USP1 as a potential partner of Bcr-Abl oncoprotein

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Bcr-Abl protein is a result of reciprocal translocation between chromosomes 9 and 22. There are three Bcr-Abl chimeras known so far: p190, p210 and p230. The variant p210 causes the development of chronic myeloid leukemia. It is distinguished by the presence of PH and DH domains. Based on mass spectrometry analysis, the ubiquitin specific protease 1 (USP1), which is a potential candidate for interaction with the PH domain of Bcr-Abl oncoproteins, has been identified. USP1 belongs to the group of cysteine proteases and acts as a deubiquitinating enzyme. USP1 consists of three domains: two intracellular peptidases and ubiquitin carboxyl-terminal hydrolase. Bcr-Abl degrades in proteosomal way because of the presence of NH2-ubiquitination site at the end of Bcr fragment. We suggest that USP1 may prevent Bcr-Abl from degradation in proteosomes by deubiquitination, which in turn might lead to the accumulation of the protein and disease progression. Thus, inhibiting USP1 can contribute to the rapid destruction of Bcr-Abl in proteosomes and prevention of its accumulation in cell.

Aim: to create recombinant genetic constructs in order to study the role of USP1 in proteosomal degradation of Bcr-Abl.

Materials and methods. For amplification of USP1 fragment the following oligonucleotide primers have been chosen: USP1 fwd (AATTGCCTGGTGTCATACCTAGTG) and USP1 rev (GAGAGACCAATAATATCCAGTAGC). Genetic construct pCMV-XL5-USP1 (obtained from the Department of Molecular Genetics' bank, IMBG) has been used as the matrix plasmid. The components of PCR conditions were set according to the manufacturer's instructions (Thermo Scientific). We cloned the *USP1* gene into vector pUC18 by Sma1 site. Then the *USP1* gene was cut from pUC18-USP1 at the KpN1 and EcoRI restriction sites and subcloned to pCMV-HA and pECFP C-3-USP1. To check the availability and orientation of the insert the following analytical methods were used: restriction, PCR.

Results. PCR sequence of *USP1* was amplified with expected size (2343 bp). After ligation reaction the pUC18-USP1 genetic construction was obtained. Then, after subcloning pCMV, the HA-USP1 and pECFP C-3 constructs for mammalian expression were obtained. The identity of obtained pUC18-USP1, pCMV HA-USP, pECFP C-3-USP1 to the expected results, namely the absence of mutation, the correct reading frame, was confirmed by PCR and restriction.

Conclusions. 1. The ubiquitin specific protease 1 is a potential candidate for interaction with the Bcr-Abl protein. The activity of the USP1 protein may contribute to the accumulation of oncoproteins in the cell that affects the disease progression.

2. We created pUC18-USP1 bearing the *USP1* gene. We have made pCMV HA-USP1 and pECFP C-3-USP1 constructs which will be used for the mammalian expression of USP-1.

3. Protein expressed from these constructs will be used for studying the Bcr-Abl – USP-1 interaction and subsequent experiments.

Novel L-lactate conductometric biosensor based on lactate dehydrogenase/pyruvate oxidase/NAD^+-modified interdigiated platinum electrodes

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Aim. Development and evaluation of the conductometric biosensor with a bi-enzyme membrane for sensitive and selective detection of L-lactic acid.

Methods. Conductometry, drop coating, cross-linking.

Results. We propose and report conductometric L-lactate selective conductometric sensor based on lactate dehydrogenase (LDH)/pyruvate oxidase (PyrOx)/NAD^+ bio-selective membrane (BSM), fabricated using enzymes Layer-by-Layer drop coating and cross-linking via glutaraldehyde vapors. L-lactate oxidation via LDH- and PyrOx-catalysed reactions, resulting in the charged ions (acetate, H^+ and HCO_3^-) generation and causing the conductivity increase in the BSM, is the basis of this work. The dependency of the developed biosensor output signals on pH and buffer concentration as well as operational/storage stability and selectivity/specificity were investigated. The limit of detection for L-lactate, calculated as three times the signal to noise ratio, was equal to 0.025 mM.

Conclusions. An original and sensitive conductometric biosensor based on LDH and PyrOx enzymes with excellent electrochemical properties was developed for L-lactate determination in model samples.

Investigation of intramolecular dynamics and conformational changes of eukaryotic tyrosyl-tRNA synthetase in complexes with substrates

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Tyrosyl-tRNA synthetase (TyrRS) is one of the key enzymes of protein biosynthesis, which catalyzes specific aminoacylation of the homologous $tRNA^{Tyr}$. Mammalian TyrRS consists of two structural modules: the N-terminal catalytic module (mini-TyrRS) and C-terminal cytokine-like module (C-module). Local conformational changes of the TyrRS contribute to the enzyme functioning, but their nature and specific role have not been studied yet. The mini-TyrRS contains three tryptophan residues (Trp40, Trp87, Trp283) which could serve as intrinsic probes sensitive to the enzyme structure. This, in turn, allows investigation of the mini-TyrRS intramolecular dynamics and monitoring local conformational transitions in the protein structure.

The aim of this work was to study dynamic aspects of mini-TyrRS functioning and characterization of local conformational changes due to the enzyme/substrate interaction.

Methods. Recombinant proteins were obtained by bacterial expression in *E.coli* BL21(DE3)*pLysE* using standard methods. The fluorescence spectroscopy has been used to explore the intramolecular dynamics of the mini-TyrRS in solution and study conformational changes of the isolated protein. The visualization and analysis of the Trp residues local environment have been performed using the PyMOL 1.3 program.

Results. According to our analysis the Trp residues of the mini-TyrR are partially solvent-exposed. The ratio of the solvent accessible surface area (ASA) of the Trp side-chain in the native protein to the ASA in the "random coil" state for Trp40, Trp87 and Trp 283 is 8.8%, 62.1%, 56.6%, respectively. We also characterized the microenvironment of Trp40, Trp87 and Trp283 in the 5 Å layer around the Trp residues. The computational analysis has shown that there are seven residues in the region of Trp40: five hydrophobic and two hydrophilic residues; seven residues in the region of Trp87: five hydrophobic and two negatively charged (Glu88, Glu91) residues; six residues in the vicinity of Trp283: three negatively charged (Asp280, Glu281, Asp308), and two positively charged (Arg279, Lys282).

To characterize properties of the tryptophans environment in the mini-TyrR we employed fluorescence quenching by three quenchers: *neutral* molecule (acrylamide), I^- *anion* (KI salt) and Cs^+ *cation* (CsCl salt). The quenching constants, $K_{s\text{-}v}$, were calculated from the slope of Stern-Volmer plot and for mini-TyrRS they are: 13.22±0.5 M^{-1}, 6.26±0.2 M^{-1}, 3.75±0.2 M^{-1} for acrylamide, I^- and Cs^+, respectively; while for free L-tryptophan the $K_{s\text{-}v}$ constants are 16.37±0.01 M^{-1}, 12.94±0.01 M^{-1}, 2.87±0.01 M^{-1}, respectively. The relative quenching efficiencies (RQE) of mini-TyrRS fluorescence as compared to free L-Trp are 80% for acrylamide, 48% for I^-, and 129% for Cs^+.

