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Changes in expression of oncogenes and methylation of tumor suppressor genes in urine from patients with prostate cancer

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Background. Prostate cancer is one of the most common type of cancer diagnosed worldwide and in Ukraine particularly. Today the problem of its early detection is unsolved. The use of epigenetic markers, namely, the detection of tumor suppressor genes methylation, is a promising tool to assess the risks of the disease and its early diagnosis. Along with that, we investigate fundamental features of prostate cancer, such as expression of specific oncogenes. **The aim** of this study was to examine methylated and unmethylated status of genes *PTEN, GDF15, NKX3.1, RASSF, CDH1* and *KRT18* in the urine of patients with prostate cancer for choosing potential combination of markers and to evaluate the level of gene expression of *Aurora A, Aurora B, Aurora C, BRAF, EGF* and *YWHAZ* in the urine of patients with prostate cancer. **Methods.** Samples of urine had been taken from patients with diagnosed prostate cancer. For methylation analysis, DNA was precipitated from the samples with CTAB and then extracted by standard phenol/chloroform/isoamyl alcohol method. Bisulfite conversion of isolated DNA was provided using EZ DNA Methylation–Lightning Kit (Zymo Research). Methylation status was detected by PCR analysis using two pairs of primers for methylated and unmethylated gene. Results were visualized using agarose gel electrophoresis. For gene expression analysis, RNA was isolated from urine cells according to standard method using TRIzol reagent. cDNA was synthesized from 1 mg of isolated RNA. Expression levels were detected by RT-PCR analysis. The data were statistically analyzed. **Results.** We found the presence of hypermethylation of *PTEN* with the most high sensitivity value (87.5%) among all samples (n=16). The lowest sensitivity value of methylation (30.76%) was detected in *KRT18* gene. We identify correlation between methylation status of *NKX3.1* and *PTEN* genes with sensitivity value 73.3% and *RASSF* and *PTEN* genes with sensitivity value 80%. All three genes were methylated in 71.4 % of samples (n=14). The results of relative expression of oncogenes demonstrated that differential expression of all five genes could be identified in both urine and plasma. Among gene expression inside Aurora kinase family, the expression level of *AurB* and *AurC* genes was significantly higher than the expression of *AurA*. The cumulative expression of *AurB* and *AurC* genes was higher than the expression of *AurA* in 17 out of 22 prostate cancer samples. We observed positive correlation between expression of *AurC* and *BRAF* genes (rs=0.688, p=0.01), *BRAF* and *EGF* genes (rs=0.719, p=0.01) and *YWHAZ* and *AurC* genes (rs=0.591, p=0.01). **Conclusions.** It was concluded there is a necessity to increase the sample size for further research of potential combination of markers including *NKX3.1, PTEN* and *RASSF.* Significantly higher levels of *AurB* and *AurC* genes expression among three Aurora kinases genes indicates the malignant transformation in studied samples. Correlations between expression of the *AurC* and *BRAF* genes and between the *YWHAZ* and *AurC* genes lead to the conclusion that there is joint and/or mutual regulation of expression of these genes and that these results confirm the research on cell lines about ability of Aurora kinase C to replace Aurora B protein.

Hydrogen peroxide in biotic elicitor effect in wheat plants

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Aim. Biotic elicitors are compounds associated with a phytopathogen attack in plants and one of the most ecologically safe ways to induce non-specific tolerance against fungal diseases. The aim is to research an ability of hydrogen peroxide (HP) to be used as an indicator of the biotic elicitors influence on wheat plants under fungal infections stress. **Methods.** The content of endogenous hydrogen peroxide (HP) was measured in leaves by the sulfate-titanium method. Winter wheat plants (cv. Poliska 90, Oberig myronivskij, Svytanok myronivskij) and spring wheat (Simkoda mironivska, Struna mironivska) were inoculated with Septoria tritici Rob et Desm. leaf blotch infection, Puccinia recondita Rob.et Desm. f.tritici Eriks. et JJenn. (Puccinia triticina Eriks.) wheat rust infection at booting phase three days after 0,1 mM solution of oxalic, citric, succinic and ferulic acids as biotic elicitors and 0,5 mM solution of sodium nitroprusside as donor of NO treatment. The powdery mildew disease caused by Erysiphe graminis f.sp. tritici DS Em. Marchal and Alternaria spp. infection were detected in the field trials. The extent of disease development according to Saari-Prescott scale, morphometric parameters and yield structure were analyzed. **Results.** It is shown that treatment with biotic elicitors and NO donor reduced the disease symptoms on 1-3 points, stimulated the processes of morphogenesis and increased crop by 15-25%. The reaction of HP in wheat plants depended on effectiveness of biotic elicitor, genetic characteristics of different cultivars and type of phytopathogen nutrient acquisition strategies. **Conclusions.** The data obtained suggest that a level of endogenous HP could be used for biotic elicitors in plants as an indicator for the function of antioxidant defense system and metabolic pathways that counteract with other non-specific immunity reactions such as cell wall synthesis. **Acknowledgements.** Thanks for help to Lysova G.M., PhD in biology, head of laboratory of agricultural plants immunity to diseases, Institute of Plant Protection of NAAS of Ukraine.

Effect of oligoribonucleotides-D-mannitol complexes on the expression of pro-inflammatory and profibrotic genes at the thioacetamide-induced liver fibrosis

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Background. Hepatic fibrosis is a common consequence of chronic liver injury caused by a variety of etiological factors. It is associated with inflammation, oxidative stress, necrosis and ends with cirrhosis, liver cancer, or liver failure. Oligoribonucleotides-D-mannitol complexes (ORNs-D-mannitol) display a vast spectrum of biological effects, including cellular metabolism stimulation with activation of endogenous synthesis of regulatory proteins, stimulation of reparation processes. **Aim.** The aim of this study was to investigate the protective effect of the ORNs-D-mannitol on liver fibrosis. **Methods.** Mice received thioacetamide (TAA) (200 mg/kg, intraperitoneal) thrice weekly, for 8 successive weeks to induce liver fibrosis. The ORNs-D-mannitol (200 mg/kg, per os) was administered orally during TAA intoxication. Body weights and mortality mice were assessed during the experiment. At the end of the experiment, oxidative stress, inflammatory and profibrogenic markers were evaluated. Additionally, liver histopathology was determined. **Results.** The results of the research showed that treatment with the ORNs-D-mannitol attenuated TAA-induced liver fibrosis in mice. Histological analysis showed that the ORNs-D-mannitol application noticeably mitigated fibrosis and improved the function of the liver. Furthermore, the ORNs-D-mannitol prevented TAA oxidative stress. The ORNs-D-mannitol decreased TBA-reactive products, carbonyl derivatives levels and myeloperoxidase activity by 60.6, 35 and 52% respectively in comparison to control thioacetamide in the liver cells. In addition, these complexes increased protein and non-protein thiol groups levels, and glutathione-S-transferase and glutathione peroxidase activities compared to the TAA-treated mice. During TAA-induced liver fibrosis it was found that the ORNs-D-mannitol reduced the the mRNA expression level of pro-inflammatory (*Il-6, Tnf-α*) genes by 70 and 76 % respectively, compared to the mice with TAA. Furthermore, the ORNs-D-mannitol suppress the HSCs/myofibroblasts activation by reduced expression of markers α-SMA, Col-1, and *Tgf-β1* in the liver. **Conclusions.** The ORNs-D-mannitol could ameliorate the effects of TAA-induced liver fibrosis in mice by inhibiting oxidative stress, expressions of pro-inflammatory cytokines and profibrotic markers.

