JOINT MEETING
OF THE 25th ANNUAL CONFERENCE
“MODERN ASPECTS OF BIOCHEMISTRY
AND BIOTECHNOLOGY”
&
2nd CONFERENCE FOR YOUNG SCIENTISTS
OF THE DIVISION OF BIOCHEMISTRY, PHYSIOLOGY
AND MOLECULAR BIOLOGY NATIONAL ACADEMY
OF SCIENCES OF UKRAINE
6-9 June 2017, Kyiv, Ukraine

Sections:
1. Biomaterials
2. Bioactive Compounds
3. Carcinogenesis
4. Gene Expression
5. Metabolites and Correction of Metabolic Processes
6. Proteomics and Protein Functions
7. Molecular Basis of Physiological Functions
Lactate dehydrogenase (LDH: EC 1.1.1.27) is the most clinically important dehydrogenase of those occurring in human serum. The application of serum LDH is relevant in the diagnosis of myocardial infarction (late detection), hemolytic anemia and prognostic serum biomarker and independent predictive factor of median survival in patients with oncology.

Lactate dehydrogenase catalyzes the reversible reaction: L-lactate + NAD$^+$ $\leftrightarrow$ pyruvate + NADH.

New electrochemical assay for LDH detection in the drop (50 µL) based on of screen-printed carbon modified electrode for NADH determination is proposed. Portable electrochemical sensors can be a good alternative to the other methods for LDH determination due to low cost, simplicity of operation and providing rapid screening for early cancer diagnostics.

This study included the development of high-performance nanocomposite sensor for the NADH determination and optimization of conditions, method and procedure for LDH determining using the obtained chip. Carbon screen-printed electrode (SPE) (DropSens), poly (allylamine hydrochloride) (PAH), NAD$^+$ and NADH, L-Lactic Dehydrogenase (Sigma-Aldrich), L-lactic acid (AppliChem) was used in this investigation. Activity of LDH samples was controlled by spectrophotometry. The amperometry, cyclic voltammetry (CV) (DropSens 400), chronoamperometry and chronocoulometry were applied for investigation of the sensor electrochemical properties.

As was found the modification of carbon electrode surface by nanocomposite film of DND with PAH resulted in the shift of the optimal value of applied potential close to 0.45 V, and improved the sensitivity of NADH determination three times.

Optimal conditions for the LDH assay were estimated as follow: 0.1 M phosphate buffer solution, pH 7.5, with NAD$^+$ (10 mM) and L-lactate (80 mM), sample pre-incubation (5 min) at 37 °C. Principal possibility of LDH activity detection in the linear range 1-25 U/L with sensitivity 2.98 µA/U/L was shown by CV method.
Neurodegenerative diseases and amyloidoses are connected with the formation and accumulation of beta-pleated protein aggregates (amyloid fibrils) in different tissues. Fluorescent probes are among the convenient tools for the detection and study of biological macromolecules, particularly proteins and their aggregates. In the case of low concentration of biomolecules, detection sensitivity provided by the probes could be increased by metal enhancement of the dye fluorescence.

Previously we proposed trimethine cyanine dyes as amyloid-sensitive probes for fibrillar aggregates detection. Such amyloid-specific dyes are considered to bind with a fibril via placing into the row formed by beta-pleats of polypeptide chains.

Here we study the efficiency of metal enhancement of the fluorescence intensity for the cyanine dyes complexed with fibrils. For this, the dye and dye-fibril complex were deposited on the polyvinylpyrrolidone (PVP)-covered silver-island films (SIF) on glass. We explored the trimethine cyanine dye D-51 with N-sulfoalkyl substituent giving high fluorescent response in aqueous solution upon binding both to insulin and lysozyme fibrils (up to 70 times).

In order to estimate the level of metal enhancement of the dye fluorescence intensity, we compared intensities of the dye deposited on PVP-covered glass and PVP-covered SIF. The shape of the fluorescence emission spectra of the dye on PVP and SIF-PVP remains the same as in aqueous solution with maximum near 576 nm that corresponds to dye monomers (so dye molecules does not aggregate on surface). Fluorescence intensity of the free dye deposited on Ag-covered surface was demonstrated to be 5.2 times higher as compared to the glass surface without SIF. As for the dye complexed with insulin fibril, the 6-fold metal enhancement of fluorescence was shown, while in the case of fibrillar lysozyme this enhancement was estimated to be little bit lower (about 3.5 times).

Thus deposition of the free dye and its complexes with fibrils on PVP-covered SIF results in the metal surface enhancement of D-51 dye fluorescence intensity and could be used to enhance the signal at low concentrations range. Besides, the origin of the fibrils could affect the intensity of this enhancement.

This work was supported by TUBITAK Grant #114Z391.
Extracellular Membrane Vesicles (EMV) play a role in intercellular communication, transportation, and protection of microorganisms. Microbial EMVs may be used as scaffolds for smart drug delivery or as vaccines against infectious diseases. It is still unknown whether the size of natural vesicles might be an essential factor that determines how easily they can diffuse in a tissue. The purpose of this study was to know how stress factors affect the size of membrane vesicles in kombucha, known as a probiotic polymicrobial culture.

A wide range of EMV with sizes of 30-1200 nm has been isolated from kombucha culture. Total preparation of extracellular vesicles, including outer membrane vesicles and post-Golgi vesicles showed round-shaped size distributions according to transmission electron microscopy (TEM) records. Size estimation of total vesicleome has been done by DLS approach (Distribution Light Scattering, Malvern Instrumental Ltd); values of found peaks at 180 and 220 nm were consistent with TEM measurements. In kombucha culture grown with anorthosite rock, the range of size distribution of EMVs was more narrow (55-1055 nm) than in the control variant (without anorthosite) (35-1080 nm) with the peak of 160 nm. Derived count rate in both variants showed that the total putative number of membrane vesicles produced by kombucha community members in the presence of anorthosite may exceed control (without anorthosite) 20 times. The 16-fold increased amount of released vesicles has been detected after a low-dose high-speed electron irradiation of the culture. It should be emphasized that the average size of membrane vesicles produced by the stressed microbial cells (both mineralized and irradiated) was reduced. The same tendency was observed in kombucha culture specimens exposed outboard of International Space Station in a 25 month space-flight experiment, the returned samples produced smaller average size vesicles (91 nm) in more narrow range of size distribution (68-105 nm). It may be suggested that physical properties of the EMVs may affect the way they mediate intercellular communication.
**IN SITU SURFACE FUNCTIONALIZATION OF IRON OXIDE MAGNETIC NANOPARTICLES (IONPS) WITH NATURAL AMINO ACIDS: A POTENTIAL CARRIER FOR BIOMEDICAL APPLICATIONS**

*H. KHEIRI MANJILI, A. SHARAFI*

Zanjan University of Medical Sciences, Iran; e-mail: ali.lahiji@yahoo.com

In this work we reported the synthesis of various natural amino acids (AAs) coated iron oxide magnetic nanoparticles (IONPs) under one-pot reaction in an aqueous medium.

Several AAs, which were made up of hydrophilic and hydrophobic groups were selected to study their effects on size, morphology and toxicity of IONPs. Functionalized IONPs were characterized by X-ray diffraction (XRD), differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM) and Transmission electron microscopy (TEM) techniques. Cellular toxicity of IONPs was also investigated on HFF2 and HEK-293 cell lines.

Natural AAs coated IONPs show the possibility of using this nanoparticles in the development of *in vitro* and *in vivo* biomedical fields due to do not possess a toxic effect, good ζ-potential and related small and narrow size distribution.

The results show that so prepared IONPs are biocompatible.
Dysfunctional endothelial cells (vasculature) play a key role in the pathogenesis of a range of human diseases. The endothelium alterations are directly involved in peripheral vascular diseases, stroke, heart diseases, diabetes, chronic kidney failure, tumor growth, venous thrombosis, endothelial dysfunction implicated in tissue injury caused by ischemia-reperfusion, etc. The aim of our study was to assess the expansion rate of endothelial progenitor cells isolated from peripheral blood (PB-EPCs) with the use of two different isolation protocols; to use cultured autologous PB-EPCs for 3D bone tissue engineered equivalent manufacturing for critical size bone defects’ restoration.

Peripheral blood specimens (per 20 ml, heparinized, from 20 donors) were obtained by venipuncture. Specimens were seeded in T75 flasks directly (group 1, n = 10) or via PB-MNCs obtaining and seeding with use of Histopaque-1077 (group 2, n = 10). PB-EPCs were cultured in EGM-2MV medium containing growth factors (Lonza) in multi-gas incubator at 37 °C, saturated humidity, 5% CO₂ and 5% O₂. Colony-forming capacity, phenotype, karyotype and capillary-like structures formation in Matrigel (Corning) have been determined for expanded EPCs cultures.

PB-EPCs cultures were obtained only from 15 donors per 20 (6 in group 1; 9 in group 2). Cultured PB-EPCs possess characteristic morphology and CD31+CD34+CD73+CD105+CD309+D90-CD45-HLA-DR- phenotype. When seeded in Matrigel, all cultures formed capillary-like structures. Colony-forming capacity was higher in Histopaque-1077-processed than in directly seeded blood specimens: 7.8 ± 3.5 (n = 9, group 2) vs 0.6 ± 0.4 (n = 6, group 1) colonies per T75. In group 2 over 24 days 22.6 ± 1.0×10⁶ PB-EPCs were obtained vs 7.0 ± 2.0×10⁶ over 46 days for group 1. The obtained autologous PB-EPCs cultures have been used for pre-vascularization of 3D bone tissue engineered equivalents which have been applied in patients with combat-related limb trauma aimed to restore the critical size bone defects.

Cell isolation method has a significant effect on the efficiency of PB-EPCs growth rate and expansion. For further broad clinical use of PB-EPCs, the development of humanized xeno-free media is necessitating.
ENDOMETRIAL MULTIPOTENT MESENCHYMAL STROMAL CELLS FOR REGENERATIVE MEDICINE APPLICATION: ISOLATION, CULTURING, MORPHOLOGICAL AND FUNCTIONAL PROPERTIES STUDY

A. ZLATSKA1,2, A. RODNICHENKO2, N. TODOSENKO1, O. GUBAR2,4, Y. KOTLIK2, V. SHUPLETSOVA1, Z. KOROP2, O. SEDNEV3, S. NOVIKOVA1, R. VASYLIEV1,2
1State Institute of Genetic and Regenerative Medicine, NAMS of Ukraine, Kyiv;
2Biotechnology laboratory ilaya.regeneration, Medical company ilaya®, Kyiv, Ukraine;
3I.Kant Baltic Federal University, Kaliningrad, Russia;
4Institute of Molecular Biology and Genetic, NAS of Ukraine, Kyiv, Ukraine;
e-mail: alenazlatcka@gmail.com

The endometrium is a unique structure that is able to complete self-renewal over the month cycle, and undergoes these changes over 400 times during women's reproductive life. A significant regenerative potential is due to the presence of stem cells in the endometrium, such as mesenchymal, epithelial and endothelial progenitor cells. Endometrium is a promising object for MSCs isolation for their further use in regenerative medicine and ART. The aim of the study was to isolate, expand and characterize the endometrial MSCs from minimal endometrial biopsy for further endometrium hypoplasia treatment.

The endometrium biopsy \((n = 10)\) was obtained at diagnostic hysteroscopy in the first phase of the menstrual cycle from women with endometrial hypoplasia and previously unsuccessful ART cycles. In all cases, a voluntary written informed consent was obtained from the patients. Endometrial fragments were dissociated by enzymatic treatment for 1 h in 0.05% collagenase IA and 0.05% pronase. The cells were cultivated in DMEM:F12 supplemented with 10% FBS, 2 mM L-glutamine and 1 ng/ml FGF-2 in a multi-gas incubator at 5% CO\(_2\) and 5% O\(_2\). The following assays were done: clonogenic potential (CFU test), phenotype by flow cytometry, karyotype (GTG-banding), directed multilineage differentiation potential and growth factors/cytokines production by Bio-Plex Pro Human 27-plex assay (BioRad).

Primary population of endometrial cells was heterogeneous and contained cells with fibroblast-like and epithelial-like morphology. We used the 3rd passage cells for characterization when the majority of cell population had fibroblast-like morphology. The cells possessed CD49f + CD73 + CD90 + CD105 + CD140a + CD140b + CD146 + CD166 + CD31-CD34-CD45-CD106-CD184-CD227-CD326-HLA-DR-Lgr5-phenotype. They were capable of direct osteogenic, adipogenic and chondrogenic differentiation. The cells showed 35.7±6.2% colony forming efficiency and a tendency to 3D spheroid formation in colonies. The GTG-banding assay confirmed the stability of eMSC karyotype during long-term culturing (up to P10). After 48 h incubation period in serum-free medium eMSC secreted following proteins: cytokines – IL-1ra (74.6 ± 9.5 pg/ml), IL-6 (29.8 ± 8.3 pg/ml), IL-8 (138.5 ± 33.3 pg/ml), IL-10 (9.6 ± 5.5 pg/ml) and IFNγ (55.9 ± 3.8 pg/ml); growth factors – VEGF (92.2±19.8 pg/ml), GM-CSF (132.2 ± 51.1 pg/ml) and FGF-2 (17.8±4.3 pg/ml); chemokines – IP-10 (39.9 ± 3.3 pg/ml) and MCP-1 (41.1 ± 6.7 pg/ml).

Thus, obtained endometrial MSCs meet minimal ISCT criteria for MSCs, such as adherence to plastic in standard culture conditions, expression of typical phenotype markers and ability for the directed differentiation in vitro. They also produce a range of cytokines, chemokines and growth factors, which make them a perspective object for the use in the regenerative medicine application, e.g. endometrium hypoplasia and Asherman’s syndrome treatment.
The interest in traditional medicinal plants study increased dramatically last years. This interest is associated with the new data obtained in scientific investigations of medicinal plants including their antioxidant, anti-inflammatory, antiviral, anticancer activities. The features of medicinal plants can be used for establishment of new medicines for treatment of different diseases.

*Artemisia annua* L. (*Asteraceae*) was the “hero” of 2015-th year Nobel Prize due to Youyou Tu discoveries concerning a novel therapy against malaria. Different compounds known as biologically active ones have been extracted from *A. annua* plants. Sesquiterpenes, flavonoids, coumarins, phenolic compounds were studied in the plants. *A. annua* extracts possess antimicrobial, anti-inflammatory, antioxidant activities etc.

“Hairy” root culture can be obtained via *Agrobacterium rhizogenes*-mediated transformation. It is considered to be an alternative way for producing valuable plant-derived compounds because these transgenic roots have been reported to synthesize large variety of secondary metabolites.

The aim of this work was to study the effect of genetic transformation on flavonoids accumulation and compared to the content of the compounds in some *A. annua* “hairy” root lines.

*A. annua* “hairy” roots were obtained earlier and were cultured on the hormone free half strength Murashige and Skoog basal medium. Plant material was collected after 1 month of cultivation, lyophilized and mashed into powder. The amount of total flavonoids in the 70% ethanol extracts was determined using modified Aluminium chloride colorimetric method. Rutin was used as standard and the results were expressed as rutin equivalents (RE) in milligram per gram of dry weight.

Flavonoids content in *A. annua* “hairy” root lines varied from 27.5±0.53 mg/g RE to 35.6±1.38 mg/g RE. The compounds were accumulated in transgenic roots in amount greater than in roots and leaves of the control plants. So, the genetic transformation has led to the increase of flavonoids content in *A. annua* “hairy” root lines.

Our investigation approved that transgenic roots of *A. annua* obtained via *A. rhizogenes*-mediated transformation can accumulate flavonoids in greater amount than non-transformed plants. The data obtained suggest the possibility of using of *A. annua* “hairy” root culture as a source of flavonoids.

Acknowledgements. Part of publication’s results is based on the research provided by the grant support of the State Fund for Fundamental Research, Ukraine (No 73/83-2016).
PREPARATION, CHARACTERIZATION, PHARMACOKINETIC STUDIES, IN VITRO AND IN VIVO DELIVERY OF ARTEMISININ LOADED PCL-PEG-PCL MICELLES

H. KHEIRI MANJILI, A. SHARAFI, H. DANAFAR

Zanjan Pharmaceutical Biotechnology Research Center, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran; e-mail: ali.lahiji@yahoo.com

Artemisinin (ART) has anti-inflammatory, antimicrobial, antioxidant, anti-amyloid and antitumor effects, but its application is limited due to its low water solubility. To improve the bioavailability and water solubility of artemisinin, we synthesized four series of poly (ε-caprolactone) - poly (ethylene glycol) -poly (ε-caprolactone) (PCL-PEG-PCL) tri-block copolymers.

The structure of the copolymers was characterized by HNMR, FTIR, DSC and GPC techniques. ART was encapsulated inside micelles by a single-step nanoprecipitation method which leading to the formation of ART/PCL-PEG-PCL micelles. The obtained micelles were characterized by dynamic light scattering (DLS) and atomic force microscopy (AFM).

The results showed that the zeta potential of ART/ micelles was about -15.4 mV and its average size was 83.22 nm. ART was encapsulated into PCL-PEG-PCL micelles with a loading capacity of 18.62 ± 0.42% and entrapment efficacy of 89.23 ± 1.41%.

The MTT assay showed that bare PCL-PEG-PCL micelles is non-toxic to MCF7 and 4T1 cancer cell lines whereas the ART/ PCL-PEG-PCL micelles showed a specific toxicity to both cancer cell lines. Pharmacokinetic study in rats revealed that in vivo drug exposure of ART was significantly increased and prolonged by intravenously administering ART-loaded micelles when compared with the same dose of free ART dissolved in acetone. Furthermore, in vivo results demonstrated that this micellar formulation significantly increased drug accumulation in tumors.

The polymeric micellar formulation of ART based on the amphiphilic block copolymer PCL-PEG-PCL could provide a desirable process for ART delivery.
A STUDY OF LIPID METABOLISM REGULATING EFFECT OF POLYPHENOLIC COMPOUNDS ISOLATED FROM GEORGIAN GRAPE SAPERAVI VARIETY ON IN VITRO MODEL OF NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

N. KULIKOVA, T. KACHLISHVILI, M. KSOVRELI,
M. MUSERIDZE, D. GABRUASHVILI, M. BEZHUASHVILI

Agricultural University of Georgia, Tbilisi, Georgia;
e-mail: n.kulikova@agruni.edu.ge

Nonalcoholic fatty liver disease (NAFLD) is one of the most common liver diseases. The over-accumulation of triglycerides in the liver is a hallmark of NAFLD. The pathogenesis of NAFLD is not entirely understood. To date, the only effective treatment of NAFLD is caloric restriction (CR), which is difficult to achieve. Resveratrol – a natural polyphenol, has been shown to mimic the beneficial effect of CR, delaying the onset of a variety of age-related diseases in mammals. Recently, studies in mice found that either CR or resveratrol protected the liver from fat accumulation induced by high fat diet. However, detailed mechanisms mediating resveratrol effects remain unclear. Low grade chronic inflammation, caused by liver-resident macrophages plays an important role in pathogenesis of NAFLD. Engagement of Toll-receptors (especially TLR4) is essential for the initiation of inflammatory process. Therefore, the relationship between metabolic state and low-grade chronic inflammation is important in understanding the progression from steatosis to severe stages of NAFLD. The main aim of our study was to study an effect of different polyphenols – resveratrol, ε-viniferine and trans-piceid extracted from Georgian grape Saperavi variety, on both: lipid metabolism and inflammatory process, utilizing different in vitro models of NAFLD: monolayer (2D) system and three-dimensional system – hepatic spheroids.

