Local conformational changes and hydrophobic sites formation in eukaryotic tyrosyl-tRNA synthetase studied by fluorescence probes and computational modeling

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Tyrosyl-tRNA synthetase (TyrRS) is one of the key enzymes of protein biosynthesis, which catalyzes the specific aminoacylation of homologous $tRNA^{Tyr}$. Local conformational changes of TyrRS contribute to the enzyme functioning, but their nature and specific role are not studied yet. The first stage of protein folding involves the formation of the molten globule state, but the physiological significance of such conformation is not fully understood. Usually, the molten globule state is characterized by higher exposure of hydrophobic regions, which allows to monitor this state by fluorescent probes.

Aim of this work was to study the local conformational changes in the N-terminal catalytic module of TyrRS (mini-TyrRS) and possible formation of molten globule state by fluorescent probes binding.

Methods. Recombinant proteins were obtained by bacterial expression in *E.coli* BL21(DE3)*pLysE* by standard methods. The binding of fluorescent probes 1,8-ANS and bis-ANS by mini-TyrRS were performed in order to monitor the formation of hydrophobic regions in the protein in the temperature range of 25–42ºC. Computational modeling of the binding of 1,8-ANS and bis-ANS on the surface of *Hs* TyrRS was performed with AutoDock Vina program.

Results. The interaction of 1,8-ANS and bis-ANS fluorescent probes with TyrRS resulted in the significant increase of fluorescence intensity due to the binding to hydrophobic sites on the protein surface. The unstable bis-ANS binding with mini-TyrRS was detected at 25 ºC, but the temperature increase resulted in a more stable dye binding. This indicates the formation of nonspecific hydrophobic sites on the protein surface at 25ºC temperature. Hence, increasing of the temperature to 42°C induced conformational changes, which lead to the formation of specific bis-ANS binding sites on TyrRS surface. Computer simulation of molecular docking of 1,8-ANS and bis-ANS to the *Hs*TyrRS revealed several binding sites, one of which is common for the both dyes. The common region was formed by Arg16, Lys265, Phe269, Tpr290, Tyr292, and the Ser273, Ala291, Val293 for bis-ANS only. The energy of interactions of 1.8-ANS and bis-ANS to this hydrophobic region of TyrRS was about -6.1 and -5.7 kcal/mol, respectively.

Conclusion. Temperature-induced local conformational changes of TyrRS were studied by 1,8-ANS and bis-ANS fluorescent probes binding. The difference in the affinity and binding sites numbers on the mini-TyrRS surface was observed in the 25-42ºC temperature range. Therefore, 1,8-ANS and bis-ANS are sensitive fluorescent probes in order to detect the formation of hydrophobic sites and possible molten globule state in proteins, especially at the neurodegenerative diseases.

Structural and energetic properties of the four biologically important configurations of the A·T and G·C DNA base pairs: comparative quantum-chemical analysis

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Aim. The main purpose of this work is to show and quantum-chemically substantiate that a necessary and sufficient condition for the transition of the G·C Watson-Crick (WC) base pair into the standard biologically important configurations, characteristic for the A·T base pair, namely – Hoogsteen (H), reverse Hoogsteen (rH), WC and reverse WC (rWC), is its tautomerisation into the G*·C* Löwdin's (L) base pair with WC geometry formed by mutagenic tautomers. **Methods.** Quantum-chemical modeling at the MP2/6-311++G(2df,pd)//B3LYP/6-311++G(d,p) level of theory in the free state (ε = 1) and in the continuum with a low dielectric constant (ε = 4) corresponding to hydrophobic interfaces of protein–nucleic acid interactions. **Results.** It was established for the first time, that H, rH, WC and rWC configurations of the A·T and G·C DNA base pairs are isoelectronic and isomorphic structures with similar dynamic properties. Based on these results, a non-ionisation mechanism of the Hoogsteen "breathing" of the G·C DNA base pair, namely transformation of the tautomerised G*·C* L base pair with WC geometry, stabilized by the three O6H…N4, N3H…N1 and N2H…O2 H-bonds, into the electroneutral G*·C* H base pair, stabilized by the three O6H…N4, N3H…N7 and C8H…O2 H-bonds, has been postulated. It is suggested that such scenario is activated only in those cases, when DNA is not located in aqueous solution, but operates together with proteins, and cytosine protonation at the N3 atom is precluded.

Moreover, it was shown for the first time, that the G*·C* L DNA base pair can acquire as the A·T WC DNA base pair four biologically important configurations, namely – WC, rWC, H and rH. This fact demonstrates rather unexpected role of the tautomerisation of one of the WC DNA base pairs, in particular, *via* double proton transfer: exactly the G·C→G*·C* tautomerisation allows to overcome steric hindrances for the implementation of the above mentioned configurations. Geometric, electron-topological and energetic properties of the H-bonds that stabilise the studied base pairs, as well as the energetic characteristics of the latters are presented. **Conclusions.** This study has shown for the first time, that the G*·C* L DNA base pair can acquire as the A·T WC DNA base pair four biologically important configurations, namely – WC, rWC, H and rH. Based on these results, the tautomeric mechanism of the Hoogsteen "breathing" [1], established experimentally [1,1], was suggested.

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Design, synthesis and evaluation of 2-phenylisothiazolidin-3-One-1,1-Dioxides as a new class of human protein kinase CK2 inhibitors

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Aim: The main goal of this work was identification of 2-phenylisothiazolidin-3-one-1,1-dioxides as a new class of human protein kinase CK2 inhibitors.

Methods: In order to reach that goal we used molecular modeling approach in combination with combinatorial synthesis and high throughput screening.

Results: We have described the synthesis and *in vitro* evaluation of 64 new 2-phenylisothiazolidin-3-one-1,1-dioxide derivatives as a new class of CK2 inhibitors. We have identified this class of compounds using flexible docking of the virtual library consisted of 1037 members [1]. The optimization based on biological screening data and molecular modeling led to the identification of 3-{[2-chloro-4-(1,1-dioxido-3-oxoisothiazolidin-2-yl)benzoyl]amino}benzoic acid, a potent inhibitor of human protein kinase CK2 (IC_{50} = 1.5 μM).

Its binding selectivity was checked with the usage of two serine/threonine (ASK1, Aurora A1) and one tyrosine protein kinase (FGFR1). It was found that 3-{[2-chloro-4-(1,1-dioxido-3-oxoisothiazolidin-2-yl)benzoyl]amino}benzoic acid *in vitro* exhibited selectivity towards inhibition of CK2.

Using the biochemical tests and molecular docking we have investigated the relationship "chemical structure-biological activity" (SAR) of 2-phenylisothiazolidin-3-one-1,1-dioxides. As a result, the binding mode of the synthesized compounds with CK2 ATP-acceptor site has been proposed.

Conclusions: Hereby, we have identified 2-phenylisothiazolidin-3-one-1,1-dioxides as a new class of human protein kinase CK2 inhibitors using molecular modeling, combinatorial synthesis and high throughput screening techniques.

Keywords: 2-phenylisothiazolidin-3-one-1,1-dioxides, CK2 inhibitors, Molecular Docking.

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Comparative conformational analysis of 2'-deoxyguanosine and 2'-deoxyadenosine with their oxidized analogs: a quantum chemical study

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Aim. To compare conformational ability of dG and dA and their eight position oxidized nucleosides (8-Oxo-7,8-dihydro-2'-deoxyguanosine, 8oxodG and 2'-deoxy-8-oxo-adenosine, 8oxodA), thus, to answer a biologically important question "Could oxidized bases in comparison with non-modified easier become DNA-like syn-conformations?"

Methods. The methods of the quantum-chemical density functional method on the MP2/6-311 + + G (d, p) / / DFP B3LYP/6-31(d, p) theory level in isolated state (ε=1) were used in this work.

