Colocalization of USP1 protein and PH domain of Bcr-Abl oncoprotein in HEK293 cells

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Ubiquitin specific protease 1 (USP1) is a deubiquitination enzyme. USP1 protein was previously identified as one of 23 candidates for interaction with the PH domain of Bcr-Abl oncoprotein.

Aim: To investigate and analyze the colocalization of the protein USP1 and the PH domain of Bcr-Abl oncoprotein in cells.

Methods: Bioinformatic analysis (Estimation of protein Expression and Solubility, NetPhos2.0 Server, Disphos, KinasePhos and cNLS mapper software), PCR, ligation, methods for isolation and purification of DNA, restriction, USP1 protein expression in HEK293T and K562 cell lines, fluorescence and confocal microscopy, image analysis using ImageJ software (Fiji Is Just version).

Results: *USP1* was amplified by PCR (expected size - 2343 bp). After performing a ligation reaction the pUC-USP1 genetic construct was obtained. Then the *USP1* fragment was subcloned to create the pCMV-HA-USP1 and the pECFP-C3-USP1 genetic constructs. The accuracy of the pUC18-USP1, pCMV-HA-USP1 and the pECFP-C3-USP1 constructs was verified by PCR and restriction.

After optimization of transfection conditions the USP1 protein expression in HEK293T and K562 cell lines has been achieved. It has been shown that the USP1 protein is localized basically in a cell nucleus. The PH domain has been found throughout the cell, but the main localization is also the nucleus. The obtained results of co-expression of the USP1 protein and PH domain were analyzed graphically by scatterplots and in quantitative terms by Pearson correlation coefficient (PCC) and Manders correlation coefficient (MCC). On scatterplots the cluster of points was around a straight line that indicates the proportional co-distribution of the USP1 protein and the PH domain. Pearson's correlation coefficient ranges from 0.38 to 0.58 units that suggest noticeable correlation between the protein USP1 and the PH domain. The Manders correlation coefficient ranges from 0.8 to 1 unit, indicating a high level and even a full overlap of the USP1 protein and the PH domain. The difference between the figures can be explained by the fact that MCC strictly measures co-occurrence that is independent of signal proportionality, whereas PCC provides an effective statistics, but it indirectly (and sometimes poorly) measures the quantity that is typically at the heart of most analyses of co-localization in cell biology.

Conclusions:

1. The pUC18-USP1, pCMV-HA-USP1 and the pECFP-C3-USP1 constructs were obtained. Effective the USP1 protein expression in cell lines has been achieved. The main USP1 protein localization in the cell nucleus has been detected.

2.The correlation between the USP1 and PH domain may indicate their co-localization in cell, as well as their joint distribution and/or interaction with similar molecular complexes.

Intramolecular dynamics and conformational changes in mammalian tyrosyl-tRNA synthetase

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Tyrosyl-tRNA synthetase (TyrRS) is one of the key enzymes of protein biosynthesis, which catalyzes specific aminoacylation of homologous $tRNA^{Tyr}$. Mammalian TyrRS is composed of two structural modules: N-terminal catalytic core (mini-TyrRS) and an EMAPII-like C-terminal domain separated by a long flexible linker. The conformational changes of TyrRS contribute to the enzyme functioning, but their nature and specific role are not studied in details. 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 changes in the protein structure.

The aim of this work was to study the dynamic aspects of the mini-TyrRS functioning and to characterize local conformational changes of the enzyme.

Methods. The recombinant TyrRS protein was obtained by expression in the *E.coli* BL21(DE3)*pLysE* cells. Fluorescence spectroscopy has been used to explore the intramolecular dynamics of the mini-TyrRS in solution and to characterize protein conformational changes. Since 3D structure of the full-length bovine TyrRS is still unknown, we have performed structural modeling using Modeller v9.14 software. Molecular dynamics (MD) simulation of bovine TyrRS was carried out using GROMACS 4.5 (FF GROMOS 53a6) in the MolDynGrid virtual laboratory (*http://moldyngrid.org*). The Distributed Analyzer Script (DAS) was used for analytical tools automation (Savytskyi et al., 2011).

Results. According to the analysis of the bovine TyrRS 3D structure, Trp residues are partially solvent-exposed. The solvent accessible surface areas (SASA) of Trp residues of the mini-TyrRS (Trp40, Trp87 and Trp283) are ~ 7%, 29% and 57%, respectively. The local environments of Trp40, Trp87 and Trp283 were characterized within cutoff 5 Å around these Trp residues. Our computational analysis revealed 15 residues in the environment of Trp40: 9 hydrophobic and 3 hydrophilic residues; 10 residues in the region of Trp87: 6 hydrophobic and 3 negatively charged (Asp75, Glu88 and Glu91) residues; and 6 residues in the vicinity of Trp283: 2 negatively charged (Asp280 and Glu281) and 2 positively charged (Arg279 and Lys282).

