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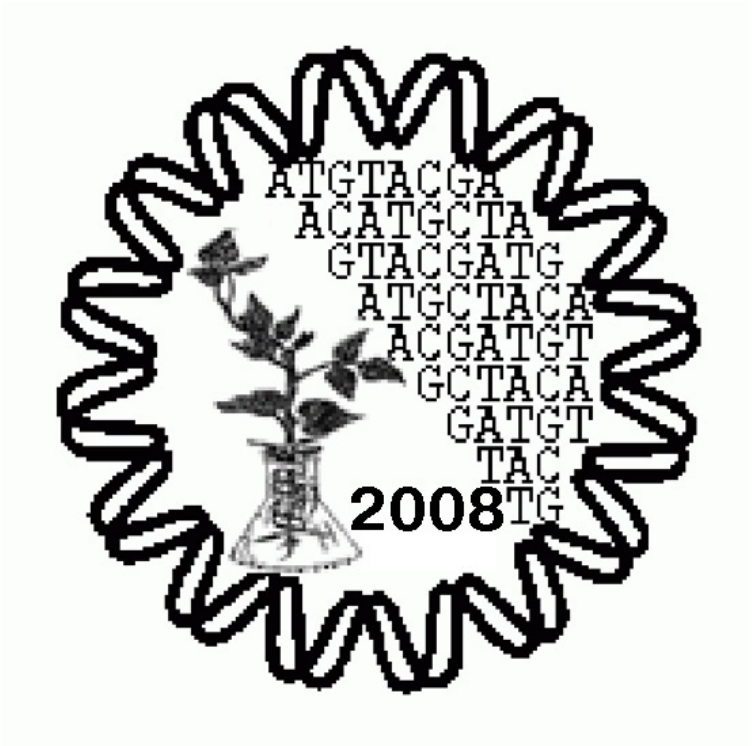

Conference of Young Scientists Dedicated to the 35th Anniversary of Institute of Molecular Biology and Genetics of NAS of Ukraine

Collection of Theses

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Study on the structural basis of the prokaryote-type prolyl-tRNA synthetase from *Enterococcus faecalis* editing activity by the methods of site directed mutagenesis

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An insertion domain of the most of prokaryotic prolyl-tRNA synthetases (ProRS) exhibits post-transfer editing activity. The mechanism of tRNA-dependent editing by ProRS has to be defined.

The present work is aimed to study the structure of the active site of enterobacteria *E. faecalis* ProRS (ProRSEF) editing domain. On the grounds of the putative structure of the editing domain active center, based on the ProRSEF crystal structure and computer modeling data [1], following amino acids positions have been chosen for the site directed mutagenesis (alanine scanning): E218, T257, K279, G331, S332, G334, H366. In the ProRSEF gene, cloned previously, mutations have been obtained by QuickChange® method (Stratagene). Mutant genes have been checked by sequencing. Mutant proteins were overexpressed in *Escherichia coli,* BL-21 Star strain. The purification procedure included graded salting-out, chromatography on DEAE-sepharose and chromatography on Toyopearl HW-60. The purity of the proteins was checked by $OD_{260/280}$ ratio and by electrophoresis in PAAG, and in all cases was over 95 %. The aminoacylation activity was determined for all ProRSEF mutants. To check editing activity of the ProRSEF mutant forms by alanyl-tRNA hydrolysis, a chimeric tRNA, cognate for both prolyl- and alanyl-tRNA synthetases, was created. Determination of the mutant forms editing activity allowed to reveal next amino acid residues, important for the editing process: K279, G331, H366. The comparison of these data with structural data enabled us to suppose that K279 is involved in substrate positioning in the editing active center, G331 takes part in putative catalytic water molecule binding and activating, and H366 maintains G331 in the right conformation. The existence of a bound water molecule in the editing active center was predicted by computer modeling.

In summary, seven mutant forms of ProRSEF with mutations in the editing domain have been obtained, and their editing activity has been compared with the wild type of ProRSEF. Three amino acid residues, important for the editing activity, K279, G331 and H366, have been revealed. The hypothesis is proposed, that the water molecule, hydrolyzing alanyl-$tRNA^{Pro}$, is positioned in the active center of the ProRS editing domain.

1. Crepin T., Yaremchuk A., Tukalo M., Cusack S. (2006) Structure, 14, 1511– 1525.

Synthesis of 2-phenylisothiazolidin-3-one 1,1-dioxides as inhibitors of human protein kinase CK2

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Protein kinase CK2 (Casein Kinase 2) is an extremely pleiotropic Ser/Thr kinase with high constitutive activity. The observation of CK2 deregulations in various pathological processes suggests that CK2 inhibitors have a therapeutic value, particularly as anti-neoplastic and antiviral drugs.