Results. The complete conformational family of 8oxodG containing 95 conformers with relative Gibbs energy lying in the range of 0-8, 56 kcal / mol and the family of 8oxodA consisting 87 structures with 8.74 kcal / mol under standard conditions were shown for the first time. Comprehensive structural features of conformers and characteristics of intramolecular H-bones stabilizing the conformers are presented. 8oxodG and 8oxodA data were compared with similar data for dG and dA for the first time.

Conclusions. Oxidation of dG and dA at the C-8 position triggering their lightweight ability to become syn conformated in comparison with canonical nucleosides was shown for the first time. These results are useful to explain the structural mechanism of mutagenic action at eight position oxidation of purine bases of DNA.

In silico design of protein kinase FGFR1 inhibitors as potential anti cancer drugs

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Aim. Fibroblast Grow Factor Receptor 1 (FGFR1) tyrosine kinase plays an important role in cell proliferation and angiogenesis control. Enhanced protein kinase activity due to activating mutations or overexpression has been implicated in several angiogenic pathologies and different cancers. Therefore, discovery of inhibitors of FGFR1 kinase has substantial potential therapeutic value. The aim of the present study is search for FGFR1 inhibitors with antiproliferative activity, which can be used for anticancer drug development.

Methods. Receptor-based virtual screening of 100000 chemical compounds collection was used to search for novel FGFR1 inhibitors. Docking of the compound collection was performed with program Autodock. Consensus scoring, checking of hydrogen bonding with a kinase hinge region and visual inspection were used for docking results filtering. *In vitro* inhibition tests were carried out with γ-^{32}P-ATP assay. The antiproliferative activity was measured by MTT assay.

Results. From virtual screening several novel FGFR1 inhibitors were found. This work presents the results of testing 4 inhibitors classes. The most active compound among 4-phenyl-1H,4H-pyrano[2,3-c]pyrazoles was 4-[4-(1,3-benzothiazol-2-yl)-5-methyl-1H-pyrazol-3-yl]-6-propylbenzene-1,3-diol (**1**) with IC_{50} 8.5 μM. The most active compound among thieno[2,3-b]pyridines was 3-amino-6-cyclopropyl-4-(thiophen-2-yl)thieno[2,3-b]pyridine-2-carboxamide (**2**) with IC_{50} 1.8 μM. The most active compound among 4-phenyl-1H,4H-pyrano[2,3-c]pyrazoles was 6-amino-4-(2,5-dimethoxyphenyl)-3-methyl-1H,4H-pyrano[2,3-c]pyrazole-5-carbonitrile (**3**) with IC_{50} 2.5 μM. The most active compound among (2Z)-3-(3-phenyl-1H-pyrazol-4-yl)prop-2-enenitriles (2E)-2-(1,3-benzothiazol-2-yl)-3-(3-phenyl-1H-pyrazol-4-yl)prop-2-enenitrile (**4**) with IC_{50} 1.8 μM. Compounds 4-[4-(1,3-benzothiazol-2-yl)-5-(trifluoromethyl)-1H-pyrazol-3-yl]benzene-1,3-diol (**5**), 3-amino-6-(4-chlorophenyl)-4-(4-methoxyphenyl)thieno[2,3-b]pyridine-2-carboxamide (**6**), **3** and **4** were tested on lung cancer cell line A549 for antiproliferative activity. All tested compounds inhibited proliferation, the most active compound was **6**.

Conclusions. In this study four new classes inhibitors of protein kinase FGFR1 with antiproliferative activity were found. The identified classes can be used for further development of FGFR1 inhibitors and anticancer drugs.

Role of PH domain of BCR/ABL fusion protein in cytoskeleton remodeling.

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Background: Reciprocal translocation between short arms of 9 and 22 chromosomes leads to generation of fusion gene *BCR-ABL*. To date, three forms of such fusion genes were identified and named according to molecular weight of their products: p185, p210, and p230. Difference in molecular weights is due to the absence or presence of certain domains of BCR protein, such as Pleckstrin-homology (PH) and Dbl-homology (DH) domains. The different forms of BCR-ABL protein are associated with different myeloproliferative disorders: p185 – with acute lymphoblastic leukemia, p210 – with chronic myelogenous leukemia, and p230 – with true thrombocytopenia. A shorter form of BCR-ABL leads to a more aggressive phenotype of disorder, while p210 and p230 lead to more mild and slow development. Therefore, it is important to determine the role of above mentioned domains in signaling and regulatory cellular pathways.

According to the earlier research of Miroshnichenko D. et. al. 23 protein-protein interactions were identified between DH-PH part of BCR and various cellular proteins of K562 cell line. Among them, several may be grouped by functional similarity. For instance, cortactin (CTTN), cytokeratin type 10 (KRT10), tubulin beta 1 chain (TUBB1), and Collagen IV alpha-1 polypeptide (COL4A1) are involved in cytoskeletal remodeling, membrane signaling, and cell adhesion. As BCR-ABL p210 is associated with abnormal cytoskeletal behavior, altered adhesion, and localization on cell cortex, a role of PH domain in these processes should be determined and previous data on protein-protein interactions verified by more sensitive methods. **Aim**. To determine cellular localization and protein-protein interactions of PH domain of BCR protein with COL4A1, CTTN, KRT10, and TUBB1 proteins. **Methods.** pCMV-SPORT6-based constructs with full coding sequences of COL4A1, KRT10, and TUBB1 were acquired from Open Biosystems. Construct with full CTTN sequence was kindly provided by Pontus Aspenstrom (Karolinska Institute, Sweden). CTTN and TUBB1 were amplified by PCR with specific primers and ligated to pBluescriptSKII(+) on EcoRI-BamHI and EcoRV sites, respectively. Further, CTTN was subligated to pGEX4T2 on EcoRI-NotI, to pCMV-myc on EcoRI-BglII, and pECFP-C3 on EcoRI-BamHI. TUBB1 was subligated to pGEX4T3 on SalI-NotI, pcDNA4HisMaxC on EcoRV-NotI, and pECFP-C3 on HindIII-EcoRI. Coding sequence of COL4A1 was cut from pCMVSPORT6 on AscI-HindIII and ligated to pECFP-C1 on XhoI(blunted)-HindIII sites. KRT10 was subligated from pCMVSPORT6 on EcoRI-NotI to pGEX4T2 and pCMV-HA, and on EcoRI-SalI to pECFP-C3. **Results.** The genetic constructs for bacterial and mammalian expression were created and verified. **Conclusions.** The derived vectors are suitable for further protein production and consequent protein-protein interaction analysis by far-Western blot, Coimmuno-precipitation and FRET.

Interconnection between functional activity and subcellular localization of mTOR kinase

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Introduction. The mammalian target of rapamycin (mTOR) is a highly conserved serine/threonine kinase. It is a member of several signal transduction pathways, but the most studied one is PI3K/Akt/mTOR signaling network. mTOR takes part in the regulation of translation initiation, autophagy, ribosome biogenesis, transcription, actin reorganization, cell-cycle progression, and cell survival. mTOR hyperactivation was detected in a number of tumors, namely the carcinomas of thyroid gland, breast cancer, prostate and stomach malignancies. Moreover, mTOR inhibitors are regarded as anti-cancer drugs.

Aim. The main goal of our work is to investigate the role of mTOR subcellular localization in functioning of 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 antibodies to the N-terminal part, C-terminal part and central region of mTOR kinase were produced and tested by western blot, and immunocytochemistry in the presence of mTOR antigenic polypeptide. Subcellular localization was investigated by double immunofluorescent analysis and confocal microscopy. For more careful visualization of the nucleoli immunonucleochemistry was applied.

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-terminal part of kinase detected fibrils-like structure. That is why, co-localization 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.

Conclusions. The data about the subcellular localization of mTOR kinase in mammalian cells are incomplete and contradictory. In our project we showed cytoplasmic localization of mTOR that is in accordance with the results of other authors. However, for the first time we revealed strong co-localization of mTOR and cytokeratins in human breast cells, and presence of mTOR in the nucleoli of malignant breast tissue and MCF-7 cells. It could be explained by the existence of several splicing isoforms of mTOR kinase. Further research will help to verify this hypothesis and broaden our knowledge about mTOR signal transduction pathway.