Protein unfolding induced by the chemical denaturants such as urea and guanidine hydrochloride (GdnHCl) is a common approach to study the protein stability. Also, it was shown that low concentrations of GdnHCl can cause the protein stabilization by eliminating the strains in a protein caused by the electrostatic interactions of charged groups on its surface (Povarova et al., 2010). Unfolding of the mini-TyrRS by GdnHCl and urea was monitored by the change in intrinsic protein fluorescence. The values of Gibbs energy exhibited a linear dependence on the GdnHCl and urea concentrations. The Gibbs energy of unfolding of the mini-TyrRS by denaturants at 25 °C was -10.63±0.74 kJ/mol for GdnHCl and -12.35±0.97 kJ/mol for urea. The mid-points of unfolding the mini-TyrRS were 1.59 M of GdnHCl and 2.62 M of urea respectively.

The influence of interferon alpha on abundance of *Pick1, Grin3a* and *Gabra2* mRNAs encoding synaptic proteins in nervous system

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Background: Interferon alpha (IFNA) is a cytokine with antiviral, antiproliferative and immunomodulatory activities. It is widely used for 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 IFNA conducted in our lab has revealed three new genes encoding the synaptic proteins in nervous system - protein kinase C-alpha binding protein (PICK1), N-methyl-D-aspartate receptor subunit 3A precursor (GRIN3A) and gamma-aminobutyric acid receptor subunit alpha2 (GABRA2).

Aim of the study: to determine how the syntheses of the above mentioned mRNAs together with *Ifnas*, *Eif2ak1* and *Oas1* are distributed among the different regions of mouse brain and whether IFNA changes the distribution and the abundance of mRNAs.

Methods: Experiments were performed with C57BL/6 female mice 6 and 7 week old. Human IFNA, $2 \cdot 10^4$ U per mouse, was administered transnasally. After 24 h the mice were decapitated. Each brain was divided into 6 functional regions: olfactory bulb, pons and medulla, cerebellum, midbrain, hippocampus and cortex. Total RNA from each region was isolated using TRIZol reagent. RNA was treated with DNase I and retrotranscribed to cDNA with RevertAid Reverse Transcriptase in the presence of luciferase RNA as external control. The PCR reaction in 25 µl contained 10 µl 2.5 PCR mix (Syntol, Russia), 2.5mM MgCl2, 0.3 µM forward and reverse primers and 5 µl cDNA. The data were normalized according to Luc recovery. Statistical analysis was carried out in R statistical software.

Results: The abundance of *Eif2ak1, Ifnas* and *Oas1* mRNAs was at the level $1.5 \cdot 10^4$ copies/µg of total RNA while other mRNAs of interest, *Grin3a, Pick1* and *Gabra2a* are represented by approximately $4 \cdot 10^5$ copies/µg of total mRNA and Gabra1a - $9 \cdot 10^6$ copies/µg of total RNA. *Gabra1a* is ubiquitously distributed throughout the brain, other genes are more expressed in hippocampus brain region. After transnasal administration of IFNA the abundance of *Eif2ak1, Ifnas, Oas1, Grin3a, Gabra2a* did not change substantially while that of *Gabra1a* and *Pick1* mRNAs in medulla and pons were respectively 2.3 and 1.5 times those in control brain.

Conclusions: The abundance of *Gabra1a, Gabra2a, Grin3a* and *Pick1* mRNAs at the background of *Ifna, Eif2ak1,Oas1* mRNAs was determined for the first time in six regions of brain from control mice and those after intranasal administration of IFNA. The abundance of mRNAs encoding synaptic proteins was nearly 10 times higher than that of typical antiviral responders. The highest abundance of all mRNAs was detected in hippocampus. The administration of IFNA induced up-regulation of *Gabra1a* and *Pick1* mRNAs abundance in pons and medulla.

Investigation of CHI3L1 and IGF1R interaction in mammalian cells

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The *CHI3L1* gene, encoding the chitinase-3-like protein 1, is well known for its 5-fold higher expression level in glioblastoma, one of the most common and fatal malignant brain tumors in humans. However, neither CHI3L1 precise function nor its molecular mechanisms in the development of glioblastoma are well described. There is no exact data about the cell-surface receptor, which might mediate the action of CHI3L1.The functional mitogenic similarity and synergistic physiological effects of CHI3L1 and insulin-like growth factor 1 (IGF1) suggests that these proteins might mediate their functions through the binding of insulin-like growth factor 1 receptor (IGF1R) on a cell membrane. Therefore, a current work is focused on the investigation of the CHI3L1 and IGF1R possible interaction.

Aim: To investigate the possible CHI3L1 and IGF1R interaction in mammalian cells.

Methods: Co-transfection, co-immumoprecipitation, Western blot and immunofluorescent analysis.

