненому 0,1 мг/л БАП та 0,5 мг/л 2,4-Д.

Таким чином, нами введено *I. pumila* в культуру *in vitro*. Виявлено високу ефективність проростання насіння за умов його попередньої холодової стратифікації, а також наступної висадки скарифікованого насіння на живильне середовище МС/2. Асептичні проростки активно росли на середовищі МС/2. Проведені експерименти з адаптації рослин до умов закритого ґрунту виявили високий рівень приживання рослин. Розроблена методика може бути використана в програмах збереження та відновлення природних популяцій *I. pumila*.

**ПРОДУКТИВНІСТЬ ГЕНЕТИЧНО ЗМІНЕНИХ РОСЛИН ПШЕНИЦІ ОЗИМОЇ
(*TRITICUM AESTIVUM* L.) ЗА ДІЇ ОСМОТИЧНОГО СТРЕСУ**

Комісаренко А.Г., Михальська С.І., Курчій В.М.
Інститут фізіології рослин і генетики НАН України,
Україна, 03022, м. Київ, вул. Васильківська, 31/17

E-mail: mykhalskasvitlana@gmail.com

На сьогодні залучення новітніх біотехнологічних розробок дає можливість прискорити селекційний процес, направлений на отримання стрес-стійких форм сільськогосподарських рослин. Особливу увагу в даному напрямку досліджень привертає пшениця озима, яка вважається основною зерновою культурою і від її врожайності залежить вирішення продовольчої проблеми населення багатьох країн. Великі надії у підвищенні врожайності та якості продовольчого зерна покладаються на генетичну інженерію, яка може створювати трансгенні сорти з кращими адаптивними властивостями до несприятливих умов довкілля.

Актуальним напрямком досліджень є створення нових форм рослин пшениці озимої шляхом генетичної трансформації з використанням генів, що контролюють метаболізм вільного проліну, поліфункціональної амінокислоти, яка може брати участь в складних процесах адаптації/стійкості рослин. Показана можливість підвищення стійкості рослин до осмотичних стресів при збільшенні вмісту вільного проліну за рахунок часткової супресії ендогенних генів проліндегідрогенази, шляхом посттранскрипційного сайленсингу РНК.

В результаті *Agrobacterium*-опосередкованої трансформації *in planta* з використанням штамів *A. tumefaciens* LBA4404 і AGLO, що містять плазмиду pVi2E з дволанцюговим РНК-супресором гена проліндегідрогенази, (люб'язно надану доктором біологічних наук, чл. кореспондентом РАН Кочетовим О.В., Інститут цитології і генетики Сибірського відділення РАН, м. Новосибірськ) отримані біотехнологічні рослини пшениці озимої сортів Фаворитка і Достаток.

Позитивним наслідком генетичної трансформації можна вважати отримання рослин із трансгенним статусом в яких поєднується функціональність перенесеного гена з продуктивністю рослин за дії стресових факторів. При цьому аналіз структури врожаю дозволяє встановити закономірність і залежність його формування від різних факторів зовнішнього довкілля. Оскільки, урожайність зернових культур перебуває у прямій залежності від числа колосків у колосі, то однією з найбільш критичних є фаза росту рослин в яку формується їх кількість. Тому саме в період, що припадає на кінець кушіння та початок виходу в трубку, рослини піддавали дії осмотичного стресу, який створювали припиненням поливу протягом 10 діб. За допомогою дослідження елементів структури продуктивності визначали вплив дефіциту вологи на урожайність рослин пшениці озимої.

За дії осмотичного стресу спостерігалась достовірна різниця за показниками продуктивності на користь біотехнологічних рослин. Слід відзначити, що у трансгенних форм пшениці сорту Достаток в умовах недостатнього забезпечення вологою маса зерна з рослини перевищувала показники контролю за тих же умов майже у 2,5 рази.

Таким чином, створення біотехнологічних рослин пшениці озимої з частковою супресією гена проліндегідрогенази є перспективним напрямком підвищення їх продуктивності за умов водного дефіциту.

ІОНИ ВАЖКИХ МЕТАЛІВ У БІОТЕХНОЛОГІЇ ПШЕНИЦІ ОЗИМОЇ

Броннікова Л.І., Сергєєва Л.Є.

Інститут фізіології рослин і генетики НАНУ, 03022, Україна, Київ, вул. Васильківська, 31/17

E-mail: Zlenko_lora@ukr.net

Суттєві (кардинальні) погіршення довкілля спричиняють підвищену потребу у формах рослин, які були б здатні протистояти «викликам» природи. Особливо це стосується сільськогосподарських культур, а саме пшениці озимої. Отримання нових сортів із поліпшеними показниками може бути прискорене у разі застосування новітніх біотехнологій.

Розвиток рослин за стресових умов забезпечується за рахунок реалізації різних механізмів стійкості. Ключові позиції серед них посідають реакції клітинного рівня. Охарактеризувати їх найбільш адекватно можливо, застосовуючи клітинні культури під час вирощування за різних умов. Піддаючи клітинні популяції дії різних стресових чинників стає можливим вирізнити реакції стресу від реакцій адаптації. Події, спрямовані на підтримання життєдіяльності клітин за стресових умов стають метою наукових наробок.

Клітинна селекція є вдалим методом відбору та вивчення форм рослин із підвищеним рівнем стійкості до абіотичних стресів. Нами запропоновано ідею використання катіонів важких металів у клітинній селекції для підвищення осмотичності рослин. Серед значної кількості токсичних у залишкових кількостях катіонів важких металів ми обрали катіони кадмію (Cd^{2+}) та барію (Ba^{2+}) з огляду на їхні хімічні властивості. Ці іони впливають на різні аспекти осмотичного статусу клітин.

Іони Cd^{2+} здатні суттєво змінювати водний баланс рослин. Іони Ba^{2+} є фізіологічними конкурентами катіонів K^+ . Обидві критичні події мають місце за умов дії засолення та водного дефіциту. З огляду на ці обставини були створені селективні системи для отримання клітинних ліній пшениці озимої із підвищеним рівнем стійкості до осмотичних стресів *in vitro*. До культуральних середовищ додавали летальні для клітинних культур дикого типу дози вказаних іонів. Із незрілих зародків різних генотипів пшениці озимої індукували первинний калус, який нарощували на оптимізованих живильних середовищах. У подальшому отримували суспензійну культуру клітин, яку пасажували на селективні середовища із іонами важких металів. Із одиничних генетично змінених клітин із частотою 10^{-6} формувались стійкі первинні колонії, що організовували стійкі до токсичних іонів клітинні лінії. Відібрані стійкі клітинні варіанти досліджувались за умов прямої дії засолення (солі морської води) або водного стресу (маніт).

Отримані клітинні лінії відзначались комплексною стійкістю до всіх стресових чинників.

**ОСОБЛИВОСТІ ЕКСПРЕСІЇ АКВАПОРИНУ *PIP2;1* В КОРЕНЯХ ГІБРИДІВ
ZEА MAYS ЗА УМОВ ВОДНОГО ДЕФІЦИТУ**

Ірина Овруцька, Шевченко Г.В., Овчаренко Ю.В.

Інститут ботаніки ім. М.Г. Холодного НАН України, вул. Терещенківська 2,
м. Київ, 01601, Україна

E-mail: ovrutska@meta.ua

Посуха є одним з основних абіотичних стресів, що впливають на виробництво сільськогосподарської продукції в усьому світі, і важливим завданням є дослідження розвитку рослин, які стійкі до посухи, а також механізмів їх посухостійкості. Рослини, які зростають в посушливих районах виживають в суворих умовах навколишнього середовища використовуючи адаптивні механізми опору. Відомо, що однією із первинних мішеней зовнішнього стресу є цитоплазматична мембрана клітин, яка реагує на нестачу вологи функціональними перебудовами структурних компонентів. Стале функціонування ЦМ в умовах нестачі вологи забезпечується складом ліпідного бішару та активністю аквапоринів. Аквапорини забезпечують транспорт води крізь цитоплазматичну мембрану і відіграють суттєву роль у водному балансі клітин, рівень експресії їх генів може свідчити про реакцію рослин на водний стрес. Проводили вегетаційні дослідження по вирощуванню рослин *Zea mays* чотирьох гібридів: Достаток та Флагман (посухостійкість висока) та Переяславський і Яхта (посухостійкість помірна) вітчизняної селекції. Рослини зростали у вегетаційних посудинах у піщаному субстраті протягом 21 діб, у контрольному варіанті, за умов оптимальної вологості субстрату 70%, та у дослідному варіанті, за умов водного дефіциту при вологості субстрату 30% протягом 10 діб, що є критичним періодом за вологістю. Дослідження були проведені у 3 біологічних повторах. Використовували біохімічні, молекулярно-біологічні, морфометричні, статистичні методи. Досліджено експресію гену аквапорину *PIP2;1*, ліпідний склад плазмалем і морфологічні показники коренів гібридів кукурудзи. У посухостійких гібридів Достаток та Флагман, за умов водного дефіциту, відбувається посилення експресії аквапоринів та неспецифічне збільшення кількості стеринів (Достаток), які разом забезпечують водоутримуючу здатність плазмалем. У гібриду Переяславський, як протидія зневодненню, підвищується співвідношення ФХ/ФЕ та кількість стеринів і фосфоліпідів. Водний стрес не впливає на довжину коренів та кількість ненасичених жирних кислот, а експресія гену *PIP2;1* навіть дещо пригнічується. Цікавим виявився гібрид Яхта – у якого збільшувалася і довжина коренів, і співвідношення ФХ/ФЕ, кількість фосфоліпідів і ненасичених жирних кислот, проте, експресія аквапоринів не зазнавала змін. Підсумовуючи дані щодо ліпідного складу плазмалем коренів, морфологічних показників та експресії гену *PIP2;1* можна зробити висновок про те, що у різних гібридів посухостійкість опосередковується як фізичними властивостями плазмалем, так і експресією генів аквапоринів, відповідальних за водоутримуючу здатність клітин в умовах водного дефіциту. Посилена експресія *PIP2;1* є характерною ознакою посухостійкості гібридів кукурудзи Достаток та Флагман, а також дозволяє розглядати експресію генів аквапорину *PIP2;1* як молекулярного маркера в селекції кукурудзи на посухостійкість.

УЧАСТЬ ЛІГНІНУ В АДАПТАЦІЇ РОСЛИН ОЧЕРЕТУ ДО ГРУНТОВОЇ ПОСУХИ

Олена Недуха

*Інститут ботаніки ім. М.Г. Холодного НАНУ, вул. Терещенківська 2,
м. Київ, 01601, Україна*

E-mail: o.nedukha@hotmail.com

Методом лазерно конфокальної мікроскопії та біохімічними методами досліджували участь лігніну в адаптації рослин *Phragmites australis* (Cav.) Trin. ex Steud, що зростали у воді та на суходолі (в зоні Києва), до природної ґрунтової посухи. Встановлено, що вміст лігніну у листках очерету залежав від типу тканин та клітин, а також від умов зростання рослин. Найбільш чутливими до посухи виявилися клітинні стінки провідних пучків та склеренхіми листових пластинок, Аналіз локалізації лігніну в листах повітряно-водних рослин очерету у фазі бутонізації-цвітіння в присутності специфічного флуоресцентного індикатора ауреміну показав яскраво жовту флуоресценцію в клітинних стінках верхньої та нижньої епідерми, клітинах провідних пучків та склеренхіми. У клітинах мезофілу та моторних клітинах флуоресценція лігніну була слабо виражена у обох екотипів очерету. Найвища інтенсивність флуоресценції ауремін+лігнін комплексу у листках повітряно-водного екотипу була у клітинах верхнього епідермісу; у листках суходільного екотипу - у клітинних оболонках провідних пучків. Порівняння відносного вмісту лігніну (по інтенсивності його флуоресценції) у периклінальних та антиклінальних оболонках основних клітин епідермісу виявило, що клітини адаксіального епідермісу незалежно від екотипу містили більше лігніну, ніж клітини абаксіального епідермісу. У листках суходільного екотипу очерету також виявлено достовірне збільшення лігніну у паренхімних клітинах обкладинки, склеренхіми та судин провідних пучків. Враховуючи, що лігнін забезпечує непрохідність води по апопласту, особливо по провідних пучках, а також формує захисний бар'єр для патогенів, можна припустити, що посилений синтез лігніну у суходільних рослин очерету забезпечує адаптацію цього виду до природної посухи.