Investigation of protein-protein interactions of the adaptor protein TKS4

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The adaptor protein Tks4 is a key protein of invadopodia and podosomes – actin-rich membrane structures that facilitate cell movement and extracellular matrix degradation and lead to cell invasion. The **aim** of our research was to identify new partners of Tks4 via testing its interaction with potential partner proteins of the intersectin protein family (ITSN), reorganizators of the actin cytoskeleton neural Wiskott-Aldrich syndrome protein (N-WASP), and the verprolin protein family as well as with adaptor protein Tks5.

Methods. The cDNA fragments encoding the SH3 domains of Tks4 were cloned into the vector for bacterial expression pGEX-4T-2. The recombinant GST-fused SH3 domains of ITSN1, ITSN2 and Tks4 were expressed in *E.coli* $XL1^{Bluetet}$ and purified by affinity chromatography. Interactions of the SH3 domains with proteins overexpressed in HEK293 cells were analyzed using GST pull-down assay and Western blotting.

Results. We showed that the SH3A, C and E domains of ITSN1 and ITSN2 bind Tks4. The SH3 domains of ITSN effectively precipitated with a wild type Tks4 and a mutant for the three sites of phosphorylation. The results of pull-down experiments demonstrated that four SH3 domains of Tks4 bound weakly to N-WASP, known component of invadopodia and podosomes. Among the proteins of verprolin family only CR16 (Corticoid Regulated) interacted with SH3_2 and SH3_3 domains of Tks4. No interaction was observed in pull-down assays with GST-fused SH3 domains of Tks4, overexpressed WIP (WASP Interacting Protein) and WIRE (WIP-related).

Tks4 contains several proline-rich motifs. We have found that the SH3 domains of Tks4 could interact with its proline-rich motifs and therefore mediate inter- or intramolecular interactions of Tks4 molecules. It was also shown that the SH3 domains of Tks4 bind another member of the Tks protein family, Tks5. It may be supposed that these interactions can regulate activity of Tks proteins.

Conclusions. The adaptor protein Tks4 is crucial for the formation of invadopodia – key structures for cancer invasion. We have shown that Tks4 interacts with scaffolding proteins ITSN1, ITSN2, Tks5 as well as with reorganizators of the actin cytoskeleton N-WASP and CR16.

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Hematopoietic progenitor cells from native and cryopreserved placental tissue and umbilical cord blood: comparative analysis

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Objectives. The main goal of our study was to show the possibility of obtaining viable hematopoietic progenitor cells (HPCs) from native and cryopreserved placental tissue and compare their phenotype with phenotype of HPCs from umbilical cord blood.

Materials. The tissue of human term placenta was cryopreserved by conventional methods. Native and cryopreserved placental tissues were enzymatically digested and cells harvested for FACS analyses. FACS was performed with the next list of antibodies: anti-CD34, anti-CD45, anti-CD45RA, anti-CD90, anti-CD31, anti-CD235, anti-CD7, anti-CD19, anti-CD33 (all from BD, USA). CFU analysis was performed with culture medium MethoCult (StemCell Tech., Canada).

Results. The cells populations with phenotype $CD34^{low}CD45^{low}$ and $CD34^{hi}CD45^{low}$ were present in placental tissue. The level of CD90 expression on HPCs from placental tissue was significantly higher than on HPC from cord blood namely 24.5 % (6,2-49,8) and 2.2 % (0,08-10,5) respectively. The expression of CD31 on HPCs from placental tissue was higher comparing to HPCs from cord blood. We demonstrated some differences in the percentage of micropopulations of erythroid, lymphoid and myeloid progenitor cells derived from placental tissue and cord blood. In contrast we didn't observe difference in the of ratio granulocyte-monocyte, monocyte, granulocyte and erythroid colonies formations in cell culture of HPCs from umbilical cord blood and placental tissue. Furthermore we obtained viable HPCs from cryopreserved placental tissue. The content of the HPCs ($CD34^{+}CD45^{low}SSC^{low}$) among all $CD45^{+}$ cells of native and cryopreserved placental tissue were 0,66 % (0,36-1,05) and 1,11 % (0,18-2,82) respectively. CFU analysis of cells revealed the presence in cryopreserved placental tissues of precursors of granulocytes, monocytes and erythrocytes.

Conclusion: The placental tissue contains HPCs that differ in expression of some markers comparing with HPCs from cord blood. Cryopreservation of placental tissue is prospective method for preservation of HPCs.

Temporal gene expression profiling in rat hepatocytes treated with quasi-physiological dose of interferon alpha

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Background. Interferon alpha (IFNα) is a pleiotropic multifunctional cytokine that has a key role in early immune events. It is produced at the minimal level in healthy liver and up-regulated by viral RNA, LPS and damage-associated molecules. Knowing how a cell responds to IFNα in dynamic is important for understanding its function. Herein for the first time we examined the gene expression profile in primary rat hepatocytes treated during two time periods - 3 and 6 hours, with quasi-physiological dose of IFNα similar to that locally produced in regenerating rat liver after partial hepatectomy.

Methods. We incubated primary rat hepatocytes with 250u/ml of rat IFNα. We used gene expression profiling with Affymetrix rat genome array 230 2.0 (Affymetrix, USA) followed by computational analysis of promoter regions of differentially expressed genes to identify the signaling pathways that are engaged by IFNα.

Results. We identified 28 and 124 differentially expressed up-regulated genes which reveal the cell-specific traits of response and distinctive dynamics. From the beginning the up-regulation of genes responsible for autophagy, ISGylation, inhibition of translation and transition of mitochondrial dNTP synthesis to salvage pathway and chemokine CXCL10 production comes to the fore. Altogether they manifest the transit from the initial mode of cellular activity to the resource-conserving one, the usage of preexisting proteins and the attraction of the cells of putative hepatic environment for the development of response. The later changes are associated with the expansion of spectrum of differentially expressed genes, increasing magnitude of response and emerging interplay between activating and inhibiting factors which regulate the signal transduction, transcription and the activity of putative cells of hepatic environment. The classical Jak/STAT/ISGF3, Jak/STAT, PI3K and p38 signaling pathways are engaged in IFNα induced response of hepatocytes.

Conclusion. The short-term treatment of hepatocytes with quasi-physiological dose of IFNα induces the self-autonomous changes in hepatocytes and those referring to the hepatocytes potential input to the whole hepatic response. The interplay between activating and inhibiting factors of immune response is a characteristic feature of hepatocytes reaction to specified IFNα treatment.

Morphological and growth properties of human cell line 4bl and its stem potential

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Morphology of cells and growth properties are important characteristics of cell line, so the **aim** of this research was to study these peculiarities of the cell line 4BL. **Materials and methods.** We investigated the original cell line 4BL, obtained from peripheral blood of healthy donor, which has successfully passed through the Heyflick limit and was cultivated for more than 220 passages. For morphological studies cells were vitally stained by neutral red. To analyze the growth properties cells were seeded on 10 cultural flasks with 10^5 cells on each. Every 24 hours, the cells were removed from the surface of the flask and the total number of cells was calculated. Stem potential of the cell line 4BL to differentiate into adipogenic, osteogenic and myogenic types was investigated by growing cells on specific induction mediums.

Results. The cell line 4BL consists of two main morphological types of cell: fibroblast-like and epithelioid cells. Growth curves are characterized by almost complete absence of lag-phase, double-step log-phase and double-step decline phase with the formation of two peaks with maximum number of cells that resembles the damped oscillations.

The cell line 4BL maintains original cytoarchitectonics in culture at different passages: the cells are non-random placed on the surface of the culture vessels and often form circular and half-circular cellular associations. The latter fact may indicate the stem potential of cell line, which was confirmed by induction of cells to adipogenic, osteogenic and myogenic differentiation.