We have developed series of 2-phenylisothiazolidin-3-one 1,1-dioxides as new class of compounds with high CK2 inhibitory activity. This class of inhibitors was identified by high-throughput docking of the virtual library of compounds in the ATP-binding site of human CK2. Synthesis of the compound series was carried out using combinatorial synthesis techniques, such as parallel synthesis in wheaton sample vials with product isolation bycentrifugation. Series of 55 compounds were tested *in vitro* for CK2 inhibitory activity. It was found that *N*-(3-acetylphenyl)-2-chloro-4-(4-methyl-1,1-dioxido-3-oxoisothiazolidin-2-yl)benzamide has IC_{50} value of 20 M. To perform further structure optimization, another 7 compounds of this class were synthesized. Five of them showed IC_{50} value less than 20 M. The most active compound is 4-{[2-chloro-4-(1,1-dioxido-3-oxoisothiazolidin-2-yl)benzoyl]amino}-2-hydroxybenzoic acid with IC_{50} value of 3 M.

Thus, we suppose that the represented compounds are promising class of novel CK2 inhibitors. To obtain more active compounds of this class, we plan to continue QSAR modeling and structure optimization of the 2-phenylisothiazolidin-3-one 1,1-dioxide scaffold.

Association between CAG repeats number in the exon 1 of androgen receptor gene and impaired spermatogenesis

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Androgens are required for male sex determination, development and spermatogenesis. The androgen activity is mediated by an androgen receptor. The androgen receptor (*AR*) gene is located on chromosome Xq11-12. The *AR* gene has a repetitive DNA sequence in the exon 1 that encodes a polyglutamine tract. Within the normal polymorphic range this $(CAG)_n$ tract length is inversely related to the transcriptional activity of the *AR* gene.

The aim of our research was to study the association between CAG repeats number in the exon 1 of *AR* gene and impaired spermatogenesis.

DNA isolated from blood samples of 158 infertile males (with azoospermia and oligozoospermia) was amplified by PCR targeting the *AR* $(CAG)_n$ tract. DNA isolated from blood samples of 124 fertile males served as a control. A fragment analysis of Cy5-labeled PCR products on an automated DNA analyzer «A. L. F.-express» was used to calculate the CAG repeats number. To determine correct number of the CAG repeats three different alleles were sequenced using ABI 3130 Genetic Analyzer. The nomenclature of alleles in our study corresponds to the CAG repeats number.

The CAG repeats number of *AR* gene in the infertile male group was more widely distributed than in control croup. There was a significant difference in CAG alleles in the infertile males versus controls ($p = 0.023$). Short alleles contained 7 CAG repeats were detected only in males with azoospermia. Long alleles contained 32 and 33 CAG repeats were detected only in males with oligozoospermia. These alleles were not found in control group. Severely oligozoospermic males had longer CAG repeat tract than azoospermic males. The results of our study allow to suppose that short CAG repeats can lead to disease of androgen-dependent tissues. On the other hand, males with longer alleles of *AR* gene within the normal range of CAG repeats may have decreased *AR* function that results in reduced spermatogenesis.

Thus, the molecular-genetic analysis of the CAG repeats number in *AR* gene as well as genetic counseling are very important for patients with male infertility, especially if they are included in an assisted reproductive technologies program.

Production and characterization of monoclonal antibody specific to Fibroblast Growth Factor Receptor 1 (FGFR1)

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Fibroblast growth factor receptor 1 is a transmembrane receptor activated by FGF-1 and by related FGF-2. It belongs to the large class of tyrosine kinase receptors.

In this study, we report the production and characterization of monoclonal antibody against FGF-1 receptor. The antigen for mouse immunization has been chosen by Bcepred software. Chosen sequence encoding loop II-III for FGFR1 extra cellular domain was cloned into pGEX4T1 and pET42a vectors and expressed in bacteria. Recombinant proteins were purified by electroelution from gel and by NiNTA affinity chromatography. GST-loopII-IIIwas used as antigen for mouse immunization whereas His-GST-loopII-III was used in primary hybridoma screening by ELISA and Western blot. Primary screening allowed us to select 14 positive clones, which have been checked for their crossreactivity with FGFR3wt. 10 clones showed no crossreactivity. Among them there are two hybridoma clones, which could specifically recognize endogenous FGFR1 in Western blot of NIH3T3L1 cell lysates. The selected positive clones were subcloned twice using a limiting dilution method.