To create an in vitro model of NAFLD, in case of 2D system, lipid-overload was induced by adding a mixture of oleic/palmitoleic acids to monolayer culture of either mouse hepatoma cell line – Hepa 1-6, or mouse macrophages cell line – RAW264.7 (as in the fatty liver, liver-resident macrophages are also exposed to FFA-rich environment). For the creation of 3D spheroids, Hepa 1_6 cells have been cultured in 96-well plate, covered with non-adhesive agarose gel and lipid accumulation was triggered as described above. In both 2D and 3D systems, the effect of stimulation with different concentrations of stilbenoids was assessed by flow cytometric measurement of lipid content and ROSes level, also the surface expression of TLR4 has been studied by immunophenotyping.

According to our results, in 2D cultures, a stimulation of lipid-loaded hepatocytes and macrophages with low concentration (10 μM) of both resveratrol and trans-piceid induced a decrease in lipid accumulation and ROSes level. In case of hepatocytes, this effect was accompanied by a decrease in the surface expression of TLR4, but in macrophages the above-mentioned modulation of TLR4 surface expression has not been seen. In 3D cultures a similar effect was detected in case of higher concentrations of polyphenols (30 μM). Based on the obtained data, we hypothesize, that the molecular pathway of lipid-load reduction, caused by polyphenols, depends to some extent on the modulation of TLR4 signaling pathway.
Study of novel [1,10]phenanthroline based cyanine dyes as fluorescent probes for nucleic acids

M. Kuperman1, V. Kovalsk2, D. Kryvorotenko3, E. Kinski4, O. Varzatskii5, S. Yarmoluk1, A. Mokhir2

1Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv;
2Organic Chemistry II, Friedrich-Alexander-University of Erlangen-Nuremberg, Erlangen, Germany;
3Institute of General and Inorganic Chemistry, NAS of Ukraine, Kyiv;
e-mail: mvkuperman@gmail.com

Analysis of nucleic acids (NA) is required for a wide range of biomedical applications. One of the most used approaches for both qualitative and quantitative NA detection is based on dyes, which have initially weak emission in unbound state and strong emission when NA-bound. Cyanine dyes are known as the most effective probes for NA sensing.

Here, a series of monomethine, trimethine and styrylcyanine dyes based on novel [1,10]phenanthroline heterocycle (FT1–FT5) was synthesized and characterized as potential fluorescent probes for nucleic acids detection. The spectral properties of these dyes both in absence and in presence of dsDNA/RNA were studied by fluorescent and absorption spectroscopy. The cell staining experiments were done on HL-60 cell line using flow cytometry and fluorescence microscopy.

The [1,10]phenanthroline based cyanine dyes are weakly fluorescent (0.5-17 a.u.u) in unbound state. Their excitation/emission maxima are in the ranges of 481-633 and 555-651 nm, respectively. The trimethine dyes FT4 and FT5 have comparably small Stoke shifts (11 and 18 nm, respectively), while for monomethine FT1 dye it is large (93 nm). Upon the binding to dsDNA/RNA their emission intensity rises up to 51 times (for monomethine benzothiazole derivative FT1 with RNA), excitation/emission bands shift (up to 51 nm). The strongest fluorescence intensity in complexes with dsDNA and RNA was observed for the trimethine benzothiazole derivative FT4. Quantum yield value of FT4 in complex with dsDNA is 1.5%, the binding constant is estimated as $K_b = 7.9 \times 10^4 \text{M}^{-1}$ that is typical value of intercalating molecules. The trimethine dyes FT3 and FT4 demonstrate strong proneness to aggregation in aqueous buffer. It was shown, that the dye excess leads to formation of the dye aggregates on DNA surface. The applicability of red-emitted FT3 and orange-emitted FT4 dyes for use in flow cytometry as stains for living cells was demonstrated. Fluorescent imaging of HeLa cells by FT3 and FT4 dyes has shown that both dyes are cell permeable but target different components in the cells. FT4 stains RNA rich components – the nucleoli and most probably the cytoplasmic RNA, while FT3 stains cellular organelles located near the nuclei (probably mitochondria or lysosomes), but not the nucleic acids.

Thus, novel [1,10]phenanthroline based cyanine dyes have potency as probes for NA sensing in vitro; their ability to stain components of living cells is demonstrated.
APPLICATION OF DON-1R DRUG IN THE TECHNOLOGY OF LIVE FEED CULTIVATION FOR FISHES

O. KUSHNIRYK¹, O. KHUDYI¹, L. KHUDA¹, M. MARCHENKO², V. NOVIKOV²

¹Yuriy Fedkovych Chernivtsi National University, Chernivtsi, Ukraine; ²Lviv Polytechnic National University, Lviv, Ukraine; e-mail: kushniryk-olga@email.ua

Searching for effective ways to improve the growth rate of the live feeds in aquaculture is a question of present interest. One among such approaches is to use substances with a stimulating effect. In pond aquaculture γ-crotonolactone-containing drug “DON-1R” is frequently used for prevention and treatment of aeromonosis. It was noticed that in addition to the therapeutic effect the drug enhances the level of natural feeds in ponds. In this regard, the possibility of DON-1R use during live feed cultivation was tested on the example of ü Simocephalus vetulus (Müller). The nutrient composition and hydrolytic enzymes activity of cultured organisms in addition to their productivity rate were evaluated as live feed is a source both of nutrients and hydrolytic enzymes for fish larvae.

The cultivation of S. vetulus was performed using yeast Saccharomyces cerevisiae as a feed substrate. Different concentrations of DON-1R have been applied to investigate its influence. The productivity of zooplankton’s culture was defined by specific growth rate (µ/day). The content of total lipids and proteins was determined in cultivated live feed. The total lipase activity was defined by Sklyarov’s unified method. The total proteolytic activity was studied at pH 4.8, 7.4 and 9.0 by Anson’s modified method. Amylase activity was determined by Caraway’s method.

In accordance with obtained results, the highest growth indicators of S. vetulus monoculture were noted during DON-1R application at a concentration of 66.8×10⁻⁶ ml/l, while the increase in the drug concentration to 100.2×10⁻⁶ ml/l in the cultivation medium did not result in significant rise of specific growth rate. In live feeds cultivation an important issue is avoiding the deterioration of their nutritional value during the increase in biomass accumulation rate. It was shown that the total proteins content in S. vetulus from the experimental and control groups did not differ significantly (513 mg/g and 576 mg/g of dry weight, respectively). Instead, crustaceans, cultivated with DON-1R, were characterized by higher total lipid content – 275 mg/g in comparison to 175 mg/g in zooplankton from the control group. Despite the established advantages of DON-1R application, it was found its inhibitory effect on enzymatic hydrolytic activity: lipase activity decreased .6 times and α-amylase – 2.7. The study of total protease activity at neutral (pH 7.4) and alkaline (pH 9.0) pH values showed its decrease 1.5 and 7 times, respectively. At the same time the proteolytic activity at acidic pH values (pH 4.8) decreased by 70 times.

Therefore, the use of DON-1R during zoooplankton cultivation enhances the growth rate in S. vetulus monoculture, while deterioration of the nutrient composition in feed organisms is not observed. However, the use of DON-1R is accompanied by inhibition of hydrolytic activity in the feed zooplankton.
Plasma membrane Ca\(^{2+}\),Mg\(^{2+}\)-ATPase (PMCA) functions as a fine tuner of cellular calcium concentration and plays a pivotal role in the termination of Ca\(^{2+}\) signal in uterine myocytes. Regarding its critical contribution to the maintenance of Ca\(^{2+}\) homeostasis, this enzyme can be an important pharmacological target. It was shown that calix[4]arene C-90 (100 µM) decreased PMCA activity by 75% with no significant influence on the activity of other plasma membrane ATPases. Indeed, other calixarene derivatives can be more effective inhibitors of PMCA, and calix[4]arene C-956 could be one of them.

The effect of different calix[4]arenes (C-715, C-772, C-960, C-716, C-957, C-975, C-90, C-956) on PMCA activity as well as the influence of calix[4]arene C-956 on membrane ATPases (Mg\(^{2+}\)-ATPase, Ca\(^{2+}\)-ATPase, Na\(^{+}\),K\(^{-}\)-ATPase) were tested in plasma membrane fraction isolated from pig’s myometrium. Using confocal microscopy and laser correlation spectrometry, we studied the effect of calix[4]arene C-956 on [Ca\(^{2+}\)]\(_i\) in uterine myocytes loaded with Ca-sensitive probe Fluo-4 AM and its effect on the effective hydrodynamic diameter of myocytes, respectively.

The first our task was to determine the most efficient inhibitor of PMCA activity among above-mentioned calixarenes. Using enzymatic assay, we demonstrated that calix[4]arene C-956 had the most prominent inhibitory effect on specific enzyme activity (I\(_{50}\) = 15.0 ± 0.5 µM, n = 5). In order to test selectivity of calix[4]arene C-956 the specific enzymatic activity of membrane ATPases was determined in the presence of 100 µM calixarene in the incubation medium. The results showed that this calixarene decreased specific plasma membrane Ca\(^{2+}\),Mg\(^{2+}\)-ATPase activity by 79.2% but had no statistically significant influence on other ATPase activities proving selectivity of calix[4]arene C-956 action.

It was also demonstrated that application of 20 µM calix[4]arene C-956 into uterine myocytes caused a temporary increase in intracellular Ca\(^{2+}\) concentration. Interestingly, during 2.5 minutes [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 by 45% that could be due to contraction of myocytes.

Thus, calix[4]arene C-956 is a selective inhibitor of PMCA which is more effective than other calixarenes and therefore is a promising compound for modulation of smooth muscle contractility.
THE EFFECT OF BACTERIOPHAGAL DSRNA ON ARGinine METABOLISM OF RAT MICROGLIAL CELLS EXPOSED TO HYPOXIA IN VITRO

L. OPEIDA¹, G. FELDMANE², K. O. STEPURA¹, M. P. RUDYK¹

¹Educational and Scientific Centre Institute of Biology and Medicine, Taras Shevchenko National University of Kyiv, Ukraine;
²Latvian Biomedical Research and Study Centre, Ratsupites, Latvia;
e-mail: opeida28021992@gmail.com

Larifan is a pharmaceutical product containing a heterogeneous population of natural origin double-stranded RNA (dsRNA). It is an agonist of TLR3. Larifan exhibits an antineoplastic effect by the modulation of functions of immune cells including macrophages. Tumor hypoxia dramatically influences tumor-associated macrophage functions and shifts their metabolism to alternative (M2) phenotype, which is characterized by the decrease of the production of inflammatory mediators including NO. In vitro studies have shown that hypoxic M2 macrophages can be reprogrammed by the use of TLR agonists to promote tumor regression. It is accepted that glioma associated macrophages are characterized by the restricted capacity to shift their metabolic profile in response to polarizing agents. The aim of the work was to investigate the effect of Larifan on arginine metabolism in rat microglial cells (MC) exposed to hypoxia in vitro.

Rat MC were cultured under normoxic (21% O₂) or hypoxic (3% O₂) conditions for 24 h. After this cells were treated with Larifan (at the concentrations of 200 μg/ml) or Larifan+bacterial LPS (50 μg/ml) for 18 h in normoxic and hypoxic conditions, respectively. To characterize arginine metabolism nitrite level was assayed by the Griess reaction, arginase activity was measured in cell lysates by colorimetric method.

The 24 h exposure of MC to hypoxia in vitro led to the 2-fold increase of MC arginase activity along with the decrease of NO generation as compared to normoxic cells. Larifan used alone moderately stimulated arginase activity of both normoxic and hypoxic MC and did not influence their NO production. Used in combination with bacterial LPS Larifan intensified the inhibitory effect of the latter on arginase activity of hypoxic MC. Stimulatory effect of LPS on NO production by hypoxic MC was hampered by Larifan.

Thus, hypoxia shifted arginine metabolism in MC to arginase pathway. Larifan synergized with bacterial LPS to decrease arginase activity in hypoxic MC, but it interfered with the stimulatory effect of LPS on NO generation in these cells.
MAGHEMITE (γ-Fe₂O₃) NANOPARTICLES AS A POTENTIAL SORBENT OF EXTRACELLULAR GLUTAMATE IN SYNAPTIC CLEFT

K. PALIYENKO, T. BORISOVA

Palladin Institute of Biochemistry, NAS of Ukraine; Kyiv;
e-mail: kostyaram@gmail.com

In the mammalian central nervous system, amino acid and its form glutamate play a primary role as a key excitatory neurotransmitter. Changing its extracellular concentration in the synaptic cleft it is possible to control physiological state of brain. Thus, in this work attention was paid to interaction of γ-Fe₂O₃ NPs with glutamic acid on the level of model in similar buffer solution which used for nerve brain terminals isolation and further dimensional analysis of formed aggregates.

Interaction of NPs with glutamate was occurred in distilled water and buffer solution. For sorption determination was used radiolabeled L-[¹⁴C] glutamic acid. Different concentrations of NPs were used for determination concentration dependent correlation. After mixing glutamate solution with NPs, it was centrifuged 15 min at 13,400 rpm. With help of scintillation counter sorption was measured in the aliquots of the supernatants by liquid scintillation counting with scintillation cocktail ACS and was expressed as percentage of total amount of radio-labelled neurotransmitter absorbed.

For the investigation of NPs’ behavior in different media was chosen size change determination. The function of particle’s size distribution was investigated with the help of laser correlation spectrometer ZetaSizer3 Malvern Instrument.

These nanoparticles show quite good ability to glutamate sorption at different concentrations, the average sorption of glutamate by nanosized particles at concentration of 3 mg/ml is 94%.

Mean value of intact NPs’ size is 171 nm. Mean value of NPs’ size is 156 nm. 0.5 nM solution of L-[¹⁴C] glutamic acid didn’t cause aggregation. After addition of NPs solution into media containing different chelating agents, sizes significantly increased. Mean value of NPs’ size is 564 nm.

It has been found during experiment that nanoparticles elicit sorptive properties towards glutamate and in distilled water sorption equals 94% at NPs’ concentration 3 mg/ml. However, it becomes seriously limited in work buffer solution due to the presence of NaH₂PO₄ and to a lesser degree HEPES that are the necessary constituents. Thus, it should be find out more convenient biological medium for synaptosomes storage and NPs sorption ability study in vitro. Dimensional analysis has shown that NPs in standard salt solution form huge aggregates and do not work correctly. Small amounts of glutamate do not cause changing in sizes.
GOLDEN ROOTS PLATFORM: THE IN VITRO GENETICALLY ENGINEERED CULTURES FOR ARTEMISININ PRODUCTION IN ARTEMISA SIEBERI, A. DIFFUSA AND A. BIENIS

A. SHARAFI, H. KHADIJEH BAGHERI, K. MANJILI, H. DANAFAR

Zanjan Pharmaceutical Biotechnology Research Center, School of Pharmacy, Zanjan University of Medical Sciences, Iran;
E-mail: sharafi.a@gmail.com

WHO recommends artemisinin-based combination therapies (ACTs) as the most effective choice to treat malaria. As artemisinin cannot be synthesized chemically in an economically feasible way due to their complex chemical structure, we suggest genetically transformed root cultures as an alternative production system. High stability of the production of secondary metabolites is an interesting characteristic of hairy root cultures.

We used the leaves of one month sterile plants as explants in inoculation medium of Agrobacterium rhizogenes strains A4, ATCC15834, MSU440, and A13 (MAFF-02-10266) for 6 min. The explants were transferred to a modified co-cultivation MS mediums for 48 h. The explants were placed on MS medium supplemented with 400 mg/l cefotaxime. The genomic DNA was extracted from transformed root tissue using CTAB DNA isolation method in order to show the integration of T-DNA of A. rhizogenes in transgenic roots. Molecular analysis of transformed root lines was confirmed by PCR using specific primers of the rolB gene.

The results showed a significant increase in transformation frequency when the strain MSU440 was used. Artemisinin content in genetically transformed root cultures was detected by HPLC analysis.

In the present study, an efficient hairy root induction system for Artemisia sieberi, A. diffusa and A. bienis was developed through Agrobacterium rhizogenes-mediated transformation as an alternative approach for artemisinin production.
DIRECT ORGANOGENESIS AND IN VITRO REGENERATION OF MEDICINAL PLANT PLANTAGO LANCEOLATA

A. SHARAFI, S. RAHAMOUZ, K. BAGHERI, H. DANAFAR

Zanjan Pharmaceutical Biotechnology Research Center, School of Pharmacy, Zanjan University of Medical Sciences, Iran; e-mail: sharafi.a@gmail.com

Plantago lanceolata is a medicinal plant which has valuable secondary metabolites including iridoid glycosides, phenylpropanoid glycosides, flavonoids and phenylcarboxylic acids. The iridoid glucosides Aucubin and Catalpol are the main constituents which have biological activities such as anti-inflammatory (as a specific inhibitor of NF-ƙB) and cytotoxic activity. Efforts in the molecular regulation of secondary metabolites biosynthesis are restricted by the lack of efficient protocols for plant regeneration. In this study, we succeeded in in vitro regeneration of this plant.

Leaf, hypocotyl and root explants excised from in vitro P. lanceolata were cultured on Murashige and Skoog (MS) medium supplemented with various plant growth regulators benzylaminopurine (BA), α-naphtalenacetic acid (NAA) and 3-indolyl acetic acid (IAA) at different concentrations. The obtained direct and adventitious shoots were transferred to root induction medium containing 0.1 mg/l IBA for 10 days. The plantlets were placed to free hormone MS medium for more growth.

Callus induction was obviously appeared on most of explants after 2 sub-cultures. After two weeks, shoot organogenesis appeared in shoot induction media. The highest regeneration frequency was obtained using MS medium containing 2 mg/l BA and 0.5 mg/l NAA. It was revealed that hypocotyl and root explants are better than leaf explants.

The present protocol prepares a simple and rapid regeneration system for P. lanceolata in short period via direct and adventitious shoot induction. In vitro regeneration could be used as a strong tool in genetic transformation to produce transgenic medicinal plants.
ANTIBACTERIAL EFFECT OF PFAFFIA PANICULATA AGAINST SOME PATHOGENIC BACTERIA

A. SHARAFI, M. MALEKINJAD, S. RAHAMOUZ HAGHIGHI, A. MEIMANDIPOUR

Zanjan University of Medical Sciences, Iran; Zanjan University, Iran; National Institute of Genetic Engineering and Biotechnology, Iran; e-mail: sharafi.a@gmail.com

Pfaffia paniculata (belonging to Amaranthaceae family) is named Brazilian ginseng which plays a major role in the revival of traditional medicine. Despite the useful role of its root in many ailments such as anti-diabetes, anti-ulcer and anti-cancer, we investigated anti-bacterial activities of roots of P. paniculata against some strains of bacteria.

The antibacterial activity of extracts of P. paniculata was assessed by disc diffusion method on Bacillus cereus, Kelbsiella pneumoniae, Proteus vulgaris, Salmonella typhi and Escherichia coli. Extract powder was solved in dimethyl sulfoxide (DMSO) and then filtered. Antibacterial effects of the extracts tested at different concentrations (2.5, 5, 10, 20, 40, 60, 80 and 100 mg/ml). Kanamycin (1 and 10 mg/ml) and DMSO was used as positive and negative control respectively.