Conclusions. Regardless the number of passages, fibroblast-like and epithelioid cells are two main morphological types of the cell line 4BL, remaining its ability to form cycle structures at the surface of culture vessels. Growth curves have graded character. The cell line 4BL has stem potential due to its ability to differentiate in adipogenic, osteogenic and myogenic types.

Green fluorescent protein chimeric mouse model

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The term “microchimerism” (Mc) indicates the coexistence, in the same organism, of genetically distinct populations of cells or DNA derived from different individuals. This commonly occurs in patients with transplantations or transfusions and termed iatrogenic Mc. Naturally there is bi-directional transfer of cells between mother and child during pregnancy, and the presence of a mother’s cells in her child and “vice versa” has been termed maternal-fetus Mc. This phenomenon may play an important role in autoimmune diseases, cancer and tissue repair.

Aims: The question of sensitive methods of Mc determination and monitoring after transplantation/transfusion is still important. The biological significance of harboring Mc in the "host" organism requires further investigation. Human studies are often limited by the number of samples for analysis. In this case chimeric mouse model is an important tool in biological research. We have observed that Green fluorescent protein (GFP)-based detection used as a reporter for patterns of gene expression and the origin of cells in transplantation experiments.

Methods: We used FVB.Cg-Tg(CAG-EGFP)B5Nagy/J – transgenic mouse strain that will allow us to analyze foreign cells in recipient organism using fluorescent microscope, flow cytometry and western-blot analysis. The method based on PCR analysis for determination of *DNA* that codes for the target protein in mouse strain was also used.

Results: The GFP transgenic (Tg) FVB.Cg-Tg(CAG-EGFP)B5Nagy/J mouse strain as donor was performed with recipient Balb/c mouse strain. Graft survival of the donor-derived GFP(+) cells was analyzed by flow cytometry. The western-blot analysis was used for the GFP gene expression.

Conclusions: The GFP-based detection technology can be used to locate and quantify Mc cells in mouse model after transplantation/transfusion and during pregnancy. So in our investigations FVB.Cg-Tg(CAG-EGFP)B5Nagy/J mouse strain was used as universal donor of foreign material in GFP chimeric mouse model. Such animal model is suitable for further studies of Mc.

Conformational adaptations of catalytic loop in complexes of *M. tuberculosis* tyrosyl-tRNA synthetase with different substrates according to the molecular dynamics

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Tyrosyl-tRNA synthetase from *M. tuberculosis* (*Mt*TyrRS) is an enzyme that belongs to class I of aminoacyl-tRNA synthetases, that catalyzes the attachment of tyrosine to cognate $tRNA^{Tyr}$ at the preribosomal protein synthesis step. *Mt*TyrRS is not able to cross-recognition and aminoacylation of human cytoplasmic $tRNA^{Tyr}$, therefore this enzyme may be a promising target for development of novel selective inhibitors as new antituberculosis drugs.

Methods: In order to study the conformational mobility of *Mt*TyrRS active center we have performed 100 ns molecular dynamics (MD) simulations of pure dimer of the enzyme and its complexes with different substrates. Crystalline 3D structure of *Mt*TyrRS dimer (PDB code 2JAN) was used to complete the missing amino acid residues at the A-subunit (M1-M4 and G424) and B-subunit (M1-M4 and P81-D93) by ModLoop server. The tyrosine in the *Mt*TyrRS active center was obtained by superposition with crystal *E.coli* TyrRS (1X8X), ATP - *T. thermophilus* TyrRS (1H3E), tyrosyl-adenylate - *E.coli* TyrRS (1VBM), and inhibitor SB219383 - *S. aureus* TyrRS (1JII). All MD simulations of *Mt*TyrRS dimer were performed at 310 K using GROMACS program package with the AMBER99SB-ILDN force field. MD simulations were calculated at the Ukrainian National Grid clusters using the services of MolDynGrid virtual laboratory (http://moldyngrid.org).

Results: It was found that in the course of MD the KMSKS catalytic loop fluctuates between open and semi-open conformations in unliganded state and in complex with the tyrosine. With the ATP in the active site the loop adopts semi-open conformation interacting with the ligand. In the presence of larger tyrosyl-adenylate or SB-219383 inhibitor the loop adopts the conformations more similar to the closed one. Our results are in good correlation with the previous work devoted to *S. aureus* TyrRS [Li et.al., 2008].

Conclusion: Fluctuations of *Mt*TyrRS with different substrates studied by MD simulations show conformational adaptations of the KMSKS catalytic loop depending on a ligand in the active center.

Epigenetic markers in plasma of colorectal cancer patients

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Colorectal cancer (CRC) is the third commonly diagnosed cancer that causes 400 000 deaths worldwide. The most sensitive modern diagnostic tool of CRC is colonoscopy which is painful procedure and can not be recommended for petient with altered topography of colon. Thus, development of less invasive tools for screening of CRC is actual problem of the modern oncology.

To detect concentration of cell free circulating DNA in plasma the qRT-PCR was used. Methylation status of *LRRC3B*, *APC*, and *FHIT* genes in cell free circulating DNA of samples was determined by using methyl-specific PCR with subsequent melting curve analysis.

It was shown that mean-value of plasma DNA is statistically higher in CRC patients than healthy donors ($p < 0.01$). Thus the mean-value concentration of cell free circulating DNA in plasma of CRC patients was 17.57 ± 3.43 ng/mL whereas 7.07 ± 0.84 ng/mL for healthy donors. Accordingly with it the upper cut-off value of the free circulating DNA concentration was 17.74 ng/mL of plasma of healthy donors. Therefore there were 8 out of 22 samples of CRC which fell into criteria as samples with abnormally increased DNA level.

We have revealed hypermethylation of *APC*, *FHIT* and *LRRC3B* genes in in 48 % (10/21), 71 % (15/21) and 67 % (14/21) of tumor samples, correspondingly. Altogether hypermethylation at least one of the selected genes was detected in 95 % (20/21) of samples. Using MSP with subsequent melting curve analysis we have previously detected methylated fragments of *APC*, *FHIT* and *LRRC3B* genes in plasma of 19 % (4/21), 29 % (6/21) and 14 % (3/21) of CRC patients, respectively. We have identified hypermethylation at least in one of the selected genes in 48 % (10/21) of plasma samples for CRC patients.

We have proposed that two stage verification might be applied for CRC screening. These stages include measurement of cell-free circulating DNA with following detection of methylated fragments of *APC*, *FHIT* and *LRRC3B* genes in plasma of CRC patients. Overlapping of the mentioned approaches allowed increasing sensitivity of studied panel up to 95 % in CRC detection. An approach based on *APC*, *FHIT* and *LRRC3B* genes did not show of 100 % sensitivity in registration of CRC which is essential for prevention of this death disease. Therefore we suggested that higher sensitivity could be achieved by further extension of gene panel for identification of methylated DNA in plasma of CRC patient.

4'-hydroxyflavone, as inhibitors of protein kinase CK2. Search, optimization and biological testing

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Aim. CK2 is a pleiotropic serine/threonine protein kinase that plays an important role in a number of cellular processes. Over expression and hyper activation of this enzyme associated with many diseases. Thus, development of new potent and selective CK2 inhibitors is a task of great importance which provides a powerful tool to extend our knowledge about CK2 function as well as to regulate its activity in both in case of health and disease. In this work we developed and researched new synthetic inhibitors of protein kinase CK2 among flavones derivatives.

Methods and Results. To search for new inhibitors of CK2 the receptor-based virtual screening of 150,000 organic compounds library has been used. Followed in vitro tests (γ – ^{32}P -ATP method) revealed that 19 flavones out of 49 selected by virtual screening display the ability to inhibit activity of CK2 (IC_{50} < 30 μM). Structure-activity relationships of flavones derivatives have been studied and binding mode of this chemical class has been predicted to realize further compounds chemical optimization. 28 derivatives of 4'-hydroxyflavone have been synthesized as a result of directed optimization, 80% of them have sub-micromolar activity against CK2. The most active compounds were FNH68 (IC_{50} = 0,01 μM) – 6,8-dichloro-2-(4-hydroxy-3-methoxy-phenyl)-chromen-4-one and FNH79 (IC_{50} = 0,004 μM) – 6,8 dybromo-2-(4-hydroxy-3-methoxy-phenyl)-chromen-4-one.