Results: Firstly, the 293 cells line were transiently co-transfected with a recombinant plasmid constructions, encoding CHI3L1 and IGF1R (pCMV-FLAG/pGFP-N1_*IGF1R*; **pCMV-FLAG_*CHI3L1*/pEGFP-N1_*IGF1R;*** pCMV-FLAG_*CHI3L1*/pEGFP-C1). The effectiveness of the transfection was proved using Western blot analysis. The CHI3L1 and IGF1R interaction was assayed by co-immunoprecipitation using the polyclonal antibodies to GFP on the protein A agarose beads. The results demonstrate an effective CHI3L1 and IGF1R binding in transiently co-transfected with the *CHI3L1* and *IGF1R* 293 cells lysates opposed to the 293 cell lysates, co-transfected with either *IGF1R* and an empty vector or *CHI3L1* and GFP. Similar results were obtained after 293 cell total lysates co-immunoprecipitation using the anti-FLAG agarose beads.

Conclusions: The immunofluorescent analysis of CHI3L1 and IGF1R in the paraformaldehyde fixed cells using the polyclonal antibodies to CHI3L1 indicates the proteins co-localization in 293 cells after *CHI3L1* and *IGF1R* overexpression.

Thus, the results obtained seem to suggest IGF1R as a potential receptor for CHI3L1 and create prerequisites for further scientific research.

Interconnection between subcellular localization and functional activity of mTOR kinase

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Background. Mammalian Target of Rapamycin (mTOR) is a serine/threonine protein kinase. mTOR plays an important role in multiple cellular events, such as protein biosynthesis, growth, proliferation and survival. Nowadays, the mTOR inhibitors are considered as perspective anti-cancer and anti-aging drugs. It was shown that rapamycin inhibited the migration and invasion of malignant cells, but the mechanism of this phenomenon is not fully understood.

Aim. The main goal of our work is to investigate a role of the mTOR subcellular localization in functioning of the mTOR signal transduction pathway in normal and malignant human breast tissues, and in cultured MCF-7 cells.

Methods. The advantage of our research is the use of several types of anti-mTOR antibodies, generated to different epitopes of mTOR molecule. In our laboratory the antibodies to the N-terminal part, C-terminal part and central region of mTOR kinase were generated and tested. Subcellular localization was investigated by double immunofluorescent analysis and confocal microscopy. For more careful visualization of the nucleoli immunonucleochemistry was applied. Different human epithelial cell lines, and also histological sections of the normal and malignant human breast tissues were used in the research. To verify interconnection of mTOR kinase and cytokeratins we performed co-immunoprecipitation and proximity ligation assay (PLA).

Results. The immunochemical reaction revealed predominantly cytoplasmic localization of mTOR. A more sensitive immunofluorescent analysis allowed detecting additional nucleolar localization of mTOR, which was confirmed by colocalization with ribosomal S6 protein in the MCF-7 cells and malignant human breast tissue, but not in the normal samples. The antibodies to the N-terminus of kinase detected fibril-like structure. That is why, colocalization with tubulin, actin, and cytokeratins was examined, and, as a result, strong co-localization with cytokeratins was observed in MCF-7 cells, normal and malignant breast tissue. 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 the anti-mTOR antibodies precipitated keratins from lysates of MCF-7 cells. The 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. We have also revealed the presence of mTOR in the nucleoli of the malignant breast tissue and MCF-7 cells.

Identification of protein-protein interactions between NPRL2 and components of MARS complex

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Macromolecular complexes, which include aminoacyl-tRNA synthetases are the hallmark of higher eukaryotes. The largest complex MARS consists of nine enzymes and three auxiliary proteins. Besides the synthesis of aminoacyl-tRNA, aminoacyl-tRNA synthetases also perform non-canonical functions participating in the processes of translation, transcription, splicing, repair of DNA and others. According to the unpublished data of group leaded by Dr. Svetlana Dokudovskaya (IGR, France), the protein NPRL2, part of the complex GATOR1, which acts as an inhibitor of mTORC1 complex, interacts with complex MARS, but it is not clear with which component. This interaction could regulate its activity.

Aim: The main goal of this work was to identify the specific partners of recombinant protein NPRL2 in MARS complex, starting from methionyl-, glutaminyl-, lysyl-, aspartyl-tRNA synthetases and p43.

Methods: To determine the interaction between NPRL2 and some components of MARS we decided to cotransfect HeLa cell cultures with vectors containing the open reading frames of NPRL2 fused to a FLAG-tag (pc5FLAG/TUSC4) and of selected synthetase (constructions were based on pCMV6-XL5 vector) by using transfection reagent Effectene. Cotransfection lasted 48 hours, after which the fractions of total proteins were incubated with affinity matrix Anti-Flag M2, containing immobilized antibodies to Flag-tag. Protein fractions after elution from the matrix were analyzed by Western-blot analysis.