Our results revealed that ethanolic extracts have an inhibitory effect on E. coli and no effect on other bacteria. Three concentration (60, 80 and 100 mg/ml) of P. paniculata extracts showed significant inhibitory activity in compared with antibiotics. The mean of inhibitory of three concentration (10, 11 and 12.5 mm of inhibition zone) was more than kanamycin at 1 mg/ml concentration (8.5 mm) but were not better inhibitory than kanamycin at 10 mg/ml concentration (21 mm) on E. coli.

So we can conclude that this plant has antibacterial properties. The result can be related to the nature of the compounds found in this plant. Based on this result, P. paniculata can be effective at higher doses for the control of E. coli.
PREPARATION OF S- AND R-ENANTIOMERS OF 3-ACETOXY-7-BROMO-5-PHENYL-1,2-DIHYDRO-3H-1,4-BENZODIAZEPIN-2-ONE USING PIG LIVER CARBOXYLESTERASES

Ye. SHESTERENKO, Yu. SHESTERENKO

A.V. Bogatsky Physico-Chemical Institute, Odesa, Ukraine; e-mail: shesterenko@mail.ru

Carboxylesterases (EC 3.1.1.1) are the most studied enzymes, catalyzing the enantioselective hydrolysis of an exceptional range of acyclic, carbocyclic and heterocyclic compounds, including 3-hydroxy-1,4-benzodiazepin-2-one esters. Nevertheless, the limitations of the carboxylesterase usage are the high cost of commercial enzyme and its single usage. Therefore, the application of more economical partially purified carboxylesterase as a component of pig liver microsomal fraction, as well as development of its immobilization on polymeric carriers are urgent tasks.

The aim of the present work was the isolation of carboxylesterase preparations from the pig liver, their immobilization on polymeric carriers and studying their biochemical and physico-chemical features to conduct enantioselective hydrolysis of 3-acetoxy-7-bromo-5-phenyl-1,2-dihydro-3H-1,4-benzodiazepin-2-one.

An accessible method of immobilization of pig liver microsomal fraction in agar was developed. The biocatalyst with 90% preservation of the initial esterase activity and widening of the pH-optimum of the immobilized preparation (pH 6.0-8.0) was obtained.

Enantioselective hydrolysis of the studied compound using immobilized microsomal fraction was conducted in optimized reaction conditions. The S-enantiomer of substrate was obtained ([α]20D = +116.9, c = 1 in chloroform). The biocatalyst was used for the enantioselective hydrolysis of the ester for 5 cycles of usage in a batch process.

According to the modified method, cytosolic carboxylesterase was isolated from the pig liver.

The belonging of the enzyme to the carboxylesterase family was indicated by the complete inhibition of its activity by the selective carboxylesterase inhibitor bis(p-nitrophenyl) phosphate and the results of electrophoresis.

It was shown, that the regioselectivity of the obtained protein fractions was significantly different. The specific activity of the most active with β-naphthyl acetate fraction (Rf 0.05) was 64 times higher than its α-naphthyl acetate hydrolytic activity.

Using the isolated cytosolic carboxylesterase, under the developed conditions, enantioselective hydrolysis of 3-acetoxy-7-bromo-5-phenyl-1,2-dihydro-3H-1,4-benzodiazepin-2-one with obtaining of R-enantiomer of substrate ([α]20D = +117.2, c = 1 in chloroform) was conducted. This indicates an opposite enantioselectivity of the pig liver cytosolic carboxylesterase compared with the carboxylesterase of the microsomal fraction.
Melanin pigmentation of skin plays the most important role in the protection of organism against UV-irradiation, capable to induce serious pathological states, including cancerous diseases of skin. But the excessive accumulation of melanin brings to toxic melanodermia, melasma, lentigo and other skin lesions and is the important current dermatological and cosmetological problem.

Tyrosinase (EC 1.14.18.1) is the key enzyme of skin melanin pigment biosynthesis. In spite of certain progress in investigation of natural and synthetic tyrosinase inhibitors, the urgency of such studies is high, because the existing inhibitors are in some cases unstable, expensive, toxic, require complex methods of synthesis or isolation from natural sources.

Tyrosinase of Agaricus bisporus was isolated according to modified method. It was found, that addition of PEG-4000 during extraction promotes the 3-fold decrease of polyphenols content. It is known, that the products of endogenous polyphenolic compounds oxidation are inhibitors of tyrosinase, their removal allowed increasing tyrosinase activity by 25%.

The search of new inhibitors of tyrosinase among the wide range of compounds, including derivatives of benzoic acid, 3-chloro-1,4-naphthoquinone, dipicolinic acid, etc. The studied substances did not display the inhibitory effect at concentration of 0.1-0.5 mmol/dm³.

It is known, that the natural substrate of mushroom tyrosinase is 1,8-dihydroxynaphthalene, thus it was supposed that the 2,7-dihydroxynaphthalene may be a promising inhibitor of enzyme activity.

It was shown, that the concentration of half-maximal inhibition (IC₅₀) of tyrosinase monophenolase activity by 2,7-dihydroxynaphthalene (96.5 μmol/dm³) is close to that of kojic acid (60.75 μmol/dm³) – a classic inhibitor of melanogenesis. It was found, that 2,7-dihydroxynaphthalene exerts inhibitory action only on monophenolase activity of tyrosinase in contrast to kojic acid, which inhibits both monophenolase and diphenolase enzyme activity.

For the first time the influence of hydroxybenzylidene aminophenols on tyrosinase activity was studied. It was found, that 4-hydroxybenzylidene-4-aminophenol and 2-hydroxybenzylidene-2-aminophenol possess the inhibitory ability, which 5- and 23-fold (IC₅₀ – 11.2 and 2.6 μmol/dm³, respectively) exceeds that of kojic acid. The studied hydroxybenzylidene aminophenols similarly to 2,7-dihydroxynaphthalene, influence only the monophenolase activity of enzyme.
Cancer is a morphologically and molecularly heterogeneous disease. Among different cancer types, the breast cancer is the most common one, and it is the leading cause of women death worldwide. Breast tumors belonging to the same intrinsic subtype could have different response to therapy, but reasons of this are still not clear. Moreover poor disease outcome after chemotherapy is often caused by resistance formation to most of commonly used drugs. Effectiveness of anti-neoplastic agents is not fully understood and could be influenced by DNA repair activity. RAD50 protein plays a key role in DNA double strand breaks repair (DSBs), it is crucial to safeguard genome integrity. The aim of this study was to determine whether RAD50 was capable of being a prognostic marker of tumor cells response to chemotherapy.

To directly investigate the association of chemotherapeutic drugs and gene expression or copy number alterations (deletion – log2<-0.3; gain – log2>0.3) of RAD50 in breast cancer, we analyzed the cell line expression and CNA data in 59 breast cancer cell lines; data was taken from Cancer Cell Lines Encyclopedia (https://portals.broadinstitute.org/ccle/home). The response information (IC₅₀) to 12 anti-cancer drugs, namely 5-fluorouracil, carboplatin, doxetaxel, doxorubicin, gemcitabine, lapatinib, methotrexate, mitomycin, oxaliplatin, paclitaxel, tamoxifen, vinblastine, were downloaded from Genomics of Drug Sensitivity in Cancer (http://www.cancerrxgene.org) and Cancer Cell Lines Encyclopedia. We determined the association between mRNA expression of RAD50 and response to drugs as well as between CAN and response to drugs using Pearson correlation and Wilcoxon-Mann-Whitney test. The analysis revealed a significant association between the mRNA expression of RAD50 and sensitivity to vinblastin in breast cancer cell lines (correlation = 0.3625; p-value 0.0215). Correlation directly in cell lines with basal like subtype was stronger and more significant than in not differentiated cohort (correlation = 0.6340; p-value 0.0199). Resistant (mean = 7.787; 25% of available cell lines with highest IC₅₀) to vinblastine cell lines have significantly higher mRNA expression (p-value = 0.0029) than sensitive (mean = 6.989; 25% of available cell lines with lowest IC₅₀). Analysis of cell lines sensitivity to chemotherapeutic compounds taking into account CNA showed a significantly better response to vinblastine in cell lines with deletions (p-value = 0.0143) than in cell lines with diploid RAD50 copy number.

Our data suggests that RAD50 might be a predictive marker in determining the benefit of vinblastin chemotherapy. However, further studies are needed to clarify the outputs using a larger sample group and more in-depth in vitro, in vivo and ex vivo studies.

This study was supported by RSF (project 15–15–20032)
ADAPTOR PROTEIN RUK/CIN85 INDUCES GENOMIC REPROGRAMMING IN BREAST CANCER CELLS AND THEREBY INCREASES THEIR MALIGNANCY

I. HORAK1, D. SHYTIKOV1, L. KNOPFOVA2, L. BORSIG3, L. DROBOT1

1Palladin Institute of Biochemistry of NAS of Ukraine, Kyiv;
2Masaryk University, Brno, Czech Republic;
3University of Zurich, Zurich, Switzerland;
email: iryna.horak@gmail.com

Adaptor proteins serve as molecular platforms for multimolecular complexes assembly and thereby regulate cell signaling. The information from public databases and our previous results demonstrated that tumors (including breast cancer) are characterized by increased expression of adaptor protein Ruk/CIN85. This ubiquitously expressed adaptor is involved in dynamic control of cell signaling in space-dependent manner and plays critical role in several cellular processes, such as ligand-induced endocytosis of RTKs, intracellular vesicular trafficking, adhesion, motility, and survival. In the present study we investigated the potential mechanisms of of adaptor protein Ruk/CIN85 involvement in breast cancer invasion and metastasis.

As a model we used mouse 4T1 breast adenocarcinoma cells with stable overexpression (RukUp cells) and downregulation (RukDown cells) of Ruk/CIN85. In vitro motility was investigated by scratch test and invasion - by Boyden chamber assay. The efficiency of RukUp and RukDown cells extravasation and metastasis in vivo was studied by using syngeneic mouse model. Gene expression was analysed by Real-time PCR.

We demonstrated that overexpression of Ruk/CIN85 increases both motility and invasiveness of 4T1 cells. Using animal model it was shown that RukUp cells are characterized by elevated ability to produce lung metastasis, while the effectiveness of RukDown cells metastasis was significantly suppressed. These changes in tumor cells behavior were accompanied by the differential expression of EMT-related genes, including vimentin, E-cadherin, SNAI1, Zeb-1, Zeb-2, Lcn2.

The obtained data suggest that adaptor protein Ruk/CIN85 is a critical regulatory component involved in EMT of breast cancer cells that arise through the reprogramming mechanisms.
METHYLATION OF GPX3 AND TIMP3 GENES OF DNA OF TUMORS IN PATIENTS WITH RENAL CELL CARCINOMA

K. SHCHERBYK, K. ONYSHCHENKO, I. SKRYPKINA, L. PERETA, O. BANAS

Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv; e-mail: katiashcherbyk@gmail.com

Approximately 250,000 cases of renal cell carcinoma are diagnosed every year in the world, and 100,000 patients die of the disease. Cancer is associated with a variety of molecular genetic changes. Hypermethylation of tumor suppressor genes is often associated with developing cancer and it can be used as biomarkers for early detection of the presence of cancer as well as for monitoring patients during and after therapy. It was indicated that tumor genomic DNA can be used for detection of hypermethylation of cancer marker genes, which have been applied in cancer risk assessment, early detection, prognosis, and prediction of response to cancer therapy.

The purpose of our study is to determine methylation of GPX3 and TIMP3 suppressor genes of tumors in patients with renal cell carcinoma.

The research was performed on biopsies of the tumor and surrounding tissues of fifty patients with clear cell renal cell carcinoma. Bisulfite modified genomic DNA was amplified using real-time quantitative methylation-specific polymerase chain reaction with specific primers for GPX3 and TIMP3 tumor suppressor genes.

Analysis of the results showed methylation of CpG islands of GPX3 gene in 43 (86%) renal cancer tumor tissue samples and in 14 (28%) tissue samples around the tumor. At the same time methylation of the TIMP3 promoter was detected in 6 (12%) tumor DNA tissue samples and in 2 (4%) DNA tissue samples around the renal cancer tumor.

To sum up we observed hypermethylation of the GPX3 promoter and low methylation level of CpG islands of TIMP3 gene. The obtained results indicate that hypermethylation of genomic DNA of GPX3 gene are cancer-specific changes.

Publications are based on the research provided by the grant support of the State Fund for Fundamental Research (project 0116U006539).
Enhancers are central regulators of spatiotemporal patterns of gene expression during development. Accessing and predicting enhancer activity in the genome is therefore the primary objective towards understanding of the complex regulatory networks underlying cell differentiation and development.

In this study, we utilized several genomic approaches to access the potential rate of enhancer activation throughout the time course of Drosophila embryonic development and characterize the dynamic properties of enhancers such as Pol II binding, enhancer RNA (eRNA) transcription, and enhancer-specific epigenetic marks. In particular, we performed ChIP-seq on initiating and elongating forms of Pol II during mesoderm and nervous system development and complemented this approach with several types of RNA-seq, such as strand-specific ribo-depleted RNA-seq and PRO-cap which enables highly sensitive identification of nascent capped transcripts. To access chromatin dynamics, we made use of the publicly available data such as ChIP-seq (H3K4me1 and H3K4Ac27) and DNase-seq.

We have found that genome-wide initiating Pol II occupancy is highly dynamic over open chromatin regions in mesoderm and neuronal development and is associated with active enhancers. We show that eRNA transcription is significantly correlated with the timing of in vivo characterized enhancer activity and transcription factor binding in fly embryo tissues, consistent with previous studies in mammalian cell culture systems. We characterize specific properties of Drosophila eRNA, such as abundance and directionality. Finally, we show that eRNA can be used for prediction of putative active enhancers and assign potential enhancer activity to a large set of thousands of previously uncharacterized regions within Drosophila genome.
INVESTIGATION OF PROMOTER REGION POLYMORPHISM OF TASAP-A1 GENE IN THE COLLECTION OF WINTER WHEAT (TRITICUM AESTIVUM L.) VARIETIES AND LINES

A. FAMINA¹, S. KULINKOVICH², S. MALYSHEV¹, O. URBANOVICH²

¹Institute of Genetics and Cytology, NAS of Belarus;
²Scientific and Practical Center of NAS of Belarus on Agriculture, Minsk, Belorussia;
e-mail: lyzosoma@mail.ru

TaSAP1 is a member of the stress association protein (SAP) gene family from wheat. It is involved in response to several abiotic stresses, including drought, salt and cold. TaSAP-A1 gene is located on chromosome 7A like the loci affecting yield-related traits, including thousand-kernel weight (TKW), spike length (SL) and the total number of spikelets per spike (TNSS). According to Chang et al., TaSAP-A1 gene is also associated with these traits. The highest polymorphism level was revealed in the promoter region of TaSAP-A1 gene. The objective of this study was to investigate the promoter region polymorphism of TaSAP-A1 gene and evaluate the association between its alleles and yield-related traits in the collection of winter wheat varieties and lines used in Belarusian breeding.

The promoter region polymorphism of TaSAP-A1 gene has been investigated in a collection of 72 varieties and lines of wheat used for the breeding process in Scientific and Practical Center of NAS of Belarus on agriculture (Zhodino). TKW, SL and TNSS measurements were conducted in the laboratory of winter wheat of the above institution. DNA was isolated from grains by the method proposed by Plaschke et al. Isolation was carried out from two kernels for each cultivar or line. An analysis of promoter region polymorphism of TaSAP-A1 gene was conducted according to the method proposed by Chang et al., 2013, with modifications.

On the basis of conducted investigation of TaSAP-A1 promoter region in the presence of 5 bp indel at position -1.810 bp and 39bp indel at position -1.637 bp using Sap5 and Sap39 markers, respectively, and a SNP (A-C) at position -2.606 bp using Sap2606 marker all investigated varieties and lines were assigned to 4 haplotypes. Among them, 17 (22.7%) belonged to haplotype I, 34 (47.2%) – to haplotype II, 13 (18.1%) – to haplotype II and 8 (11.1%) – to haplotype IV. In investigated collection an averaged TKW was 51.5 g. Among the cultivars and lines with haplotype I it amounted 51.1g, with haplotype II – 51.4 g, with haplotype III – 51.9 g and with haplotype IV – 52.6 g, that confirm the literature data about haplotypes III and IV being a superior haplotypes for TKW. An averaged SL was 9.1 cm and among the cultivars and lines with haplotype I it amounted 9.4 cm, with haplotype II – 9.0 cm, with haplotype III – 9.0 cm and with haplotype IV – 8.9 cm. An averaged TNSS among investigated cultivars and lines was 17.8: among the cultivars and lines with haplotype I it amounted 18.1, with haplotype II – 17.9, with haplotype III – 17.4 and with haplotype IV – 17.4. It confirms the literature data about haplotype I and being a superior haplotype for TNSS compared with haplotypes II, III and IV.

Thus, the results of investigation of promoter region polymorphism of TaSAP-A1 gene in the winter wheat varieties and lines used in Belarusian breeding provides useful information for marker-assisted selection for yield-related traits.
BEST PRACTICES FOR GENE EXPRESSION MICROARRAY AND RNA-SEQ DATA ANALYSIS IN INTEGRATIVE STUDIES

A. FROLOVA, M. OBOLENSKA

Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv; e-mail: a.o.frolova@imbg.org.ua

Modern gene expression study design usually implies operating with multiple sources of information. Such sources are not limited by simple control-experiment groups of samples produced in one go, but also comprised from datasets made within different timeframes and by different technologies. Moreover, last trends in massive gene expression data analysis shifted towards integrative approaches, where data from different experiments are combined directly or through meta-analysis to increase statistical significance of results. Here we summarized our experience in gene expression microarray and RNA-seq data analysis to provide optimal pipeline for joint data processing of both types.

Necessary steps for quality control analysis of raw microarray and RNA-seq data were compiled. Time and technology dependent batch-effect and its negative influence on differential expression analysis were studied. We confirmed, that Illumina BeadChips, which contain several single arrays, each targeting one sample, are subject to unnecessary technical variations, especially if combined with RNA amplification and isolation batches. Based on quality control results global normalization methods for RNA-seq should be accompanied with specific methods such as RUVseq or svaseq to minimize the effect of different amount of RNA and library preparation. General-purpose Empirical Bayes methods such as ComBat can be applied to both microarray and RNA-seq data. Since microarrays contain ambiguous probesets to genes representations, we identified the best strategy for probesets selection to obtain more uniform result between different platforms and when comparing with RNA-seq data. Such strategy rely on actual probesets signal intensities contrast to mapping probe sequences to genome. Finally the methods based on estimating the expression from RNA-seq reads overlapping the microarray probe regions were compared with RNA-seq transcripts quantification on gene level with feature counts and normalization to counts per million (CPM) in log scale with voom method.

We highlighted important steps and difficulties in gene expression microarray and RNA-seq data analysis and provided guidelines and scripts to further facilitate integrative studies.
Publication of gene expression raw data in open access to online resources like NCBI or ArrayExpress made it possible to use these data for cross-experiment integrative analysis and make new insights into biological phenomena. However, most popular of the present online resources are meant to be archives rather than ready for immediate access and interpretation databases. Data uploaded by independent contributors is not standardized and sometimes incomplete and needs further processing before it is ready for the analysis. Hence, the need for a specialized database appears.

Given in this article is the description of the database that was created after processing a collection of 33 relevant datasets on pre-eclampsia-affected human placenta. Data processing includes the choice of relevant experiments from ArrayExpress database, the experiment sample attributes standardization according to MeSH term dictionary and Experimental Factor Ontology and the completion of missing data using information from the corresponding articles and authors.

