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Contents

Dynamic modelling of CFTR receptor maturation in cystic fibrosis: What controls the receptor concentration in the plasma membrane?

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Key words: CFTR, cystic fibrosis, endoplasmic reticulum, quality control, folding, trafficking

Motivation and Aim: Cystic fibrosis (CF) is the most common autosomal recessive lethal disorder. Approximately 85 % of cystic fibrosis patients express the F508del mutation in the transmembrane conductance Regulator (CFTR) gene [1]. We aimed 1) to model mechanisms of CFTR maturation in order to understand how F508del mutation may lead to Cystic fibrosis and 2) to use the model for network targets capable to compensate the disease symptoms.
Methods and Algorithms: Experimental datasets of time-dependent receptor distribution in various cell lines were obtained from the Biosystems and Integrative Sciences Institute of the University of Lisbon. A detailed network diagram describing basic principles of CFTR maturation was built in CellDesigner 4.4. The corresponding dynamic model, based on a system of rate and balance equations, was constructed in COPASI. Reaction rates were described by mass action kinetics. Metabolic Control Analysis imbedded into COPASI was applied to compute the distribution of flux and concentration control.
Results: Our model allowed reconstructing the dynamics of receptor maturation *in silico* for both healthy and mutant cells. Now the model is being validated by experimental data. Our preliminary data may predict intracellular distribution of receptor concentrations, both for healthy cells and for mutants. Metabolic control analysis suggested that the main control of the receptor concentration in plasma membrane may not reside not at the level of receptor transport into the plasma membrane.
Conclusion: Our model reveals a paradox: the receptor concentration in plasma membrane (where the receptor should work) may not be controlled by the insertion of the receptor into the plasma membrane itself. Receptor concentration in the plasma membrane may rather be controlled by early "upstream" events of its maturation.
Acknowledgements: We thank the European project CORBEL and ISBE.NL colleagues for support.

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Association rs189037 in the ATM gene with bronchial asthma taking into account environmental factors

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Key words: calcium overload, rhythm disturbances, cardiac mechanics

Motivation and Aim: Bronchial asthma (BA) belongs to the group of multifactorial allergic diseases, with a pronounced genetic component. In this study, the involvement into predisposition to BA of rs189037 in the ATM gene was studied. The ATM gene encodes a kinase that is involved in many biological processes, both to maintain DNA integrity, and to form immune responses (negative regulation of B-lymphocyte proliferation, production of immunoglobulins, V (D) J-recombination) [1, 2].
Methods and Algorithms: The analyzed groups are represented by Russian individuals (BA patients and population sample of Tomsk are 134 and 307 people, respectively). Genotyping was performed using the SNaPshot-analysis on the ABI Genetic Analyzer 3730 platform. The studies were executed on the basis of the Center for Collective Use of Scientific Research Equipment and Experimental Biological Material "Medical Genomics" of the Institute of Medical Genetics of Tomsk National Research Medical Center RAS. The analysis of associations of genotypes with different physiological states was carried out with application of the standard methods of the statistical analysis (χ^2, OR with 95 % CI); statistically significant differences were considered when $p < 0.05$.
Results: As a result of the study, the presence of a significant effect of environmental influences on the expressiveness of rs189037 associations with BA was shown. In the general group of patients, the risk effect was shown for the G allele (OR = 1.48 (CI: 1.10–1.99), $\chi^2 = 6.68$, $p = 0.01$) and the GG genotype (OR = 1.67 (CI: 1.01–2.76), $\chi^2 = 4.03$, $p = 0.045$). Accounting for the analysis of the presence/absence of environmental pressure (smoking, parasitic invasion) has allowed the following conclusions. The risk effect of the G allele (OR = 2.13 (CI: 1.45–3.12), $\chi^2 = 15.93$, $p = 0.00007$) and the GG genotype (OR = 1.67 (CI: 1.01–2.76), $\chi^2 = 4.03$, $p = 0.045$), and also – the protective effect of the AA genotype (OR = 0.37 (CI: 0.18–0.75), $\chi^2 = 8.24$, $p = 0.0041$) is manifested only in the presence of the heavy environmental stocking. Into the group of patients with isolated asthma, the tendency to a protective effect of the GG genotype is shown (frequency at patients is onto 7.85 % lower, than in control and onto 27.12 % lower, than in the weighed group) and predisposing to AG (frequency in patients above 8.13 % with respect control).
Conclusion: Thus, environmental factors play a significant role in the manifestation of the effects of rs189037 in the ATM gene on the development of asthma.

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Model melanocortin obesity: immunohistochemical examination of cyclooxygenase-2 and macrophages in the kidney of Ay mice

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Key words: kidney, obesity, cyclooxygenase-2, macrophages

Motivation and Aim: Cyclooxygenase-2 (Cox-2) is an inducible enzyme mediating induction of prostaglandins under physiological and pathological conditions. In kidney, Cox-2 is constitutively expressed in macular densa (MD), ascending limb of loop of Henle and medullar interstitial cells. Kidney Cox-2 expression is strikingly upregulated during inflammation with type 2 diabetes and other pathological conditions. Mice with melanocortin obesity syndrome, heterozygous for dominant lethal mutation Agouti-yellow (Ay-mice) are characterized by type 2 diabetes in adult state. However, renal tissue damage in diabetic nephropathy in these animals has not been sufficiently studied. The aim of this work was to evaluate influence of melanocortin obesity on the severity of inflammatory reaction in the Ay-mice kidney.
Methods and Algorithms: The kidneys of male diabetic C57 Bl/6j Ay (Ay) mice and control C57 Bl/6j (Bl) mice were investigated at 8 month age. Left kidney was quickly removed, cut longitudinal, placed in Tissue-Tek O.C.T. medium (Sacura) and frozen in liquid nitrogen. Samples were stored at –20 °C. 10 μm tissue sections were obtained on a freezing microtome of Microm HM505H series. Sections were double-stained with rabbit anti-Cox-2 antibody (sc-1747) and rat anti – CD68 antibodies – (AbCam) for detection of macrophages. Sections were photographed by fluorescent microscope Axioskop 2 plus (Zeiss) and analyzed with AxioVision program in CMA ICG SB RAS.
Results: The size of renal glomeruli in 8-month-old Ay mice was significantly greater than in control group Bl ($p < 0.01$). It is typical for initial stages of diabetic nephropathy [1]. Kidney expression of immunoreactive protein Cox-2 (irCox-2) was detected not only in MD and ascending limb of Henle loop, but also in glomeruli, tubular epithelium, and in interstitial cells indicating an increase in inflammation in both Ay and Bl mice. Kidney CD68 macrophages were detected near large blood vessels, around glomeruli and in interstitium between tubules in both genotypes. Only in Ay mice we found infiltration of macrophages in the glomeruli that is typical for diabetic nephropathy. Our dates is in accordance with the results obtained in the diabetic KK Ay mice [1, 2].
Conclusion: Thus, we found melanocortin obesity in Ay mice results in a significant increase in the size of kidney glomeruli and the appearance of macrophages in them. The data obtained indicate the presence of morphological signs of diabetic nephropathy in 8-month-old male Ay mice.
Acknowledgements: Supported by the RFBR (17-04-00912) and Budget Project No. 0324-2018-0016.

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Ultrastructutal organization of hepatocytes in distant tumor growth

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Key words: hepatocarcinoma-29, hepatocytes, autophagy, volume density of organelles

Motivation and Aim: The liver is the most common site of distant metastases with tumor growth of different localization. At the same time, the liver, as the central organ of detoxification and metabolism, is most susceptible to the toxic effects of malignant growth products. In connection with the steady growth of oncological morbidity, it is actual to study structural rearrangements in the liver as a result of tumor growth in distant organs, with the purpose of correcting its condition to maintain the body's homeostasis.
Methods and Algorithms: In the experiment on CBA mice, structural changes in the liver were studied during the development of experimental hepatocarcinoma-29, which was transplanted into the hip´s region [1]. Ultrastructural changes in Hepatocytes was studded by electron microscope JEM 1010. Statistical processing of the results was performed using the STATISTICA software package v. 6 (StatSoft Inc., USA). The significance of the differences was assessed using the Mann–Whitney U test. A level of significance of 5 % ($p < 0.05$) was adopted for all tests.
Results: The decrease in the volume density of the cytoplasm of hepatocytes, the volume density of mitochondria, the endoplasmic reticulum, lipid inclusions, and the increase in the volume density of lysosomal structures in the dynamics of tumor growth were shown. All stages of intracellular autophagic degradation were detected by electron microscopy: the presence of autophagosomes, autophagolysomes and secondary lysosomes in the cytoplasm of hepatocytes. In autophagosomes fragments of the cytoplasm, glycogen, mitochondria, fragments of the endoplasmic reticulum with ribosomes were observed.
Conclusion: The data obtained indicate that in conditions of distant tumor growth in the liver non-selective autophagy develops to maintain intracellular homeostasis of hepatocytes, as well as energy and trophic homeostasis of the organism.

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Regenerative technologies in the treatment of knee osteoarthritis

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Key words: osteoarthritis, arthroscopy, PRP, hyaluronic acid, ultrasonography

Motivation and Aim: Drugs with chondroprotective properties and the technique of arthroscopy (AS) are actively used in the treatment of osteoarthritis (OA). Growth factors are increasingly used to stimulate the repair of various tissues injured. Platelet-rich plasma (PRP) consists ones and may be useful in OA-healing. Nevertheless, the effectiveness of PRP in case of knee OA is described only in a few reports. The aim of the study was to evaluate the differences between three technology of knee OA treatment: hyaluronic acid/PRP intra-articular injection and method of therapeutic AS.
Methods and Algorithms: Twenty women (age 60 ± 5.8 yrs) were included in the study. All patients gave written informed consent before enrollment and had knee OA 2 or 3 Kellgren-radiologic stage. The manipulations had been performed on a one knee joint. Patients had been divided into 3 groups. All ones were treated by hyaluronic acid intra-articular injection. Seven patients (1st group) were without any additional treatment. AS was carried out for 6 peoples (2nd group). For 7 patients (3rd group) PRP intra-articular injections were used in common with medical AS. PRP was obtained by collection of 75 ml of venous blood into Plasmolifting™ tubes. The material was centrifuged within 6 min at 3800 rpm. Supernatant with platelets was transferred into a new tube and was centrifuged within 15 min at 3600 rpm to give PRP. Further, supernatant without platelets was removed and PRP solution was resuspended by patients' plasma to give 5 ml. Activation of RPR was carried out by lysing the platelet membranes by double freezing (–20 C)/thawing (+37 C). The PRP activated was filtered through 0.22 nm membrane. Solution obtained was resuspended by patients' plasma to give 9 ml and was frozen into 3 tubes (3 ml each). The PRP activated was injected intraarticular on 2nd, 9th and 16th days after AS. Cartilage thickness was assessed by ultrasound in three zones (in the upper pole of the patella, in the medial and lateral condyles of the tibia) at intervals of three months. Descriptive statistics, Wilcoxon T-test were used for data analysis. The differences were considered statistically significant at $p < 0.05$.
Results: The growth of cartilage in the region of the upper pole of the patella was found in the 1st group (medians 2.3 vs 2.7, $p = 0.041$). An increase of cartilage thickness in the medial condyle of the tibia was found in patients from 2nd group (median 1.45 vs. 2.05, $p = 0.041$). Treatment with PRP hadn't shown any advantage over other methods used.
Conclusion: The clinical effectiveness of intra-articularly hyaluronic acid therapy and surgical methods of OA-treatment (medical AS) have been confirmed by ultrasound visualization. PRP injection technology needs additional investigation with longer follow-up period.
Acknowledgements: Supported by the state task on the program of search scientific researches FASO of the Russian Federation.

IL4 (rs 2243250) gene polymorphism depending on the clinical features psoriatic disease

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Key words: psoriasis, psoriatic arthritis, single nucleotide polymorphisms, immunogenetics, cytokine

Motivation and Aim: Psoriatic disease is a multifactorial inflammatory disease affecting not only skin as psoriasis (PS), but the whole body. One of the severe form of it is Psoriatic arthritis (PsA). Interleukin 4 (IL4) gene is one of the key candidate genes as a result of the encoding of the immunoregulatory cytokine in PS and PsA [1, 2]. Polymorphisms of the promoter region of the *IL4* gene, including C-590T (rs2243250), are important in psoriatic disease [3]. We analyzed the frequency of distribution of genotypes of the promoter region C-590T (rs2243250) of *IL4* gene depending on the clinical course of the psoriatic disease.
Methods and Algorithms: We formed 4 cohorts Europeoids: 1 – PS with *C/C* genotype ($n = 31$), 2 – PS with *C/T* and *T/T* genotypes ($n = 18$), 3 – PsA with *C/C* genotype ($n = 30$), 4 – PsA with *C/T* and *T/T* genotypes ($n = 18$). Genotyping of SNPs of *IL4* genes was performed by PCR and restriction fragment length analysis. Genotype frequencies were tested for deviation from Hardy–Weinberg equilibrium (HWE) by Fisher's exact test.
Results: The index of severity of psoriasis (PASI) is statistically significantly lower in PS with *C/C* genotype in comparison with PS with *C/T* and *T/T* genotype and PsA with *C/C* genotype, $p1,2 = 0.01$, $p1,3 = 0.007$. In PsA with *C/C* genotype was associated with a longer course of the disease in comparison with PS with *C/C* genotype, $p1,3 = 0.01$. It was determined that complaints of skin pruritus were statistically significantly more frequent in PsA with genotype *C/C* than in PS with the same genotype, $p1,3 = 0.02$, $OR\ 1,3 = 4.11$ (95 % CI = 1.00–18.09). It was found that in PsA with *C/C* genotype, a lesion of the scalp with an area more than 30 % is statistically significantly more frequent in comparison with PS with same genotype, $p1,3 < 0.01$, $OR1,3 = 7.19$ (95 % CI =2.25–22.95). In patients with *C/T* and *T/T* genotypes, it was established that the body mass index (BMI) was statistically significantly higher in the PsA group compared to PS, $p2,4 = 0.005$.
Conclusion: Polymorphic variants of the promoter region C-590T (rs2243250) of *IL4* gene determine the genetic basis of individual differences in the clinical course of the psoriatic process: severe forms of PS associated with *C/T* and *T/T* genotypes and severe forms of PsA – with *C/C* genotype. Consequently, further study of the polymorphism of cytokine genes that cause a change in the level of the final product will allow improving preventive measures to reduce the risk of developing severe forms of psoriatic disease in the population.

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Possible mechanisms of therapeutic effects of mesenchymal stromal cells conditioned medium on the model of cryptorchidism in rats

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Key words: MSC, paracrine effects, cryptorchidism, SSC, stem cells niche

Motivation and Aim: Today, male infertility is responsible for 40–50 % of infertility cases in pairs, but the mechanisms of male infertility are generally idiopathic. Hence, one of the possible therapy approaches might be maintenance of normal spermatogonial stem cells (SSC) microenvironment. Mesenchymal stromal cells (MSC) is an inherent component and regulator of different stem cell niches, particularly SSC. It is assumed that paracrine activity of MSC might influence angiogenesis or viability and function of specific cells supporting spermatogenesis named Sertoli and Leydig cells. Thus, we investigated probable mechanisms of MSC effect on SSC niche reconstruction using a model of abdominal cryptorchidism.

Methods and Algorithms: The testicular injury was performed on mature Wistar male rats using abdominal cryptorchidism model. The therapy with subtunical injection of MSC or MSC conditioned medium (MSC-CM) was conducted after 2 weeks, and the effect of this therapy was evaluated 30 and 90 days later using morphometric analysis, histochemistry and immunohistochemistry methods.

Results: We demonstrated that the size and weight of testes increased after 30 days, and achieve normal parameters 90 days after injection of MSC as well as MSC-CM. It should be noted that, despite the positive trend in recovery of the testicle, significant changes of the number of blood vessels in the experimental groups were not observed compared to control. On the other hand, the number of fibrotic tubules in groups with MSC-CM therapy decreased more than 6-fold compared to control groups, while in the groups with MSC therapy the antifibrotic effect was significantly lower. Also we have shown that the number of tubules with intensive proliferation increases in the experimental groups. At the same time, hyperplasia and the proliferation of interstitial cells, particularly Leydig cells, was much lower in the experimental groups. Moreover, renewal of spermatogenesis, right up to the final active forms achieved only in group with MSC-CM therapy.

Conclusion: We have shown the effectiveness of MSC therapy for restoration of the SSC niche. We found that the angiogenic factor does not influence spermatogenesis recovery, while the antifibrotic potential of paracrine secretion of MSC contributes to testicle recovery. We also showed formation of the normal testicular ultrastructure, that contains Sertoli cells, a pool of proliferating spermatogonia cells, including spermatozoa, and interstitial “resting” Leydig cells.

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Design of vaccine constructs capable of inducing a cross protective effect against different strains of the influenza virus A

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Key words: universal influenza vaccine, artificial polyepitope immunogens, conserved epitopes of flu proteins, DNA-vaccine constructs; virus like particles

Motivation and Aim: Given the high influenza virus variability the task of influenza vaccines development requires the use of new non-traditional approaches aimed at construction of new-generation efficient and safe vaccines capable of providing protection against broad range of influenza virus subtypes. In this study we used an approach based on computer design of artificial protein-antigens comprising conservative T- and B-cell epitopes of influenza virus antigens potentially capable of inducing immune response to influenza viruses of different subtypes.

Methods and Algorithms: Design of polyepitope T-cell antigens was carried out using NetMHC and TEpredict/PolyCTLDesigner software based on conservative T-cell epitopes identified in influenza virus proteins HA, NA, M1, NP, PA, PB1, and PB2. The design of B-cell vaccine constructs was carried out using two artificial antigens designed on the basis of conservative fragments of hemagglutinin stem of influenza viruses H1N1 of H3N2, respectively, as well as based on conservative virus protein M2. Designed antigens were used to obtain two variants of vaccine constructs, i. e. DNA-vaccines and virus-like particles (VLP) exposing constructed B-cell antigens in the compound of lentivirus particles.

Results: We evaluated immunogenic and protective properties of the designed vaccine constructs using BALB/c mice. The obtained results revealed that immunization of mice with a combination of DNA-vaccine constructs encoding designed T-cell immunogens provides at least 37.5 % protection of mice against infection both with virus A/California/4/2009 (H1N1) and with virus A/Aichi/2/68 (H3N2). Immunization of mice with a combination of DNA-vaccine constructs encoding conservative fragments of hemagglutinin stem and conservative protein M2 provided protection of immunized animals against infection with pathogenic strain A/Aichi/2/68 (H3N2) and failed to protect mice against infection with pathogenic strain A/California/4/2009. When we used VLP as a delivery system for target B-cell antigens they provided cross-protective immunity against infection of animals both with virus A/California/4/2009 (90 % protection) and with virus A/Aichi/2/68 (50 % protection).

Conclusion: The obtained results established methodological platform for constructing vaccines against highly variable viruses based on rational (computer) design of artificial antigens comprising conservative T- and B-cell epitopes of virus proteins capable of inducing immune response to influenza virus of different subtypes.

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"Mining" for the alpha-1-antitrypsin deficiency in the population of Serbia – experience in the implementation of an integrative diagnostic algorithm

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Key words: alpha-1-antitrypsin deficiency, population of the Republic of Serbia, diagnostic algorithm

Motivation and Aim: Alpha-1-antitrypsin deficiency (AATD) constitutes an important genetic risk factor for the premature chronic obstructive pulmonary disease (COPD) or/ and hepatic disorders. The condition often goes undetected, thus limiting the possibilities for therapeutic and preventive interventions. In order to enhance the detection of AATD in the population of Serbia, an algorithm was proposed, integrating clinical indications for testing with a standardized laboratory procedure [1]. The aim of the study was to evaluate the implementation of the AATD diagnostic algorithms using published data.
Methods and Algorithms: International standards for diagnosing and managing AATD provided clinical indications for testing. A laboratory procedure was based on the joint recommendations of the experts from three different testing centers in Europe [1]. The results based on the algorithm implementation, published either as articles or abstracts in peer-reviewed international journals with impact factor, were considered eligible.
Results: Two articles and two abstracts were identified. The review [1] reported the frequency of 6.7 and 19.2 %, respectively, for the AATD affected individuals and heterozygous carries, in a group of 120 participants selected between 2007 and 2012, according to clinical indications for AATD presence. In the study using integrative laboratory algorithm in a group of 50 patients with the premature COPD [2] high prevalence of AATD (14.0 %) and carriers (20.0 %) was reported, with two rare alleles described – one of them for the first time in the Serbian population. Furthermore, in a group of 25 children it was shown that additional indications, both clinical and a family history of AATD might be taken into account [3]. Also, a pilot evidence was obtained in seven families affirming that testing the asymptomatic parents and siblings of children clinically suspicious for AATD might be a rational approach [4].
Conclusion: The published results can be considered as encouraging, but confirmation in larger studies is necessary prior to their recommendation for common practice.

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Neuroprotective effects of lithium carbonate in conditions of tumor growth

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Key words: hepatocarcinoma-29, neuron structure, behavior of animals, lithium carbonate, accumulation, neuroprotection

Depression is commonly comorbid in cancer patients and has detrimental effects on disease progression. Evidence suggests that biological mechanisms may induce the onset of cancer-induced depression. One of the neuroprotective agents and drugs used to treat neurodegenerative diseases is lithium. At the same time, its antitumor effects are also shown [1, 2]. The neuroprotective effects of lithium carbonate in conditions of peripheral tumor growth were analyzed.

Methods and Algorithms: The experimental study was carried out on male mice of the CBA line. Hepatocarcinoma-29 cells were used to induce tumor growth. Lithium carbonate in physiological solution was administered *per os* at a dose of 125 mg/kg body weight. Ultrastructural changes in the neurons of the prefrontal cortex was studded by electron microscope JEM 1010. The behavior of animals under tumor growth conditions was determined by using the LABORAS™ system. Lithium accumulation was carried out by using Agilent 7500A Inductively Coupled Plasma Mass Spectrometer. Statistical processing of the results was performed using the STATISTICA software package v. 6 (StatSoft Inc., USA). The significance of the differences was assessed using the Mann–Whitney U test. A level of significance of 5 % ($p < 0.05$) was adopted for all tests.