Results: We have found optimal cotransfection conditions with pc5FLAG/TUSC4 and pCMV6-XL5 vectors for HeLa cells at DNA to Effectene ratio - 1 mkg to 6.75 mkl and ratio between two plasmids - 1 to 2. The proteins of interest were extracted from the total protein fraction and the interactions between them were checked. We showed that methionyl-, glutaminyl-, lysyl-, aspartyl-tRNA synthetases and p43 do not interact with NPRL2 when coexpressed in HeLa cells under standard conditions. Methionyl-tRNA-synthetase binds to the affinity matrix Anti-Flag M2 in unspessific manner. The same results we obtained for glutaminyl- and aspartyl-tRNA synthetases. No interractions we observed in the pull-down assays with Flag-fused NPRL2 and lysyl-tRNA synthetase or p43.

Conclusions: We have shown that NPRL2 does not interact with such elements of MARS complex as methionyl-, glutaminyl-, lysyl-, aspartyl-tRNA synthetases and protein p43. A possible interaction with another component of MARS has to be checked.

Cytogenetic and Molecular Analisys of *Deschampsia antarctica* Desv. from the maritime antarctica

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Aim. *Deschampsia antarctica* Desv. is a dominant taxon of vascular plants that currently occurs along Antarctic Peninsula and on the adjacent islands. It is well known that the stressful environmental factors can influence a plant genome by causing changes in the chromosome number and structure, and an increase in genetic variation. However, the genome of Antarctic hairgrass is still poorly investigated at cytogenetic and molecular levels. Therefore, the objective of our research was to study the variation in the genome of *D. antarctica* plants at both the chromosomal and molecular levels.

Methods. Plants cultured *in vitro*, which are originated from the Argentine Islands region (Darboux, Galindez, Skua, Great Yalour Islands and Rasmussen Cape) of Maritime Antarctic, were used for analysis. The chromosome number was determined in the preparations of root apical meristem cells, counterstained by DAPI. The localization of 5S rDNA and 25S rDNA loci was determined by dual-colour fluorescence *in situ* hybridization (FISH). The molecular-genetic analysis was carried out by PCR using 8 ISSR- and 2 IRAP-primers.

Results. Cytogenetic analysis revealed that most of *D. antarctica* plants from the Argentine Island region have 26 chromosomes in their karyotype. Mixoploidy was found in plants from the Darboux Island (2n = 13-27) and the Great Yalour Island (2n = 13-39). FISH analysis revealed ten 5S rDNA sites and four 25S rDNA sites in the samples from Darboux, Skua, Galindez Islands, and Rasmussen Cape. Whereas, a greater number of rDNA loci (twelve of 5S rDNA and six of 25S rDNA) were identified in the plant from Great Yalour Island. The molecular-genetic analysis demonstrated a low genetic diversity of *D. antarctica* in the investigated region. Furthermore, the differences between the samples with different chromosome number fall within the range of the differences between the plants with chromosome number typical for this species (2n = 26).

Conclusions. Cytogenetic analysis of the *D. antarctica* plants revealed the intraspecies chromosomal polymorphism, which was also confirmed by FISH analysis. The number and localization of rDNA sites were determined in karyotype of this species for the first time.

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

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Aim. O^6-methylguanine-DNA methyltransferase (MGMT) is the DNA repair enzyme that removes alkylation adducts from the O^6-guanine in DNA. It prevents point mutations and cell death, but this protein can cause the cancer cell resistance to the alkylating chemotherapy.

Today, the combination therapy of many types of cancers is widely used in clinic. For example, the chemotherapy and hormone therapy are used together to treat breast, endometrial, kidney, brain and other cancers. During the combination therapy the hormonal medicines such as glucocorticoids, estrogens, progesterone and their antagonists are used, but only glucocorticoids (e.g. dexamethasone) are known to up-regulate the *MGMT* transcription and to cause the resistance of cancer cells to the alkylating agents. However, there is a lack of data about effects of other hormones used in clinic on the *MGMT* transcription and the chemotherapy efficacy. Therefore, the aim of this study was to search the hormone response elements (HREs) within the human *MGMT* promoter and to investigate the regulation of the gene expression by their ligands.

Methods. To predict HREs within the promoter region of the human *MGMT* gene (acc. number at GenBank X61657.1, 1157 bp) we performed *in silico* analysis using different programs including JASPAR, Cister, LASAGNA-Search, MAPPER, NHR-scan, NUBIScan, Paint, PROMO, PromoterScan, SignalScan, SiteGA, Tfscan, TESS, TFSEARCH and Tfsitescan programs.

HEK293, Hep-2, MCF7 and HepG2 cells were treated with β-estradiol and progesterone. Total cellular RNA and proteins were isolated using TRI Reagent (Sigma Aldrich, #T9424) and purified according to Chomczynski method. We used the RT-qPCR assay to determine a level of the *MGMT* mRNA. The relative *MGMT* gene expression was quantified versus two reference genes selected for each model by us in previous experiments using geNorm algorithm. The relative *MGMT* expression at the protein level was analysed using Western blotting.

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

According to the RT-qPCR data, the estrogen tends to down-regulate the *MGMT* transcription at low and high concentrations. We observed the same influence of estrogen on the *MGMT* expression at the protein level at the same range of concentrations.

