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Glioma tumor markers CHI3L1 and CHI3L2 in cell signaling and fate

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Aim. Serial Analysis of Gene Expression revealed *CHI3L1* and *CHI3L2* among the genes with the most pronounced changes of expression in tumor cells. In contrast to CHI3L1, which has been characterized as a marker of inflammatory diseases, the features of its closest homologue – CHI3L2 were poorly described. Because they are closely related in size and sequence, we summarize the research on CHI3L1 as a background for the CHI3L2 investigation. *CHI3L1* gene expression increases significantly under the pathological conditions such as inflammation or tumors, notably glioblastoma. It was shown that CHI3L1 protein stimulated the growth of connective tissue cells similarly to IGF1. This mitogenic activity is mediated through the MAPK and PI-3K signaling cascades. **Methods**. To examine if CHI3L2 can activate the MAPK pathway by phosphorylation of Erk1/2 similarly to EGF, we used HEK293 and U373 cell lines. The results obtained suggest that Erk1/2 phosphorylation was stimulated in these cells by addition of CHI3L2 or CHI3L1 in dose- and time-dependent manner. To determine whether CHI3L2 and CHI3L1 can enhance mitogenesis, the cell proliferation and [^{3}H]thymidine incorporation were evaluated. **Results**. Unexpectedly, in contrast to CHI3L1, dose dependent decreasing in the measured parameters values was observed in HEK293 and U373 cells. In both cell types the treatment with CHI3L2 gave more sustained MAPK pathway activation than CHI3L1, with prolonged phospho-Erk-1/2 nuclear accumulation in HEK293 cells. Moreover, our results indicate that CHI3L2 inhibits proliferative action of CHI3L1 and IGF-1 in the tested cell lines. **Conclusions**. The obtained results demonstrate the influence of CHI3L2 and CHI3L1 on the duration of MAPK cellular signaling and phosphorylated ERK1/2 translocation to the nucleus. In contrast to the activation of Erk1/2 phosphorylation by CHI3L1 that leads to a proliferative signal, the activation of these kinases by CHI3L2 inhibits cell mitogenesis and proliferation.

tRNA-assisted editing by *Enterococcus faecalis* Prolyl-tRNA synthetase

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Aim. Some aminoacyl-tRNA synthetases (ARSases) require a special mechanism of hydrolysis of their own mis-synthesized product, referred as editing, for amino acid specificity maintanance. In the course of pretransfer editing, hydrolysis of mis-synthesized aminoacyl-adenylate occurs, and posttransfer editing means hydrolysis of aminoacyl-tRNAs. Certain ARSases, particularly bacterial-type ProRSs, can edit using both pathways. Previously we have described the bacteria *E. faecalis* ProRS (ProRS*Ef*) pretransfer editing against alanine induction in the presence of $tRNA^{Pro}$. The aim of our present work was further examination of ProRS*Ef* tRNA-dependent pretransfer editing and determination of

tRNA role in posttransfer editing. **Methods.** 2'-deoxy and 3'-deoxy $tRNA^{Pro}$ were obtained by enzymatic approach. For the estimation of editing activity rate, a test based on ATP hydrolysis in the presence of alanine, and a test based on alanyl-$tRNA^{AlaPro}$ deacylation, were used. Steady-state parameters of ATP hydrolysis by ProRS were determined by varying concentration of alanine. Amino acid activation rate was estimated by the ATP-PPi exchange test. **Results.** The catalytic constant of ProRS*Ef* tRNA-dependent pretransfer editing k_{cat} is 0.292 ± 0.023 s^{-1}, and thereby it largely exceeds nonenzymatic hydrolysis rate constant k_{obs}, that equals 0.000780 ± 0.000166 s^{-1}. Contrary to wild type tRNA, 2'- and 3'd A76 tRNA derivatives are practically unable to induce pretransfer editing. In addition, 2'd A76 tRNA, when aminoacylated with alanine, can not be deacylated by ProRS, but appears to beprotected by it even from nonenzymatic hydrolysis. At the same time the 2'd A76 tRNA acceptor activity is significantly less affected, than the functions related to editing. The presence of tRNA does not increase either proline or alanine activation rate. **Conclusions.** Pretransfer tRNA-dependent editing is a catalytic process, that occurs in the ProRS*Ef* aminoacylating active center and is mediated by 2'- and 3'-OH groups of tRNA A76. The role of tRNA in this process consists in assisting alanyl-adenylate hydrolysis, but not alanine activation. Besides, 2'-OH group is not required for aminoacylation, and so, for tRNA binding in the active site. At the same time, 2'-OH of tRNA A76 plays the central role in catalytic mechanism of posttransfer editing.

Photosynthetic activity of *Kalanhoe daigremontiana* sheltered by bacterial consortium under stressful conditions

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Aim. To evaluate bacterial protecting effect on photosynthetic activity of *K. daigremontiana* Hamet & Perr. plants or adventitious plantlets under different stressful conditions. **Methods**. A dual culture of *Klebsiella oxytoca* IMBG26 and *Paenibacillus* sp. IMBG156, *Pseudomonas fluorescens*, *Methylobacterium* sp. was applied for model *K. daigremontiana* plant protection in the Mars-simulation chamber HUMILAB (DLR Berlin) on Mars-like soil or earth soil. Chlorophyll *a*/*b* fluorescence emission from living leaves has been measured with a pulse amplitude-modulated fluorometer (Heinz WALZ GmbH, Germany). **Results**. There were no differences in PA in hypobaria (10 mbar) and ambient pressure, as well as between treated with bacteria and control plants. However, a difference was seen between variants of kalanchoe exposed to high CO_2 (95 %) concentration. The PA was higher in presence of bacteria, although under a high CO_2 concentration in the atmosphere PA dropped in both variants (inoculated and noninoculated), in contrast to PA under low pressure. The PA of kalanchoe plants grown in martian regolith stimulant (MRS) or earth soil under simulated martian conditions were lower than under normal earth conditions. The positive effect of bacterial inoculation on plant accommodation to adverse simulated martian conditions was more pronounced in kalanchoe grown on MRS and depended on bacterial species, especially, under rigorous conditions of cooperative action of low pressure and high content of CO_2. Under a combination of gamma irradiation and following the near-Mars UV-radiation of kalanchoe impact the Dual on PA was a gamma-quant-dose-dependent: at a low dose of γ-rays (30 Gy) the effect of UV on PA was higher than at a higher dose (70 Gy) when effect on PA was practically unnoticeable. **Conclusions**. Kalanchoe acquired better defensive capacities to wit stand adverse conditions which corresponds to more efficient photosynthetic activity after treatment with bacterial consortium. The results support the concept of microbial community usage for growing plants under stressful conditions.

Molecular dynamics of deoxyribonucleoside triphosphates sealed in water solution

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In spite of immense size of the genetic information carrier in living organisms, it is copied with great fidelity. On average only few mistakes occur during reproduction of the mammalian genome composed of DNA of 3 billion base pairs. In addition, DNA is synthesized incredibly fast, its polymerization velocity fluctuates in the range from 500 nucleotides/s in bacteria to 50 nucleotides/s in mammals. High accuracy of replication as well as its high velocity is provided with special mechanisms editing mistakes. Currently only some stages of this process are investigated experimentally and theoretically. In particular, among the investigations of conformational flexibility of DNA structural components, the studies on isolated or microhydrated DNA bases are mainly presented. Analogous studies on the conformational flexibility of nucleosides are less known, and there are only few reports on the deoxyribonucleotides. In the present research the conformational flexibility of deoxyribonucleoside triphosphates (dNTPs) is investigated. Namely, the conformational behavior of canonical dNTPs (deoxyadenosine, deoxycytidine, deoxyguanosine, and deoxythymidine triphosphates) was studied by the methods of classical molecular dynamics. The AMBER-99SB force field as well as TIP3P water model (several thousand water molecules) ha-

ve been applied. The following aspects were explored: 1) Synanti transition dynamics; 2) C2'-endo-C3'-endo-transition and existence of other deoxyribose component conformations; 3) dynamics of conformers distribution for med by rotation around torsion angles of deoxyribonucleotide and phosphate links; 4) distribution of dihedral angles in dNTPs phosphate tails depending on composing nitrogen base; 5) influence of water solution representation (TIP3P via dielectric continuum) and charge compensation of dNTP. The results of this work are compared with those available in the literature for the nucleotides, nucleosides and DNA bases. The similarities and differences are discussed.