Conclusions. Fluorescence quenching of mini-TyrRS demonstrates that all three Trp residues are accessible for the used quenchers, which is supported by our computational ASA analysis. The protein fluorescence quenching curves are linear, which means that quenching of fluorescence is a dynamic process. The enhanced relative fluorescence quenching efficiency of Cs^+ cations and decreased RQE of I^- anions could be explained by negatively charged surrounding of some Trp residues in the protein structure, which can attract the cationic and repulse anionic quenchers. This assumption corroborated by our computational analysis data indicated that the microenvironment of at least two Trp residues contains electronegative residues in the vicinity: Trp87 (Glu88, Glu91) and Trp283 (Asp280, Glu281, Asp308). Interestingly, the residues in a static protein structure are tightly packed, however the RQE of acrylamide is high, RQE=80%. The observed high efficiency of quenching, i.e. an ability of acrylamide molecules to penetrate deep into the protein structure could be a result of quite large dynamic fluctuations of the mini-TyrRS structure.

Cloning murine interferon alpha in E.coli and optimization of its output in the soluble form

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Background: Interferon alpha (IFNα) is a cytokine with antiviral, antiproliferative and immunomodulatory activities. It is widely used in the treatment of viral hepatitis and hematological malignancies. Despite its efficiency it has side effects of unknown mechanism like depression, headache, fever, myalgia etc. The bioinformatical genome-wide search for target genes of IFNα conducted in our lab has revealed three new genes encoding the proteins of nervous synapses. We suggest the involvement of these genes in the development of side effects of IFNα and intend to verify this idea.

Aim of the study: to clone the murine *IFNα* gene and express the protein in the sufficient amount for *in vitro* and *in vivo* experiments.

Methods: The amplified coding sequence of *mIFNα,* type 11 (BC116870) was inserted in the expression plasmid pET-24a(+) downstream of the IPTG–induced T7 promoter and cloned in Rosetta (DE3) *E.coli* cells. The basic cultivation medium: 30mM NaCl, 7 mM NH_4Cl, 9 mM $MgSO_4$, 0,5% w/v Yeast Extract, 1,1% w/v Tryptone, 0,2% w/v Glycerol. The basic lysis buffer: 25 mM HEPES pH 7.0, 500 mM NaCl, 10% w/v Glycerol, 0.025% w/v NaAzide, 0.5% w/v CHAPS, 10 mM $MgCl_2$. To optimize the output of soluble mIFNα11 the fractional factorial design 2^{7-4} with resolution IV was applied. The effect of seven variables: T °C of cultivation (25 – 37 °C), pH of the medium (6.8 – 7.4), concentration of glucose (0 – 1% w/v), trehalose (0 – 200 mM) and glycine (0 -200 mM) in the medium and glycine (0 – 200 mM) and CTAB (0 – 1% w/v) additives in the lysis buffer was explored. The amount, identity and proper folding of the soluble IFNα were controlled by 12% SDS–PAGE and the resistance of the Gasser's ganglion cells to the vesicular stomatitis virus. The statistical analysis was carried out in the *R* statistical software (R version 2.8.0, http://www.rproject.org). The significant effect was considered at $p \leq 0.05$.

Results: The IFNα output in dependence of seven variables may be described by multiple regression model $y=A+B\cdot x_1+C\cdot x_1^2+D\cdot x_2+E\cdot x_3+F\cdot x_5+G\cdot x_6+H\cdot x_7$ with $R^2=0.91$, where x_1 - x_7 – tested variables, A - H – the corresponding effect. The statistically significant influence on the output had T °C of cultivation and the concentration of three carbohydrates in the medium while the optimal ones were 200 mM glycine added to the basic medium and cultivation at 25°C. A 19 kDa protein similar to mature mIFNα11 was identified in the initial homogenate and supernatant; both revealed a specific antiviral activity; the yield of the soluble mIFNα11 was ~20 mg/L.

Conclusions: The fractional factorial design for optimization of the recombinant protein output is a useful approach saving the time and cost of experiment. The pET-24a(+)–mIFNα11 recombinant is a promising construction for mIFNα11 output in the soluble form and in appropriate amount for *in vitro* and *in vivo* experiments.

New approaches for inferring gene regulatory network and their application

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Introduction. The gene regulatory network (GRN) reflects the interaction between its various elements, genes or proteins, and provides the most complete information on the regulation of cell functioning. The significant problems the scientists encounter are an accuracy of reconstruction together with validation and computational complexity of the GRN inference, which means that the time required to reconstruct GRN increases very quickly as a size of the data grows. Though various formalisms are already proposed to reconstruct GRN the main challenges still remain.
Aim was to improve the accuracy of inference algorithms by applying ensemble method and to validate obtained results using synthetic data based on the biological networks topology.
Results. The network inference methods have complementary advantages and limitations in different contexts, which suggests that combining the results of multiple inference methods could be a good strategy for improving predictions.
We studied different ways how to combine graphs (union, intersection) as well as edge scores and how particular combinations affect the validation of the resulting networks. In order to get reconstructed networks we used different inference software. One of such softwares is BNFinder – a tool for inferring optimal Bayesian networks, which has been already successfully applied not only to the reconstruction gene regulatory networks, but also to the linking expression data with sequence motif information, identifying histone modifications connected to the enhancer activity and to the predicting gene expression profiles of tissue-specific genes. As a result we got a new parallel version of BNFinder, which we successfully tested in the Ukrainian GRID infrastructure.
In addition to BNFinder we used MiNET R package, which implements information-theoretic approaches to the infer gene regulatory networks: ARACNE, MRNET, MRNETB, CLR. Test dataset, it provides, contains 100 samples and 50 genes generated by the publicly available SynTReN generator using a yeast source network. Complementary to this validation of algorithms we used the expression profiles with corresponding golden networks provided by DREAM initiative - a Dialogue for Reverse Engineering Assessments and Methods. The topologies of networks we used are generated by extracting modules from the known *in vivo* gene regulatory network structures such as those of *E.coli* and *S.cerevisiae*.
The best result was obtained by the combination of two different algorithms – BNFinder and MRNET. The characteristics of this combination (AUROC - area under receiver operating characteristic and AUPR - area under precision recall) appeared substantially better than those of each specified methods and their combinations.
Conclusion. Here we present the results of this study with the best-scored ensemble method – undirected graph union with linear combination of scores.

Discovery and characterization of N-phenylthieno[2,3-d]pyrimidin-4-amines as inhibitors of FGFR1

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Aim. Fibroblast grow factor receptor 1 (FGFR1) is an important anti-cancer target that plays crucial role in oncogenesis and oncogenic angiogenesis. FGFR1 has been shown to be frequently amplified or overexpressed in various cancers, including breast, lung, prostate cancers, myeloproliferative disorders. The role of FGFR1 in cancerogenesis makes it potential therapeutic target for the treatment of cancers. Thienopyrimidine derivatives have been known as protein kinase inhibitors. The aim of the present study was to develop thieno[2,3-d]pyrimidines-based FGFR1 inhibitors and test their selectivity and antiproliferative activity.

Methods. The methods of the combinatorial synthesis, semiflexible docking, γ-^{32}P-ATP assay and MTT tests were used in this work.