Conclusions. We predicted novel cis-regulatory HREs within the human *MGMT* gene promoter using *in silico* analysis. We have observed the down-regulation of the gene expression by low and high concentrations of β-estradiol.

Cloning, expression and purification of D-Tyr-tRNA^{Tyr}-deacylase from *Thermus thermophilus*

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Introduction. D-amino acids were detected in various living organisms from bacteria to mammals. Although most D-amino acids cannot be incorporated into proteins, D-amino acids still have toxic effects in both prokaryotes and eukaryotes. Aminoacyl-tRNA synthetases (aaRS), being specific to L-amino acids, ensure the first step of exclusion of D-amino acids. However, the stereospecificity of these enzymes is not absolute: several aaRS have been found to charge tRNAs with D-amino acids. D-Tyr-tRNA^{Tyr}-deacylase (DTD) is the conservative enzyme, found in all domains of life, which ensures an additional checkpoint in the recycling of misaminoacylated D-Tyr-tRNA^{Tyr}. DTD does not hydrolyze stereospecific L-amino acids, being peculiar to D-amino acids. The structural bases of such specificity as well as mechanism of hydrolysis realized by DTD are considered.

Aim. Cloning and sequencing of the *T. thermophilus* D-Tyr-tRNA^{Tyr}-deacylase (DTD) followed by the creation of genetically engineered constructs for high protein expression in *E.coli* cells and its purification.

Methods. The gene of D-Tyr-tRNA^{Tyr}-deacylase from *T. thermophilus* was cloned into pET15b, pET28b, pET29b vectors (under control of T7 promoter and lac-operator) and pProExHTb vector, controlled by Trc promoter and lac-operator. All constructions were expressed in BL21(DE3)pLys and Rozetta *E. coli* strains. pET-based DTD constructions displayed low expression levels in BL21(DE3)pLys, and Rozetta strain could not provide effective cultivation. In contrast, pProExHTb-DTD showed a high expression level.

Results. The obtained His-tagged DTD was purified by three chromatography columns: 1) affinity chromatography on Ni-NTA Sepharose Fast Flow (GE Healthcare); 2) ion-exchange chromatography on Q-Sepharose Fast Flow (Pharmacia) and 3) gel-filtration column Superdex 75 10/300 GL (Amersham Biosciences). His-residues were cut off by TEV (tobacco etch virus) protease.

Activity of the enzyme was tested. The addition of DTD caused the decrease in the level of D-Tyr-tRNA^{Tyr}, generated *in situ*, and did not have an influence on the level of L-Tyr-tRNA^{Tyr}. On the other hand, an excess accumulation of AMP was observed, when TyrRS, tRNA^{Tyr} and DTD were incubated in the presence of D-Tyr, but not in the presence of L-Tyr, suggesting the editing process against D-Tyr.

Conclusions. D-Tyr-tRNA^{Tyr}-deacylase from *T. thermophilus*, cloned into pProExHTb vector, and purification procedure permit to obtain enough amounts of the target protein for further structural and functional investigations, which have been developed.

Mass-spectrometric investigation of complexation between RNA and mannitol.

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Aim. It is known that the drugs based on ribonucleic acid (RNA) are widely used in clinical practice as therapeutic agents. The antiviral and anti-inflammatory drug Nuclex is one of the most effective RNA-based drugs. According to our previous studies, RNA as a component of the drug forms a complex with mannitol. Therefore, the main goal of our study was to investigate a possible chemical nature of this complex.

Methods. Pyrolytic mass spectrometry approach (PMS) allows estimation of the structural peculiarities of complex composition by analysis of their thermal destruction products.

Results. Mannitol is a resistant substance with the discharge maximum of volatile components at 430°C under the thermal destructing conditions.

Under these conditions, mannitol formed 27 volatile products with the total ion current (TIC) 111 a.u. Our studies revealed that at the of 200°C the TIC of Nuclex is in 2.6 times higher, the number of ion fragments (IF) - 3 times higher in comparison with the original RNA. The Nuclex thermal destruction speed at the first stage is much higher than for RNA, whereas the thermal destruction velocity at these condions stage is almost identical. Maximum discharge of Nuclexvolatile products at the second stage is shifted by 20°C to a higher temperature compared to the original RNA. In spite of the fact that the number of IF at extreme points (for RNA - 253 ° C, for Nuclex - 270 ° C) is almost the same, the J-value for Nuclex is in 1.4 times higher than for RNA. The total ion current of volatile components and their quantity is by 2 times lower for Nuclex in comparison to mannitolat 430°C. The mass spectrum of Nuclex contains all IF, which are detected in the mass spectrum of RNA at the relevant temperature. It should be noted that the third most intense fragment in the mass spectrum of Nuclex at 200°C is volatile fragment with m / z = 86, which is absent in the mass spectra of the parent compounds.