The model for the investigation of novel ovarian cancer marker – sodium-dependent cotransporter NaPi2b

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Ovarian cancer is the most common gynecologic cancer that is usually far advanced before it is diagnosed and thus patients have a poor prognosis and survival rate. Identification and characterization of novel ovarian cancer markers is important for understanding the molecular mechanisms of malignant transformation and for the development of novel diagnostic and immunotherapeutic approaches in gynecologic oncology.

Recently, we have identified the sodium-dependent phosphate transport protein 2b (NaPi2b) as a new ovarian cancer antigen based on the immunoscreening of ovarian cancer cell line (OVCAR3) cDNA library with MX35 monoclonal antibodies obtained at Ludwig Institute for Cancer Research by mice immunization with ovarian carcinoma cells.

The human protein, NaPi2b encoded by SLC34A2 gene is involved in the homeostasis of inorganic phosphate. NaPi2b is a membrane protein with the NH_2- and COOH-termini located on the cytoplasmic side of the membrane, 8 transmembrane domains and a large extra cellular loop (188–361 aa). Previously, we have generated monoclonal antibodies (L2/20) against the large extra cellular loop (188–361 aa) of NaPi2b and determined a region of the transporter molecule thatincludes the epitope for these antibodies (311–340 aa) which happened to be the same as for the MX35 antibodies.

The present study was aimed to create a model for the investigation of functional activity of a new marker of ovarian cancer – sodium-dependent phosphate transporter NaPi2b, in malignant cells. The stable cell lines expressing wild type and mutant forms of NaPi2b were created by stable transfection of HEK293 cells. We have chosen the mutations of NaPi2b that according to the data base search could be potentially associated with ovarian cancer. There are deletion of 6 aa in C-terminus and point mutation T330V in the extra cellular loop of NaPi2b. The expression of NaPi2b by stable cell lines was confirmed by Western-blot analysis using tag-specific and anti-NaPi2b antibodies.

In addition we have shown that amino acid substitution T330V resulted in loss of NaPi2b recognition by both antibodies in Western-blot analysis that could be explained by destruction of the epitope for anti-NaPi2b antibody. The experimental system described here will have strong implications for the further investigation of the NaPi2b function in normal and pathological states.

Thienopyrimidinone derivatives as a new class of protein kinase CK2 inhibitors

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Signal transduction by phosphorylation of proteins is an important mechanism that is responsible for the most of physiological and pathological processes in the cell. Protein phosphorylation is realized by protein kinases – enzymes, which regulate such essential events as cell growth and proliferation. Enhanced activity of protein kinases is often associated with a number of disorders, including carcinogenesis, inflammation and viral infections. Therefore, the creation of protein kinase inhibitors is of significant value in modern drug discovery industry.

Here we represent a novel class of inhibitors of protein kinase CK2 – an enzyme playing, in particular, crucial role in the suppression of cell apoptosis that is one of the causes of tumor development. At the first stage, we performed docking of virtual compound library (20,000 compounds in total) using crystal structure of the ATP-binding site of CK2. 100 thienopyrimidinone derivatives selected by docking engine were synthesized. The synthesis was carried out in the following way. 2-Amino-3-carbethoxy-thiophenes were obtained by the Gewald reaction. Their thermal condensation in formamide gave 3-H-thienopyrimid-4-ones that were further alkylated in 3-position of pyrimidine moiety. Most of the incorporated side chains contained carbalkoxy-groups that were hydrolyzed into carboxylic acids.

At the next stage, the set of synthesized compounds was tested *in vitro* towards CK2. The testing results revealed that about one-third of the investigated compounds displayed IC_{50} values from 15 to 30 мМ. Further structural optimization of the thienopyrimidinone scaffold allowed toobtain more active derivative with IC_{50} of 2.5 мМ.

Hence, using docking technique and organic synthesis methodologies, a new class of CK2 inhibitors was identified. These inhibitors may be of interest for further structural optimization and evaluation of biological activity.

FSH receptor and *INH 1* genes association analysis with diminished ovarian reserve in women

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Objectives: Premature ovarian failure (POF) is a secondary gonadotrophic amenorrhoea affecting 1–3 % of females. FSH (follicle stimulation hormone) and its receptor (FSHR) play a major role in the development of follicles and regulation of steriogenesis in the ovary. Mutations in the FSHR might theoretically lead to an impaired signal transduction and thereby to a diminished ovarian reserve. Genes encoding the three inhibin subunits can be proposed as candidates for POF due to its role in the negative feedback control of FSH. We investigate Asn680Ser and Thr307Ala transitions in FSHR gene and Ala257Thr transition in *INH 1* gene as markers for diminished ovarian reserve in women: with clinical POF diagnosis and women with poor response (less than 4 oocytes after standard protocol of FSH stimulation) – «poor responders».