Results. A series of 33 thieno[2,3-d]pyrimidines derivatives have been synthesized and their biological activities as inhibitors of FGFR1 kinase were evaluated. The SAR study showed that substitutions with phenyl in the 5- or 6- positions of the thienopyrimidine heterocycle and substitutions with metahydroxyl group in N-phenyl ring increased the FGFR1 inhibition activity. Molecular modeling studies revealed important structural features of thieno[2,3-d]pyrimidines for binding affinities towards FGFR1 kinase domain. The most active compounds were 3-({6-phenylthieno[2,3-d]pyrimidin-4-yl}amino)phenol and 3-({5-phenylthieno[2,3-d]pyrimidin-4-yl}amino)phenol (IC_{50} 0.16 and 0.18 μM respectively). These compounds displayed a good selectivity profile against a panel of 6 kinases. These active FGFR1 inhibitors have also antiproliferative activity in human myeloma cell line KG1 with GI_{50} 26.2 and 16.2 μM respectively.

Conclusions. Thieno[2,3-d]pyrimidines derivatives were discovered as potent and selective FGFR1 kinase inhibitors with anticancer activity. The obtained results can be used in further development of FGFR1 inhibitors.

Role of cytoskeletal reorganization in BCR/ABL-induced signaling pathways.

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Background. Cytoskeleton reorganization is one of the ways, in which the cell coordinates its architecture with the signaling pathways. BCR protein is involved in the membrane stretching and following cytoskeleton changes during axon guidance. The presence of DH and PH domains in the BCR is important for this purpose. The first one has GTPase activity which is necessary for the molecular switching and the second one is known for its lipid-binding activity and involvement in cytoskeleton reorganization. However, little is known about a specific role of the PH domain of BCR, and how it acts as a part of BCR/ABLp210, the fusion protein with constitutive tyrosine-kinase activity that is a triggering factor for the chronic myeloid leukemia development. Previously D. Miroshnichenko et al. showed that it is able to bind several proteins from K562 lysate, that are a part of the cytoskeleton or involved in its reorganization.

Aim. The aim of our research was to verify whether the PH domain of BCR interacts with cortactin and keratin 10.

Methods. For recombinant protein expression, the genetic constructs from previous study were used. Specifically, pcDNAhisMaxC-PH and pECFP-CTTN were used for PEI transfection of 293T cells. For bacterial expression, pET32a-PH, pGEX4T2-CTTN, and pGEX4T2-KRT10 were employed. The sequences of PH domain and KRT10 were expressed using IPTG induction at 22^0C, and the sequence of CTTN was expressed at 37^0C using autoinduction according to Strudier et al. The affinity purification of his-tagged PH was done with Ni-NTA agarose, the GST-tagged KRT10 and CTTN were purified using glutathione sepharose. A theoretical chance of protein solubility in *E.coli* was calculated according to the logistic regression model of Diaz and Harrison. The expression and purification efficiency of his-tagged PH domain and GST-tagged KRT10 and CTTN was estimated by PAGE and Western-blot. The expression of ECFP-CTTN was estimated by the wide-field fluorescent microscopic analysis.

Results. Western-blot confirmed the efficient purification of PH domain from *E.coli* lysate, and expression of PH in 293T cell line. The efficiency of purification of GST-CTTN from *E.coli* lysate was confirmed by PAGE and Western Blot. The expression of ECFP-CTTN was confirmed, it is localized predominantly at the cell periphery. GST-KRT10 was insoluble when expressed in *E.coli* despite the optimization of expression temperature, *E.coli* strain, additives to lysis buffer and growth media. A theoretical chance of GST-KRT10 insolubility in *E.coli* is 95%.

Conclusions. The obtained recombinant GST-cortactin and his-PH can be used for far-Western blot analysis of a possible protein-protein interaction. The localization of ECFP-CTTN indicates its possible role in the cytoskeleton-membrane reorganization. A partial expression of KRT10 in *E.coli* is a strategy to overcome insolubility. Co-immunoprecipitation of his-PH and ECFP-CTTN may be performed to determine the presence or absence of interaction inside the cell.

Gene expression signature for glioblastoma subtypes and participation of *CHI3L1* gene in malignant transformation

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Aim. Glioblastoma is the most aggressive intracranial malignancy characterized by high invasiveness, recurrence, and poor response on chemo- and radiotherapy. Heterogeneous character of glioblastoma eliminates the value of single molecular markers. Data from high-throughput gene expression analysis allows to identify characteristic gene expression profiles (signatures), associated with specific tumor properties, that could be used in clinics for diagnosis and prognosis. «Mesenchymal» subtype of gliobastoma is characterized by overexpression of the set of genes, and among them the gene, encoding chitinase-3-like protein 1 (*CHI3L1*), involved in abnormal cell proliferation and tumor angiogenesis. Clarification of the mechanisms of *CHI3L1* regulation and structure-functional characterization of the protein could shed light on the process of malignant transformation.

Methods. We used cluster analysis and self-organizing map approach using data on gene expression to search for glioblastoma intratumor differences. To study regulation of CHI3L1 level by p53, we overexpressed *TP53* in U87 glioblastoma cell line, treated U87 cells with resveratrol to activate p53 pathway or sirtinol to increase p53 stability. We used homology modeling and site-directed mutagenesis to detect regions of CHI3L1, responsible for the cell growth in soft agar.

Results. Distribution of glioblastoma on two subgroups on the basis of the expression of 416 genes, selected in our work, could represent two routes of glioma development, leading to the formation of the subtype with increased expression of either «proliferative» or «proneural» genes. Ectopic expression of *TP53* in glioblastoma U87 cells led to decreased CHI3L1 protein level. This effect was also achieved either by activation of p53 by antitumor agent resveratrol or by inhibiting p53 degradation mediated by sirtuin SIRT1, using specific inhibitor sirtinol. To study structural peculiarities of CHI3L1, we compared 3D structures of CHI3L1 and its closest homolog CHI3L2, and showed the presence of unique positively charged amino acid cluster 144-RRDKQH-149, located on the protein surface of CHI3L1. Site-directed mutagenesis of potential CHI3L1 heparin-binding region revealed that residues Arg144, Arg145, and Lys147 are crucial for the binding. Soft agar assay demonstrated that mutation in heparin-binding site significantly decreased colony formation efficiency after stable transfection of 293 cells.

Conclusions. We demonstrated the existence of «proliferative» and «proneural» glioblastoma subtypes, that could be determined on the basis of the expression of 416 genes. As p53 was found to decrease *CHI3L1* expression in glioblastoma cells, inactivation of p53 in tumors could lead to the overexpression of *CHI3L1*, and, consequently, increase CHI3L1-mediated pathological effects, such as abnormal cell proliferation and angiogenesis. *CHI3L1* can enhance malignant properties of 293 cells after its ectopic expression, and its heparin-binding site can be responsible for substrate-independent cell growth.

Characteristics of new spliced isoform of ribosomal protein S6 kinase 1 – S6K1Δ15

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The ribosomal proteins S6 kinases (S6K1 and S6K2) are members of the PI3/Akt/mTOR pathway and play an important role in the transcription, translation, cell growth/size and metabolism. Recently, the link between expression of S6K1 splicing isoform-2 (p31) and oncogenic transformation of cells has been reported (*Karni 2007, 2013*). We have identified another spliced isoform of S6K1 with a deleted last exon (called S6K1Δ15) and confirmed its expression in cell lines and tissues at mRNA level.