Conclusion. We can conclude that ribonucleic acid, which is main component of Nuclex, forms complex with mannitol.

Study on 2'-5'A_3 binding to human protein S100A1

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Aim. The current study was aimed at broadening our unrstanding on how 2'-5'A_3 affects the S100A1 conformation and structure.

Methods. To achieve this goal we applied the Small angle X-ray scattering (SAXS) technique.

Results. As follows from the measurements, in all cases, with and without 2'-5' A_3, the human S100A1 protein presented as a homodimer with giration radius (R_g) around 2.1 ± 0.5 nm and 2.3 ± 0.5 nm (Figure 1A). The small differences detected for R_g are in the experimental error and the native 3D structure is essentially the same. Analysis of Fourier transformation and P(r) reconstruction suggested that 2'-5' A_3 oligoadenylates interact with S100A1 in Ca^{2+}-loaded form providing the appearance of clearly visible shoulder in higher distances.

Conclusion. We hypothesize that these data could serve as a direct evidence for 2'-5'A_3 binding to S100A1 molecule as a relatively long tail. On the other hand, it is possible that the protein conformation is being changed upon binding of 2'-5'A_3, which is in complete agreement with our previously obtained data.

Rational design of small molecule inhibitors of protein kinase Ask1

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Protein kinase ASK1 is a promising therapeutic target because the increased activity of this enzyme is associated with the development of a number of human diseases. For example, ASK1 was identified as an essential component in the neuronal death signaling pathway in Alzheimer's disease and amyotrophic lateral sclerosis. It was revealed that ASK1 is involved in pathogenesis of myocarditis, cardiac fibrosis and heart failure. *In vivo* studies support the role of ASK1 in septic shock. Therefore, highly active and selective inhibitors of ASK1 may be good candidates for drug development for the treatment of these diseases.

Aim. The aim of this work was the rational design of low-molecular ASK1 inhibitors and investigation of their interactions with the amino acid residues in the active site of ASK1 using computer modeling approaches.

Methods. At the first step of work we have performed receptor-based virtual screening of the library, containing about 270,000 organic compounds, using DOCK software package. After the docking followed by visual inspection of the best-scored ligand binding poses, the most promising 186 compounds from different chemical classes have been selected for the kinase assay study. *In vitro* experiments revealed that 5-(4-Chloro-phenyl)-4-(furan-2-carbonyl)-3-hydroxy-1-(6-methoxy-benzothiazol-2-yl)-1,5-dihydro-pyrrol-2-one inhibited ASK1 with an IC_{50} of 4.2 μM.

In order to examine the structure-activity relationships (SAR) of the derivatives of 1-benzothiazol-2-yl-3-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-one, 33 derivatives of this class were synthesized and tested. It was found that the most active compound 1-(6-Fluoro-benzothiazol-2-yl)-3-hydroxy-5-[3-(3-methyl-butoxy)-phenyl]-4-(2-methyl-2,3-dihydro-benzofuran-5-carbonyl)-1,5-dihydro-pyrrol-2-one (BPyO-34) inhibited ASK1 with IC_{50} of 0,52 μM.

Results. It has been observed that the presence of benzothiazol in the compound structure is important for the inhibitory activity toward ASK1. According to the computer simulations, this heterocycle is involved in hydrophobic interactions with the adenine-binding region of ASK1 active site. It was revealed that the oxygen atom of 3-hydroxy-1,5-dihydro-pyrrol-2-one forms a hydrogen bond with the amino group of Val757, which is located in the hinge region of ASK1. It has been demonstrated that the chemical structure of the substituent, which interacts with the hydrophobic pocket I, is important for the inhibitory activity toward ASK1.

The selectivity studies of the most active ASK1 inhibitors were performed *in vitro* using a panel from six protein kinases. It has been shown that BPyO-34 and a number of other active compounds demonstrate a good specificity toward ASK1.

Conclusions. Therefore, the combination of virtual screening approaches and biochemical studies allowed us to identify novel class of ASK1inhibitors, namely, 1-benzothiazol-2-yl-3-hydroxy-5-phenyl-1,5-dihydro-pyrrol-2-ones.

Temozolomide promotes the diverse genome and phenotype changes of glioblastoma cells

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Temozolomide (TMZ) is a cytotoxic DNA-methylating drug for treatment of the patients with newly diagnosed glioblastoma (GB). The short- or long-term TMZ treatment of tumor cell lines induced heterochromatin reorganization, changes in DNA, transcriptome, proteome, kinome and metabolome, remodeling of the entire electron transport chain, changes in morphology, proliferation, adhesion and migration/invasion. Such profound changes definitely indicate the complex multilevel cellular defense against TMZ cytotoxicity and cannot be explained by any accepted molecular mechanism of TMZ resistance. On the other hand, chromosome instability (CIN) can drive genomic and (epi)genetic heterogeneity, rewire and create new genetic networks due to the dynamic alterations of transcriptome/proteome, and eventually generate the diverse tumor cell phenotype variants, which are the basis for cancer evolutionary selection.