A database of more than 1000 samples contains sufficient sample-wise metadata for them to be arranged into relevant case-control groups. Metadata includes information on biological specimen, donor's diagnosis, gestational age, mode of delivery etc. The average size of these groups will be higher than it is in separate experiments. This will reduce experiment bias and enhance statistical accuracy of the subsequent analysis such as search for differentially expressed genes or inferring gene networks. The article concludes with the guidelines for the microarray experiment metadata uploading for future contributors.
The endoplasmic reticulum stress is an important component of tumor growth, including glioblastoma. IRE1 (inositol requiring enzyme-1) signaling pathway of endoplasmic reticulum stress is a central mediator of the unfolded protein response and inhibition of this signaling pathway leads to a suppression of glioma growth through down-regulation of proliferation processes as a result of metabolic reprogramming of cancer cells. The endoplasmic reticulum stress controls the expression of numerous regulatory and proliferation related genes, which are responsible for glioma growth. We have studied the effect of inhibition of IRE1 signaling enzyme on the expression of a subset of genes encoding important regulatory proteins in U87 glioma cells.

We have used U87 glioma cells and their sub-line stably transfected with cDNA-construct expressing dnIRE1 (without kinase and endoribonuclease activities). The expression levels of BRCA1, DEK, BCL2L1, COL6A1, TPD52, GLO1, HOMER3, and STC2 mRNAs as well as ACTB mRNA were measured in these glioma cells by real-time quantitative polymerase chain reaction.

It was shown that the expression level of breast cancer 1 early onset (BRCA1), a nuclear phosphoprotein which coordinates a diverse range of cellular pathways and transcriptional regulation to maintain genomic stability, tumor protein D52 (TPD52), which inhibits growth and metastasis in renal cell carcinoma cells through the PI3K/Akt signaling pathway, and stanniocalcin 2 (STC2) mRNAs is significantly up-regulated (+83, +135, and +514%, correspondingly) in U87 glioma cells by inhibition of IRE1 signaling enzyme in comparison with the control cells. At the same time, the expression level of collagen, type VI, alpha 1 (COL6A1), which play an important role in tumorigenesis, DEK oncogene (DEK), glyoxalase I (GLO1), which promotes tumor growth, and homer homolog 3 (HOMER3), which is implicated in diverse biological functions, is strongly down-regulated (-72, -18, -91 and -36%, correspondingly) in glioma cells without IRE1 signaling enzyme function. It was also shown that hypoxia up-regulated the expression level of COL6A1, TPD52, and STC2 mRNAs (+60, +91, and +604%, correspondingly) and down-regulated – BRCA1, DEK, and GLO1 mRNAs (-51, -28, and -12%, correspondingly) in control glioma cells and that IRE1 inhibition modifies the effect of hypoxia on the expression of COL6A1, BCL2L1, HOMER3, and STC2 mRNAs.

Thus, the expression of most studied genes is responsible for IRE1-mediated endoplasmic reticulum stress signaling in gene specific manner. Therefore, the changes in expression level of genes encoding BRCA1, DEK, BCL2L1, COL6A1, TPD52, GLO1, HOMER3, and STC2 proteins possibly reflect metabolic reprogramming of glioma cells by IRE1-mediated endoplasmic reticulum stress signaling and correlate with suppression of glioma cell proliferation upon inhibition of the IRE1 signaling enzyme.

Acknowledgments. I would like to express my gratitude to my research supervisor Prof. Oleksandr Minchenko and also to all colleagues from Molecular Biology Department of Palladin Institute of Biochemistry for their help in experiments.
The endoplasmic reticulum stress is an important component of tumor growth, including glioblastoma. IRE1 (inositol requiring enzyme-1) signaling pathway of endoplasmic reticulum stress is a central mediator of the unfolded protein response and inhibition of this signaling pathway leads to a suppression of glioma growth through down-regulation of proliferation processes as a result of metabolic reprogramming of cancer cells. The endoplasmic reticulum stress controls the expression of numerous regulatory and proliferation related genes including tumor suppressors, which are responsible for glioma growth. We have studied the effect of inhibition of IRE1 signaling enzyme on the expression of a subset of genes encoding important proliferation related proteins in U87 glioma cells.

We have used U87 glioma cells and their subline stably transfected with cDNA-construct expressing dnIRE1 constructs without kinase and endoribonuclease activities. The expression levels of NAMPT, HSPB8, RAB5C, BIRC5, PSAT1, KRT18, CLU, GPI, and TSPAN13 genes as well as ACTB as reference gene were measured in these U87 glioma cells by real-time quantitative polymerase chain reaction and Western-blot analysis.

It was shown that the expression level of nicotinic acid phosphoribosyltransferase (NAMPT), a key enzyme in the biosynthesis of nicotinamide adenine dinucleotide, which is involved in many important biological processes, heat shock 22 kDa protein 8 (HSPB8), which is involved in carcinogenesis, tetraspanin 13 (TSPAN13), RAB5C, member RAS oncogene family (RAB5C) mRNAs is significantly down-regulated (-95, -86, -82, and -77%, correspondingly) in U87 glioma cells by inhibition of IRE1 signaling enzyme in comparison with the control cells. Inhibition of IRE1 enzyme had significantly lesser suppressive effect on the expression of baculoviral IAP repeat containing 5 (BIRC5), phosphoserine aminotransferase 1 (PSAT1), and clusterin (CLU) mRNAs (-40, -61, and -54%, correspondingly).

At the same time, the expression level of keratin 18 (KRT18), which plays an important role in cell proliferation, and glucose-6-phosphate isomerase (GPI), which is involved in glycolysis, is up-regulated (+170 and +30%, correspondingly) in glioma cells without IRE1 signaling enzyme function. It was also shown that hypoxia up-regulated the expression level of most studied genes and that IRE1 inhibition modifies the effect of hypoxia on their expression.

Thus, the expression of most studied genes is responsible to IRE1-mediated endoplasmic reticulum stress signaling in gene specific manner. Therefore, the changes in expression level of genes encoding BRCA1, DEK, BCL2L1, COL6A1, TP53, GLO1, HOMER3, and STC2 proteins possibly reflect metabolic reprogramming of glioma cells by IRE1-mediated endoplasmic reticulum stress signaling and correlate with suppression of glioma cell proliferation upon inhibition of the IRE1 signaling enzyme.

Acknowledgments. I would like to express my gratitude to my research supervisor Prof. Oleksandr Minchenko and also to all colleagues from Molecular Biology Department of Palladin Institute of Biochemistry for their help in experiments.
IRE1 MEDIATED REGULATION OF THE EXPRESSION OF A SUBSET OF NUCLEAR GENES ENCODING MITOCHONDRIAL PROTEINS IN U87 GLIOMA CELLS

O. RIABOVOL1, D. O. MINCHENKO1, O. RATUSHNA1

1Palladin Institute of Biochemistry, NAS of Ukraine, Kyiv;
2Bohomolets National Medical University, Kyiv, Ukraine;
e-mail: olenariabovil@gmail.com

Mitochondria play an important role in the regulation of tumor growth and apoptosis through numerous metabolic pathways. The functional activity of mitochondria is controlled through numerous nuclear-encoded mitochondrial proteins and most of these factors and enzymes are responsible for metabolic reprogramming of mitochondria in cancer as well as in other diseases. The endoplasmic reticulum stress is an important component of tumor growth and inhibition of IRE1 (inositol requiring enzyme-1) signaling pathway, which is a central mediator of the unfolded protein response, leads to a suppression of tumor growth through down-regulation of the angiogenesis and proliferation processes. This stress contributes to the expression profile of many regulatory genes resulting in proliferation, apoptosis, angiogenesis, and mitochondrial functions. We have studied the effect of IRE1 inhibition on the expression of a subset of nuclear genes encoding mitochondrial proteins in U87 glioma cells.

We used U87 glioma cells and their subline stably transfected with cDNA-construct expressing dnIRE1 (no kinase, no endoribonuclease). The expression level of NNT, NR3C1, FAM162A, PRSS15 and ETHE1 mRNAs as well as ACTB mRNA were measured in U87 glioma cells by real-time quantitative polymerase chain reaction.

It was shown that the expression level of nicotinamide nucleotide transhydrogenase (NNT), glucocorticoid receptor (NR3C1), and pro-apoptotic protein FAM162A (family with sequence similarity 162 member A) mRNAs is significantly up-regulated (+64, 50, and 176%, correspondingly) in U87 glioma cells by inhibition of IRE1 signaling enzyme in comparison with the control cells. At the same time, the expression level of mitochondrial ethylmalonic encephalopathy 1 (ETHE1), which represents a sulfur dioxygenase that localizes within the mitochondrial matrix and suppresses p53-induced apoptosis, is strongly down-regulated (-76%) in glioma cells without IRE1 signaling enzyme function. It was also shown that IRE1 inhibition decreases the expression level of mitochondrial serine protease 15 (PRSS15), also known as hLON ATP-dependent protease, which mediates the selective degradation of misfolded, unassembled or damaged polypeptides in the mitochondrial matrix and participate in the regulation of mitochondrial gene expression.

The expression of all studied genes is responsible for IRE1-mediated endoplasmic reticulum stress signaling in gene specific manner, because IRE1 knockdown significantly affects their expression. Therefore, the changes in expression level of nuclear genes encoding NNT, NR3C1, FAM162A, PRSS15, and ETHE1 proteins possibly reflect metabolic reprogramming of mitochondria by IRE1-mediated endoplasmic reticulum stress signaling and correlate with suppression of glioma cell proliferation upon inhibition of the IRE1 enzyme function.
A dvent of the next-generation sequencing (NGS) of DNA-analysis at the beginning of the 1980’s brought about a revolution in biology and medicine.

Frederick Sanger was the first scientist to report the basic principles for determination of amino acid and nucleotide sequence of DNA and RNA in 1977.

But the determination of the whole human genome as the fundamental capability of sequencing was not suggested at the time. An international consortium of 16 laboratories accomplished this task in 2003.

To remain up-to-date with evolving technology in the field of DNA-analysis and human identification SSRFC of MIA of Ukraine introduced Ion Torrent PGM™ sequencer in 2016. It enables us to get a robust data about DNA sequence in record-breaking time. The instrument can simultaneously analyze more than a dozen samples in less than 24 hours.

Depending on its processor configuration it is capable of determining from 10 million to 1 billion base pairs with high precision.

Ion Torrent PGM™ enables us to sequence specific stretches of genome of various organisms, search for somatic and hereditary mutations, analyze expression of genes and noncoding RNA, conduct metagenomic analysis by the sequence of 16S pRNA as well as analysis of protein-DNA interactions (ChIP-Seq), Y-DNA and mtDNA.

In 2016-2017 we conducted a number of studies with Ion Torrent PGM™ that demonstrated the efficiency of mtDNA sequencing for establishing maternal relationship between parents and children as well as between siblings.

The results obtained are reproducible, repeatable and robust and have the following advantages over the results of Sanger mtDNA sequencing:

– mitotypes are more informative and discriminative since the whole mtDNA chain is sequenced (~16570 nucleotides) which is not the case with the Sanger method (~830 nucleotides);
– it takes 10 times less messenger DNA to get the result which allows us to analyze samples with extremely low DNA concentration.

Quantitative and differential analysis of gene expression plays a key role in performing various research tasks particularly in correlating gene expression with phenotype which can result in establishing such human characteristics as sex, age, color of hair, eyes, skin, etc.
Gravitropism (geotropism) is an ability of roots and various above-ground plant organs to change their growth in response to gravity. Under alteration of gravity vector the asymmetric growth of upper and lower halves of an organ is stimulated and its gravitropic bending occurs. A key factor in mediation of the gravitropic bending is redistribution of phytohormone auxin between cells of gravistimulated organs. Such auxin redistribution in the above-ground organs induces extrusion of protons into the apoplast, acidification and weakening of the cell wall, “acid growth” of cells of the lower half of an organ and its subsequent bending. At the same time, the sensitivity of expression of genes associated with auxin metabolism and signaling to gravistimulation remains poorly understood.

The aim of this research was to evaluate influence of gravistimulus on expression of genes associated with auxin signaling and transport such as some isoforms of small auxin upregulated RNA (SAUR) gene family, auxin receptor TIR1, auxin efflux and influx transporters LAX1 and SiPIN1.

Influence of gravistimulus on gene expression was determined in the apical leaves of tomato plants. For gravistimulation the experimental group of tomatoes were turned by 90° so their stems were horizontal and exposed at different time intervals. Relative quantitative real-time RT-PCR was used to measure the change in mRNA expression levels of target genes.

It was found that expression of SAUR15, SAUR58, TIR1, LAX1, and SiPIN1 was increased in 15 min after the beginning of gravistimulation. The expression of SAUR15, SAUR58, SiPIN1, TIR1 remained high at the late stages of observations, meanwhile the expression of LAX1 was decreased.

The obtained results suggest the changes in expression of target genes at the transcriptional level can play an important role in development of gravitropic response in plant leaves.
The aim of the present study was to investigate DGAT1, Alpha-lactalbumin and Ghrelin genes polymorphisms and their associations with milk production and compositions in buffaloes of Mazandaran Province using PCR-RFLP and PCR-SSCP methods. In this study the blood samples were collected from 100 buffaloes and DNA was extracted using modified salting out extraction protocol. A fragment of DGAT1 gene with the length of 411 bp including part of exon 3, and a fragment of Ghrelin gene with the length of 402 bp amplified by polymerase chain reaction (PCR). In order to PCR products were digested with CfrI and Hpa II restriction enzymes for DGAT1 and ghrelin genes, respectively. Polymorphism at Alpha-lactalbumin gene was detected by single strand conformation polymorphism (SSCP) method. In order to study association of studied genes with milk production and composition on buffalo, from GLM procedure of SAS software was used. The PCR or digested PCR products were electrophoresed on 1.5% agarose and 12% polyacrylamide gel. In the RFLP method for DGAT1 gene, only K allele and for ghrelin two allele’s i.e. T and C were found. In this population TT, TC and CC genotypes have been identified with the 0.55, 0.3 and 0.15 frequencies, respectively. T and C allele’s frequencies were 0.7 and 0.3. Using SSCP method, four banding patterns were found at Alpha-lactalbumin site with frequencies of 0.17, 0.66, 0.15 and 0.02 for AB, BC, BB and CC, respectively. A, B and C allele’s frequencies were 0.085, 0.565 and 0.35, respectively. The results showed significant effect of Ghrelin gene on milk fat, protein and dry matter percentage. These traits were more favorable in the group of individuals with CC genotype, which may indicate an advantage of T allele over C. The Alpha-lactalbumin genotype was not significantly affected the studied. In general, results indicated possible potential for using DGAT1, Alpha-lactalbumin and ghrelin genes in marker-assisted selection programs.
Unbalanced or high-caloric diet can cause numerous pathologies, including the metabolic syndrome. The metabolic syndrome is a complex of hormonal and metabolic disorders that have pro-atherogenous complications. The major factor of that is the alteration in blood lipoprotein profile.

The aim of the research was to investigate changes in lipoprotein metabolism indices under experimental metabolic syndrome model.

Activities of key enzymes of lipogenesis and lipoprotein content were measured in liver homogenates on male Syrian hamsters fed with high-caloric diet. Despite more favorable blood serum lipid profile of animals at the age of 4 weeks, after being fed by high-caloric diet subsequent metabolic syndrome and dyslipidemia develops in all animals regardless of age. An increase in triglycerides level in blood under the metabolic syndrome is a key factor in atherogenic dyslipidemia formation. We established direct correlation between the levels of free fatty acids in blood serum of the animals receiving a high-energy diet and ApoB-lipoproteins in the liver. Free fatty acids levels also correlate with triglycerides and ApoB-lipoproteins in blood serum of experimental animals. Hydrolysis of triglycerides in high-density lipoprotein particles by hepatic lipase leads to their rapid removal from blood circulation. We also observed the decrease of lipoprotein lipase activity in blood serum of young hamsters that were fed with high-caloric diet. We established that hepatic lipase activity in blood serum of the experimental hamsters was increased regardless of age. The increase of hepatic lipase activity is one of key factors in development of an atherogenic dyslipidemia under the obesity and metabolic syndrome.

Lipoprotein profile of male hamsters gets less favorable with age. Increased levels of free fatty acids and triglycerides, as well as decreased levels of cholesterol-rich high-density lipoproteins were observed in hamsters under the experimental metabolic syndrome. Under the high-caloric diet atherogenic dyslipidemia develops in experimental animals regardless of age.
Obesity is a complex metabolic disorder often associated with insulin resistance (IR) as well as type 2 diabetes. Chronic hypernutrition and high fat diet rich in saturated fatty acids leads to molecular changes in insulin sensitive tissues (the liver, muscle and adipose tissue), impairment in insulin signaling and following dyslipidemia. Leptin is an important adipose tissue–derived hormone that has been shown to be involved in pathophysiological mechanisms of diabetes. That is why the aim of our study was to investigate the free fatty acid (FFA) composition of adipocytes and plasma leptin level of obesity-induced IR rats and its changes induced by the N-stearoylethanolamine (NSE).

The experimental model was induced by the 6-month high-fat-diet (HFD). NSE was administered as water suspension per os at a dosage 50 mg/kg of body weight daily during 2 weeks. Adipocytes were isolated from abdominal fat using Type I Collagenase solution. Adipocytes lipid extract was separated on the fractions by thin-layer chromatography. Free fatty acids composition was analyzed by gas-liquid chromatography. The fatty acids desaturase activity Δ9-D was estimated using product-to precursor index (oleinic/stearic acids ratio). Plasma leptin level was measured using ELISA. Experimental data were processed statistically using Student’s t-test. The statistical significance was determined for \( P < 0.05 \).

The investigation of FFA content demonstrated that the content of saturated FFA significantly increased in adipocytes of obese IR rats compared to control. The assay of unsaturated FFA showed a statistically significant growth in monounsaturated and bi-unsaturated FFA content whereas the level of polyunsaturated FFA decreased dramatically in IR-group in comparison to control. The plasma leptin content growth was also observed in IR rats in comparison to control. NSE administration had a considerable effect on normalization of FFA composition and caused statistically significant decrease in plasma leptin level in IR rats.

It was demonstrated, that obesity-induced IR caused by prolonged HFD leads to impairment in adipocytes FFA profile and is followed by the considerable increase of leptin content in plasma. As far as NSE administration normalized FFA composition of adipocytes and plasma leptin level, we can consider NSE as a prospective agent for the treatment of obesity.
Section 5. Metabolites and Correction of Metabolic Processes

**STRAINS PSEUDOMONAS FLUORESCENS ONU541, BACILLUS MEGATERIUM ONU542 ARE PROMISING PRODUCERS OF SURFACE-ACTIVE METABOLITES AND DESTRUCTORS OF PETROLEUM PRODUCTS**

O. GORSHKOVA, O. VOLIUVA.CH, M. SHTENIKOV, T. GUDZENKO

Odesa I. I. Mechnikov National University, Ukraine; e-mail: 7872930@mail.ru

Methods for eliminating oil pollution, based on their decomposition by non-pathogenic microorganisms, are recognized as effective and ecologically safe. The degree of purification is increased in the case of the production of biosurfactants by microorganisms. The urgent task of ecobiotechnology remains the search for new oil-oxidizing microorganisms capable of producing biosurfactants on a cheap nutrient medium. The purpose of the work is to screen microorganisms isolated from the oil-contaminated soil of Zmiiny Island, promising in the biotechnological plan for the production of surface-active metabolites and the destruction of petroleum hydrocarbons.