Results: The motional activity of animals with tumor growth was significantly reduced on the 20th day of the experiment. The phenomena of neurons cytoplasm swelling, decreasing of volume density of transport vesicles, autophagosomes, late endosomes and free polysomal ribosomes were noted. After oral administration of lithium carbonate the increasing of volume density of free polysomal ribosomes complexes, transport vesicles, autophagosomes, late endosomes and lysosomes were revealed. The accumulation of lithium in the brain was determinate. The motional activity of animals was restored.

Conclusion: Peripheral tumor growth affects on the behavior and ultrastructural organization of neurons of the prefrontal cortex of experimental animals. Oral administration of lithium carbonate leads to the accumulation of lithium in the structures of the brain, an increases the motional activity of animals and activates neuron systems of vesicular transport and degradation.

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Investigation of meningioma samples by multi-omics analysis

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Key words: high-resolution mass spectrometry, phospholipids, biomarkers, meningioma, database

Motivation and Aim: Rapid and reliable tumor tissue identification during surgical operations is a challenging problem especially in neurosurgery, where the tissue is formless, and precision of tumor/normal tissue border determination is vital. Recently we presented a tissue profiling method based on a combination of electrospray ionization and liquid extraction of phospholipids directly from tumor tissue, which allowed obtaining a mass spectrometric profile in less than a minute [1]. For rapid tumor type identification, we proposed a method based on a search through a reference database containing mass spectra of samples characterized by histopathological methods. To validate the results of tissue profiling we identified the features that forming the molecular profiles of meningioma samples by high-resolution mass spectrometry, which differs them from healthy brain tissue.

Methods and Algorithms: Tumor samples were collected from dissected tissues (brain tumors) during neurosurgical operations in the N.N. Burdenko Scientific Research Neurosurgery Institute. Unmodified brain tissue samples were collected in the N.N. Burdenko Scientific Research Neurosurgery Institute during surgery of patients with drug-resistant epilepsy. New special spray-from-tissue ambient ion source [1] was used for tumor samples profiling. For classification of tumor samples by lipid profiles a database filled with the results of the investigation from more than 100 brain tissue samples was created. High-resolution mass spectrometry data were obtained using Thermo LTQ FT Ultra mass spectrometer. To increase the reliability of the analysis, we have combined our mass-spectrometry profiles of lipids with data from transcriptomics and proteomics databases similar to [2].

Results: A set of features that differ tumor from healthy brain samples were identified and compared to the literature.

Conclusion: Simultaneous analysis of multi-omics data lead to identification of reliable lipid features, which differ in meningioma and healthy brain sample.

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Potential genetic markers of Alzheimer's disease, which play a significant role in aging

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Key words: Alzheimer's disease, MALDI-TOF mass-spectrometry, SNP, Russian population

Motivation and Aim: It is well-known that Alzheimer's disease (AD) is a progressive neurological disorder characterized by slow progressive memory loss due to a gradual loss of brain cells. Alzheimer's disease is now considered a very serious public health problem. Although scientists know how brain cells of persons with AD are affected, and additionally understand some of the genetic explanations of the disease, the precise cause of this disease is still unclear. Earlier we have developed method of multiplex genotyping of polymorphic markers of genes associated with cognitive abilities and various neurodegenerative and neuropsychiatric traits such as schizophrenia, AD, bipolar disorder, and attention-deficit hyperactivity disorder [1]. In this study we have improved method and performed genotyping of samples from AD case–control set.

Methods and Algorithms: In study 190 patients with AD and 711 healthy controls, matched to the patients by age, gender, and ethnicity were included. 62 SNPs were genotyped by MALDI-TOF mass-spectrometry using MassARRAY Analyzer 4 (Agena Bioscience™). Allele specific ORs and associated p-values were calculated.

Results: Statistically significant differences in allele frequencies of 7 SNPs between AD patients and control group were detected: rs1532278 at CLU gene (OR = 1.28, $p = 0.04$), rs2616984 at CSMD1 gene (OR = 1.33, $p = 0.02$), rs3818361 (OR = 1.47, $p = 0.002$) and rs6656401 at CR1 gene (OR = 1.41, $p = 0.008$), rs429358 (OR = 1.85, $p = 0.00005$) and rs769449 at APOE gene (OR = 1.76, $p = 0.0006$), rs3772130 at FBXO40 gene (OR = 1.39, $p = 0.02$).

Conclusion: In the present study we observed the associations of genetic variants of the CSMD1, CR1, APOE, CLU and FBXO40 genes with AD in the Russian population. Previously, we reported about rs2616984 at CSMD1 gene [2] and rs3772130 at FBXO40 gene [3] as markers of cognitive functions in norm or in preclinical stages.

Acknowledgements: The work was supported by the Russian Science Foundation (grant No. 16-15-00020).

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Computer method for image enhancement in fish diagnostics

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Key words: fluorescence *in situ* hybridization (FISH), chromosome analysis, image analysis

Motivation and Aim: Causes of many hereditary and congenital diseases are chromosome abnormalities. One of the ways to detect these changes is a method of FISH with chromosome-derived DNA probes. The problem connected with its usage is a large number of repeated sequences in chromosomes. This paper presents method for visualization and analysis of chromosome-specific DNA sequences in metaphase chromosomes, which helps to solve this problem without suppression of repetitive DNA hybridization.
Methods and Algorithms: The VISSIS (visualization specific signal *in silico*) method [1] developed previously was significantly improved. The method treated the images of two-color FISH with chromosome-derived microdissected DNA probes. Certain steps of image processing have become semi-automated: a segmentation of chromosomes, a detection and rejection of interphase nuclei and stain debris. The following additional procedures were applied: automate filtration of DAPI channel (median filter), new procedure of normalization intensity of signals, a separate detection and processing of bright regions in the image (Bright objects are detected using threshold methods). The ratio of mean signal intensities in the point belonging to the medial-axis of the objects was used as a new feature for classification of chromosomes. The effectiveness of the method was assessed as signal-to-background ratios (SNR) [2].
Results: The method was successfully tested to identify specific FISH signal without suppression of repetitive DNA hybridization. SNR for processing images is comparable to the fluorescence signal of probes hybridized under standard conditions (addition of Cot-1 DNA and preannealing). SNR for processing images is within the range from 4 to 21. The efficiency of the method depends on the sets of DNA-probes. SNR for processing images negatively correlates with differences in SINE/LINE content in the chromosomes, from which DNA–probes were obtained (Spearman and Kendall tau correlation coefficients, $p < 0.001$).
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The use of mesenchymal stem cells and erythropoietin in lower limb ischemia

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Key words: mesenchymal stem cells, erythropoietin, proliferation, migration, lower limb ischemia

Motivation and Aim: Multipotent mesenchymal stem cells are used to stimulate angiogenesis. Mesenchymal stem cells (MSCs) are capable of secreting proangiogenic and cytoprotective factors [1]. Studies have shown that the introduction of MSC promotes angiogenesis and repair of ischemic tissue [2]. The problems of effective implantation of MSC can be solved by improving the functional properties of cells previously preconditioned with growth factors. The aim of this study was to evaluate the regenerative potential of MSC in combination with erythropoietin in rat lower limb ischemia models.
Methods and Algorithms: The work was carried out on male rats Wistar. MSCs were obtained from the bone marrow cells of the femurs. Critical lower limb ischemia was modeled by a ligation of the left femoral artery. The development of ischemia and the effect of MSC on the severity of ischemia were recorded using laser Doppler flowmetry. Rats after ischemia modeling in the muscles of the tibia at 5 points were injected with MSC at 250×10^3 cells/point (MSK group), MSC at 250×10^3 cells/point and erythropoietin 6.68 IU/point (MSK + EPO group), 0.9 % NaCl solution (saline group); the control group did not receive any drugs.
Results: Introduction of MSC and MSC with erythropoietin resulted in a statistically significant increase in the index of microcirculation efficiency after 24 h compared to the control group and the saline group ($p < 0.05$). On the 7th day after the injection, the microcirculation efficiency in the foot was higher in the MSK group with erythropoietin compared to other groups, as well as in the MSC group compared to the control group and the saline group. It was found that the treatment of critical lower limb ischemia with MSC and with the addition of erythropoietin contributed to a statistically significant increase in the microcirculation rate in the foot compared to the same parameter in the control at different times ($p < 0.05$).
Conclusion: The obtained data on the treatment of MSC with erythropoietin indicate the acceleration of angiogenesis processes against the background of experimental critical lower limb ischemia in rats.
Acknowledgements: The reported study was supported by RFBR, research project No. 16-34-00076 mol-a.

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Modified sorbents for pharmacology

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Key words: sorbents, modifiers, lithium, silver, melatonin

Motivation and Aim: The inclusion of porous sorbent carriers in the recipe of dosage forms allows to deliver the active pharmaceutical ingredients (APIs) (including prolonged) to the therapeutic zone and to sanitize the body from toxic agents by hemosorption (through blood), enterosorption (per os), application sorption (impact on damaged skin), cosmetic hygiene sanation [1].

Methods and Algorithms: Evaluation of the effectiveness of modified sorbents (silver-containing, melatonin-containing, lithium-containing sorbents) developed in Research Institute of Clinical and Experimental Lymphology was carried out in an animal experiments using histological, biochemical, immunological, and physiological methods. Results on the lithium-containing sorbent for enteral administration were obtained in pre-clinical studies on the full cycle (including embryotoxic and teratogenic effects) in accordance with standard methods [2]. The significance of the obtained results was evaluated using conventional statistical processing methods [3, 4].

Results: A normotimic drug with prolonged release of lithium shows greater efficacy and safety (compared to the standard lithium carbonate) in the prophylaxis and treatment of psychoemotional disorders. The safety of the polyfunctional silver-containing preparation "AlSi/Ag" with detoxification properties during long-term enteral administration has been experimentally confirmed. Its pronounced antibacterial effect against Salmonella enteritidis and its toxins has also been confirmed. The adaptogenic, lymphotropic, cytoprotective effectiveness of the melatonin-containing silica-alumina sorption complex, which ensures prolonged action of melatonin in various experimental pathologies, including diabetes, has been shown (in comparison with melatonin).

Conclusion: Immobilization of biologically active molecules on porous carriers allows to obtain highly effective and safe drugs, which have an additional detoxifying effect.

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A search for molecular relationships between bronchial asthma and hypertension as an example of comorbid diseases

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Key words: asthma and hypertension, SNPs, association analysis, eQTL, mass spectrometry

Motivation and Aim: Previously *in silico* we have identified genes which can be involved in comorbidity of asthma and hypertension [1]. As a result of this analysis, we identified top genes for experimental validation including *TLR4*, *CAT*, *IL10*, *CST3*, *ICAM1*, *IRF6*, *AKT1*, *NFKB1*, *PNP*, *SELL*, *CCL5*, *IL2RB*, *IDS*, *FOS*, *NT5C2*, *BHLHE40* and other genes. In these genes, we chose eQTL SNPs for the study of association with bronchial asthma, hypertension and their combination.

Materials and Methods: Genotyping of eQTL SNPs (n = 34) was carried out in patients with BA (n = 145), hypertension (n = 147), comorbidity of both diseases (n = 137) and healthy individuals (n = 141) using MALDI-TOF mass spectrometry. All individuals were ethnic Russians living in Tomsk region (Russia). Association analysis was carried out using logistic regression.

Results: The SNPs rs11032700, rs480575, rs7130331, rs12587456 were associated only with bronchial asthma; rs7026297 with hypertension; and rs7026297, rs7025144 with the comorbidity of hypertension and bronchial asthma. The SNPs associated with comorbidity of asthma and hypertension (rs7026297, rs7025144) regulate the *TLR4* gene expression; thus, the study provides evidence in support of the role of the *TLR4* in the development of comorbidity of bronchial asthma and hypertension.

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Serum levels of adipokines in type 2 diabetic subjects: the relationships with adipose tissue distribution and microvasculature

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Key words: diabetes, obesity, adipose tissue, adipokine, total body composition

Motivation and Aim: Dysfunction of adipose tissue (AT) is considered to play an important role in the development of metabolic disorders and complications in type 2 diabetes [1–3]. The relationships between the function, distribution and vascularization of AT are needed to be clarified. The aim of the study was to determine the relationships between serum levels of adipokines, body fat distribution, and the density and ultrastructure of blood and lymphatic microvessels in subcutaneous AT in type 2 diabetic subjects.

Methods and Algorithms: We observed 125 patients, including 82 ones with obesity, and 30 lean non-diabetic individuals. The concentrations of leptin, resistin, visfatin, adipsin, and adiponectin in the fasting serum were determined by Multiplex analysis. The fat mass and AT distribution was assessed by DEXA. The samples of subcutaneous abdominal AT were obtained with the knife biopsy in 25 patients and in 15 healthy subjects. Immunohistochemistry for biomarkers CD-34, podoplanin and LYVE-1 was applied to identify the blood and lymphatic microvessels.

Results: Patients with diabetes, as compared to control, had significantly higher levels of leptin ($p = 0.004$), resistin ($p < 0.0001$), adipsin ($p < 0.0001$) and visfatin ($p = 0.0003$). The concentrations of leptin, resistin and adipsin were associated with total fat mass. The levels of resistin and adiponectin demonstrated relationships with truncal and central abdominal fat mass. In obese diabetic subjects an increase in the volume and numeral density of lymphatic vessels was observed. The swelling of cytoplasm, mitochondria, rough endoplasmic reticulum and reduced content of micropinocytic vesicles was revealed in lymphatic capillaries. The increase in leptin levels in patients with type 2 diabetes and obesity was associated with a decrease in the volume and numeral density of the blood and lymph microvessels in the subcutaneous fat ($r = – 0.63$, $p = 0.02$; $r = –0.55$, $p = 0.05$). The level of resistin was negatively correlated with the numeral density of lymphatic vessels ($r = –0.59$, $p = 0.03$).

Conclusion: The levels of circulating adipokines in patients with type 2 diabetes are related differently with body fat distribution and AT microvessel density.

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The use of melatonina in patients older than 60 years in the preoperative period

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Key words: Postoperative delirium, melatonin

Motivation and Aim: The population of elderly and senile patients who need surgical treatment is becoming more numerous. Therefore, the problem of safe medical care for this population becomes an important and urgent task, including surgery for hip and knee replacement [1]. Postoperative delirium (POD) is a serious complication that develops in almost all areas of surgery, more often in the elderly and elderly [2]. Carrying out an operation in consciousness requires creating psychological comfort and reducing anxiety. For these purposes, anxiolytics of the benzodiazepine series are used in the perioperative period. However, anxiolytics of the benzodiazepine series are an additional risk factor for the development of postoperative delirium in elderly and senile patients. The search for alternative drugs, with possible neutral impact on cognitive functions, would reduce the risk of POD and the severity of cognitive impairment in the early postoperative period. To reduce anxiety and improve sleep, the use of melatonin is promising and correct [3]. Aim: To evaluate the efficacy and safety of melatonin as a premedication drug before surgery, endoprosthetics of large joints performed under combined spinal epidural anesthesia. *Methods and Algorithms*: Pilot randomized trial. Patients of the surgical department of the NIIKEL clinic – a branch of ICG of SB RAS planned for endoprosthetics of large joints, are randomized into two groups: patients of the control group (benzodiazepine series anxiolytics) and patients of the study group (melatonin). Anesthesia method – combined spinal-epidural anesthesia (local anesthetic – ropivacaine), with postoperative prolonged epidural analgesia. The quality of sleep on the night after the appointment of melatonin is assessed using the diary of the patient. Before the operation, the initial level of cognitive functions is assessed using the Montreal scale. Further evaluation is performed prior to the appointment of premedication and at 1, 2, 3 days after surgery with the help of the mini-COG test. The number of scores on test scales and their dynamics, cases of postoperative delirium development and other early postoperative complications in both groups are fixed. To confirm postoperative delirium, Nursing delirium screening scale (Nu-DESC) is used; the patient is evaluated in the first five days of the postoperative period. During the operation, the sedation level is fixed using the Ramsey scale. After surgery, the patient subjectively assesses the pain syndrome using a visual analog scale (VAS) for the first five days.
Results: An analysis of the diaries of patients with melatonin indicates a deep and qualitative sleep on the eve of the operation. The level of sedation during surgery in patients with melatonin is fixed at 2–3 on the Ramsey scale. Perioperative anesthesia was adequate, there was no difference in study groups.
Conclusion: At this stage of the study, data were obtained on the sufficient sedative and hypnotic effect of melatonin in patients under conditions of combined spinal epidural anesthesia, which is not inferior to the effect of benzodiazepines. The data obtained on the effectiveness of melatonin in the prevention of postoperative delirium require further study.

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Morphology and proteomic analysis of human placental exosomes

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Key words: placenta, exosomes, proteins, size-exclusion chromatography

Motivation and Aim: Various placental cells secrete exosomes during pregnancy. Exosomes are 40–100 nm vesicles of endosomal origin containing RNA and different proteins. The placental exosomes are important in intercellular communication and immune function. A key role in the exercise of the biological functions of exosomes is played by their different proteins and RNA. In this study, we investigate multiple proteins from human placenta exosomes.
Methods and Algorithms: In this work the exosomes were isolated from placentas of healthy women by differential centrifugation and ultrafiltration. Exosome preparations were additionally purified by size-exclusion chromatography from impurity proteins. Morphology of vesicle preparations was characterized by transmission electron microscopy (TEM).
Results: According to the results of TEM the best protocol of isolation exosomes from human placenta includes the combination of differential centrifugation, ultrafiltration and size-exclusion chromatography. Electron microscopy revealed the presence of spherical vesicles, with a typical cup-shape and diameters ranging from 40 to 100 nm, microparticles without membranes (20–60 nm) were also observed within the preparations after size-exclusion chromatography. TEM with anti-CD63 and anti-CD81 immune labeling demonstrated the presence of exosomes in 40–100 nm membrane particles. Exosome proteins were identified before and after size-exclusion chromatography by MALDI MS and MS/MS spectrometry of protein tryptic hydrolysates derived by 2D electrophoresis. Purified exosomes contained only from ten to twelve different major proteins: secreted proteins, iron transport proteins, cytoskeleton-related proteins, tetraspanins.
Conclusion: Our results demonstrating the decrease of number of major proteins identified in preparations of exosome after size-exclusion chromatography may be important in further studies of exosome functions. Also, in the future the study of placental exosome proteins from healthy women will allow us to establish the differences from exosomes isolated from placentas of women with pathologies of pregnancy and to develop new fundamental approaches for diagnosis and therapy of pregnancy pathologies.
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The possibility of colonization of spongy collagen plate by mesenchymal stromal cells

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Key words: spongy collagen plate (SCP), mesenchymal stromal cells (MSC), bone marrow, adipose tissue

Motivation and Aim: The development of tissue engineering requires the active introduction of new materials that are as close to their biological and physicochemical properties as possible to human tissues. A key role in the processes of tissue repair and regeneration is played by stem cells, in particular mesenchymal stromal cells (MSCs). Many problems can be solved by transplantation of cells together with a biodegradable substrate, or scaffold, which degrades during the regeneration of the tissue to form non-toxic products. The collagen materials are largely correspond to these requirements. Thanks to the biocompatibility, the ability to provide cell adhesion, fibrous structure, mechanical strength, good compatibility with other materials, low toxicity and antigenicity, collagen is a highly promising material for scaffolding [1]. A spongy collagen plate (SCP) is a new modification material from reconstructed collagen structured with using a special temperature regime.

Methods and Algorithms: The biocompatibility assessment was carried out using MSCs isolated according to standard protocols from bone marrow and adipose tissue of rat, as well as from aspirate of human adipose tissue. All scaffold specimens, 8 mm in diameter, were seeded with 2–4 passage cells in amount of 1×10^6 cells and cultured during 24 days in DMEM medium supplemented with 20 % fetal calf serum (for MSC from adipose tissue) or αMEM medium supplemented with 20 % fetal calf serum (for MSC of bone marrow). The results of the experiments were assessed by light microscopy after staining histological sections with hematoxylin-eosin, and also after staining with Hoechst dye using fluorescence microscopy.

Results: On the 24th day of cultivation, cells from all three sources successfully populated the SCP, located as a dense layer over the scaffold surface and penetrated into the deeper layers of the collagen sponge. Inside the scaffold there were quite a few cells with oval nuclei laid out on the fibers. During the cultivation period the scaffold significantly decreased in size, the fibers were thinned and replaced by proliferating cells.

Conclusion: Successful colonization of SCP by the bone marrow- and adipose tissue-derived MSC suggests that it is a promising scaffold for the creation of tissue engineering constructs and requires further research. A further analysis of the applicability of the scaffold studied is needed for a longer duration of cultivation and stimulation of differentiation in the osteogenic direction [2].

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25 leading laboratories in Russia and abroad are successfully using GenSeq™ DNA Library Kit v1.1 for targeted sequencing on the routine basis

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Key words: targeted sequencing, NGS, DNA Library Kit, GenSeq, highly multiplexed PCR

GenSeq™ DNA Library Kit v1.1 was introduced to the market in March 2017 on the IX Conference of Molecular Diagnostics, Moscow.

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The role of Wip1 phosphatase in tumor cells, in the tumor microenvironment and anti-cancer therapy

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Key words: anti-cancer therapy, immunotherapy, tumor microenvironment, DNA repair, cell death

Motivation and Aim: Cancer is one of the leading causes of death in the world. The potentiation of anti-cancer therapy efficiency is an essential task of modern oncology. Phosphatase Wip1 is frequently overexpressed in cancer cells [1]. Additionally, the activating Wip1 mutations found in immune cells of lung cancer patients [2]. Both alterations observed in cancer patients associated with poor prognosis: the increased levels of Wip1 in tumor cells can positively affect the tumor growth and compromise the tumor response to anti-cancer therapy; the activating mutations in immune cells can negatively affect the tumor microenvironment.

The proposed project aim is to decipher the mechanisms of Wip1 phosphatase actions in tumors and tumor microenvironment for the developing a new anti-cancer strategy.

Methods and Algorithms: Using several models of tumorigenesis in mice and patient-derived xenografts (PDX) we have analyzed the impact of Wip1 mutations that affected its levels or activity on tumorigenesis and response to anti-cancer therapy.