The aim of this study was to analyze CIN (clonal and non-clonal chromosome aberrations (CCAs/NCCAs) in response to the long-term TMZ treatment of U251, T98G and C6 glioma cell lines and its impact on the cell phenotype changes (karyotype-phenotype evolution).

Methods. Conventional cytogenetics was used for the analysis of CCAs/NCCAs, array comparative genome hybridization to determine copy number alterations (CNAs); cells growth was evaluated by proliferation test; colony formation efficiency (CFE) by soft agar assay, migration by wound healing scratch test and cell invasion by transwell assay.

Results. TMZ treatment significantly increased CIN in U251 and T98G cells but reduced in C6 cells. The analysis of proliferation in high- and low-glucose medium revealed distinct sensitivity and heterogeneous response of cells to the glucose concentration reduction and suggested that in the different metabolic microenvironments the TMZ-treated cells can demonstrate opposite, superior or inferior, growth in comparison to the original cells. The analysis of CFE showed no difference between U251 and U251TMZ1/U251TMZ2. However, U251TMZ2 formed more colonies than U251TMZ2. CFE of T98GTMZ cells was higher than that of parental T98G cells, whereas C6TMZ and C6R2TMZ formed less number of colonies than parental C6 and control C6R1 cells, respectively. U251TMZ1 migrated faster than parental U251 but no difference in migration was observed between U251 and U251TMZ2. No difference in migration was also detected between T98G and T98GTMZ, whereas C6TMZ and C6R2TMZ migrated slower than parental C6 and control C6R1 cells, respectively. In contrast to migration analysis, transwell invasion assay demonstrated lower and higher invasion rate of U251TMZ1 and U251TMZ1, respectively, in comparison to parental U251 cells. Similar to migration analysis, no difference in an invasion rate was observed between T98G and T98GTMZ.

Conclusions. The evolution of genome landscape under TMZ therapy results in the diverse tumor cell phenotype variants, either less or more aggressive than original tumor cells.

Thermal stability analysis of RNA mixtures with sugars alcohol

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Aim. RNA-based drugs are actively implemented in practical medicine during last years. The combination of nucleic acids with different substances like D-mannitol, sorbitol and lactose, leads to changing their biological activity and efficiency. D-mannitol and sorbitol have only two differ in the orientation of the hydroxyl group on carbon. While similar, the two sugar alcohols have very different sources in nature, melting points and uses.

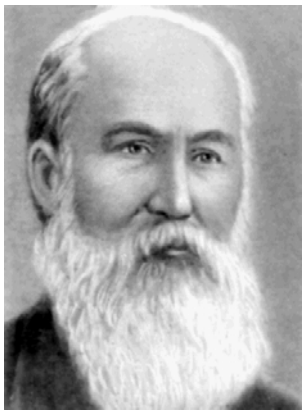
Methods. One of the most commonly used techniques for the investigation of RNA thermal stability are UV absorbance melting curves and TDS analysis. A thermal difference spectrum (TDS) is obtained by recording the difference between UV absorbance spectra of the unfolded and folded states of nucleic acids at temperatures above and below its melting point. All experiments were performed on spectrophotometer Specord PLUS 210 with the peltier temperatured cell holder.

Results. The thermal stability analysis of RNA and its mixture with alcohol sugars D-mannitol, sorbitol and lactose was performed. Typical for RNA a hyperchromic effect with melting temperature in the region near 50^0C is observed. This effect is connected with the double-stranded fragment dissociated and single-stranded fragments formation due to the hydrogen bonds breaking during melting process. The addition of sugars to RNA leads to the increasing of hyperchromic effect in comparison with pure RNA in case of sorbitol and its decreasing for lactose. Therefore, we suppose that sorbitol promotes formation of double-stranded fragments in RNA-sorbitol mixture while lactose slightly destroys them. In turn for D-mannitol-RNA mixture the hyperchromic effect is almost absent. It means that this mixture mainly contain single-stranded RNA. It is well known that lowering the temperature after RNA melting leads to its hybridization. The characters of "cooling curve" are similar for both pure RNA and RNA-sugar mixture. It means that the addition of alcohol sugars have no effect on RNA renaturation process. The changes that occurred in the RNA secondary structure during its melting from 25 to 75^0C were irreversible.

As the TDS spectra provided a distinctive spectroscopic signature for each nucleic acid structure we used it for the detection of RNA conformational changes in the RNA mixtures with D-mannitol. The comparison of RNA's TDS spectra and its mixture with sugar alcohol revealed significant changes in the RNA structure caused by D-mannitol.

Conclusions. The melting curves and TDS analysis of RNA-D-mannitol mixture showed that the presence of D-mannitol promotes the formation of RNA single-stranded structure. In this way mannitol can provide the antiviral activity of RNA-based drug Nuclex.