Synthesis of new 1-[2-(alkylamino)-pyridine-4-il] imidazo[1,5-*a*] quinoline-3-carboxylic acid derivatives as inhibitors of protein kinase ck2

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Background. Protein kinase CK2 belongs to important regulators of apoptosis and is involved in the stress signaling pathways of the cell. Due to the ability of CK2 to phosphorylate a number of substrates implicated in the oncogenesis process and increased activity of this enzyme in highly proliferative cells allow us to consider protein kinase CK2 as an essential factor in tumor formation. Therefore, CK2 is a perspective molecular target and highly active and selective inhibitors of this enzyme can be the basis for the development of anticancer drugs. Earlier, using rational drug design approach we have found inhibitors of CK2 kinase among derivatives of imidazo[1,5-*a*]pyridine-1-carboxylic acid which are substituted at the third position. **Results.** We have synthesized a number of new derivatives of imidazo[1,5-*a*] quinoline carboxylic acid from the corresponding amides of 2-aminoquinoline. The next step was to replace the chlorine atom with fragments of aliphatic amines. The reaction of trifluoroacetylation of the resulting substances followed by the halophormic cleavage of the trifluoroacyl group gave the corresponding 1-[2-(alkylamino)-pyridin-4-yl]-imidazo[1,5-*a*]quinoline-3-carboxylic acids. **Conclusions.** Using the above described method we have synthesized and characterized 10 new derivatives of imidazo[1,5-*a*]quinoline carboxylic acid which can be taken for further biochemical testing of their inhibitory activity toward protein kinase CK2.

Gene *GART* is an object for elucidation of the mechanisms and functional role of alternative polyadenylation

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Background. Cleavage and polyadenylation are essential parts of mRNA processing in eukaryotes. The genes containing multiple polyadenylation signals produce multiple transcript variants with different length. About 70% of human genes generate isoforms due to alternative polyadenylation. Most of alternative polyadenylation signals are situated in 3' most exon and do not impact on a coding sequence of transcripts. Our work was focused on the gene *GART* which encodes the protein with three enzymatic activities in a row of ten enzymatic reactions of *de novo* purine synthesis. Utilization of alternative polyadenylation signal in 11th intron leads to production of a shorter isoform with single enzymatic activity. The biological role of short isoform or specific regulatory mechanisms of its expression remain unclear. **Aim.** The aim of the study was to determine the prevalence of short isoform in tissues with different proliferative activity and unravel its biological function if any. **Methods.** mRNA abundance of the GART transcripts was determined by RT-qPCR, the level of the GART isoforms - by western blot analysis. Cell line with knockout *GART* was obtained from HeLa cells using CRISPR. Rate of purine synthesis was assayed by cultivating HeLa cells with [$^{13}C_2$]-Glycine and detecting the products – adenine, guanine and hypoxanthine with HPLC-MS. Bioinformatic analysis of the GART isoforms ratio in different human tissues was performed using the data from the quantitative atlas of polyadenylation (Derti *et al.* 2012; Gruber *et al.* 2016) and applying AfterQC 0.9.3, FastQC 0.11.5 and Bowtie2 2.3.2 tools. **Results.** mRNA abundance of the short monofunctional *GART* isoform was 4 times higher than a long variant in placenta from the first trimester and almost the same in the third one. Corresponding proteins were present in placental samples, their quantities being higher in the beginning of pregnancy. The HeLa cell line with knockout *GART* was created; it does not produce both isoforms of GART and prevent the whole *de novo* purine synthesis. Using data from NGS of 3' ends of polyadenylated transcripts the prevalence of the short and long GART transcripts was determined in five human tissues.The short:long isoform ratio positively correlated with the rate of proliferation estimated by expression of the cell cycle specific genes such as PCNA. **Conclusions.** The positive correlation between the short:long ratio of *GART* isoforms and the rate of cell proliferation was demonstrated in several human tissues. The created HeLa cell line with knockout *GART* is on the way for elucidation of the functional activity of a short isoform.

The effect of chondroitin sulfate on level of glutathione system in the serum of rats during monoiodoacetate-induced osteoarthritis

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Background. The glutathione system plays a major role in an organism defense against reactive oxygen species. Glutathione exists in the organism in reduced (GSH) and oxidized (GSHS) states. The enzymes of the glutathione system are glutathione peroxidase (GPO), glutathione transferase (GT), glutathione reductases (GR) *etc.* A high reactivity of the glutathione system ensures its participation in metabolic reactions aimed at supporting cellular homeostasis and protection against oxidative stress, including osteoarthritis (OA). Chondroitin sulfate (Cs) is well recommended symptomatic slow-acting drugs for OA. **Aim.** In this study, we investigated the effect of CS on the glutathione system in an animal model of osteoarthritis. **Methods.** We used a single injection of MIA through the infrapatellar ligament of Wistar male rats to start OA model; the control group got saline instead. The therapeutic groups got an intramuscular injection of CS daily from 2nd to 29th days. Sampling was provided on the 30th day. Total GPO activity was determined by the accumulation of GSHS. GT activity was measured by the accumulation of optically active conjugates. GR activity was evaluated by decreasing of the NADPH+ protein content. The amounts of GSH and GSHS were determined by the spectrofluorometry method using benzendicarboxaldehyde under different pH values of media. **Results.** MIA-induced OA caused decreased levels of glutathione system, comparing to the control group. Introduction of CS induced a significant alteration; all levels have approached to the control level but did not reach it. **Conclusions.** Further studies will be directed to the search for synergistic substances that enhance the effect and efficiency of CS.