Methods: The FSHR variants Asn680Ser and Thr307Ala, and Ala257Thr transition in *INH 1* gene were analyzed by PCR and RFLP. Statistical analysis was performed by ^{2} test, Fisher's exact test, likelihood-ratio test and Expectation-Maximization algorithm.

Results: The frequency of Ala307-Ser680 (AS) allele of *FSHR* gene was significantly higher both in POF group and in «poor responders» group comparing to control group. The carriers of *INH 1* gene Ala257Thr transition predominated in the «poor responders» group. Quantity of oocytes after stimulation ovulation in women with *INH 1* gene Ala257Thr transition was significantly decreased in comparison to patients without such mutation.

Our data shows the prevalence of *FSHR* gene AS allele in both patients groups: group of POF patients (45.7 %) and «poor responders» group (52.8 %), comparing to control group (35.1 %).

Conclusions: Our data about *FSHR* and *INH 1* genotype association with ovarian reserve and response to FSH represent that the best stimulation protocols can based on the individual's genetic profile in order to reduce side-effects and costs and improve the delineation stimulation protocols.

Cytotoxicity of homocysteine and its transsulfuration pathway in human placenta

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Background: Elevated level of homocysteine (Hcy) increases the risk of developmental defects and placenta-mediated diseases, such as preeclampsia, spontaneous abortion, etc. The problem is whether placenta has some metabolic resources to withstand the Hcy loading. Our previous data have revealed the unexpectedly high cysteine/glutathione (Cys/GSH) ratio = 3:1 in human placenta contrasting with 1:6 in maternal blood and 3:2 in embryonic liver. These data together with relatively high correlation between Hcy and Cys content ($k_s = 0.5$, $p < 0.001$) served as a basis to advance a hypothesis that transsulfuration pathway via cystathionine- -synthase (CBS) is active in human placenta.

Here we aimed to analyze the influence of increased concentration of Hcy supplemented with folic acid or not on the proliferation in the villous explants and to examine the presence of CBS in human placenta.

Methods: Placental explants (10–25 mg of villous tissues) were obtained from normal term placentas (38–40 weeks) and from abortive material (6–11 weeks). Explants were cultivated in DMEM/F12 in the presence of 20, 40, 80 μM Hcy with or without 20 nM folic acid. Cultures were run for 48 h at 37 °C and 5 % CO_2, 20 % oxygen. After cultivation the explants were embedded in paraffin and immunohisto chemically (IHC) stained for Ki-67, and cytokeratin 18-neo-epitope (M30). Proliferation index was calculated as the number of Ki-67 positive nuclei in cytotrophoblast cells per 100 μm of the villous circumference. The presence of CBS was examined by Western blot and IHC analyses with corresponding antibodies.

Results: Proliferation index in the first trimester explants is higher than in the term explants (3,8 vs. 0,6). The cultivation with increasing concentrations of Hcy (20, 40 and 80 μM) is followed by gradual decrease of proliferation index (0.53, 0.48 and 0.4 in the term explants and 3.3, 2.7 and 2.0 in the first trimester explants). Higher concentrations of Hcy retard the nuclei transition from cyto- to syncytiotrophoblast and provoke apoptotic processes (higher M30 staining). The addition of folic acid at the background of 40 and 80 μM Hcy slightly increases proliferation index till 2.96 and 2.20 in the first trimester explants and till 0.50 at 40 μM Hcy in the term placenta explants. The CBS was detected in the first trimester and term placental explants.

Conclusions: The increased level of Hcy decreases the proliferation index in placenta explants, induces the tissue destruction and apoptosis. The additional folic acid slightly reverses the cytotoxic effect of Hcy. We suppose that the elevated level of Hcy induces the disbalance of methylation processes and DNA synthesis. We expect that Hcy may be trans-sulfurated in human placenta. A role of this pathway in the placental physiology merits further investigation.

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ITSN adaptor protein family: specificity of partners interaction

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Human intersectins (ITSN 1 and 2) are members of a conserved family of adaptor proteins encoded by two paralogous genes. ITSN1 is known to participate in multiple cellular processes including endocytosis, mitogenic signaling, actin cytoskeleton rearrangements and apoptosis while the function of ITSN2 is to be elucidated. In this work we intended to examine interaction of ITSN2 SH3 domains with protein partners that could be common to ITSN1 or different. For this purpose cDNAs encoding ITSN2 SH3 domains (SH3A–E) were cloned into *pGEX4T3* vector, proteins were expressed, purified in *E. coli* system and used for *in vitro* analysis. Pull-down experiments were carried out using mouse brain lysates (for dynamin 1 and SOS1 proteins) or lysates of MCF7 cells overexpressing adaptor protein CIN85/Ruk and ubiquitin ligase c-CBL.