Identification of target sites of microRNA and TTP in 3'UTRs of human intersectin 1 mRNA

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Aim. To find and experimentally confirm potential target sites for different posttranscriptional regulators in 3'UTRs of ITSN1 and its partners mRNAs. **Methods**. Computational analysis, molecular cloning and western-blot analysis. **Results**. Recently we have found potential target sites for microRNAs in 3'UTRs of ITSN1-S mRNA. We constructed deletion mutants and have found complete absence of expression in luciferase assay for one of them where target sites for microRNA miR-10a and ARE-binding protein TTP have been deleted. Then computational analysis of 3'UTRs of mRNAs of ITSN1 and its endocytic partners was performed and several target sites for different microRNAs and one common site for miR-10a in 3'UTRs of SNAP23 and ITSN1-S mRNAs have been found. We plan to create a construct with 3'UTR of SNAP23 mRNA to investigate possible effect of miR-10a target site. To explore possible impact of potential target sites for miR-10a and ARE-binding protein TTP on 3' UTR of ITSN1-S mRNA we have created construct based on *pTKluc* vector with insert of 500 nt region of 3'UTR of ITSN1-S mRNA bearing potential target sites for miR-10 a and TTP. We have also obtained «pseudo-3'UTR» of ITSN1-S mRNA construct with insert of 1500 nt sequence from *pRL-TK* vector without regulatory elements. This construction will be used for comparison of 3'UTR and pseudo-3'UTR of ITSN1 mRNA expression. TTP is the ARE-binding protein that negatively regulates translation of mRNAs with AREs in 3'UTRs. Brooks et al. found that ITSN1-S could be potentially regulated by TTP via its binding to 3'UTR of ITSN1. We have analyzed full-length 3'UTR of ITSN1-L and found similar site for TTP. We have performed western-blots with construction based on *pCDNA*3.1 vector with insertion of TTP in different cell lines but found no expression. Then we created another construct based on *pCMV-HA* vector that showed expression in HEK293 cells. We have also cloned 1060 nt region of 3' UTR of ITSN1-L mRNA with potential target site for TTP. Cotransfections of constructs with TTP and 3'UTRs of both forms of ITSN1 mRNAs and luciferase assays will be held in the near future. **Conclusions**. We have found targets for several microRNAs and TTP protein in 3' UTRs of ITSN1-S and ITSN1-L mRNAs. To validate these targets in luciferase test constructs with inserts of pseudo-3'UTR of ITSN1-S mRNA and 500 nt region of 3'UTR of ITSN1-S mRNA were created. For TTP targets validation we created construct with TTP CDS insert and found its expression in HEK293 cells. We also cloned 1060 region of 3'UTR of ITSN1-L mRNA with potential TTP target in *pTKluc* vector. Cotransfections and luciferase assays with all these constructs will be performed.

Search for novel fluorescent dyes for detection of amyloid fibrils using QSAR methodology

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Aggregation of proteins into insoluble amyloid fibrils plays a key role in the development of a number of neurodegenerative diseases including Alzheimer's and Parkinson's diseases. Thus, study on amyloid fibril formation and its inhibition is of medical importance owing to the lack of protein assembly inhibitors that might be used as therapeutic agents for treating amyloid-associated diseases. Although the fluorescence based assays are widely used for examination of anti-amyloidogenic agents, only a limited number of dyes are applicable for this method. For searching the dyes sensitive to ASN oligomers we used quantitative structure activity relationship (QSAR) methodology, which is widely known in ligand search for biological targets in the absence of information about a binding site structure, and also in the case when to allocate a concrete, accurately defined site is not obviously possible. First, we studied previously obtained data on dyes – synuclein oligomers, and basing on this information we created training set of dyes sensitive to ASN oligomers. The regression analysis with use of back propagation neural networks has been applied. We have built a statistical model with good characteristics ($R2 = 0.82$, $P < 0.02$). From a set of 923 descriptors only 23 were selected for a model construction by the method of PCA (principal component analysis) Concordantly to this model, three dyes were selected and tested as fluorescent probes. One of them enhanced the intensity of fluorescence by 20 times in the presence of ASN oligomers. There is a lack of evidence to the presence of special binding sites for inhibitors on amyloid fibrils or their intermediates. Thus, the QSAR approach appeared the most

appropriate method for identifying potential dyes for amyloid fibrils. From three candidates for *in vitro* testing one shows an ability to be an amyloid dye in aggregation assay with ASN oligomers in fibril formation experiment and will be used for further optimization.

Computational modeling of human Cytochrome P450 2E1 interaction with ethanol

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Aim. Human Cytochrome P450 2E1 (CYP2E1) is an ethanol-inducible heme-containing monooxygenase. The induction occurs due to the stabilization of spatial structure of enzyme that leads to its accumulation in the cell. The modeling of the interaction of the cytochrome P450 2E1 active site with ethanol molecule gives us an opportunity to understand the mechanisms of stabilization of the protein spatial structure. Therefore, the aim of the work was the development and optimization of the spatial model of «CYP2E1-ethanol» complex. **Methods**. In this work we used the model of human Cytochrome P450 2E1 spatial structure, previously created by us. The docking of ethanol molecule into the enzyme active site was carried out using ArgusLab program. To optimize the resulting «CYP2E1-ethanol» complex, the 1 ns molecular dynamics (MD) simulation was performed (NAMD program, NVE-ensemble, 300K temperature, water environment). To simulate the mechanisms of CYP2E1 interaction with ethanol the 20 ns long MD simulation was carried out at the same conditions. MD analysis was performed using the VMD and VegaZZ programs. **Results**. Human Cytochrome P450 2E1 metabolizes the substrates which differ considerably in mass and size (from the solvents, such as chloroform and ethanol, to fatty acids). The enzyme active site is located inside the protein globule and has an average volume of 2 nm^3. A set of possible binding sites were found during the docking of ethanol in the enzyme active site. From this set a «CYP2E1-ethanol» complex was chosen which had the highest binding energy (–4.91 kcal/mole) and the shortest distance between the molecules of heme (atom of iron) and ethanol (~ 0.5 nm). During optimization of the resulting complex both the orientation of ethanol relative to the heme and the enzyme spatial structure changed little. The 20 ns MD simulation did not lead to significant changes in the spatial structure of the protein, but revealed an additional possible binding site of ethanol in the active center, which was on the same plane with the heme molecule at a distance of 0.9 nm from the atom of iron. Such ethanol location allows heme to interact simultaneously with one more ligand. **Conclusions**. The computational model of the «CYP2E1-ethanol» complex spatial structure was made. The molecular dynamics simulation showed a high stability of the enzyme spatial structure. Moreover, the ethanol location, which allows heme to interact simultaneously with one more ligand, was found. The results obtained will allow us to simulate the processes with the simultaneous presence of two substrates in the CYP2E1 active site.