IP_3Rs and LCC-channels coexpression in cardiomyocytes' nuclear membrane

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Background. Nucleus functioning as Ca^{2+}-store is done by ion channels in the inner and outer nuclear membranes. Large Conductance Cation Channels (LCC-channels) were described in the nuclear membrane of central neurons [Marchenko *et al.*, 2005], cardiomyocytes [unpublished results] and other cells types. LCC-channels are permeable to K^+, Na^+, but impermeable to Ca^{2+} and Cl^-. We first assumed the role of LCC-channels in Ca^{2+}-signalling [Marchenko *et al.*, 2006]. The essence of the hypothesis is that when Ca^{2+} ions are released from the store, an oppositely directed flow of positively charged ions is needed to neutralize an excess charge. Otherwise, the release of Ca^{2+} from the store will stop as the calcium equilibrium potential is reached. Such LCC-channels function makes a membrane easily permeable for K^+ ions. **Aim.** Our aim was to examine the expression of Ca^{2+}-release channels (IP_3Rs, RyRs) in the nuclear membrane of cardiomyocytes. **Methods.** The experiments were conducted on cardiomyocytes nuclei of 3-week old Wistar or Fisher rats. Nucleuses were isolated as described earlier [Kotyk *et al.*, 2018]. We registered single-channel ion currents using Patch clamp technique (voltage-clamp mode in excised patch or nucleus-attached configuration). **Results** were analyzed using Clampfit and Origin. **Results.** We have found that the inner nuclear membrane of rat cardiomyocytes contains IP_3Rs (with conductance 384 pS) which are co-expressed with LCC-channels. But we detected no ryanodine receptors - the most abundant intracellular Ca^{2+} channels of cardiomyocytes - in the nuclear membrane. Thus, the cardiomyocytes nuclear membranes which contain a large amount of LCC-channels, also have high expression of IP_3Rs. **Conclusions.** Our data indicate that the nuclear envelope of cardiomyocytes is a Ca^{2+} store, which is sensitive to inositol 1,4,5-trisphosphate, but in contrast to sarcoplasmic reticulum of these cells, it does not contain ryanodine receptors. Therefore, the regulation of Ca^{2+} release in the nucleus differs from that in the cytoplasm. Numerous LCC channels may be involved in Ca^{2+} signaling and may provide a route to counterflow cations and thereby facilitate Ca^{2+} release from the nuclear envelope. Our future goals include identification of LCC channel amino acid sequence and protein structure. Considering involvement of LCC channels in Ca^{2+} signalling, we expect that they are important for overall survival of the organism, therefore further mouse knock-out studies are necessary to confirm their importance. LCC-channels may also be involved in diseases associated with muscle degeneration and certain neurodegenerations. Further studies on LCC channels may provide a novel understanding of Ca^{2+} signalling in cells, as well as they may become novel targets for treatment of the above mentioned diseases.

The target sites of splicing factors affiliated with alternative 3'-UTR of *GART* gene

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Background. *De novo* purine biosynthesis consists of ten reactions, catalyzed by six enzymes among which the gene *GART* is responsible for the 2^{d}, 3^{d} and 5th reactions. The *GART* mRNA and corresponding protein are represented by two isoforms – the long trifunctional and the short monofunctional. The latter is responsible for the 2^{d} reaction and is produced by alternative polyadenylation (APA) within 11th intron. The regulatory factors of this APA and the functional activity of short isoform if any are largely unknown. Among different potential candidates for regulatory role like are RNA binding proteins (RBP), micro RNA, long non-coding RNA *etc.* We have focused on RBPs, which are ubiquitously involved in APA of 3'-most exon. **Aim.** The aim of the study was to search for potential target sites of RBPs within 3'-UTR of the short *GART* transcripts and compare them with those within canonical 3' UTR of the trifunctional *GART* mRNA. **Methods.** Analysis of 3' UTR of a short and full-size *GART* mRNA for the presence of potential binding sites of RBPs was conducted by the service of RNA-Binding Protein DataBase (RBPDB). RBPDB is a collection of *in vitro* and *in vivo* experimental observations of RNA-binding sites for RBPs. **Results.** Several putative binding sites for RBP were found in alternative 3'-UTR like 5'-AAAGAAH-3' (570 bp from 5'ss) for SRSF10; two 5'-GGACH-3' (309 bp, 596 bp) for YTHDC1, a m^{6}A-dependent regulator of alternative splicing and five 5'-YHWHH-3' sites (304 bp, 311 bp, 376 bp, 563 bp and 593 bp from 5'ss). YTHDC1 promotes exon inclusion in targeted mRNAs through recruiting splicing factor SRSF3 while blocking the SRSF10 mRNA binding. SRSF10 mRNA exhibits a large degree of diversity. It may prevent the binding of YTHDC1/SRSF3 complex and support exon skipping and repress splicing being dephosphorylated or activate the splicing being phosphorylated. By contrast, 3'-UTR of the full *GART* contains only one binding site for SRSF1 factor of normal splicing. **Conclusions.** Identification of putative binding sites characteristic for alternative splicing in the 11th intron of *GART* points to the high probable interaction of alternative polyadenylation and alternative splicing in the *GART* gene and creates the basis for verification of this finding.

Identification of the interaction between scaffold protein ITSN1 and RNA-binding protein SAM68

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Background. Different cellular processes including proliferation, growth, migration are regulated by scaffold proteins that modulate formation and functioning of the protein complexes. ITSN1 is a scaffold that regulates endocytosis, cytoskeleton remodeling, and signaling processes. ITSN1 is implicated in cell growth and survival regulating the major mitogenic and apoptotic signaling pathways. Nuclear localization of ITSN1 suggests a possible role of ITSN1 in the gene expression and mRNA maturation. Several high-throughput screening identified RNA-binding proteins as potential partners of ITSN1 whereas the relevance of the interactions in a cell remains unknown. **Aim.** The aim of the current work was to analyze the interactions between ITSN1 and several RNA-binding proteins. **Methods.** GST pull-down. ITSN1 SH3 domains fused to GST-tag were used to precipitate proteins WBP11, SAM68 and LARP6 fused to GFP from HEK293 lysates. Western blot analysis was used to detect the proteins. *Microtubule bench assay*. A protein of interest (bait) fused to microtubule-associated domains of Tau is brought onto microtubules in living cells whereas the presence of a protein partner (prey) on microtubules reveals an interaction between bait and prey. For the purpose, HeLa cells were co-transfected with constructions encoding proteins of interest fused to Tau-RFP and GFP. A co-localization analysis was performed using fluorescent microscopy. *Proximity ligation assay*. Fixed HeLa cells were incubated with primary antibodies specific to ITSN1 and SAM68, secondary antibodies conjugated with oligonucleotides and fluorescent probes. Detection of fluorescent signals was performed using fluorescent microscopy. **Results.** The interactions between ITSN1 SH3 domains and RNA-binding proteins containing proline-rich regions were analyzed using GST pull-down. It was shown that ITSN1 SH3 domains bind WBP11, SAM68 and LARP6 *in vitro*. However, microtubule bench assay confirmed the interaction between ITSN1 and SAM68 only, as recombinant protein $ITSN1_{SH3}$-RFP-Tau co-localized with SAM68-GFP on microtubules. The reciprocal analysis of cells expressing recombinant proteins SAM68-RFP-Tau and GFP-ITSN1 or its truncated forms showed the co-localization between SAM68 and full-length ITSN1 or forms containing SH3 region. The data reveal the interaction between two proteins in a cell whereas SH3 domains mediate the binding. Proximity ligation assay confirmed that the proteins ITSN1 and Sam68 are localized in close proximity in HeLa cell nuclei, demonstrating that ITSN1 and SAM68 interact in a nucleus. **Conclusions.** Using *in vitro* and in vivo techniques, we have shown that the scaffold protein ITSN1 interacts with the RNA-binding protein SAM68. The interaction is mediated by ITSN1 SH3 domains occurring predominantly in a cell nucleus. The data suggest a potential role of ITSN1 in the processes of SAM68-mediated RNA maturation serving as a link between nucleus-specific processes and signaling processes in a cytoplasm.