ДОСЛІДЖЕННЯ МОРФОЛОГІЧНИХ ОЗНАК АУТОФАГІЇ В КОРЕНЯХ ПРОРОСТКІВ
ARABIDOPSIS THALIANA В УМОВАХ ЗМІНЕНОЇ ГРАВІТАЦІЇ

Шадріна Р.Ю., Блюм Я.Б., Ємець А.І.

Інститут харчової біотехнології та геноміки НАН України,

Україна, 04123, м. Київ, вул. Осиповського, 2А

E-mail: ruslanashadrina@gmail.com

Аутофагія – один з катаболічних процесів в еукаріотичних клітинах, під час якого відбувається деградація внутрішнього вмісту клітини, відпрацьованих білків та органел [1, 2]. Ряд раніше отриманих результатів свідчать про те, що аутофагія задіяна під час росту та розвитку рослини та реалізується в клітині у відповідь на стрес [3, 4]. Зокрема відомо, що рослини в умовах мікрогравітації зазнають значних морфологічних та фізіологічних змін. Саме тому метою дослідження був аналіз впливу умов зміненої гравітації на розвиток аутофагії у клітинах коренів проростків *A. thaliana* на різних часових проміжках (10-15 діб) вирощування.

Як об'єкт дослідження використовували проростки *A. thaliana* екотипу Columbia Col-0. Після стерилізації насіння було висаджене на стандартне середовище Мурасіге і Скуга (МС) [5] у чашки Петрі. Частина рослин була поміщена до кліноштату з режимом обертання 2 об/хв протягом 10-15 діб при 22°C та довжині світлового періоду 14 год на добу. Насіння контрольних рослин поміщали у культуральну кімнату для подальшого проростання та культивування. Візуалізацію аутофагосом проводили за допомогою люмінесцентної мікроскопії з використанням флуоресцентного барвника монодансилкадаверину (MDC) – загальноживаного маркеру аутофагосом. Рівень ацидифікації клітин оцінювали за допомогою флуоресцентного барвника акридин-оранжевого (АО).

Раніше нами були отримані дані, які свідчать про те, що за умов кліноштатування протягом 6–10 діб вже на 6 добу культивування відмічається розвиток аутофагії у коренях проростків *A. thaliana*. Разом з тим, найбільш інтенсивний розвиток аутофагії в зоні кореневого чохла відповідав 9 добі досліджень. У зв'язку з цим було проведено оцінку морфологічних показників розвитку аутофагії протягом 10-15 діб культивування проростків *A. thaliana* за умов зміненої гравітації. Отримані результати підтверджують той факт, що вже на 11 день кліноштатування відбувається адаптація коренів проростків до дії стресового фактору. Рівень ацидифікації клітин був високим на часовому проміжку 10-11 доба, але спадав, починаючи від 12 доби культивування. Дані результати демонструють, що кліноштатування є стресовим фактором, який активує аутофагію в проростків *A. thaliana*, проте на часовому проміжку 10-15 діб відбувається зниження рівня аутофагії, що може свідчити про адаптацію рослинних клітин до умов мікрогравітації.

1. Reggiori F., Klionsky D.J. Autophagic processes in yeast: mechanism, machinery and regulation. *J. Genetics*. 2013. Vol. 194. P. 341-361.
2. Yoshimoto K., Ohsumi Y. Unveiling the molecular mechanisms of plant autophagy—from autophagosomes to vacuoles in plants. *J. Plant Cell Physiol*. 2018. Vol. 59(7). P. 1337–1344.
3. Demidchik V., Tyutereva E.V., Voitsekhovskaja O.V. The role of ion disequilibrium in induction of root cell death and autophagy by environmental stresses. *Funct. Plant Biol.*, 2017, 45(1):28-46.
4. Stefan W. R., Augustine M. K. Autophagy: an integral component of the mammalian stress response. *J. Biochem. Pharmacol. Res.*, 2013, 1(3), P. 176–188.
5. Murashige T., Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *J. Physiol. Plant*. 1962. Vol. 15. P. 473-497.

6th Ukrainian Congress for Cell Biology with international representation

*June 18-21, 2019,
Yaremche*

Session 6

Biology of stem cells and specialized cells and tissues

INTRAVENOUSLY INJECTED MESENCHYMAL STEM CELLS PENETRATE THE BRAIN AND TREAT INFLAMMATION-INDUCED BRAIN DAMAGE AND MEMORY IMPAIRMENT IN MICE

Olena Lykhmus

Poster 1

Lykhmus O.Y.¹, Koval L.M.¹, Voytenko L.P.¹, Uspenska K.R.¹, Deryabina O.G.², Shuvalova N.I.², Kordium V.A.^{2,3}, Ustymenko A.², Kyryk V.², and Skok M.V.¹

1 – Palladin Institute of Biochemistry NAS of Ukraine, Kyiv

2 – State Institute of Genetic and Regenerative Medicine NAMS of Ukraine, Kyiv

3 – Institute of Molecular Biology and Genetics NAS of Ukraine, Kyiv

E-mail: olenalykhmus@gmail.com

The use of mesenchymal stem cells (MSC), which can differentiate into multiple cell types, including neurons, is an attractive idea of regenerative medicine, in particular, for neurodegenerative disorders like Alzheimer disease (AD). Neuroinflammation is regarded as one of the pathogenic factors of AD. Previously we showed that mice regularly injected with bacterial lipopolysaccharide (LPS) possessed the AD-like symptoms like episodic memory decline, elevated amounts of amyloid beta peptide A β (1-42) and decreased levels of nicotinic acetylcholine receptors (nAChRs) in the brain. In the present study, we aimed to investigate whether pathogenic effect of LPS on the brain and behavior of mice can be prevented or treated by injection of MSCs or MSC-produced soluble factors. Fluorescently-labelled MSCs, injected intravenously, penetrated the brain of LPS-treated mice. MSCs co-injected with LPS prevented episodic memory impairment, A β (1-42) accumulation and nAChR decrease in the brain and brain mitochondria caused by LPS. Their mitochondria did not release increased amounts of cytochrome *c* under the effect of Ca²⁺ as did mitochondria of LPS-only-treated mice. Moreover, MSCs could reverse the pathogenic symptoms developed 3 weeks after LPS injection. Cultured MSCs produced IL-6 in response to LPS and MSCs effect *in vivo* was accompanied by additional stimulation of both micro- and macroglia. Xenogeneic (human) MSCs were almost as efficient as allogeneic (mouse) ones and regular injections of human MSC-conditioned medium appeared to be almost as efficient as injection of MSCs themselves. These data allow suggesting MSCs as a potential therapeutic tool to cure neuroinflammation-related cognitive pathology.

BANKING OF POOLED HUMAN OLFACTORY EPITHELIUM-DERIVED MESENCHYMAL STEM CELLS FOR APPLYING IN CELL THERAPY

Natalia Antonevich

Poster 2

Natalia G. Antonevich¹, Andrei Y. Hancharou¹, Alena G. Rynda², Darya V. Babrukevich²

1 – The Institute of Biophysics and Cell Engineering of National Academy of Sciences of Belarus, Belarus, Minsk, Akademicheskaya st., 27

2 – The Republican Research and Practical Center for Epidemiology and Microbiology, Minsk, Belarus, Minsk, Filimonova st., 23

E-mail: antonevich.n@gmail.com

Introduction. Olfactory epithelium (OE) contains tissue-specific mesenchymal stem cells (MSCs), which possess broad differentiation potential and immunomodulatory activities comparable to well-studied bone marrow and adipose tissue derived MSCs. The advantage of OE as a source of MSCs for cell therapy is that biopsy can be taken from people of any age and gender under local anesthesia without any adverse consequences. Nevertheless, cell cultures vary in their therapeutic potential depending on the donor's individual health status. It has been shown that bone marrow MSCs obtained from donors with severe systemic diseases, for example, with lupus erythematosus, have aberrant profile of gene expression and reduced ability to suppress immune cells and therefore cannot be used in autologous transplantations. Obviously, in such cases transplantation of allogenic cells from healthy person is more preferable. The recent tendency in cell therapy is to produce biomedical cellular product based on pooled cultures in order to standardize cell material and avoid considerable variations in its properties. The aim of the current study was to choose the OE-MSCs with the highest immunosuppressive activity, produce pooled culture and bank them for long-term storage.

Methods. Olfactory mucosa samples were taken from 15 patients with non-inflammatory diseases of nasal cavity. OE-MSCs were expanded using DMEM/F12 media supplemented with 10% FBS and cryopreserved at passage 2 (P2). Cells recovered from cryopreservation were used to obtain pooled cultures OE-MSCs, OE-MSCs from three individual donors were pooled in equal proportion (1:1:1) after individual culturing at P3 and expanded as pooled cultures to P4–P5. OE-MSCs were assayed for phenotype (CD71, CD90, CD105, CD45, CD31), population doubling time (PDT). All cultures were routinely tested for microbiological contaminants (aerobic and anaerobic bacteria, fungi, herpesviruses HSV-1, HSV-2, EBV, HHV-6). At P4–P5 proliferative potential and immunosuppressive activity of pooled OE-MSCs were analyzed in comparing with monocultures. Immunosuppressive activity of MSCs were measured as follow: peripheral blood mononuclear cells (PBMC) isolated from donor's blood were primed with CFSE, co-cultured with OE-MSCs (1:20) for 3 days with PHA additions, and then CD3⁺ cells were assayed. Nonparametric statistical analysis was used.

Results. As was shown, all obtained OE-MSCs were CD73⁺CD90⁺CD105⁺CD31⁻CD45⁻, PDL was 32,7(28,2–36,2) h at P3. The ability of mono OE-MSCs to inhibit mitogen-induced proliferation of CD3⁺ T cells was shown, suppression was 1.55 (1.33–1.75) times comparing to control. The MSCs cultures which inhibited T-cell proliferation more than 1.5 times (n=6) were chosen to prepare pooled cultures (n=4, different combination). There was not revealed significant difference in both PDL and suppression activity in relation to PHA-induced proliferation of CD3⁺ T cells between mono (n=6) and pooled cultures (n=4). Tested pooled culture were banked for long-term storage (5×10⁷ cells/each culture, P4).

Conclusion. OE-MSCs with high proliferative and immunosuppressive potential were chosen for producing of pooled OE-MSCs, pooled cell biomass was cryopreserved for prospective applying. Pooled OE-MSCs possesses immunosuppressive properties and may possibly be used in cellular immunotherapy of immune system disorders, which include autoimmune diseases, and prevention of organ and tissue rejection.

IDENTIFICATION OF HEMATOPOIETIC STEM CELLS IN CRYOPRESERVED AND LYOPHILIZED HUMAN CORD BLOOD LEUKOCONCENTRATES

Lutsenko O.D., Ostankov M.V., Yampolskaya Ye.Ye., Grisha I.G., Ostankova L.V., Sokil L.V., Goltsev A.M.

Institute for Problems of Cryobiology and Cryomedicine National Academy of Sciences of Ukraine, 23, Pereyaslavska str., Kharkiv, Ukraine, 61016

E-mail: cryopato@gmail.com

At present, the problem of using human cord blood as an alternative to bone marrow and peripheral blood as a source of hematopoietic stem cells (HSC) attracts the interest of scientists. However, the use of cord blood in a combined treatment of diseases of different etiologies involves the use of cryopreserved or freeze-dried cells. However, the effect of cryopreservation and lyophilization on the state of HSC subpopulations of varying levels of differentiation has still remained uncertain.