Results: We determined the effect of Wip1 levels on senescence, cell death and autophagy in response to chemotherapeutic drugs. Additionally to genetic approach to manipulating with Wip1, we have used Wip1 specific small molecule inhibitor, GSK 2830371, to confirm results obtained in genetically modified mice or cell lines.

In tumor microenvironment we have analyzed how the modulation of Wip1 levels in immune cells affected tumorigenesis and immunotherapy. We established a conditional knockout mouse model with deletion of Wip1 in the different compartments of the hematopoietic system and studied consequences of Wip1 depletion on tumor microenvironment and tumorigenesis. We have determined the levels of Wip1 expression in various immune cells (lymphocytes, macrophages, neutrophils) in the tumor microenvironment and periphery (bone marrow, blood, spleen). We estimated the potentiating effect of Wip1 inhibition on the modern anti-cancer immunotherapy with inhibitors of immune checkpoints (anti-PD1, anti-CTLA-4) that was recently approved for the treatment of cancer patients.

Conclusion: In conclusion, we have discovered the mechanisms of Wip1 action in tumor cells and tumor microenvironment and proposed recommendations for improving current anti-cancer strategies using modulation of Wip1 activity levels.

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Drug-drug interactions severity prediction based on xenobiotic structural formulas and PASS prediction algorithm

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Key words: drug-drug interactions, DDI, drug-metabolizing enzymes, xenobiotics, PASS

Motivation and Aim: When several drugs are co-administrated, a drug-drug interaction (DDI) phenomenon may appear. Many DDIs are due to changes in the metabolism of drugs. In this case, DDI is manifested by the effect of one drug on the biotransformation of other drugs, its slowdown (in case of inhibition of drug-metabolizing enzymes) or acceleration (in case of induction of drug-metabolizing enzymes), which leads to change in the pharmacological action of co-administrated drugs [1]. The severity of DDIs can be classified by applying different classification systems. One of the most advanced is OpeRational ClassificAtion (ORCA) system for the classification of DDI, created for physicians to assess the risk of co-administration of two drugs [2]. ORCA divides DDI into five classes: contraindicated (class 1), provisionally contraindicated (class 2), conditional (class 3), minimal risk (class 4), no interaction (class 5).

Methods and Algorithms: For computer prediction of the severity of DDI, we collected a training set consisting of approximately 4000 pairs from 500 drugs that belong to classes 1–3 of DDI in case of co-administration. The prediction of DDI classes is based on a combination of modified substructural MNA descriptors – PoSMNA (Pairs of Substances Multilevel Neighbourhoods of Atoms), and a classification algorithm implemented in the PASS (Prediction of Activity Spectra for Substances) software [3].

Results: The average invariant accuracy of prediction, calculated in the leave-one-out and 20-fold cross-validation procedures, were approximately 0.9.

Conclusion: drug-drug interactions severity prediction based on xenobiotic structural formulas and PASS prediction algorithm shows accuracy sufficient for the practical application in the fields of the biomedicinal and pharmaceutical chemistry. Such prediction before the clinical experiences can offer great benefits to pharmaceutical companies in drug designing as well as physicians and patients.

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Selective killing of cancer cells by ultrasound assisted by biopolymer-coated metal nanoparticles

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Key words: metal nanoparticles, biopolymer coating of nanoparticles, ultrasound treatment of cancer

Motivation and Aim: Cancer is a leading cause of death worldwide. Traditional physical methods of cancer treatment, such as radiation therapy, including electromagnetic ionizing radiation, elementary particle ionizing beam radiation (electrons, protons, and neutrons), and non-ionizing radiation (photons, microwaves, and radio waves) have numerous undesirable side effects and cannot provide fully efficient treatment of the decease. The ionizing radiation is intended to be directed only at the tumor. Moreover, ionizing radiation itself may cause DNA mutation in normal cells, causing these cells to become cancerous. Non-ionizing radiation therapies are mainly based on hyperthermia in tumors as a result of the higher sensitivity of tumor cells to heat than their normal counterparts. But these methods are also does not have sufficient selectivity.
Methods and Algorithms: In our work we use high intensity ultrasound treatment enforced by addition of biopolymer-coated various metal nanoparticles in tissue or cell culture. In our study we used gold, silver, palladium, mercury, platinum, bismuth, wolfram, and some other metal nanoparticles covered by biologically inertial specific polymer shells.
Results: Combination of ultrasound and metal nanoparticles leads simultaneously to the strengthening of the destructive effect of ultrasound irradiation, and the selective toxicity of nanoparticles for cancer cells. This allows decreasing the power of the ultrasound radiation and expands the irradiated area without damaging the surrounded normal tissues. Proper selection of biopolymer shells, as far as the size and composition of metal cores provide the selective killing of cancer cells in the main tumor and in the metastases without negative impact to the surrounded normal tissues and perceptible side effects. This work is the continuation of previous studies [1, 2].
Conclusion: We found that nanoparticle-assisted therapy of cancer by the high intensity ultrasound irradiation significantly increase the efficiency of the treatment, allows reducing the intensity of irradiation, and decreasing the side effects.
Acknowledgements: This work was supported by Ministry of Science and Technology of Taiwan (MOST 102-2113-M-001-002-MY5 and NHRI-Ex107-10603EI).

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Cancer stem cells: Emergent nature of tumor emergency

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Key words: Krebs-2 tumor initiating cells, pluri-/multipotent stem cells, stemness, stem niches, "generalized cellular stress", pluripotent/stem phenotype

A functional analysis of 167 genes overexpressed in Krebs-2 tumor initiating cells was performed. In the first part of the study, genes belonging to the three functional groups that determine the malignant phenotype of cancer cells were identified. These groups represent the following features of tumor cells: proliferative self-sufficiency, invasive growth and metastasis, and multiple drug resistance. Malignancy of cancer stem cells was found to be provided by the same genes that provide the stemness of normal pluri-/multipotent stem cells. These results suggest that the malignancy is simply the ability to maintain the stem cell specific genes expression profile, and, as a consequence, the stemness itself regardless of the controlling effect of stem niches. In the second part of the study, three stress factors combined into the single concept of "generalized cellular stress," which are assumed to activate the expression of these genes, were defined. In addition, possible mechanisms for such activation were identified. The data obtained suggest the existence of a mechanism for the *de novo* formation of a pluripotent/stem phenotype of tumor cells.

Real-time lipidomic analysis of biological tissue samples

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Key words: neurosurgery, mass-spectrometry, lipidomic analysis

Motivation and Aim: Neurosurgery remains up to date the most effective modality of solid brain tumors treatment. The accurate determination of tumor margins is challenging, due to the difficulties in distinguishing between healthy and affected brain. Nowadays, the most available methods of intraoperative visualization, such as CT, fluorescence diagnostics or ultrasound have certain limitations coming from their inherent characteristics and patients individual specificities, which may strongly restrict the application of these methods and influence the analysis of the obtained data. Mass-spectrometry is a new promising approach for biological samples identification. We propose a new direct ESI with the inline capsular extraction of biological tissue samples for lipidomic analysis, which allows obtaining mass spectra for a period of time of less than 1 minute.
Methods and Algorithms: The system consists of disposable capsules with quick connect fittings and ESI source. And it has been tested at Thermo LTQ-Orbitrap XL and Bruker esquire 3000 plus with custom developed ESI atmospheric ion funnel interface. The Rattus norvegicus mice brain samples were used for tuning method and as a negative control. All human samples were provided by the Burdenko Neurosurgical Institute. Pathological tissues were dissected during the neurosurgical procedure. Mass-spectrometry data were normalized, scaled, transformed, features were extracted from data and aligned for further analysis. All data manipulations were performed in R environment with custom script available by request from authors. Significant features were detected by shrinkage discriminant analysis, classifiers were developed with lasso-regression.
Results: In one of our previous works, we announced the direct spray-from-tissue ionization method, which was shown to be useful in distinguishing between different grades and types of tumor. New capsular extraction ESI system that can be used with any mass spectrometer with ESI interface, and requires less than 1 minute for analysis. Spectra obtained with new system characterized by more reproducible spectra, which allow increasing of classification accuracy. Various solvent mixtures for extraction have been tested and described. Data validation is conducted by compound identification using precise masses from the MS profile, MS/MS, and isotopic distribution structure analysis. The method demonstrates good stability and reproducibility. The data were compared with previous results. The method can be improved for automatic operation and applied for real-time identification of tissue type during surgery, also does not require special operator skills in mass-spectrometry.
Conclusion: The new method of lipidomic extraction is proposed and tested.
Acknowledgements: Supported by the RSF (No. 16-15-10431).

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Fluctuating asymmetry of gene expression in embryos derived by *in vitro* fertilization

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Key words: fluctuating asymmetry, *in vitro* fertilization, gene expression, immunization

Motivation and Aim: Fluctuating asymmetry refers to small random deviations from perfect symmetry in bilaterally paired structures. It is thought to reflect an organism's ability to cope with genetic and environmental stress during development. Usually morphometric parameters are used for evaluation of fluctuating asymmetry rate. Here, we introduce new approach for assessment of gene expression fluctuating asymmetry. As an intriguing developmental model we use mouse embryos derived by *in vitro* fetilization (IVF). There is evidence that IVF-derived individuals show higher frequencies of metabolic dysfunctions and cardiovascular disease. Research on this relative young cohort in terms of genetic stability is of great interest.
Methods and Algorithms: All experiments were performed on outbred CD1 mice in specific pathogen free conditions. 16-day embryos were obtained from the following experimental groups:
Control: fertilization of intact females by intact males in natural conditions.
IVF: standard IVF of oocytes derived from superovulated females by spermatozoa from intact males, incubation of embryos in culture media for 3 days, and further transplantation to surrogate mothers at day 3.5.
IVF-i: standard IVF of oocytes derived from superovulated females by spermatozoa from males on 7th day of immunization with hemocyanin, incubation of embryos in culture media for 3 days and further transplantation to surrogate mothers at day 3.5.
SVO: 3.5 day embryos derived from superovulated females covered by intact males were transplanted to surrogate mothers at the same day (no incubation of embryo in culture media)
SVZ: zygotes derived from superovulated females (day 0.5) were incubated in culture in IVF-i group dispersion rate was comparable to that of control group.
Conclusion: Prolonged incubation *in vitro* during IVF is an important factor which can increase rate of gene expression fluctuating asymmetry at early developmental stages. We propose male immunization as possible approach for overcome of negative effect of IVF procedure on gene expression stability in development.
Acknowledgements: Supported by the RFBR (No. 18-34-00493).

Clinical case: rare mutations in the *CYP1B1* gene in a patient with primary congenital glaucoma

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Key words: primary congenital glaucoma, CYP1B1 gene, mutation

Motivation and Aim: Primary congenital glaucoma (PCG) is the most common cause of blindness among children. Many PCG cases occur sporadically, but family cases are also known. Mutations in the cytochrome P4501B1 (*CYP1B1*) gene are the major cause of PCG [1]. The aim of this study was to identify mutations in the *CYP1B1* gene in the patient with PCG (P01) and her relatives.
Methods and Algorithms: The proband (P01) and available members of her family, including healthy mother (P00) and daughter (P03) and sister with PCG (P02), were exposed to Sanger direct DNA sequencing in order to analyze the *CYP1B1* gene exons and splice sites.
Results: Two rare heterozygous Arg444Gln (CGA>CAA) and Arg444Stop (CGA>TGA) substitutions were discovered in the *CYP1B1* gene in the probands P01 and P02. Only Arg444Stop substitution was identified in healthy mother P00 and her granddaughter P03.
Conclusion: In our study, we identified two rare mutations in the same codon of the *CYP1B1* gene in two sisters with primary congenital bilateral glaucoma from the same family. Both of sisters have severe form of the disease with more than 3 surgical procedures including enucleation of the bling eye. The carriers (P00 and P03) with one of these mutations have no symptoms of PCG with normal ocular pressure.
Acknowledgements: The reported study was supported by the Russian Foundation for Basic Research (project No. 18-315-00297).

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Analysis of transcripts and expression of *hox* genes in the holothuria *Eupentacta fraudatrix* during regeneration

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Key words: Hox genes, Echinodermata, sea cucumber; gene expression, regeneration

Motivation and Aim: Regeneration is a biological process, which is one of the most important adaptations for the survival of the organism [1]. Many genes of development play an important role in regeneration. Thus, the *hox* genes play a fundamental role in the structuring of the body in Metazoa, they participate in the formation of expression patterns necessary for the proper tissue formation in embryogenesis or regeneration [2]. The alternation of various *hox* genes expression determines the correct formation of organs. To date, the *hox* genes in holothurians remain poorly understood. The aim of this work was to determine the complete sequences of transcripts of all *hox* genes and to study the expression of *hox9/10* and *hox11/13a* in the process of regeneration of aquapharyngeal complex (AC) in *Eupentacta fraudatrix*.
Methods and Algorithms: In studies we used the adult *E. fraudatrix* in normal and at different stages of regeneration. To establish the complete transcript sequences of the *hox* genes in *E. fraudatrix*, the 5′- and 3′-Step-Out RACE PCR method was used. A study on changing gene expression was carried out with qPCR using *ef1α* and *tubulin* as reference genes. Confirmation of the correct identification of genes was carried out using the construction of phylogenetic trees.
Results: In *E. fludatrix* the expression of 8 *hox* genes: *hox1*, *hox3*, *hox5*, *hox7*, *hox8*, *hox9/10*, *hox11/13a*, *hox11/13c* occur during regeneration and in normal. Next, we examined the expression level of *hox9/10* and *hox11/13a* in the regeneration of AC in *E. fraudatrix* using real-time PCR. From the 3rd to the 7th day, the level of *hox9/10* expression increases 5 times from the normal level, by the 10th day there is a decrease, and by the 14th day the expression again increases 4 times and by 20th day gradually decreases, reaching the normal. During the regeneration of AC in *E. fraudatrix*, the expression of *hox11/13a* initially increases 4 times from normal from the 3rd to 5th day and on the 7th day it still remains at a high level. A sharp decrease in the level of expression occurs on the 10th day, and then by the 20th day the expression of *hox11/13a* gradually decreases, recovering to normal.
Conclusion: As a result of our studies, we identified the transcript sequences of 8 *hox* genes. Transcripts of the *hox* genes in *E. fraudatrix* have the greatest homology with *hox S. purpuratus* (50–57 %) и *hox A.japonicus* (60–70 %). The activity of the *hox11/13a* gene reaches its maximum on the 5th–7th day after the evisceration, the *hox9/10* gene has its maximum of expression on the 7th day of regeneration. Apparently, the detected *hox* genes take an active part in the regeneration process of AC in *E. fraudatrix*.

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Hox genes in the holothurian *Eupentacta fraudatrix* and their participation in the regeneration

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Key words: *Hox* genes, Echinodermata, *Eupentacta fraudatrix*, gene expression, regeneration

Motivation and Aim: Restoration of damaged or lost body parts and functions after trauma is typical of almost all modern organisms. Among invertebrate animals, representatives of Echinodermata are distinguished by pronounced regeneration abilities [1]. Holothurian *Eupentacta fraudatrix* was chosen as a model object for studying the mechanisms of regeneration. Currently, morphology and cellular mechanisms in the process of regeneration in *E. fraudatrix* are described in detail, however, the molecular mechanisms underlying the restorative morphogenesis are poorly understood. Previously, when analyzing the regenerating rudiments of the aquapharyngeal complex (AC) in *E. fraudatrix*, transcripts of *hox* genes were detected [1]. The Hox cluster is a group of related genes through which the body plan (head-chest-tail) is determined and set during embryo development [2]. The aim of this work was to determine the complete transcripts sequences of all *hox* genes and to study their expression during the regeneration of the AC in the holothurian *E. fraudatrix*.
Methods and Algorithms: In studies, *E. fraudatrix* were used in normal and at different times of regeneration: 3, 5, 7, 10, 14 and 24 days after evisceration. To determine the complete sequence of transcripts of the studied genes, the 5′- and 3′-Step-Out RACE method was used. Gene activity dynamics was analyzed using the qPCR method.
Results: As a result of our studies, we have identified the following sequences of gene transcripts: *hox1*, *hox3*, *hox5*, *hox7*, *hox8*, *hox9/10*, *hox11/13a*, *hox11/13c*. Next, we examined the expression level of individual genes during regeneration of the AC. On the third day *hox5* already reaches its maximum, which is 30 times higher than normal, there is a 7-fold increase in the expression of *hox8* and a 6-fold decrease in the expression of *hox1* on the 5th day. At the same time, *hox5*, *hox8* are restored to the normal level, and on the 7th day there is a 17-fold increase in *hox5* and a 3-fold increase in *hox8*. On the 10th, 14th and 24th days, *hox8* gradually decreases, *hox1* reaches the norm, and *hox5* gradually decreases its activity, but even on the 24th day it is still 6 times higher than normal.
Conclusion: 8 *hox* genes in *E. fraudatrix* are expressed. Transcripts of genes have a great similarity with homologous genes: *S. purpuratus* (81 %), *A. japonicus* (74 %). The detected *hox* genes are involved in the regeneration process of the AC in the holothurian *E. fraudatrix*.

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DNA methylation level in regulatory regions of mtDNA and three mitochondria-related nuclear genes in atherosclerosis

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Key words: mtDNA, DNA methylation, mitochondria, atherosclerosis

Motivation and Aim: Epigenetic regulation of gene expression can contribute to the common diseases, including atherosclerosis. Mitochondrial dysfunction and oxidative stress are pathogenetic factors for the atherosclerosis development. The aim of the study was to estimate DNA methylation level in mtDNA D-loop and mitochondria-related nuclear genes (*POLG*, *TFAM*, *PPARGC1A*), in connection with atherosclerosis.

Methods and Algorithms: The study included 50 patients with carotid atherosclerosis (blood sample and excised carotid atherosclerotic plaque for each patient) and 14 asymptomatic volunteers as control group (blood sample). After sodium bisulfite DNA treatment, a part of mtDNA D-loop, promoter regions of *TFAM* and *PPARGC1A*, and CpG island in exon 2 of *POLG* were amplified with bisulfite sequencing primers (designed with MethPrimer 2.0). The PCR products were used for NGS library preparation with Nextera XT kit (Illumina). The libraries were sequenced on Illumina MiSeq platform. Sequencing reads were aligned on converted reference sequences, and methylation levels were calculated as C/(C+T) ratio, with minimum 150x coverage. Methylation levels between the samples were compared by Mann-Whitney U test.

Results: Cytosine methylation levels in mtDNA D-loop and in *TFAM* promoter were very low (mean level less than 2 %) and did not differ between the samples, except for several positions. In contrast, for *POLG*, methylation level was 86 %, varying from 36 % to 97 % among 45 studied CpG sites. In the patients, *POLG* methylation level in blood was about 5 % higher than in the plaques (significant differences for 39 CpG sites), but there were almost no differences between blood samples from patients and healthy individuals. For *PPARGC1A*, 12 CpG sites were studied, and 8 of them differed between blood and plaques of the patients ($p < 0.0001$); mean level was 13 % (1.3 %–34 %) in plaques, 26 % (1.6 %–72 %) in patients' blood, and 28 % (1 %–76 %) in controls. Methylation level increased toward 5′ end of the studied region.

Conclusion: We evaluated DNA methylation level in main regulatory region of mtDNA (D-loop), as well as in three genes functionally related to mitochondrial function, in blood vs plaque samples of patients, and in healthy individuals. *POLG* and *PPARGC1A* regions have lower methylation in the plaques than in blood of the patients. These differences may reflect tissue-specific epigenetic response to the oxidative stress and mitochondrial dysfunction in atherosclerosis.