The Aim

To examine oncogenic activity and place of S6K1Δ15 in PI3K/mTOR/S6K signaling pathway as well as to determine its subcellular localization.

Methods

The investigation was performed on model HEK293 cells with stable expression of S6K1Δ15. To define subcellular localization of S6K1Δ15 we used immunofluorescent microscopy and nuclear/cytoplasm fractionation. The proliferative activity and viability under serum starvation were examined by MTT and LDH tests respectively. Soft agar assay with subsequent data estimation using *Image J* software has been used to evaluate anchorage-independent cell growth. To determine the signaling activity of S6K1Δ15 we analyzed an effect of rapamycin (specific inhibitor of mTOR) treatment and serum starvation on phosphorylation of S6K1 and its substrate - S6 protein by Western blot in cells expressing S6K1Δ15.

Results

No significant impact of S6K1Δ15 overexpression on proliferative activity of cells was found. However, the expression of studied S6K1 isoform caused an increase in cell viability under serum starvation compared to the control cells (transfected by empty vector). The efficiency of colony formation of the cells overexpressing S6K1Δ15 was slightly higher (15.4%) than the control cells (12.7%) but the size of colonies formed by the cells overexpressing the spliced isoform was smaller than in control. Using cells fractionation we determined that the major part of recombinant protein S6K1Δ15 was located in cytoplasm. Additionally, the inhibition of nuclear export by leptomycin B, did not lead to the accumulation of S6K1Δ15 in nucleus. However, according to the data of IF microscopy, S6K1Δ15 was detected in both cytoplasm and nucleus. Unexpectedly, we found that the phosphorylation of T389 S6K1Δ15 was not sensitive to the rapamycin treatment or serum starvation as opposed to the full-length forms of S6K1. At the same time the phosphorylation of S6 protein remained sensitive to rapamycin and starvation despite the presence of phosphorylated S6K1Δ15.

Conclusions

S6K1Δ15 did not show evident oncogenic activity, though it could slightly increase an ability of cells to anchorage-independent growth and to survival under the serum starvation. The regulation of S6K1Δ15 and its activity toward a substrate had obvious differences in comparison with well-known S6K1. The protein of S6K1Δ15 is located preferably through the cytoplasm, but existence of the nuclear pull cannot be excluded.

Colocalization of mTOR kinase and cytokeratins in the human epithelial cells

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Background. Mammalian Target of Rapamycin (mTOR) is a serine/threonine protein kinase, which integrates signals from hormones, growth factors, cytokines and nutrients. mTOR plays an important role in multiple cellular events, such as protein biosynthesis, growth, proliferation and survival. Nowadays, mTOR inhibitors are considered as perspective anti-cancer and anti-aging drugs. It was shown that rapamycin inhibited migration and invasion of malignant cells, but the mechanism of this phenomenon was not fully understood. For the first time we revealed and clarified the colocalization of mTOR kinase and cytokeratins in the human epithelial cells.

Aim. Investigation and confirmation of the mTOR kinase colocalization with the cytokeratins.

Methods. Anti-N-terminal mTOR antibodies were generated in our laboratory and tested by western blot and immunocytochemistry in the presence of the antigen polypeptide. Different human epithelial cell lines, such as MCF-7, MCF-10A, HeLa, HepG2, A549, and also histological sections of the normal and malignant human breast tissues were used in the research. Colocalization of mTOR and intermediate cytokeratins was studied by double immunofluorescent analysis and then visualized with confocal or fluorescent microscopy. To verify interconnection of mTOR kinase and cytokeratins we performed co-immunoprecipitation and proximity ligation assay (PLA).

Results. Earlier we revealed the strong colocalization of mTOR kinase and cytokeratins in the human MCF-7 cell line using the anti-N-terminal mTOR antibodies, generated in our laboratory. To confirm obtained data, we have shown that anti-N-terminal mTOR antibodies, specifically recognized mTOR kinase in western blot and immunofluorescent analysis. To exclude the possibility of cross reaction of the antibodies against N-terminus of mTOR and cytokeratins (№ 4, 5, 6, 8, 10, 13, 18) the amino acid sequences of N-terminal region of mTOR (24-120 a.a.) and every mentioned cytokeratins were compared using protein BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). There was no significant homology of compared sequences. Confocal microscopy revealed colocalization of mTOR kinase and cytokeratins in a set of human cell lines and epithelial cells of the human breast tissue samples. Also the use of a series of the alternative fixation and permeabilization protocols did not alter the link between mTOR kinase and keratins. Comparison of the anti-N-terminal mTOR antibodies, generated in our laboratory, with commercially available ones have shown that both tested antibodies recognized mTOR kinase at the fibrils of the intermediate filaments. Co-immunoprecipitation revealed that different types of anti-mTOR antibodies precipitated keratins from lysates of MCF-7 cells. Obtained data were also confirmed by PLA.

Conclusions. For the first time we discovered and confirmed the colocalization of mTOR kinase and cytokeratins in the set of the human cell lines, normal and malignant breast tissue samples.

Comparative analysis of immunophenotype and *in vitro* differentiation potential of hematopoietic progenitors cells from fetal hematopoietic tissues

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Aim. The aim of our study was to investigate the immunophenotype characteristics and potential to differentiation *in vitro* of hematopoietic progenitor cells (HPCs) from placental tissue in comparison with HPC from cord blood and fetal liver.

Methods. All tissues were received according to the women's informed consent. Term and fetal placental tissues were enzymatically digested, fetal liver was mechanically digested and cells from all sourses were harvested for FACS analyses with the antibodies: anti-CD34, anti-CD45, anti-CD45RA, anti-CD90, anti-CD31, anti-CD235, anti-CD7, anti-CD19, anti-CD33, anti-CD14 (BD, USA). CFU analysis was performed with culture medium MethoCult (StemCell Tech., Canada) and agar-containing medium.

Results. It has been shown that HPCs from placental tissue were characterized by phenotypic heterogeneity unlike cord blood and fetal liver, namely they contain the populations such as $CD34^{+++}CD45^{low}$/-, $CD34^{++}CD45^{low}$/- (very early progenitors that express CD133), $CD34^{+/low}CD45^{low}$/-, $CD34^{++}CD45^{+}$ and $CD34^{+/low}CD45^{+}$ (late progenitors). Majority of HPCs from placental tissue and umbilical cord blood remained uncommitted in contrast to fetal liver. Placental tissue in compare to cord blood had higher amount of later myeloid ($CD34^{+}CD45^{low}CD14^{+}SSC^{low}$) and erythroid ($CD34^{+}CD45^{low}CD235^{+}SSC^{low}$) progenitors, tendency to higher content of T- lymphoid ($CD34^{+}CD45^{low}CD7^{+}SSC^{low}$) progenitors and similarities in the amount of myeloid ($CD34^{+}CD45^{low}CD33^{+}SSC^{low}$) and B-lymphoid ($CD34^{+}CD45^{low}CD19^{+}SSC^{low}$) progenitors. Fetal liver had higher level of myeloid, T- lymphoid and B-lymphoid progenitors in compare to placenta and cord blood and the same level of erythroid progenitors. Placental HPCs have similar potential for differentiation *in vitro* in comparison with cord blood HPCs as gave rise to BFU-E, CFU-E, CFU-M, CFU-G, CFU-GM and CFU-GEMM. For the first time we have shown that umbilical cord blood and placental tissue in addition to BFU-E and CFU-E contain high proliferative HPCs that give rise to the flat erythroid colonies in semisolid mediums.