Phosphorylation state of mitogen-regulated sites in the ribosomal protein S6 kinase 1 isoform, p60-S6K1, at different cell growth conditions

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Aim. To estimate phosphorylation status and response to growth factor stimulation of the p60-S6K1 isoform expressed in p85/p70-S6K1 knockout HEK-293 cells. **Methods.** CRISPR/Cas9 gene editing, immunoprecipitation, immunofluorescence, Western blotting. **Results.** The CRISPR/Cas9 technology was applied to shut off expression of the major p85- and p70-S6K1 isoforms to create HEK-293 cells expressing exclusively p60-S6K1. The specificity of S6K1 gene editing was confirmed by Western blot, immunofluorescent and immunoprecipitation analysis. To assess phosphorylation status and growth factor response of the mitogen-regulated sites in p60-S6K1 (Ser^{371}, Thr^{389}, Thr^{421}, and Ser^{424}) the generated HEK-293 cells were subjected to starvation in serum-depleted conditions followed by 1 h serum stimulation. Since p60-S6K1 has a truncated N-terminus lacking a binding site for mTOR Complex 1, activity of which is critical for Thr^{389} phosphorylation, we could not observe any traces of Thr^{389} phosphorylation in p60-S6K1. As the data indicate, p60-S6K1 exhibits a response to serum stimulation similar to that of p85/p70-S6K1 when phosphorylated at Ser^{371} showing a sharp increase in a phosphorylation level following 1 h serum stimulation. Despite similarity of kinetics of Ser^{371} phosphorylation, Ser^{371} in p85/p70-S6K1 becomes phosphorylated to a lesser extent in response to mitogenic stimulation compared to Ser^{371} in p60-S6K1. However, the kinetics of p60-S6K1 phosphorylation at Thr^{421}/Ser^{424} seems to be different from that of phospho-p60-Ser^{371}, as 1 hour of serum stimulation is not sufficient for any increase in phosphorylation of Thr^{421}/Ser^{424} compared to a serum-deprived level. In addition, the level of p60-S6K1 phosphorylation at Ser^{371}, Thr^{421} and Ser^{424} was lower compared to the major p85/p70-S6K1 isoforms, although expression of the p60-S6K1 protein in HEK-293 generated in the current study can be comparable to that of p70-S6K1 expressed in wild-type HEK-293. **Conclusions.** Phosphorylation pattern of p60-S6K1 and kinetics of growth factor-mediated activation by phosphorylation differ from that of the p85- and p70-S6K1 isoforms. As a consequence, p60-S6K1 could have different functional properties and play a part in regulation of distinct cellular processes compared to the major p85- and p70-S6K1 isoforms.

Status of the liver mitochondria at damage of miocardium

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Aim. Mitochondria, which are the centers of energy in the cell, often suffer from the effects of drugs. The effect of ischemic heart disease and pathology of the liver on the state of liver mitochondria is not sufficiently studied. However, in the ischemic processes, these organelles play an important role. The changes in activity of the liver mitochondrial enzymes at the pituitrin-isadrin induced myocardial ischemia and correction of this pathological condition with the drugs "α-Ketoglutarate" and "Inspra" are of special interest. **Methods.** The white rats of 4 months age weighing 150-190 g. were used. Experimental damage to the myocardium was performed according to the model of Belenichev and others [2012] using combination of pituitrin (AB "Endocrine", Lithuania) and isadrin (Sigma-Aldrich, USA). As corrective drugs α-Ketoglutarate (SQP, Sweden), "Inspra" (Pfizer PJM, France) were used. The manipulation with animals was carried out in accordance with the rules of the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1986). The mitochondrial fraction of the liver was obtained by differential centrifugation in the sucrose gradient [Wieckowski *et al.*, 2009]. The statistical analysis of the obtained results was carried out by ANOVA. **Results.** In case of pituitrin-isadrin damage the destructive effects of oxidative processes are observed, accompanied by a decrease in the total protein in the mitochondrial fraction of the rat liver. At the same time, the activity of aspartateaminotransferase and alanineaminotransferase in the mitochondrial fraction increase. An increase in the number of TBA-active products in the mitochondrial fraction of the liver is shown. Thus, the increased permeability of the external mitochondrial membrane is observed which is accompanied by the oxidation of the cytochrome C complex with the cardiolipin, and as a consequence a decrease in the number of cytochrome C. At the same time, there is an increase in the activity of antioxidant protection enzymes such us superoxide dismutase and catalase. **Conclusions.** The use of α-Ketoglutarate and the drug "Inspra" contributed to a decrease in the number of nitrogen-containing metabolites that were formed in the damaged myocardium, as evidenced by a decrease in the activity of aminotransferases. In addition, the use of α-Ketoglutarate effectively restores the integrity of mitochondrial membranes, which is confirmed by the reduction of cytochrome C and the reduction of TBA-active products. But the use of the drug "Inspa" more effectively influenced the antioxidant system, namely decreased activity of enzymes such as catalase and superoxide dismutase.

Tmprss2-erg dependent gene expression changes and possible fusion mechanism in prostate tumors

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Aim. To analyze the relative expression (RE) of several androgen receptor-associated genes in prostate tumors, related to the presence or absence of the *TMPRSS2-ERG* fusion; and to examine a putative correlation between the gene expression and clinical characteristics, to define a possible molecular mechanism of the *TMPRSS2-ERG* fusion in prostate cancer. **Methods.** The relative expression levels of 13 genes (18 transcripts) were analyzed by a quantitative PCR in 37 prostate cancer tissues with different Gleason score, 37 paired conventionally normal prostate tissue (CNT) samples and 21 samples of prostate adenomas with the presence/absence of the *TMPRSS2-ERG* fusion detected earlier. **Results.** We have found that 3 genes, namely *ESR1*, *PCA3* and *INSR B* are differentially expressed in adenocarcinomas with the absence and presence of the *TMPRSS2-ERG* fusion, compared with the paired CNT. An analysis of the gene expression pattern in adenomas, CNT and adenocarcinomas showed that 5 genes, namely *AR* (2 isof), *ESR1, PRLR, SRD5A2, PCA3* were differently expressed, depending on the presence or the absence of the *TMPRSS2-ERG* fusion. We have characterized by bioinformatics methods a possible mechanism of *TMPRSS2-ERG* fusion formation in prostate tumors. That could be not only a genetic cause, but also the transcription-mediated gene fusion. **Conclusions.** We have found the specific differences in the expression of the steroid and peptide hormone receptors, metabolic enzymes depending on the presence/absence of the *TMPRSS2-ERG* fusion in prostate adenocarcinomas, CNT and adenomas. We have identified by bioinformatics methods the transcription-mediated gene fusion mechanism that we intend to confirm by molecular methods.