Using GST pull-down assays we showed the interaction of ITSN2 SH3 domains with dynamin 1, molecule thought to drive the endocytosis late events by interacting with multiple endocytic proteins and phospholipids. Dynamin 1 strongly bound to SH3 A, C and with highest affinity to SH3E domain but not to SH3 B and D. No significant difference was observed comparing ITSN 1 and 2 SH3 domains interaction with dynamin 1. The ability of multiple SH3 domains in ITSN2 to bind dynamin 1 suggests that ITSN2 can cluster several dynamin molecules during endocytosis.

The interaction of ITSN1 SH3A domain with Ras exchange factor SOS1 was reported. It is also known that expression of ITSN1 is detected in both proliferating and differentiating neurons, while ITSN2 is mainly expressed in the latter. Given that we analyzed ITSN2 participation in signal transduction mechanisms through SOS1 binding. We showed that ITSN2 SH3 A, C and E domains interact with SOS1 but the affinity is less when compared to that of ITSN1 SH3A domain. The results obtained indicate the role for ITSN2 in linking endocytosis and signal transduction pathways.

Considerable differences were observed while examining ITSN2 interactions with ubiquitin ligase c-CBL and adaptor protein CIN85/Ruk. Comparing to ITSN1 only two of ITSN2 SH3 domains (SH3 C and E) were involved in binding to c-CBL *in vitro*. It is worthwhile to mention that SH3A domain is the most divergent one when ITSN 1 and 2 are compared which might impose differences in its ability of binding partners. The results of pull-down assays showed that only SH3A domain of ITSN2 binds to CIN85/Ruk with low affinity. This allowed us to suggest that interaction of ITSN2 with CIN85/Ruk does not occur *in vivo* since there is an intramolecular interaction of CIN85/Ruk own SH3A domain with its own proline-rich region.

Using *in silico* prediction we identified new ITSN interacting partner semaphorin 6A (Sema6A) implicated in retrograde signaling and cytoskeletal rearrangements during neuro- and organogenesis. The interaction of Sema6A with ITSN is mediated by SH3A domain of ITSN2 and SH3 A, C and E domains of ITSN1.

In this study we first demonstrated *in vitro* interaction of ITSN2 with endocytic GTPase dynamin 1, guanine nucleotide exchange factor for Ras SOS1, ubiquitin ligase c-CBL, adaptor protein CIN85/Ruk, new intersectins interactor Sema6A and showed differences in binding properties of ITSN 1 and 2 with these proteins.

This work was supported by INTAS Ref. Nr 05-1000004-7762 and grant of NASU for young scientists.

Interactions of human glutathione S-transferase P1 promoter with placental nuclear proteins

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Background: GSTP1 is a phase II detoxification enzyme, which participates also in intracellular signal transduction, molecular transport and NO storage. Down-regulation of *GSTP1* expression in human placenta is associated with pregnancy disorders. The goal of this work was to recognize how *GSTP1* promoter realizes its regulatory function in human placenta. We investigated the level of promoter methylation and the interactions of its binding sites with placental nuclear proteins.

Materials and methods: DNA from placenta was purified, treated with bisulphate and the level of methylation was assessed by methylation-specific PCR. The DNA-microarray database was screened for the placental expression of transcription factors, known to regulate this gene in tumors. The region of human *GSTP1* promoter was PCR-amplified and cloned. After excision from plasmid it was end-labeled with -^{32}P-ATP and used in competitive EMSA with placental nuclear extracts. Synthetic oligonucleotides corresponding to ARE, iARE, NF- B-like, NF- B, CRE and GATA-binding sites were annealed, end-labeled with -^{32}P-ATP and used in EMSA with placental nuclear extracts and competitive oligonucleotides corresponding to the consensus sequences for AP-1, Mafs, ER , RAR , NF- B, CREB and GATA transcription factors.

Results: The computer analysis revealed the specific pattern of transcription factors expression in human placenta, represented by highly expressed GATA2, GATA3, Fos-B, Nrf3 and MafK, and moderately expressed c-Fos, Juns, Mafs, ER , RAR and NF- B. Competitive EMSA with promoter fragment provided the evidence that ARE and NF- B-like sites are involved in the interactions with placental nuclear proteins. Using EMSA with synthetic oligonucleotides we revealed that ER and Maf proteins, but not AP-1 and RAR, interact with *GSTP1* ARE and inner ARE elements, NF- B factor binds to NF- B and NF- B-like elements, and GATA proteins to GATA element.