Somaclonal variation in *Gentiana lutea* L. tissue and organ culture: RAPD-analysis

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The aim of this study was to investigate somaclonal variation in the course of non-organized growth and regeneration of *G. lutea in vitro* by RAPD-PCR method. The specimens of *G. lutea* from mountain valley Lems'ka, Chornogora ridge of Ukrainian Carpathians were examined: regenerants generated via direct organogenesis *in vitro*, initial callus of stem origin, six month old morphogenic, six month and one year old non-morphogenic calli of root origin, isolated root culture. Every culture *in vitro* was compared with the original two month old aseptic donor-plant. Dendrogram of genetic fidelity for *G. lutea* specimens was constructed by FAMD 1.21 beta according to genetic distances of Jaccard (D_J). For RAPD analysis 11 primers were used providing the synthesis of clearly reproducible amplicons. Total number of amplicons made up 114.26 (22.8 %) among them appeared to be monomorphic for each object. The culture *in vitro* specimens in genetic fidelity dendrogram were distributed into two clusters with the first being formed by donor-plant, initial callus and «direct» regenerant, while the second presented morphogenic, non-morphogenic calli and culture of isolated roots. The «direct» regenerant was proved to be the nearest to donor-plant. The genetic distance between them according to Jaccard amounted to 0.091 and was the least among all specimens of the sample. Individual branch was formed by the initial stem callus (D_J = 0.139). The genetic distance from donor-plant to six month old morphogenic culture was as much as 0.197, increasing for six month old non-morphogenic one to 0.239 and as the latter growth to one year it increased up to 0.264. Morphogenic and non-morphogenic calli of the same duration of culturing, which were maintained in the media differing by the phytohormone composition nutrients, formed joint cluster; the genetic distance between them comprised 0.108. Isolated root culture produced individual ramification within the other cluster distinguishing from both the initial plant (D_J = 0.239) and morphogenic as well as non-morphogenic calli (D_J = = 0.125–0.257). Genetic distances of culture *in vitro G. lutea* (D_J = 0.091–0.264) was about twice less than intrapopulation polymorphism between 15 wild growing plants

from the original population (D_J = 0.161–0.532). Hence, the somaclonal variability of *G. lutea* tissue and organ cultures was explored by RAPD analysis. The regenerant-plants showed the lowest level of genetic changes whereas the one year old non-morphogenic cultures displayed the highest rate. The genome variability was found to increase as the duration of culturing rose, however, it does not extend beyond the confines of intrapopulation polymorphism.

The influence of cytokines on the *MGMT* gene expression in human cells *in vitro*

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Aim. Enzyme O^6-alkylguanine-DNA alkyltransferase (MGMT) repairs the most mutagenic and carcinogenic primary DNA lesions induced by alkylating agents. The expression of *MGMT* gene encoding this repair enzyme has been already known to be influenced by its promoter hypermethylation, polymorphisms in the gene structure and DNA breaks. Moreover, there are some literature data about the regulation of the *MGMT* expression by cytokines IF-β, IF-γ + IL-1β, IL-24. The aim of our work was to study a possible influence of cytokines LIF, SCF, IL-3, IFN-α2b and EMAP II on *MGMT* gene expression in the human cell cultures. **Methods**. We used the next cell lines: A102 (fibroblasts), CB-1 (umbilical cord blood stromal cells), 4BL6 (cells derived from peripheral blood), and Hep-2 (laryngeal cancer). The cells were treated with exogenous cytokines in different concentrations. The identification of MGMT in cell protein extracts was performed by Western blot analysis. **Results**. In our previous study it has been shown that MGMT protein may exist in cell cultures in two forms: the unmodified one with molecular mass of 22–24 kDa and the modified form of 48–50 kDa. Therefore both protein forms were taken into account in our studies. The treatment of Hep-2 cells with the cytokine LIF in concentration of 20 ng/ml led to the disappearance of unmodified form while the amount of modified one did not change. After the treatment of 4BL6 cells with IL-3 (20 ng/ml) and SCF (20 ng/ml) the protein in unmodified form disappeared completely, and the amount of modified protein considerably decreased. Thus, we may suppose that these cytokines down-regulate the expression of *MGMT* in human cells *in vitro*. IFN-α2b in the concentration of 20 U/ml up-regulates the expression level of *MGMT* gene in A102 cells and down-regulates it at the concentration of 200 U/ml in CB-1 cells. Cytokine EMAP II was the inductor of *MGMT* gene expression in A102, CB-1, 4BL6 cell lines in the range of concentrations from 0.2 to 20 мg/ml. **Conclusions**. Taking into account these results we can conclude that cytokines LIF, SCF, IL-3, IFN-α2b and EMAP II are able to influence the *MGMT* gene expression at the protein level in human cells *in vitro*.

Role of PH and C2 domains of Bcr protein in development of Ph-positive leukemias

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Background. Bcr-Abl protein, the product of Philadelphia chromosome, is believed to be a key factor for malignant transformation in case of Ph-positive leukemias. There are three forms of this hybrid protein (p190, p210, p230) that correlate with different types of leukemia and differ in the presence or absence of the DH, PH and C2 domains of normal Bcr protein. Detection of interaction of these domains with proteins and lipids may give a new knowledge about molecular events that occur in the cells during their malignant transformation and can form a basis for the development of new therapeutic strategies to conquer these leukemias. The previous studies resulted in identification of some proteins-candidates (Cortactin and FBP17) that possibly can bind PH domain of Bcr protein, but these data must be confirmed by further research. A role of the C2 domain of Bcr protein is still unknown. **Aim**. Our study was focused on the evaluation of interaction between the PH domain of Bcr protein and Cortactin as well as between the PH domain and FBP17. Besides, the creation of effective system for bacterial expression of the recombinant C2 domain of Bcr protein has been planned. **Methods**. For estimation of interaction of Cortactin and FBP17 with the PH domain of Bcr protein, the co-transfection of HEK293 cells with appropriate vectors (*pRK5myc*-Cortactin, *pJ3H*-FBP17, *pEGFP*-PH) has been performed, with following coimmunopreciptation. The PCR product that contained the sequence encoding the C2 domain of Bcr protein was cloned into *pET28c* vector with standard genetic engineering procedures. The effectiveness of cloning was confirmed with sequencing the DNA construct. The C2 domain was expressed in Rosetta strain of *E. coli*, the protein being purified with NiNTA system. **Results**. Co-immunoprecipitation did not show any binding between the PH domain of Bcr protein and Cortactin and FBP17. The sequence of C2 domain of Bcr protein was successfully cloned in *pET28c* vector, expression of the protein was optimized and the recombinant protein was purified. **Conclusions**. A negative result on the interaction of Cortactin and FBP17 with the PH domain of Bcr protein has to be confirmed with other methods. The purified recombinant C2 domain of Bcr protein can be used for detection of the binding of this domain with proteins, lipids, and Ca^{2+} ions

Analysis of genetic markers of mouse strains for microchimerism study

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It has only recently become obvious that the phenomenon of microchimerism (Mc) exists in many mammals, including humans. Mc may be a result of iatrogenic interventions such as transplantation or transfusion, however, the most common one is certainly a naturally acquired microchimerism. The derivation of Mc is extraordinarily wide. This phenomenon has been suggested to contribute some success into clinical practice of transplantation. Mc is a subject of rather great current interest for a number of reasons. The greatest interest is associated with the Mc's origin and destiny of the foreign cells. The aim of this study was searching for an animal model to learn Mc phenomenon, to examine experiment conditions and follow donors material. Mice are the most examined and useful objects in laboratory studies. That is why we chose them for our researches. As the first important step, we have to select the donor and recipient for obtaining a necessary model, which includes genotyping mouse strains. Microsatellite Eb loci on 17 chromosome were analysed using PCR in two mouse strains: ICR/IMBK, BALB/c. The PCR results showed strains contamination, therefore, we started to obtain pure-breeding strain, according to this locus. It is already known that interleukin 3 (IL-3) is one of the natural growth factors. This cytokine promotes the proliferation and differentiation and spontaneous fuse of haematopoietic cells. The adult cells also can fuse spontaneously in the presence of IL-3. This function might be useful for our study. We cloned this cytokine, conducted the clone selection, incubated the culture – protein's producer strain, the protein was isolated and purified. In this way we have prepared the base for the subsequent experiments in fundamental research on the phenomenon of MC .