The purpose of this work was to compare the CD34⁺ HSC content and the subpopulations of these cells with varying degrees of differentiation (CD34⁺CD38⁻, CD34⁺CD38⁺) in cryopreserved and lyophilized human cord blood leukoconcentrates (HCBL).

HCBL was obtained by sedimentation of erythrocytes as a result of the dextran-60 supplement to cord blood. HCBL was cryopreserved by a two-stage program using the method of Tsutsayeva AA et al. (1998) with a programmed freezer UOP-6 (Special Designing and Technical Bureau with Experimental Unit of IPCC of the NAS of Ukraine). The HCBL was frozen-dried according to the method of Goltsev AM (2016) using UZV-2 device (Special Designing and Technical Bureau with Experimental Unit of IPCC of the NAS of Ukraine). The samples were rehydrated by adding physiological saline to cells. After rehydration, the resulting suspension was evaluated by the count of the nucleated and viable cells (trypan blue, propidium iodide). The number of CD34⁺CD38⁻, CD34⁺CD38⁺ cells, mean fluorescence intensity of CD34 before and after cryopreservation or lyophilization was evaluated with FACSCalibur (BD Biosciences, USA) flow cytometer, using monoclonal antibodies – FITC Mouse Anti-Human CD34, PE Mouse Anti -Human CD38 (BD Biosciences, USA).

After HCBL cryopreservation or lyophilization, the ratio of hematopoietic precursors of varying degrees of differentiation was changed relative to the fresh one. In the cryopreserved HCBL, a relatively high number of CD34⁺CD38⁻ cells was kept and the number of CD34⁺CD38⁺ cells was elevated. In the lyophilized HCBL, more differentiated elements, CD34⁺CD38⁺, were predominant, and their number was higher than in fresh and cryopreserved material. The obtained results call for the evaluation of the clonogenic properties of either cryopreserved or freeze-dried HCBL in order to predict its therapeutic potential.

EFFECT OF COOLING ON SYNTHESIS OF NUCLEAR PROTEINS IN CULTURE CELLS

Strona V.I.

*Institute for Problems of Cryobiology and Cryomedicine National Academy of Sciences of Ukraine,
23, Pereyaslavka str., Kharkiv, Ukraine, 61016*

E-mail: vstrona@ukr.net

The development of adaptation reactions in a cell under the action of low temperatures and during the rehabilitation period after cooling is determined by the activation of the cell genetic apparatus. Knowledge of the mechanisms of cooling action on the genome functioning will allow to actively intervene in certain stages of cryopreservation, preparing the cells for the action of an extreme factor, i.e. deep cooling.

Studies were performed in cells of a transplanted calf kidney culture. The cell monolayer was cooled down to +4 °C. Then the culture medium was replaced with fresh (cooled down to +4 °C) one, wherein the labeled precursors of DNA, RNA, proteins (H³-thymidine, C¹⁴-orotic acid, C¹⁴-chlorella protein hydrolyzate) were introduced. Cells were kept at +40 °C for 1-24 hours (continuous labeling). After a certain time interval, the vials with cell monolayer were selected. Cells were subjected to chemical fractionation using a modified Schmidt-Tanhhauser method, specific radioactivity was determined in DNA, RNA and protein fractions. The concentration of these components was calculated spectrophotometrically.

Deep hypothermia has been shown to leads to complete inhibition of DNA synthesis, which remains at a level of 0.6-0.1% throughout the entire cooling period. At the same time, protein synthesis was 12-15% of the control; during the cold exposure (during the day), the increase in protein synthesis in cells was not observed. RNA synthesis, which was 3-5% of the control in the first 1-3 hours of cooling, then increased up to 15% by 5 hours of incubating the cells at +4° C and remains at this level for about 24 hours. The RNA content in the cells decreased in the first hours of cooling (5 hours) down to 83%, and then, from the moment of the increase in RNA synthesis, gradually accumulated in the cells and by 7 hours of cooling, its content was 133% of the control.

With a decrease in temperature from +37°C down to +4 °C, the synthesis of not only cellular, but also of nuclear proteins was inhibited. The specific radioactivity of proteins of isolated nuclei was 98% of the specific radioactivity of total proteins of cells, i.e., the proteins, accumulating in the nuclei, were mainly synthesized. When the temperature dropped down to +4° C (within 2 hours), the synthesis of nuclear proteins was 35% of the control. The introduction of labeled amino acids into the medium with cooled cells led to a decrease in the synthesis of cellular proteins down to 12% of the control and an increase in the synthesis of nuclear proteins up to 58%, i.e., more than one and a half times the level of synthesis in the process of cell cooling.

During the rehabilitation period of cooled cells (culturing at +37°C for 24-48 hours), the normal mitotic index was not restored, the number of abnormal mitoses was increased (from 16% in control up to 38% after cooling).

APPLICATION OF HYDRATED FULLERENE C₆₀ DURING HYPOTHERMIC STORAGE OF BLOOD

Falko O.V., Lipina O.V., Chyzhevskiy V.V.

*Institute for Problems of Cryobiology and Cryomedicine National Academy of Sciences of Ukraine,
23, Pereyaslavska str., Kharkiv, Ukraine, 61016*

E-mail: o.v.falko@gmail.com

Donor's blood after hypothermic storage is widely used in transfusiology. To increase the efficiency of existing technologies of hypothermic blood storage and to expand their capabilities, the search for new and safe substances capable to exert a protective effect under adverse temperature conditions remains topical. Hydrated fullerene C₆₀ (C60FWS) being nanoscale compound has a unique biological feature. Due to its special physicochemical properties, it is able to participate in biological processes as a regulator of reactive oxygen species and an acceptor of free radicals [Shcherbakov AV, 2011; Ivanov VK, 2009], preventing a cell damage against oxidative stress. This circumstance was a prerequisite for the study, the purpose of which was to evaluate the effect of C60FWS on human blood during hypothermic storage. Blood was collected from healthy donors' cubital vein. Sodium citrate was used as an anticoagulant. The collected blood was stabilized for 4 hrs at 20 °C, and then divided into three experimental groups: 1 – blood, with the addition of a physiological solution of sodium chloride (control); 2 and 3 - blood, with the addition of C60FWS solutions at a final concentration of 1×10^{-5} M and 1×10^{-7} M, respectively. The investigated solutions were added to the blood in a 1: 1 ratio. C60FWS solutions were prepared with physiological sodium chloride solution. After adding fullerene solutions, the blood samples were stored at 4 °C for 7 and 14 days. Blood safety was estimated by the degree of erythrocytes hemolysis of the studied blood samples placed in hypotonic solutions of sodium chloride. Below the experimental data on the hemolysis value of erythrocytes (%) for a hypotonic solution of sodium chloride with 0.5% concentration are presented. It was shown that the level of erythrocytes hemolysis increasing depended on the period of hypothermic storage of blood and the concentration of fullerene solution. Storage of the control blood samples at 4 °C for 7 days led to a rise in the hemolysis rate of erythrocytes from $10.0 \pm 0.1\%$ up to $40.2 \pm 13.4\%$, and the longer storage (14 days) revealed an increase in the erythrocytes hemolysis rate up to $67.6 \pm 9.3\%$. The fullerene C60FWS addition as a protective component to the blood led to a slowdown in the hemolysis rate of erythrocytes during its hypothermic storage. It was shown that after hypothermic blood storage for 7 days in the presence of a C60FWS solution (group 2) at a final concentration of 1×10^{-5} M, the erythrocytes hemolysis rate was $12.5 \pm 0.3\%$ (in the control $43.8 \pm 3.7\%$). The study of blood samples after an increase in their storage time up to 14 days revealed that the hemolysis rate of erythrocytes made $24.3 \pm 4.4\%$, which was almost 3 times lower than in the control samples ($67.6 \pm 9.3\%$). Hypothermic storage of blood at a decreased final concentration of fullerene C60FWS to 1×10^{-7} M showed that on day 7 of storage the hemolysis rate of erythrocytes was $15.3 \pm 0.5\%$, and on day 14 it made $48.5 \pm 4.7\%$. Thus, the use of a solution of hydrated fullerene C60FWS in hypothermic storage of human blood contributed to its preservation, as evidenced by a decrease in the erythrocytes hemolysis rate.

INFLUENCE OF NANOCRYSTALLINE CERIUM DIOXIDE ON VIABILITY OF ESCHERICHIA COLI BACTERIA AFTER HYPOTHERMIC STORAGE

Vysekantsev I.P., Buriak I.A., Falko O.V., Chyzhevskiy V.V.

Institute for Problems of Cryobiology and Cryomedicine National Academy of Sciences of Ukraine, 23, Pereyaslavskaya str., Kharkiv, Ukraine, 61016

E-mail: buriakiryna@gmail.com

To date, the most effective way of storage of different cells is cryopreservation and-hypothermic storage in multicomponent media. During the effect of low temperatures, the cells are exposed to various physical and chemical factors that can cause their irreversible injury. One of these factors is oxidative stress. Reducing the negative effect of oxidative stress on cells can be achieved by adding the antioxidants to the protective medium. The use of nanocrystalline cerium dioxide (NCD), which is known to significantly exceed the degree of antioxidant activity if compared with existing antioxidants (ascorbic acid, tocopherol, amino acids, etc.) is prospective. We have shown the effectiveness of NCD application in the studies on cryopreservation of mouse fibroblasts and microalgae *Spirulina platensis*. It is known that the mandatory condition for biomedical application of various chemicals is the absence of their toxicity to different biological objects. Microorganisms are the convenient test objects for the study of toxicity. The purpose of this work was to determine the effect of NCD in a concentration of 0.2 g / L on cells of *Escherichia coli* (*E. coli B*) bacteria during their hypothermic storage. Bacteria of *E. coli B* were grown for 24 hours under standard conditions. Afterwards the suspensions of microorganisms were prepared with sterile physiological saline of sodium chloride (NaCl). The cell concentration was 10^8 CFU / ml. To evaluate the effect of NCD on *E. coli B* bacteria, NaCl (control) or NCD (experimental) solution were added to the suspension. The final concentration of NCD in the suspension made 0.2 g / L. Control and experimental specimens were placed in a refrigerating chamber and stored at 4 °C for 5 days. The viability of bacteria in the samples was evaluated by the indices of colony formation on agar plates. It was established that during hypothermic storage for 5 days the viability of *E. coli B*, both in the control and experimental samples, did not significantly change and remained at the initial level. Thus, nanocrystalline cerium dioxide at a concentration of 0.2 g / L did not affect the viability of *E. coli B* bacteria during their hypothermic storage at 4 °C for 5 days. This indicates the possibility of using nanocrystalline cerium dioxide at the mentioned concentration for cryopreservation of cells, including microorganisms. In further studies, we are planning the experiments on cryopreservation of *E. coli* cells under the protection of nanocrystalline cerium dioxide.

WHETHER NANOCRYSTALLINE CERIUM DIOXIDE AND HYDRATED FULLERENE AQUEOUS SOLUTIONS AFFECT *SPIRULINA PLATENSIS* CELL CULTURE TOXICALLY?

Goloiad M.O.