Kinetic studies of FNH68 and FNH79 showed that they are ATP competitors. Inhibition constants are 3,5 nM and 1,8 nM, respectively. Initial *in vitro* tests of the most active compounds (FNH68 and FNH79) on four serine/threonine (ASK1, JNK3, Aurora A and Rock 1) and three tyrosine protein kinases (FGFR1, Met and Tie2) revealed remarkable specificity towards CK2.

Conclusion. New synthetic inhibitors of protein kinase CK2 among class flavones have been received by the methods of computer modeling and chemical optimization. These compounds are more active compared to their natural analogues. High efficiency of developed inhibitors provides a basis for their further use in biological research and for development of therapeutic agents.

Metagenomic analysis of domesticated kombucha multi-microbial culture

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Aim. To screen microbial community of domesticated kombucha microbial culture (known as tea "fungus") using analysis of metagenomic data.

Methods. DNA barcoding based on pyrosequencing data. Phylogenetic and cultural analyses.

Results. Probiotics occupy a wide niche within this sector because of keeping gut health and boosting immune system, memory and mental sharpness. Kombucha beverage is becoming increasingly popular around the world today as a prophylactic and healing agent. Kombucha culture (KC) is an example of a multi-microbial community with mutualistic relationships between its members. Bacterial and fungal barcoding followed by alignment with sequences from public databases revealed 2 bacterial (*Proteobacteria* and *Firmicutes)* and 1 fungal phyla (*Ascomycota*) in the KC, and an unprecedented number of yeast operational taxonomic units (OTUs) were detected. The acetobacteria of the *Gluconacetobacter* and *Gluconobacter* genera dominated in KC metagenome in both phases of the kombucha micro-system (a pellicle and a cultural liquid) with a few bacterial species of the *Gluconacetobacter* genus and *Gluconobacter oxydans*. Also there were plenty of unknown but clonal bacterial and fungal species, meaning that the blast scores were not sufficient to reliably assign them to specific taxonomic units, but several conserved patterns of blast hits were observed suggesting that these unidentified reads had originated from several sources of unknown species not represented in the used reference databases. However, the core community was represented by the 13 most abundant OTUs of bacteria and yeast. It was found that the most heavily sequenced bacteria and yeast in KC were not almost related to the cultured species

Conclusions. The results of pyrosequence-based DNA barcoding showed that Ukrainian ecotype of kombucha culture contains uncultivable representatives of yeast which where not described earlier. As some of they represented major populations, we may speculate that these species are true kombucha 'aborigens', but are uncultivable and non-identified. In general, our study exhibited a lower representation of cultured species in respect to whole members of KC. Cultivable forms of the bacterial community members well matched with the pyrosequencing data, however, some OTUs were not proven by traditional cultural analysis.

Embrionically induced β-catenin haploinsufficiency in heart inhibits adult myocardium development and leads to fetal genes expression upregulation.

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Aim. Though canonical WNT/β-catenin signaling is inhibited in adult heart it is well known that its activation is critical for heart adaptation to different changing conditions. The main aim of our work is to determine the significance of cardiac ablation of β-catenin during cardiogenesis for adult heart formation.

Methods. Using conditional knock-out approaches we have generated mice with cardiospecific β-catenin haploinsufficiency. The routine PCR was used for genotyping of the experimental animals. The 1, 3 and 6 months old mice were used in our research. For morphological analysis the body weight/heart weight ratio was estimated. Standard histological (HE- and MT-staining) methods were implemented to analyze the paraffin sections. The quantitative RT-PCR was used for analyzing the level of *ANP, BNP, β-MHC* and *α-MHC* expression. The data were normalized to *GAPDH* gene expression.

Results. In our research we focused on the role of β-catenin in adult myocardium functioning, hypertrophic response and adaptation to stress and aging. We have monitored the influence of embrionical β-catenin ablation in mice cardiomyocytes on heart development and canonical Wnt signaling activity. As a result we have revealed that β-catenin haploinsufficiency leads to fetal gene upregulation (*ANP, BNP, β-MHC*) in mice of 1 and 3 months age but without morphological abnormality of heart tissue. Meanwhile we have observed the tendency to heart development attenuation under β-catenin haploinsufficiency condition in adult mice (3 months). Analyzing the next time point (6 months) we have registered the decrease in expression of the hypertrophy response (*ANP, BNP*) genes in comparison to control mice of the same age group while *β-MHC* and *α-MHC* genes were still upregulated.

Conclusion. We have observed that cardiac ablation of β-catenin during cardiogenesis leads to attenuation of adult heart development and fetal genes expression violation.

Development of dermal equivalents contained living cells for the treatment of massive burns of human skin

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Aim. We plan to create new dermal equivalents for therapy of extensive skin damages, for example massive burns. The skin equivalents will the bioengineered wound coverings with immobilized human cells. These cultured cells secrete various growth factors. Besides various additional components in composition of scaffolds also influence positively the tissue regeneration in the wound area. So our dermal equivalents will be able to accelerate tissue regeneration, stimulate the engraftment of donor grafts and have a bactericidal effect. **Methods.** As a cell component of the skin equivalents we used: 1) cell line 4BL, which was obtained in the Department of Human Genetics (IMBG NASU) from peripheral blood cells; 2) human multipotent mesenchymal stromal cells isolated from human placenta. In this work we develop dermal equivalents on the basis of different natural and synthetic materials. We focus our attention on three types of scaffolds for making new skin equivalents. The first variant is porous polyacrylamide hydrogels, which are synthesized using acrylamide and acrylonitrile. We introduce various additional components in these hydrogels and investigate biocompatibility of obtained specimens and their influence on cell vital functions and wound healing processes. For that we use microscopic examinations and cytochemical staining. The second type of scaffolds consists of two natural compounds: collagen and gelatin. We are patenting this type of bioconstruction. After that they could be produced at the conditions of our laboratory for experimental purposes. The third type of the scaffold developed is the mucous film that is created with a symbiotic complex of bacteria and yeast named as Medusomyces gisevi (so called tea fungus or kombucha). This film contains a natural polysaccharide chitosan, which promotes healing of wounds. Besides this film does not require complicated equipment for synthesis and can be obtained easily. Now we are carrying out experiments with laboratory mice. The burns are made on mouse skin and we are using the bioconstructions “membrane/cells” for treatment of wounds. **Results.** Microscopic examinations on the cell immobilized on polyacrylamide hydrogels have shown that the cells are adsorbed on the surface of gel systems without changing their morphology. It should be noted that the best parameters for cultured cells are the ratio of the hydrogel monomer units "acrylamide: acrylonitrile = 5: 3". All three types of the scaffolds obtained are biocompatible and support cell viability on their surface for some days before application. Our current experiments with laboratory mice are in progress. **Conclusions**. The preliminary data allow us to hope for successful development of new bioengineered dermal equivalents for the treatment of massive burns or other extensive damages of human skin.

Transcriptional profiling of normal and cancer prostate cell lines

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Background: Prostate cancer is the fifth most common cancer in the world. Prostate-specific antigen (PSA) is generally accepted as a clinical biomarker for this disease. The increased concentrations of PSA can be detected upon emergence of prostate cancer and also in the development of prostate adenoma, prostatitis and other inflammatory processes. This marker alone could not discriminate aggressive tumors from non-aggressive, however. There is an opinion that some of localized tumors will never progress, therefore would not need any surgery. Unfortunately, with the help of existing markers it is difficult to distinguish dormant tumors from aggressive. The better tools are needed to diagnose different forms of prostate cancer and to identify patients who will benefit from intervention.