Conclusions: Binding of ER , Maf, NF- B and GATA proteins to the corresponding binding sites of promoter, but not CpG methylation is responsible for the regulation of *GSTP1* transcription in human placenta. We suppose that *GSTP1* in human placenta is a responsive gene for estrogens and intracellular redox state.

Optimization of bioselective elements operation of multibiosensor for toxins detection

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The investigation presents the development of highly sensitive and selective multibiosensor based both on a number of immobilized enzymes as bioselective elements and the matrix of ion-selective field effect transistors as transducers of biochemical signal into the electric one. To develop bioselective elements of multibiosensor, such enzymes as acetylcholinesterase, butyrylcholin esterase, urease, glucose oxidase, and three-enzyme system (invertase, mutarotase, glucose oxidase) were used. Obtained bioselective elements were shown to demonstrate high sensitivity to corresponding substrates in direct enzymatic analysis, which lasted 10 min. Dynamic range of substrate determination (0.1 mM–1.5–10 mM) was shown to depend on enzymatic system and to differ specifically in upper threshold. Current work presents the investigation on the dependence of multibiosensor response to pH, ionic strength and buffer capacity of the solution; optimal conditions for simultaneous operation of all bioselective elements of the multibiosensor were selected; the data on cross-influence of substrate of all enzymes used were obtained. The developed multi-analyzer was shown to demonstrate sufficient signal reproducibility. Therefore multibiosensor turned out to be suitable for real sample analysis.

Application of Real-Time PCR for detection of mutations

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The traditional methods for detection of mutations such as restriction analysis, SSCP, DGGE, direct sequencing are labour-intensive and time-consuming. The use of Real-Time PCR techniques can essentially shorten the time of analysis and avoid contamination of probes that is very important for molecular-genetic diagnostics. Our aim was to develop a closed-tube system for the scanning of mutations using Real-Time PCR techniques: melting analysis of amplicons and allele-specific Real-Time PCR. We studied the 3 bp deletion dF508 in exon 10 of *CFTR* gene using melting analysis. We designed specific oligonucleotide primers and optimized the PCR conditions for obtaining amplification product of 44 bp for the DNA sample without deletion and 41 bp for the sample with dF508. It was shown that SYBR Green I dye is stabilizing the DNA duplex, thereby increasing the melting temperature of the complex. SYBR Green I dye is supplied with the concentration 10000 . We determined that the optimal dye concentration was 5 . The difference in the T_m between the normal sample and the sample with dF508 was 0.9–1 °C. The samples were checked by heteroduplex analysis to confirm the presence of dF508. We have also studied deletions/insertions in genes *BRCA1/2* and *CHEK2* – the susceptibility genes for hereditary breast cancer: *BRCA1* 5382insC, *BRCA1* 185delAG, *BRCA1* 4153delA, *BRCA2* 6174delT, *CHEK2* 1100delC using the allele-specific Real-Time PCR. This method allows discriminating heterozygous carriers of mutations using two pairs of primers, one of which is homologous to normal DNA sample and the other one – to the DNA with mutation. We designed specific oligonucleotide primers and optimized the PCR conditions for amplification with SYBR Green I. We used samples with abovementioned mutations as positive controls in our study. Thus, we believe that the developed assays for detection of mutations using melting analysis and allele-specific Real-Time PCR in combination with direct sequencing can be used for molecular-genetic diagnostics and screening programmes.

Design of protein kinase CK2 inhibitors based on the p-quinone arylsulfonelimides scaffold

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During recent decade the inhibitors of protein kinases draw great attention as anticancer and antiviral drug candidates. The protein kinase CK2 (casein kinase 2, CK2) is known to show enhanced activity in wide range of tumors, inhibit cell apoptosis and is used by many viruses for their own protein phosphorylation. Therefore, the inhibitors of CK2 are considered prospective for pharmacological application.

We developed a novel class of CK2 protein kinase inhibitors – halogen derivatives of p-quinone arylsulfonelimides. Flexible docking in ATP-binding site of CK2 was used for selection of 50 compounds from virtual library of about 1000 p-quinone arylsulfonelimide derivatives. About 30 compounds from selected ones were synthesized and tested *in vitro* against CK2. Some of them showed high inhibitory activity (IC_{50} from 0.5 to 20 M).