Screening of microorganisms is carried out to reduce the equilibrium value of surface tension (Wilhelmi method) of liquid bacterial cultures and the appearance of emulsified capacity of their supernatants, by ability to destroy petroleum hydrocarbons. The ability of microorganisms to produce biosurfactants is evaluated depending on the presence of organic components (peptone, yeast extract, glucose) in M-9 medium. Biotechnologically promising non-pathogenic strains of microorganisms for the comparative analysis of their fatty acid profiles using the Sherlock MIDI system are identified as Pseudomonas fluorescens ONU541, Bacillus megaterium ONU542. The residual content of petroleum hydrocarbons in bacterial suspensions is determined by IR spectrometry in the wave number range of 2700-3200 cm⁻¹.

**Results.** Strains *P. fluorescens* ONU541, *B. megaterium* ONU542 in medium M-9 in the absence of peptone and yeast extract in its composition for five days produce metabolites with surface-active properties to a greater degree than in the presence of these organic components. This ability for soil strains of *P. fluorescens* ONU541, *B. megaterium* ONU542 increases from 12.1 to 28.9% and from 17.2 to 28.0%, respectively. It has been established that the strain *P. fluorescens* ONU541 produces both cell-bound and extracellular biosurfactants, *B. megaterium* ONU542 strain is mainly extracellular biocurfactants. The emulsified properties of the supernatants obtained, estimated by the emulsification index (E24, %), are high and in relation to sunflower oil make over 50%. An experimental test of the ability of selected strains to utilize petroleum hydrocarbons with an initial concentration of 1000 mg/l showed that both strains, when cultured in M-9 medium, decompose oil hydrocarbons for 30 days at 30 °C by 60.3-74.6% taking into account the correction for control samples.

The isolated non-pathogenic strains identified by the fatty acid composition of cellular lipids as *P. fluorescens* ONU541, *B. megaterium* ONU542, can be recommended for use in biotechnologies of environmental purification from chronic oil contamination.
Synthetic surfactants are a part of all domestic and most industrial wastewater. 95-98% of the total amount of detergents used in Ukraine are synthetic detergents based on surfactant mixtures, which, as a rule, are characterized by low biological decomposition and because of their chemical nature have a negative impact on water bodies. Getting into water, synthetic surfactants actively participate in the processes of redistribution and transformation of other pollutants, activating their toxic effect. Therefore, for today, the actual task of biotechnologists remains the search for new microorganisms with increased resistance to these compounds and the ability to destroy them. The purpose of this work is to screen microorganisms isolated from the sea water of the Odesa coast in the zone of splash (psammocontura), resistant to the action of deca (ethylene glycol) monooctylphenol ether (nonionic surfactant, Triton X-100) in 2016, and to carry out biotechnological indicators for selection of the most active strains of microorganisms-destructors of compounds with phenolic fragments.

To isolate a specific microbiota, a "hungry" agar was used, in which Triton X-100 was added at a concentration of 1%. The cultivation was carried out at a temperature of 30 °C for 30 days. The presence of growth on Petri dishes and the zones of medium enlightenment indicated not only the stability of strains to Triton X-100, but also indicators of destructive activity—the ability of microorganisms to use a surfactant as the sole carbon source.

The results of the research indicate that strains Nos. 64 and 65, isolated from the pore water of the psammocontura in the area of discharge of urban wastewater into the Black Sea aquarium, exhibited a high level of resistance and destructive activity with respect to Triton X-100. After 7 days, on the nutrient medium containing a nonionic surfactant, the appearance of bacterial colonies was registered in an amount of 3-9 colony-forming units, respectively, after 10 days their number increased 4-7 times, and after 30 days reached 58 (strain No. 64) and 83 colony-forming units (strain No. 65). Among the strains (Nos. 66-68) isolated from the pore water of the psammocontura in a relatively clean area of the water area (at the Hydrobiological Station), only strain No. 66 had a high destructive potential with respect to Triton X-100 in comparison with strains No. 67 and No. 68. The number of colonies grown on "hungry" agar on the 30th day of cultivation of strain No. 66 reached 42 colony-forming units.

The screening of microorganisms isolated in 2016 from the coastal zones of the Black Sea made it possible to select three biochemically active strains (No. 64, No. 65, No. 66) resistant to deca (ethylene glycol) monooctylphenol ether. Selected strains of microorganisms are non-pathogenic, can be used in biotechnologies for cleaning the environment from nonionic surfactants and other organic compounds with phenolic fragments. Their identification is planned in the future.
Arterial hypertension is compulsory component of the metabolic syndrome as well as abdominal obesity and hyperglycemia. Genetic predisposition and food habits are the main factors determining progress of arterial hypertension. Increased nutrition value of food and fast-digesting carbohydrates (glucose, fructose etc) induce changes of metabolism, thus, provoke metabolic syndrome. Additional changes are about to form namely endothelial dysfunction as soon as in young and middle age. However, individuals with metabolic syndrome not always change their diet and continue consume meat products like sausages and so on. Thus, development of new functional food product with antihypertensive effect is priority task for food industry. Quercetin is flavonoid with hypotensive effect that might be effectively extracted from onion skin that is secondary raw. We investigated the effects of meat raw with onion skin extract on glucose and heart function of isolated heart in spontaneously hypertensive rats (SHR) kept at high-fructose diet.

Five groups were studied: Wistar rats, SHR, SHR+fructose, SHR+fructose+meat with quercetin (2,25 mg per rat), SHR+fructose+meat with onion skin extract. Fructose was administered as 25% solution in drinking water. Meat was administered per os, 1 g per rat daily for 3 month. After sacrificing the rats at the end of the treatment, glucose was measured in arterial blood. Rat isolated hearts were perfused by Langendorff preparation. Contractile function was evaluated as left ventricle developed pressure (LVDP) and its first derivative (dP/dt).

Glucose was higher in blood of SHR by 59% (8.84 ± 0.3 mmol/l) compared to Wistar (5.56 ± 0.86, Р < 0.01). Fructose supply increased glucose in SHR only by 5% (9.3 ± 0.4 mmol/l). Glucose level was not affected by quercetin; however, onion skin extract decreased the level of glucose by 14%. LVDP and dP/dtmax were significantly higher in SHR. Fructose decreased dP/dtmax and dP/dtmin by 20% and 21% respectively, increased coronary flow by 30% in SHR. However, onion skin extract as well as quercetin significantly decreased LVDP, dP/dtmax and heart work compared to SHR (Р < 0.01) normalizing these parameters to the values in Wistar rats.

Our data indicate that long-term intake of meat with onion skin extract is safe and reveal hypotensive and hypoglycemic potential that might be used for development of new functional food product.
Nowadays, obesity is one of the most common pathological conditions in the world and according to World Health Organization (WHO) the overweight bothers more than 30% of the planet. It is known that obesity occurs on a background of violations of all types of metabolism in the body, but especially of the lipid metabolism and later its exacerbate effect. Endocrine glands including thyroid, have a regulatory effect on the metabolism of lipids, lipolysis and lipogenesis process, controlling the use of lipids as energy material. Deficiency of thyroid hormones is common for the regions of endemic iodine deficiency. Iodine deficiency refers to the states that serve to develop of dyslipidemia, atherosclerosis, abdominal obesity, endothelial dysfunction and other complications. The consequence of reducing the synthesis of thyroid hormones due to iodine deficiency is a dysfunction of several organs and systems, including the immune, antioxidant and free-radical processes. Iodized salt, iodine-containing tablets and dietary supplements, which contain inorganic iodine in the form of iodide or iodate, are often used to prevent hypothyroidism. Biologically active iodine supplements as a part of food supplement of Jodis-concentrate, which is similar organic iodine and safe and effective for learning thyroid gland becomes more and more popular. Therefore, the aim of research was to determine some indicators of free-radical oxidation in the blood of rats with experimental alimentary obesity (EAO) on the impact of inorganic and biologically active iodine.

The study was conducted over 45 days on 24 white female Wistar rats weighing 160-180 g, which were divided into four groups of 6 animals each. The 1st group (a control group) animals had a typical diet. Animals of 2nd, 3rd and 4th groups had EAO (Maruschak, 2016). Animals of the 3rd group were intragastric administered in the form of inorganic iodine as potassium iodide in medicine "Iodomarin" (IM) as of 0.4 μg of potassium iodide per kg of body weight a day, and animals in group 4 received biologically active iodine in the composition of "Jodis-concentrate" (J-K) as of 0.1 ml (0.4 μg of iodine) per kg of body weight a day. The blood plasma of rats was studied and it was determined the concentration of conjugated diene (cD), TBA-active products (TBp) on the background of spectrophotometric method.

Changes of free radical oxidation were found in blood plasma of researched groups of rats compared to controls. Thus, on the 45th day of the experiment plasma of rats with EAO had CD content 1.36 times higher, and the content of TBp –1.42 times higher as compared to the control group. The contents of CD and TBp in the blood plasma of the 3rd group of animals (EAO + IM) decreased, respectively, 1.13 and 1.16 times, and the animals of 4th group (EAO + J-K) 1.24 times and 1.29 times compared to animals of the 2nd group (EAO). So, biologically active iodine in the composition "Jodis-K" largely reduces CD and TBp in the blood plasma of rats with EAO (the 4th group), inorganic iodine in the "Iodomarin" (the 3rd group).
The plant research model to investigate plant-bacteria-environment interactions under mercury contamination conditions was developed. The research sources of material were coltsfoot (*Tussilago farfara* L.), a mercury hyperaccumulator plant, and coltsfoot endosymbiotic bacteria. Plants were collected from areas uncontaminated or postindustrial, contaminated with Hg, and bacteria were isolated from coltsfoot tissues, respectively. The research covered: two-seasonal collecting of coltsfoot samples as well as obtaining callus tissue and organogenesis, analyses of Hg concentration in different plant organs, determination of antioxidant properties of tissues extracts; isolation and identification of endosymbiotic bacterial strains of *T. farfara* as well as optimization of their culturing conditions and determination of Hg resistance and growth kinetics with and without Hg.

The content of Hg in soil and in tissue of different organs collected in spring (inflorescence stem, rhizome) and autumn (young and old leaves) was measured by cold vapor atomic absorption spectroscopy (CVAAS). Obtained results showed, that coltsfoot tissues contain on average 0.14 mg Hg/kg d.m. on uncontaminated and 82 mg Hg/kg d.m. on contaminated area, young leaves – 17 mg/kg d.m. and old leaves 72.5 mg/kg d.m. The antioxidant properties of contaminated and uncontaminated leaves were determined by ABTS·+ radical cation decolorization assay. Results demonstrated that there were no statistically significant differences between antioxidant properties of tissue from uncontaminated and Hg contaminated areas and reached the values 52.0-55.5% inhibition/0.1 mg of d.m. The 14 strains of bacteria colonizing plant tissue were isolated. Isolates were identified by 16S rRNA sequencing and banked. The most of strains isolated from uncontaminated plants did not show the resistance to applied Hg concentration (0.01% (w/v)).

To determine the effect of selected endosymbionts on Hg hyperaccumulation, antioxidant properties as well as secondary metabolites production by *T. farfara*, callus and subsequently sterile *in vitro* plant culture were obtained. Callus induction of coltsfoot was performed on MS medium containing 2 mg/l Picloram+2 mg/l BAP. The organogenesis on MS including BAP resulted in obtaining plantlets, subsequently cultured on MS medium.
ACCUMULATION OF ROS IN THE PRESENCE OF EXOGENOUS H₂O₂ IN HUMAN CORD BLOOD NUCLEATED CELLS DURING CRYOPRESERVATION

O. MYKHAILOVA

Institute for Problems of Cryobiology and Cryomedicine, NAS of Ukraine, Kharkiv;
E-mail: olga.o.mykhailova@gmail.com

Reactive oxygen species (ROS) are constantly generated under normal conditions in cellular metabolism. However, the cell has an extensive direct (interception) or indirect (oxidative damage reversal) antioxidant defense system for ROS inactivation. When the level of ROS exceeds the cell defense mechanisms and redox homeostasis is altered, the state of oxidative stress develops. In this state an accumulation of ROS high concentrations is observed and it can cause peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition, activation of programmed cell death pathway and ultimately leading to death of the cells.

Cryopreservation of cord blood (CB) units is the only way for long-term storage of hematopoietic stem cells (HSC) that is widely and successfully used in clinical practice for the treatment of many diseases. It is important to understand that homeostasis disruption can occur at each stage of cryopreservation technology: isolation of total nucleated cells (TNCs), which contain HSC, TNCs treatment with CPA and, especially, at freeze-thawing stage.

Thus, in this study, we assessed the redox state of TNCs at each stage of cryopreservation technology by monitoring of the intracellular ROS in the presence or absence of exogenous H₂O₂.

Dextran-isolated TNCs were cryopreserved with 5% DMSO. For redox state assessment, TNCs were incubated in the presence of 0.01 or 0.025 mM H₂O₂. Intracellular content of ROS was evaluated by flow cytometry (FACS Calibur, BD, USA) using DCFH2-DA in terms of geometric mean fluorescence intensity (MFI).

It was shown that after 15 min of incubation of whole CB and dextran-isolated TNCs with different concentration of exogenous H₂O₂ the MFI was reduced to the control level in non-treated samples. Further incubation with H₂O₂ led to more pronounced rise in MFI versus the control: 1.8 times for CB and 3.0 times for isolated TNCs. For DMSO-treated TNCs, MFI decreasing was less pronounced in first 15 min and did not reach the control remaining 3-times higher level regardless of H₂O₂ concentration. Continued incubation resulted in a 4-fold increase in the MFI versus the control level.

For freeze-thawing samples, a decrease in MFI index in first 15 min after H₂O₂ adding was not observed. Moreover, the prolonged incubation with hydrogen peroxide led to MFI decline: from 3.5 to 1.9-fold for 0.01 mM H₂O₂ and from 6.5 to 3.0-fold for 0.025 mM H₂O₂. This kind of cell response can indicate a delayed recovery of antioxidant defense system after cryopreservation. However, in the dynamics up to 45 min, the MFI index decreased to the control level in samples without exogenous H₂O₂ and became comparable with MFI index in the whole CB.

Thus, cryopreservation did not result in a significant redox homeostasis disruption in CB TNCs and activity of antioxidant system remained at the level close to the control in the whole CB.
Mitochondrial membrane potential (Δψm) plays an important role in the functioning of cardiomyocytes as the motive force for ATP synthase, which provides the energy for the contraction and relaxation of the heart. The prolonged fructose intake increases the risk of metabolic diseases (diabetes, obesity, arterial hypertension, reduced mitochondrial biogenesis), however, the effect of short fructose intake is under debate. In our research, we studied the effect of fructose diet on function cardiac mitochondria of spontaneously hypertensive rats (SHR).

Our research was conducted on six-month-old male Wistar, SHR and SHR kept at 25% fructose in drinking water for 3 months. Cardiac mitochondrial then were isolated and incubated in the medium at 37 °C. Mitochondrial respiration was induced by sodium succinate addition and registered by Clark electrode. Mitochondrial membrane potential was measured with lipolytic cation methyltriphenylphosphonium (TPMP+) and TPMP+-sensitive electrode. Δψm was calculated by the Nernst equation. Proton leak was titrated 3 times by sodium malonate additions. The ROS generation in mitochondria was measured: generation rate of superoxide radical (O2−) was determined by oxidation of cytochrome c and generation rate of hydroxyl radical (·OH) was measured using 2-deoxy-D-ribose. Data were analyzed by Mann-Whitney U-test.

Δψm of SHR cardiac mitochondria was significantly decreased (163 ± 0.7 mV vs 169 ± 1.3 mV in Wistar, P < 0.01), however, oxygen consumption did not change indicating uncoupled oxidative phosphorylation. It was accompanied by increased ROS generation, namely O2− generation rate was 9.7 ± 1.7 vs 5.2 ± 1.1 nmoles/min/mg in Wistar, P < 0.05. Fructose intake significantly increased Δψm of cardiac mitochondria of SHR (168.4 ± 1.1 mV, P < 0.01) and improved oxygen utilization by decreasing oxygen consumption (167 ± 6 vs 188.3 ± 5.6 nmoles O2/mg protein/min in SHR, P < 0.05). Additionally, ROS generation was attenuated under fructose intake: O2− generation rate was decreased (4.2 ± 1.0 vs 9.7 ± 1.7 nmoles/min/mg in SHR) as well as ·OH− generation rate (1.4 ± 0.1 vs 3.5 ± 0.4 in SHR, P < 0.05). Proton leak at 157 mV was 87.5 ± 6.4 in Wistar, 112 ± 8 in SHR and 89.7 ± 3.3 nmoles O2/mg protein/min under fructose intake, P < 0.05.

Thus, three months of fructose intake improves mitochondrial respiratory chain functioning and decreases of ROS generation in spontaneously hypertensive rats.
BIOMARKERS FOR SPECIES-SPECIFIC DETECTION OF A SOIL STRAIN OF BACILLUS MEGATERIUM 22-DESTRUCTOR OF ORGANIC COMPOUNDS

O. GORSHKOVA, M. SHTENIKOV

Odesa I. I. Mechnikov National University, Ukraine;
e-mail: 7872930@mail.ru

The progress of environmental biotechnology depends critically on the formation and constant replenishment of the stock of cultures of microorganisms possessing the ability to decompose and utilize toxic compounds has been studied. The aim of the work is to offer reliable criteria - biomarkers for species-specific detection of a non-pathogenic strain of a microorganism isolated from an oil contaminated soil that has an oxidizing ability with respect to most organic compounds (petroleum products, synthetic surfactants, phenols).

The fatty acid analysis of the strain under investigation was carried out by comparing it with known standards using an automatic microorganism identification system Sherlock (MIDI, USA) based on the Agilent 7890 gas chromatograph (Agilent Technologies, USA).

The dominant in the fatty acid profile of the bacterial strain 22 were the isomers of saturated fatty acids (56.6%), of which 16.9% and 23.3% were the C13:0 and C15:0 isomers. The total content of isomers of saturated fatty acids with an odd number of carbon atoms in the hydrocarbon radical (43.3%) was 3.25 times higher than the total isomer content of saturated fatty acids with the even number of carbon atoms in the hydrocarbon radical (13.3%). Among the fatty acids of the normal structure, fatty acids with the even number of carbon atoms in the hydrocarbon radical are found, namely: lauric (dodecanoic) C12:0, myristic (tetradecanoic) C14:0 and palmitic (hexadecanoic) acids C16:0 in an amount of 1.1, 6.6 and 2.6%. The fraction of the acids C13:0 anteiso and C15:0 anteiso from the total area of the peaks was 3.1 and 5.2%. With the total content of saturated fatty acids - 75.2% from the number of cellular lipids of the strain 22 under study, their exaggeration with respect to unsaturated fatty acids (17.8%) is quite noticeable. A characteristic feature is that with the same number of carbon atoms in the hydrocarbon radical (C16), the total proportion of saturated fatty acids C16:0 (2.6%) and C16:0 iso (4.8%) is 1.78 times less than the fraction of unsaturated C16 fatty acids: 1 w6c/C16:1 w7c (13.3%). The saturation factor (Kras), calculated from the ratio of total saturated fatty acids to unsaturated fatty acids, can serve as a biomarker value, for strain 22 it is 4.2. Biomarkers for detection of selected biochemically active strain 22 with organic compounds are hydroxy acid C15:0 2OH (ω = 0.6%) and a fragment of hydroxy acid C14:0 3OH, ω (C14:0 3OH/C16:1 iso 1) = 3, 3%.