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Possibilities of lymph nodes phytostimulation in the old age

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Key words: lymph node, trace elements, gerontology, phytotherapy

Motivation and Aim: The most priority in medicine is the problem of providing an immune protection and increase in nonspecific resistance of an organism at elderly and senility age [1]. First of all, search of ways of control of functions of lymphatic system is necessary for counteraction to aging. It is possible to make it by means of lymphotropic technologies of preventive medicine if to consider the concept of the lymphatic region [2]. The purpose is studying influence of phytotherapy on structure and function of the lymph node which underwent age changes.
Methods: In the experiment used 160 albino rats that were divided into groups of young and old animals. The mesenteric lymph node is chosen as a research object. We used the original herbal remedy (phytocomposition) to improve the function of the lymph node. We conducted a histologic research of mesenteric lymph nodes. We defined the content of trace elements (Mn, Fe, Cu, Zn, Se) in a mesenteric lymph node by means of the radiofluorescence analysis with use of the synchrotron radiation (RFA SI). Morphometric data processing was performed with licensed statistical software package StatPlus Pro 2009, AnalystSoft Inc.
Results: Age-related changes in mesocolic lymph node reflect the general process of ageing. These changes of lymph node are associated with reduction of structural and functional compartments and with excessive manganese content and deficiency of trace elements (iron, zinc, and selenium). There is a decrease in drainage and immune function of lymph nodes in the elderly and senile age. We have realized the idea to control the lymphatic system functions using phytotherapy. Phytotherapy provides improved drainage and immune functions of the lymph node by increasing the size of functional compartments, intensification of cellular proliferation and mitigating the deficiency of the main trace elements [1–3]. There is the formation of new lymphoid follicles after phytostimulation.
Conclusion: Phytotherapy has a structural and modifying effect, which is important for improving the non-specific resistance of the body at the late stage of ontogenesis. This result is of practical importance for the optimization of endoecological rehabilitation.
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New lymphotropic properties of herbal remedy Silymarin at liver pathology

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Key words: lymphatic system, lymph node, liver, toxic hepatitis, phytotherapy, Silymarin

Motivation and Aim: The concept of the lymphatic region provides structural and functional unity of the "organ – lymphatic system" complex [1]. Such approach does necessary search of herbal remedies which at the same time influence both organ, and lymphatic system. Correction of disturbances and optimization of inadequate functions of lymphatic system are important, and in a row a case an indispensable condition of effective therapy of organ pathology. The research aim is to study lymphotropic properties of herbal remedy Silymarin for correction of liver pathology [2].
Methods: The experiment is executed on 80 rats males Wistar. We did an intraperitoneal injection of tetrachloride carbon in a dose 1mg/kg and created model of acute damage of a liver (40 animals). We entered previously Silymarin of 120 mg/kg within 2 weeks to a half of animals. There were 20 intact rats in control group. The liver and lymph nodes investigated by a histologic method with a morphometry. The obtained data were analyzed by means of the statistical program StatPlus Pro 2009, AnalystSoft Inc.
Results: Experimental hepatitis is the reason of a necrosis of hepatocytes that reduces function of a liver. At the same time, there is a reduction of B-and T-dependent zones and lymphatic sinuses of a lymph node that is a morphological sign of immune and drainage insufficiency. We confirmed hepatoprotective effect of Silymarin [3] that was characterized by reduction of the area of hepatocytes necrosis (by 2.2 times). Degree of hepatoprotective effect depends on the response of lymphatic system after Silymarin. It is noted, that the function of a lymph node is more active, the injury of a liver is less. Preventive introduction of Silymarin changes reaction of structural and functional zones of a lymph node to development of toxic hepatitis. Silymarin increases the areas of a paracortex (in 1.2–1.3 times) and a lymphatic sinus (in 1.2–1.5 times). Change of these functional compartments demonstrates increase in an immune response on cellular type and strengthening of drainage function of a lymph node. The effect of action of Silymarin considers the combined activity of a liver and a regional lymph node.
Conclusion: Silymarin possesses the difficult mechanism of action at acute toxic hepatitis. Silymarin has hepatoprotective effect [2] but also Silymarin shows lymphotropic properties to strengthening drainage and immune functions of a lymph node. Observed effects expand Silymarin area in clinical practice.
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Characterization of cell division in primary hippocampal cultures derived from kaiso deficient mice

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Key words: neuron progenitors, kaiso, nestin

Motivation and Aim: Development of central nervous system (CNS) is accompanied by dynamic proliferation of neuron progenitor cells. Neuron progenitor cells differentiate then into neurons and glia. During early stages of CNS development intermediate filament protein nestin is expressed by dividing cells and also is commonly used as neuron progenitor marker. Kaiso is a transcription factor that regulates gene expression, controlling cell division and apoptosis, as well as signaling pathways Notch/Hes and Wnt/β-catenin that are crucial in cell proliferation and differentiation during development [1, 2]. Although kaiso is widely expressed throughout the mammalian brain [3], its role in cell division during brain development is poorly understood. Here we study *in vitro* effect of kaiso deficiency on cell division.
Methods and Algorithms: We have used primary cultures derived from the hippocampus of 17.5 days old embryos of wild type (WT) and kaiso knockout (KO). Cells were plated at equal density $1{,}5 \times 10^5$ cells/cm^2 (Neurobasal 21203, L-glutamine 1 μM, B-27 supplement, penicillin-streptomycin 50 μg/mL GIBCO Laboratories). Cells were fixed with PFA 4 % in PBS at 4 h, 24 h, 48 h and 72 h after plating and stained for neuron progenitor marker – nestin and cell nuclei with DAPI. Total cell number and nestin expressing cell number were counted and averaged from several micrographs (Axio Imager 2, Carl Zeiss, 40x objective lens).
Results: The number of cells at the start of the experiment (4 h after plating) was not significantly different between WT and KO culture (WT 192 ± 15; KO 181 ± 16, $p < 0.05$). Total number of cells increased from 4 h to 72 h, however we found greater total cell number at 72 h after plating in WT culture (WT 317 ± 20; KO 257 ± 10, $p < 0.01$). We have calculated the number of nestin expressing cells in both WT and KO derived hippocampal primary cultures. Our preliminary results show that the number of nestin expressing cells is different between cultures derived from KO and WT mice at 4 h, 48 h and 72 h after plating.
Conclusion: The result shows that the number of nestin expressing cells may be different in cultures derived from KO mice compared with cultures derived from WT mice. This may indicate that kaiso is involved in the mechanism of division of neural progenitor cells during development. However kaiso deficit seems to decrease the total number of cells in culture *in vitro* 72 h after plating.
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Nanoparticles of manganese oxide induce stress granule formation in human glioblastoma cells

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Key words: stress granule, nanoparticle, manganese oxide, glioblastoma, oxidative phosphorylation

Motivation and Aim: Nano sized objects – man-made and naturally occurring are interacting with living organisms in everyday life, but their effect on cellular level remains largely uninvestigated. We have shown *in vitro* that an array of inorganic nanoparticles (NP) absorbed intrinsically disordered RNA-processing proteins, which also take part in stress granule (SG) formation [1]. Stress granule is a dense formation consisting of a majority of cell mRNA and associated proteins that form in cell cytosol under stress. SG formation is an energy dependent process [2]. Our aim was to study the mechanism by which SG formation occurs in cells treated with NP.

Methods and Algorithms: This study was performed on human glioblastoma cell line U87. Cells were cultured for 24 h. Both commercially available and produced in the lab inorganic NP were used in the experiments. Nanoparticles of $Ru(OH)_2$, Co_2O_3, C, CeO_2, Mn_3O_4 (50–70 μg/ml) were diluted in cell growth medium and incubated for 15 min up to 48 hours with U87 cells. Immunocytochemistry with eIF3 η marker was used to visualize cells with SG. For NP visualization in cell structures, ultrathin sections of the cells fixed in 2.5 % glutaraldehyde, postfixed in 1 % OsO_4 and embedded in Epon812, were examined under JEOL-1400 transmission electron microscope. Standard Cell Mitochondrial Stress Test and Glycolysis Stress Test were measured using Seahorse XFp Analyzer (Aligent). Cell viability was estimated using Viacount reagent (Merck Millipore) on GUAVA cell counter (Merck Millipore).

Results: We have observed significant increase in percentage of cells with SG under Mn_3O_4 NPs treatment (2 h incubation, 1:100 dilution in growth medium) (0.7±0.3 % control; 13.6±1.9 % Mn_3O_4 and 15.9±2.2 % Mn_3O_4 with sodium citrate; ONEway ANOVA $p < 0.001$). Using electron microscopy, we established that glioblastoma cells incorporated NP into cellular vesicles. Predominantly NP remained in vesicular cell structures for at least 24–48 h. Stress granule formation is ATP dependent; therefore, we have tested influence of Mn_3O_4 NP onto energy metabolism of U87 cells: oxidative phosphorylation and glycolysis. Incubation with Mn_3O_4 NP (2 h, 37 °C) *showed* a significant reduction in U87 cells oxygen consumption rate in comparison with control, which indicates lower oxidative phosphorylation rate. Within the same time, no difference in glycolytic rate was observed. Cells remained viable up to 48 h incubation with Mn_3O_4 NP. A significant reduction in cell viability (16 %) was observed after 72 h incubation with Mn_3O_4 NP.

Conclusion: Glioblastoma U87 cells form stress granules under Mn_3O_4 NP treatment. This process is likely induced by Mn_3O_4 NP influence onto oxidative phosphorylation. How do Mn_3O_4 NP, that are largely remaining in cell vesicles, can affect oxidative phosphorylation remains to be investigated.

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The spectrum of common and rare *CYP1B1* gene variants in Russia patients with congenital and juvenile open angle glaucoma

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Key words: primary congenital glaucoma, juvenile glaucoma, CYP1B1 gene, mutation, polymorphism

Motivation and Aim: Childhood glaucoma is a rare severe neurodegenerative disorder, which can cause blindness. Specific mutations in the cytochrome P4501B1 (*CYP1B1*) gene are associated with autosomal recessive primary congenital glaucoma (PCG) and some cases of juvenile open angle glaucoma (JOAG) [1, 2]. The aim of this study was to identify the spectrum of common and rare *CYP1B1* gene variants in Russian patients with PCG and JOAG.

Methods and Algorithms: Unrelated patients with PCG and JOAG were examined. Substitutions in the *CYP1B1* gene were analyzed using Sanger sequencing. The phenotypic variability was compared between the carriers of pathologic mutations and non-carriers. The frequencies of the polymorphisms were compared with aged healthy control and patients with aged open angle glaucoma.

Results: We found no novel mutations in patients with PCG. Common sequence variants of the c.-1 -12C>T, Arg48Gly, Ala119Ser, Leu432Val, Asn453Ser, Asp449Asp substitutions were identified in both PCG and COAG groups. A total of four previously described rare mutations (Gly387Lys, Pro437Leu, Arg444Gln and Arg444Stop) were found in the *CYP1B1* gene. The carriers with Arg444Gln substitution had a more severe phenotype compared with other patients.

Conclusion: We described the spectrum of pathogenic and common sequence variations between Russian patients with PCG and COAG. The frequencies of pathogenic mutations are low in studied patient groups.

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The association of single nucleotide variations Rs6582147 and Rs2136810 with sudden cardiac death

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Key words: sudden cardiac death, single nucleotide variation, genome-wide association study, GWAS

Motivation and Aim: Cardiovascular diseases are responsible for approximately 17 million deaths every year in the world, approximately 25 % of which are sudden cardiac death (SCD). The molecular genetic markers of SCD are studied to create an effective diagnostic system of predisposition and prophylactic of SCD, especially to people without cardiac diseases [1]. Single nucleotide variations rs6582147 and rs2136810 were identified in own genome-wide association study (GWAS) as associated with SCD [2]. The aim of this work is confirm the association of rs6582147 and rs2136810 with SCD in a case-control study.
Methods and Algorithms: The SCD group was formed using the European Society of Cardiology criteria for SCD ($n = 360$, mean age 53.0 ± 9.2 years, men – 76.9 %, women – 23.1 %), the control group was selected according to sex and age from the DNA bank of project Health, Alcohol and Psychosocial factors In Eastern Europe (HAPIEE), Multinational MONItoring of trends and determinants in CArdiovascular disease (MONICA) ($n = 402$, mean age 52.9 ± 9.1 years, men – 69.7 %, women – 30.3 %). DNA was isolated by phenol-chloroform extraction. Genotyping was done by PCR followed by analysis of restriction fragment length polymorphism. The data were statistically processed using χ^2 test according to Pearson, two-sided Fisher's exact test with Yates' correction for continuity.
Results: The genotype frequencies of rs6582147 and rs2136810 are according to Hardy–Weinberg equilibrium in the control group (χ^2=2.43; 0.25, respectively). Genotype GT of rs6582147 is associated with protective effect against SCD ($p = 0.011$, OR = 0.67, 95 % CI 0.49–0.91). Genotype TT of rs6582147 is risk genotype for SCD ($p = 0.002$, OR = 1.60, 95 % CI 1.20–2.14). Genotype GG of rs2136810 is risk genotype for SCD ($p = 0.049$, OR = 1.37, 95 % CI 1.01–1.87). Genotype GA of rs2136810 is associated with protective effect against SCD in group over 50 years old ($p = 0.045$, OR = 0.64, 95 % CI 0.42–0.98).
Conclusion: Single nucleotide variations rs6582147 and rs2136810 are associated in with sudden cardiac death in the sample of people died suddenly.
Acknowledgements: Supported by scholarship of the President of the Russian Federation.

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Molecular-genetic phenotype of experimental breast cancer

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Key words: breast cancer, luminal type, N-methyl-N-nitrosourea

Motivation and Aim: Breast cancer (BC) in women is in second place in prevalence worldwide, among all newly detected cases of cancer. Therefore, experimental animal models of BC are used for study development and treatment of BC. So, experimental BC model in rat often used for researcher the development of BC because the main steps of pathogenesis and progression, and also, the histology are similar to BC in humans. *N*-methyl-*N*-nitrosourea (MNU) is a DNA alkylating agent which can induce a mammary tumor, and does not require metabolic activation to exert mutagenic effects, and also, the tumors had a more aggressive potential. Molecular-genetic phenotype of chemically induced of BC in rats, including the expression of steroid receptors, proliferation markers, is today a standard approach for diagnosis, treatment and prognosis of BC in humans, not sufficiently studied.
Materials and Methods: In the experiment were used rats-female Wistar 10–12 week-old, weighted 250–300 g. MNU (Sigma) was administered into second mammary gland on right side to 30 Wistar rats at 30 mg/kg body weight dissolved in warm physiological saline and acidified to pH 5.0 with acetic acid, once per week 5 times. In control group animal was given an identical volume of physiological saline. 24-weeks after MNU administration, the animals were observed daily and palpated one a week to detect the induction of the tumors. 24-weeks after administration MNU tumor specimens (thorax mammary glands number 2 on right side) were collected. All tumors were carefully excised, then piece of tumor was fixed in 10 % formalin for histologic or immunohistochemical evaluation. Immunohistochemistry to detect the estrogen receptor-α, Her2/neu, progesterone receptor and Ki-67 was performed on 5-μm-thick formalin-fixed paraffin embedded sections cut onto coated slides. The antibodies used were E115 (Rabbit Polyclonal primary antibodies, Novus Biologicals) to identify Estrogen receptor α, rabbit polyclonal antigens (Novus Biologicals) to identify Progesterone receptor, SP3 (Rabbit monoclonal antibody, Labvision) to identify Her2/neu receptor, and MIB-5 (mouse antigen, Dako) to identify Ki-67. These primary reagents were followed by a standard streptavidin-biotin-peroxidase 3/39 diaminobenzidine immunohistochemical technique.
Results: In the 30 rats, first palpable tumor appeared in the 6^{th} week in four of the rats after MNU administration. In the 12^{th} week of the experiment tumor was palpated in 10 rats (30 %), whereas in the 18^{th} week and 24^{th} week of the experiment, respectively in 60 % and 90 % of the rat had detectable tumors. In the 10 % of rat intermammary gland administration of MNU don't caused development tumor. Macroscopic structure of the mammary gland tissue was composed of fibrotic strands with a small inclusion of fat tissue. Macroscopic structure of the newly formed tissue had the appearance of a node within indistinct borders of the growth, and in some animals with ulcerations and calcification of the skin over the hearth of tumor growth. Histologically in the excised tumors revealed infiltrative duct cancer with precancerous changes in edge zones, intra-ductal proliferation. It was noted the formation of the ductal, glandular cribriform structures lined by moderately pleomorphic epithelium with variable mitotic activity; tubular structures predominated, some areas present mixed nests, trains and groups of individual cells with less formalized channels until their disappearance. In, almost all of the analyzed tumors, expression of estrogen receptor-α (ER-α) and Her2/neu wasn't detected, whereas expression of progesterone receptor (PG) and Ki-67 was detected. The proportion of positive to PG cells was 2/3, score 4. The intensity of staining of the cell was average, points 2. Total score of the evaluation of expression by Allred DC (proportion score + intensity score) 6. Among tumor cells 45 % of them were positive to Ki-67, a marker of proliferative activity.
Conclusion: In summary, intermammary administration of MNU possessed to breast cancer development with similarities to human luminal B phenotype.

Evaluation of circulating tumor cells with epithelial-mesenchymal transition properties in the blood of breast cancer patients with different effect of neoadjuvant chemotherapy

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Key words: circulating tumor cells, EMT (epithelial-mesenchymal transition), neoadjuvant chemotherapy (NACT), breast cancer

Motivation and Aim: CTCs are a heterogeneous population. Some cells are cancer stem cells, other cells are in an EMT (epithelial-mesenchymal transition) state, and most of the cells do not have EMT and stemness properties [1, 2]. It was shown that neoadjuvant chemotherapy (NACT) on breast cancer does not act on CTCs in the EMT state [3]. Therefore, the aim of our study is to estimate CTC with EMT properties before starting treatment in the blood of patients with breast cancer with a different effect NACT.
Methods and Algorithms: The prospective study includes 30 patients with invasive breast cancer T2-4N0-3M0 aged 32 to 60 years admitted for treatment in Cancer Research Institute, Tomsk National Research Medical Center. Neoadjuvant chemotherapy was carried out for 14 patients. 16 patients were in the group without NACT. Venous blood samples taken before and after biopsy Venous blood samples (5 mL) were collected in tubes containing heparin and used for examination within 2 h.
The various pools of circulating tumor cells were determined using monoclonal antibodies to EpCam, CD44, CD45, CD24, N-cadherin, labeled with different fluorochromes on flow cytometry BD FACSCanto ™ II. The obtained data were processed using variation statistics. Assessment of the normal distribution of the results was performed using the Kolmogorov-Smirnov test. The significance of differences was assessed using the nonparametric Mann–Whitney test (for independent samples) and the Wilcoxon (Z) test (for dependent samples).
Results: The study showed that patients with a complete and / or partial tumor regression after NACT level CTC with sign EMT in the blood before biopsy was significantly lower than in patients who have no observed response to treatment (stabilization and/or progression) ($p = 0.02$).
Conclusion: Thus, the level of CTC with a sign of EMT before treatment is associated with the effect of NACT. The results are useful for determining tactics and creating a personalized approach to the treatment of patients with invasive breast carcinoma.
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Serum markers in head and neck squamous cell carcinoma

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Key words: head and neck squamous cell carcinoma, chronic hyperplastic laryngitis, early diagnosis, metastases, prognosis

Background and Aim: Head and neck squamous cell carcinoma (HNSCC) is characterized by an advanced stage at the time of diagnosis, high aggressiveness and poor survival [1]. Therefore, the search for the most relevant biomarkers related to HNSCC is of great importance [2]. The aim of the study was to analyze the serum concentrations of protein phosphatase, Mg2+/Mn2+ Dependent 1B (PPM1B), cofilin-1 (CFL1), adenylyl cyclase of associated protein 1 (CAP1), alpha 2-macroglobulin (A2MG), and circulating proteasomes (c-proteasomes) in patients with HNSCC and in patients with chronic laryngeal and hypopharyngeal diseases with histologically proven dysplasia (CLHD, DII-III).
Methods and Algorithms: The study included patients with HNSCC, who did not receive any treatment ($n = 69$, T1-4N0-3M0), patients with CLHD, DII-III ($n = 12$) and healthy volunteers ($n = 11$). Serum levels of PPM1B, A2MG, CFL1 and CAP1 were measured by ELISA assay using Aunthos Reader 2020 microplate readers. Chymotrypsin-like activity (CLA) and caspase-like activity (CsLA) of circulating proteasomes were measured using fluorogenic kinetic assays [3]. Statistical analysis of the results was performed using Statistica 6.0 software.
Results: The differences in serum levels of CFL1 and CAP1 between the control group and patients with early-stage HNSCC (T1N0Mo) and in CAP1 and PPM1B between the control group and patients with CLHD were found ($p \leq 0.05$). Approximately 2-fold increase in the CAP1 and CFL1 levels and 1.5-fold increase in the PPM1B level were observed in patients with HNSCC (T1N0M0) compared to patients with CLHD, DII-III ($p \leq 0.01$ and $p \leq 0.05$, respectively). No differences in the A2MG serum level between the study groups were found. Serum CAP1 and A2MG levels correlated significantly with the presence of metastases in patients with HNSCC: the CAP1 level was lower and the A2MG level was higher in patients with HNSCC (T2-3N0M0) than in patients with HNSCC (T2-3N1-2M0) ($p \leq 0.02$). In patients with HNSCC (T1N0M0), chymotrypsin-like activity (CLA) of proteasomes was 1.5 times higher than that observed in patients with CLHD, DII-III ($p \leq 0.05$). A strong positive correlation between serum levels of CAP1 and CFL1 ($r = 0.9$) and mild correlation between serum levels of CAP1 and PPM1B ($r = 0.4$) and between chymotrypsin-like activity (CLA) of proteasomes and CAP1 ($r = 0.3$) were found in HNSCC patients.
Conclusion: Our results are consistent with other recent studies, which indicate that the studied proteins participate in the development of HNSCC [3–5]. These results show promise for using CAP1, CFL1 and PPM1B concentrations as well as proteasome chymotrypsin-like activity to monitor precancerous lesions in patients with CLHD, DII-III. The use of serum levels of CAP1 and A2MG could be helpful for monitoring metastases in patients with HNSCC.
Acknowledgements: Supported by the RFBR (No. 17-04-00198A).

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The relationship of the genes expression of actin-binding proteins with metastasis in squamous cell carcinoma of the head and neck and non-small cell lung cancer

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Key words: non-small cell lung cancer, head and neck squamous cell carcinoma, metastases, prognosis

Motivation and Aim: Metastasis is associated with the ability of tumor cells to move [1], which is characterized by changes in the protein composition, including actin-binding proteins (ABP): cofilin (CFL1), proflin (PFN1), ezrine (EZR) and adenylyl cyclase-associated protein 1 (CAP1) [2]. The involvement of ABP in the progression of non-small cell lung cancer (NSCLC) and squamous cell carcinoma of the head and neck (SCCHN) has been little studied. Therefore, the aim of the work was to study the relationship between the level of ABP genes expression in tissues of patients with NSCLC and SCCHN with a metastatic process.

Methods and Algorithms: The expression of ABP genes (CFL1, PFN1, EZR, CAP1) in paired tissue samples of patients with NSCLC (T2-3N0M0, $n = 15$; T2-3N2-3M0, $n = 10$) and SCCHN (T2-3N0M0, $n = 14$; T2- 3N2-3M0, $n = 9$) were evaluated using SYBR Green I in real time PCR in relation to normal tissue. There was used the "housekeeping" gene of the GAPDH enzyme (glyceraldehydes-3-phosphate dehydrogenase) as the referee gene. Statistical processing of data was carried out using the "STATISTICA 8.0" software package.

Results: There was found a change in the level of ABP mRNA in the tumor tissue of SCCHN and NSCLC depending on the presence of metastases (N0 vs. N1). In the tumor tissue of NSCLC (T2-3N1M0), the mRNA expression level of all studied proteins increased ($p = 0.05$). In patients with SCCHN (T2-3N1M0), there was a multidirectional change in the expression of ABP mRNA level. The EZR expression level significantly decreased and the level of CAP1 expression increased ($p = 0.05$).

Conclusion: In general in our study we showed the dependence of the ABP mRNA expression level on the presence of metastases in patients with SCCHN and NSCLC. The data obtained indicate the involvement of ABP in the processes of tumor progression in NSCLC and SCCHN, which does not contradict the literature data [3].