Aim: In the present study the gene expression profiles in androgen-sensitive non-aggressive and androgen-insensitive aggressive cell lines were compared with the aim to find the new putative biomarkers of aggressive prostate tumors.

Methods: Quantitative PCR (Q-PCR) was used to analyzed expression of 65 genes at the mRNA level in the aggressive (DU145, PC3) and non-aggressive (LNCaP) prostate cancer cell lines.

Results: An increase of expression of a set of genes (*IL8, IL1b, PLCB1, PREX1, THBS4, SPP1*) was observed in more aggressive cell lines. These genes are involved in the different cellular signaling pathways, such as NF-kB (*IL1b, IL8*) and chemokines (*PLCB1, PREX1*), and also in the processes of cell and focal adhesion (*THBS4, SPP1*).

Conclusions: We have found that aggressive and non-aggressive prostate cell lines differ in cell adhesion, invasion and/or metastasis, and inflammatory signaling pathways. Genes that showed decreased levels of expression could be putative tumor suppressor genes. Genes that showed an elevated expression could be putative oncogenes.

Oligoribonucleotides affect the expression of some genes encoding the innate immunity system components

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Aim: Natural and synthetic oligoribonucleotides are known to possess immunomodulating, antiviral and anti-inflammatory properties. In this respect, the aim of our study was to explore the gene expression of antiviral innate immunity system components under the influence of oligoribonucleotides.

Methods: In our study RT-PCR method was applied.

Results: Significant increase in the *IFNα* and *IFNβ* genes expression was observed upon the oligoribonucleotides injection into mice for preventive and treatment purposes, similar to virus infected animals. At the same time, the alteration of gene expression did not occur in the animals of control group. The *MX1* gene expression increased almost 190 times, *OAS1* – 19 times and *NOS2* – 7 times after oligoribonucleotides were injected for prevention. When oligoribonucleotides were injected as a treatment drugs we observed the 150 times increase of *MX1* gene expression, *OAS1* – 180 times, and *NOS2* – 18,5 times. In contrast to these genes, the expression of *RNAse L* gene decreased 1,4 times when oligoribonucleotides were used as a preventive and 1,6 times when they were used as a treatment drugs. The similar tendency in expression of these genes was observed for influenza infected animals.

Conclusion: Thus, for both animals, infected with the influenza virus, and for animals injected with the oligoribonucleotides, we observed the significant increase in the expression of some genes of innate immunity system enzymes.

Flexible structure and interdomain interactions in human tyrosyl-tRNA synthetase studied by molecular dynamics simulations

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Tyrosyl-tRNA synthetase (TyrRS) is the key enzyme of protein biosynthesis, which catalyzes the aminoacylation of $tRNA^{Tyr}$. The full-length TyrRS has no cytokine activity, but its proteolytic cleavage reveals IL8-like activity of the N-terminal catalytic module and EMAP II-like activity of non-catalytic C-terminal domain (Wakasugi and Schimmel, 1999; Kornelyuk et al., 1999). Earlier, it was found that the ELR-motif (E91, L92, R93) in TyrRS is responsible for IL8-like cytokine activity (Wakasugi and Schimmel, 1999), but the 3D structure of the full-length human TyrRS (*Hs*TyrRS) is still unknown. **Aim**. In this work we performed the modelling of 3D structure of *Hs*TyrRS and studied its compactization by molecular dynamics simulations (MD). **Methods.** All computations were performed using the grid-services of MolDynGrid virtual laboratory (http://moldyngrid.org). The model of three-dimensional structure of *Hs*TyrRS was constructed in Modeller 9.7 using templates (PDB codes: 1N3L, 1NTG and 1OPL for interdomain linker). The best 10 structures were selected and six independent 100 ns MD trajectories of *Hs*TyrRS were computed using GROMACS 4.0.5 software and G43a1 force field. **Results**. Our MD simulations produced compact final structures of *Hs*TyrRS, where both C-modules were bound to the dimer of N-modules. There is a pronounced asymmetry in the binding of C-modules, which explained by their almost independent motions The main "binding hot spot" on the N-terminal module contains the residues: E25-K26, E29-R34, G46-K47, Y79-L89, R93, R135, V139, K154, E157-H158, E175-D180, F192-V208, E227-K231, L235-C250, K243, F328-P342. The strongest binding energy of ~1000 kJ/mol was observed for the second C-module in simulation 1 and for the first C-module in simulation 6. In trajectories 1 and 6, only one of the C-modules binds strongly, whereas in trajectory 4 both C-modules exhibit almost identical binding energies of ~400–500 kJ/mol. In contrast, the binding energies in simulations 2, 3 and 5 were much smaller (300–400 kJ/mol). **Conclusions.** Our data suggest that *Hs*TyrRS in solution exists in a number of compact asymmetric conformations, which differ significantly by their general rigidity, mobility of C-modules and the strength of their binding to the dimer of N-modules. The orientation of bound C-modules is rather unspecific while there is a pronounced set of binding hot spots on the surface of N-modules. In our MD simulations we observed the hydrogen bonds formation between R93 of ELR cytokine motif and A340 and E479 residues in C-module. These data support the idea that the full-length TyrRS lacks its cytokine activity due to the interactions between N-terminal and C-terminal modules and protection of ELR cytokine motif.

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Mesenchymal and trophoblast progenitor cell subpopulations in the culture of placental multipotent mesenchymal stromal cells

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Introduction. Placenta is an attractive source of multipotent mesenchymal stromal cells (MMSC) for needs of regenerative medicine. The origin of placental MMSC remains unknown though the hypothesis about perivascular origin has been recently published. We previously showed that placental MMSC contain various populations of cells that express trophoblast-specific markers.

The **aim** of this study was investigation of immunophenotype and expression of some genes in the subpopulations of mesenchymal and trophoblast cell in culture of placental MMSC.

Methods. Placental MMSC were obtained by classical culture methods, the placental cells were phenotyped by flow cytometry and immunocytochemistry, gene expression was analyzed by RT-PCR. The expression of vimentin, chorionic gonadotropin, HER2 and cytokeratin 7 in the placental tissue was investigated by immunohistochemistry and flow cytometry.

Results. Populations of colony-forming cells with immunophenotype $Vim^+CG^+HER2^+CK7^-CD90^+$ and $Vim^+CG^+HER2^+CK7^+CD90^+$ were present in the culture of placental MMSC. It was established mRNA expression of the *EOMES*, *CDX-2* genes that is characteristics of trophoblast stem cells, and mRNA of the *POU5F1* gene, which is a marker of embryonic stem cells. In placental tissue we found the placental MMSC-like populations with immunophenotype $CD90^+CD73^+CD45^-CD34^-CD14^-$- and $CD90^-CD73^+CD45^-CD34^-CD14^-$ but we did not observe the vimentin-positive stromal cells, which express both chorionic gonadotropin and cytokeratin 7.

Conclusions. Placental MMSC simultaneously express mesenchymal and trophoblast markers and contain populations of trophoblast and mesenchymal stem cells.