*Institute for Problems of Cryobiology and Cryomedicine National Academy of Sciences of Ukraine,
23, Pereyaslavska str., Kharkiv, Ukraine, 61016*

E-mail: o.v.falko@gmail.com

Over the past 20 years, the rapid development of nanotechnology has allowed the creation of innovative nanomaterials with fundamentally new properties and huge potential to be used in biology, medicine and pharmaceuticals. However, the problem of the toxicity of these compounds remains a serious problem. Undoubtedly, the controversy and misunderstanding in this regard are due to the fact that the study of biological properties of aqueous solutions of nanoparticles does not always fully take into account the conditions of synthesis, methods for stabilizing them in colloidal systems, particle size, transport path ways to biological targets, etc. We used water-soluble form of hydrated unmodified fullerene C₆₀ FWS in our studies, namely, the concentrate "Fullerene C₆₀ hydrated" (C₆₀FWS) with initial concentration of 144 mg/L (10⁻⁴M) and a solution of nanoparticles of nanocrystalline cerium dioxide (NCD) in final concentration of 0.02 g/L with a particle size of 2 nm obtained by wet synthesis [Kavok N. et al., 2017]. We believe that one of the promising biological objects for studying the toxic effects of nanocrystalline materials may be *Spirulina* microalgae. An essential point in favor of its application as a test object is the ability of blue-green algae to act as a sensitive bioindicator of the first link of trophic chain in toxicological studies of environmental pollution [Bryantseva Yu.V. et al., 2005]. To determine the effect of the studied nanoparticles on *Spirulina platensis* cells, we used a functional loading method, namely, the transfer of cells to a distilled water from the nutritional Zarrouk's medium (with up to 11.5 pH). After wash-out from the nutrient medium, the microalgae were divided into the following experimental groups: 1- storage in distilled water; 2- storage in distilled water with the addition of C₆₀ FWS; 3 – storage in distilled water with the addition of NCD; 4 – storage in Zarrouk's culture medium. The morphofunctional state of *Sp.platensis* cell culture was assessed using the methods of vitals staining, counting and recording of the spectra of own fluorescence of cells, as well as the growth of microalgae biomass. The obtained results indicate that aqueous solutions of cerium dioxide with a particle size of 2 nm and a concentration of 0.02 g/L as well as hydrated fullerene C₆₀ FWS with a concentration of no higher than 2x10⁻⁵M do not exert a toxic effect on cells of *Sp. platnesis*. It is known that solutions of fullerene and nanocrystalline cerium dioxide, due to their special physical and chemical properties, can participate in biological processes as a regulator of oxygen reactive species and the acceptors of free radicals [Shcherbakov AB et al., 2011], this outlines the ways of possible application of these nanoscale compounds in a variety of biotechnological processes.

EFFECT OF CRYOPROTECTIVE AGENTS AND LOW TEMPERATURES ON PRODUCTION OF REACTIVE OXYGEN SPECIES IN HUMAN ERYTHROCYTES

Nina Zemlianskykh, Regina Migunova, Liubov Babiychuk

*Institute for Problems of Cryobiology and Cryomedicine National Academy of Sciences of Ukraine,
23, Pereyaslavska str., Kharkiv, Ukraine, 61016*

E-mail: nzemliansky@gmail.com

Cryoprotective agents (CPAs) are able to maintain the structure of protein macromolecules, membranes and cells in a state close to the native one under the stressful conditions. However the physicochemical properties of the cryoprotectant solutions significantly differ from the physiological characteristics, that induces structural and functional changes in various subcellular systems. One of the possible manifestations of the cryoprotectant influence may be changes in the structure of hemoglobin and the consequent activation of oxidative processes. More significant changes in ROS production may occur after the cell cryopreservation because during the freeze-thawing, despite the presence of cryoprotectants, some cells are still subjected to lethal or sublethal damage.

The study was aimed to examine the CPAs and low temperature effects on the ROS production in human erythrocytes. The estimation of ROS in erythrocytes was performed by flow cytometry with FACS Calibur (Becton Dickenson, USA) on the fluorescence data of DCF originated from DCFH-DA (2',7'-Dichlorofluorescein diacetate) after its oxidation in a cells with hydrogen peroxide. The cryopreserved erythrocytes were under the protection of a mixture of 3.25 M glycerol and 0.2 M mannitol, as well as 0.2 M polyethylene glycol with m.w. 1500 (PEG). Freezing was performed in liquid nitrogen (-196 °C), while heating was carried out at 42 – 44 °C. The results were analyzed using the software “WinMDI 2.8”. Changes in the intensity of DCF fluorescence in erythrocytes were characterized by the value of the median of the distribution histograms. Statistical processing of the results was performed using the software “Statgraphics plus 2.1 for Win”

Incubation of human erythrocytes in 2 M glycerol caused a slight increase in DCF fluorescence, while in a glycerol-mannitol medium the intensity of ROS production was lower than in the control samples. Such changes may be stipulated by a slowdown in the intensity of metabolic processes and protein stabilization in the glycerol presence, that affects the possibility of ROS production in cells. In addition, both components of the cryoprotectant mixture have the free radical scavenger properties, which can explain the decrease in ROS level in erythrocytes during incubation, since these substances compete with DCF for interaction with H₂O₂. Freezing of human erythrocytes in the glycerol presence, as well as its removal after thawing, do not affect the intensity of ROS production processes.

Incubating human erythrocytes in PEG presence led to a significant intensification of ROS production, which can reduce cell stability during freeze-thawing. After freezing the intensity of ROS production even more enhanced that attested sublethal cell lesions. However, PEG removal and the concomitant lysis of injured cells led to the normalization of ROS production processes.

Thus, changes in the rate of ROS production in erythrocytes under the CPA effect and freeze-thawing can have a significant regulatory effect on the membrane-cytoskeleton complex state and cell stability under stressful conditions of cryopreservation.

ASSESSMENT OF EFFICIENCY OF CRYOPROTECTANT MIXTURES CONTAINING VARIOUS ANTIOXIDANTS

Babiychuk L.O., Makashova O.Ye., Zubov P.M., Zubova O.L.

Institute for Problems of Cryobiology and Cryomedicine National Academy of Sciences of Ukraine, 23, Pereyaslavska str., Kharkiv, Ukraine, 61016

E-mail: Olena.makashova@gmail.com

The use of cord blood (CB) hematopoietic progenitor cells (HPCs) is firmly established in practical medicine as an effective method for treating diseases of various genesis. This has led to the development of protocols of low-temperature storage and the cryobanks network. DMSO at 7.5-10% concentrations is the most common cryoprotectant for cryopreservation of CB nucleated cells (NCs) including HPCs. However besides cytoprotective effects, DMSO may also cause the accumulation of reactive oxygen species (ROS) during cell cryopreservation with following initiation of apoptosis and cell death. Accordingly, the promising approaches to the cryopreservation may be supplementation of medium with antioxidants which are able to "trap" free radicals and reduce the intensity of free radical oxidation production at all stages of freezing and thawing. Among these antioxidants glutathione (GSH), N-acetyl-L-cysteine (NAC) and ascorbic acid (AA) can be used.

The purpose of the study was assessment of cell viability and quantification of CB NCs with ROS excess compared to control after their cryopreservation in medium containing DMSO and different antioxidants.

In the work for CB NCs' cryopreservation we used 7.5% DMSO and different antioxidants (Sigma-Aldrich (USA)) at their final concentrations as follows: AA - 0.1 mM; NAC - 10 mM; GSH - 1 mM. The viability and the amount of cells with ROS excess were determined with flow cytometry (FACS Calibur (BD, USA)) using 7AAD and DCF fluorescent dyes, respectively.

Analysis of the NCs/HPCs viability showed that after cryopreservation under DMSO protection without antioxidants this index decreased by 30/35%, respectively. This may be due partly to ROS production, as the level of cells with ROS excess increased by 6-8% during freezing.

Assessment of viable CB NCs/HPCs after cryopreservation with DMSO and antioxidants showed that the supplementing 7.5% DMSO solution with 0.1 mmol AA ensured getting of viable NCs/HPCs up to 73/77%, respectively. When NAC was used at 10 mM concentration this index was up to 69/78%, while in the sample without the addition of antioxidants were obtained 57/65% of viable NCs/HPCs, respectively. Determining the amount of viable NCs/HPCs which were cryopreserved in samples with 7.5% DMSO and 1 mM GSH after thawing showed an increase of this index by 20/21%.

Quantification of cells with ROS excess indicated that in the group with AA application this index did not differ from the similar group when the antioxidant was not added. In samples cryopreserved with a 7.5% DMSO supplemented with the antioxidants NAC (10 mM) or GSH (1 mM), this index decreased by 12-13% compared to data when any antioxidant was not added.

Thus, the results indicated that antioxidants such as NAC and GSH contributed to an increase in their viability and decrease in the level of cells with ROS excess, while AA did not show antioxidant effect during the NCs cryopreservation. Cryoprotective solutions containing GSH at a concentration of 1 mM and 7.5% DMSO were the most effective.

ЕКСПРЕСІЯ НЕЙРАЛЬНИХ МАРКЕРІВ У КУЛЬТУРІ КЛІТИН, ОТРИМАНОЇ ЗІ СПІНАЛЬНИХ ГАНГЛІЇВ НЕОНАТАЛЬНИХ СВИНЕЙ

Алі С.Г., Божок Г.А.

*Інститут проблем кріобіології і кріомедицини НАН України,
вул. Переяславська, 23, м. Харків, Україна, 61016*

E-mail: bozhokgaru@gmail.com

На теперішній час у медико-біологічних дослідженнях сформувався запит на використання в експериментах так званих трансляційних моделей на великих тваринах (translational large animal model) задля максимального наближення до фізіологічних характеристик людини. В цьому відношенні свиня розглядається як один з придатних видів модельних тварин. Культури клітин зі спінальних гангліїв (СГ) є перспективним джерелом нейральних клітин-похідних нервового гребеню (Li et al., 2007; Tongtako et al., 2017). Однак досі не було зроблено спроби отримання та вивчення основних фенотипових характеристик клітинних культур, отриманих зі СГ свиней. Мета дослідження – вивчити експресію нейральних маркерів у культурі клітин, отриманої зі спінальних гангліїв неонатальних свиней.

В експериментах використовували СГ поросят віку P0-P1. Суспензію клітин отримували ферментативним методом. Клітини висівали в концентрації $0,5 \times 10^4$ кл/мл і культивували в чашках Петрі з поверхнею, обробленої полі-D-лізином, при 37°C в атмосфері з 5% CO₂ у живильному середовищі α -MEM з додаванням 2% НейроМакс та антибіотиків. На 8-му добу культивування спостерігалось утворення мультиклітинних сфероїдів, які пересівали та продовжували культивувати у середовищі α -MEM з 10% фетальної телячої сироватки. Після отримання конфлуентного моношару зразки культур фіксували та забарвлювали за протоколом непрямого імунофлуоресцентного мічення з першими антитілами до глутамін-синтетази (ГС), S-100 та β -III-тубуліну (ТубВ3) та відповідними другими антитілами, кон'югованими з флуоресцентною міткою. Мікрофотозйомку здійснювали за допомогою флуоресцентного мікроскопу Carl Zeiss Axio Observer Z1. Аналіз зображень проводили з використанням програми Axio Vision Rel. 4.8.

Встановлено, що в культурі клітин, отриманої зі СГ неонатальних свиней, спостерігається висока експресія одного з основних специфічних маркерів сателітних гліальних клітин (СГК) – глутамін-синтетази. Позитивне мічення антитілами до ГС було відмічено у більш, ніж 95% клітин, які мали морфологічні ознаки СГК: малий розмір, веретеноподібну форму клітини, незначний шар цитоплазми, щільне інтенсивно базofilне ядро та два коротких відростки. Нами не було виявлено специфічного забарвлення жодних клітин з антитілами до S-100. Однак цікаво, що у певної частини клітин спостерігалася експресія ТубВ3, при цьому позитивно забарвлювалися клітини іншого морфологічного типу: великі розпластані мультиполярні клітини з крупним ядром овальної форми.

Порівнюючи власні результати з даними, які були отримані у культурі клітин зі СГ мишей (Tongtako et al., 2017), можна відзначити, що клітини СГ неонатальних свиней, також як і мишей, експресують ГС. Однак, на відміну від культури зі СГ мишей, в культурі клітин зі СГ свиней спостерігається експресія ТубВ3. При цьому морфологічно тип клітин, який позитивно реагує з антитілами до ТубВ3, не належить до нейронів. Це може бути ознакою присутності у культурі клітин зі СГ свиней прогеніторних клітин-похідних нервового гребеню, які за певних умов диференціювання здібні експресувати ТубВ3 (Li et al., 2007).