According to the obtained fatty acid composition, deciphered using the Library RTSBA6 6.21 program Version 6.2. Of the Sherlock MIDI system, the microorganism studied was identified with a high similarity index to the genus Bacillus megaterium 22. Reliable biomarkers were detected for species-specific detection of the soil strain of B. megaterium 22-destructor of organic compounds.
THE ROLE OF FERULIC ACID IN ELICITATION
OF TRITICUM AESTIVUM RESISTANCE
TO SEPTORIA TRITICI INFECTION

I. ZHUK¹, G. M. LISIOVA², L. O. KUCHEROVA²

¹Institute of Cell Biology and Genetic Engineering, NAS of Ukraine, Kyiv;
²Institute of Plant Protection, National Academy
of Agricultural Sciences of Ukraine;
e-mail: iren_v_zhuk@ukr.net

Ferulic acid is an important compound for lignin biosynthesis. As an initiation site of plant cell wall structure it plays a key role in defense system against pathogenic agents. Ferulic acid also may be a biotic elicitor, the compound that activates nonspecific plant disease resistance to fungal infections. The usage of biotic elicitors for elicitation of defense responses may prevent increased environmental pollution by pesticides.

The aim of research was to analyze in field trials participation of ferulic acid in elicitation of winter wheat Triticum aestivum L. resistance to Septoria tritici.

Content of endogenous hydrogen peroxide, peroxidase, catalase and ascorbatperoxidase activities were measured in elicitor-treated and inoculated by S. tritici winter wheat plants (cv. Poliska 90) during different ontogenetic phases. The extent of disease development, morphometric parameters and yield structure were analyzed. The results were statistically analyzed by ANOVA.

The data obtained suggest that ferulic acid induced defense responses in winter wheat against S. tritici blotch agent. Initiation of defense responses in elicitor-treated plants occurred shortly. Hydrogen peroxide content was enhanced in elicitor-treated plants. Ascorbatperoxidase activity also increased in last wheat leaves treated by ferulic acid and infected by S. tritici. But peroxidase activity decreased and catalase stabilized under ferulic acid action in last wheat leaves infected by S. tritici. It is shown that the effect of ferulic acid stimulated plant growth and development, increased the grain quantity of winter wheat cv. Poliska 90 in field trials. It is estimated by Saari-Prescott scale that the ferulic acid influence decreased disease symptoms in winter wheat plants and enhanced the Triticum aestivum resistance Septoria tritici infection.

Ferulic acid could be used as biotic elicitor. Elicitation of biochemical nature of induced defense responses revealed the increased hydrogen peroxide content and changes of antioxidant enzyme activities for redox signal transduction, plant defense responses, lignin biosynthesis and mechanical strengthening of the plant cell walls.
The management of neoplastic cell cultures of neuroectodermal origin, as a rule, is carried out in accordance with the clear protocols attached by the cell culture banks when acquiring cell lines from them. These requirements allow accurate reproduction of the results of studies on these cell lines, rapid production of cellular responses to the agent under study, while maintaining the standard culture conditions. However, in some cases, the introduction of changes in cultivation conditions makes it possible to achieve great success in in vitro studies in view of a more vivid extrapolation of phenomena to the living organism of mammals.

So the cell line of glioma C6 of neuroectodermal origin is widely used as a model of cellular responses of fibroblast and astrocyte-like cells. The general culture conditions for conducting the confluent culture of this line are described as the DMEM or RPMI medium containing 10% fetal bovine serum (FBS). However, given that this cell line consists of four subtypes of cells (astrocyte-, fibroblast-, oligodendrocyte-like, and epithelioid cells) that can be differentiated with specific antibodies to the glial acid fibrillar protein (GFAP) and galactocerebroside.

Then in some cases it is advisable to apply the conditions of the C6 line in which the majority of cells will differentiate according to the type of astrocyte-like cells.
ORTOPHOSPHATE EFFECT ON PROTEOLYTIC ACTIVITY OF SUPERNATANTS OF CHLORELLA VULGARIS CELL HOMOGENATES

I. IL’YUCHIK, V. NIKA

Polessky State University, Pinsk, Belarus; e-mail: nikandrov.vitaly@gmail.com

It was earlier shown that the brain and liver mitochondrial fraction of mice did not cleave fibrin. The fibrinolysis was shown in the presence of inorganic orthophosphate – Pi. The increase of proteolytic activity in the presence of Pi was also demonstrated on some lymphoblast cell lines. And, judging by data of the inhibitory analysis, this effect was not bound to a resynthesis of the ATP. It allowed us to put forward the idea about existence of the ATP-independent pathway of proteolysis stimulation by Pi – ”phosphatic effect”. Further it was shown that inorganic orthophosphate (0.001-0.06 M) increased the activating function of streptokinase, urokinase, or tissue activator of a plasminogen by 50-250% and, in general, – 1.2-12.0 times lysis of a number of proteins by trypsin (T), α-chymotrypsin (cT), subtilisin (S), papain (Pa), metalloprotease of bacilli (MP), and at ≤0.004 M pepsin (Pep) as well. In higher concentration phosphate activity of Pep was sharply decreased. It suppressed lysis of Pap gelatin, gelatin and casein of MP by 40-50%. It turned out that fibrinogenolitic activity of a number of opportunistic microorganisms strains was shown only in the presence of inorganic orthophosphate.

The aim of the present work – manifestation of the feature of Pi effect on proteolytic activity of cell water-soluble fraction of a photosynthesizing alga Chlorella vulgaris.

Researches are executed on Ch. vulgaris cells, the strain of IBCE C-19 (algas’ collection of Institute of Biophysics and Cell Engineering of NAS of Belarus). Ch. vulgaris grew up in the conditions of periodic culture on the Tamiya medium at the continuous bubbling of suspension of cells air – 25 l/h; t = 25–26 °C; illuminating intensities on a vessel surface – 32 W/sq.m; to a photoperiod (light/darkness) – 12 h/12 h. After the 7th day of culture growth, we measured the cells concentration, selected their aliquots, washed with distilled water three times. Cells were homogenized with bidistilled water on ice, homogenates were centrifuged within 10 min, at 8000 rpm, at 4 °C. Proteolytic activity was determined by lysis of a fibrinogen or casein in a thin agar layer as it was earlier described. Concentration of proteins was 10 g/l, and agar – 10 g/l. As solvent for preparation of protein-agar plates we used deionized water to which Na_{2}HPO_{4}+KH_{2}PO_{4} aliquots were added. All experiments were made no less than five-fold. Results are processed statistically with calculation of a t-student criterion (Statistica-6).

Supernatants of homogenates of Ch. vulgaris cells were capable of cleavage of both proteins and in the absence of inorganic orthophosphate though the casein was hydrolyzed less intensively, than fibrinogen: 20.3 ± 0.9 and 24.5 ± 0.9 mm², respectively. However, in the presence of inorganic orthophosphate the proteolysis significantly differed. At 0.001-0.009 M Pi concentration the fibrinogenolytic activity of supernatant was reduced by 12-37% whereas at effector concentration of 0.15 and 0.45 M it increased by 21 and 27%, respectively. Changes of caseinolytic activity had a three-phase character. At concentration of an effector of 0.001 and 0.003 M this activity increased by 68 and 84%, respectively, at concentration of Pi of 0.009 M it decreased by 37%. It was noted that at Pi concentration between 0.03-0.06 M the second phase of activity increase by 51–63%

Therefore, as well as it is shown earlier, the effect of Pi depends on substrate protein. In this case, the complex concentration dependence on the effector action and the zone of proteolytic activity inhibition was observed. The causes of such picture need further researches.
ROLE OF THE proHB-EGF HEPARIN-BINDING DOMAIN IN DIPHTHERIA TOXIN BINDING

O. KRYNINA, K. MANOILOV, A. LABYNTSEV

Palladin Institute of Biochemistry, NAS of Ukraine, Kyiv;
e-mail: OlyaKrynina@gmail.com

Heparin-binding EGF-like growth factor (HB-EGF) is a member of the EGF family of growth factors. This protein binds heparin and heparan sulfate proteoglycans (HSPGs) with high affinity. Interaction with HSPGs provides HB-EGF recruitment to cell surface and stabilizes the complex with epidermal growth factor receptor (EGFR). Transmembrane precursor of HB-EGF (proHB-EGF) is the only known diphtheria toxin (DT) receptor in eukaryotic cells. It is known that the site for DT binding is located in the EGF-like domain of proHB-EGF. However, participation of the other structural parts of proHB-EGF in interaction with toxin is still unclear.

The aim of this work was to investigate the influence of heparin-binding site of HB-EGF on interaction with DT.

To reduce ability of proHB-EGF to interact with heparan sulfate, positively charged amino acids in heparin-binding domain were substituted with non-polar alanine using PCR-driven overlap extension technique. Mutant proHB-EGF sequence was cloned to plasmid vector pEGFP-N1 that allows eukaryotic cell expression of fluorescently-labeled proteins. The resulting (pEGFP-N1-proHB-EGF-mut) and reference (pEGFP-N1-proHB-EGF) constructions were used to transfect Vero cells to obtain stable expressing population of cells. Transfected cells were treated by recombinant Eschrichia coli-produced B-subunit of DT fused with red fluorescent protein mCherry.

It was determined that expression of heparin-binding deficient form of proHB-EGF was 15% lower compared to full-size form of proHB-EGF. The intensity of B-subunit binding to mutant form of proHB-EGF was 30% lower compared to non-mutated proHB-EGF.

Obtained results may suggest that the loss of heparin-binding ability of HB-EGF reduces the intensity of ligand-receptor complexes formation. We suppose that proHB-EGF interaction with HSPGs is responsible for efficient toxin internalization by mammalian cells.
Hemoglobin has been previously used as a model protein in numerous studies concerning calcium alginate microspheres preparation, their properties as well as effectiveness of their application as a delivery system were estimated. One of the restrictions in protein loaded microsphere usage is protein oxidation during hypothermic storage. Earlier it has been established that heme, which is known to be the main hemoglobin functional cite, undergoes oxidation under certain storage conditions. Hydrogen peroxide (H$_2$O$_2$) is known to be one of the reactive oxygen species acting as a key intermediate for oxidative stress development. Thus the reaction between hemoglobin and hydrogen peroxide results in degradation of the protein heme. Herewith the addition of antioxidant enzyme, catalase, to the hemoglobin solution has been shown to limit the extent of this reaction. Catalase was also reported to be successfully encapsulated in alginate microspheres without losing its functional activity. The constant ratio between hemoglobin concentration and catalase activity was demonstrated in erythrocytes. The isoelectric points of these proteins lay in the same pH area (6.8 for human hemoglobin, 7.0 for human blood catalase). Therefore, our assumption was that the effective encapsulation of hemoglobin and catalase into alginate microspheres will occur under the same conditions.

Hemoglobin and catalase loading in alginate microsphere were carried out by ionotropic gelation. Hemoglobin concentration in hemolysate was assessed spectrophotometrically at 540 nm. Hemoglobin degradation in microspheres in the presence of H$_2$O$_2$ (6 mM) was evaluated recording absorbance at 413 nm. H$_2$O$_2$ concentration was assessed using ammonium molybdate.

It has been established that the amount of encapsulated hemoglobin correlates with the hemolysate concentration. In these conditions the catalase activity, determined by the level of peroxide in the solution and by ability to prevent hemoglobin degradation in microspheres, strongly depends on hemoglobin concentration.

Experimental data have revealed the possibility of obtaining microspheres containing both, hemoglobin and catalase. In so doing catalase was shown to protect hemoglobin against peroxide deleterious action. Herewith, the usage of high hemoglobin concentration decreases the level of catalase incorporation into alginate microspheres.
Mutagenesis probe into editing mechanism of D-aminoacyl-tRNA deacylase from Thermus Thermophilus

M. Rybak, A. Rayevsky, O. Kovalenko, M. Tukalo

Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv;
e-mail: mariia.rybak@gmail.com

D-aminoacyl-tRNA-deacylase (DTD) exists as an additional checkpoint in the machinery of protein biosynthesis control. It hydrolyzes the ester bond between D-amino acids and tRNA, having mistakenly bound during the activation step. DTD is specific toward different D-aminoacyl-tRNA substrates (D-Tyr, D-Phe, D-Trp, D-Asp-tRNA) (Calendar and Berg 1967; Soutourina, Plateau et al. 1999; Zheng 2009), strictly discriminating the chirality of amino acid (Ahmad, Routh et al. 2013). Interestingly it was recently found that this enzyme could also cut achiral Gly from its cognate tRNA and misacylated tRNAAla (Routh, Pawar et al. 2016; Pawar, Suma et al. 2017). This fact suggests DTD’s role in glycine deacylation, preventing from its cellular toxicity. Despite the interest to this enzyme, its editing mechanism still requires analysis and investigation.

We performed molecular modelling by AUTODOCK and Modeller and molecular dynamics (MD) simulations by Gromacs and VMD based on reported crystal from Plasmodium falciparum bound to substrate analogue D-Tyr-3AA (Ahmad, Routh et al., 2013). The results after 5ns were analysed for site-directed mutagenesis studies. Mutagenesis was done by QuickChange Stratagene Kit. All substitution mutants were tested in deacylation assay with α-[32P]-radiolabelled-tRNATyr.

The comprehensive analysis of wild-type enzyme and its mutants’ activity divided them into 3 groups: 1) with significantly decreasing editing activity (Q78A, F79A, Y125A, G137A, P138A, P138H); 2) with significantly increasing activity (Y125F, A127M); 3) with the same activity comparing to WT (S77A, V124A, A127V, V139A). Those mutants belong to several conservative elements from prokaryotes to eukaryotes: -SFQL- and -Gly-cis-Pro- (enantioselective) motif, and some separate amino acids from proposed catalytic site (Y125, A127). Steady-state kinetic parameters of the deacylation reaction were determined for WT, Y125A, Y125F and S77A, showing 10-fold less (Y125A) and 100-fold higher (Y125F) catalytic velocity than WT DTD.

Based on the results of mutagenesis experiments and MD data, a preliminary idea for deacylation mechanism catalysed by DTD was suggested.
Snake venoms are the natural source of proteases targeted to fibrinogen and fibrin (fibrinogenases) that can be used for obtaining functionally active fragments of fibrinogen for further study in vitro as well as for the direct defibrination in vivo. The aim of the present study was to determine the target of proteolytic action of fibrinogenase from the venom of Agkistrodon halys halys (Halygenase) and study the effect of proteolysis on fibrinogen-dependent aggregation of platelets.

Halygenase was purified from the crude venom of Agkistrodon halys halys using two-step chromatography on Blue-Sepharose followed by Q-sepharose substrate specificity of Halygenase was determined by chromogenic substrate assay using S2251 (D-Val-Leu-Lys-pNA), S2238 (H-D-Phe-Pip-Arg-pNA), S2765 (Z-D-Arg-Gly-Arg-pNA). Products of hydrolysis were characterized by SDS-PAGE under reducing conditions with following Western-Blot using monoclonal antibodies ІІ-5С and 1-5А with epitopes in Аα20-78 and in Аα504-610, respectively. Fibrinogen fragments cleaved by Halygenase were detected by MALDI-TOF analysis using a Voyager-DE and identified using software “Peptide Mass Calculator”. Aggregation of washed platelets was studied using aggregometer SOLAR-2110.

Amidase activity assay showed that the halygenase was mostly specific towards peptide bonds formed by C-group of lysine. It was shown that it preferentially cleaved the Аα-chain of fibrinogen splitting off the peptide with apparent molecular weight of 20 kDa. Western-Blot analysis using monoclonal antibody recognized the cleaved fragment as the C-terminal part of Аα-chain of fibrinogen. MALDI-TOF followed by in silico analysis with “Peptide Mass Calculator” identified this peptide as fragment Аα414-610 of fibrinogen molecule. It was shown that in the presence of fibrinogen desАα414-610 the rate and speed of platelet aggregation were decreased by 63 ± 11% and by 40 ± 7%, respectively in comparison to aggregation in the presence of native molecule.

It was demonstrated that protease from the venom of Agkistrodon halys halys preferentially cleaves the peptide bond АαThr-Glu-Lys413-Leu414 of fibrinogen Аα-chain thus cleaving-off the C-terminal half of αC-domain. Its proteolytic action dramatically impaired fibrinogen-dependent aggregation of washed human platelets. Thus we can consider protease from the venom of Agkistrodon halys halys as a prospective agent for study of interaction between fibrinogen and platelets and possibly as the useful tool for anti-aggregatory action in vivo.

Acknowledgment. Prof T. Platonova for her support in the development of the ideology of experiments. To Dr E. Lugovskoy for his help with the production and selection of antibodies used in the study.
Aminoacyl-tRNA synthetase complex-interacting multifunctional protein 1 (AIMP1/p43) is an auxiliary component of aminoacyl-tRNA synthetase complex of higher eukaryotes. Outside of the complex AIMP1/p43 shows pleiotropic cytokine activity, modulates the proliferation of different types of cells, suppresses angiogenesis and stimulates apoptosis and inflammation. Since the spatial structure of full-length protein has not been established yet, the specific physical nature of protein conformational changes and their contribution to the functional activity of the AIMP1/p43 protein remain largely unknown.

The purpose of this work is the AIMP1/p43 protein nanosecond dynamics studies at the temperature interval from 25 to 50 °C by steady state fluorescence spectroscopy technique. AIMP1/p43 was expressed in the bacterial system using pET28b vector and Escherichia coli BL21(DE3) cells. The protein has been purified to homogeneity by metal-chelating chromatography. In this work we used the intrinsic fluorescence of the single tryptophan residue Trp271 in the AIMP1/p43 structure as a probe to monitor the protein conformational change during thermal denaturation. The dynamics of the microenvironment of Trp271 was investigated by the red-edge excitation shift effects.

The fluorescence emission spectrum of AIMP1/p43 on excitation at 295 nm reveals a maximum position at 331 ± 1 nm that indicates the mainly buried state of fluorophore in the protein globule. A significant gradual red-edge excitation shift effect which is about 9 ± 1 has been detected in the range of the excitation wavelengths from 290 till 304 nm at 25 °C. The magnitude of the red-edge excitation shift begins decreasing at 37 °C and practically disappears at 48 °C. The disappearing of magnitude indicates the disruption of the Trp271 specific microenvironment in native protein globule in this temperature range.

Based on the obtained results it is proposed that such conformational rearrangement of the Trp271 microenvironment which began at physiological temperature may be essential for the following binding with the partner molecules during recognition process.
FUNCTIONING AND PHYSIOLOGICAL ROLE OF NICOTINIC ACETYLCHOLINE RECEPTORS IN MITOCHONDRIA

K. USPENSKA, O. LYKHMUS, M. SKOK

Palladin Institute of Biochemistry, NAS of Ukraine, Kyiv; e-mail: kate.uspenska@gmail.com

Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels, which regulate synaptic transmission in muscles and neurons, as well as cell viability and proliferation in many non-excitable cells. Previously we reported the presence of α3β2, α4β2 and α7(β2) nAChRs in mitochondria, where they control the early stages of mitochondria-driven apoptosis like cytochrome c release under the effect of Ca\(^{2+}\) or H\(_2\)O\(_2\). Mitochondrial nAChRs function in ion-independent manner by activating intramitochondrial kinases through conformational changes caused by the binding of either agonist or competitive antagonist. The aim of the present study was to delineate structural requirements for activation of different signaling pathways in mitochondria and to evaluate physiological role of mitochondrial nAChRs.

Experiments have been performed in mitochondria isolated from rodent liver, lung or carcinoma and from human thymus by differential centrifugation according to standard procedures. The level of nAChR subunits in mitochondria detergent lysates was studied by Sandwich-ELISA using subtype-specific antibodies and the apoptogenic effect of Ca\(^{2+}\), H\(_2\)O\(_2\) or wortmannin was evaluated based on the level of cytochrome c released from liver mitochondria.