Application of potentiometric urease biosensor for urea analysis in real samples

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A determination of urea is of great interest in biomedical and clinical analysis. Urea is a main and final product of protein metabolism. It is a bio-molecule, which is often monitored in blood serum as an indicator of renal function. The normal level of urea in human serum is between 1.7 and 8.3 mM. In patients suffering from the renal insuffiency the urea concentration can increase up to 100 mM. Moreover, urea is widely distributed in nature and its analysis is also of considerable interest in agro-food chemistry and environmental monitoring. The traditional methods for urea determination generally involve the formation of coloured complexes with urea or with ammonia obtained by decomposition of urea catalyzed by urease. However, these methods generally involve complex reactions and require pretreatment of samples. Therefore, more simple, sensitive and inexpensive methods are required for determination of urea concentration. **Aim**. The main goal of the work is application of the biosensor for detection of urea concentration in real samples. **Methods**. We propose for urea detection the potentiometric biosensor developed in our laboratory [1] based on pH-sensitive field-effect transistors and immobilized urease. **Results**. The present research is focused on application of urease biosensor for urea analysis in human serum and blood dialysate. Urea was measured in 10 blood serum samples by enzymatic method and the biosensor developed. A comparative analysis of two methods has shown good correlation of the results ($R = 0{,}92$). The urea concentration was also determined in the samples of dialysis fluid. The dynamics of changes of urea concentration in dialysis samples of a patient over a period of dialysis was obtained. The developed urea biosensor demonstrates high reproducibility, operational and storage stability during analysis in real samples. **Conclusions**. The possibility of effective application of the potentiometric urease biosensor for urea detection in the blood serum and dialysis liquid has been shown.

1. *Gorchkov D. V., Poyard S., Soldatkin A. P., Jaffrezic-Renault N., Martelet C.* Application of the charged polymeric materials as additional permselective membranes for improvement of the performance characteristics of urea-sensitive ENFETs 2. Urea determination in blood serum // Materials Science and Engineering C.–1997.–**5**, N 1.– P. 29–34.

Endocytic adaptor intersectin 1 interacts with cytoskeletal protein STOP, associated with synaptic transmission

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Aim. *ITSN1* is one of the candidate genes to be involved in the development of Down syndrome phenotype as well as in early stages of Alzheimer's disease. This gene encodes multifunctional adaptor protein intersectin 1, which is implicated in clathrin-mediated endocytosis, actin cytoskeleton rearrangements and signal transduction. *ITSN1* is characterized by high level of expression in neurons. Moreover, several *ITSN1* alternative splicing events occurring predomi-

nantly in neuronal cells were reported. The objective of this work is to elucidate a role of intersectin 1 in neuronal functioning, specifically in synaptic transmission. **Methods**. Molecular cloning, recombinant protein expression and purification, pull-down assays, MALDI-TOF mass spectrometry, immunoprecipitation, transfection of rat hippocampal neurons primary culture, fluorescent laser scanning confocal imaging. **Results and Conclusions**. In order to determine the composition of neuronal intersectin 1-related protein complexes we performed search for novel intersectin 1 neuron-specific interacting partners. For this purpose, we performed GST pull-down assay with mouse brain lysate using intersectin 1 SH3 domains as a bait. Bound proteins were subjected to MALDI-TOF mass spectrometry analysis. For the SH3A domain of intersectin 1 a band of 125 kDa was observed. It was identified as STOP (stable tubule-only polypeptide). This protein is a main factor that determines Ca^{2+}/calmodulin-regulated microtubule cold and drug stability and also takes part in generation of synaptic plasticity. Since glial cells contain STOP isoforms with smaller molecular weight; we concluded that STOP is a neuron-specific binding partner of intersectin 1. To confirm these results we performed several independent experiments. GST pull-down assay revealed that STOP can bind the SH3A and with less affinity the SH3C and SH3E domains. Intersectin 1-STOP complexes were co-immunoprecipitated from mouse brain lysate using anti-STOP and anti-ITSN1-EH2 antibodies. Finally, we determined subcellular localization of these proteins in rat primary hippocampal neurons using both fluorescent antibodies and recombinant FP-tagged proteins. We found that intersectin 1 and STOP partially co-localize in both cell body and neurites. Additional experiments will be performed to elucidate the functional meaning of this interaction. This work was supported by The State Fund for Fundamental Researches and Joint Project Programme of European Scientific Unions «Early Stages of Human Oncological, Autoimmune and Neurodegenerative Diseases».

The ITSN1-22a isoform is specifically polyubiquitinated on its C-terminal domain

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Aim. Intersectin 1 (ITSN1) is an adaptor-protein involved in membrane traffic, regulation of clathrin-coated vesicles formation and synaptic vesicle recycling. The *ITSN1* gene encodes variety of isoforms, ITSN1-s and ITSN1-22a are among them. ITSN1-s protein consists of two N-terminal EH domains, Coiled-coil region (CCR) and five Src-homology domains (SH3A-E). ITSN1-22a consists of two EH-domains, CCR, SH3A domain and specific C-terminal domain (CTD). Recently we have shown that ITSN1-s undergoes monoubiquitination whereas ITSN1-22a is being multiply ubiquitinated. In this work we investigated ubiquitination of ITSN1-22a isoform. **Methods**. To study mode of ITSN1-22a ubiquitination we co-expressed Omni-ITSN1-22a with HA-tagged ubiquitin or its mutant KO-UB-HA that is unable to form polyubiquitin chains. Also we obtained Omni-ITSN1-22aK978R mutant using site-directed mutagenesis to investigate the possible role of single lysine residue specific of ITSN1-22a isoform for polyubiquitin chain formation. Recombinant proteins were immunoprecipitated with anti-Omni antibodies with subsequent immunoblotting. HA-tagged ubiquitin conjugated to ITSN1-22a isoform was detected with anti-HA antibodies. **Results**. Co-expression of ITSN1-22a with the KO-UB-HA that is unable to form polyubiquitin chain and subsequent immunoblotting revealed decrease in amount of the multiply ubiquitinated isoform. Thus we suggested that ITSN1-22a isoform underwent polyubiquitination, rather than multiubiquitination. The next step was to study a possible role of Lys978 in ITSN1-22a ubiquitination. Substitution of the single lysine residue (K978R) specific of ITSN1-22a significantly affected ubiquitination of the isoform. However, the signal corresponding to monoubiquitinated form was still detected. **Conclusions**. Thus we suggest that ITSN1-22a undergoes combined type of ubiquitination. It is monoubiquitinated similarly to ITSN1-s and is polyubiquitinated upon residue K978R located in CTD.

Intermolecular hydrogen bonds in specific binding of stavudine triphosphate to HIV-reverse transcriptase active site

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The aim of work was to understand molecular mechanisms of biological activity of stavudine (3'-didehydro-2'-3'-dideoxythymidine, d4T), a nucleoside analogue reverse transcriptase inhibitor. To achieve this purpose we resorted to the hybrid quantum mechanical/molecular mechanical (QM/MM) modeling of d4T triphosphate (d4TTP) in the HIV-1 reverse transcriptase active site. The similar simulations were also performed for canonical nucleoside thymidine triphosphate (TTP). X-ray crystal structure of the catalytic HIV1-RT/DNA/TTP complex (pdb ID 1RTD) was taken for investigation *in silico*. Two sets of QM/MM calculations (one with d4TTP in the nucleotide binding site and the other with TTP) were carried out. Minimized solvated and neutralized molecular systems were subjected to canonical ensem-

ble (NVT) molecular dynamic simulation lasting 100 ps with the Amber99SB-ILDN force field. The position restraints were imposed on the atoms of HIV1-RT/DNA/dTTP complex to equilibrate solvent molecules. The output systems were used for further QM/MM investigation. QM calculations were performed using B3LYP/3-21G/6-31G* method. Hydrogen bonds (H) were identified by means of geometrical criteria. Both d4TTP and TTP in the binding site of the HIV-RT form various types of H-bonds with the amino acid residues as well as with the surrounding water molecules. The former H-bonds are mainly of NH··O type, where NH group of an amino acid interacts with oxygen atoms (O) located in nucleoside triphosphates. This lace of the H-bonds facilitates the Pα-Oβ3 bond dissociation, dramatically enhancing the HIV-RT polymerization activity. The intramolecular O3'H··Oβ1 H-bond, which is formed between TTP and HIV-RT, deserves a special attention. It can be inferred that the lack of the O3'H hydroxyl group in d4TTP and therefore of the O3'H··Oβ1 bond inhibits its incorporation into DNA nascent chain in comparison with its natural analogue. It should be also noted that H-bonds between nucleoside triphosphate group and positively charged tail of Lys65 play an important role in the binding process, since replacement of Lys65 with Arg residue (K65R) causes resistance to some nucleoside analogues, devoid of the O3'H group.