Detection of methylation of tumor supressor genes and identification of expression profile of non-coding rnas in samples of urine from patients with bladder cancer

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Aim. Bladder cancer is one of the most common cancers in the world, and the problem of its early diagnosis is still unresolved. The use of epigenetic markers, especially the detection of methylation of tumor suppressor genes and up-regulated non-coding RNAs, is a promising tool to assess the risk of the disease and its early detection. The aim of this study was to identify and evaluate the level of gene expression of two lncRNA*s* genes *PANDAR* and *BCYRN1* and to identify whether methylated and unmethylated forms of *GDF15*, *MYO3A*, *RASSF1A* and *NKX3-1* genes-oncosupressors appears in urine samples of patients with bladder cancer. **Methods.** We collected urine from patients with bladder cancer (n=36) and used urine samples from healthy people (n=4) as a control. DNA was precipitated by CTAB and isolated by using a standard phenol/chloroform/isopropanol approach. The presence of methylated and unmethylated forms of the *GDF15*, *MYO3A*, *RASSF1A* and *NKX3-1* genes promoters was detected by PCR and agarose gel electrophoresis after the procedure of DNA bisulfate conversion. Also 28 urine samples from patients with bladder cancer were gathered before surgical invasion. We isolated RNA using a standard approach with phenol and guanidine thiocyanate from urine samples. cDNA was synthesized and taken to qPCR reactions. The data were statistically analyzed. **Results.** We found the presence of methylated and unmethylated forms of all four *GDF15*, *MYO3A*, *RASSF1A* and *NKX3-1* genes among the studied patients. The analysis of the data showed that the highest value of the presence of only the methylated form among the four genes was found in the promoter region of *NKX3-1* gene in 83.3% cases, *RASSF1A* gene in 66,6%, *MYO3A* gene in 47.2% cases. However, the *GDF15* gene had no methylated forms in any of the analyzed sample of urine but had both forms in 66,6% cases. It was shown that genes had never had only unmediated forms or had but in a lowest frequencies. The results also demonstrated that the differential expression of the *PANDAR* and *BCYRN1* genes, which code lncRNAs as PANDAR and BC200, respectively, can be identified in urine samples from people with diagnosed bladder cancer. **Conclusions.** It was concluded that the panel of *RASSF1A* and *NKX3-1* genes is the most statistically significant in assessing the frequency of methylated and unmethylated forms for primary diagnosis of bladder cancer. Also we have found that there is the differential expression of PANDAR and BC200 in urine samples from patients with bladder cancer.

Influence of the magnetic field of biogenic magnetic nanoparticles (bmns) and diffusion on vesicular transport

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Background. Intracellular transport of vesicles is mainly powered by special rotor proteins (kinesins and dyneins) which move along the microtubules of tubulin. It is considered that globular "head" of protein moves in random way under the impact of diffusion where energy of ATP spends on creating bound with tubulin. On the other hand, we showed that scattering magnetic field of the chain of BMN could affect the trajectory of vesicle movement. **Aim.** The purpose of this work is to simulate the behavior of ordinary and magnetically targeted vesicles in a scattering magnetic field created by chain of BMNs while linear (kinesin or dynein) transport is processed. **Methods.** Main idea of the mathematical model is to measure the time of vesicle linear movement from a center of cell to a membrane with the average kinesin or dynein transport considering the each step diffusion shift (Gaussian distribution with zero center) and impact of magnetic field. Mathematical model was developed using Python programming language. For reducing time wasting Numba library was used. **Results.** According to the results, magnetically targeted vesicles which magnetic sensitiveness higher than 10^{-4} could be effectively catched by chain of BMNs. **Conclusions.** The main conclusion is that it is necessary to consider the influence of the magnetic field of BMNs chain within using magnetically targeted bio-objects in drug delivery.

Methyltransferase activity under the influence of oligoribonucleotides and their derivatives

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Aim. DNA methylation is a prerequisite for the realization of the functions of cellular "immunity" by regulating the expression of genes during the development of pathological states. RNA-based oligoadenylates drugs that can bind and affect the work of epigenetic regulators and transcriptional proteins through interaction with regulatory domains can be used as safe analogs. At this stage of our investigation, we studied the ability of oligoribonucleotides and their derivative to affect the methyltransferase activity. **Methods.** The studies on methyltransferase EcoRI *in vitro* were performed with the native DNA phage λ c1857s7 by the methyl sensitivity restriction method. Docking simulations were run at AutoDock Vina. The study of thermodynamic parameters of the interaction of methyltransferase with oligoribonucleotides was carried out on a NanoITC isothermal calorimeter. **Results.** As the 2'-5' oligoribonucleotides are known to most effectively associate with enzyme domains and can circulate in the body for a long time, we have been investigating precisely this group of oligonucleotides. As a control, we used yeast RNA, a yeast RNA complex with D-mannitol in a ratio of 1:2.5 (Nuclex) and 3'-5'A_3. In the course of the research, it was found that total RNA does not possess any modifying effect on the methylation process with the participation of the EcoRI methyltransferase. At the same time, the complex of yeast RNA with D-mannitol and 3'-5'A_3 showed an inhibition effect. An identical result was observed when used 2'-5'A_3epoxy, 2'-5'A_3-NH_2 and 2'-5'A_3cord. The analysis of molecular docking were carried out on 3'-5' and 2'-5' forms, on the ribonucleic and deoxy variants oligonucleotides with length from 1 to 10 monomers. In addition to natural oligonucleotides, cordycypin, epoxy, and amino derivatives were also studied. The highest total free energy of complexation was observed for 5 monomer drugs. There was also a decrease in the entropy component and an increase in the enthalpy component of the reaction required to enhance the specificity of the protein-ligand interaction. In the case of using 2'-5'A_3amino we assume the presence of two binding sites. **Conclusions.** We suppose that the effect of inhibition of the enzyme EcoRI by core oligoadenylate in broad range concentrations can be explained by the synergistic effect of the enzyme conformation and stacking interaction with a change in the secondary structure of the DNA that connected with the methylation. The highest total free energy of complexation was observed for 5 monomer drugs. Our results are important for revealing the molecular mechanism of the RNA-based compounds epigenetic action.

The effect of chondroitin sulfate on levels of IL-1β, TGF-α and *Ptgs2* in the serum of rats during MIA-induced osteoarthritis

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Background. Prostaglandin-endoperoxide synthase 2 (Ptgs2) is involved in the conversion of arachidonic acid to prostaglandin H2 and collaborate with inflammation. Cytokines like interleukin (IL)-1β and tumor necrosis factor (TGF)-α take apart in Nf-Kb pathway and directly associate with inflammatory diseases. In this study, we applied an animal model of monoiodoacetate (MIA)-induced osteoarthritis (OA) to cause inflammation and check the effectiveness of chondroitin sulfate (CS) action on the level of inflammation markers. **Methods.** We used a single injection of MIA through the infrapatellar ligament of Wistar male rats to start OA model while control group got saline solution instead. The therapeutic groups got an intramuscular injection of CS daily from 2nd to 29th days. Sampling was provided on the 30th day. The concentration of IL-1β and TGF-α were measured in serum by enzyme-linked immunosorbent assay (ELISA), following the standard protocol (Biotrak ELISA System, Healthcare, USA). The expression level of gene *Ptgs2* was assessed with real-time PCR, using commercial set Thermo Scientific Verso SYBR Green 1-Step qRT-PCR ROX Mix (Thermo Scientific, Lithuania). **Results.** MIA-induced OA provoked an increase of IL-1β by 1.7 times and TGF-β by 1.3 times, comparing to control. Introduction of CS caused a decline of both parameters by 1.3 and 1.4 times respectively, comparing to OA group. Further research showed an essential increase of the *Ptgs*2 expression by 8 times comparing to the control group. The levels of IL-1β, TGF-β and *Ptgs*2 expression in therapeutic and control groups have not varied significantly. **Conclusion.** Thus, the CS therapy has positive trends to limit the activity of inflammation markers in animals with MIA-induced OA. The further studies on humans are of great interest.