1. Li H.-Y. et al., Stem cells. 2007, 25:2053–2065.

2. Tongtako W. et al., Sci Rep. 2017, 7(1):13915.

ЛІОФІЛІЗОВАНИЙ ЛЕЙКОКОНЦЕНТРАТ КОРДОВОЇ КРОВІ В ТЕРАПІЇ ЕКСПЕРИМЕНТАЛЬНОГО ГОСТРОГО ГНІЙНОГО ПЕРИТОНІТУ

Гольцев К.А.^{1,3}, Пархоменко К.Ю.³, Криворучко І.А.², Луценко О.Д.¹, Шевченко О.М.², Ажгибесов К.А.³

1 – Інститут проблем кріобіології й кріомедицини НАН України, вул. Переяславська, 23, м. Харків, Україна, 61016

2 – Харківський національний медичний університет, пр. Науки, 4, м. Харків, Україна, 61022

3 – КНП ХОР "Обласна клінічна лікарня", просп. Незалежності, 13, м. Харків, Україна, 61058

E-mail: cryopato@gmail.com

Гострий гнійний перитоніт (ГПП) залишається однією з найважливіших проблем сучасної невідкладної хірургії. Незважаючи на суттєві досягнення в його діагностиці та лікуванні, результати терапії не задовільні, а летальність при цій патології висока (20-90 %). У зв'язку з тим, що ГПП є однією з форм прояву імунозапальної реакції організму хірургічних хворих показано застосування імуномодуючої терапії. У даному дослідженні для такої терапії використовували ліофілізований лейкоконцентрат кордової крові людини (ЛЛККЛ).

Мета дослідження – оцінити вплив ЛЛККЛ на імунний статус і показники крові щурів в комплексній терапії ГПП.

Матеріали і методи. Експерименти проводили на щурах-самцях лінії Вістар, 6 міс. віку, масою 180-200 г. Моделювали ГПП після апендектомії укладаючи перев'язаний червоподібний відросток у черевній порожнині (ЧП). Через 24 години усім дослідним тваринам проводили релапаротомію і санацію ЧП охолодженим до 4-6 °С 0,02% водним розчином фурациліну. Ліофілізували ЛККЛ (ЛЛККЧ) за методом Гольцева А.М. і співавт. (2016 р). Тварини були розподілені на групи: 1. Здорові (контроль); 2. ГПП; 3. ГПП + ампіцилін (40 мг/кг маси тіла) під час релапаротомії; 4. ГПП + введення ЛЛККЛ (по 0,3 мл, 5-6*10⁶ клітин); 5. ГПП + ЛЛККЛ + ампіцилін. Аналіз імунологічних показників проводили на 1, 3, 5 та 7-у добу.

Результати. Включення в комплексну терапію ЛЛККЛ з антибіотиком супроводжувалося істотним поліпшенням імунологічних показників у щурів з ГПП. Найбільшу імунокоригуючу чутливість до ЛЛККЛ серед регуляторних субпопуляцій Т- лімфоцитів (CD4⁺ і CD8⁺) проявляли CD4⁺ клітини, їхня кількість підвищилася, у зв'язку з чим на 3-ю добу нормалізувалися показники імунорегуляторного індексу, які вірогідно не відрізнялися від контролю. Мало місце істотне зниження рівня IgA, IgG, IgM і ЦІК на 7-у добу. Терапія в такому сполученні позитивно впливала на цитокіни, підвищуючи показники ІЛ-10 та пригнічуючи активацію ІФН-γ. У щурів, яким під час релапаротомії вводили антибіотик або тільки ЛЛККЛ, такого ефекту не спостерігали.

Висновок. Експериментально доведено, що комплексна терапія ЛЛККЛ з антибіотиком давала більш виражений позитивний результат у порівнянні із застосуванням тільки антибіотика або тільки ЛЛККЛ.

КЛІТИННА ТЕРАПІЯ КОРДОВОЮ КРОВ'Ю ЕКСПЕРИМЕНТАЛЬНОГО ГЕНІТАЛЬНОГО ГЕРПЕСУ

Стецишин В.Г., Останкова Л.В., Луценко О.Д., Гриша І.Г., Гаєвська Ю.О.
*Інститут проблем кріобіології й кріомедицини НАН України,
вул. Переяславська, 23, м. Харків, Україна, 61016*

E-mail: cryopato@gmail.com

Встановлено, що сучасна терапія генітального герпесу (ГГ) припускає пригнічення окремих ланок репродукції вірусу простого герпеса-1 (ВПГ-1). Водночас розвиток ГГ впливає на імунну систему (ІС) хворого. Тому актуальною проблемою лікування ГГ, є необхідність застосування препаратів із системною імуномодулюючою активністю.

Мета – оцінити ефективність імуномодулюючої терапії експериментального ГГ кріоконсервованою (кККЛ) та ліофілізованою (лККЛ) кордовою кров'ю людини.

Матеріали і методи. Експерименти виконані на 6- ти місячних щурах - самках лінії Вістар, масою 180 – 200 г. Моделювали ГГ внутрішньоочеревинним введенням 1 мл ВПГ-1 (титр 1:20 000) протягом 5-и діб. Кріоконсервували ККЛ за методом Цуцаєвої А.А. і співавт. (1998 р.). Ліофілізували ККЛ за методом Гольцева А.М. і співавт. (2016 р). Регідратували лККЛ фізіологічним розчином (1:1). На 5-у добу після індукції ГГ щурам внутрішньоочеревинно вводили кККЛ або лККЛ по 0,5 мл в дозі 5×10^6 клітин. Ін'єкцію ацикловіра (АЦВ) в дозі 50 мг/кг проводили 1раз/добу протягом 3-х діб. Групи тварин: 1.Здорові (контроль); 2. ГГ; 3. ГГ + АЦВ; 4. ГГ + кККЛ; 5. ГГ + лККЛ, 6. ГГ + кККЛ + АЦВ; 7. ГГ + лККЛ + АЦВ. Визначали кількість Т-лімфоцитів (CD3, CD4, CD8, CD16, CD25) методом імунофлуоресценції; рівень цитокінів (ІЛ-10, ФНО- α , ІФН- γ), концентрацію імуноглобулінів IgM, IgA методом ІФА; та клінічний аналіз крові. Ефективність лікування ГГ оцінювали на 7, 14 та 21-у добу від початку терапії.

Результати. Отримані дані свідчать про виражений імуномодулюючий вплив як лККЛ, так і кККЛ на ІС щурів з ГГ. Проведене комплексне лікування щурів групи 6 і 7 більшою мірою, ніж групи 4 і 5, сприяло усуненню диспропорції в показниках Т- клітинної ланки імунітету. Починаючи з 7-ї доби у тварин групи 6 і 7 спостерігали відновлення імунорегуляторної функції Т-клітин і продукції протизапальних цитокінів; зниженню гіперактивації ЦІК, продукції специфічних імуноглобулінів IgM, IgA та нейтрофильних гранулоцитів. У щурів групи 4 і 5 показники клітинної та гуморальної ланки імунітету відповідали значенням контролю на 14-у добу.

Висновок. Розроблено й патогенетично обґрунтовано застосування комплексного метода лікування експериментального ГГ введенням кККЛ або лККЛ із АЦВ.

КРІОКОНСЕРВОВАНІ КЛІТИНИ ФЕТАЛЬНОЇ ПЕЧІНКИ В ЛІКУВАННІ ЕКСПЕРИМЕНТАЛЬНОГО АТОПІЧНОГО ДЕРМАТИТУ

Леонова Л.А., Останков М.В., Бондарович М.О., Ямпольська Є.Є., Останкова Л.В.
*Інститут проблем кріобіології і кріомедицини НАН України,
вул. Переяславська, 23, м. Харків, Україна, 61016*

E-mail: cryopato@gmail.com

Серед кожних захворювань atopічний дерматит (АД) через далеко не ясний етіопатогенез, хронічного плину й пов'язаних із цим терапевтичними проблемами, займає особливе місце серед алергічних захворювань. Ступінь важкості перебігу та порушення показників імунного статусу (ІС) у пацієнтів є основою для розробки методів терапії АД. Регуляція імунних механізмів, що лежать в основі розвитку АД відкриває нові можливості в розробці патогенетичних методів терапії цього захворювання.

Мета роботи – оцінити вплив кріоконсервованих клітин фетальної печінки (кКФП) на показники клітинної та гуморальної ланок імунітету у щурів з АД.

Матеріали та методи. Були обстежені щури лінії Вістар у віці 6 місяців, масою 180-200 г. Ініціювали АД нанесенням на шкіру спини (3x3 см²) 5% спиртово-ацетонового розчину динітрохлорбензолу протягом 21 доби. Кріоконсервували КФП за методом Гольцева А.М. і співавт. (1992 р.). Щури були розподілені на групи: 1. Здорові (контроль); 2. АД; 3. АД + преднізолон; 4. АД + кКФП по 0,5 мл, 5*10⁶ клітин. Імунологічне обстеження включало оцінку показників концентрації субпопуляцій лімфоцитів (CD3, CD4, CD8, CD16, CD19) в шкіряних біоптатах методом проточної цитофлюориметрії. В сироватці крові оцінювали показники: імуноглобулінів (IgA, IgE, IgG); цитокинового статусу – інтерлейкінів (ІЛ-2, ІЛ-4, ІЛ-6, ІЛ-10), ІФН-γ методом імуноферментного аналізу, та ЦІК - спектрофотометричним методом.

Результати дослідження. Алергійна відповідь при АД виникає в органі-мішені, шкірі і має Т- клітинний імунний механізм. Так в шкірі щурів з АД спостерігалася інфільтрація Т-лімфоцитами, що секретують прозапальні цитокини (ІЛ-4, ІЛ-6, ІЛ-10 і ІФН-γ), відбувалося ушкодження епітелію, що є наслідком алергійного запалення тканини. Застосування кКФП знижувало тканинну гіперреактивність, відбувалося пригнічення ознак алергійного запалення, перебудови характеру клітинної й цитокинової відповіді. Лікування введенням кКФП супроводжувалося зростанням загальної кількості Т-лімфоцитів, зменшенням кількості Т-хелперів і підвищенням Т-супресорів, що привело до нормалізації імнорегуляторного індексу, а також зниження кількості В-лімфоцитів та НК на 7-у добу. В сироватці крові спостерігали зростання ІЛ-2, та зниження ІЛ-4, ІЛ-6, ІЛ-10 і ІФН- γ; підвищення рівня IgA, та зниження IgE, IgG та ЦІК. Преднізолонова терапія до такого ефекту не призводила.

Висновок. Експериментально доведено, що використання кКФП позитивно впливає на показники клітинної та гуморальної ланок імунітету при лікуванні АД.

ВПЛИВ ТРАНСПЛАНТАЦІЇ КЛІТИН КОРДОВОЇ КРОВІ НА РОЗВИТОК ПОСТІНСУЛЬТНИХ ПРОЦЕСІВ У ГОЛОВНОМУ МОЗКУ ЩУРІВ

Лебединець В.В.^{1,3}, Останков М.В.¹, Лебединець Д.В.^{2,3}, Дубрава Т.Г.¹

1 – Інститут проблем кріобіології й кріомедицини НАН України, вул. Переяславська, 23, м. Харків, Україна, 61016

2 – Харківський національний університет ім В.Н. Каразіна

3 – Центральна клінічна лікарня Укрзалізниці, м. Харків

E-mail: cryopato@gmail.com

Клітинна терапія застосуванням кордової крові (КК) вважається перспективним підходом для корекції наслідків ряду патологій, у тому числі й ішемічному інсульті (ІІ). Лікування ІІ КК принципово відрізняється від традиційної медикаментозної терапії, для якої характерні симптоматичний підхід і короткочасність впливу. В той час як застосування КК в терапії ІІ, у випадку успішної інтеграції в тканину реципієнта, може робити позитивний ефект протягом тривалого часу. Тому використання КК як терапевтичного агенту при ІІ дає надію на відновлення структури і функції головного мозку, втрачених в результаті розвитку патологічного процесу.