Stoichiometric model of folate-related one-carbon unit metabolism in human placenta

R. R. Rodriguez, I. S. Lushchyk

Background. Folate-related one-carbon unit metabolism (FOCM) is a complex metabolic network with the two interlocking cycles – tetrahydrofolate (THF) and methionine (Met), that tightly connected with the synthesis of purines and pyrimidines, all reactions of methylation, particularly DNA and histones, synthesis of cysteine (Cys) and therefore with the vital functions of the cell. The impairment in FOCM is associated with pre-eclampsia, one of the major complications of pregnancy and the leading cause of maternal and fetal morbidity and mortality, and manifests itself by elevated level of homocysteine (Hcy), the inertmediate in Met cycle, in the blood. Placenta plays a pivotal but still not specified role in pathogenesis of pre-eclampsia. Our previous study of FOCM revealed the disbalance between metabolites of Met cycle and folates in pre-eclamptic placentas and the decline of proliferation and increase of apoptosis in placental explants cultivated with elevated concentrations of Hcy. The objective of our study is to construct the stoichiometric model of FOCM in human placenta, simulate the pre-eclamptic phenotype and analyze the behavior of the model. **Methods**. To gain this objective we analyzed cross-databases? and conducted literature search for placental-specific expression and activity of FOCM enzymes, compiled a list of reactions with stoichiometric coefficients comprising 9 reactions of folate cycle in cytosol, 4 of methionine cycle, 2 of transsulfuration, 4 of glutathione metabolism, 3 of taurine synthesis along with 8 reactions of in- and output of external metabolites. Metatool software and COBRA toolbox were used to construct the matrix, FOCM model, detect the minimal set of reactions (elementary modes, EM) sufficient for the network function in steady state and model simulation according to pre-eclamptic placental phenotype. **Results**. We have obtained a set of EM of FOCM, the relative weight of each metabolic pathway in normal and pre-eclamptic placenta. The simulation of the processes by introduction of elevated level of Hcy, reduced capacity of methylenetetrahydrofolate reductase, diminished concentration of methionine results in reduced capacity of pathway responsible for purine synthesis; increase of transsulfuration pathway but with the diminished synthesis of taurine, the antagonist of Hcy; and the substantial changes in GSH metabolism. **Conclusi- ons**. The comparison of obtained results with the experimental data has shown: the reduced capacity of the pathway of purine synthesis under the conditions simulating the pre-eclamptic phenotype may explain the down-regulated proliferation mentioned above; the increased capacity of the transsulfuration pathway after simulation may be one of the causes of oxidative stress in pre-eclamptic placenta due to the cysteine oxidation. Therefore, the model provides some ideas for hypotheses and outlines the experiments to confirm or reject them.

Optimization of composition of glucose biosensor modified with carbon nanotubes

N. S. Rogaleva

Aim. To optimize composition and analytical characteristics of the previously developed amperometric glucose biosensor based on screen-printed electrodes and glucose oxidase (GOx) membrane modified with carbon nanotubes [1]. **Methods**. Screen-printed electrodes C220AT and portable bipotentiostat μStat 200 («DropSens») were used for the glucose biosensor development and investigation by cyclic voltammetric and amperometric methods. Aminated and carboxylated multi-walled carbon nanotubes (MWNT-NH_2, MWCNT-COOH) were used for GOx layer modification. MWCNT-COOH nanotubes have been also used for the modification of electrode surface prior to enzyme immobilization. The methods of enzyme immobilization were as follows: cross-linking with BSA in glutaraldehyde vapour and carbodiimide method. To control the suspension quality, the transmission electron microscope (TEM) images we-

re made using the JEM-1230 TEM model («JEOL», Japan). **Results**. Integration of nanotubes into the bio-selective membrane without preliminary electrode surface modification resulted in an extension of the linear dynamic range of glucose determination and led to the biosensor sensitivity enhancement, while modification of the gold electrode surface by carboxylated nanotubes caused only linear dynamic range extension without any visible changes in biosensor sensitivity. It has been noted that the concentrations higher than 0.1 % for GOx and 0.25 % for nanotubes, are not effective in improvement of the biosensor signal. At the same time, an increase in nanotubes content in bio-selective membrane up to 6.0 % causes an enhancement of signal/noise ratio and substantial decrease of detection limit. **Conclusions**. It has been shown that integration of MWCNT-NH_2 into GOx membrane of the biosensor is an effective approach for extending the linear dynamic range of glucose determination and improving sensitivity of the biosensor designed. The main analytical characteristics of the optimized glucose amperometric biosensor were determined to be as follows: time of base line stabilization – 800 s, response time – 80 s, sensitivity – 6.6 μA/mM, detection limit – 0.05 mM, linear dynamic range – 0.1–6.0 μM. High operational stability during 5 h of measurements was demonstrated. Study on storage stability showed a sensor signal being 88 % of initial value after 35 days.

1. *Biloivan O. A., Rogaleva N. S., Korpan Ya. I.* Optimization of bioselective membrane of amperometric enzyme sensor on basis of glucose oxidase using NH_2-modified multi-wall carbone nanotubes // Biopolym. Cell.–2010.–**26**, N 1.–P. 56–61.

NKIRAS1, *PRICKLE2* and *PPM1M* genes alterations in human renal cell carcinomas

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Renal cell carcinoma (RCC) is the most common malignant kidney tumor in adults. We chose the most predominant type, clear cell carcinoma, for this study. The identification of genetic/epigenetic changes and gene expression pattern in RCC might lead to the identification of the set of putative disease markers. Using *Not*I microarrays we subsequently confirmed that *NKIRAS1* showed the highest degree of genetic/epigenetic changes. *PPM1M* and *PRICKLE 2* demonstrated hemizygous deletion/methylation in 38.5 and 40 % cases respectively. To validate the results of *Not*I microarray analysis we studied the expression level, methylation status and copy number. In our work we used the frozen material of a surgically removed tumor and surrounding normal tissues as a control. For global scanning of chromosome 3p we used *Not*I microarray. Bisulfite sequencing was used for studying DNA methylation. Q-PCR was performed with SYBR Green Mastermix («Fermentas», Lithuania) and *TBP* gene was used as a reference. Copy numbers of *NKIRAS1* gene and its expression level were estimated by the $2^{-\Delta\Delta CP}$ method of relative quantification. Analysis of *NKIRAS1* gene expression was performed in 12 tumor samples by Q-PCR. *NKIRAS1* expression was down-regulated in 73 % of RCC samples (8 of 11) compared to surrounding normal tissue. High grade tumors (3 and 4) showed lower expression of *NKIRAS1* at the mRNA level than tumors of low grade (1 and 2). Analysis of copy number of the *NKIRAS1* gene was carried out in 19 tumor samples. Changes in the copy number of *NKIRAS1* gene were observed in 64 % (9 of 14) of RCC samples. 9 samples were considered as hemizygous deletions. 5 samples were considered as normal. The expression level of *PPM1M* was determined in 10 RCC samples. The decrease in expression level was detected in 4 samples. These data confirm in fact *Not*I microarrays analysis. The *PRICKLE 2* expression level was estimated in 9 samples. It decreased in 8 samples from 9. This gene does not contain CpG islands, so to explain such expression level we will provide deletion analysis. For *PPM1M* gene the methylation status will be assessed. We also plan to investigate a possibility to use *NKIRAS1* as a diagnostic marker for renal cell carcinoma. The data on expression in the investigated samples validate *Not*I microarrays analysis for *PPM1M*, *PRICKLE 2* and *NKIRAS1* genes. The copy number analysis explicates the decreased expression of *NKIRAS1*.