Conclusions. The investigation have shown the presence of HPCs in different stage of differentiation and all types of lineage HPCs in placental tissue similarly to cord blood and fetal liver. Such facts evidence that placental hematopoiesis continue during all term of gestation. Placental tissue contains significantly lower number of lineage committed HPCs in compare to fetal liver but significantly higher in compare to cord blood that makes placenta an attractive source of HPCs for medicine.

Regulation of the O^6-methylguanine-DNA methyltransferase (MGMT) transcription by hormones

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Aim. The human DNA repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT) removes alkylation adducts from the O^6-guanine in DNA, preventing point mutations and cell death. On the other hand, it plays a crucial role in the resistance of cancer cells to alkylating agents of chemotherapy. Combination of the chemotherapy and the hormone therapy is widely-used for the treatment of many types of cancer. For example, glucocorticoids, estrogens, progesterone and their antagonists are used during treatment of breast, endometrial, kidney, brain and other cancers. Currently, little is known about effects of hormones on the *MGMT* transcription. Only glucocorticoids (e.g. dexamethasone used in clinic) are known to up-regulate the *MGMT* transcription and cause resistance to the alkylating chemotherapy.

Therefore, the aim of this study was to search hormone response elements (HREs) within the human *MGMT* promoter.

Methods. We performed *in silico* analysis to predict HREs within the promoter region of the human *MGMT* gene (acc. number at GenBank X61657.1, 1157 bp). We used the JASPAR Core database, as well Cister, LASAGNA-Search, MAPPER, NHR-scan, NUBIScan, Paint, PROMO, PromoterScan, SignalScan, SiteGA, Tfscan, TFSEARCH and Tfsitescan programs.

We used the RT-qPCR assay to determine levels of mRNA coding for the human MGMT protein in HEK293, Hep-2 and HepG2 cell lines after treatment with hormones. Nine housekeeping genes (*ACTB*, *B2M*, *GAPDH*, *18S*, *TBP*, *TOP*, *HMBS*, *YWHAZ*, *RPLPO*) were used to detect the most suitable reference genes for each model. To select them obtained results were analysed by NormFinder software (Andersen et al., 2004), as well as GeNorm algorithm (VBA macros for Microsoft Excel and *qbase+* software at Biogazelle). *RPLPO* and *HMBS* are the most stable during cell treatment by estrogen.

Results. We predicted several novel HREs within the *MGMT* promoter, localization of which was confirmed by two and more programs. Among them are such, which bind homodimers and/ or heterodimers of steroid hormone receptors, including glucocorticoid receptor, progesterone receptor, estrogen receptor, as well as thyroid hormone receptor-like factors.

We confirmed two predicted by Harris and colleagues (Harris et al., 1991) glucocorticoid responsive elements in positions 28-42 and 63-77 of X61657.1, as well as predicted many novel ones. Also, we revealed potential binding sites for progesterone and estrogen receptors.

Conclusions. Thereby, we predicted novel cis-regulatory HREs within the human *MGMT* gene promoter using *in silico* analysis. To study their functional role in the regulation of this gene transcription, we designed promoter constructs for the luciferase assay.

Recombinant CK2a and CK2α' subunits differ in their sensitivity to same inhibitors

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CK2 is a serine/threonine protein kinase that have tetrameric structure consisting of two regulatory (β) and two catalytic (α and/or α') subunits. Despite the similar enzymatic properties of the last ones in experiments *in vitro* they have different localization in the cell and exhibit differences in tissue-specific expression and function. It has recently been shown that CK2α and CK2α' are involved in various pathological conditions independently of each other. [1]. Therefore, isoform-selective inhibitors of CK2 will be a significant tool for studying of individual catalytic subunits role in cellular processes and its regulation.

Aim. The aim of our research is to evaluate of activity of protein kinase CK2 submicromolar inhibitors from different chemical classes towards its catalytic subunits.

Methods. Evaluation of activity of 19 potent CK2 inhibitors was carried out on the previously obtained CK2α and CK2α´ recombinant proteins.

Results. The biochemical experiment shown the same compounds had different effect on catalytic subunits. The most isoform-selective inhibitor was 4`-hydroxyflavone derivative with IC_{50} = 0.020 μM (CK2α) and 0.003 μM (CK2α′). To explain this difference the complexes of this compound with CK2α and CK2α´ ATP-binding site have been analysed with molecular modelling methods.

Conclusions. Thus, obtained results can be used to further optimization and development of CK2 isoform-selective inhibitors.

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Genes with altered expression in prostate cancer as putative biomarkers of invasion and metastasis

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Introduction: Prostate-specific antigen screening is used for prostate cancer diagnosis, but there is a need for more specific biomarkers to distinguish dormant from metastatic tumors at an early stage. Prostate cancer cell lines with different invasion and metastatic ability could be valuable models for such investigations.

Aim: to find differentially expressed genes in prostate cancer cell lines with different invasion and metastatic ability and in adenoma and prostate carcinoma as putative biomarkers of cancer invasion and metastasis.

Methods: Q-PCR was used to analyze expression of 65 cancer-related genes in androgen-dependent (AD) LNCaP and androgen-independent (AI) DU145, PC3 prostate cancer cell lines compared to normal PNT2 cell line. Cancer PathFinder RT2 Profiler PCR array (84 genes) was used to determine difference in gene expression between LNCaP and PC3 cell lines. *Not*I-microarray was used to examine gene expression in prostate adenoma and carcinoma biopsy samples.

Results: Expression of 29 genes was changed in LNCaP cell line, 20 genes - in DU145 and 16 genes - in PC3 compared to PNT2. Most changes undergo genes of cell adhesion (*CDH1*), invasiveness and metastasis (*IL8, CXCL2*), cell cycle control (*P16, CCNE1*). These genes might be used to create diagnostic panels for invasive metastatic prostate tumors. 36 from the 84 investigated genes have altered expression in PC3 compared to LNCaP. Genes involved in angiogenesis (*PDGF, TGFB1, THBS1*), invasiveness and metastasis (*MET, MMP1, PLAU*), and counteracting apoptosis (*BCL2, BCL2L1*) were over-expressed in PC3 cell line. Among them 7 genes (*MET, MMP1, PLAU, SERPINE1, EPDR1, TGFB1, VEGFA*) were selected for further investigations in cancer invasion and metastasis. Genes for detection of prostate adenoma, AI and AD carcinoma (*BHLHE40, FOXP1, LOC285205, ITGA9, RBSP3*) and discrimination between prostate adenoma and carcinoma (*FAM19A4, CAND2, MAP4, KY, LRRC58*); AI and AD carcinoma (*LOC440944/SETD5, VHL, CLCN2, OSBL10/ZNF860, LMCD1*) according to obtained *Not*I-microarray data were selected.