Мета дослідження – оцінити морфологічні зміни в головному мозку, моторні й когнітивні навички у щурів при ІІ на тлі лікування кріоконсервованою (кККЛ) або ліофілізованою кордовою кров'ю людини (лККЛ).

Матеріали й методи. Експерименти проводили на щурах-самцях лінії Вистар 6 міс. віку, масою 210-250 г. Моделювали ІІ оклюзією сонної мозгової артерії (СМАо). Через 6 годин після СМАо щурам одноразово внутришньочеревинно вводили кККЛ або лККЛ по 0,5 мл в дозі 5×10^6 клітин. Церебралізін розчиняли у фізіологічному розчині і вводили внутришньочеревинно по 0,1 мл/100 г маси тварини. Гістологічні препарати мозку готували за стандартною методикою, серійні зрізи 5- 7 мкм фарбували гематоксилін - еозином і люксолом. Морфометрію виконували після мікроскопії й створення цифрового зображення за допомогою мікроскопа Zeiss Primo Star, x 100, 400, 900. Неврологічний статус оцінювали по поведженню тварин у тестах "Відкрите поле" і "Хрестоподібний лабіринт".

Результати. На моделі ІІ у щурів було показано, що введення лККЛ, також як і кККЛ, прискорює плин реакції асептичного запалення, сприяє виживанню нейронів у зоні ішемічної півтіні, поліпшенню васкуляризації ішемізованої зони й збереженню цілісності судинного сплетіння. Терапія ІІ введенням лККЛ або кККЛ дозволяє зберегти вірогідно більшу кількість життєздатних нейронів у нервовій тканині, прилеглою безпосередньо до зони травматичної півтіні, а також сприяє більш швидкому й повному відновленню моторних і когнітивних навичок у щурів з ІІ в порівнянні із застосуванням церебралізіна.

Висновок. Результати експериментального дослідження застосування лККЛ, також як і кККЛ, можуть бути використані для розробки нових терапевтичних підходів, заснованих на принципах клітинної терапії, для лікування пацієнтів з ІІ.

ОСОБЛИВОСТІ СТРУКТУРНО-ФУНКЦІОНАЛЬНОЇ ОРГАНІЗАЦІЇ СТОВБУРОВИХ РАКОВИХ КЛІТИН ЗАЛЕЖНО ВІД ЛОКАЛІЗАЦІЇ І РОЗМІРІВ ПУХЛИНИ

Бондарович М.О., Челомбитько О.В., Останкова Л.В., Гольцев А.М.
*Інститут проблем кріобіології й кріомедицини НАН України,
вул. Переяславська, 23, м. Харків, Україна, 61016*

E-mail: cryopato@gmail.com

Розвиток і метастазування раку молочної залози (РМЗ) значною мірою залежить від рівня структурно-функціональної організації стовбурових ракових клітин (СРК). Відомо, що найбільш канцерогенними в розвитку РМЗ є СРК CD44^{hi}. У групу прогеніторних, але більше диференційованих входять CD44⁺24⁻ і CD133⁺-клітини. Визначення вмісту СРК в молочній залозі (МЗ) і пухлині має важливе теоретичне і практичне значення.

Мета роботи - визначити особливості структурно-функціональної організації СРК в МЗ і пухлині.

Матеріали і методи. Експерименти проведені на мишах-самках лінії С3Н і СВА 13 місячного віку, масою 18-20 г. Тварини були розподілені в групи: 1. СВА (контроль); 2. С3Н без пухлини; 3. С3Н з пухлиною (1-1,5 см²); 4. С3Н з пухлиною (3-4 см²). Кількість CD44^{hi}, CD44⁺24⁻ і CD133⁺-клітин визначали в МЗ та в пухлині методом проточної цитофлуориметрії (FACSCalibur, США) з використанням моноклональних антитіл («BD Pharmingen», США).

Результати. В МЗ мишей групи 2 без клінічних ознак РМЗ збільшувалася кількість клітин CD44⁺24⁻ (в 2 рази) і CD133⁺ (в 3 рази), а також спостерігалася експансія CD44^{hi} клітин. Поява пухлини у мишей групи 3 супроводжувалася підвищенням кількості CD44⁺24⁻ клітин і значущим зниженням вмісту CD133⁺ клітин в МЗ. При цьому концентрація CD44^{hi} клітин в МЗ залишалася на рівні групи 2. Збільшення кількості CD133⁺ клітин в МЗ мишей групи 2 та зниження в МЗ і пухлині у тварин групи 3 свідчать про неоднозначний вплив цієї субпопуляції на розвиток пухлини. При дослідженні вмісту СРК у тварин групи 4 було показано, що відносна кількість клітин CD44⁺24⁻ в МЗ знижувалася в порівнянні з групою 3, а з групою 2 значущих розходжень не виявлено. У той же час кількість CD133⁺-клітин залишалася на рівні значень групи 3, а вміст CD44^{hi} клітин був вірогідно нижче ніж в групах 2 і 3. В пухлині мишей груп 3 і 4 відзначено вірогідно нижчий вміст клітин з фенотипом CD44⁺24⁻, CD133⁺, ніж в МЗ. При цьому вірогідної кореляції між розміром пухлини і вмістом клітин з цим фенотипом не виявлено. Відносна кількість найбільш канцерогенних CD44^{hi}-клітин у пухлині збільшувалася по мірі її росту і була в 3 рази вищою у мишей групи 4 порівняно з групою 3. Це може бути пов'язано зі збільшенням вмісту індукованого гіпоксією фактора (HIF-1) в тканинах.

Висновки. Встановлено факт кількісної і якісної експансії СРК залежно від місця локалізації і розміру пухлини.

АДОПТИВНЕ ЗАСТОСУВАННЯ ТОЛЕРОГЕННИХ ДЕНДРИТНИХ КЛІТИН - АЛЬТЕРНАТИВНИЙ ТЕРАПЕВТИЧНИЙ ПІДХІД ДО ЛІКУВАННЯ РЕВМАТОЇДНОГО АРТРИТУ

Гольцев А.М., Дубрава Т.Г., Ямпольська К.Є., Бабенко Н.М., Гаєвська Ю.О., Бондарович М.О.
*Інститут проблем кріобіології й кріомедицини НАН України,
вул. Переяславська, 23, м. Харків, Україна, 61016*

E-mail: yampi@ukr.net

Проблема лікування аутоімунних захворювань (АІЗ), зокрема, ревматоїдного артриту (РА) є однією з актуальніших у біомедицині на протязі багатьох років. Важливою складовою патогенеза РА є дисрегуляція функції імунної системи, з порушенням толерантності до власних антигенів і розвитком аутоімунних реакцій. Відомо, що значима роль у пригніченні аутоімунної відповіді належить регуляторним Т-клітинам (Treg), вміст і функція яких знижена при РА. Перспективним методом корекції таких порушень є індукція антиген-специфічної толерантності, у формуванні якої важливе місце займають толерогенні дендритні клітини (толДК).

Метою дослідження було вивчити вплив толДК на стан Т-регуляторної ланки імунітету у мишей з ад'ювантним артритом на різних етапах його розвитку

Експерименти виконували на мишах лінії СВА/Н масою 20-24 г. Толерогенні ДК отримували з моноклеарних клітин (МНК) кісткового мозку при культивуванні у середовищі RPMI-1640 з 10% ембріональною телячою сироваткою, ГМ-КСФ, ІЛ-4 та дексаметозоном. Ад'ювантний артрит (АА) індукували у мишей субплантарним введенням повного ад'юванта Фрейнда. Оцінку інтенсивності запального процесу здійснювали по динаміці набряку суглоба, що характеризується індексом артриту. Вміст серомукоїда в сироватці крові визначали турбодиметричним методом. Кількість сіалових кислот визначали по методу Гессе й оцінювали в умовних одиницях. Аутологічні толДК, у дозі 5×10^5 кл/мишу вводили внутрішньовенно на 7, 14 і 21 добу розвитку АА. У якості контролів тваринам з АА вводили дексаметазон (КРКА, Словенія) 0,022 мг/мишу й МНК 5×10^5 кл/мишу. Через 7 діб після введення толДК визначали клінічні показники та вміст Treg, які ідентифікували у селезінках тварин за допомогою моноклональних антимишачих антитіл: CD4, CD25, FOXP3 («BD», США).

Встановлено, що рівень терапевтичної ефективності толДК визначався строком їх введення. У гострий період розвитку артриту (7-а доба) терапія ДК виявилася не ефективною. Найбільший імунокоригуючий ефект проявляли толДК введені на 14-у добу розвитку патології, що сприяло підвищенню кількості Treg-клітин ($CD4^+CD25^+FOXP3^+$) і нормалізації клінічного статусу тварин (показників сіалових кислот, серомукоїдів та індекса артрита). Даний факт є підтвердженням можливості використання толДК, отриманих *ex vivo* для лікування АА.

ДОСЛІДЖЕННЯ МЕТАБОЛІЧНОЇ АКТИВНОСТІ ФІБРОБЛАСТІВ ПІСЛЯ 2D ТА 3D КУЛЬТИВУВАННЯ

Моїсєєв А.І., Божок Г.А.

Інститут проблем кріобіології й кріомедицини НАН України,

вул. Переяславська, 23, м. Харків, Україна, 61016

E-mail: bozhokgaru@gmail.com

Відомо, що альтернативною моношаровій культурі в якості інформативних модельних систем можуть бути мультиклітинні сфероїди (МС), в яких умови функціонування фібробластів максимально наближені до умов *in vivo*. Тому дослідження безпосередньо впливу об'ємного культивування клітин на їхні метаболічні показники у порівнянні з моношаровою культурою, є актуальним завданням.

Дослідження виконані на лінії клітин L929, отриманої після 4 пасажів з кріоконсервованої культури, що зберігалася при температурі -196°C . Клітини культивували в живильному середовищі DMEM/F12 з додаванням антибіотиків (200 Од/мл бензилпеніциліну, 200 мкг/мл стрептоміцину та 10% фетальної телячої сироватки) при 37°C в атмосфері з 5% CO_2 . Мультиклітинні сфероїди (МС) отримували на чашках Петрі з площею поверхні росту $22,1\text{ см}^2$, які попередньо обробляли 2% розчином агару для утворення низькоадгезивної поверхні. Посівна концентрація фібробластів для моношарової та об'ємної культури становила 2×10^5 кл/мл. Культивування проводили протягом 7 діб, на 3-ю добу здійснювали заміну середовища. Спектр низькомолекулярних речовин білково-пептидної природи, що містяться в зразках середовищ після 7 діб культивування, оцінювали за допомогою рідинної гель-хроматографії. У якості контролю використовували – живильне середовище на основі DMEM/F12 та 10% фетальної телячої сироватки

Встановлено, що хроматографічні профілі усіх середовищ (контрольного і після 2D і 3D культивування) мали по 10-11 основних піків в діапазоні молекулярних мас (498 – 12000 Да). Пептидний склад контрольного середовища значно відрізнявся від культивування клітин в 2D і 3D умовах за кількісним і якісним складом пептидів з молекулярними масами (5657-5691 Да); (3184 Да); (1045 – 1067) та (490-498 Да).

Дослідження щодо кількісного і якісного складу середовищ у залежності від умов культивування встановили відмінності пептидного складу середовищ у діапазоні молекулярних мас (4345-3791 Да), (1600 Да); (705-718 Да); (490-498 Да). Доведено, що в умовах 3D культивування кількість пептидів з молекулярною масою (1067-1045 Да) та (705-718 Да) збільшувалась в 4 і 7,2 рази відповідно відносно даних показників після моношарового культивування. Необхідно також відзначити той факт, що після культивування фібробластів в умовах 3D встановлена поява нових піків у діапазоні молекулярною масою 1600 Да, які не були визначені в контролі і після 2D культивування. Для решти піків після порівняльного аналізу встановлено характерне зменшення вмісту речовин після 7-добового культивування фібробластів як в 2D, так і в 3D форматі відносно показників контрольного середовища.