Starting p-arylsulfonamidophenols were prepared via reaction of arylsulfochlorides with p-aminophenol derivatives in presence of triethylamine or sodium hydrocarbonate. Oxidation of p-arylsulfonamidophenols to p-quinone arylsulfonelimides was carried out using potassium bichromate in 20 % sulphuric acid or lead tetraacetate. Halogenation of quinoneimine moiety was conducted by action of corresponding hydrogen halogenides. This reaction was accompanied by reduction of quinoneimide ring into amidophenole that was oxidated again under the same reaction conditions. The resulted halogen derivatives of p-quinone arylsulfonelimides were tested *in vitro* using -labeled ATP.

The halogen derivatives of p-quinone arylsulfonelimides with two or more halogen atoms substituted in quinoneimide moiety showed higher inhibitory activity. Substituents in arylsulfonyl moiety have lesser influence on the inhibitory activity. Further optimization of this scaffold will be performed.

COTRASIF: genomics tool for systems biology

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Summary: A new tool has been developed (COTRASIF, conservation-aided transcription factor binding site finder) for the genome-wide identification of the putative transcription factor binding sites (TFBS) in eukaryotic gene promoters.

Motivation and aim: Promoter analysis and TFBS identification are essential for the understanding of gene regulatory networks. Increasing specificity of the TFBS prediction in eukaryotic gene promoters is a challenging task for bioinformatics.

Based on our previous research, we observed better specificity of the TFBS search when comparing promoters of orthologous genes of the evolutionary close species (e. g. rat and mouse).

Our aim was to develop an easy-to-use web-tool for genome-wide identification of putative TFBS with enhanced results quality.

Methods and algorithms: COTRASIF is built upon the semi-automatic importer of promoters from the Ensembl genome annotation database. Currently COTRASIF has 11 genomes available (including the human, rat, and mouse genomes).

Promoters are defined as 800 bp upstream from transcription start site, plus the 5' UTR coding sequence. For the initial TFBS search, either classical position-weight matrix (PWM) approach or the recently developed HMM-based (hidden Markov models) search method were used, For PWM method, frequency matrices are needed as input; for HMM – a list of at least 3 known sequences of the TFBS, plus an optional position frequency matrix.

Initial search results can be further analyzed using the built-in gene orthology filter. The orthology information is automatically obtained from the Ensembl Compara genome alignments database. If the putative TFBS is present in the promoters of the genes of both orthologous genes being analyzed, then it has higher probability of being functional.

Results: We developed a web-accessible tool (conservation-aided transcription factor binding site finder, COTRASIF) for the genome-wide conservation-aided TFBS search.

Further development includes: addition of new genomes; integration of the Gene Ontology category enrichment functional analysis (hypergeometric and Bayesian); more formats of results output; specialized web-API (application programming interface) for enabling easy use of COTRASIF by other tools.

Availability: COTRASIF is freely available at http://biomed.org.ua/COTRASIF/.

Interspecies polymorphism of gentiana l genus. Members: the results of RAPD-analysis

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Gentiana L. – the biggest genus of the family *Gentianaceae* Juss. (~ 400 species) which is presented by 10 species in Ukraine. The genus scope and classification are treated differently by various authors due to both insufficient characterizations of *Gentiana* species and problems with the genus boundaries determination. As many problems concerning the systematics and evolution of *Gentiana* L. genus are still unsolved the genetic and molecular analysis of gentians is of special importance.

The aim of the work was to study gentians interspecies variability by means of the RAPD-PCR technique.

To perform a genetic analysis of the intact plants *G. lutea*, *G. punctata*, *G. acaulis*, *G. asclepiadea*, *G. cruciata*, *G. pneumonanthe* and *G. verna* 27 decamer RAPD-primers were used. Following preliminary screening there were chosen 19 primers, which generated unique amplification 1–14 fragments for each object within the range of 2.7–0.3 kb. Overall, it was taken into account 460 fragments. Polymorphism of RAPD-spectra is proved by presence or absence of individual amplicons and varying intensity of fluorescence for some of the homologous fragments.

The results of the RAPD-analysis revealed the gentians interspecies polymorphism. We failed to disclose any amplicon shared by all the objects involved. The only fragment of 720 bp being amplified with A01 primer was shared by each species except for *G. verna*. *G. punctata* showed much higher number of this amplicon as compared with the other studied species.

On the whole, RAPD-analysis demonstrated some peculiarities of *G. verna* which according to the electrophoretic profiles of amplification products differs substantially from the rest of species. The nearest to *G. lutea* appeared to be *G. punctata* species, to *G. asclepiadea – G. pneumonanthe.* According to Ho and Liu classification (1990) these pairs of species are included into *Gentiana* and *Pneumonanthe* sections, respectively.