Development of method of cultivation of mesenchymal stem cells from umbilical cord matrix with preservation of the cells with reduced attachment rate

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Aim*:* The aim of present work was to develop the method of optimization of mesenchymal stem cells from umbilical cord matrix (UC-MSC) cultivation with preserving the spontaneously detached cells, which evaded the impact of the standard passing procedure on the surface apparatus, and to test this method for cultivation of the UC-MSC under physiological oxygen tension. Cultivation of mesenchymal stem cells (MSC) from various sources is associated with the loose of the features relevant for their use in clinical practice. One of the reasons is the damaging of the cell surface proteins during standard passing procedures. Recent studies have developed various variants of cultivating MSC without obligatory detachment. It is also known that during the specific phase of the cell cycle, prior to the division, cell's attachment to the substrate is decreased. Preserving these non-attached cells may appear a helpful addition to the accepted passing procedures. **Methods**: UC-MSC were cultivated up to 3 passages under CO_2 incubator conditions. When the monolayer confluence reached ~70 %, the cells of the control groups were passed using the mixture of trypsin (0.25 %) and versene (0.02 %) (1:1). In the experimental group, while replacing the cultural medium with the fresh portion, the medium where the culture was maintained (probably containing spontaneously detached cells) was collected, transferred into a new flasks with the addition of fetal bovine serum and FGF2. Two days after the first detection of attached cells the medium was replaced by the fresh portion. The same was done for UC-MSC cultivated under nitrogen-based and argon-based gas mixtures containing 3 % oxygen. Cell numbers and morphology were analyzed at each passage. **Results***:* Two days after the medium transfer 10–20 cell's "attachment places" can be noticed on the bottom of the culture flask. Approximately two weeks after the first detection of cells, the clones reached the size and confluence sufficient to be passed using standard methods. The morphology of the cells in these cultures was typical for UC-MSC of initial passage. The degree of surface markers expression was similar to that in cultures, replated by the standard method.

The method was also tested for UC-MSC cultivated under nitrogen-based and argon-based gas mixtures containing 3 % oxygen. The cells obtained from cultures maintained in both gas mixtures had a slightly higher proliferation rate.

Conclusion*:* The method of keeping the medium with spontaneously detached cells helps to preserve MSC population which avoided the negative effect of the standard passing procedure. Cultivation under physiological oxygen concentrations appeared to enhance the proliferative potential of cultures obtained by this method.

Design of protein kinase CK2 inhibitors based on the 3-alkyl substituted 2-quinolone scaffold

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Aim. The activity of protein kinase CK2 (casein kinase 2) increased in a wide range of tumors. It inhibits cell apoptosis and is used by many viruses for their own protein phosphorylation. Therefore, the potent and selective inhibitors of CK2 would be important compounds for the development of clinical agents. Recently some 4-quinolone derivates as effective CK2 inhibibitors have been developed [1]. Therefore we have chosen their structure analogs – 2-quinolone derivatives for investigation of inhibitory activity against CK2. **Methods and Results.** In order to discover CK2 inhibitors we have performed flexible docking of virtual library containing 3000 derivatives of 2-quinolone in CK2 ATP-binding site. The most promising 112 compounds have been selected for chemical synthesis. These compounds were divided into four general sub-classes: amides of quinolone-3-ylacetic- and quinolone-3-ylpropanoic- acids, amides and sulfonamides of quinolone-3-ylmethane- and quinolone-3-ylethane-amines. Synthesis of 3-alkyl substituted 2-quinolone derivatives was based on the 2-chloro quinoline obtaining method. Treatment of aliphatic carboxylic acids anilides with Vilsmeer-Haak complex in phosphorus oxychloride leads to intramolecular cyclization with 2-chloroquinoline ring formation. As we previously reported some new 3-alkyl substituted 2-quinoline derivatives were synthesized by optimization of this method in high yields [2, 3]. Series of new 3-alkyl substituted 2-quinolone derivatives were obtained by chlorine atom hydrolyses in 2 position of quinoline with water acetic acid treatment. Building blocks were synthesized after removing protected groups. Resulting screening compounds were obtained by high-throughput combinatorial synthesis. Synthesized derivatives of 3-alkyl substitute 2-quinolone were taken for the kinase assay study. *In vitro* experiments revealed four compounds with inhibitory activity toward CK2 in the micromolar range. Several important structural features of 2-quinolone derivatives were identified as a result of SAR analysis. It was found that the increasing of the side-chain length in 3 position of 2-quinolone derivatives leads to the decreasing of inhibitory activity against CK2. **Conclusion.** Some novel CK2 inhibitors have been developed on the base of the 3 – alkyl substituted 2- quinolone scaffold.

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2'-5'-linked oligoadenylates alter the conformation of S100A1 and S100B proteins

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Aim. In order to broaden the understanding of biological action of dephosphorylated 2'-5'-linked triadenylates (2'-5'A_3), this study was aimed at searching for additional biological targets being under their regulating impact. We showed previously, that 2'-5'A_3 binding to Calmodulin – multifunctional Calcium ions transducer – caused considerable (~ 3 orders of magnitude) increase of its Ca^{2+} affinity (Tkachuk et al., 2011). Therefore, we suggested a possibility of 2'-5'A_3 interaction with other Ca^{2+}-binding proteins – S100A1 and its close structural homologue – S100B.

Methods. For these purposes we used the Circular Dichroism Spectroscopy method, as it provides valuable structural data. CDNN deconvolution software was used for the extraction of protein's secondary structure content.

Results. In this study we have obtained the evidence for interaction between 2'-5'A_3 and Ca^{2+}-binding proteins in both *apo*- and *holo*-form. It was proved by the occurrence of conformational changes within protein's secondary structure upon interaction with both 2'-5'A_3 and its epoxy-modified analogue. It was shown, that in both cases of S100A1 and S100B interaction with 2'-5'A_3, 10 % of their helical structure content converts to randomly coiled elements. This value may be considered as insignificant, but, on the other hand, it may serve as an evidence for the existence of concrete binding site for 2'-5'A_3.

Conclusion. Our earlier proposed suggestions about the possibility of interaction between 2'-5'A_3 and Ca^{2+}-binding proteins – S100A1 and S100B – was proved by the experimental data obtained. It turned out, that such binding may occur even in Ca^{2+}-free solution, though the interaction of S100A1 and S100B with target peptides/inhibitors was shown to occur only in the presence of Ca^{2+} according to the literature sources. This important feature indicates, that 2'-5'A_3 are capable of regulating the Ca^{2+}-signal transducing proteins in both Ca^{2+}-free and Ca^{2+}-saturated states.

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Constitutive expression of *CHI3L1* oncogene promotes chromosome instability in immortalized 293 cells

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Multiple genetic rearrangements, including whole chromosome and gene copy number gains and losses, chromosome translocations, gene mutations are necessary for establishing the malignant cell phenotype. Previously, we and others have demonstrated that increased *CHI3L1* gene expression stimulated cellular mitogenic and proliferative properties, enhanced migration, invasion, radio- and chemoresistance of tumor cells, capacity for anchorage-independent growth in soft agar, promoted tumor growth, and angiogenesis. The aim of this study was to establish the link between transforming properties of oncogene *CHI3L1* and changes in karyotype of 293 cells with stable expression of *CHI3L1*.

Conventional cytogenetics and array comparative genome hybridization (aCGH) were used to describe chromosome abnormalities in cell lines.

We found that constitutive expression of *CHI3L1* promoted chromosome instability (CIN) in 293 cells. Modal number of chromosomes in 293_*CHI3L1* cells was distinct from that in transfected control 293_pcDNA3.1 and parental 293 cells. Interline whole chromosome heterogeneity was manifested. A number of new distinct marker chromosomes were observed in *CHI3L1*-expressing cells in two independent experiments. aCGH revealed significant difference between the spectra of cytoband gains and losses in 293_*CHI3L1* and control cells.

Here it was established the link between transforming properties of oncogene *CHI3L1* and changes in karyotype of 293 cells with stable expression of *CHI3L1*. Deregulation of oncogenic pathways triggers and collaborates with CIN during transformation and tumorigenesis. Relationship between CIN and cancer genes explains why such large number of cancer genes was identified (Cancer Gene Census) and why hundreds of oncogenes with diverse functions are characterized by the same ability to transform a cell or aggravate tumorigenicity.

Construction and expression of deletion mutants of translation elongation factors eEF1Bα, eEF1Bβ, eEF1Bγ.

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In higher eukaryotes, translation elongation factor 1B (eEF1B) exist as a stable high molecular weight complex comprising three different subunits: alpha, beta and gamma. The part of this complex involves also tightly bound valyl-tRNA synthetase. eEF1Bα and eEF1Bβ subunits catalyze GDP-GTP recycling on eEF1A, whereas eEF1Bγ is believed to be a structural constituent. Up to date, the structure of the complex is unknown and the mode of interaction between the subunits was not studied in details.