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Gene set enrichment analysis of prefrontal cortex and hippocampus of social defeat stressed mice reveals physiological, developmental and metabolic pathways involved in mood disorders

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Key words: gene expression changes, GSEA, social defeat stress model

Motivation and Aim: Since stress is the most common risk factor contributing to the onset and progression of mood disorders, animal models are often used to determine gene that are involved in the pathological mechanism. Changes in gene set enrichment occurred in prefrontal cortex on different stages of social defeat stress model such as anxious stage (10 day), transitional stage (15 day) and depressed stage (20 day) were analyzed. Here we apply that gene set enrichment analysis (GSEA) of regulatory or signaling network that are significantly enriched with expressed genes on different stages of social defeat stress modeling is a valuable tool for investigation potential drug targets for therapy of mood disorders [1].
Methods and Algorithms: Standard sensory contact protocol for social defeat stress modeling on 9 C57BL/6J strain mice was conducted. Briefly, each animal was placed in cage with aggressive mice equipped that was removed for 10 minutes each day. As a result 4 groups ($n = 3$) were used: anxious (defeated for 10 days), transitional (15), depressed (20) and control (without consecutive experience of agonistic interactions). RNA sequencing was performed on HiSeq1500 with standard protocols for extraction, library preparation, quality check and sequencing. Pathways in resulting groups were established by GSEA protocol and additionally grouped by similarity of core genes [2].
Results: Total of 40 upregulated and 98 downregulated GO pathways and 4 upregulated and 12 downregulated KEGG pathways were found in anxious group. Total of 186 downregulated GO pathways and 4 downregulated pathways were found to be enriched in transitional group.Total of 73 upregulated GO pathways, 37 upregulated and 1 downregulated KEGG pathways were found in depressed group.
Observed changes that were conservative between brain regions can be described as follows: major disruption of neurotransmitter signaling in anxious group, followed by astrocyte function downregulation in transitional group and ended by differential specific reactions of tissues in depressed group. These changes are mostly mediated by variety of signaling pathways downregulations and are usually accompanied with changes in cell adhesion, inflammation and ion transport systems.
Conclusion: Study shows that social defeat stressed modeling in mice leads to changes in expression of genes associated with main signaling pathways, neurotransmitter systems, ions homeostasis, astrocytes function, apoptosis, adhesion and immune response and propose potential drug targets for treatment of associated with this model conditions
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Impact on the structure of thymus and regionary lymph nodes adjuvant therapy using preparation double-stranded DNA human Panagen

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Key words: thymus, lymph nodes, breast cancer, chemotherapy, panagen

Motivation and Aim: In organism bearing tumor, there is violation of various stages of immune response, including involution of thymus, which accompanies development of many tumors human and animals and can form basis for development of T-cell immunodeficiency in the body. The study of morphological features of one of the central immune organs (thymus) and its regional lymphatic node in malignant tumors, tumor removal, chemotherapy, and the introduction in treatment of adjuvant therapy of preparation double-stranded DNA human Panagen may influence on methods of treatment and to evaluate actions of known and new anticancer cytotoxic preparation.

Materials and methods: In the experiment were used rats-female Wistar. Breast cancer was modeled by introduction of N-methyl-N- nitrosourea 5 times, every 7 days subcutaneously in area 2 th mammary gland on the right. After 6 months, formed a breast cancer (adenocarcinoma). Performed resection of breast cancer, chemotherapy according to the scheme of CMF after 6 months from the start of the experiment. The preparation double-stranded DNA Panagen was administered intraperitoneally, once daily, for 14 days – after 3 h after injection of cyclophosphamide. Was performed histological research of thymus and anterior mediastinal lymph node.

Results: After resection of breast cancer and chemotherapy in the thymus, in comparison with the group with breast cancer without treatment, reduced area of cortical substance, gland tissue, density of cellular elements of the parenchyma and will increase area of medulla, connective tissue, number of immunoblasts, macrophages. The barrier – fiin areas responsible for cellular and humoral immunityltration function of the anterior mediastinal lymph node is reduced: volume of sinus system increased, reduced volume of lymphoid nodules with germinative centers and timus-dependent zone, reduced proliferative activity of lymphoid cells in areas responsible for cellular and humoral immunity, reduced the number of macrophages in all zones. After adjuvant therapy with introduction of double-stranded DNA Panagen it was revealed morphological signs of activation of lymphoid and epithelial components of the thymus: area of cortex and medulla, glandular and connective tissue corresponded to the values of intact animals, increased number of small lymphocytes in central part of the cortical substance and macrophages in all zones thymus. In the anterior mediastinal lymph node, at decrease of the volume sinuses of medulla the increased volume and number lymphocytes of thymus-dependent zone, the volume of mantle zone and germinative of centers lymphoid nodules, at decrease in their proliferative activity.

Conclusion: Thus, preparation double-stranded DNA Panagen causes activation of activities of lymphoid and epithelial components of the thymus, affects the maturation of lymphocytes in respective zones of the thymus and migration of lymphocytes from the thymus and influences on activity of local immune response and barrier-filtration properties of regionary lymph nodes of the thymus.

Structure of regional and remote lymph nodes at treatment of chemically induced breast cancer using double-stranded DNA human Panagen

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Key words: lymph nodes, breast cancer, chemotherapy, panagen

Motivation and Aim: With metastasis main method of treating cancer is chemotherapy, which exacerbates imbalance in immune system, exerting a damaging effect on lymphoid tissue. The study of morphological features of regional and distant from primary tumor site lymph nodes at different methods of treatment of malignant tumors (combination of resection tumor, chemotherapy and introduction of double-stranded DNA Panagen) can have of great prognostic value an integrated approach to methods of treatment and evaluation of action of known and new anticancer cytotoxic drugs.

Materials and Methods: In the experiment were used rats-female Wistar. Breast cancer was modeled by introduction of N-methyl-N- nitrosourea 5 times, every 7 days subcutaneously in area 2 th mammary gland on the right. After 6 months, formed a breast cancer (adenocarcinoma). Performed resection of breast cancer, chemotherapy according to the scheme of CMF after 6 months from the start of the experiment. The preparation double-stranded DNA Panagen was administered intraperitoneally, once daily, for 14 days – after 3 h after injection of cyclophosphamide. Was performed histological research of axillary (caudal from 4 nodes) and mesenteric lymph nodes.

Results: In adjuvant therapy without use of preparation Panagen in axillary lymph nodes, in comparison with the group with breast cancer without treatment, revealed reduction of number metastases identified structural features reduce of transport function studied lymph nodes, increase of activity humoral immune response in axillary lymph nodes and cellular immunity in mesenteric lymph nodes. After introduction in the adjuvant therapy of preparation double-stranded DNA Panagen, in contrast to adjuvant therapy without use of preparation Panagen, in axillary lymph nodes revealed structural features of reduce of transport functions, increase activity cellular immune response and decrease activity of humoral immune response, in mesenteric lymph nodes – increased transport function, activity of cellular immunity and recovery of activity of humoral link of immunity.

Conclusion: Inclusion of preparation Panagen in adjuvant therapy promotes the increase of leukostimulating activity, of anticancer effect of this therapy at treatment of experimental breast cancer.

Technologies of blood saving with the use of tranexamic acid in myomectomy

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Key words: tranexamic acid, iron carboxymethyltosate, uterine fibroids

Motivation and Aim: The main method of treating uterine fibroids is laparoscopic myomectomy. Preservation of reproductive function in women after the operation of myomectomy is an important medical and social task [1]. This category of women may differ in that anemia (20–60 % of patients) arising from metrorrhagia is determined before and after surgical treatment. Anemic syndrome causes the development of hemic hypoxia with the development of secondary metabolic disorders. Therapy of anemia in patients with uterine myoma is a difficult task. Since, against the background of recurrent menometrorrhagia, a vicious circle is forming – due to the severity of the patient's condition there are no conditions for conservative myomectomy, and the treatment of anemia, for a short period before the next bleeding – is ineffective [2]. Therapy of anemia with donor blood components has its drawbacks: the risk of contracting viral infections, the possibility of severe blood transfusion complications. Therefore, the strategy of the perioperative period in this group of patients should include methods for correcting anemic syndrome and blood saving technologies. The aim of the study was to evaluate the effectiveness of iron carboxymethyltosate for the correction of anemia and tranexamic acid as an inhibitor of fibrinolysis in myomectomy.
Methods and Algorithms: Standard therapy group (*n* 30). In the preoperative period, patients do not receive therapy for anemic syndrome. If necessary, compensation for blood loss will be performed by infusion solutions and, according to indications, blood products. Group therapy (*n* 30) iron deficiency anemia in the preoperative period of iron carboxymethyltosate, with the introduction of inhibitors of fibrinolysis. Assessment of the volume of hemorrhage, hemoglobin, erythrocytes, hematocrit, platelets, lactate, glycemia, use of components of the donor blood was carried out at the four control points.
Results: In the group with infusion of tranexamic acid, blood loss was 28.6 % less than in the group or standard therapy. In the standard therapy group, in 13 % of cases there was a need for intraoperative transfusion of homologous blood. Patients of the other group escaped blood transfusions.
Conclusion: Therapy of iron deficiency anemia in the preoperative period with iron carbomethyltosate in combination with intraoperative administration of fibrinolysis inhibitors has allowed to avoid transfusions of homologous donor blood.

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New indicator for edema evaluation

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Key words: lymphedema, impedance

Motivation and Aim: The magnitude of excess volume of extracellular fluid is an important characteristic of edema of any genesis. The value of specific resistance of soft tissues is inversely proportional to the volume of fluid contained in them.
Methods and Algorithms: We use a device for measuring the active component of the electrical conductivity of biological tissues and liquids, allowing us to perform a selective evaluation of extracellular fluid. A four-electrode contact pad with 4 cm distance between the active electrodes is used, which ensures that the results are obtained predominantly from the subcutaneous fat [1]. To assess the nature and severity of soft tissue swelling, we suggest using an impedance fluid index (IFI), the value of which corresponds to the volume of extracellular fluid per 1000 ml of tissue in the zone of interest. This index is calculated by the following formula: $S/Vt \times 1000$, where S is the siemens (the reciprocal of the ohm, 1*/ohm*), Vt is the volume of the tissue segment being studied. The proposed index is more convenient for use, since its value does not depend on the shape of the contact pad used, and is in direct proportion to the true value of the extracellular fluid volume. 28 women with secondary (postmastectomy) lymphedema of the upper limb were examined. Impedansometry was performed at the beginning and at the end of treatment. Measurements were performed at four symmetrical points on the diseased and healthy limbs: the middle third of the shoulder, the lower third of the shoulder, the upper third of the forearm and the middle third of the forearm.
Results: At admission, the average index of IFI on the intact limb was 0.63 ± 0.04, and on the affected one – 1.13 ± 0.09. Thus, a statistically significant difference in the volume of extracellular fluid in the soft tissues of the upper limbs was found, consisting in a larger volume on the side of the lesion, the asymmetry was 35.6 ± 8.4 %. At the end of the course of treatment IFI on the intact limb remained unchanged – 0.65 ± 0.06. IFI on the affected extremity at discharge was 0.86 ± 0.09, the asymmetry of the indicator was 15.9 ± 9.8 %. Thus, a distinct positive dynamics was registered, consisting in a decrease in the volume of extracellular fluid in the soft tissues of the edematous limb. The data obtained corresponded to the observed clinical results.
Conclusion: The results demonstrate the informativeness and convenience of the proposed indicator when assessing the effectiveness of treatment and the initial status in the case of unilateral lesion. Using IFI in combination with other methods will predict the outcome of treatment and help develop an individual treatment program. Development of normative indicators is required. It is promising to obtain similar results when measuring the impedance of the whole limb tissues and their individual segments.

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Search for a signals of genetic adaptation to high-mountain living in populations of Dagestan

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Key words: compressing autoencoder, genomic data, neural networks

Motivation and Aim: Identification of genetic signals of natural selection using modern genomic and bioinformatic approaches is an important and urgent problem of human genetics. One of the fundamental problems is to analyze the genetic structure of the gene pool of various populations of indigenous people in high mountain areas, for various gene systems, in order to identify the relationship between genetic diversity and climatic conditions of their residence, in terms of genetic adaptation to high altitude conditions and the detection of traces of natural selection and bioinformatic analysis of the biological processes involved in mechanisms of genetic adaptation to high-mountain living.
Methods and Algorithms: We used data of genotyping of chips, obtained from populations of Dagestan living in high and lowland areas [1], containing data on more than 550,000 different SNPs. Using the software package R and the geonames library, there was a certain height above the sea level of the village where the material was collected. Using data on genotype frequencies in each population and altitude above sea level, a correlation coefficient was calculated and SNPs with significant correlation were annotated on different databases using the WebGestalt tool.
Results: We have detected significant correlation of 1663 SNPs with altitude above sea level in Native populations of Daghestan. Two genome regions shows the greatest accumulation of such SNPs are: 9q21.13 (genes TMC1, PCSK5, RORB, TRPM3) and 3p14.2 (genes SYNPR, LINC00698, CADPS, FHIT), as well as many other genes. The WebGestalt annotation of phenotypic manifestations of genes with accumulation of such SNPs showed the following categories: broad phalanges of the hand, broad long bones and aggressive behavior. The following pathways were identified: glutamatergic synapse, glycosaminoglycan biosynthesis, sulfur metabolism, dopaminergic synapse, purine metabolism, focal adhesion, amphetamine addiction and cell adhesion molecules. The main biological processes are the cell-cell adhesion via plasma-membrane adhesion, neuron development, regulation of neuron projection development, ion transmembrane transport and its regulation.
Conclusion: We suppose that genetic diversity in the substantial part of the human genome in Dagestan populations were shaped by adaptation to high-mountain living.
Acknowledgements: This work was supported by the Russian Foundation for Basic Research (grant No. 16-34-60222).

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Continuous real-time observation of behavior of adipose-derived multipotent mesenchymal stromal cell by means of cell-IQ phase-contrast microscopy

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Key words: stem cells, in vitro, morphology, motility, cell division, immunophenotype, secretion

Motivation and Aim: Multipotent mesenchymal stromal cells (MMSCs) are capable to migrate into inflammation and regeneration foci [1]. Continuous monitoring of MMSCs is a promising tool of cellular biology, biotechnology and tissue engineering to study *in vitro* stem cells behavior alone, as well at contacts with other cells (e. g., macrophages) and/or scaffolds for tissue engineering. Human adipose-derived MMSCs (hAMMSCs) culture (cell morphology, motility, cell division, immunophenotype, and secretion) has been investigated with the help of Cell-IQ v2 MLF integrated platform.

Methods and Algorithms: 70 μL suspension (5×10^4 viable karyocytes) of the cells was applied into the center of the well of 12-well plastic plates, and cells were allowed to adhere in a moist chamber for 120 min. Nonadherent cells were washed, and the wells were carefully filled with 1.5 mL of a nutrient medium DMEM/F12 (1:1) without osteogenic additions. Cells were cultured for 14 days at 100 % humidity in a 5 % CO_2 atmosphere at 37 °C until a monolayer formation. In each well, 4 points were chosen for Cell-IQ microscopy around the initial drop of cell suspension. Cell culture images were captured every 45 min. Automatic analysis with the Cell-IQ Imagen software was conducted.

Results: The cells were positively stained with alizarin red (osteoblasts), alcian blue (chondrocytes), or oil red (adipocytes). Attached cells expressed CD73, CD90, and CD105 markers, mainly. Thus, the cells corresponded to the morphological criteria of MMSCs. The Cell-IQ system has allowed establishing 182 μm/h linear velocity of free (until the cell contacts) motility of spindle or fibroblast-like cells. Maximum number of cells achieved 136 cells per field of view; 13–24 % of cells divided each 1–3 h until a monolayer was formed. Chemokine cooperation between hAMMSCs and poor macrophages intermixture was proposed.

Conclusion: Cell-IQ could be useful for *in vitro* real-time imaging of MMSCs and/or their morphofunctional response to natural or artificial (scaffolds) microenvironments.

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Generation of 293FT cell lines deficient in base excision repair and mismatch repair by CRISPR/Cas9-mediated genome editing

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Key words: CRISPR/Cas9, base excision repair, mismatch repair, knockout cell line, 293FT

Motivation and Aim: DNA constantly undergoes chemical modification due to endogenous and exogenous mutagens [1]. Base excision repair is the major guard of genome integrity [2]. Mammalian apurinic/apyrimidinic endonuclease 1 (APEX1) and DNA polymerase β are the most important players of this repair pathway as mice and cell lines deficient in these proteins are not viable. Mismatch repair corrects the replication machinery mistakes [3]. Disruption of *MSH2* gene involving in recognition of mismatch is associated with heredity nonpolyposis colorectal cancer. The aim of our study is to generate *APEX1*, *POLB*, *MSH2* knockout cell lines utilizing the newly established tool for genome editing – CRISPR/Cas9.
Methods and Algorithms: sgRNA is the key component of CRISPR/Cas9 system. The sgRNA design has been carried out using bioinformatic online tool "benchling.com". Three sgRNAs have been choosen for each target. Thereafter each spacer sequence has been cloned into a pX458 vector. Created constructs have been transfected into 293FT cell line using lipofectamine 3000 followed FACS of GFP-positive cells. The efficiency of gene disruption for each Cas9-sgRNA complex has been evaluated using TIDE method based on Sanger sequencing data. For further study sgRNAs with the highest targeting efficiency have been utilized.
Results: sgRNAs with 14.9 %, 39.2 and 29.6 TIDE efficiency score for *APEX1*, *MSH2* and *POLB* corresponding have been taken for generation 293FT knockout cell lines. 133 clones for *APEX1*, 114 clones for *MSH2* and 36 clones for *POLB* have been obtained. Restriction fragment length polymorphism of target loci has been exploited for detection of gene editing events.
Conclusion: Currently, we have verified the presence of frameshift mutation in clones for *APEX1*, *POLB* and *MSH2* knockout cell lines using TIDE method.

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Maturity-onset diabetes of the young due to *HNF1B* mutation: a case report

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Key words: diabetes; gene; hepatocyte nuclear factor-1β

Motivation: Maturity-onset diabetes of the young (MODY) is a group of monogenic disorders characterized by autosomal dominantly inherited non-insulin dependent form of diabetes classically presenting in adolescence or young adults before the age of 25 years. The MODY is a rare cause of diabetes (1 % of all cases) and is frequently misdiagnosed as type 1 diabetes or type 2 diabetes [1, 2]. Mutation in HNF1B, the hepatocyte nuclear factor-1β (HNF-1β) gene, results in MODY type 5, which is characterized by gradual impairment of insulin secretion [3]. The HNF1B plays a crucial role in the regulatory networks that control pancreatic multipotent progenitor cells expansion, acinar cell identity, duct morphogenesis and generation of endocrine precursors [4]. Here we present a clinical course of MODY5 manifested as gestational diabetes in women with *HNF1B* mutation.

C*ase presentation*: A moderate hyperglycemia in a 27-year-old non-obese woman was detected in the first trimester of the third pregnancy in November 2015. Diagnosis of gestational diabetes was established, and insulin therapy was initiated (detemir and aspart 12 U/day). After delivery for two months blood glucose levels returned to normal. Then, hyperglycemia up to 10–15 mmol/l had returned. As metformin and glimepiride therapy was non-effective, insulin detemir was restarted (8 U/day), in combination with sitagliptin and empagliflozin. In October 2016, pancreatic islet and glutamate decarboxylase antibodies were negative, C-peptide level was 553 pmol/l (298–2350 pmol/l) at fasting and 1129 pmol/l after the meal. In September 2017 mutation in *HNF1B* gene was revealed (*rs138986885*), therefore, MODY5 was diagnosed. Thereat, blood glucose levels elevated up to 15–16 mmoL/l, and C-peptide decreased to 83 pmol/l. After metformin and empagliflozin have been withdrawn and basal-bolus insulin therapy with glargine U300 24 U/day and glulisine 22 U/day was initiated, glycemic targets were achieved. No other *HNF1B*-associated clinical phenotypes, including genital malformations or kidney involvement, were revealed in this subject. The same mutation in *HNF1B* was found in normoglycemic mother and daughter of proband. Additionally, it was revealed that proband is the carrier of heterozygous variant of *rs2476601* in the protein tyrosine phosphatase, non-receptor type 22 gene (*PTPN22*), which is associated with increased risk of type 1 diabetes. The combination of these SNPs can be associated with the clinical course of diabetes in this subject.

Conclusion: The present case demonstrates the clinical course of diabetes with progressive insulin deficiency in MODY5 patient.

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Serum levels of WISP1/CCN4 in subjects with type 2 diabetes: the relationships with body fat distribution and adipose tissue dysfunction

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Key words: diabetes, obesity, adipokine, adipose tissue

Motivation and Aim: Dysfunction of adipose tissue (AT) is considered to play an important role in the development of metabolic disorders and complications in type 2 diabetes [1, 2]. Wnt1-inducible signaling pathway protein 1 (WISP1), also known as CCN4, is a member of the CCN family of secreted, extracellular matrix associated signaling proteins. Recently WISP1 was validated as a novel adipokine that may play a role in linking obesity to inflammation and insulin resistance [3]. The data on WISP1 in diabetes are scarce [4, 5]. The aim of our study was to assess the relationships between the circulating WISP1 and the levels of other adipokines, inflammatory markers, fat mass and fat distribution, and parameters of glycemic control in type 2 diabetic subjects.

Methods: We observed 156 patients, 45 M/111 F, from 41 to 80 years of age (median 61 years), including 102 subjects with obesity. The levels of WISP1, high-sensitivity C-reactive protein (hsCRP), alpha1-acid glycoprotein (AGP), and macrophage inflammatory protein 1alpha (MIP-1alpha) were measured in the fasting serum by ELISA. Serum concentrations of leptin, resistin, visfatin, adipsin, adiponectin, IL-6, IL-8, IL-18 and TNF-alpha were determined by Multiplex analysis. Twenty four non-obese non-diabetic subjects, matched by age and sex, were acted as control. The fat mass and distribution was assessed by DEXA. The mean diameter of adipocytes was estimated in the samples of subcutaneous AT in 25 patients. Glucose variability (GV) parameters were derived from continuous glucose monitoring.