Conclusions: Obtained results of gene expression in prostate cancer cell lines evidence that main signaling pathways involved in transition to AI type of prostate cancer are cell adhesion, cell cycle control, angiogenesis, invasion and metastasis pathways. Genes with altered expression in AI prostate cancer cell lines PC3, DU145 compared with AD cell line LNCaP might be putative biomarkers of prostate cancer invasion and metastasis. However, all selected genes need further validation.

Comparative analysis of nuklex and nucleinat influence on the expression of some genes encoding the innate immunity system components

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Aim. Oligoribonucleotides, dsRNA and ssRNA interact with receptors and trigger signaling pathways that lead to the activation of transcription factors that initiate gene transcription of interferon, 2'-5'OAS / RNase L system, protein kinases, cytokines and other factors of innate immunity. The aim of our study was to compare the Nuclex and Nucleinat influence, on gene expression of some genes encoding the innate immunity system components. These substances were produced from yeast oligoribonucleic acid.

Methods. The gene expression of *IFNα*, *IFNβ*, *MX1*, *OAS1* and *RNASEL* were studied in lung cells of influenza virus infected mice by RT-PCR method.

Results. Significant increasing of the *IFNα* and *IFNβ* genes expression was observed upon the Nuclex injection into mice for prevention and for treatment, in comparison with the control group of animals. Besides, the expression of *IFNα* gene was increased in 2.5 times, when Nucleinat was injected for prevention and in 1.25 when it was injected for treatment, in comparison with animals which were treated by Nuclex . It is interesting to note, that the expression of *IFNβ* gene in a case, when the animals were injected by Nucleinat, in comparison with the Nuclex, was in two times higher, for both, prevention and treatment. It is important to note the presence of differences in the expression of *Mx1* gene in mice that received these substances. Separately, when Nuclex was injected for prevention, the expression of this gene was in 1.4 times higher than in the case of Nucleinat prevention. When these substances were injected to animals infected with influenza virus for treatment, the *Mx1* gene expression was higher more than in two times than, in case, when Nuclex was used. The *OAS1* gene expression increased almost in 19 times when Nuclex was injected for prevention, in comparison with the control group of mice. When Nucleinat was treated for prevention, the *OAS1* gene expression was increased in 1.6 times compared with animals which were treated by Nuclex. In contrast, the *RNASEL* gene expression decreased in the case of Nuclex and Nucleinat injection. It should be noted that the injection of Nucleinat for treatment leads to the decreasing of *RNASEL* gene expression in 1.4 times, compared with the Nuclex injection. The similar tendency in expression of these genes was observed for influenza infected animals.

Conclusion. These features of gene expression of mice infected with influenza virus show us the differences in the typical clinical manifestations of Nuclex and Nukleinat drugs. Despite of their common origin from yeast ribonucleic acid Nuclex exhibit antiviral properties and Nucleinat - to immunomodulatory properties.

Clinoptilolite application in the conductometric biosensors

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At present ammonium is widely determined in clinical medicine, water quality monitoring, and research (e.g., the accurate measurement of ammonium is fundamental to understanding of nitrogen biochemistry in aquatic ecosystems). Determination of urea and arginine, compounds of the diagnostic relevance for variety of the metabolic disorders, has become possible due to just determination of ammonium ions generated in the enzymatic decomposition of the formers (with urease and arginase/urease reactions respectively) by the electrochemical sensors. Therefore the search of the selective probes for ammonium is of great importance for the development of the highly sensitive and reliable sensors for determination of ammonium itself and improvement of performance of the enzyme biosensors.

Aim. The analytical characteristics of the RClt-based urease biosensor and the RClt-based biosensor for arginine were studied and compared.

Methods. The scope of this study is the influence of the raw form of the natural zeolite Romanian clinoptilolite (RClt) on conductivity of the ammonium- and sodium-based aqueous solutions, phosphate buffer solution, and phosphate buffer solution, in which ammonium was injected afterwards. Coefficients of selectivity of a RClt-modified pair of electrodes were calculated in the aqueous solutions of NH_4^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, and Al^{3+} ions and the phosphate buffer (according to the Fixed Interference Method).

Results. It was shown that zeolite being introduced to 0.01 mM NH_4NO_3 and 0.01 mM $NaNO_3$ aqueous solutions significantly increased the overall conductivity of the solutions (8.3 times and 13.4 times respectively). The progressive increase in the conductivity of the obtained suspensions (suspension “ammonium+zeolite” and suspension “sodium+zeolite”) was observed with time and at the increasing concentrations of both ions in the distilled water comprising zeolite (5% m/v). However the introduction of zeolite to the phosphate buffer solution (5 mM KH_2PO_4-Na_2HPO_4) decreased the conductivity of the buffer, and the ammonium ions injected to this suspension further did not cause the perceptible shift in the background conductivity. This was only observed when the concentration of ammonium in the buffer reached the point of 0.2 mM testifying for the retention of the ammonium-dependent signal due to the competitive ion-exchange processes on zeolite if compared with the conductivities of the pure buffer solution at the same ammonium concentrations (the G growth in the buffer solution with no zeolite was observed when the ammonium concentration in the buffer reached the point of 0.04 mM).

Conclusion. Comparison of coefficients of selectivity of the RClt-modified pair of electrode shows that Na^+ and K^+ ions have the interference effect on the ammonium uptake onto RClt in the aqueous solutions but not in the buffer solution. Incorporation of RClt to the selective elements of the conductometric biosensors for urea and arginine results in the significant increase in the biosensor’s sensitivity.

Conformational changes of human tyrosyl-tRNA synthetase in the complex with tyrosyl-adenylate studied by molecular dynamics simulations

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Tyrosyl-tRNA synthetase (TyrRS) is a key enzyme of protein biosynthesis, which catalyzes the aminoacylation of $tRNA^{Tyr}$ via tyrosyl-adenylate intermediate formation. Once formed, the aminoacyl-adenylate is stabilized by specific interactions at the enzyme active site.

Aim. In this work we have studied the conformational changes in human tyrosyl-tRNA synthetase induced by tyrosyl-adenylate formation using computational MD simulations.

Methods. 100 ns MD simulations were performed using grid services of the MolDynGrid virtual laboratory (http://moldyngrid.org/). Three-dimension structure of *Hs*TyrRS was constructed in Modeller 9.7 using structure templates (PDB codes: 1N3L, 1NTG and 1OPL for interdomain linker). Tyrosyl-adenylate intermediate from the crystal structure of tyrosyl-tRNA synthetase of *Bacillus stearothermophilus* (PDB code: 3TS1) was used as a template. The full-length *Hs*TyrRS structure in the complex with tyrosyl-adenylate was constructed and optimized in AutoDock 4 and MGLTools 1.5. Localization of the potassium ion (K^+) corresponds to the coordinates from crystal structure of human mini-TyrRS (PDB code: 1Q11). The 100 ns MD trajectory of *Hs*TyrRS with tyrosyl-adenylate and K^+ complex was computed using NAMD 2.10 software in Charmm27 force field. The Distributed Analyzer Script was used for analytical tools automation (Savytskyi et al, 2011).