Методом рідинної гель-хроматографії в проведених дослідженнях були встановлені відмінності кількісного і якісного складу речовин білково-пептидної природи в середовищах моношарового і тривимірного культивування фібробластів. Особливу увагу привертають результати щодо розбіжності за кількістю пептидів у залежності від умов культивуванні в діапазоні молекулярних мас (705-1067 Да), що може бути наслідком активного синтезу біологічно активних речовин, зокрема ростових факторів. Таким чином, результати роботи свідчать про суттєві відмінності синтетичних процесів клітин у залежності від умов культивування і частково пояснювати перевагу щодо функціонального потенціалу клітин після об'ємного відносно з моношарової культури фібробластів, в проведених нами раніше дослідженнях. Однак, для поглиблення розуміння щодо метаболічних особливостей фібробластів після об'ємного культивування необхідні подальші дослідження цього питання, зокрема з'ясування речовин у молекулярному діапазоні мас (705 – 1607 Да).

АНТИАПОПТОТИЧНА ДІЯ НЕЙРОПЕПТИДІВ В УМОВАХ ХОЛОДОВОГО СТРЕСУ *IN VIVO* ТА *IN VITRO*

Олександр Гулевський, Наталія Моїсеєва, Ольга Горіна, Іван Щенявський

Відділ холодової адаптації, Інститут проблем кріобіології і кріомедицини НАН України, вул. Переяславська 23, м. Харків, Україна, 61016

E-mail: profgulevskyy@gmail.com

На сьогоднішній день з літературних джерел відомо ряд біологічно активних сполук, а саме – пептидів, які проявляють антиапоптотичну дію, в тому числі і в умовах холодового стресу *in vivo* та *in vitro*.

В нашому дослідженні були використані опіоїдні пептиди: лей-енкефалін, кіоторфін та неокіоторфін, які синтезуються в головному мозку зимосплячих тварин і забезпечують їх стійкість до холодового стресу. Оскільки одним з механізмів загибелі клітин гомойотермних тканин при холодовому стресі, як *in vivo*, так і *in vitro*, може бути апоптоз, в наших дослідженнях було вивчено вплив вищевказаних речовин опіоїдної природи на розвиток апоптотичних процесів в тканинах експериментальних тварин (печінка, нирки, селезінка, слизова оболонка кишечника) та в лейкоцитах. Розвиток апоптозу в тканинах після холодового стресу *in vivo*, який відтворювався шляхом охолодження тіла лабораторних щурів до 20 °С та наступного саморозігріву до фізіологічного рівня, оцінювали за ступенем фрагментації ДНК, а в лейкоцитах – методом флуоресцентної мікроскопії з використанням забарвлення флуорохромами акридиновим помаранчевим та етидіум бромідом. Антиапоптотичну дію нейропептидів в умовах холодового стресу *in vitro* вивчали на лейкоцитах донорської крові за морфологічними показниками клітин та з допомогою їх забарвлення флуоресцентними барвниками: Hoechst 33342 і пропідіум йодид (PI). Холодовий стрес викликали послідовною інкубацією проб в різних температурних умовах: 15 хв при 37 °С, потім 15 хв на льодовій бані, потім знову 15 хв при 37 °С.

Було встановлено, що введення експериментальним тваринам перед холодовим стресом синтетичного аналогу лей-енкефаліну (препарату «Даларгін») в дозі 100 мкг/кг ваги тварини вірогідно попереджувало фрагментацію ДНК в клітинах селезінки та слизової кишечника. Введення антагоніста опіатних рецепторів препарату «Налоксон» (500 мкг/кг ваги тварини) запобігало антиапоптотичній дії препарату «Даларгін». Подібний ефект при холодовому стресі *in vivo* у відношенні лейкоцитів продемонстрували нейропептиди кіоторфін та неокіоторфін в тому ж дозуванні.

В експериментах *in vitro* було встановлено, що додавання в середовище лей-енкефаліну у концентрації 10⁻⁹ Моль/л сприяло вірогідному зменшенню відсотка клітин з морфологічними ознаками апоптоза (пікноз ядра, фрагментація ядра, блебінг) у порівнянні з контролем. При використанні іншого нейропептиду – кіоторфіну кількість клітин з морфологічними ознаками апоптозу і некрозу вірогідно зменшувалась при його концентраціях в інкубаційному середовищі: 10⁻⁷Моль/л–10⁻⁸Моль/л–10⁻⁹Моль/л, з найбільшим впливом на відсоток апоптотичних клітин в концентрації 10⁻⁹Моль/л. Ідентична тенденція була встановлена і з застосуванням флуоресцентних ДНК-барвників Hoechst 33342 та PI.

Таким чином, в результаті проведених досліджень можна прийти до висновку, що попереднє введення в організм експериментальних тварин або в середовище інкубації лейкоцитів лей-енкефаліну або нейропептиду кіоторфіну зменшує прояв апоптозу клітин. Даний ефект є результатом взаємодії вказаних нейропептидів з опіатними рецепторами, про що свідчить інгібування антиапоптотичної дії лей-енкефаліну «Даларгін» та кіоторфіну антагоністом опіатних рецепторів препаратом «Налоксон».

ВПЛИВ ГІПОТЕРМІЧНОЇ ІНКУБАЦІЇ В КОНСЕРВУЮЧИХ РОЗЧИНАХ НА ЕКСПРЕСІЮ ЕПІТОПУ GAL- α -1,3-GAL У ТКАНИНІ АОРТИ НЕОНАТАЛЬНИХ ПОРОСЯТ

Богуславський К.І., Алабедалькарім Н.М.

*Інститут проблем кріобіології і кріомедицини НАН України,
вул. Переяславська, 23, м. Харків, Україна, 61016*

E-mail: alkarimru@gmail.com

Епітоп Gal- α -1,3-Gal (α -Gal) є галактозним залишком, який входить до складу глікокаліксу клітинних мембран. Біосинтез цієї структури поширений у тваринному світі, однак є відсутнім в організмі людини. Висока експресія α -Gal- епітопу приводить до гіпергострого відторгнення тканин свині при ксенотрансплантації. У зв'язку з цим виготовлення біопротезів на основі тканин тваринного походження потребує видалення α -Gal-епітопу. На сьогоднішній час існують технологічні прийоми елімінації епітопу з біологічної тканини, однак всі вони мають свої недоліки, тому актуальним є розробка нових способів зниження експресії α -Gal-епітопу.

Мета роботи – вивчення експресії α -Gal-епітопу після інкубації тканини аорти неонатальних поросят у розчинах, які використовуються у клінічній практиці для гіпотермічного зберігання (ГЗ) органів при трансплантації, а саме – розчинів НТК (Custodiol) та Коллінза в модифікації (Євро-Коллінз).

Фрагменти аорти (ФА) поміщали на 2, 7 та 24 години при 4°C в розчини НТК або Євро-Коллінз. Після цього ФА фіксували та піддавали стандартній методиці приготування кріостатних зрізів та забарвлення за протоколом прямого іммунофлуоресцентного мічення з FITC-кон'югованим ізолектином BSI-B4, який специфічно зв'язується з α -Gal-епітопом. Ядра клітин у тканині контрастували Hoechst 33342. В якості контролю використовували нативні ФА. Флуоресценцію аналізували на мікроскопі Carl Zeiss Axio Observer Z1. Аналіз фотографій здійснювали за допомогою програм для обробки зображень Zeiss LSM та Photoshop. Для аналізу відбирали мікрофотографії, що були отримані за однакового збільшення, інтенсивності збуджувального лазера, розміру пінхолу. Відносну інтенсивність флуоресценції зразків розраховували після перетворення мікрофотографій, що були отримані під час запису в каналі зеленої флуоресценції, на бітову карту (bitmap-аналіз). Вимірювали середньозважений рівень яскравості зображення і нормалізували його щодо яскравості фону, який брали за нульову точку. Показник інтегральної щільності, визначений за допомогою Hoechst 33342, використовували як показник кількості ядер клітин у тканині аорти.

Нативні ФА характеризувалися стійким міченням з ізолектином BSI-B4. Морфометричний аналіз дозволив встановити, що відносна інтенсивність флуоресценції значуще не змінюється при гіпотермічній інкубації ФА в розчині НТК на всіх випробуваних термінах. На відміну від цього, в розчині Євро-Коллінз вже після 2 ч ГЗ спостерігається зменшення інтенсивності ФА на 50% в порівнянні з нативними зразками. При подальшій інкубації даний показник знижується на 70 %.

Таким чином, нами встановлено зменшення експресії α -Gal-епітопу в тканині аорти неонатальних поросят після ГЗ в розчині Євро-Коллінз, що в подальшому дає можливість розробки протоколів перфузії і короткострокового зберігання органів тваринного походження для використання їх з метою виготовлення біопротезів

Index of authors

6th Ukrainian Congress for Cell Biology with international representation

|

***June 18-21, 2019,
Yaremche***

6th Ukrainian Congress for Cell Biology with international representation

- Abrahamovych M. 14
Abrahamovych O. 14
Andreev I. 136
Andreieva Y. 83
Andrushyshyna I. 45, 118
Antonevich N. 157
Babicheva V. 50
Babiychuk L. 163, 164
Babrukevich D. 157
Bahniuk O. 67
Barabasz W. 130
Batyuk L. 104, 105, 108,121
Bednarska S. 38
Bednarzak M. 78
Bekere L. 35
Bentrad V. 120
Berest V. 104
Berezhnoy A. 27
Berezka K. 75
Berger W. 91
Beschasnyi S. 26
Bezdeniezhnykh N. 95
Biliavska L. 47, 149
Bilonozhko Yu. 136
Bilyavska N. 140
Bisenieks E. 35
Blashkiv T. 17
Blume Y. 8, 128, 132, 136, 137
Bobak Y. 101, 106
Bondarenko M. 108
Borbuliak M. 75
Borikun T. 109, 111
Boyeva S. 46
Bratiichuk D. 85
Brieieva O. 110
Broda D. 133
Brokowska J. 6
Brykov V. 127
Buchynska L. 110
Bulbotka N. 74, 76
Buriak I. 161
Burlaka A. 114
Buziashvili A. 125
Chabanenko O. 42
Chaka O. 34
Chekhun V. 109, 111, 112, 118
Chen O. 107
Chyzhevskiy V. 160, 161
Chornyi S. 101
Chovpan H. 105
Chrzanowski G. 133
Cysewski D. 9
Cyske Z. 18, 19
Dankevych L. 144
Daugelavičius R. 35, 36, 37, 82, 88
Demchuk O. 8, 137
Demkiv O. 70, 72
Deryabina O. 156
Dijke P. 12
Dmytruk K. 13, 63, 64, 65, 66, 73, 74, 75, 76,
79, 81, 82, 83, 84, 85
Dmytruk L. 80
Dmytruk O. 10, 74, 76
Domina E. 113
Drobot L. 11, 20, 102
Druzhina M. 113
Dubrovska A. 107
Duburs G. 35
Dulak J. 9
Duzh A. 99
Dzanaieva L. 13, 81
Dziedzic A. 71
Dzugan M. 38
Fafula R. 22, 44
Falko O. 160, 161
Fayura L. 73, 80
Fayura O. 14
Fediuk O. 140
Fedorovych D. 64, 73, 80
Fickers P. 7, 71
Filonenko S. 53
Finiuk N. 67
Gaffke L. 6, 18, 19
Galalytė D. 37
Ganusevich I. 114
Gayda G. 70, 72
Gerashchenko D. 102
German O. 145
Glavin O. 113
Glushchenko N. 110