Шановні колеги цьогорічну конференцію ми присвячуємо видатному науковцю Миколі Феофановичу Кащенко



Кащенко Микола Феофанович

(7 травня 1855, Московка — 29 березня 1935, Київ) – видатний біолог, ембріолог, селекціонер, доктор медицини (1884) та зоології (1901), приват-доцент порівняльної анатомії (1884), заслужений професор (1909), академік ВУАН (1918).

Народився 7 травня 1855 р. в с. Веселому Олександрійського повіту Катеринославської губернії (нині – с. м. т. Веселе Запорізької області). Упродовж 1875–1880 рр. навчався на медичних факультетах імператорських Московського та Харківського університетів, після закінчення якого отримав звання повітового лікаря. Вивчав курс зоології, порівняльної анатомії й ембріології на природничому відділенні фізико-математичного факультету (1880–1881), працював приватним асистентом ембріологічного кабінету (1881–1882) Імператорського Харківського університету. Паралельно у 1881–1882 рр. працював лікарем у приватному жіночому пансіоні мадам Бауман (м. Харків) та помічником директора земської повивальної школи, де читав курси анатомії, гістології, ембріології, вів акушерську практику.

У 1884–1885 рр. викладав курс порівняльної анатомії на фізико-математичному факультеті, у 1885–1886 рр. – курс гістології й ембріології на медичному факультеті Імператорського Харківського університету. У 1886–1888 рр. стажувався в Німеччині та Італії, де працював у лабораторіях В. Гіса, Г. Вальдейєра, О. Гертвіга, на зоологічній станції А. Дорна в Неаполі.

Після повернення із-за кордону М. Ф. Кащенко розпочав роботу у Томському університеті, де працював екстраординарним професором (1888–1891), ординарним професором та завідувачем (1891–1912) кафедри зоології і порівняльної анатомії медичного факультету, директором Зоологічного музею (1889–1912) та ректором університету (1893–1895).

М. Ф. Кащенко був дійсним статським радником (1902), членом Комісії з реорганізації вищих навчальних закладів у Санкт-Петербурзі (1903), почесним мировим суддею Томського міського управління (1907–1912).

Після переїзду до Києва упродовж 1912–1921 рр. працював професором кафедри зоології сільськогосподарського факультету Київського політехнічного інституту. М. Ф. Кащенко входив до складу Комісії з розробки законопроекту зі створення Всеукраїнській академії наук (1918). Упродовж 1918–1935 рр. працював у ВУАН. Був головою Фізико-математичного відділу (1918–1919), завідувачем Кафедри акліматизації, Зоологічного кабінету (1918–1921), директором Акліматизаційного саду в Києві (1918–1935), організатором і першим директором Зоологічного музею (1919–1926), членом Комісії з вивчення природних багатств України та Комітету з вивчення фауни України.

Був почесним членом Західносибірського товариства сільського господарства (1925), Омського товариства плодівництва (1926), Мінусинського товариства краєзнавства (1927); членом Міжнародного анатомічного товариства, Московського товариства любителів акваріуму і кімнатних рослин, Київського товариства сільського господарства, товариств природодослідників при Київському, Московському, Петербурзькому, Томському та Харківському університетах та ін.

М. Ф. Кащенко є автором понад 200 наукових праць, присвячених вивченню проблем ембріології хребетних тварин і людини, гістології, мікроскопічної техніки, теріології, герпетології, археологічної палеонтології, зоології, акліматизації та селекції рослин в умовах Сибіру і України. Здійснив дослідження з акліматизації плодових і квіткових рослин, був одним з основоположників наукового плодівництва в Сибірі. Вивів багато сортів яблук (Багряна Кащенко, Бугристе наливне, Сибірське біле плямисте, Сибірське золото, Сибірська зоря, Сибірська зірка, Сибірська янтарка та ін.). Встановив низку нових для науки видів хребетних тварин.

Здійснив декілька наукових експедицій: на річку Об (1890), на Барабинські озера (1891), до центрального Алтаю (1898), уздовж сибірської залізниці між містами Омськ, Томськ та Красноярськ (1899), до приалтайських степів (1900) та ін., в яких досліджував сибірську фауну.

Організував Західносибірське товариство сільського господарства (1898), дослідні акліматизаційні сади в Томську (1902), у садибі Київського політехнічного інституту (1915). Ініціював створення багатьох наукових видань: «Вісник Фізико-математичного відділу», «Праці Акліматизаційного саду», «Український зоологічний журнал», «Записки Фізико-математичного відділу» та ін.

Нагороджений орденами Св. Станіслава II ступеня (1894), Св. Ганни II ступеня (1899), Св. Володимира IV ступеня (1906) та III ступеня (1909), срібною медаллю «В пам’ять царювання імператора Олександра III».

М. Ф. Кащенко помер 29 березня 1935 р., похований на Лук’янівському цвинтарі м. Києва.

Ім’ям вченого названа вулиця в Голосіївському районі м. Києва (1955).