One of the purposes of this work is to explain how all three subunits interact with each other to form the complex. For that, we created series of deletion mutants for all subunits and expressed them in bacteria. Further, all recombinant truncated proteins will be purified and their interactions will be studied *in vitro* to define domains responsible for the complex formation.

Conservative protein domains in the structures of all three subunits were delineated by multiple sequences alignment using ClustalW. Corresponding DNA primers were generated to amplify respective fragments of eEF1Bα, eEF1Bβ and eEF1Bγ coding regions. DNA fragments encoding truncated mutants of eEF1Bα and eEF1Bβ were cloned into pGEX6P1 vector in frame with GST-tag. To produce eEF1Bγ deletion mutants with N-terminal His-tag the respective DNA fragments were cloned into pET28a(+) vector. The sequences of all obtained constructs were verified. All recombinant eEF1Bα and eEF1Bβ mutants were found to be soluble when produced in BL21Gold bacterial strain at 37°C. In contrast, production of soluble eEF1Bγ mutants was efficient only in Rosetta strain at 20°C. eEF1Bγ deletion derivatives were purified by affinity chromatography on Ni-NTA resin and their aggregation state was analyzed by gel filtration on a Superose 6 column. Two from four deletion mutants of eEF1Bγ were found to form soluble aggregates, while two others behaved as monomers judging by gel filtration technique.

As a result, we created and produced in bacteria a set of soluble mutants of eEF1Bα, eEF1Bβ and eEF1Bγ subunits. eEF1Bγ deletion mutants were purified and can be now used to test their interactions with full length eEF1Bα and eEF1Bβ proteins *in vitro*. Purification of eEF1Bα and eEF1Bβ mutants is in progress.

Ammonium ion nanocomposite sensor fabricated at copper-plated/nafion/polyaniline screen-printed carbon electrodes

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Aim. Modification of the screen-printed carbon electrode with a new, easy-to-handle and inexpensive nanocomposite layers for highly sensitive and selective detection of ammonium ions.

Methods. Electrodeposition, electropolymerization, cyclic voltammetry, chronoamperometric detection.

Results. For the first time, we propose and report amperometric ammonium ions selective sensor based on Copper/Polyanyline (PANI) nanocomposites, which were fabricated using Layer-by-Layer electrodeposition techniques. The NH_4^+ ions diffuse into the PANI layer and provoke the reduction of PANI on the Copper-plated screen-printed carbon electrodes at a potential of about −450 mV (vs Ag). Moreover, electrodeposited Copper displays catalytic properties towards ammonium ions at the same reduction region as for PANI and leads to the substantial improvement of the fabricated sensor electrochemical response. The experimental result reveals that the Cooper/PANI nanocomposite sensor exhibits much higher sensitivity (325 nA/(per 0.1 mM mm^2)) than that of PANI and Cooper alone, and shows approximate linearity over a range of target analyte concentration from 5 to 100 μM with the limit of detection equal to 1×10^{-6} M of NH_4Cl (signal/noise ratio = 3, n = 10).

Conclusions. A simple and inexpensive Copper/Polyaniline-based chemosensor for ammonium determination with excellent electrochemical properties has been developed.

Investigation of amino acids of the human cytochrome P450 2E1 active center critical for interaction with substrates

V. Kitam

Aim. The microsomal ethanol-induced cytochrome P450 2E1 (CYP2E1) belongs to the superfamily of hem-containing monooxygenases that are responsible for the biotransformation of many endogenous compounds and xenobiotics. This enzyme catalyzes oxidative metabolism and metabolic activation of wide range exogenous organic substrates including drugs, environmental toxicants, procarcinogenic and carcinogenic chemicals. The major part of them are low molecular weight compounds. Using computational methods of docking of ligands into cytochrome P450 2E1 active site we have identified some amino acids that specifically interact with substrates or inhibitors (Kitam and Chashchyn, 2010). In order to verify obtained data we performed computational site-directed mutagenesis of the enzyme by changing one of the identified critical for protein-ligand interactions amino acids Phe298 for Ala (alanine screening) in its structure.

Methods. Computational methods of molecular dynamics (NAMD program, CHARMM forcefield, standard NVT-ensemble, 310K, 0,15mmole KCl) were used for modeling of possible crucial changes in CYP2E1 spatial structure caused by such aminoacid replacement. In order to investigate the influence of this amino acid substitution on the enzyme's catalytical activity experimentally and compare obtained results with computational data we introduced codon amino acid Ala instead of codon amino acid Phe in the human CYP2E1 cDNA coding region in position 298 using PCR site-directed mutagenesis method and cloned this modified cDNA in *E. coli* expression vector pCWOri+ (Gillam, Guo and Guengerich, 1994).

Results. Molecular dynamic of this mutated protein and its complexes with p-nitrophenol (substrate) and 4-methylpirasol (inhibitor) showed that such amino acid substitution did not affect the overall protein structure, slightly influenced on its interaction with inhibitor and strongly influenced on its binding with substrate. This data confirmed previous information obtained from docking studies. The evaluation of catalytic activity of the expressed in *E.coli* DH5α cells recombinant protein have shown 2-fold decrease in its p-nitrophenol hydroxylation activity related to the unmodified protein.

Conclusions. The experimentally obtained results showed close agreement with our computational data revealing the significant role of Phe298 in interaction of CYP2E1 active site with substrates thus evaluating and verifying the quality of the computational methods used in our work and demonstrating the reliability of our modeling experiments.

Synthesis of 2-amino-3-(1H-benzimidazol-2-yl)-1-aryl-4, 5-dihydro-4-oxopyrroles derivatives as potential FGFR1 kinase inhibitors

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Aim. The fibroblast growth factor receptor kinase (FGFR1) is member of a family tyrosine receptor proteinkinase. The main role of FGFR1 in postnatal life is regulation of angiogenesis process, which is necessary for tumor growth. Therefore kinase inhibitors can be appropriated for effective treatment of a whole number of diseases, including oncogenic.

There have been described development of FGFR1 inhibitors among 2-amino-3-(1H-benzimidazol-2-yl)-1-aryl-4, 5-dihydro-4-oxopyrroles derivatives, which have been found in a virtual screening.

Methods. Receptor-based virtual screening has been used in search for FGFR1 kinase inhibitors. Consequently, 2 compounds with activity IC_{50} less than 25 μM have been obtained. The number of close structural analogs have been synthesized for the purpose of optimization and tested for activity of FGFR1 enzymatic inhibition. Eventually, 27 compounds were chosen, obtained and tested. Two compounds shown the activity IC_{50} less than 1 μM.

All 2-amino-3-(1H-benzimidazol-2-yl)-1-aryl-4,5-dihydro-4-oxopyrroles derivatives were synthesized according to the next schema. Firstly, benzimidazol-2-yl acetonitriles have been obtained through cyclocondensation reaction of o-phenylendiamine and ethyl cyanoacetate. Then active methylene group of benzimidazol-2-yl acetonitrile was acylated with chloroacetyl chloride and product was used to obtain 2-amino-3-(1H-benzimidazol-2-yl)-1-aryl-4, 5-dihydro-4-oxopyrroles derivatives as desired products.

Results. Structure-activity relationship study showed that the greatest effect had meta-substitution of aryl fragment especially with hydroxyl group. At the same time, para-substitution of phenyl appreciably reduced the FGFR1 inhibitory activity of our compounds. Cause of this reducing can be related to steric obstacles, which para-substitutes made. The compound with methylated Nitrogen atom of benzimidazolyl has shown low-activity inhibitor, as well. This fact gave to understand the necessity of availability of hydrogen bond donor here.

Conclusions. Accordingly, 2-amino-3-(1H-benzimidazol-2-yl)-1-aryl-4,5-dihydro-4-oxopyrroles derivatives were synthesized and tested *in vitro* as human FGFR1 kinase inhibitors firstly.