Predicting model of regulatory relationships between long non-coding RNAs ANRIL/XIST, microRNAs and DNA methylation associated with human cancers

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Background. Long noncoding RNAs (lncRNAs) are defined as a class of important heterogeneous ncRNAs with the length more than 200 nucleotides. The recent study suggested that lncRNAs ANRIL and XIST participate in development of several unisex tumors via binding disease-specific microRNAs. However, a resource linking DNA methylation, an essential epigenetic regulator and disease biomarker with lncRNAs is still lacking. DNA methylation is a fundamental feature of epigenomes that can affect expression of protein coding or non-coding transcripts. **Aim.** The aim of research is to predict miR-lncRNA (ANRIL and XIST) interactions in several selected cancers, also to predict relationships between long non-coding RNAs and DNA methylation *in silico* for creation a model of epigenetic regulation lncRNAs of interest. **Methods.** We used LncRNADisease, Lnc2Cancer, Diana Tools to analyze present expression data of lncRNAs ANRIL and XIST for selected diseases *in silico*. We used HMDD v2.0 and Mir2Disease databases to find possible involvement of target microRNAs in various diseases. And we used online tools Lnc2Meth for identification of differential methylation patterns for lncRNAs ANRIL and XIST. **Results.** Using Lnc2Meth we identified differential methylation patterns for ANRIL and XIST in some types of oncology. Using computational analysis of disease-associated microRNAs (Diana Tools), we predicted potential microRNAs for sponge activity of ANRIL (miR-15,-125,-7 family) and XIST (miR-30,-15 family). Then, we used HMDD v2.0 and Mir2Disease databases to find a possible involvement of these microRNAs in various cancers. We created our prediction model of potential regulatory network of long noncoding RNAs ANRIL and XIST, that gives an opportunity to see a more systematic picture of epigenetic regulation. **Conclusions.** We create the prediction model of potential regulatory network of long noncoding RNAs ANRIL and XIST and in the future we will continue updating and integrating the data content into this model. We will experimentally validate the predicted microRNA sites in ANRIL and XIST 3'UTR, and also further experimentally validate the predicted differentially methylated regions.

Development an electrochemical biosensor system for the determination of lactate and pyruvate concentration in human serum

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Aim. Pyruvate and lactate are key molecules in many metabolic pathways, and measurements of their concentrations are very useful in clinics for the assessment of the patient's state and prognosis of the probability of shock and death. Thus, the aim of the present work is the development of a biosensor system for determination of lactate and pyruvate in human blood serum. **Methods.** The biosensor system contains two electrochemical biosensors. The biosensors consist of enzymes (lactate oxidase and pyruvate oxidase) immobilized onto the surface of amperometric disc electrodes using covalent cross-linking with glutaraldehyde in the presence of bovine serum albumin. The three-electrode scheme of amperometric analysis was used. **Results.** The created biosensor system exhibited high sensitivity to lactate and pyruvate. The linear range of lactate determination was from 5 μM to 350 μM, and linear range of pyruvate was from 10 μM to 5000 μM. An influence of the solution parameters (ionic strength, pH, buffer capacity) on the biosensors operation was investigated. We also studied the cross-influence of substrates and cofactors on biosensors. The concentration of lactate and pyruvate in blood samples of human serum was analyzed. The reproducibility of analysis of blood serum samples and an impact of sample dilution was evaluated. Also the results were compared with those obtained by the spectrophotometric method. **Conclusions.** The biosensor system is highly sensitive and selective; it can be utilized for rapid and low-cost determination of the lactate and pyruvate concentrations in aqueous samples.

Manipulation of mitochondrial energy production by light

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Aim. Mitochondria are double membrane organelle, which are the main energy plants in a eukaryotic cell and are essential for us to be alive. Adenosine triphosphate (ATP), which is the "molecular unit of currency" of intracellular energy transfer, is synthesized in the mitochondria using the H^+ gradient across the mitochondrial inner membrane. The main idea of the research is to manipulate energy production processes by light. Our hypothesis is that energy production should be reduced by light when the light-sensitive H^+ channels are artificially targeted to the inner membrane of mitochondria, whereas it should be increased when the light-sensitive H^+ pumps are targeted. For this goal we engineered Chrimson, an algal rhodopsin that is selectively permeable to H^+[1],to be targeted to the inner membrane of mitochondria. **Methods.** 1. Design of a plasmid. The constructed plasmid encoding Chrimson was fused with modified enhanced yellow fluorescent protein (eYFP) at the C-terminal end as a reporter, and the MitoX protein at the N-terminal end as a targeting signal for the inner membrane of mitochondria (mt-Chrimson -eYFP). 2. Expression in cell culture. HEK293cells were transfected with the plasmid using Effectene transfection reagent. Twenty four hours post-transfection, the cells were replated onto the collagen-coated glass coverslips and served for the immunohistochemistry. 3. Immunohistochemistry. Outer membrane of mitochondria was identified with the anti-TOM22 antibody labeled with Alexa 548 (red fluorescence). The distribution of Chrimson-eYFP was identified by the anti-GFP antibody with Alexa 488 (green fluorescence). **Results.** After 48 h after transfection the eYFP fluorescence was distributed in the small intracellular structures reminiscent of mitochondria. However, some eYFP-expressing cells looked apoptotic. Immunohistochemical analysis demonstrated that the distribution of eYFP was coincident with that of mitochondria. **Discussion.** It is suggested that the mt-Chrimson –eYFP was targeted to mitochondria as expected. However, the precise localization in mitochondrion needs to be specified in future. It is necessary to investigate the functional significance of mt-Chrimson -eYFP on the pH gradient across the inner membrane as well as on the ATP generation. For instance, targeting H+ pump to the inner membrane leads to production of the cellular energy (ATP) from light without consumption of O_2, that can be used for the growth facilitation in plants and animals. Moreover, the reduced production of CO_2 will prevent the global warming. On the other hand, the same technique using H^+ channelrhodopsin and light should facilitate the energy consumption and the heat production. It could be applied for the treatment of obesity and the control of body temperature in future.