Results. Root mean square deviation (RMSD) analysis shows relaxation period after 20 ns of time. Tyrosyl-adenylate binds at the active site via hydrogen bonds interactions with more than 10% of time: Thr42 – 76.08%, Asp173 – 76.08%, Tyr39 – 71.24%, Trp40 – 46.14%, Gln170 – 42.46%, Ala43 – 16.85%, Asn212 – 16.33% and Val215 – 13.49%. The lowest values (~ 0.06 nm) of root mean square fluctuation (RMSF) in active site were observed for the catalytic KMSSS loop in monomer A in the complex with substrate, while in monomer B without substrate they were much higher (~ 0.2 nm). A novel antiparallel β-sheet formation at the Ala355-Val363 region of the interdomain linker was revealed for 3-100 ns time interval. Also, the β-turn formation in Pro365- Arg367 region was revealed for 40-90 ns time interval at the interdomain linker.

Conclusions. The conformational changes at both the active site and the interdomain linker of human tyrosyl-tRNA synthetase in the complexes with substrates were observed during MD simulations. The active site of monomer A in the complex with substrates reveals more compact conformation with lower values of RMSF in comparison with free monomer B. Some local conformational changes (antiparallel β-sheet and turn formations) have been observed at the linker. These findings support the idea that the secondary structure formation in the interdomain linker could take a part in the interdomain compactization in human tyrosyl-tRNA (Savytskyi et al, 2013).

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Characterization of MMSC cultures from umbilical cord matrix cultivated in various gas mixtures and under standard conditions

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Aim. The aim of present work was to study the changes in MMSC from umbilical cord matrix (UC-MMSC) cultures, cultivated in various gas mixtures, and to determine optimal conditions of cultivation, using the data about proliferative potential, morphology, and the characterization of surface markers expression and reactive oxygen species production.

Methods. From the first passage UC-MMSC were plated on plastic flacks and Petri dishes, d=35 mm, at a density 15,000 per dish and 50, 000 per flack, and cultivated for 4 passages under nitrogen-based gas mixture (3% oxygen, 4% carbon dioxide, 93% nitrogen - NM) and argon-based gas mixture (3% oxygen, 4% carbon dioxide, 93% argon - AM) for 7 days before replating. Control group was maintained under standard CO2 incubator conditions. Cell numbers, morphology and the surface markers (CD90, CD73, CD105) were analyzed at each passage. Reactive oxygen species (ROS) formation in cells was detected by using a fluorescent probe, 2',7'-diclorofluorescin diacetate.

Results. Compared to the cultures maintained under atmospheric oxygen concentration, the UC-MMSC, both in AM and NM, had higher proliferation rates, the effect slightly more pronounced in NM. Cells' morphology in both gas mixtures was less heterogeneous compared to that in CO_2 incubator at every analyzed passage. The surface marker analysis showed that both AM and NM prevented loosing surface markers by UC-MSC population at 3-4 passage, AM slightly more effective. The measurement of ROS production showed no correlations between passages and used gas mixtures, and varied depending on donor.

Conclusions. Under standard cultivation conditions the decrease of surface marker protein expression and the proliferation rate can be observed, alongside with the morphology of cultures becoming more heterogeneous, that shows the degenerative processes in culture. Using both gas mixtures helped to preserve the proliferation rate and morphological characteristics of culture. The effects of mixtures differed: AM preserved the levels of surface markers expression, and NM appeared to have beneficial effect on preventing the proliferation rate decrease and morphological changes in cultures. ROS production showed no correlation with passage, and appeared to be more donor-dependent.

Expression of recombinant human Calmodulin in insect cells and its purification

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Aim. It has been shown earlier, that dephosphorylated 2'-5'-linked triadenylates (2'-5'A_3) are capable of tuning the Ca^{2+}-binding properties of Calmodulin (CaM) – crucial participant of Ca^{2+} mediated signaling. Binding of 2'-5'A_3 to this protein caused significant (3 orders of magnitude) change in its affinity to Calcium ions (Tkachuk et al., 2011). For the purposes of more detailed analysis of this phenomenon, namely usage of NMR for locating the binding site for 2'-5'A_3, it has been decided to obtain the recombinant human CaM.
Usage of initially obtained pET21a plasmid vector (L. Kovacic), fused with the gene of human CaM1, for the transformation of *E. coli* BL21(DE3), was not successful. We hypothesize, that it could be due the C-terminal location of the 6xHis-tag. As it has been shown by the Western Blotting, the application of the anti-6xHis antibodies at the favorable concentrations, provided by the developers, resulted in nearly transparent bands on the nitrocellulose. That, in turn, led us to the conclusion, that the product of the plasmid at the unusually high concentrations was toxic for bacterial cells, which caused cleavage of either the His-tag, or the part of the C-terminal domain within Calmodulin globule, containing the His-tag.
After sequencing the plasmid our hypothesis was proved, since no signs of cloning mistakes or mutations were present. At this point we decided to use another expression system, which would not react to the presence of the abnormal CaM concentration in the same way.
Methods. In order to achieve this goal, CaM1 gene was first amplified, using the pET21a plasmid vector that we possessed. After that, it was cloned into the destination vector, suitable for further co-infection of the insect cells with the *Autographa californica* nucleopolyhedrovirus (AcMNPV) DNA. High affinity metal chromatography was used afterwards in order to extract the protein from insect cell lysate.
Results. Firstly, we have managed to clone the CaM1 into the destination vector, which later was used for transformation of DH10Bac *E. coli* cells, containing the baculovirus "shuttle" vector. Recombinant vector, containing CaM1, was thus generated. Secondly, we used this recombinant product to infect the sf9 insect cells, derived from the pupal ovarian tissue of the fall army worm *spodoptera frugiperda*, where the CaM1 was overexpressed for 72 hours. Thirdly, we have extracted and purified the CaM from the cell lysate with the use of High affinity metal chromatography, namely Ni-NTA resin. Further analysis on polyacrylamide gel electrophoresis has shown, that the purity of the protein was around 95%.

Conclusion. We have managed to obtain the highly pure recombinant human protein Calmodulin, expressed in the insect cells and suitable for further usage in various biochemical and biophysical experiments. We believe, that the usage of an eukaryotic organism as an expression system is strongly suggested, since it allows to avoid undesirable post translational protein modifications, which are common for prokaryotic systems.
Firstly, we are planning to apply the CD spectroscopy to investigate the impact of 2'-5'A_3 binding on Calmodulin's secondary structure. Later, we will carry out the study of more precise investigation of the Ca^{2+} affinity changes, caused by 2'-5'A_3 binding. Taking into account the CD data obtained, we will make a decision concerning the usage of Small angle X-ray scattering (SAXC) for further investigation of the 2'-5'A_3–CaM complex.

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Expression of *CHI3L1* in plasmid vector, vector DNA itself, and cytotoxic drug temozolomide promote karyotype and phenotype evolution of tumor cells

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Chromosome instability (CIN) is the driver and catalyzer of cellular immortalization, malignant transformation, metastasis, and drug resistance. CIN refers to the rate of karyotype changes in cell population and implies both the clonal (CCAs) and non-clonal (NCCAs) chromosome aberrations, which comprise the whole chromosome and segmental chromosome instability (translocations, breaks, deletions, and amplifications).