It was found that the binding of nAChR-specific type 2 positive allosteric modulators (PAMs) was sufficient to attenuate cytochrome c release from mouse liver mitochondria in the absence of orthosteric agonist. Moreover, binding of α7 nAChR-specific PAMs PNU120596, PAM-2 or 4BP-TQS prevented the apoptogenic effect of Ca\(^{2+}\), while β2-specific desformylflustrabromine was effective against wortmannin-induced cytochrome c release. We suggest that α7 nAChR subunits regulate CaK-MII-dependent, while β2 subunits control PI3K-dependent signaling pathways in mitochondria.

We have found increased levels of α7 nAChRs in mitochondria of human thymoma compared to normal thymus and in Lewis carcinoma compared to normal mouse lung. Experiments with mitochondria of regenerating liver in rats demonstrated the increase of not only α7-, but α3-, α4- and, especially, α9-containing nAChRs 3-6 h after partial hepatectomy resulting in increased mitochondria resistance to 0.1-0.9 μM Ca\(^{2+}\) and 0.1-0.5 mM H\(_2\)O\(_2\). These data demonstrate a physiological significance of mitochondrial nAChRs to support the proliferating cell viability in the course of liver regeneration or tumor growth.
OXIDIZED LIPIDS SUPPRESS AMYLOID FIBRIL FORMATION: SEARCHING FOR A MECHANISM

K. VUS1, V. TRUSOVA1, G. GORBENKO1, P. KINNUNEN2

1V. N. Karazin Kharkiv National University, Ukraine;
2Aalto University, Finland;
e-mail: kateryna_vus@yahoo.com

Preventing of amyloid fibril formation in vivo has long been a focus of extensive studies, since this process plays a key role in a molecular etiology of Alzheimer’s disease, type II diabetes, systemic amyloidosis, etc. However, effective strategy of treating these diseases is still not developed.

This study was aimed at assessing the inhibiting effects of oxidatively modified phospholipids, viz. 1-palmitoyl-2-azelaoyl-sn-glycero-3-phosphocholine (PazePC), and 1-palmitoyl-2-(9′-oxononanoyl)-sn-glycero-3-phosphocholine (PoxnoPC) on the protein fibrillization.

Lipid dispersions of PazePC and PoxnoPC in sodium phosphate buffer were prepared using a bath sonicator. Alternatively, the extrusion technique was employed to obtain liposomes composed of 80 mol% phosphatidylcholine (PC) and its mixtures with PazePC (20 mol%) or PoxnoPC (20 mol%), referred to here as PazePC20 and PoxnoPC20, respectively. The lysozyme (insulin) fibrillization was initiated at pH 2 (7.4), 60 °C, at lipid concentration 16 µM. The kinetic parameters for amyloid fibril formation, viz. lag time, apparent rate constant for the fibril growth (k) and maximal fluorescence of the dye (Fmax), were obtained by approximation of the time dependence of the Thioflavin T (ThT) fluorescence intensity at 480 nm with the sigmoidal curve.

It appeared that Fmax values of lysozyme- (insulin-) bound ThT were about 20% (86%) lower in the presence of PazePC20/PoxnoPC20, as compared to those in control samples. The revealed effects were attributed to the decrease in the extent of amyloid fibril formation. The inhibition of lysozyme aggregation was accompanied by the reduction of the lag time and increase of the k values, while the opposite effect was observed for insulin. Furthermore, lipid vesicles, containing PazePC and PoxnoPC, inhibited protein aggregation into mature fibrils, unlike lipid dispersions, highlighting the important role of the polar surfaces of the lipids in the reduction of the protein fibrillogenesis. The obtained results point to significant impact of PazePC-lysozyme hydrophobic interactions on the inhibition of the protein fibrillogenesis. In turn, Shiff bases could be formed between insulin monomers and PoxnoPC domains of the lipid vesicle, thereby stabilizing an aggregation-resistant protein conformation.

In conclusion, our findings provide a basis for comprehensive testing of oxidized lipids as potential anti-amyloid agents.
MOLECULAR BASIS OF PHYSIOLOGICAL FUNCTIONS

REDISTRIBUTION OF DNA LOOP DOMAINS DURING TRANSCRIPTION ACTIVATION AND MALIGNANT TRANSFORMATION AS REVEALED BY COMET ASSAY

M. CHOPEI, V. OLEFIRENKO, K. AFANASIEVA

Taras Shevchenko National University of Kyiv, Ukraine;
e-mail: mariana.chopei@gmail.com

Recent studies indicate that DNA loop domains are not only the key element at higher level of chromatin organization but also involved in various functional activities within cell nucleus. In addition to conservative loop domains (e.g., associated with nuclear lamina), many loops may appear as a result of transcription regulation, DNA replication and repair. Therefore, the distribution of the loop domains between different fractions varies in dependence on functional state, phase of the cell cycle and cell type. The aim of this study was to investigate peculiarities of the loop domain organization in lymphocytes at different stages of their activation and in human glioblastoma cells.

Human lymphocytes were isolated by centrifugation in density gradient and transformed with interleukin-2 to obtain lymphoblasts. Human glioblastoma cells (T98G) were grown in DMEM and then synchronized at G1 phase of cell cycle by incubation in serum-free medium for 48 hours. The organization and redistribution of DNA loop domains in these cells were investigated using kinetic approach of single-cell gel electrophoresis (the comet assay). We estimated the DNA amount in the comet tails and contour length of the largest loops in the tails depending on the electrophoresis duration.

We observed significant differences in the kinetics of DNA exit during electrophoresis between all the cell types investigated. First of all, for T98G cells and lymphoblasts at 44th hour after transformation (G2 phase of cell cycle) the decreasing in the maximum amount of DNA that can migrate into the comet tail was observed in comparison with inactive lymphocytes (G0 phase) and lymphoblasts at 24th hour after transformation (G1 phase). This difference was probably due to a decrease in the number of loops with contour length from 50 to 150 kb. Secondly, the amount of small (up to 30 kb) surface loops was almost two-times higher for both types of lymphoblasts in contrast with lymphocytes. Moreover, the correlation between the DNA amount in the comet tails and the tail length implies that the contour length of the loops is distributed exponentially and distribution parameter, the loop density, is dependent on the cell type.

Our findings indicate that the comet assay may be applied to detect some DNA loops redistribution in cells with different transcriptional activity or at various stages of cell cycle.
The mechanisms of HIF3α subunit effect on adaptation to physical exercise remain to be uninvestigated.

In our experiments we knocked down Hif3α using siRNA to study rats endurance capacity. Real-time PCR analysis was performed for quantitative evaluation of HIF3α, IGF1, GLUT-4 and PDK-1 in m. gastrocnemius, m. soleus, in the lung and heart tissues. Mitochondrial respiratory function and electron microscopy were performed.

The knockdown of Hif3α using siRNA increases the time of swimming to exhaustion 1.5 times.

The level of mitochondrial NAD- and FAD-dependent oxidative pathways is decreased, however efficiency of phosphorylation is increased after HIF3α siRNA treatment. Some destructive changes in muscle tissue were detected in animals with siRNA-inducing silencing of Hif3α.

Optimization of oxidative phosphorylation in mitochondria and increase of HIF dependent gene (Pdk-1) expression explain the effect of Hif3α silencing that led to the significant increase of endurance capacity of rats.
Bacteriophages are regarded as promising tools for the control of bacterial pathogens. Viruses with broad host range, able to infect not only different strains, but even genera of bacteria are referred as polyvalent. Two bacteriophages of Podoviridae family, E105 and TT10-27, obtained from amylovora-like bacteria Erwinia “horticola” and isolated from plant material, affected with fire blight (Erwinia amylovora), respectively, are polyvalent. They perform productive infection in 2 different species of bacteria: both phytopathogenic E. “horticola” and plant-associated Pantoea agglomerans.

Determination of the mechanisms underlying the expansion of host range is a keystone of phage characterization and gives insight into virus/host interaction details. To reveal them, phages’ DNA was sequenced and analyzed. Methods of DNA sequencing and Bioinformatics were used.

Sequence analysis revealed that E105 belongs to genus of phiKMV-like phages. Its DNA composition is highly unique and shares high percentage of similarity only with 1 genome in GenBank, of Pantoea phage LIMElight (NC_019454.1). E105 DNA of 43,856 bp with direct exact terminal repeats is comprised of 55 ORFs, 54 of which are protein coding sequences (CDS) and 1 is a tRNA gene. They are placed on plus-strand and can be divided into early/ middle and late genes regions. Phage DNA possesses high GC% content – 54.46%.

Phage TT10-27 is a representative of N4-like phages; its close relatives are Erwinia amylovora phages Frozen (NC_031062.1) and Ea9-2 (NC_023579.1). Its DNA is larger (74,143 bp), features direct various repeats, contains 90 ORFs, including 4 tRNA genes and at least 1 gene of bacterial origin; 86 CDS are transcribed from plus-strand (early and middle genes) and minus-strand (late genes). Phage DNA codes heterodimeric T3/T7-like RNA polymerase, as well as virion RNAP, ORF68, huge protein of 3491 aa that is packed into virion along with DNA. GC % content of DNA is 46.8%.

Though phages’ E105 and TT10-27 DNA sequences reveal no significant similarity to each other, they both code for a protein, responsible for interaction with host cell surface that possesses depolymerase activity. ORF47 (E105) of 857 aa and ORF83 (TT10-27) of 881 aa reveal homology to EPS-depo (extracellular polymeric substances/exopolysaccharide depolymerases) of various Erwinia phages of Podo-, Myo- and Siphoviridae families. E105/TT10-27 EPS-depo protein sequences align in C-terminal region (responsible for interaction with host), and share no similarity in N-terminus, that is responsible for protein attachment to phage virion. Predicted tertiary structure of C-termini of both proteins coincides with crystal structures of hydrolases (galacturonidases).

Broad host range of viruses E105 and TT10-27 is determined by their attachment apparatus, mainly its EPS-depolymerase activity. Thus, phages of different genera, with different morphology, strategies of transcription/replication share similarly organized EPS-depo protein that allows for their polyvalent feature. Further study of these phage-coded depolymerases can provide the background for antibacterial tools construction against phytopathogens.
CARDIOPROTECTIVE EFFECTS OF PAG ADMINISTRATION

R. FEDICHKINA, Y. GOSHOVSKA, V. SAGACH

Bogomoletz Institute of Physiology, NAS of Ukraine, Kyiv; e-mail: fedichkina@biph.kiev.ua

Hydrogen sulphide (H$_2$S) is gaseous transmitter that causes many effects in organism including reduction of infarct zone of myocardium and improving vasorelaxation. Exogenously applied H$_2$S donors protect the heart against ischemia-reperfusion (I/R). In cardiovascular system H$_2$S is produced from amino acid L-cysteine mainly by cystathionine-gamma-lyase (CSE). CSE can be inhibited with DL-propargylglycine (PAG). Earlier we demonstrated that L-cysteine alone had no significant effect on cardiac function, however, PAG increased resistance of myocardium to ischemia. Pre-treatment of PAG and L-cysteine manifested in strong cardioprotective effects. We decided to investigate how biochemical indexes changed in heart tissue. Six month Wistar rats were divided into four groups: 1 – control, 2 – hearts after 20 min of ischemia and 10 min of reperfusion (I/R 20/10), 3 – PAG (11.3 mg/kg 10 min) + L-cysteine (121 mg/kg 30 min) without I/R and 4 – PAG+L-cysteine after I/R 20/10. The hearts were isolated by Langendorff preparation. The heart tissue was examined for superoxide radical, hydroxyl radical, peroxide, peroxide radical and diene conjugates content, nitrate, nitrite and H$_2$S levels, iNOS, eNOS and H$_2$S-synthesizing enzyme (CSE+CBS) activity. I/R caused oxidative stress in terms of increased ROS production. The 2.4-fold increase in diene conjugates indicated intensified lipid peroxidation in I/R cardiac samples probably due to the significant increase in hydroxyl radical (˙OH) generation rate. That was absent in I/R group pretreated with PAG+L-cysteine. Lower activity of H$_2$S producing enzymes and H$_2$S level was observed in group 4 vs 3. The activity of constitutive synthesis of NO decreased 5.5 times in 2 group and was 2-fold renewed in 4 vs 2. NO production changed in the same manner. There was neither increase in iNOS activity nor NO$_3$-levels elevation in group 4. PAG+L-cysteine significantly decreased reactive oxygen species production and diene conjugates. Thus, pre-treatment with PAG and L-cysteine showed strong protection against oxidative stress and reperfusion injury induced by ischemia.
THE ROLE OF C-DI-GMP IN THE PSEUDOMONAS AERUGINOSA RHAMNOLIPIDS BIOSYNTHESIS

M. GALKIN, S. SEMENETS, D. DUSHENKOVSKII, V. KLANDNITSKII

Odessa I. I. Mechnikov National University, Ukraine;
e-mail: volandaron@ukr.net

**Biological Context**

![Biological Context Image](image)

**Text Content**

 Bis-(3-5')-cyclic dimeric guanosine monophosphate (c-di-GMP) is in the spotlight of the scientists as the result of last achievement of microbial genomics and great interests in microbial communities. Cytoplasmic c-di-GMP is a bacterial secondary messenger, that regulates numerous of physiological processes: cell-to-cell communication, biofilm formation, motility, virulence, etc. Depends on concentration of this regulator bacteria shifts its life-form from motile to sessile (biofilm formation). It is found that c-di-GMP affects all stages of the biofilm formation process in *Pseudomonas aeruginosa* from the beginning of adhesion to biofilm decay. This compound regulates biosynthesis of matrix components, quorum sensing signal molecules, biosurfactants. That fact, that the direction of many processes change in bacterial cells depends on c-di-GMP, led to hypo- and hyperproduction strains construction. Their use allows extending knowledge in the role of this compound in many bacterial cells processes and the possibility to use this molecule as an instrument for biofilm formation control.

The aim of this work was to study the ability to produce rhamnolipids by *P. aeruginosa* strains with different levels of c-di-GMP synthesis. This was used mutant strains: PA01 pJN2133 with very low biosynthesis of c-di-GMP and PA01 wsp F1 with overproduction of this second messenger. For comparison, using wild-type strain of *P. aeruginosa* PA01. The incubation was carried out in LB medium at 37 °C with shaking at 150 rpm/min.

The results show that during the first five days of incubation rhamnolipids content in the environment was maintained at a level that was reached after 24 hours. This pattern of accumulation of surfactant was inherent in all three strains. But for further incubation (the seventh day) the picture changed. When strains PA01 and PA01 pJN2133 rhamnolipids content increased significantly compared to the previous study period (5 days), 3 and 4 times, respectively. The level of surfactant biosynthesis strain PA01 wsp F1 has not changed. It should also be noted that the strain with low level of c-di-GMP in all study periods produces more rhamnolipids than *P. aeruginosa* PA01. On the seventh day the difference between them was 60%.

Previously we found that *P. aeruginosa* PA01 pJN2133 cell is more mobile in comparison to *P. aeruginosa* PA01 cell and have low adhesion and biofilm formation abilities. We assumed that these features of the strain PA01 pJN2133 are due to its ability to synthesize large amounts of rhamnolipids. This was confirmed in this study.

*P. aeruginosa* strains with reduced biosynthesis c-di-GMP are potential candidates for the biosurfactant production.
Hypothermia might induce damage as well as increase of cardiac tolerance to ischemia. Such divergence obviously depends on the degree of temperature increase. Hyperthermia-induced cardiac tolerance is greatly determined by ROS and heat shock proteins implications. On the other hand, one of the main targets for cardioprotection is mitochondrial permeability transition pore (MTPP), which is not described in hyperthermia. We studied two regimes of hyperthermia in order to estimate the dependence between hyperthermia effects at heart function and MTPP opening.

We used Wistar male rats aged 6 months. Isolated hearts were perfused by Langendorff preparation with on-line registration of left ventricular developed pressure (LVDP), coronary flow and evaluation of oxygen utilization by the myocardium. Hyperthermia was modeled as the increase of perfusion solution temperature from 36.5 to 39.5 °C or 41.5 °C during 15 min before 20 min total ischemia and 40 min of reperfusion (I/R). “Opened/closed” state of MTPP was evaluated by UV-measuring of the levels of mitochondrial factor (MF) which released from the inner mitochondrial space into solutions outflow from the coronary vessels of the isolated heart.

In our experiments I/R strongly depressed cardiac contractile activity of isolated rat hearts \((n = 5)\). At the 40th min of reperfusion the average values of LVDP, dP/dtmax and coronary flow recovered only to 30, 35 and 75%, respectively from the initial values. However, the contractile activity of hearts which underwent 15 min of 41.5 °C hyperthermia was not renewed at all \((n = 4)\). On the contrary, hearts which underwent 39.5 °C hyperthermia \((n = 6)\) showed 68%, 71%, 90% recovery of LVDP, dP/dtmax and coronary flow, respectively. At the 5th min of reperfusion oxygen cost of myocardial work was increased 11.3 times compared to 3.2 times in 39.5 °C hyperthermia pretreated hearts. The MF level in outflow solutions collected at the 1st min of reperfusion was significantly lower compared to non-hyperthermia hearts.

Thus, 41.5 °C hyperthermia depresses the heart function and aggravates reperfusion injury of isolated rat heart, whereas 39.5 °C hyperthermia reveals cardioprotective effect in terms of contractile activity restoration and optimization of oxygen utilization by ischemized myocardium and greatly decreases MTPP opening at the reperfusion.
The nuclear membrane forms a semi-permeable barrier for the movement of molecules and ions. Transport between the cytoplasm and nucleoplasm occurs through a large number of nuclear pores and ion channels with different biophysical properties.

In this work we have studied the biophysical properties of the ion channels in the nuclear membrane of cardiomyocytes. Nuclei from cardiomyocytes were isolated by homogenization. Single ion channels were recorded from nucleus-attached and excised patches of the nuclear membrane in the voltage-clamp mode of the patch-clamp technique.

We have registered many ion channels with different properties in the nuclear membrane of cardiomyocytes, including Lcc-channels, and inositol-1,4,5-trisphosphate receptors. Our results indicate that Lcc-channels were selective for monovalent (K' and Na') cations and demonstrated voltage dependence. At positive potentials, the activity of these channels is significantly more intense than at negative potentials. In symmetrical KCl solution, the slope conductance of the Lcc-channels in the nuclear membrane of cardiomyocytes was 209 ± 13 pS (n = 44). Considering biophysical properties such as conductance, kinetics and voltage dependence, we conclude that in the nuclear membrane of cardiomyocytes are expressed similar or identical Lcc-channels, which we had identified and described earlier in the nuclear membrane of neurons.

We have also first registered IP3Rs in the native nuclear membrane of cardiomyocytes, which differ in their properties from all other isoforms of IP3Rs. The slope conductance of the IP3Rs in the nuclear membrane of cardiomyocytes in symmetrical KCl solution was 384 ± 5 pS (n = 4). All known types of IP3Rs are blocked by Ca²⁺ in concentrations less than 1 μM, however, our experiments showed that IP3Rs of cardiomyocytes were not blocked by high concentration of Ca²⁺ (up to 100 μM) in solution. It is known that IP3R2 prevail in the nuclear membrane of cardiomyocytes, therefore we assume that we recorded receptors of type 2 in this membrane.