Detection of aberrant methylation and loss of heterozygosity of cancer suppressor genes in cfDNA as markers of renal cell carcinoma

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Aim. During recent years, many publications have suggested that cell-free DNA (cfDNA) might be a cancer biomarker. In samples from patients with different types of malignant tumors, genetic and epigenetic changes that are associated with cancer development and progression have been detected in cfDNA. Therefore, we tested markers that are based on epigenetic and genetic changes in order to interrogate aberrant DNA methylation and the loss of heterozygosity (LOH) of distinct genomic regions, respectively. **Methods.** Blood plasma and tumor tissue were obtained from 89 clear cell RCC (ccRCC) patients who received a nephrectomy. The concentrations of cfDNA in blood plasma were determined by measuring the fluorescence level of intercalated SYBR Green I dye and by quantitative real-time PCR (qPCR) of the β-actin gene. For LOH analysis DNA was amplified using PCR and analyzed by capillary electrophoresis. The methylation status of the different genes was determined by the methylation-specific polymerase chain reaction (MS-PCR), by using primers to modified sequences of genes CpG islands. **Results.** Receiver operating characteristic (ROC) analysis showed that the concentration of cfDNA can be used as a diagnostic feature for the detection of renal tumors. AUC obtained for qPCR analysis was slightly higher (0.8049, p=0.0012) than for the SYBR Green I fluorescence measurements (0.7679, p=0.0044). The frequency of LOH in tumors DNA was found at 15% at D3S966 and 21% at D3S1568, which corresponded to the *RASSF*1 gene (locus 3p21.3), 32% at D3S1038, corresponding to the *VHL* gene (locus 3p25–26). Simultaneous deletions were detected for two markers in 15% of the samples. MS-PCR for the first loci of gene *RASSF*1 (*RASSF*1A) revealed that 72% of the tumor's genomic DNA (tgDNA) was methylated, while in cfDNA this figure was 68%, of the second loci of *RASSF*1 (*RASSF*1C) – 48% of the tgDNA and 20% in cfDNA, of *LRRC3B* – 56% of the tgDNA and 42% in cfDNA, of *GPX*3 – 74% tgDNA and 46% in cfDNA, of *PCDH*8 – 78,6% of the tgDNA and 57,2% in cfDNA, of *RUNX*3 – 57% tgDNA and 41% in cfDNA APC - 81% tgDNA and 73% in cfDNA, of *TIMP*3 -13% of the tgDNA, p14 *ARF* – 25% tgDNA, p16 – 28% tgDNA. **Conclusions.** In summary, microsatellite markers with the high level of LOH must be verified in cfDNA in future studies. The concentration of cell-free DNA in plasma and methylation of specific genes can be a significant addition to serological tumor markers in the identification of patients with ccRCC. However, further studies need to be performed to evaluate their diagnostic value.

Obtaining and characterization of the IL-7-CBD fusion protein

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Background. Investigations of interleukin-7 (IL-7) and IL-7 receptors (IL-7R, complex of the CD127 and CD132 chains) have shown that their levels vary in different diseases such as viral infections (HIV, CMV, HCV), multiple sclerosis, Type 1 diabetes and others. Monitoring of IL-7R concentrations appears to be of an important prognostic value. IL-7 genetically fused with cellulose-binding domain (CBD – a component of the cellulase complex of *Clostridium cellulovorans*) can be a perspective component of the test-systems for IL-7R detection. IL-7-CBD can be also used as a ligand for purification of polyclonal antibodies specific to IL-7. **Aim.** To obtain the IL-7-CBD fusion protein in *Escherichia coli*, and to prove functionality of both parts of the conjugate. **Methods.** The DNA sequences encoding human IL-7 and CBD were genetically fused and subcloned into pET24a(+) expression vector under the control of T7 promoter and upstream of the vector-derived 6xHis-tag. *pET24-IL7-CBD* was transformed into *E. coli*, protein synthesis was induced with IPTG and with auto-induction protocol. Refolding of solubilized IL-7-CBD was developed with modifications of dialysis strategies. The protein functions were tested in ELISA and cellulose binding analysis. **Results.** Usage of the auto-induction protocol provided significantly higher protein yield as compared to IPTG induction. The protein of interest was obtained in the form of inclusion bodies. The immobilized-metal affinity chromatography was used for purification of the solubilized protein with subsequent renaturation of IL-7-CBD. The evaluation of the bifunctional activity of the obtained fusion protein after renaturation was performed by binding IL7 with specific antibodies in ELISA and CBD with cellulose. The IL-7-CBD fusion protein was affinity immobilized on the microcrystalline cellulose CC31. Purification and immobilization of IL-7-CBD were essentially one step, thus significantly reducing the cost of production. The resulted affinity medium was successfully used for purification of polyclonal antibodies. **Conclusions.** The fully functional IL-7-CBD fusion protein was obtained and applied for the purification of high specific polyclonal antibodies. The IL-7-CBD fusion protein and purified polyclonal antibodies can be used in capture (sandwich) immunoassays. In addition, our results may potentially contribute to the development of the diagnostic tools for IL-7R detection.

Implication of S6K1 isoforms in the regulation of cell motility

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Background. Ribosomal protein S6 kinase 1 (S6K1) is a member of PI3K/mTOR/S6K signaling pathway. S6K1 takes part in transcription, translation, ribosomal biogenesis, and cell survival. Recent studies have shown that S6K1 may participate in the regulation of cell migration, but the precise mechanism is not known yet. In mammalian cells S6K1 has a range of isoforms: p85S6K1, p70S6K1, p60S6K1, p31S6K1, that vary in structure and features. Mostly studied are p85S6K1, p70S6K1, and p31S6K1 isoforms, while nothing is known about the role of p60S6K1 in the cell. **Aim.** The main aim of this project was to generate MCF-7 cell lines with altered expression of different S6K1 isoforms (//MCF7 and //MCF-7) and to study their involvement in cell migration. **Methods.** CRISPR/Cas9-system was used to target S6K1 gene in MCF-7 cells. Western-blot analysis was applied to confirm knockout of isoforms expression. Cell morphology of MCF-7 cell lines was studied by Romanovsky-Giemsa staining and immunofluorescent reaction. Cellular proliferation was estimated by conducting the mitotic index analysis. Scratch assay was used to investigate the migration potential of the cells *in vitro*. **Results.** We generated MCF-7 cell lines with down regulation of all S6K1 isoforms (//MCF-7) or only p85S6K1 and p70S6K1 (//MCF7). It was revealed that //MCF7 cells had fibroblast-like elongated shape, while MCF-7 wild type and //MCF-7 had typical epithelial-like cell morphology. Mitotic index analysis revealed an increased amount of dividing // MCF7 cells, comparing to wild type cells. *In vitro* scratch assay showed that //MCF-7 had higher migration activity, while //MCF-7 migrated slower than wild type MCF-7 cells. **Conclusions.** MCF-7 cell lines with knockdown expression of different S6K1 isoforms were generated and characterized. The obtained data showed that alterations in S6K1 isoforms expression affect the cell adhesion and migration. //MCF-7 and //MCF7 cell lines have be used for further studies of the functional peculiarities of S6K1 isoforms.