Aim. To study the patterns of CCAs/NCCAs after genotoxic stress (integration of foreign DNA into genome, overexpression of cancer-associated gene *CHI3L1*, and long-term treatment with cytotoxic drug temozolomide) and to establish the link between changes of karyotype and malignant phenotype, we used a panel of cell lines with different intrinsic pattern of CCAs/NCCAs.

Methods. Cell lines were karyotyped using Giemsa differential staining of chromosomes. To evaluate the individuality of cell lines and to establish the patterns of CCAs/NCCAs, the following karyotype parameters of 20 metaphases were analyzed: total line-specific chromosome number, total number of marker chromosomes per cell line, line-specific marker chromosomes, total number of NCCAs per cell line, total frequencies of NCCAs per cell line, and variations of non-clonal markers between individual cells in cell line. Array comparative genome hybridisation (aCGH) was applied to elucidate sub-chromosomal changes. Phase-contrast morphology analysis, MTT proliferation test, growth in soft agar assay, invasion assay, qRT-PCR, and Western blotting were used for phenotype analysis.

Results. Overexpression of the transfected oncogene *CHI3L1*, foreign DNA integration itself into genome, or temozolomide treatment promoted karyotype and phenotype changes. Karyotypes of cell lines after genotoxic stress evolved stochastically and were individual with different patterns of CCAs/NCCAs. The pattern of CCAs/NCCAs of cells depended on the nature of genotoxic stress and intrinsic pattern of CCAs/NCCAs of cells. Phenotype changes paralleled karyotype evolution, were unpredictable and diverse in the derivatives of cell lines.

Conclusion. Karyotype changes and heterogeneity determine the complex malignant phenotype of tumor cells.

The interaction of truncated forms of translation elongation factor eEF1Bγ with eEF1Bα and eEF1Bβ.

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Introduction. Elongation factor-1 (eEF1), which is responsible for aminoacyl-tRNA transfer to the ribosome, consists of two components: eEF1A - a G-protein forming a ternary complex with aminoacyl-tRNA and eEF1B - a guanine-nucleotide exchange factor. eEF1B – is a complex that includes α, β and γ subunits. Both eEF1Bα and eEF1Bβ catalyze the GDP/GTP exchange on eEF1A, whereas eEF1Bγ is a structural subunit. In higher eukaryotes, the eEF1B complex can also contain a unique enzyme, the valine-tRNA synthetase (VRS-eEF1H complex). Several models of VRS-eEF1H organization have been proposed, but they are contradictory to each other. Thus, the general purpose of my work is to define the mode all subunits interact with each other to form the complex. Here, we present the results of the expression and purification of several eEF1Bγ truncated forms and their interaction with eEF1Bα and eEF1Bβ.

Methods. All recombinant truncated forms of eEF1Bγ were expressed in Rosetta DE3 bacterial strain and purified by the affinity chromatography (Ni-NTA, glutathione sepharose or MBPTrap columns). The aggregation state of eEF1Bγ deletion mutants and their interaction with protein partners was analyzed by gel filtration on Superose 6 column.

Results. We expressed and purified to homogeneity a set of truncated forms of eEF1Bγ. Unfortunately, some of them formed soluble aggregates that did not dissociate on monomers even after denaturation-renaturation procedure. However, we succeeded in obtaining in monomeric state of two C-terminal fragments of eEF1Bγ comprising amino acids 263-437 and 228-437 which correspond to C-terminal domain and C-terminal domain with linker region, respectively. The N-terminal fragments of eEF1Bγ comprising amino acids 1-33 and 1-230 (N-terminal domain) fused with glutathione S-transferase were obtained in the form of dimers. Other truncated forms of eEF1Bγ comprising amino acids 1-93, 1-165, 33-437 and 93-437 were obtained as high molecular weights aggregates. We showed that both C-terminal fragments of eEF1Bγ interacted with neither eEF1Bα nor eEF1Bβ subunits. Whereas, the N-terminal domain of eEF1Bγ formed stable complex with both eEF1Bα and eEF1Bβ proteins simultaneously. The truncated form of eEF1Bγ containing amino acids 1-33 fuse to GST doesn't interact with either of the abovementioned partners.

Conclusions. The N-terminal domain of eEF1Bγ could not be divided on separate subdomains most probably due to the high content of hydrophobic amino acids. Both eEF1Bα and eEF1Bβ subunits interact with the N-terminal domain of eEF1Bγ simultaneously and, thus, their interaction sites are not overlapping.

Investigation of complexation in rna mixtures with mannitol, sorbitol and lactose using ir spectroscopy and chemometric data analysis

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Aim. It's well known that new immunomodulation medicine – Nucleinat contains RNA and lactose, but new antiviral medicine– Nuclex contains RNA and mannitol. The optimal ratios between RNA and sugar alcohols that caused their biological activity previously were estimated using biological methods. The main aim of our investigation is to obtain the possibility of complexation in RNA-sugar alcohol mixtures.

Methods. As IR spectra are very sensitive to structural changes caused by intermolecular interaction between components of the mixture we choose this method in our investigations. Chemometric analysis was used for the determination of quantitative information from IR spectra, such as number and concentration of species in the mixture [1,2]. Our approach is based on a three-component MCR-ALS analysis of the FTIR spectra of RNA mixtures. The graphical user interface (GUI) in the MATLAB environment developed by Tauler et al. [3] was used for calculations.

Results and Conclutions. The optimal ratio (the complexation is almost 100%) in RNA-mannitol mixture is 3:1 correspondingly. In a case of RNA-lactose mixture the complexation decreased to 30% and in RNA-sorbitol mixture – to 20%. So the optimal ratio in RNA-mannitol mixture obtained by FTIR spectroscopy and biological methods are in a good coincidence. Under these conditions we have the best complexation and biological activity at the same time. The replacement of lactose for mannitol leads to the appearance of new biological properties – antiviral activity.

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Ammonium Ion Selective Copper/Polyaniline-based Nanocomposite for Creatinine and Urea Biosensing

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Aim. Application of ammonium ions sensitive and selective nanocomposite for creatinine and urea biosensing.

Methods. Electrodeposition, electropolymerization, differential pulse voltammetry, cyclic voltammetry, chronoamperometric detection, enzyme immobilization.

Results. We propose and report amperometric urea and creatinine biosensors based on ammonium ions selective Copper/Polyanyline (PANI) nanocomposite. The proposed nanocomposite reveals high sensitivity and specificity towards ammonium ions with response range between 5 and 100 μM and a detection limit of 0.5 μM. To demonstrate its suitability as transducer in biosensors, creatinine and urea biosensors were fabricated by immobilising creatinine deiminase or urease, respectively, on the nanocomposite surface. The response range of the creatinine biosensor was 2 to 100 μM, which fits well with the normal levels of creatinine in healthy people (30-150 μM). The urea biosensor had a response range of 5 to 100 μM. A limit of quantification of 1 μM was achieved for both biosensors.

Conclusions. Ammonium ions selective Copper/Polyaniline-based chemosensor was successfully applied for the development of creatinine and urea biosensors.