Optimization of animal model for studying CYP2E1-dependent processes in the pathogenesis of type 2 diabetes

V. V. Ruschak

Aim. Pathogenesis of diabetes is connected with the development of oxidative stress. Cytochrome P450 2E1 (CYP2E1) is one of the main factors of free radicals production in the cell. The purpose of this work was to optimize animal model for studying CYP2E1-dependent processes in the pathogenesis of type 2 diabetes. **Methods**. In this study, we used a model of type 2 diabetes (A. Ulyanov, A. Tarasov, 1997), which is based on the treatment of guinea pigs with protamine sulfate (intramuscular injection of 15 mg/kg, twice a day). 25 guinea pigs were used, which were divided into 5 groups of 5 animals each. Animals of the first four groups were treated with protamine sulfate for 28 days while the 5th group consisted of intact guinea pigs (control). Euthanasia of the first group animals was performed in 24 hours after the last injec-

tion of protamine sulfate. The second group was kept in standard conditions for 14 days after the last injection of protamine sulfate in order to monitor the progress of the disease. Animals of the 3rd and 4th groups for 14 days were treated with the CYP2E1 inhibitor – quercetin (5 mg/kg) and its combination with antioxidant – ascorbic acid (20 mg/kg), respectively. For animals of all groups, the ALT and AST activity, cholesterol and glucose levels in the blood serum and the level of CYP2E1 protein in liver were determined. The morphological and histological analyses of liver and pancreatic cells were also performed. **Results**. Mild changes were found in the blood serum of the first group of animals for all the studied parameters. No structural changes in liver and pancreatic cells were identified. The animals of the second group showed the symptoms of diabetes: ~1.5-fold increase in glucose level and AST activity in serum, as well as a slight increase in ALT activity. The results of morphological and histological analyses showed focal structural changes of the pancreatic cells. In the liver we observed a decrease in glycogen content, which indicates a disorder of carbohydrate metabolism, and an increase in Kupffer cell activity, which indicates the presence of local inflammatory processes. The CYP2E1 protein level in liver was 1.5-fold increased. Introduction of CYP2E1 inhibitor, especially in combination with an antioxidant, increased the number of functionally active β-pancreatic cells (histological analysis) and normalized the main biochemical parameters of blood serum. **Conclusions**. According to the results of biochemical and histological analyses, the symptoms of type 2 diabetes appear on the 14th day after the last injection of protamine sulfate. The development of the disease is accompanied by increased CYP2E1 protein level in the animal liver. Using CYP2E1 inhibitor at type 2 diabetes leads to an increase in the number of functionally active β-pancreatic cells and normalization of the main biochemical parameters of blood serum.

Novel conductometric bienzyme biosensor for L-arginine determination based on arginase and urease

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In recent years, the metabolism of L-arginine into urea and ornithine *via* the arginase family of isozymes has emerged as an important pathway in numerous physiological and pathophysiological conditions, including lung disease, vascular dysfunction in diabetes, endocrine system diseases, and colorectal cancer. L-arginine level in biological fluids is usually determined by ion exchange and thin layer chromatography, spectrophotometry, colorimetry, radiometry, and by means of enzymatic endpoint analysis. Most of these methods, however, are tedious and time consuming, require expensive equipment or complex sample pre-treatment. Currently, biosensors seem to be the most promising tools for clinical analysis, owing to their high selectivity and suitability to be used in complex media. Thus, the aim of the work was to develop a biosensor for L-arginine detection. Conductometric bienzyme biosensor for L-arginine determination has been developed on the basis of co-immobilization of arginase (E.C. 3.5.3.1) and urease (E.C. 3.5.1.5) in a single bioselective membrane. The immobilization of both working and reference sensors on the sensitive surfaces of thin-film interdigitated electrodes was optimized applying two methods: cross-linking with glutaraldehyde and entrapment in PVA-SbQ polymeric membranes. To optimize the performance of the biosensor, the dependencies of its analytical signal on pH, buffer capacity and ionic strength of the working buffering system were studied. Optimum pH for the L-arginine biosensor was found in phosphate buffer solution (PBS), as pH 6.0. An increase in buffer capacity and ionic strength of PBS resulted in wider dynamic linear range of L-arginine detection. The developed biosensor was evaluated by determination of the sensitivity, linear and dynamic range, detection limit, apparent Michaelis-Menten constant and response time. The appropriate values were 5.7 μS/mM, 0.01–4 mM, 0.01–9 mM, 10^{-6} M, 2.2 mM and 120 s, respectively. Study on operational stability of the biosensor showed that during 600 min of continuous performance the biosensor did not lose its initial sensitivity, and some increase in the biosensor response, observed with time, was referred to ammonium accumulation within the bioselective membrane. The storage stability studies revealed an increase of ~ 30 % in the biosensor response in first 10 days with the following constant activity during at least one month. Therefore, the bienzyme biosensor for L-arginine determination can be considered as a promising analytical device for application in real samples analysis to perform accurate and patient-specific diagnostics.

Peculiarity of *MGMT* gene expression in tumour and normal human cells

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Aim. Effectiveness of chemotherapy by alkylating agents depends on the expression level of repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT) in tumour cells. High level of MGMT expression provides resistance to alkylating drugs, and cancer cells with the lack of MGMT expression are very sensitive to this treatment. The single-stranded DNA breaks, promoter hypermethylation and mutation of *MGMT* gene causes decrease of its expression. According to literature, different types of normal and tumor

cells show different levels of MGMT expression. The objective of this project is to research the peculiarity of MGMT expression in different types of cancer and normal human cells. **Methods**. We used standard lines of tumour cells, probes of peripheral blood leukocytes of donors and patients, samples of tumour tissues from heart myxomas and malignant gliomas (glioblastomas). The clinical materials was provided by the Institute of Cardiovascular Surgery and Institute of Neurosurgery. Proteins in cell extracts from studied samples were separated by electrophoresis in PAAG and analyzed by Western blotting. Blots were probed with monoclonal antibodies («Novus Biologicals, Littleton, Co», USA) against MGMT. **Results**. It has been shown the higher level of MGMT expression in tumour cells of standard lines (Hep-2, U937, Mcf-7, SK-Hep) comparing with the studied normal human cells. MGMT is present in tumour cells of those lines in regular (24 kDa) and «heavy» (50 kDa) forms. In the rank of samples of donor blood leukocytes MGMT has been detected only in the modified form. Until now we have collected 65 samples of tumour tissues of different origin, which were cryopreserved. The electrophoretic spectra of some samples of tumour tissues were shown to be very different. They had different number of bands, their position and density. The proteins with molecular mass less than 19 kDa dominated in all protein spectra of cultivated cells, but not in tumours. All studied tumour spectra showed the dominated bands in 40–80 kDa region. **Conclusions**. It has been created a collection of tumour tissue samples and probes of blood leukocytes of donors and patients. The higher level of MGMT expression has been shown in human tumour cells of standard lines comparing with normal cells.