Identifying new pathogenic genetic factors of sex development disorders using WES method

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Aim. Disorders of sex development (DSD) include clinical phenotypes such as ambiguous genitalia (1 in 4500 live births), complete XX or XY sex reversal (1 in 20,000 births) (*Baskin LS, 2001)* and represent a major pediatric concern, due to their common sequel of gonadal cancer and infertility. Up to date there are more than 80 known genes involved in DSD pathogenesis and about 960 candidates possibly implicated in the gonadal development. The aim of the research was to identify new DSD gene candidates using whole exome sequencing (WES) in trios (DSD-affected child and both parents). **Methods.** The WES analysis was performed for 7 DSD-patients (3 patients 46, XX, *SRY*-negative; 4 patients 46, XY, *SRY*-positive) from Ukraine and Poland, selected after genealogical, clinical, biochemical and cytogenetic investigation. The informed consent for participation was obtained from all patients and/or their parents. WES was performed on an Illumina HiSeq 4000 System. Read alignment was carried out using DRAGEN Germline Pipeline (Edico Genome). Sequencing reads were mapped to the human genome assembly GRCh37/hg19. Filtering was performed using VarSeq (Golden Helix). After first step of filtering we have obtained approximately from 340 to 780 variants for each trio among SNPs and indel with population frequency less than 0,01% (1000 Genome Project Database). The genotype variant lists included homozygotes, compound heterozygotes, X-linked and *de novo* variants. After segregation with genotype was performed, all variants, which coincide in affected children and healthy parents, were excluded. The rest of variants were annotated according to DSD-associated gene panel (*Fan, 2017*) and their implication in gonadal development obtained from animal models, RNA-seq studies, and known molecular pathways. For all SNPs the following databases were searched: NCBI including PubMed, OVID, GeneCards Human Gene Database. **Results.** The search resulted in shortlist of gene candidates for each patient. For *SOX3*, which were previously described as a likely pathogenic gene, new SNP (X:139586222), resulting in missense mutation (Gly/Asp) was annotated in 46, XY, *SRY*-positive. For *TYRO3* we have described genotype containing paternal SNP (15:41862801) and *de novo* 12 bp insertion (15:41865665). Both mutations may disrupt the splice sites. Other promising candidates are *NBN, AQP7, TCP10L2* and *SVIL* due to their high expression levels in gonadal tissues or relation to specific early developmental processes. **Conclusions.** The identified novel genetic variants of *SOX3* and *TYRO3* for patients with 46, XY, *SRY*-positive can be included in new DSD-target panels for diagnostic tests. The further investigation of *SOX3* and *TYRO3* mutant protein structure and functions will be important for better understanding the DSD-mechanisms.

Phthalocyanine complexes of different geometry as inhibitors of amyloid fibrillization

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Background. Pathological protein aggregation involves several disorders in pathogenesis such as neurodegenerative diseases, amyloidoses, *etc.* One of important approaches against these disorders is a search for molecules able to affect the amyloid formation. Among the efficient scaffolds against fibrillization, the macrocyclic complexes phthalocyanines have been reported. **Aim.** The goal of this work is to study relative structure metal-containing macrocyclic complexes (phthalocyanines, porphyrazines) of spatial and planar geometry for their effect on the proteins amyloid aggregation. **Methods.** For this, the fluorescent dye-based *in vitro* assay, atomic force microscopy (AFM), scanning electron microscopy (SEM), dynamic light scattering (DLS), surface enhanced Raman scattering (SERS) were used. **Results.** Axially-coordinating phthalocyanines have shown the ability to essentially affect the intensity of the insulin amyloid fibril formation and morphology of the formed aggregates depending on the nature of the coordinated ligand. We have discovered high-effective inhibitors – Hf and Zr phthalocyanines bearing quinolinium styryl fragment, that demonstrate strong fibrillization suppression; their half-maximal inhibitory concentration IC50 is equal (0.16 μM and 0.11 for μM Zr and Hf phthalocyanines, respectively). According to AFM in the presence of Hf phthalocyanine a very small amount of thin protofilaments and oligomeric aggregates was formed. Besides, high-efficient amyloid inhibitor Hf phthalocyanine bearing two chlorine atoms demonstrates several times increase of signal intensity in SERS spectra in the presence of insulin fibrils comparing to free phthalocyanine, this indicates its potency as probe for amyloids determination by SERS. We have also studied the influence of planar Zn and Mg phthalocyanines and porphyrazines on insulin fibrillization. Mg-containing compounds have more pronounced suppression effect (up to 54%), than Zn-containing ones (up to 17%). In the case of axially-coordinated phthalocyanines, the central metal atom does not affect the inhibitory properties, because it is "closed" by ligand. In the case of planar phthalocyanines and porphyrazines, central metal atom affects the intensity of inhibition. It is shown that porphyrazines led to the formation of shorter fibrils comparing to free insulin fibrillization. In the case of phthalocyanines, the high dispersion of the fibrils length is observed. So, variation in structure of used planar macrocycles affect the morphology of formed amyloid fibrils. Hydrodynamic diameters of fibrils population estimated by DLS generally correlate with fibrils size single fibril spices obtained by SEM. **Conclusions.** Overall, different modifications (axial coordination or peripheral substitution) of macrocyclic heme-like complexes, (phthalocyanines and relative compounds) could strongly affect their anti-fibrillogenic properties. We also demonstrate a potential of chemical optimization as tool for design of relative compounds with preferred properties, targeting and therapeutic efficiency.

Analysis of differential gene expression in tissues of healthy and preeclampsia-affected human placenta at different trimesters of gestation

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Background. The human placenta is a unique temporary organ that mediates tight interactions between the fetus and the maternal organism. The syndrome of preeclampsia is one of the major complications of pregnancy (7 – 10% worldwide) connected with abnormalities in the placental gene expression and is a leading cause of maternal and fetal morbidity and mortality. Open-access databases are an abundant source of the gene expression profiles on preeclampsia-affected human placenta provided by independent researchers applying various techniques (microarray, NGS) and using various platforms (Genechip and Beadschip) to obtain these profiles. **Aim.** The aim of our study is to substantially increase the statistical power of the existing gene expression data on healthy and preeclampsia-affected human placenta by merging data from different studies into the larger and more specific cross-experiment case-control groups. **Methods.** We used our previously developed database (http://194.44.31.241:24173/) as a source of gene expression profiles and sample annotation; R programming language for gene expression data merging and analysis; RMA and ComBat methods for inner- and cross-experiment technical batch effect removal; principal component analysis (PCA) methods to identify samples of similar gene expression; limma package, which implements linear models and empirical Bayes method, to find differentially expressed genes; biomaRt and massiR packages to identify fetal sex; known tissue-specific and y-chromosome markers to verify the tissue type and fetal sex, respectively. **Results.** We merged the expression data for 171 samples from 9 experiments on Affymetrix genechip platforms. According to y-chromosome markers we estimated fetal sex as female in 100 samples and as male in 71 samples. Based on presence of the overrepresented marker genes in gene expression profiles we redefined the original annotation and specified 54 samples as chorion, 26 as decidua, 66 as placenta and 23 as basal plate. Based on this pre-analysis and gestational age category we divided our experimental data into the following cross-experiment study groups: 3rd trimester chorion (preterm and term; healthy, severe preeclampsia, fetal growth retardation) - 45 samples; 3rd trimester decidua (preterm and term; healthy; preeclampsia) - 14 samples; 3rd trimester placenta (preterm and term; healthy; preeclampsia) - 56 samples; 1st & 2nd trimester decidua (healthy vs. preeclampsia) - 12 samples; 1st & 2nd trimester healthy (chorion - 9, decidua - 8, placenta - 10 samples) vs. 3rd trimester healthy (chorion - 29, decidua - 13 and placenta - 36 samples). **Conclusions.** Differential expression analysis confirmed reproducibility of original studies' results and revealed the new differentially expressed genes due to increased size and specificity of newly specified case-control groups.