Thus, genetic investigations of *G. lutea*, *G. punctata*, *G. acaulis*, *G. asclepiadea, G. cruciata*, *G. pneumonanthe* and *G. verna*, performed by the RAPD-PCR technique, revealed the interspecies polymorphism for these gentians *G. lutea* and *G. punctata,* as well as *G. asclepiadea* and *G. pneumonanthe* are the most similar judging from their electrophoretic profiles. These results are in accordance with the literature data (Ho, Liu, 1990; Yuan, 1996) relative to the systematic and evolution of these species within the *Gentiana* L. genus.

Investigation of the expression of multisubunit translation elongation factor 1 in human carcinomas

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Translation elongation factor 1 (eEF1) regulates the specific interaction of aminoacyl-tRNA with the ribosome during the elongation phase of protein biosynthesis. It is comprised of four subunits: eEF1A (eEF1A1 and eEF1A2 isoforms), eEF1B , eEF1B and eEF1B .

Previously, eEF1 was demonstrated to be related to carcinogenesis. The eEF1A1 mRNA is overexpressed in malignant tissues of pancreas, colon, breast, lung and oesophagus. The eEF1A2 mRNA is overexpressed in ovary and breast cancer. The overexpression of the eEF1B and eEF1B mRNAs was also found in certain types of human cancer. No data exist concerning the expression of different eEF1 subunits at the protein level.

In our studies, the expression of different subunits of eEF1 in samples of cardioesophageal carcinoma (CEC), renal cell carcinoma (RCC) and non-small cell lung carcinoma (NSCLC) was studied at the level of protein. Since the only available commercial antibodies for eEF1 were anti-eEF1A1/2 antibodies, we obtained the polyclonal antibodies for eEF1B , eEF1B and eEF1B , as well as antipeptide polyclonal antibodies specific exclusively for eEF1A2. Cytosolic proteins were extracted from the tissues and examined by Western blot analysis.

Our preliminary data show 1.5 to 2-fold increase in the eEF1A expression in CEC and NSCLC. Importantly, no corresponding changes in the amount of eEF1B have been observed.

Characterization of complexes of T-284 and SH-516 cyanine dyes with alpha-synuclein fibrils

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Parkinson's disease and other related disorders are characterized by the accumulation of fibrillar aggregates of -synuclein (ASN) inside brain cells. Recently we have firstly proposed fluorescent cyanine dyes for specific detection of amyloid formations. It was shown that benzothiazole mono- (T-284) and trimethinecyanines (SH-516) bind fibrillar ASN with significant enhancement of the emission intensity [1].

This study aimed to characterize dye/ASN fibril complexes by means of fluorescence lifetime analysis and atomic force microscopy. The ability of T-284 and SH-516 dyes to selectively recognize amyloid proteins of various amino acid compositions and to monitor the kinetics of proteins fibrillogenesis process was also, studied. Some prospects towards practical application of these dyes were revealed.

Total lifetime value for the free T-284 dye in aqueous solution was about 0.25 ns, while in the presence of fibrillar ASN it increased almost in order of magnitude up to 2.3 ns. Upon interaction with aggregated ASN in fluorescence decay of T-284 the additional slow component appeared, which may be related to protein-embedded dye molecules.

Studies on the dyes selectivity were carried out on various fibrillar proteins, namely insulin, lysozyme, wild-type ASN and the known Parkinson disease-related ASN mutants A30P and A53T. It was found, that both dyes exhibit fluorescence response in the presence of fibrillar proteins species, comparable to that of the classic amyloid stain Thioflavin T. T-284 appeared to be somewhat more specific to fibrillar wild-type ASN and A53T mutant. On the other hand, for trimethine SH-516 the least protein-to-protein variability was found (with the exception for lysozyme).

Both dyes appeared to have ability to follow the step-by-step transition of monomeric wild type ASN, A30P and A53T proteins into fibrils, demonstrating good results reproducibility, much better than it was observed for dye Thioflavin T. The presence of fibrils was confirmed with AFM. It was shown that quantification of the flibrillar ASN is possible in the range from 1 to about 30 g/ml using studied cyanine dyes.

Trimethinecyanine SH-516 appeared to be able to penetrate inside neuroblastoma SH-SY5Y cells, which contain aggregated ASN, and selectively increase the fluorescence intensity.

1. Volkova K. D., Kovalska V. B., Balanda A. O., Losytskyy M. Yu., Golub A. G., Vermeij R. J., Subramaniam V., Tolmachev O. I., Yarmoluk S. M. (2008). Specific fluorescent detection of fibrillar alpha-synuclein using mono- and trimethine cyanine dyes. Bioorg. Med. Chem. 16(3), 1452–1459.