Developing and testing a method of culturing human mesenchymal stem cells at low oxygen concentration

N. S. Shuvalova, V. A. Kordium

Aim. Many researchers showed that stem cells of different origin cultured in routine conditions quickly lost their capacity to differentiate into various cell types. Cultivation of stem cells in conditions resembling the ones of their natural localization is a promising method. The research data showed that maintenance of stem cells under physiological oxygen tensions (2–5 %) can preserve their features relevant for use in clinical practice. The researches, involving the mesenchymal stem cells from umbilical cord (MSC), which are considered to be a potent tool for regenerative stem cell therapy, are rare instances. The aim of our research was to develop a method of culturing MSC under low oxygen tensions and to test its effectiveness for MSC cultivation. **Methods**. Beginning with the first passage MSC were plated on Petri dishes, d = = 35 mm at a density of 15,000 per dish and cultivated for 5 days under different oxygen tensions before replating for 3 passages. At each passage the cells were counted and the cultures in some dishes were fixed with formalin and stained with haematoxylin and eosin. Nuclear-cytoplasmic ratio was determined with the Image J program. **Results**. For research of cultivation of MSC under reduced concentrations of oxygen (compared to atmospheric ones) we developed the device consisting of a gas mixer, two gas cylinders (with argon and carbon dioxide), air pump and gas analyzers. The experimental group of cultural dishes with MSC was maintained in plastic bags with thick buckle ZipLock («TESS» Ltd.) filled with gas mixture (2 % oxygen, 4 % carbon dioxide and 94 % argon) using the device. Cultural medium was previously maintained in glass bottles with a cotton-gauze plugs placed in hermetically sealed packages with gas mixture. The control group was cultured under routine CO_2 incubator conditions. MSC grown for 3 passages under 2 % oxygen showed significantly higher proliferation rates compared to those, cultured under ambient oxygen concentration. By the passage 3, MSC population grown under 2 % O_2 was less heterogeneous, and MSC were of smaller size with high nuclear/cytoplasmic ratio, compared to the ones under routine CO_2 incubator conditions, which often had «flattened» shape and low nuclear/ cytoplasmic ratio. **Conclusions**. Testing the device for culturing cells at low concentrations of oxygen showed that it fits for this purpose. The obtained data show that the population of MSC grown under 2 % O_2 preserve the features of less differentiated, «primitive» MSC (known as MSC I), which are supposed to have extended replicative age. The overall picture of heterogeneity in the culture under 2 % O_2 was less pronounced.

Fusion proteins based on single-chain antibodies and marker proteins

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Based on the DNA sequence of the genes of single-chain antibodies (single-chain fragment variable, scFv), which we had obtained before, and such marker proteins as *Escherichia coli* alkaline phosphatase with enhanced catalytic activity (bacterial alkaline phosphatase mutated, BAPmut) and red fluorescent protein mCherry, we are aiming at the construction of the genes of chimeric proteins scFv-BAPmut and scFv-mCherry, as well as obtaining these proteins by bacterial synthesis. The following methods and techniques were used in this work: bacterial cell culture, gene cloning, polymerase chain reaction, DNA electrophoresis, DNA sequencing, ELISA, Western blotting, gene expression,

protein purification, protein refolding, immunocytochemistry, and fluorescent microscopy. We chose BAP as one of the marker proteins because of the wide use of this highly sensitive and stable marker protein. Moreover, BAP exists in a dimeric form, so that its avidity redoubles after fusion with scFv. Several chimeric scFv-BAPmut proteins with different specificity were obtained by secretory expression in *E. coli*, that ensures accumulation of the protein in the periplasm and culture medium in a functionally active form. As a result of amino acid substitutions D330N and D153G, we observed a manyfold increase in the phosphatase catalytic activity as part of the chimeric complex compared to the wild-type *E. coli* protein. We demonstrated a high spicificity of the interaction between scFv-BAPmut and antigen as well as the possibility of using bacterial periplasm and culture medium in immunodetection without preliminary purification. mCherry has elevated brightness and photostability and is tolerant enough for fusion with other proteins. The genes of the scFv-mCherry fusion proteins were designed and expressed in *E. coli*. The expressed protein yield was optimized and amounted up to 500 mg of scFv-mCherry per 1 l of *E. coli* culture. The fusion proteins were obtained in milligrams quantities in a purified and soluble form with a rapid and effective on-column folding process. The immunochemical and immunofluorescent assays confirmed that both moieties of the scFv-mCherry fusion proteins retained their functional activity upon refolding. The convenience of designing the sequence encoding specific scFv, simplicity of obtaining recombinant proteins by bacterial synthesis, high sensitivity to antigen, as well as a wide spectrum of available substrates for the phosphatase open new perspectives and utility of the obtained fusion proteins in immunochemical and immunofluorescent analysis.

Role of phospholipase Cε in development of Ph-positive leukemia

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Background. Bcr-Abl, the product of a chromosomal translocation t(9;22), has been demonstrated to be a key protein responsible for the pathogenesis of Ph-positive leukemia. Depending on the breakpoint region of the *bcr* gene, three Bcr/Abl proteins have been observed: p190 Bcr-Abl, p210 Bcr/Abl, and p230 Bcr-Abl. The only structural difference between various Bcr-Abl chimeras is the presence of Dbl-homology (DH) and pleckstrin-homology (PH) domains in p210 Bcr-Abl and p230 Bcr/Abl. p210 Bcr-Abl and p230 Bcr-Abl are responsible for chronic myelogenous leukemia while p190 Bcr-Abl is associated with more aggressive acute lymphoid leukemia suggesting the important role of the DH and PH domains in leukemogenesis. Our recent data demonstrated that the Bcr-Abl PH domain binds a number of proteins including phospholipase Cε (PLCε). Previous studies have shown that PLCε is both target and regulator of the Ras superfamily of GTPases, key players in p210-Bcr-Abl-dependent leukemogenesis. Also it has been shown that PLCε transcripts are not expressed in normal peripheral blood leukocytes. However, a role of PLCε in leukemia progression is not clear yet. **Aim**. The aim of this study is to clarify a role of PLCε in the development of Ph-positive leukemia. The main tasks of the study were: to analyze the presence of PLCε transcripts in patients with myeloproliferative disorders, and to express and purify the recombinant protein related to the RA domains of PLCε. **Methods**. The cDNA was obtained from the peripheral blood leukocytes by one-step RT-PCR using total RNA as template. PLCε expression and the presence and the type of Bcr/Abl transcript were identified using gene specific primers. PLCε expression was detected using oligonucleotide primers specific to Ras/Rap-associating (RA) domains that are a unique distinguishing feature of PLCε among other phospholipase C isozymes. Bcr/Abl rearrangement was detected by nested RT-PCR analysis. Protein expression and purification were performed using standard molecular biology protocols. **Results**. We have analyzed PLCε expression in 12 patients with different MPDs. Our data show that all patients have detectable level of PLCε expression and 9 of them demonstrate the presence of p210 Bcr/Abl translocation. Also we have prepared a genetic construction for bacterial expression of RA domains of PLCε. The recombinant protein was expressed in soluble fraction and its prospective size (46 kDa) was confirmed using electrophoresis in 10 % polyacrylamide gel. **Conclusions**. Our results suggest that the expression of PLCε in peripheral blood leukocytes of patients with MPDs does not correlate with the presence of p210 Bcr/Abl, but it could be a new marker of oncogenic transformation during the leukemia development. Thr obtained recombinant RA domains can be used in further analysis of the molecular mechanisms of Bcr-Abl-PLCε signaling.

Synthesis and biological testing substituted derivatives of 1,2,4-triazynobenzotiazin-carboxylic acids

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Aim. To widen a spectrum of substituted derivatives of 1,2,4-triazinobenzotiazin-carboxylic acids (TBT), to evaluate a series of new substituted TBT as potential inhibitors of transcription and DNA relaxation, and to determine their *in vitro* antimicrobial and antiviral activity. **Methods**. Organic syn-

thesis, spectral methods (methods of NMR-spectroscopy) to confirm structures of the synthesized compounds; *in vitro* testing the synthesized compounds in a model transcription system of bacteriophage T7 DNA-dependent RNA-polymerase as well as in relaxation complex of *Escherichia coli* topoisomerase I, biological trials with gram-positive and gram-negative pathogenic bacteria, and *in vitro* testing in surrogate model of hepatitis C virus (HCV) – bovine viral diarrhea virus (BVDV). **Results**. 28 TBT compounds were synthesized. The relaxation complex of *E. coli* topoisomerase I was not sensitive to inhibition by TBT agents. *In vitro* transcription experiments revealed 13 TBT compounds effectively inhibiting RNA synthesis. Calculated IC_{50} values for 13 derivatives from TBT series were determined within 4–60 μM range and for 5 of them IC_{50} values were found within limits 5–12 μM. The number of effective inhibitors of BVDV was found among the compounds that actively suppress RNA synthesis in the T7 RNAP transcription complex. The most active proved to be TBT-11 with EC_{50} value 1.5 μM, while SI equaled to 80. It should be noted that TBT-11 inhibits RNA synthesis with IC_{50} value ~10 μM. The antimicrobial effect of the tested compounds was evaluated by measuring the inhibition zone diameters. The tested compounds demonstrated no inhibitory activity against all bacteria models that were used. **Conclusions**. We have made an assumption that TBT-11 compound may be the inhibitor of the HCV targeting the HCV RNA-polymerase.