Therefore, we have found in the native nuclear membrane of cardiomyocytes Lcc-channels. We are the first to register the inositol 1,4,5-trisphosphate receptors and to show that they are functional. Also, we found many types of ion channels with different conductivity, which have yet to be investigated.

The publication is based on the research provided by the grant support of the State Fund for Fundamental Research (F-70, project 17884).
THE EFFECT OF BUTHIONINE SULFOXIMINE ON REALIZATION OF CARDIOPROTECTIVE EFFECT OF ISCHEMIC PRECONDITIONING

D. KURNEVA1, Y. GOSHOVSKA2, R. FEDICHKINA2, V. SAGACH2

1National University of “Kyiv-Mohyla Academy”, Kyiv, Ukraine;
2Bogomoletz Institute of Physiology, NAS of Ukraine, Kyiv;
e-mail: dianakurneva21@gmail.com

The phenomenon of ischemic preconditioning (IPre) is a known powerful protective strategy that is implemented in many experimental models in vitro, in situ or in vivo. It is well established that the main target of cardioprotective effect of IPre is mitochondrial permeability transition pore (MPTP) that is opened under reperfusion. Since IPre demonstrates antioxidant effects, we hypothesized that IPre might be realized via activation of glutathione system. Thus, the aim was to evaluate the effect of glutathione synthesis inhibitor - buthionine sulfoximine – BSO at protective action of ischemic preconditioning in isolated rat heart model.

We used 6 month Wistar male rats. BSO (22 mg/kg, Sigma) was injected intraperitoneally (for 40 min). We registered cardiodynamic parameters (left ventricular pressure (LVP), dP/dt, heart rate, coronary flow) and oxygen utilization by Langendorff isolated hearts. IPre (3 episodes of 5 min ischemia and reperfusion) was provided before 20 min of ischemia and 40 min of reperfusion. The levels of mitochondrial factor (as an indicator of MPTP opening) were measured as increased UV-optical density (OD) of coronary solutions outflow the isolated hearts before and after ischemia.

The data showed that the initial values of heart indexes (LVP, heart rate, coronary flow, dP/dt and oxygen cost) did not differ in control (n = 5) and IPre (n = 7) groups as well as pretreatment with BSO (n = 7). IPre induced a significant restoration of contractile function of isolated rat heart specially in the early postischemic period: at the 10th min of reperfusion LVP was 82.5 ± 8.8% vs 32.7 ± 7.9% in control. In BSO+IPre group the LVP restored to 70.6 ± 7.9%. The relaxation index (dP/dt min) was 78.7 ± 5.8% in IPre, 89.4 ± 16.6% in BSO+IPre vs 28.0 ± 6.7% in control group. Ischemia-reperfusion induced a considerable increase of OD of outflow solutes collected at the 1st min of reperfusion with “peak” of absorbance at 245-250 nm. It was found that IPre strongly prevent appearance of the “peak”. Pretreatment with BSO slightly increased OD of coronary effluents at the 1st min of reperfusion, however, it was significantly lower than in control.

Thus, one-time administration of glutathione inhibitor BSO at least in selected dose does not eliminate the protective effect of IPre in terms of heart function restoration. Moreover, the level of mitochondrial factor was not affected by BSO treatment indicating strong realization of IPre-induced protective program.
**DIADINOXANTHIN DE-EPOXIDATION KINETICS IN ARSENIC TREATED PHAEODACTYLM TRICORNUTUM**

*S. LISTWAN, W. TOKAREK, D. LATOWSKI*

Department of Plant Physiology and Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland; e-mail: stanisezaw.listwan@student.uj.edu.pl

*Phaeodactylum tricornutum* is a marine diatom capable of conducting the reactions of the diadinoxanthin cycle. This mechanism is believed to play a crucial role in the photoprotection of algal photosystems and is based on the de-epoxidation of diadinoxanthin (Ddx) with the production of diatoxanthin (Dtx) under the intense light conditions, and the epoxidation of Dtx to Ddx, when amount of light absorbed by diatoms falls to lower level. The aim of this work was to evaluate the influence of arsenic stress on diadinoxanthin cycle. *Phaeodactylum tricornutum* was cultured in the f/2 Guillard’s medium. As a source of arsenic, sodium arsenate (Na₃AsO₄) was used. After the growth in media containing different concentrations of sodium arsenate, the cultures were exposed to intensive light and samples of media were collected after 5, 10, 15, 25 and 45 minutes of exposition. Additionally for the analysis of the rate of Dtx epoxidation, the samples after 45 min of strong light illumination were incubated for 4 hours under weak light condition. All the samples were immediately frozen in liquid nitrogen, stored in -80 °C and then the content of Ddx and Dtx was measured by HPLC with photodiode array detector. The ratio of Dtx content to the sum of Ddx and Dtx contents (DES) was determined. The effect of arsenic was observed both on epoxidation and de-epoxidation reaction in *P. tricornutum* cells. Data showed higher de-epoxidation rate, measured as the change of DES value over the course of illumination, in diatoms cultured with the addition of arsenic. Moreover, such treatment, at any tested concentration, caused the increase of DES value of non-illuminated cells, compared to the cells not treated with arsenic.
Hydrogen sulfide (H₂S) is one of the three biological gaseous mediators, which takes part in regulation of variety of functions in cardiovascular system. H₂S is produced enzymatically by three different enzymes: cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3-MST), which works in tandem with cysteine aminotransferase (CAT). Because of the fact that the last two enzymes are located in mitochondria and oxidation of H₂S also takes place in mitochondria, it becomes clear that mitochondrial origin H₂S plays a great role in the cardiac and vascular functions regulation. However little is known about the impact of mitochondrial origin hydrogen sulfide on heart resistance to calcium overload and the sensitivity of MPTP to this cation.

To investigate the role of mitochondrial origin H₂S in cardiac function under calcium overload and in Ca²⁺-induced MPTP opening in rat hearts.

In our work we used adult (5-7 months) Wistar rats. Cardiodynamic parameters such as left ventricular pressure (LVP), dP/dt, heart rate, coronary flow and oxygen consumption were registered using Langendorf isolated rat heart. Calcium load was carried by adding of CaCl₂ in perfusion solution every 10 min until the concentration of calcium increased from 1.7 to 12.5 mmol/l. Rat heart mitochondria were isolated using differential centrifugation method. MPTP opening was registered spectrophotometrically as mitochondrial swelling.

It was shown that the inhibition of mitochondrial H₂S-synthesis enzyme had a negative influence on initial cardiodynamic enzyme. In particular, the LVP and the rate of contraction and relaxation of myocardium decreased twice. Coronary blood flow decreased by 1.12 times, while the heart rate was tended to increase. We found that the hearts of experimental animals developed less powerful reaction under the calcium overload that manifested in reduced parameters of LVP, coronary flow and heart work intensity. The inhibition of 3-MST by O-CMH (in vitro and in vivo) causes significantly dose-dependent increase of Ca²⁺-induced mitochondrial swelling in adult rat heart. The highest concentration of inhibitor (10⁻³ mol/l) that were used increased this parameter 2.3 times.

H₂S which is synthesized in mitochondria has the great impact on regulation of cardiac functions, in particular on cardiac resistance to Ca²⁺ overload and on calcium-induced MPTP opening in rat heart.
Hydrogen sulfide (H$_2$S) as well as nitric oxide belong to gas transmitters’ family and play an important role in vessels’ tone regulation. It was shown that H$_2$S reveal cardio- and neuroprotective properties preventing extensive ROS generation and apoptosis. H$_2$S is synthesized from aminoacid L-cysteine by cystathionine-β-synthase (CBS), cystathionine-γ-lyase (CSE) localized in cytoplasm and 3-mercaptopyruvate sulfurtransferase, mitochondrial enzyme which is coupled with cysteine aminotransferase enzyme (CAT). CBS, CSE and CAT have pyridoxal-5-phosphate (P5P) as co-factor. Earlier we showed decrease of H$_2$S synthesis with aging, however, role of H$_2$S in vessels function in aging is still poorly understood. The aim of current work was to study the effect of P5P administration at H$_2$S synthesis and endothelium-dependent aortic smooth muscles relaxation in old rats.

We used Wistar male rats divided into 3 groups: adult (6 months), old (24 months) and old+P5P. P5P was dissolved in distilled water and administered per os in dose of 0.714 mg per kg once a day for 2 weeks. After sacrificing the rats at the end of the treatment, aorta was extracted and muscle contractile activity of aorta rings was measured with tensiometry in a chamber at 37 °C. Norepinephrine was added to induce contraction of aorta smooth muscles, and further acetylcholine perfusion was performed to induce relaxation. Additionally, content of H$_2$S was measured in aortic tissues.

Our results show that endothelium-dependent relaxation of smooth muscles was greatly impaired in old rats: the index of acetylcholine-induced relaxation was 18.4 ± 4.1% vs 66.5 ± 6.4% in adult rats (P < 0.001). After 2 weeks of P5P administration the index of aorta relaxation was 47.7 ± 4.8,% that indicates at least partial renovation of endothelium-dependent relaxation of aortic smooth muscles of old rats treated with P5P. Biochemical measurements showed that H$_2$S content was 1.6 times lower in old rat aorta compared to adult ones. However, P5P induced a significant 2-fold increase in H$_2$S content (P < 0.05) in aortic tissue.

Thus, we can conclude that endogenous H$_2$S is greatly engaged in regulation of endothelium-depended relaxation that might be stimulated by pyridoxal-5-phosphate administration in conditions of vessels’ tone dysfunction like aging, hypertension etc.
A number of studies have shown that cobalt ions at the concentrations that exceed 0.6 mg/kg/day (NOAEL – no observed adverse effect level) tend to destabilize red blood cells membranes even under single administration to laboratory animals. Works, that investigate the chronic effect of Co²⁺ ions at much lower concentrations than NOAEL on the structural stability of erythrocytes, are almost absent.

The aim of the present study was to investigate the effect of prolonged action of low doses of Co²⁺ ions on erythrocytes structural resistance to hemolysis.

The effect of intragastric administration of Co²⁺ ions in doses 0.012 and 0.06 mg/kg/day during 15 and 36 days on hemolysis of 3-month old Wistar rats’ red blood cells has been studied. The animals were divided into 5 groups. Animals from the group 1 (control group) were receiving clean water during 36 days. Groups 2 and 3 were getting CoCl₂ solutions in aforementioned concentrations during 15 days, groups 4 and 5 – during 36 days. Water and salt solutions were administered by intragastric probe daily.

Erythrocytes were obtained from the whole blood of rats. Hemolysis was induced by adding HCl to its final concentration of 0.002N. Red blood cells hemolysis kinetics was recorded by changes in the optical density of samples with registration step of 1 s. The time of erythrocytes membranes structural rearrangement before the beginning of their destruction process and the rate of their destruction were selected as the indicators of hemolysis.

According to the obtained results, the rate of the membranes cooperative destruction of the bulk of red blood cells is 1.6 times higher in experimental animals than in control group after 36 days of Co²⁺ administration in both concentrations. Furthermore, the time before the membranes cooperative destruction starts decreases by 16% under Co²⁺ administration during 36 days compared to the control.

Thus cobalt ions during acidic hemolysis enhance the destabilizing effect of HCl even at doses 10 and 50 times lower than NOAEL. The additional destabilizing effect of Co²⁺ ions increases with the dosage and duration of administration.
PROMOTING OLIGODENTROCYTES PRECURSOR CELLS PROLIFERATION AND SURVIVAL IN MULTIPLE SCLEROSIS

A. SOLIMAN¹,²

¹October University for Modern Sciences and Arts, MSA
²Euro-Mediterranean Master Program in Neuroscience & Biotechnology between Alexandria University and the University of Bordeaux.⁴, Cairo, Egypt; e-mail: ahmedo.soliman@yahoo.com

Multiple sclerosis (MS) is a neurodegenerative disease at which demyelination of the neurons happens. Microglia, lymphocytes, and macrophages are among the main causes of such effect. Oligodendrocytes Precursor Cells (OPCs) are the main targets of inflammation and immune attacks which cause their death by apoptosis; thus, not only demyelination occurs, but losing the ability of re-myelination is lost as well resulting in MS.

In mice with introduced multiple sclerosis-like disease, a combination of two drugs will be introduced integrating to positively affect OPCs proliferation and survival; therefore, a synergistic effect should be achieved. First, ‘WIN55,212-2’ chemical compound, with a cannabinoid-like effect, stimulates OPCs proliferation, has neuroprotectant effect and induces oligodendrocytes maturation. Second, Minocycline, lipophylic tetracycline antibiotic, that has anti-apoptotic effect on oligodendrocytes. The drugs will be applied to a non-viral vector, a designed dendrimer will be used. Through stereotactic intra-cranial injection, the dendrimer including the drugs in nano size, will be injected. The experiment can be monitored by using TUNEL (Terminal deoxynucleotidyl transferase dUTP Nick End Labeling) to detect oligodentrocytes cells apoptosis level.

Through the above steps and design, OPCs size and proliferation level should be better, and the reverse on apoptosis level, promoting control of the induced MS case and even an approach to make the case better.
THE IMPACT OF SIRTUIN ACTIVITY MODULATORS ON THE CUMULUS CELLS VIABILITY OF FEMALE MICE IN THE CONDITIONS OF EXPERIMENTAL SYSTEMIC IMMUNE DISORDER

M. STUPCHUK

Bohomolets Institute of Physiology, NAS of Ukraine, Kyiv; e-mail: mariastupchuk@yahoo.com

Resumption of oocyte meiotic maturation and the formation of the first polar body of oocytes is a prerequisite for normal fertilization and further development of the fetus and the ability of the ovary to ovulate; as a result oocyte with haploid sets of chromosomes gets into the fallopian tube for fertilization, which is a prerequisite for female mammals fertility. Systemic immune disorder (SID) is considered to be one of the major factors that may probably affect a female reproductive function. There is an assumption that SID is able to influence both directly, through direct action on oocytes and through indirect mechanisms – the cumulus cells viability, particularly. Thus, above-mentioned pathology is a serious problem for the development of diagnostics and therapy of the female reproductive system pathologies, caused by immune factors. Sirtuins are a class of proteins that regulate a wide range of cellular processes like aging, transcription, apoptosis, and also play an important role in stress resistance by activating oxidant-antioxidant system. As a result, they are able to correct the negative impact of various pathological processes that occur in the body, particularly systemic immune disorder on the process of meiotic maturation of oocytes. Therefore, the assessment of the impact of sirtuin activity modulators on the oocyte meiotic maturation and cumulus cells viability in female mice in the conditions of experimental SID was considered important for the research. To determine the effect of SID on ovarian function we used an experimental model of systemic immune disorder, created by immunizing experimental group of animals (mice) with antigenic suspension. Assessment of apoptotic and necrotic death of immune and cumulus cells performed using the method of in vivo dual-color fluorescent dyes of nucleic acids. Investigation of primary DNA damages (the damage index) of immune and cumulus cells was performed using gel electrophoresis of isolated cells method (the comet assay). Our results indicate that experimental systemic immune disorder in mice can cause suppression of cumulus cells viability. It is shown that the number of viable cumulus cells reduced under the experimental systemic immune disorder and the number of cells with morphological signs of apoptosis and necrosis increased, that can probably cause the further decrease of oocytes viability. We have found that sirtuins activation leads to the increase of the percentage of viable cumulus cells – 49.7 ± 0.8% (P < 0.01, n = 7) versus 39.2 ± 0.8% in the conditions of cultivation without resveratrol. Moreover, we indicated the influence of sirtuin activator – resveratrol (c = 20 μM) and inhibitor – nicotinamide (c = 5 mM) on the cumulus cells viability and it’s number of primary DNA damages. We determined the decreasement of cumulus cells DNA damage index in the conditions of cultivation with resveratrol – 3.1 and 3.4 without resveratrol in mice with experimental kidney injury. DNA damage index in the conditions of cultivation with nicotinamide – 3.5. Therefore, our data indicate the role of sirtuin activity modulators on the cumulus cells viability of female mice in the conditions of experimental acute immune kidney injury, thus increasing the likelihood of successful fertilization of the oocyte and passage of further stages of its development.
ALPHA-E-CATENIN INVOLVEMENT IN NEONATAL CARDIOMYOCYTES SIZE REGULATION

V. VASKIVSKYI¹, V. BALATSKYI¹, L. MACEWICZ⁴,
T. RUBAN², O. PIVEN²

¹Taras Shevchenko National University of Kyiv, Ukraine;
²Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv;
e-mail: vaskivskyi.v@gmail.com

Alpha-E-catenin is a key component of cell-cell adhesion, but recent studies suggest that α-catenins have more complex and diverse functions and involved in some signaling pathways. In this study, we have focused on alpha-E-catenin function in neonatal cardiomyocytes. Cells were isolated from hearts with alpha-E-catenin homozygous and heterozygous knockout and control one, and used for histological and molecular biological analysis. In our previous work, we registered heart and atria enlargement in adult mice with alpha-E-catenin depletion compared to control. In contrast to these data we have found that newborns’ cardiomyocytes with full and heterozygous alpha-E-catenin knockout were smaller (length and width) compared to control. Interesting that compared to control cells, the number of binuclear cardiomyocytes was decreased in cells with alpha-E-catenin missing. Additionally, we registered autophagy activation in cardiomyocytes with heterozygous and homozygous alpha-E-catenin deletion. We also registered violation of canonical WNT-signaling in mutant cells.

Collectively our data indicated that alpha-E-catenin has important function in newborns’ cardiomyocytes size regulation and survival, probably through regulation of canonical WNT-signaling.
Standardized methods of diagnosing the safety of food and raw materials are classical methods of food microbiology, which are time-taking, based on the phenotypic characteristics of microorganisms and are not always able to diagnose their toxigenic properties. Analytical information on the inaccuracy of indication of bacillary food poisoning, the need for a preventive analysis of the risks that aerobic and facultative-anaerobic spore-forming microorganisms of the genus *Bacillus* bear, cause the urgency of their detection by accelerated modern methods. Such diagnostics will allow producing new competitive food of guaranteed quality and microbiological safety. The work was aimed at molecular-biological diagnostics of potential causative agents of food poisonings – the contaminants of the genus *Bacillus* – according to the genetic determinants of their toxicity.

Characteristics of 9 morphotypes of contaminants in 117 food samples were studied with standardized classical methods by phenotypic properties. Samples of food for PCR were prepared by the priority method developed by us. PCR was carried out with specific primers to detect toxicity in various kinds of bacilli genes: *nhe*, *hbl*, *cyt K*.

Among the bacillary contaminants of the samples, the *subtilis-licheniformis* group is the most numerous one (20 to 37% of total bacilli count), *Bacillus megaterium* was detected in the amount of 6 to 21%, *B. pumilis* – 4 to 13%, *B. circulans* – 2 to 7%, gas-forming *Paenibacillus polymyxa* and *P. macerans* – the causative agents of bombarding spoilage – 3 to 14% and 2 to 9%, respectively, the microorganisms of the *Bacillus cereus* group (in particular *B. cereus* and *B. thuringiensis*) – 10-31% and 4-13%, respectively. Molecular genetic diagnosis showed the specificity of the contaminants in Ukraine: the presence of the *nhe* gene was detected in 100% of *B. cereus* strains, *hbl* in 60% and *cyt K* in 40% of the strains studied. It should be noted that the presence of the toxicity gene *cyt K* was established for a typical saprophyte – the strain *B. licheniformis*.

Studies of food raw materials and products have confirmed the need to improve microbiological control of product safety by introducing accelerated specific diagnostics of contaminants by molecular genetic methods. Such studies should be continued, which will allow diagnosing both traditional and emergent pathogenic contaminants.