Results: Patients with diabetes, as compared to control, had significantly higher levels of WISP1 ($p = 0.02$), leptin ($p = 0.005$), resistin ($p < 0.0001$), adipsin ($p < 0.0001$), visfatin ($p = 0.0003$), hsCRP ($p < 0.0001$), AGP ($p < 0.0001$), MIP-1alpha ($p = 0.006$) and IL-6 ($p = 0.01$). Other investigated molecules did not shown significant differences. Serum WISP1 levels demonstrated positive correlation with percentage of android fat mass ($r = 0.46$, $p < 0.001$). No association with BMI, total fat mass and mean adipocyte diameter was found. The concentrations of WISP1 demonstrated positive correlations with resistin, visfatin and MIP-1alpha levels ($r = 0.36$, $r = 0.28$ and $r = 0.47$ respectively, all $p < 0.01$), but it did not correlated with other inflammatory markers, HbA1c and estimated GV parameters. In a multiple regression analysis android fat mass was the only reliable predictor of serum WISP1 levels (beta = 0.393, $p = 0.04$).

Conclusions: In subjects with type 2 diabetes serum levels of circulating WISP1 are associated with adiposity and adipose tissue dysfunction. The relationships between WISP1 and inflammation need further investigations.

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Switching to insulin glargine 300 U/mL from other basal insulin analogues provides less 24-hour glucose variability in hospitalized patients with type 1 diabetes

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Key words: diabetes, glucose variability, insulin, glargine

Motivation and Aim: Hypoglycemia and high glucose variability (GV) remain serious problems in insulin-treated diabetic subjects [1, 2]. Insulin glargine 300 U/mL (Gla-300) consistently showed comparable glycemic efficacy with less prevalence of hypoglycemia compared with glargine 100 U/mL (Gla-100) in clinical trials [3]. In the real-world settings, switching to Gla-300 reduced the risk of hypoglycaemia in patients with type 2 diabetes when compared with those switching to another basal insulin [4]. We aimed to assess the effect of transition to Gla-300 from other basal insulin analogues on GV parameters in hospitalized patients with type 1 diabetes.
Methods and Algorithms: Twenty six diabetic subjects, 10 M/16 F, from 19 to 67 years of age (median – 44 years), HbA1c from 6.9 to 13.2 % (median – 9.4 %), were switched to Gla-300 from other basal analogues: Gla-100 (n = 15), detemir (n = 10), and degludec (n = 1). Dose titration of Gla-300 was performed in accordance with current recommendations. The GV parameters: High Blood Glucose Index (HBGI), Low Blood Glucose Index (LBGI), Mean Amplitude of Glucose Excursions (MAGE), and Lability Index (LI) were derived from two 3-day 6-point glucose profiles. Wilcoxon matched paired test was applied for comparisons. The data are shown as medians, 25–75 percentiles.
Results: At 6-12 day after transition to Gla-300 mean fasting and postprandial glucose decreased significantly (10.3, 8.1–12.2 vs. 7.8, 6.7–8.8 mmol/l, p = 0.008 and 10.5, 7.9–14.2 vs. 7.9, 6.7–9.6 mmol/l, p = 0.02 resp.). There was decrease in the values of HBGI (11.2, 6.3–18 vs. 6.9, 3.6–12.1, p = 0.01) and LI (3.4, 2.1–6.1 vs. 2.2, 1.6–3.5, p = 0.04), without significant LBGI increment (1.6, 0.5–4.0 vs. 2.7, 0.7–5.0 $(mmol/l)^2/h$, p = 0.16) and MAGE changes (5.7, 3.6–6.8 vs. 4.4, 3.3–6.0 mmol/l, p = 0.32). The mean dose of basal insulin did not change significantly (25.5, 19–32 vs. 27, 18–38 U/day, p = 0.97).
Conclusions: Switching to insulin Gla-300 from other basal insulin analogues provided less 24-hour GV in hospitalized patients with type 1 diabetes.

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Comparative analysis of protein-coding potential of mRNAs and lncRNAs based on sequence features

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Key words: mRNAs, lncRNAs, small ORFs, discriminant analysis, position weight matrix approach

Motivation and Aim: Transcription is one of the vital stages of processing of genetic information in cells leading to the generation of a rather wide spectrum of RNA types, including mRNAs and long non-coding RNAs (lncRNAs). The lncRNAs participate in regulation of transcription, translation, mRNA stability, miRNA generation, etc. Some lncRNAs may have been translated in cells, and understanding the difference between the translated and untranslated lncRNAs is currently the modern challenge in bioinformatics.
Methods and Algorithms: Reliable prediction of translation start sites (TSSs) is the keystone for identification of CDSs in transcripts. For prediction of putative TSSs, we developed a novel method for scoring an arbitrary RNA fragment. This method takes into account not only the cores of putative sites but also the nucleotide flanks surrounding the cores. We also developed a novel method for matrix derivation in the framework of the position weight matrix approach. It exploited our method for scoring the putative TSSs and can derive several matrices for representation cores of TSS.
Results: 1) By application of the proposed method to the training set of TSSs that consisted of experimentally verified sites, we obtained several matrices for representation of TSSs. We have demonstrated that the training set of TSSs was heterogeneous; therefore it is necessary to use several position weight matrices simultaneously in order to achieve high accuracy of TSS prediction. 2) For comparison of protein-coding RNA and lncRNAs, we exploited the discriminant Fisher's model and such features as lengths of CDSs as well as scores of TSSs obtained with the help of the derived matrices. This model discriminated mRNAs and lncRNAs with almost 10 % accuracy. 3) On the base of the matrices derived for TSS prediction, we identified potential CDSs in human lncRNA transcripts available in the Ensembl database (build 38). With the help of detailed statistical analysis of the predicted CDSs, we revealed that lncRNAs were saturated significantly (p-value < 0.01) by small ORFs (length < 300 nt) – the object of intensive modern investigations. In other words, the number of the predicted small ORFs essentially exceeded the number of small ORFs that can be expected at random.

An integrative framework for effective identification of functional disease-associated variants in genome-wide studies

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Key words: SNPs, functional variants, allele-specific events, complex trait disease

Motivation and Aim: With the advent of next-generation sequencing technologies boosting the number of findings, thousands of SNPs that are statistically associated with disease or traits have been identified. While the genome-wide association study (GWAS) remains a powerful and most widely used method to identify disease-associated variants, it does not directly address the biological mechanisms underlying such association signals. Further, numerous disease-associated SNPs detected by GWAS fall into the non-coding regulatory genomic regions. Therefore, detecting and functional annotating of such genetic variation, especially regulatory SNPs (rSNPs) that affect gene transcription are critical to our understanding of genotype–phenotype relationships.
Methods and Algorithms: With the above biological background, here we used our earlier-designed bioinformatic pipeline to identify rSNPs that contribute to disease by combining genetic evidence from GWAS with genome-wide maps of chromatin features (from ChIP-Seq data), gene transcription (from RNA-Seq data) and chromosome interactions (from ChIA-PET data) including in cancer, cognitive disorders and cardiovascular disease. The results were further validated using ENCODE RNA-Seq datasets and 1000genomes project.
Results: Given a list of GWAS-reported variants, we predicted disease-associated rSNPs from the earlier-identified set of 1476 rSNPs that were associated with both allele-specific binding and expression events. Among these, we note thirty-nine GWAS-reported variants for colorectal, breast, pancreatic and lung cancers, Alzheimer's, Parkinson's, inflammatory bowel disease, coronary heart disease and human blood cell traits. Functional annotation of the targeted genes highlighted the key biological pathways for malignancy, cognitive disorders and blood pressure regulation. The results revealed the involvement of processes that relate to the regulation of transcription (notably the abberant RNA splicing) in both colorectal cancer and cardiovascular risk. Notably, the targeted genes for five rSNPs (rs10445033, rs210142, rs210962, rs3744061, rs8106212) were likely relevant for future investigations on drug targets for hypertension according to the recent reports. Further, several targeted genes showed differential expression between patient and control groups for some oncological and cognitive diseases including schizophrenia and bipolar disorder.
Conclusion: Our findings expand the repertoire of functional variation in human genome and offer the biological insights into the genetic mechanisms that contribute to disease. To conclude, our bioinformatics approach provides an efficient platform for narrowing down the list of candidate SNPs prior to functional studies.
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Estimation of memory retention in *db/db* mice, a model of type 2 diabetes, under empagliflozin or combination of empagliflozin and linagliptin treatment

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Key words: type 2 diabetes, Morris test, cognitive function, empagliflozin, linagliptin

Motivation and Aim: Cognitive dysfunction is wide-spread condition associated with type 2 diabetes (T2D), insulin resistance and hyperinsulinemia [1]. The SGLT2 and DPP4 inhibitors are innovative classes of antihyperglycemic agents with numerical pleiotropic effects. The influence of these agents on cognitive function has not been studied yet. The present study estimates the effect of SGLT2 inhibitor empagliflozin and DPP4 inhibitor linagliptin on learning and memory in *db/db* mice, a model of T2D.
Methods and Algorithms: Eight-week-old male and female *db/db* mice (BKS.Cg-Dock7^{m}+/+Lepr^{db}/J) were treated with empagliflozin (10 mg/kg) or combination of empagliflozin and linagliptin (10 mg/kg of each agent), or placebo for 8 weeks. Non-diabetic heterozygous *db/+* mice were acted as control. The concentrations of insulin, glucagon, leptin and ghrelin in blood plasma were determined by Multiplex analysis, and body composition was assessed by MRI at week 0 and 8 of experiment.Short -term learning abilities and memory retention were estimated how was described previously [2]. Short-term memory retention was estimated by Morris test without platform after 4 days previews tests before the experiment. Long-term memory retention was assessed by Morris test without platform after 8 weeks of the treatment.
Results: After 4 days previews tests before the treatment, *db*/*db* mice spent less time in the opposite quarter and more time in the target quarter than *db*/+ mice ($p = 0.0002$ and $p = 0.00009$, respectively). At the end of the experiment, non-diabetic mice improved their results by decreasing time in the opposite quarter ($p = 0.002$) and increasing time in the target one ($p = 0.004$). Treatment with empagliflozin did not change the time in both quarters ($p > 0.17$ for Wilcoxon matched pair test). However, mice received combination spent less time in the opposite quarter than emagliflozin or placebo treated mice ($p = 0.04$).
Conclusion: Diabetic *db/db* mice have better short-term memory retention than non-diabetic mice. Long-term memory retention improves over time in non-diabetic mice, but does not deteriorate in diabetic mice. Combination of empagliflozin and linagliptin may improve memory retention.
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Empagliflozin and linagliptin demonstrate antifibrogenic activity in *db*/*db* mice, a model of type 2 diabetes

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Key words: type 2 diabetes, diabetic nephropathy, empagliflozin, linagliptin

Motivation and Aim: The activation of fibrogenic pathways is proven to be involved in pathogenesis of diabetic nephropathy. Among fibrogenic factors, transforming growth factor β (TGF-β) plays a pivotal role in the kidney fibrogenesis [1, 2]. Recently inhibitors of SGLT2 and DPP4 were posted as the promising therapeutic agents in diabetic kidney disease [3, 4]. Thus, we aimed to assess the effects of SGLT2 inhibitor empagliflozin and DPP4 inhibitor linagliptin on glomerular fibrosis and TGF-β expression in *db/db* diabetic mice, a model of type 2 diabetes.

Methods and Algorithms: Eight-week-old male *db/db* mice were treated with empagliflozin (10 mg/kg), linagliptin (10 mg/kg), combination of these agents, or placebo for 8 weeks. Non-diabetic heterozygous *db/+* mice were acted as control. Urine was collected before and at the end of the experiment. Renal structural changes were analyzed quantitatively from the light microscopic images. TGF-β staining in glomeruli was assessed by immunohistochemistry (*Abcam*, UK).

Results: Diabetic *db/db* mice became obese and hyperglycemic before the start of experiment and demonstrated elevated levels of leptin and insulin and increased fat percentage at week 0 and week 8 (all $p < 0.00001$). Vehicle-treated mice demonstrated higher mesangial volume and larger TGF-β-positive areas in glomeruli as compared to non-diabetic animals ($p = 0.0004$ and $p < 0.00001$ respectively). The TGF-β-positive areas correlated positively with mesangial volume ($r = 0.64$, $p = 0.0006$). Empagliflozin and linagliptin, either alone or in combination with each other, attenuated mesangial expansion ($p = 0.0008$ for empagliflozin, $p = 0.03$ for linagliptin and combination) and decreased TGF-β expression in glomeruli ($p < 0.00001$).

Conclusion: The obtained results demonstrate that empagliflozin and linagliptin could ameliorate fibrotic process in glomeruli in a model of type 2 diabetic nephropathy. The data provide further explanation for the mechanism of nephroprotective effect of SGLT2 and DPP4 inhibitors in diabetes.

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Empagliflozin and linagliptin ameliorate podocyte injury and enhance autophagy in a model of type 2 diabetic nephropathy

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Key words: type 2 diabetes, diabetic nephropathy, empagliflozin, linagliptin

Motivation and Aim: Podocyte injury is believed to be a cornerstone in pathogenesis of kidney disease in diabetes [1]. Recent data indicate emerging role of autophagy downregulation in diabetic podocytopathy [2]. Inhibitors of SGLT2 and DPP4 are considered as promising therapeutic agents in diabetic nephropathy [3, 4]. Thus, we aimed to assess the effects of SGLT2 inhibitor empagliflozin and DPP4 inhibitor linagliptin on podocyte injury and autophagy in a model of type 2 diabetes.
Methods and Algorithms: Eight-week-old male *db/db* mice were treated with empagliflozin (10 mg/kg), linagliptin (10 mg/kg), combination of these agents, or placebo for 8 weeks. Non-diabetic heterozygous *db/+* mice were acted as control. Renal structural changes were analyzed quantitatively from the light and electron microscopic images. To estimate autophagy, beclin-1 staining in glomeruli and volume density (Vv) of autophagosomes, lysosomes, and autolysosomes in podocytes were assessed by immunohistochemistry and electron microscopy, respectively.
Results: Diabetic *db/db* mice became obese and hyperglycemic before the start of experiment and demonstrated elevated levels of leptin and insulin and increased fat percentage at week 0 and week 8 (all $p < 0.00001$). Vehicle-treated diabetic mice had weak staining for beclin-1 in glomeruli and reduced autophagosome number in podocytes. Beclin-1-positive area correlated with Vv of autophagosomes and lysosomes (both $r = 0.43$, $p = 0.04$) and mean width of foot processes ($r = –0.64$, $p = 0.0008$). Under the treatment, glomerular staining for beclin-1 was increased ($p = 0.03$ for empagliflozin, $p = 0.008$ for linagliptin, $p = 0.003$ for combination). Empagliflozin and linagliptin, either alone or both, increased Vv of autophagosomes ($p = 0.04$) and autolysosomes ($p = 0.03$) in podocytes.
Conclusion: The data provide further explanation for the mechanism of nephroprotective effect of SGLT2 and DPP4 inhibitors in diabetes.
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Phenotypes of peripheral dendritic cells in patients with early rheumatoid arthritis

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Key words: Dendritic cells, rheumatoid arthritis, osteoarthritis

Motivation and Aim: Dendritic cells (DCs), a type of antigen-presenting immune cell, play an important role in the pathogenesis of rheumatoid arthritis (RA). They are involved in various mechanisms of the normal and pathological processes of immune system under wide range of diseases. DCs, a potential target for drug development, may be useful for immunopathological disorders correction. The aim of our study was to investigate the subpopulations of peripheral blood DCs (myeloid and plasmacytoid) in patients with early RA.
Methods and Algorithms: Twenty patients with early RA (duration of the disease up to 12 months) were included in the study. The mean age of patients was 57.7 years ± 13.6, the duration of the disease – 8.3–3.0 months. Fifteen patients with osteoarthritis (OA) and without inflammatory arthropathy had formed the control group: the mean age was 61.2 years ± 7.3. Analysis of the content of the B-lymphocytes, myeloid, and plasmacytoid DCs, labeled by antibodies against surface markers, was carried out using a flow cytofluorimeter (BD FACSCantoII, USA) and FacsDiva software. B-lymphocytes, subtypes of peripheral blood DCs were characterized by the following phenotypes: myeloid DCs (CD3-CD14-CD19-HLA-DR + CD11c + CD123-), plasmacytoid DCs (CD3-CD14-CD19-HLA-DR + CD11c-CD123 +), B-lymphocytes (CD19 +). Statistical data processing was carried out using standard approaches on a personal computer using Statistica 10.0 software. Two independent groups had been compared using the Mann-Whitney U test. The differences were considered statistically significant at $p < 0.05$.
Results: The count of plasmacytoid DCs was statistically significant predominated in the group of patients with early RA in comparison with the control group – 3.8 * 106/l vs. 0.85 * 106/l, respectively ($p = 0.008$). Furthermore, the difference was found in the number of cells with the phenotype B-lymphocytes: 8.6 * 106/l vs. 4.5* 106/l, respectively ($p = 0.008$). No significant differences were observed in the number of myeloid DCs.
Conclusion: These data demonstrate the difference in the DCs subtypes ratio in peripheral blood in patients with early RA compared with patients with OA. Circulating plasmacytoid DCs prevail in patients with inflammatory arthropathy. This data indicate the involvement of different DCs subpopulations in the inflammatory process. The results allow to consider the DCs as a potential target for the development of new directions in diagnosis and therapy of immunopathological processes.
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Remote monitoring for the diabetes mellitus type 1 as an effective tool to improve disease compensation

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Key words: diabetes mellitus type 1, telemedicine, remote monitoring, pump insulin therapy

Motivation and Aim: On an outpatient visit, it is not always possible to fully appreciate the variability of glycemia and its indexes, and also to make a careful correction of insulin therapy because of the lack of time for the doctor. The use of remote technologies is a very topical direction in monitoring patients with diabetes mellitus type 1. The aim is to evaluate the clinical and metabolic efficiency of remote monitoring of the children and adolescents with diabetes mellitus type 1.
Materials and methods: The study included 80 patients with diabetes mellitus type 1, aged 8–18 years (12.6±2.8), who were divided into 2 groups: 1 – patients receiving pump insulin therapy with a remote monitoring (40 people). The second group includes patients receiving therapy in the basal-bolus regimen (40 people). All patients were comparable in age and sex.
The first group patients remotely transmitted data on self-monitoring, insulin therapy and diet to the doctor for recommendations, using the program CareLink iPro-2, Guardian (Medtronic, USA). Patients from the second group were visiting a doctor at their place of residence. All patients done analysis of glycated hemoglobin (HbA1c). Using the EasyGV calculator [https://www.phc.ox.ac.uk/research/technology-outputs/easygv], the following indexes were determined: standard deviation (SD), long-term glycemic index (CONGA), hypoglycemia risk index (LBGI), hyperglycemia risk index (HBGI), the average amplitude of glycemic fluctuation (MAGE), M-value. The statistical processing of the results was carried out using the IBM SPSS Statistics 20.0.0 program. For abnormally distributed parameters, we calculated the quartiles (Me, Q1–Q3). The significance of the differences was evaluated according to the Mann–Whitney U test. Significant differences were considered when $p < 0.05$.
Results: When comparing fasting glycemia 8.8(8.2–9.7) mmol/L and before sleep 9.65(8.7–10.9) mmol/L in two groups, these measurements had a significant difference ($p = 0.001$). The average of glycemia (in group 1 – 9.55(7.85–10.65), in group 2 – 11.1(9.3–13.4)) had a significant difference ($p = 0.007$). HbA1c had a significant decrease in the measurements in group 1 patients compared to group 2 ($\chi^2 = –0.450$, $p = 0.014$). Since glycated hemoglobin does not always reliably reflect the level of compensation, an analysis of the variability parameters, which was lower in group 1 than in the 2: SD ($\chi^2 = 0.022$, $p = 0.022$), CONGA ($\chi^2 = –0.853$, $p = 0.001$), J-index ($\chi^2 = –0.504$, $p = 0{,}005$), LBGI ($\chi^2 = –0{,}451$, $p = 0.014$), HBGI ($\chi^2 = –0.053$, $p = 0.003$), MAGE ($\chi^2 = –0.480$, $p = 0.008$), M value ($\chi^2 = –0.593$, $p = 0.001$).
Conclusions: Remote monitoring of patients with diabetes mellitus type 1 is an effective method of observation and leads to a decrease in the variability of glycemia and improvement of disease compensation.

Comparative pharmacokinetic analysis of a novel prolonged release dosage form of lithium citrate in mice

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Key words: *lithium, pharmacokinetics, aluminium oxide, organosilicone polymer*

Motivation and Aim: In clinical practice use of lithium preparations is limited due to difficult adjustment of drug dosage, necessity of monitoring its concentration in blood, side effects development as a result of accumulation of lithium in a body. For the purpose of improvement of pharmacologic properties lithium is combined with other agents (for example modifying sorbent) thus it can produce longer-term and more harmless (less side reactions) effect in the long view [1, 2]. Lithium immobilization on sorption basis will allow to use sorbent as carrying agent of drugs to body. This study assessed the comparative pharmacokinetics of a novel prolonged release dosage form of lithium citrate in white outbred mature mice – males after single intragastrically administration

Methods and Algorithms: In the experiment mice were divided into two groups (8–10 animals each group) which were received lithium citrate (LC) (75 mg/kg) or complex based on lithium citrate, aluminum oxide and organosilicone polymer (LCAS) (1120 mg/kg) once intragastrically. These doses were calculated based on lithium containing at the ratio 5.6 mg/kg. Decapitation of mice was made first time after 15 minutes since study samples injection, than it was repeated after 30 minutes, 1 hour, 2 hours, 6 hours, 24 hours, 30 hours and 48 hours. Pharmacokinetic parameters and relative bioavailability were calculated based on lithium ions concentration in serum and brain, which was measured by inductively-coupled plasma atomic emission spectrometry (ICP-AES). The results were processed using STATISTICA 6.0 statistical program.