6th Ukrainian Congress for Cell Biology with international representation

- Gnatyshyna L. 28, 32, 33
Gogol S. 115, 120
Goloiad M. 162
Goltsev A. 158
Golub I. 45
Gonchar M. 62, 68, 70, 72
Gordiienko I. 119
Grabek-Lejko D. 38, 39
Granovski V. 69
Grisha I. 158
Grochot-Przeczek A. 9
Gromyko O. 40
Gudkova O. 20
Hancharou A. 99, 157
Hasiuk O. 26
Havva E. 143
Heffeter P. 91
Horak I. 20, 102
Horiunova I. 128, 134, 135
Horyn O. 32, 33
Hrynychak N. 29
Hryniv O. 75
Hudenko N. 103
Hudz N. 92
Hurmach V. 91
Iefremova U. 44
Iurchenko N. 110
Iutynska G. 48, 149
Ivanivskaya T. 119
Ivash M. 70
Jozkowicz A. 9
Kalafat L. 136
Kalashnyk O. 16
Kaleynykova O. 17
Kalme Z. 35
Kanuka A. 30
Kapusta I. 38
Karatsai O. 94
Karpets Yu. 126
Karpov P. 8, 137
Karvatskiy I. 17
Kashchak N. 91
Kashuba O. 116, 117
Kata I. 66
Kavetsky T. 68
Kavok N. 53
Kharchenko M. 148
Kharchenko T. 52
Khoma V. 28, 32, 33
Khroustalyova G. 77
Khudiakova O. 20, 102
Khyzhnyak S. 49
Kit Yu. 14
Kizilova N. 105
Klenov O. 120
Klochkov V. 53
Kloska D. 9
Kluz M. 39
Klymenko O. 127
Klyuchivska O. 67, 70
Knigavko V. 108, 121
Kolupaev Yu. 126, 143
Kopacz A. 9
Korchynskyi O. 12
Kordium V. 156
Kordyum E. 141
Kosterin S. 15
Kot K. 51, 52, 53
Kot Yu. 51, 52, 53
Kots S. 138
Koval L. 156
Kovalenko I. 30
Kovalevska L. 116, 117
Kozak T. 95
Kozak Y. 98
Krasnova L. 35
Kravets O. 43, 128
Kruk B. 13, 79
Kuliešienė N. 35, 37
Kunakh V. 139
Kunska L. 118
Kunz-Schughart L. 107
Kurlishchuk Y. 106
Kurylenko O. 63, 65, 79, 82, 85
Kus-Liškiewicz M. 71
Kutsyaba V. 72
Kuznetsov K. 27
Kyryk V. 156
Kyzym P. 27
Lagutina O. 92

6th Ukrainian Congress for Cell Biology with international representation

- Lapikova-Bryhinska T. 50
Latyshko N. 20
Legostaeva O. 145
Lehmann L. 107
Leonova N. 144
Levashov M. 34
Linder T. 75
Lipina O. 160
Loboda M. 149
Lootsik M. 40
Lories R. 12
Lozovska Y. 118
Lugova G. 143
Lukan R. 51, 52
Lukavetsky N. 14
Lukianova N. 109, 111, 112, 118
Lutsenko O. 158
Luyten F. 12
Lykhmus O. 16, 156
Lykhova O. 95
Makashova O. 164
Makovetska L. 113
Malyukin Yu. 53
Mamenko T. 138
Manig F. 107
Manko N. 40
Martinyuk V. 33
Marx H. 80
Maslenny V. 103
Mattanovich D. 80
Melnik N. 45
Meskalo O. 22
Midyk S. 49
Migunova R. 163
Mikhailenko V. 113
Mishchuk N. 33
Mitina N. 67
Moiseenok A. 98
Morozova O. 121
Motyka O. 73
Naleskina L. 118
Navrotska D. 139
Nedukha O. 141
Nesina I. 110
Nielsen J. 13
Nikolaev V. 103
Onufrovyeh O. 44
Onyshchenko G. 27
Orlova N. 42
Orlovskiy O. 120
Ostankov M. 158
Ostankova L. 158
Ozheredov S. 8, 137
Paiuk O. 67
Panchuk R. 91, 98
Pankivska Y. 47
Passoth V. 75
Pavliukh K. 64
Paziuk L. 103
Persky Ye. 51, 52, 53
Personnic N. 9
Petrovska Y. 84
Piechota-Polanczyk A. 9
Pierzynowska K. 6, 18, 19
Pikulicka A. 130
Pirko Ya. 136
Piskun R. 29
Płoch D. 71
Plokhovska S. 128, 132, 135
Podbielska M. 133
Podlacha M. 6, 18, 19
Polonska A. 51
Ponomarenko N. 121
Portnichenko V. 50
Portnychenko A. 50
Postovoitova A. 136
Povnitsa O. 47
Prokopik N. 148
Prokopiv T. 70
Prylutskyi Yu. 91
Puke M. 77
Pukhtajevich P. 138
Pykalo S. 148
Rabokon A. 136
Raksha-Slusareva O. 46
Rapoport A. 25, 77
Rarok Y. 32
Rayevsky A. 8, 137
Rędowicz M. 94
Ribak M. 49

6th Ukrainian Congress for Cell Biology with international representation

- Rohr J. 91
Rozenfelde L. 77
Rozhyna A. 30
Ruchala J. 13, 63, 64, 65, 73, 78, 79, 80
Rudny E. 39
Rukin A. 121
Rynda A. 157
Sakalauskaite S. 36
Samofalova D. 137
Sarnatskaya V. 103
Saulite L. 77
Savchuk V. 17
Semenovich D. 98
Semkiv M. 10, 66, 75, 76
Serkiz R. 70
Shandrenko S. 20
Shcherbina V. 119
Shchus A. 27
Shckorbatov Y. 27, 30
Shevchenko G. 127
Shevchuk T. 29
Shishkina N. 53
Shkarupa V. 29
Shklyarevskiy M. 126
Shkrabak O. 15
Shkuropat A. 26
Shlapatska L. 119
Shpakova N. 42
Shundel T. 46
Shuvalova N. 156
Shuvayeva G. 106
Shvydenko M. 125
Shysha E. 149
Shytikov D. 102
Sibirny A. 10, 13, 63, 64, 65, 66, 73, 74, 75, 76, 78, 79, 80, 81, 82, 83, 84, 85
Skaterna T. 102
Sklyarenko L. 115, 120
Skok M. 5, 16, 156
Skorokhyd N. 91, 98
Slusarev O. 46
Smutok O. 68
Sokil L. 158
Sorour N. 107
Souchelnytskyi S. 14
Spivak S. 8, 137
Springe G. 28, 33
Sribna V. 17
Starykovich M. 14
Stasyk O. 90, 94, 101, 106, 107
Stasyuk N. 70
Stoika R. 14, 40, 67, 89, 91, 98
Stoliar O. 28, 32, 33
Strona V. 159
Stupchuk M. 17, 21
Svydenko L. 147
Svyshch I. 73
Swacha S. 38
Szpyrka E. 133
Tahar I. 71
Tarasova I. 46
Theron C. 7
Tistechok S. 40
Tkachuk N. 43
Todor I. 118
Tomczyk M. 38
Trykhlub V. 46
Tsaryk L. 33
Tsygankova V. 149
Tsyruynyk A. 64, 73, 80
Tytova L. 48
Uspenska K. 16, 156
Ustyomenko A. 156
Utytskykh T. 121
Vaitkienė S. 35
Vandermies M. 7
Vasylenko M. 50
Vasylyshyn R. 65, 82
Vedernikov N. 77
Veklich T. 15
Virko S. 114
Voitsitskiy V. 49
Vorobets N. 146, 147
Vorobets Z. 22, 44
Vovk A. 114
Vovk O. 106
Voytenko L. 156
Voznesenskaya T. 17, 21
Vozniuk S. 48
Vydasov N. 95

6th Ukrainian Congress for Cell Biology with international representation

- Vysekantsev I. 161
Wegrzyn G. 6, 18, 19
Wrona A. 39
Wu Si. 52
Yalovenko T. 109
Yampolskaya Ye. 158
Yanish Y. 115
Yanko R. 34
Yastreb T. 143
Yavorska H. 147
Yavorska N. 146
Yehemberdinov Ye. 51
Yemets A. 8, 125, 128, 132, 134, 137
Yershova N. 42
Yurchenko T. 148
Yusko L. 103
Zadvornyi T. 112
Zagorodnya S. 47
Zaichenko A. 67
Zakalska O. 72
Zakalskiy A. 72
Zala D. 77
Zaletok S. 115, 120
Zaytseva O. 108
Zazulya A. 66, 76
Zelena L. 43
Zemlianskykh N. 163
Zhemyoda A. 138
Zubov P. 164
Zubova O. 164
Ажгибесов К. 166
Алабедацькарім Н. 174
Алі С. 165
Андрєєв І. 150
Антонєвіч Н. 100
Антонюк В. 131
Бабенко Н. 171
Барілка В. 122
Бездєнєжних Н. 96, 97
Блюм Я. 155
Богуславський К. 174
Божок Г. 31, 165, 172
Бондаренко Т. 31, 54, 59
Бондарович М. 168, 170, 171
Борис Ю. 55
Боцул О. 24
Броннікова Л. 152
Варяниця В. 58
Видасов Н. 96, 97
Воробець М. 55
Гаєвська Ю. 167, 171
Гарматіна О. 23
Глоба В. 54
Гольцев А. 170, 171
Гольцев К. 166
Гончаров А. 100
Горіна О. 173
Гриша І. 167
Гулевський О. 173
Дерев'янка С.В. 61а
Дерябіна О. 24
Дубрава Т. 169, 171
Дьоміна Е. 121
Ємець А. 155
Єршова Н. 61
Жуйкова А. 56
Завелєвич М. 122, 124
Зотова О. 123
Кишинєць Н. 86, 93
Кіт Ю. 123
Клименко С. 129
Коба Л. 56
Коваленко І. 57
Козак Т. 96, 97
Комісаренко А. 151
Конвалюк І. 142
Кордюм В. 24
Корнійчук О. 57
Корчинська О. 57
Криворучко І. 166
Кунах В. 142, 150
Курчій В. 151
Лебединець В. 169
Лебединець Д. 169
Левашова В. 87
Легач Є. 54, 59
Леонова Л. 168
Лихова О. 96, 97, 124
Логінський В. 123
Лук'янова Н. 124

6th Ukrainian Congress for Cell Biology with international representation

Лупан В. 96	Романовська С. 100
Луценко О. 166, 167	Сергєєва Л. 152
Луцик М. 55	Семіонова К. 56
Маммадов Л. 24	Слюсарев О. 60
Меркулова Ю. 86	Стецишин В. 167
Мироновський С. 123	Стойка Р. 123
Михальська С. 151	Тарасова І. 60
Можилевська Л. 142	Твардовська М. 150
Моїсєєв А. 172	Тимохіна О. 100
Моїсєєва Н. 173	Тимченко О. 86
Недуха О. 154	Топорова О. 24
Ніпот О. 56	Точиловський А. 24
Новікова О. 31, 41, 58	Фільченков О. 122, 124
Овруцька І. 153	Фіщенко В. 24
Овчаренко Ю. 153	Фіщенко О. 24
Онуфрович О. 57	Чабаненко О. 61
Орлова Н. 61	Челомбитько О. 170
Останков М. 168, 169	Чехун В. 96, 97, 124
Останкова Л. 167, 168, 170	Шадріна Р. 155
Панчак Л. 131	Шалай О. 123
Пархоменко К. 166	Шапкіна О. 56
Піскун Р. 129	Шевченко Г. 153
Побеленська Л. 59	Шевченко О. 166
Побеленський К. 59	Шкарупа В. 129
Побеленський О. 59	Шпакова Н. 61
Портниченко А. 23	Шульга М. 24
Похоленко Я. 24	Щенявський І. 173
Прохоров О. 100	Ямпольська Є. 168
Ракша-Слюсарєва О. 60	Ямпольська К. 171
Решотько Л.М. 61а	Яремін С. 24

Підписано до друку 12.06.2019 р.

Формат 60x84/8 Папір офсетний.

Гарнітура Times.

Ум. друк. арк. 23.33

Наклад 140 примірників

Друк ФОП Стадник С.О.

79034, Україна, м. Львів, вул. Навроцького, 69,

тел. (38-032) 247-99-82,

Свідоцтво держреєстру:

серія В02, №967439 від 21.09.2009 р.