Eukaryotic translation elongation factor A2 is targeted by oncosuppressive hsa-mir-663 and potential oncosuppressive hsa-mir-744

A. A. Vislovukh

Aim. The tissue-specific eukaryotic elongation factor 1A2 (eEF1A2) is a putative proto-oncogene. The overexpression of eEF1A2 has been observed in pancreatic, lung, ovarian and breast cancers. Ectopic expression of eEF1A2 induced the malignant transformation of mouse and rat fibroblasts, and its expression in human ovarian cell line ES-2 that did not normally express eEF1A2, confered a more aggressive neoplastic phenotype. However, despite an example of multiplication of eEF1A2 locus, in the majority of samples of ovarian cancer the overexpression of the gene was not dependent on the modifications in the EEF1A2 locus. Thus, deregulation of eEF1A2 expression can occur on the post-transcriptional level and we assume that the EEF1A2 expression in cancer tissues can be controlled by microRNAs. **Methods**. The levels of eEF1A2 transcript and specific microRNAs were examined by qPCR. Dual-luciferase reporter system was used to check the Influence of micro-RNAs on the expression of the reporter gene that harbors 3'UTR of eEF1A2. **Results**. *In silico* analysis of 3'UTR of EEF1A2 mRNA revealed that it may be potentially targeted by hsa-mir-661, hsa-mir-663, hsa-mir-675 and hsa-mir-744. Co-expression of eEF1A2 mRNA and abovementioned miRNAs was proved by qPCR in HeLa cell line. Using dual-luciferase reporter system we revealed that hsa-mir-663 and hsa-mir-744 downregulate the expression of the reporter plasmid harboring 3'UTR of EEF1A2 mRNA by 20 % and 50 % respectively. Since microRNAs predominantly act to decrease the target mRNA level we checked the effect of hsa-mir-663 and hsa-mir-744 on the endogenous EEF1A2 mRNA. Indeed, hsa-mir-663 was shown to decrease the EEF1A2 mRNA level by 44 % and hsa-mir-744 by 68 %. **Conclusions**. hsa-mir-663 is oncosuppressive miRNA that targets transcripts of several proto-oncogenes such as *Jun*B, *Jun*C and *TGF*β. The gene encoding hsa-mir-663 is hypermethylated in 34–86 % of cases in a series of 71 primary human breast cancer specimens. For the first time we showed that eEF1A2 transcript is also targeted by this micro-RNA. Thus, the loss of posttranscriptional control of eEF1A2 expression can be involved in cancer progression as well. Also, since no data on the function of has-mir-744 are available till now, we hypothesize that one of the roles of hsa-mir-744 is to target eEF1A2 acting as oncosuppressor.

Double-expression recombinant baculovirus vector with reporter *eGfp* and murine β-interferon genes for mammalian cells transduction

O. A. Zaharuk, O. V. Anopriyenko, I. N. Vagyna, L. I. Strokovskaya

Aim. The aim of this study was the construction of double-expression baculovirus vector with reporter *eGfp* and murine β-interferon genes and evaluation of its efficiency for murine fibroblast cells transduction. **Methods**. Recombinant baculoviruses were constructed on the basis of AcMNPV virus using Bac-to-Bac expression system. Virus was concentrated by centrifugation at 100000 *g*. The virus preparations titre after amplification and concentration comprised $(2–4)\cdot10^8$ PFU (plaque-forming units)/ml. The virus dose used to get the optimal results of transduction efficiency *in vitro* was 200 moi. A primary culture of fetal murine fibroblasts was obtained from soft tissues of 14-days long embryos by tissue enzymatic (C57Fb) desegregation. The transduction efficiency and average fluorescence intensity were measured using flow cytometer Coulter Epics XL. The expression of mouse β-interferon was estimated in interferon-titration experiments determining the inhibition of vesicular stoma-

titis virus plaque formation capacity on L929 cell culture. As a control murine β-interferon (Sigma-Aldrich) was used. **Results**. Baculovirus vector Ac-IFN-GFP containing two genes – reporter *eGfp* regulated by CMV promoter and mouse β-*Ifn* regulated by CAG expression cassette with chicken β-actin promoter – was constructed. Efficiency of murine fibroblasts transduction by the double-expression baculovirus vector was evaluated using as a control recombinant baculovirus Ac-CMV-GFP with the only reporter *eGfp* gene regulated by CMV promoter. Efficiency of murine fibroblast cells transduction by Ac-IFN-GFP virus comprised 16.2 ± ± 1.6 % and by Ac-CMV-GFP – 59.1 ± 13.1 %. Thus, we observed the decrease in transduction efficiency more than three times in case of using the double-expression recombinant baculovirus construction with murine β-interferon compared to the control single-reporter gene vector. At the same time it was observed that quantity of biologically active β-interferon for double-expression Ac-IFN-GFP construction and recombinant baculovirus with single β-*Ifn* – Ac-CAG-IFN was approximately equal and comprised (4–8)·10^4 u/ml. **Conclusions**. Double-expression recombinant baculovirus construct allows quick and convenient estimation of the efficiency of mammalian cell transduction and producing high level of biologically active mouse β-interferon in murine fibroblast cells. However, it must be taken into consideration that the β-interferon gene expression may influence the expression of a reporter gene lowering the estimation of transduction efficiency.

Study of somatic segregation in Arabidopsis cells cultured *in vitro*

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Aim. The phenomenon of somatic chromosome segregation is known to represent a chromosome separation in dividing somatic cells like the first meiotic division. But still there is no information about homologous chromosomes behavior during somatic segregation, genetic consequences and fate of cells arising as a result of this event. Investigating the cell division in cells cultured *in vitro*, Chen (2001) and Ronchi (1992) suggested that the cases of somatic chromosome segregation are associated with the cell dedifferentiation and somatic embryogenesis. The aim of our research is to study various aspects of spontaneous and induced somatic chromosome segregation in *Arabidopsis thaliana* cells cultured *in vitro*. At this stage we have optimized culture medium for *A. thaliana* to obtain high efficiency of callus induction and plant regeneration. **Methods**. Ecotypes Columbia (Col N 1093, NASC) and Landsberg *erecta* (Ler N 1298, NASC) of *A. thaliana* were used for experiments. Seeds were sterilized and germinated at 24 °C under continuous light. Callus and regenerated plants were obtained from root explants by technique of Chen (2001) (first system) and shoot-apical-tip explants of seedlings by the method of Ikeda-Iwai (2003) (second system). Cultured material was fixed on callus and somatic embryo formation stages followed with digestion in 2 % cellulose, the cells were stained with DAPI and analyzed using the microscope Zeiss Axiostar Plus. **Results**. High regeneration efficiency up to 37.5 % for Ler and 35 % for Col was observed in the first culture system. Following the procedure for the second system, the efficiency of plant regeneration was 15 % and 12.5 % respectively. The frequency of callus formation for both systems was 100 %. Since the first system had better efficiency of plant regeneration it was chosen for the cytological analysis of embryogenic callus. There were found out diploid cells with prophase chromosomes, separated into two groups, with haploid number of chromosomes (n = 5) each and also some cells with bivalent-like structures, number of which was equal to haploid chromosome set. We refer such events to somatic segregation, the frequency of which was amounted approximately to 1 %. As the chromosomes of Arabidopsis are very small, it was not easy to distinguish and estimate the events of somatic segregation by light microscopy. Therefore, we have developed a model hybrid of *A. thaliana*, the homologous chromosomes of which are marked by reporter transgenes. This system allows studying somatic segregation consequences in cell colonies cultured *in vitro* and in regenerated plants by observing transgenes expression and using DNA markers. **Conclusions**. Two somatic embryo systems were tested and optimal conditions for efficient callus induction and plant regeneration were selected for further study of somatic segregation.