Results: We determined differences between kinetics of LC and LCAS at the absorption phase: Cmax and Tmax was reducing when LC with supportive porous material were administered. Lithium concentration in serum increased since 15 minutes after administration of LC and maximum equaling 12.1 μg/ml was achieved in 2 hours. As for LCAS, lithium concentration in serum had been increasing during the first hour after its administration, maximum concentration equaling 2.80 μg/ml was achieved in an hour and it was lower than LC parameters, relative bioavailability of LCAS is 44.41 % of standard LC. Maximum concentration of lithium ions is lower by 4.3 times, than if administration of LC. Lithium concentration in brain increased half an hour later after LC administration, maximum concentration equaling 1.03 μg/g was achieved in 6 hours. LCAS increased lithium concentration in brain approximately an hour later since its administration. Maximum concentration was identified 2 hours later and was equal to 0.29 μg/g, which was 3.5 times lower in comparison with LC.

Conclusion: Performed research has proven that combining aluminium oxide and organosilicone polymer as supportive components with lithium citrate helps to maintaining a stable lithium ions concentration in blood and brain which is important for achieving positive lithium therapy effect.

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Relationship of glutamatergic and autistic gene expression in the hippocampus of male mice with disturbances of social behavior

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Key words: agonistic interactions, hippocampus, autistic spectrum disorder, glutamatergic genes

Motivation and Aim: There is evidence in support of glutamate abnormalities in autism. Our previous studies have shown that negative social experience in daily agonistic interactions leads to impairment of social behavior which is accompanied by decrease in communication, disturbance of socialization, the emergence of stereotyped behaviors in male mice which may be considered as symptoms of the autistic spectrum disorders. The aim was to study the differentially expressed glutamatergic and autistic genes in the hippocampus of animals with impaired social behaviors caused by repeated social defeat stress and, for comparison, repeated experience of aggression.
Methods: The repeated aggression and defeats in male mice were generated during 20 days. The social behaviors in animals were studied in different behavioral tests. The hippocampus, which is involved in the mechanism of autism, was sequenced at JSC Genoanalytica (http://genoanalytica.ru/, Moscow, Russia), where the mRNA was extracted using the Dynabeads mRNA Purification Kit (Ambion, USA). The Cufflinks program was used to estimate the gene expression levels in FPKM units. Bioinformatic methods were used for the analysis of differentially expressed genes in male mice.
Results: This study confirmed the results obtained earlier: disturbances in social behavior may develop in male mice under chronic intermale confrontations. So, the defeated mice exhibited an avoidance of social contacts toward unfamiliar conspecific, immobility and low communication on neutral territory. The winners demonstrated aggression and hyperactivity in this condition. These symptoms were similar to the symptoms observed in animal models of autistic spectrum disorders. Transcriptomic analysis revealed decreased expression of autistic *Shank3*, *Auts2*, *Ctnnd2*, and *Nrxn2* genes, and decreased expression of glutamatergic *Grm4* gene in aggressive animals. In the defeated mice increased expression of autistic *Shank2*, *Nlgn2*, *Ptcdh10*, *Reln*, and *Arx* genes and glutamatergic *Grik3*, *Grm2*, *Grm4*,*Slc17a7*, *Slc1a4*, and *Slc25a22* genes excluding the *Grin2a* gene were found. Correlation analysis revealed a reliable relationship between the altered expression of glutamatergic and autistic genes in the hippocampus of male mice. According to the bioinformatic STRING (string-db.org) database, the *Shank3* gene may carry out the interactions of differentially expressed genes in aggressive animals, while the *Grin2a* gene plays a key role in defeated animals.
Conclusion: The obtained results, on the one hand, confirm role of the glutamatergic system and glutamate receptors in the pathology of autistic spectrum symptoms. On the other hand, we can assume the co-expression of autistic and glutamatergic genes that can develop in the hippocampus of animals with disturbances of social behavior induced by the influence of social environment.
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Pathogenesis of cerebral cortex damage in mice during infection with seasonal influenza A virus

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Key words: influenza A/H1N1 virus, frontal cortex, somatomotor cortex, somatosensory cortex, edema, M1/M2 polarization of microglial cells

Motivation and Aim: Influenza A viruses infect various animals, from birds to humans. In addition to respiratory damage, acute viral infection in the human body can lead to the development of central nervous system damage. This is accompanied by progressive encephalopathy combined with severe cephalgia, as well as coma against the backdrop of increasing brain edema, up to a lethal outcome. However, the mechanisms of various structures of the brain damage induction are controversial: on the one hand, they can be associated with the primary response of cells in response to the presence of the virus in the CNS tissue, and on the other, result from a systemic immune response secondary to the infectious pathogen. Thus, it is necessary to study the potential of seasonal viruses as probable initiators of predetermining neural insults, which can provide neuroprotection by limiting inflammation for future CNS insults.
Methods and Algorithms: The study has been conducted on 60 6–8-week-old male BALB/c mice, divided into control and infected intranasally with 1 MLD_{50} seasonal influenza strain A/H1N1 A/Tomsk/13/2010 groups. The infection in mice was verified by the lung section histology and the virus titrating on MDCK cells. Lung and brain samples were obtained on 1, 3, 6, 10, 14, 21 and 30 days after infection. To further study the sections were treated by standard histological methods and IHC analysis with specifics primary antibodies [1]. It was performed histological evaluation of destructive changes, angiogenesis, and M1/M2 polarization of microglial cells.
Results: IHC analysis on the influenza virus antigen in brain cells and the virus titration from brain homogenate to MDCK cells was not detected. In all investigated sections extensive areas of pericellular and perivascular edema, gliocytosis and single hemorrhage zones were recorded. It should be noted the activation of neoangiogenesis with an increase in the number of CD34+ newly formed vessels. Beginning with the 3rd day of the experiment, the percentage of neurons, astrocytes and microglia cells in the state of apoptosis increased. At the same time, the microglia phenotype did not change from m1 to m2, which indicates a prolonged inflammatory response.
Conclusion: Probably destructive changes in the cortex and inflammatory response with M1 microglia polarization is secondary to the viral infection. The obtained data demonstrate the potential of non-neurotropic viruses initiate CNS lesions.
Acknowledgements: The reported study was funded by RFBR according to the research project No. 18-315-00257.

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ADRB1 gene polymorphism and pro-arrhythmic electrocardiographic patterns in the general population of Novosibirsk

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Key words: ADRB1 gene polymorphism, Brugada pattern, Early Repolarization, QRS fragmentation, Metabolic Syndrome, General Population

Motivation and Aim: Brugada, Early Repolarization and QRS fragmentation patterns have predictive significance in relation to sudden cardiac death. Adverse electrophysiological changes occur not only as a result of the rare congenital membrane channelopathies, but mostly are secondary, due to the various due to the various factors. They, in turn, may also be genetically determined. Therefore, electrical instability of the myocardium, obviously, has a multigenetic and multifactorial basis.

The objective is to study pro-arrhythmic electrocardiographic patterns association with ADRB1 gene polymorphism and the components of metabolic syndrome in the general male population of Novosibirsk.

Methods and Algorithms: The data was formed of the representative sample of 831 males aged 25-64 years from the general population of Novosibirsk (WHO "MONICA" project). For defining A145G, Ser49Gly (rs1801252) – ADRB1 gene polymorphism were random selected 195 people. Brugada, Early Repolarization and QRS fragmentation patterns were measured in accordance with recommended current criteria in 30 % sub-sample – 261 people. The study included individuals who had both genetic and electrocardiographic data – 105 people. The components of the metabolic syndrome were evaluated according to the WHO criteria. Data analysis was performed in a multivariate general linear model (GLM).

Results: QRS fragmentation in inferior leads was independently associated with ADRB1 gene polymorphism ($F = 9.3$; $p = 0.00019$), obesity ($F = 6.3$; $p = 0.014$), elevated triglyceride level ($F = 4.3$; $p = 0\ 040$) and reduced level of high-density lipoproteins ($F = 12\ 0$; $p = 0.00079$).

The frequency of the QRS fragmentation pattern in groups of individuals with different ADRB1-genotypes was as follows: in group AA – 3.9 %, in group AG – 4.3 %, in group GG – 40.0 %.

Conclusion: The results indicate the prospects of the integral approach in the analysis potential factors of occurrence of adverse electrophysiological changes in the myocardium.

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Evolutionary analysis and mathematical modeling of gene networks of energy metabolism disorders

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Key words: diabetes, metabolic pathway, gene regulatory network, evolution, phylostratigraphic analysis, mathematical modelling

Motivation and Aim: Obesity, type 2 diabetes and neurodegenerative diseases are global problems of the humankind in the XXI century. The common mechanism of these socially significant diseases is the insulin resistance syndrome associated with energy metabolism disorders. There is still a lack of understanding of evolutionary-genetic aspects of the origin of insulin resistance and associated phenotypes. The aim of the study was: (1) to analyze several gene networks of energy metabolism disorders from the evolutionary point of view; (2) to perform sensitivity analysis for mathematical models obtained from the literature; (3) to assess the relationships between the sensitivity of the dynamics of the gene network to variations in the model parameters and a number of evolutionary and functional indices of the genes that determine these parameters.

Methods and Algorithms: We obtained gene networks of diseases from KEGG database (http://www.kegg.jp/). Orthoscape software [1] was used to calculate evolutionary indices like phylostratigraphic age index of a gene and the Darwinian selection index (Ka/Ks). Mathematical models obtained from literature [2–4] were analyzed using COPASI software (http://copasi.org/). Comprehensive statistical analysis was performed with Python for data analysis (NumPy, SciPy and Pandas).

Results: Our preliminary results demonstrate that substantial role in development of energy metabolism disorders is played by genes of recent evolutionary origin, which are still under strong selection. The study reveals common evolutionary-genetic mechanisms in the development of socially significant diseases of the technogenic civilization era: obesity, type 2 diabetes, and dementia.

Acknowledgements: The study is supported by SB RAS Integration Project No. 0324-2018-0021.

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Ultrasound technologies for quantitative characterization of insulin-induced lipohypertrophy in subjects with diabetes

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Key words: diabetes, insulin, ultrasound. lipohypertrophy

Motivation and Aim: Recent studies demonstrated the applicability of ultrasound scan for diagnostics of insulin-induced lipohypertrophy [1, 2]. The aim of our study was to assess the applicability of Gray-Scale Densitometry, Strain Elastography and 3D power Doppler ultrasound for quantitative characterization of lipohypertrophy in insulin-treated diabetic subjects.

Methods and Algorithms: Eighty two adult subjects, 27M/55F, with duration of insulin therapy for more than 3 months, were recruited consecutively. Among them, 26 ones had type 1 diabetes and 56 individuals had type 2 diabetes. The visualization algorithm included Gray-Scale Densitometry with Mean Gray Value (MGV) index estimation, Strain Elastography with Strain Ratio (StR) calculation, and 3D power Doppler ultrasound with Vascularization Index (VI), Flow Index (FI), and Vascularization Flow Index (VFI) assessment. The *ELASTOGRAPHY ADVANCED 4D*, *OmniView+VCI*, *VOLUME CALCULATION II* (*VOCAL*) options were applied for imaging.

Results: Lipohypertrophy was revealed by palpation and ultrasound in 57 and 80 patients (70 % and 98 %) respectively. The aggregated ultrasound-verified lipohypertrophy square (LS) varied from 50 to 1847 mm^2 (median 370 mm^2). Most of the lipohypertrophy sites demonstrated hyperechogenicity and increased stiffness when compared to surrounding subcutaneous fat (MGV and StR indices: $p < 0.001$). The reduced vascularity in lipohypertrophy areas were confirmed by 3D power Doppler ultrasound vascular indices (all $p < 0.05$). Total LS and MGV showed weak positive correlations with daily insulin dose (both $r = 0.3$, $p = 0.006$), however in patients with type 1 diabetes LS correlated with insulin dose more closely ($r = 0.47$, $p = 0.02$). Patients receiving insulin analogues had smaller aggregated LS than those on human insulin ($p = 0.03$). The LS demonstrated positive correlations with mean postprandial glucose ($r = 0.35$, $p = 0.001$) and triglycerides levels ($r = 0.4$, $p = 0.0002$). The FI index, but not VI and VFI, correlated negatively with mean postprandial glucose ($r = –0.29$, $p = 0.01$). The levels of HbA1c showed no association with ultrasound parameters.

Conclusion: The Gray-Scale Densitometry, Strain Elastography and 3D power Doppler ultrasound provide comprehensive quantitative characteristic of the areas of lipohypertrophy in insulin-treated diabetic subjects.

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New regulatory SNP associated with colorectal cancer

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Key words: regulatory SNPs, genotyping, colorectal cancer, functional analysis

Motivation and Aim: A central goal of genetics is to understand the links between genetic variation and disease. Recent genome-wide association studies (GWASs) bring us closer to this goal, but GWASs alone are unable to pinpoint disease-causing SNPs. The noncoding SNPs, which represent the majority of GWAS SNPs, present a particular challenge [1]. Earlier we have developed a new approach based on vast amounts of genomic, transcriptomic and association data, permitted to select regulatory SNPs (rSNPs) in the human genome and to reveal potential disease drivers. The purpose of the work was to study the association of six predicted rSNPs with colorectal cancer (CRC) and to perform their selective functional analysis.

Methods and Algorithms: Case-control study involved 195 CRC patients, and 194 control individuals. Genotyping was done by the allele-specific PCR. DNA pulldown experiments followed by quantitative mass spectrometry were performed using rat liver nuclear extract and 30 bp biotin-tagged oligonucleotides containing the A or T allele at rs2072580.

Results: The major C allele (rs590352 in the promoter region of *ATXN7L3B*) was shown to be significantly associated with predisposition to CRC. Regulatory significance of rs2072580 in promoter region of *SART* gene was demonstrated: it was found that rs2072580 results in differential binding patterns of nuclear proteins to biotinylated oligonucleotide probes corresponding two alternative alleles.

Conclusion: Taken together, our results progress the understanding of the regulatory and pathological significance of rSNPs under study. Since both *ATXN7L3B* and *SART* genes encode important components of cell regulatory systems, participating in transcription control, splicing and protein degradation, rSNPs in promoters of these genes may be linked with broad system effects and so are very promising for further investigation.

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Simulation of circadian temporal organization of the lymph node system using the automated expert program SMLN

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Key words: lymphoid cells, migration, thymus, spleen, circadian rhythm

Motivation and Aim: The processes of cell migration, recirculation, proliferation, differentiation in the lymphoid system are characterized by dynamism and the presence of pronounced circadian rhythms [1]. The combination of somatic and visceral lymph nodes plays a leading role in the formation of the daily internal temporal order of the lymphoid system. Using the apparatus of mathematical modeling of the lymphocytes movement provides an integrative picture of the temporal organization of cellular and tissue kinetics of lymph nodes of different locations.
Methods and Algorithms: Quantitative results on the lymph node cell count in male mice (CBAxC57Bl/6)F1, CBA and C57Bl/6 during the 24-hour cycle were interpreted using the automated expert system SMLN. The program algorithm allows evaluating statistically the directions of movement of cellular elements in different segments of the daily cycle, their power and the average migration rate [1, 2].
Results: The formation of daily biorhythms of cellular composition, activity of drainage systems, lymphoid structures and microenvironment of lymph nodes in connection with anatomical regions of the location of lymph nodes, differences in diet, motor activity and sleep-wakefulness has been characterized. The influence of the genetic factor on the circadian space-time organization of cell movements in the lymph nodes of different localization has been revealed.
Conclusion: Thus, the use of the original automated expert system SMLN allowed to characterize the daily space-time organization of the lymph nodes as a part of the circadian organization of the lymphoid system consisting of the interchange in the 24-hour period of temporary morphofunctional complexes of activity of dynamic and stationary factors of the microenvironment, the number of lymphoid cells, vascular and sinus systems, which causes differences in the readiness of the immune system to respond to the daily cycle [3].

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Primate-specific long non-coding RNA (LncRNA) genes as regulators and drug target candidates in human cancer and diabetes

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Key words: long non-coding RNA, lncRNA, Genome-Wide Association Studies, GWAS, primate-specific genes, personalized medicine, breast cancer, metabolic disease, diabetes

Motivation and Aim: Most human genes do not encode proteins [1]. LncRNA genes are a key class of non-coding RNA genes. Over a decade ago, we exposed the lack of evolutionary conservation at lncRNA loci, highlighting primate-specific lncRNAs as putative contributors to primate-rodent phenotypic differences [2]. Unlike protein-coding genes, most human lncRNA genes are not conserved beyond primates [3–5]. Here, we tested whether primate-specific lncRNAs drive human disease phenotypes.

Methods and Algorithms: Breast cancer: we utilized RNAseq and custom microarrays to catalog the estrogen-responsive lncRNAome of human estrogen receptor positive breast cancer cells. We performed cell viability and proliferation assays (MTT and crystal violet) to quantify the impact of overexpression, and siRNA knockdowns of, estrogen-responsive lncRNAs on cellular phenotypes. Diabetes: we performed a global, disease-agnostic computational intersection of all SNPs (single-nucleotide polymorphisms) significantly associated in Genome-Wide Association Studies (GWAS) with any human disease in any published peer-reviewed study, with all exons of known lncRNAs, identifying all lncRNA-exonic SNPs associated with disease. Both projects: we used human-mouse reciprocal BLAST and BLAT of repeat-masked full-length transcripts and genomic regions. We manually annotated key gene structure elements (splice sites and polyadenylation signals) in 100-species MultiZ alignments in the UCSC Genome Browser to gauge primate-specificity of gene structures.

Results: Breast cancer: we identified 44 estrogen-induced putatively oncogenic, and 83 estrogen-repressed putatively tumor suppressive, lncRNAs. BC041455, an estrogen-repressed primate-specific lncRNA, decreased ERK phosphorylation, indicating that primate-specific lncRNAs can impact the conserved MAP kinase pathway [6]. 60 (47 %) of the 127 lncRNAs were primate-specific, with 24 originating after the prosimian split. None were segmentally duplicated or repetitive. This implicates *de novo* gene birth within a Gouldian exaptation paradigm, rather than duplications or repeat dispersions, in the origin of these lncRNAs in the common ancestor of Old and New World primates. Furthemore, we have used RNAseq, Ribo-seq, and mass spectrometry to show that persistent in-frame ribosomal stop-codon mistranslation of one of these lncRNAs yields unanticipated, primate-specific peptides with putative autoimmunity and cancer roles. Diabetes: our top hit from 20 independent public GWAS datasets was the SNP rs4841132 in the primate-specific lncRNA LOC157273, associated with a wide spectrum of metabolic-disease quantitative traits. We show that LOC157273 in primary human hepatocytes represses its nearest-neighbor gene PPP1R3B, which controls glycogen storage and hence fasting glucose levels. The minor, disease risk allele (A) appears to be unique to modern humans and Neanderthals, whereas all known Denisovan, and most non-human primate, sequences exhibit the major allele (G) at this site. We infer that changes or selective pressures during recent human evolution may have been responsible for the origin of this risk allele.

Conclusion: Primate-specific lncRNAs bridge evolution and health by contributing to human disease phenotypes. High-throughput cellular screening, as well as GWAS followed by cell-based validation of individual candidates, can be used to identify candidate disease causative lncRNAs. In vivo studies are limited by the lack of orthologs in nonprimate animal models. As evolutionarily young network nodes, and as new regulatory switches upstream of ancient networks, primate-specific lncRNAs are connected only by limited, sparse edges to older, conserved network components, because they have not yet had the time to become more deeply embedded in disease networks. Therefore, modulating them in therapeutics may yield fewer side effects than current small-molecule-based, protein-targeting drugs.

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Epigenetic changes of MMSCs under the influence of calcium phosphate coating with different roughness

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Key words: human adipose-derived stromal cells, gene expression, osteogenic differentiation, microarc calcium phosphate coating, *in vitro*

Motivation and Aim: Topography of calcium phosphate (CP) coating, stimulating osteodifferentiation of multipotent mesenchymal stromal cells (MMSCs), plays a fundamental role in the successful integration of implants for bone bioengineering. The aim of the research was to study the osteodifferentiation genes expression of in the MMSCs culture under in vitro conditions of contact with a relief CP coating simulating the state of the natural mineral matrix during the regeneration of bone tissue.

Methods and Algorithms: 3D matrices ($12 \times 12 \times 1$ mm^3) made of commercially pure titanium with a relief bilateral calcium phosphate coating with a roughness index Ra = 2.2 – 3.0 μm (model of physiological bone regeneration) and Ra = 3.1 – 4.5 μm (model of excessive (pathological) bone repair). MMSCs were isolated from human lipoaspirate (Resolution No. 7 of 09.12.2015 Local Ethics Committee, IKBFU). Cultivation of MMSCs with 3D matrices under conditions of indirect contact for 14 days in a standard culture medium based on DMEM/F12 at 37 °C, humidity 100 % in an atmosphere of 5 % CO_2. The affiliation of cells to the MMSCs pool is confirmed by the expression of CD73, 90, 105 and the ability to differentiate in 3 directions in specialized serum-free media. The level of relative gene expression (*RUNX2*, *FGF10*, *BMP2*) was studied by qPCR. Data was analyzed using IBM SPSS Statistics 20.

Results: 3D matrices with a surface roughness simulating the model of physiological bone regeneration had an inducing effect on the relative expression of the *RUNX2*, *FGF10* and *BMP2* genes, suggesting a mild epigenetic activity of the degradation products of the CP coating (calcium and phosphorus ions) against hAMMSCs. At the same time, in the experimental model of pathological bone repair, the level of relative expression of the studied genes was comparable to the control values obtained during the cultivation of MMSCs without 3D matrices.

Conclusion: The different distant (epi) genomic response of hAMMSCs to CP coatings with a different roughness range requires further study.

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Bone marrow hematopoiesis and peripheral blood parameters in mice with chronic opisthorchiasis and social stress

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Key words: mice, *Opisthorchis felineus*, social stress, blood cells, red bone marrow, hematopoiesis, biochemical blood parameters

Motivation and Aim: *Opisthorchis felineus* is neglected liver fluke causative agent of opisthorchiasis. The world's largest area of this disease is the Ob-Irtysh river basin in Russia. The parasites inhabiting in the liver bile ducts and gallbladder of the definitive host live a long time and can cause serious complications. Currently, it is impossible to imagine the life of a person without encountering any kind of stresses, be it everyday life or occupational problems. Combination of chronic opisthorchiasis and social stress is a more real situation for people. The goal of this work was to study the model of C57BL/6 mice infected with *O. felineus* larvae on the background of a social stress at final stages of the disease progression. The specific features of bone marrow hematopoiesis, peripheral blood cell composition and biochemical parameters were examined.
Methods and Algorithms: We evaluated the effects of *O. felineus* ("OP" group) and 30-day social stress (intermale confrontations, "SS" group) alone and in combination ("OP+SS" group) in inbred C57BL/6 male mice and compared these effects according to the parameters listed below. The animals exposed to neither factor formed the control group ("CON").
Results: By the end of the experiment, we have observed crucial effects of the two factors on the blood and liver of "OP" and "OP+SS" mice whose content of adult flukes in the bile ducts was as low as 3 to 6 %. Eosinophil and basophil counts increased and relative segmented neutrophil and monocyte counts decreased in "OP+SS" mice on the background of activated myelopoiesis, mainly determined by social stress. Despite depressed erythropoiesis, "OP" mice displayed no changes in the relative peripheral erythrocyte counts in the blood. On the contrary, social stress, which stimulated erythropoiesis in "SS" and "OP+SS" mice, was accompanied by a decrease in the relative erythrocyte counts and hematocrit. Hepatosplenomegaly was observed on the background of these two impacts; possibly, part of erythrocytes was sequestered by the spleen. Changes in transaminase (ALT and AST) and alkaline phosphatase activities as well as an increase in cholesterol and products of lipid peroxidation suggest a pronounced destruction of the liver in "OP+SS" mice.
Conclusion: Thus, social stress exacerbates many of the assayed parameters in the mice infected with the liver fluke.
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Erythropoietin augment therapeutic potential of mesenchymal stem cells in rat with degenerate intervertebral disc

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Key words: mesenchymal stem cells, hypoxia, puncture of the intervertebral disc, erythropoietin

Motivation and Aim: Mesenchymal stem cells are widely used in cellular therapy of degenerative diseases, including and disease of the spine [1, 2]. The effectiveness of the treatment cells depends on their survival at the site of implantation. The aim of the study was to conduct a comparative study of clinical efficacy of treatment of degeneration of the intervertebral disc in rats Wistar mesenchyme cells without and with addition of erythropoietin.

Methods and Algorithms: Mesenchymal stem cells isolated from nucleated bone marrow cells and evaluated the morphological and functional properties, puncture of the intervertebral disc was modeled degenerative process, cells were injected once into the region of the vertebral disk, the treatment effect is tracked in dynamics using magnetic resonance imaging and histological examination.

Results: It is shown that erythropoietin stimulates proliferation under hypoxia. Introduction in the damaged intervertebral disc bone marrow multipotent mesenchymal stem cells promotes an increase in height of the intervertebral disc and the activation of the reparation of the nucleus pulposus. The combination of the introduction of mesenchymal stem cells with erythropoietin, gives the best effect of cell therapy in degenerative damage to the intervertebral disc.

Conclusion: The combined application of mesenchymal stem cells with erythropoietin increases therapeutic potential.

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Autologous plasma enriched with platelet lysate for treatment in patients with age-related macular degeneration

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Key words: autologous plasma enriched with platelet lysate, age-related macular degeneration, visual acuity, optical coherence tomography

Motivation and Aim: Plasma enriched in growth factors, is widely used in medical practice [1, 2]. However, the clinical efficacy of its use in the treatment of age-related retinal integrity violations investigated enough. The aim of the study was the evaluation of the clinical efficacy of treatment of age-related macular degeneration by autologous plasma enriched with platelet lysate.

Methods and Algorithms: Autologous plasma enriched with platelet lysate was received from peripheral blood. Assessed Visual acuity, intraocular pressure, conducting optical coherent tomography eyes on the side of the pathological process.

Results: Were showed that the combination of a standard 3-port transconjunctival subtotal vitrectomy with the subsequent tamponade of the gap by the autologous plasma enriched with platelet lysate and injections of the autologous plasma enriched with platelet lysate in the area of pterygopalatine fossa on the side of the operated eyes statistically significantly promoted recovery of visual acuity in the early postoperative period (15 days) and late period (90 days) compared with patients who received only surgical treatment ($p \leq 0.05$). Treatment by the autologous plasma enriched with platelet lysate increased the closing rate of the tearing of the retina in the macular region up to 62.5 %, while only surgical treatment leads to the closure of the defect of the retina in 37.5 % of cases. It is shown that autologous plasma enriched with platelet lysate contained cytokines, growth factors and nitric oxide, which is involved in the regeneration/reparation of the retina.

Conclusion: The course of injections of autologous plasma enriched with platelet lysate in patients with age-related macular degeneration is accelerating the closure of retinal tears of the eye and improves visual acuity.

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Development and validation of experimental cholangiocarcinoma models for *ex vivo* and *in vivo* analysis

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Key words: *Opisthorchis felineus*, cholangiocarcinoma, biomarkers, model, translational medicine

Motivation and Aim: It is known that one of the most serious complications of opisthorchiasis is bile duct cancer, cholangiocarcinoma (CCA). It is an aggressive tumor that is difficult to treat and diagnose at an early stage. Recently we developed a model of experimental CCA on Syrian hamsters *Mesocricetus auratus* infected with opisthorchiasis liver fluke *Opisthorchis felineus* and treated with dimethylnitrosamine (DMN). However, the markers of the obtained tumors were not described sufficiently. The aim of this work was to validate experimental models of bile duct tumors associated with *O. felineus* invasion using markers of human CCA.

Methods and Algorithms: We used the model of experimental CCA [1], and a cell line obtained from experimental CCA. To validate CCA models standard and potential tumor markers were identified. In particular, by the methods of immunohistochemistry and real-time PCR we determined the expression of the specific markers: keratin 7 (CK7), vimentin (VIM), annexin A1 (ANXA1) and exostosin 1 (EXT1). To compare the data between groups, statistical significance was determined by Mann–Whitney U test ($P < 0.05$).

Results: In *O. felineus*-infection animal expression of CK7 was observed in the areas of bile duct proliferation, cysts, cholangiofibrosis. Additionally, VIM was intensively expressed in the areas of bile duct proliferation and periductal fibrosis. Tumors were positive for CK7 and VIM expression. The ANXA1 marker, considered as a potential marker of *O. viverrini*-associated CCA in *O. felineus*-infected hamster, was found generally in inflammatory cells and single cholangiocytes. Besides, the potential marker Ext1 was observed in vascular walls, portal area of liver and cholangiocytes in tumor tissue.

Real-time PCR showed that the expression level of all investigated markers significantly increased in tumor tissue compared to the control liver. Additionally, immunocytochemistry analysis of the cell culture revealed positive staining of all markers, especially CK7.

Conclusion: The findings indicate that in the experimental models the expression of standard and potential tumor markers corresponds to the literature data. Thereby, the models can be used for studying cholangiocarcinogenesis, as well as for searching CCA treatments and methods for early diagnosis of the disease.

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DNA methylation and expression of microRNA genes in unstable carotid atherosclerotic plaques

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Key words: DNA methylation, microRNA, atherosclerosis

Motivation and Aim: Unstable atherosclerotic lesion, unlike stable fibrous plaque, is characterized by a large lipid core, thin ruptured cap, high immune cells content, and prone to thrombosis and hemorrhage. MicroRNAs (miRs) known to be involved in pathological processes during atherogenesis, but the involvement of miRs in destabilization of atherosclerotic plaque is under-investigated [2]. To address this problem, we compared DNA methylation and expression profiles of miR genes in cells from stable and unstable atherosclerotic plaques of carotid arteries.

Methods and Algorithms: Stable (n = 8) and unstable (n = 8) samples of carotid atherosclerotic plaques were collected from 16 patients (6 males, 10 females, aged 65 ± 6 years) and verified by histopathologist. DNA and RNA were extracted from the adjacent sections of sample. DNA methylation was evaluated using microarrays Infinium MethylationEPIC BeadChip (Illumina), comprising 9906 CpG sites within ± 1.5 kb regions from known microRNA genes. MicroRNA expression in plaques was detected using NEBNext library preparation kit, HiSeq 1500 sequencer and miARma-Seq pipeline [2]. Following data analysis was performed in R and Bioconductor.

Results: We showed that 116 sites (101 miR genes) were hypomethylated and 4 sites (4 miR genes) – hypermethylated in unstable plaques compared with stable samples ($p < 0.01$). But the effect size was moderate (only 8 CpG sites had difference of beta values between groups > 0.1). We found 277 ± 44 miRs to be expressed in carotid atherosclerotic plaques. 161 miRs were shared between all samples and had expression level > 10 CPM, and 13 of them was differentially expressed between stable and unstable samples ($p < 0.05$). Only 3 miR genes (*MIR136*, *MIR149*, *MIR7704*) had both differential expression and DNA methylation, according to the results of this study.

Conclusion: Cells of unstable atherosclerotic lesion of carotid artery, compared to stable plaque, have a moderate shift in DNA methylation (commonly towards hypomethylation) in the regions of 105 miR genes and only 13 differentially expressed miRs. For most of miR genes differential DNA methylation and expression are not overlapped.

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Computational search of evolution and drug resistance determinants of HIV-1

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Key words: HIV-1 drug resistance, antiviral therapy

Motivation and Aim: Mutations in reverse transcriptase (RT) of human immunodeficiency virus (HIV) can cause the development of drug resistance to different classes of antiretroviral agents. Currently, the emergence of mutant nucleic acid sequences encoding HIV targets is one of the reasons for treatment failure [1]. RT is important target for antiretroviral therapy because it is crucial for viral life cycle and there are several RT inhibitors that are known to be used for HIV/AIDS treatment. Purpose of this study was to investigate the relationship between presence of short peptide combinations in the amino acid sequence of HIV RT and the appearance of drug resistance in particular viral variant to HIV RT inhibitors.
Methods and Algorithms: The study was performed based on the data of over 1500 HIV RT amino acid sequences and their resistance to antiretroviral agents from Stanford University HIV drug resistance database. Estimation of short peptide's contribution to drug resistance development was calculated based on frequency of peptide's occurrence in the set of resistant HIV variants and its occurrence in all set of sequences.
Results: We obtained a list of short peptides and their combinations, which made a significant contribution to the development of resistance to RT inhibitors. We found several experiments in literature, which allowed explaining the role of selected short peptides and their combination in the HIV resistance.
Conclusion: We have proposed computational method aiming at selecting the short peptides that are potentially associated with HIV resistance. The method we propose can be applied for further selection of HIV variants resistant to HIV RT inhibitors.
Acknowledgements: This work is supported by Russian Science Foundation grant No. 17-75-10187.

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Linagliptin effect on expression of apoptosis regulators Bcl-2 and Bad in *db*/*db* mice liver

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Key words: diabetes mellitus type 2, *db/db* mice male, liver, endothelial cells, hepatocytes, Bcl-2, Bad

Motivation and Aim: Lipoperoxidation and oxidative stress in liver cells are the main activators of apoptosis in obesity and diabetes mellitus type 2 (DM2). A relatively new drug for the correction of carbohydrate metabolism is linagliptin, dipeptidyl peptidase type 4 inhibitor. Its positive effects are aimed at decreasing free fatty acids flow into the liver, reducing oxidative stress and apoptosis. The aim of our study was to evaluate the expression of antiapoptotic Bcl-2 protein and proapoptotic Bad protein in liver cells of animals with the obesity and DM2 model in the treatment with linagliptin.

Methods and Algorithms: The experiments were carried out in SPF-Vivarium of Institute of Cytology and Genetics SB RAS on *db/db* mice male. Animals received linagliptin ($n = 7$) or placebo (physiological solution; $n = 7$) once a day intragastrically from the 10th to the 18th week of life. Immunohistochemical study of Bcl-2 and Bad protein expression levels was performed on liver paraffin sections using indirect immunohistochemical ABC method. Computer morphometric analysis of digital photos at magnification × 400 was carried out using program "Image J" program. The average area and solidity of colored areas were determined.

Results: The significant area of immunohistochemical staining of Bad against the background of low Bcl-2 expression was revealed in the liver of 18-week db/db mice without treatment, that created conditions for apoptosis activation in this organ of animals by this age. Linagliptin administration to db/db mice led to a decrease in Bad expression area in liver cells against an increase in BcI-2 expression area. At the same time, the area of Bad staining was still higher than Bcl-2 expression area in the group receiving linagliptin.

Conclusion: The obtained data indicate that the treatment with linagliptin partially normalizes the balance of pro- and antipoptotic proteins and this helps to reduce the development of the mitochondrial "branch" of apoptosis in the organ (in comparison with the placebo group), although it does not block its development completely.

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Apoptosis in the liver of *db/db* mice female in postnatal ontogenesis

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Key words: diabetes mellitus type 2, non-alcoholic fatty liver, *db/db* mice female, Bcl-2, Bad

Motivation and Aim: In conditions of the development of non-alcoholic fatty liver disease, programmed cell death mechanisms take an active part in maintaining cellular homeostasis. The ratio of apoptosis regulator proteins Bcl-2 and Bad, Bax determines cell sensitivity to apoptotic factors effects and is a "molecular switch" that defines whether tissue growth or atrophy will occur. The aim of our study was to evaluate expression of antiapoptotic Bcl-2 protein and proapoptotic Bad protein in liver cells of animal with the obesity model and diabetes mellitus type 2.

Methods and Algorithms: The experiments were carried out in SPF-Vivarium of Institute of Cytology and Genetics SB RAS on *db/db* mice female at age 8 ($n = 7$) and 16 ($n = 7$) weeks of life. Immunohistochemical study of Bcl-2 and Bad protein expression levels was performed on liver paraffin sections using indirect avidin–biotin ABC-peroxidase method. Computer morphometric analysis of digital photos at magnification x400 was carried out using program "Videotest Morfo 3.2". The average area and brightness of stained areas were determined (the average brightness of the staining is inversely proportional to marker concentration).

Results: In *db/db* mice aged 8 weeks the weak Bcl-2 and a Bad-positive signals were detected in heterogeneous population of sinusoidal liver cells and in single hepatocytes. By the 16th week of life there was an increase in Bcl-2 and Bad areas against the background of a decrease in their brightness (i. e., against the background of an increase in their concentrations). It is necessary to note the greater contribution of the area, rather than the concentration in change in expression of studied markers – in adult animals Bcl-2 expression area exceeded the value of this parameter for Bad by almost 2.5 times, and the average brightness change was only 1.2 %.

Conclusion: The obtained results indicate the presence of liver cells antiapoptotic protection and the creation of conditions for blocking the apoptosis mitochondrial "branch" development in *db/db* mice female under non-alcoholic fatty liver disease in postnatal ontogenesis.

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Use of next-generation sequencing (NGS) to clarify the cause of a premature newborn death: clinical case

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Key words: premature newborn, thrombocytopenia, sudden death

Motivation and Aim: The risk of death in the neonatal period in premature infants is 20 times higher than in full-term newborn [1].Therefore, to reduce infant mortality is one of the most important tasks of neonatology.

Methods and Algorithms: Analysis of the proband DNA was performed using the Illumina TruSight One sequencing panel on the MiSeq platform (Illumina, San Diego, CA). Databases for analysis and variant annotation (HGMD, dbSNP, ClinVar, ExAC, OMIM) were added using the ANNOVAR tool. The pathogenicity of missense variants was predicted using the following in algorithms: Poly-Phen-2, UMD-Predictor, MCAP-Classification.

Results: The preterm male baby was born at 28–29 weeks of gestation to a second gravida para one mother by emergency cesarean section. The obstetric history showed that the first pregnancy was with a fetus death at 20–21 weeks of gestation. This pregnancy was complicated by second trimester bleeding, and by intrauterine growth restriction discovered at 28–29 weeks' gestation. At birth the infant suffered from respiratory distress syndrome, pneumonia. Despite maximum ventilatory support, the baby had severe hypoxia and cardiac failure, anemia. Thrombocytopenia was persistent and severe despite repeated platelet transfusions. The newborn developed multiple organ failure, disseminated intravascular coagulation with diffuse bleeding, unstable hemodynamic status. He died on day 5 of life. Sequence analyses revealed the presence a pathogenic variant in exon 9 of the C8B gene (c.1282C>T/p.Arg428*ϭ NM_000066) responsible of complement component 8 deficiency and very rare primary immunodeficiency. Also, a heterozygous variant in exon 5 of the LYST gene (c.T1334G/p.F445C, NM_000081), not described earlier, was detected in the infant. This missense-variant is "possibly pathogenic" according to pathogenicity prediction algorithms. Homozygous mutations in LYST associated with some primary immunodeficiency diseases, but some study identified patients with only one allele of LYST gene mutation [2].

Conclusion: It is required to carry out genetic testing and clinical examination of the parents of the proband for further prenatal diagnosis.

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NMR and DFT study of the mechanism for increased antitumor activity of carboplatin in mixture with cucurbit[7]uril

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Key words: carboplatin, drug encapsulation, drug stability

Motivation and Aim: Encapsulation of platinum-based antitumor drugs into host molecules provides the potential to reduce the toxicity and overcome tumor resistance. Very promising results were reported for the system of cisplatin encapsulated into cucurbit[7]uril (CB7) [1]. Our work is an attempt to develop a new antitumor drug based on the mixture of CB7 with carboplatin, a second generation Pt(II)-based antitumor agent. NMR spectroscopy provides an essential opportunity to study the mechanism of interaction between carboplatin and CB7 on molecular level for better understanding of the process and advanced explanation of the observed effects. Also, DFT calculations help with interpretation of NMR data for such complex systems.

Methods and Algorithms: The effect of carboplatin, CB7, and the mixture of carboplatin with CB7 (1 : 1) on the tumor cells of the cell culture B16 and the primary culture of peripheral blood mononuclear cells (PBMCs) of healthy volunteers was studied. Cytotoxic effect was evaluated by MTT test, proliferative activity was evaluated using CFSE labeling. ^{1}H, ^{13}C, ^{195}Pt, and 2D ROESY and DOSY NMR spectra of carboplatin, CB7, and their mixture in D_2O, PBS buffer solution, and RPMI 1640 growth medium were recorded on Bruker Avance 500 spectrometer. DFT calculations for hypothetical complexes of carboplatin with CB7 were performed with Gaussian09 code (geometry optimization) and ADF code (NMR parameters).

Results: NMR data imply that CB7 induces the hydrolysis of carboplatin and encapsulates the products of the hydrolysis [2], which are known to be more cytotoxic than the initial carboplatin. Indeed, the experiments on the cell cultures show that, at some concentrations, carboplatin + CB7 mixture demonstrates higher toxicity towards tumor cells than pure carboplatin, while, towards PBMCs, the toxicity of the mixture and of the pure carboplatin is equal. The latter fact, probably, may be attributed to the protective effect of CB7.

Conclusion: The mixture of CB7 and carboplatin is a promising system for future development of an antitumor medication, which increased effectiveness is based on induced hydrolysis of carboplatin and further encapsulation of hydrolysis products.

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Software “DoctorCT” for modeling images of internal organs, based on computer and magnetic resonance imaging

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Key words: computed tomography, 3D modeling, 3D bio-printing

Motivation and Aim: Every day, the number of patients who are assigned to study with the help of computer and magnetic resonance imaging (MRI) methods is growing. When visualizing the image of computed tomography, it is difficult to separate the tumor from the organ and to calculate its volumetric characteristics. For making a decision on an operable tumor, it is necessary to build a clear 3D model of the studied material with topography, with visualization of the paths of dissection to education [1]. Modern image processing software obtained during CT and MRI is supplied with CT and MRI devices. The functions of these programs are often not enough to conduct more complex work with the data obtained, including when working with complex surgical interventions [2].
Methods and Algorithms: The main materials of our study were medical images (DICOM files) obtained through Toshiba multislice computer tomographs during 2015–2018. Algorithms for recognizing and improving the image quality of the Slicer-DoctorCT module of the software complex based on the Slicer constructor are developed. The software package contains tools for editing the model, both at the stage of creating the segmentation mask, and directly in the 3D (postprocessing) mode.
Results: A software package for reconstruction and planning of surgical intervention for resection of neoplasms of the urogenital system was developed.
Conclusion: The program complex “DoctorCT” will allow you to see more clearly the boundaries of the tumor, layer by layer to study the structure of neoplasms and cysts, to perform multilayer reconstruction of the organ based on CT and MRI.
Acknowledgments: The obtained software “DoctorCT” was successfully applied in the Stavropol Regional Clinical Consultative and Diagnostic Center for 12 operations on the kidneys and 6 operations of the prostate glands.

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Studying calcium signaling in individual suspended platelets

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Key words: blood platelets, platelet activation, calcium signalling, caged compounds

Motivation and Aim: Blood platelets are the principal component of haemostasis and also mediate other normal and pathological processes. Methods for *in vitro* studies of platelet's reactions to different stimuli, such as platelet aggregometry, have significant role in both diagnostics and fundamental understanding of platelet functions. However, activation dynamics of single platelets is usually studied in cells anchored to the surface [1, 2], which significantly alters the results.
Methods and Algorithms: We developed a method to observe calcium dynamics in single suspended platelets. Cells were loaded with Fluo-4 calcium indicator and placed under fluorescent microscope. Some cells spread over the surface, while other were floating above. Activation of platelets were made either conventionally (by the addition of ADP and epinephrine) or optically, using photolabile caged analog of epinephrine. We were able to obtain the fluorescence intensity of floating (suspended) cells using the ImageJ plug-in TrackMate [3].
Results: Dynamics of calcium spikes upon activation were measured for both spread and suspended platelets. Spread cells showed multiple oscillations, in accordance with previous studies. On the other hand, calcium spiked once in suspended cells. We also showed that photolabile analog of epinephrine can be used to trigger platelet activation optically, which significantly decreases displacement of cells compared to conventional addition of agonist.
Conclusion: The obtained results form the basis for novel single-cell technique for studying platelet activation and also indicate that spread platelets behave substantially different than in suspension.
Acknowledgements: This work was supported by Russian Science Foundation (grant No. 18-15-00049).

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Polymorphic variants in stress resistance genes and human emotional stability

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Key words: gene polymorphisms, stress resistance, psychological testing, athletes

Motivation and Aim: Emotional stability is the product of genetic and environmental interactions with a strong genetic influence [1]. It is very important to identify the genetic variation causing individual differences in stress resistance. The aim of this study was to examine the association of 9/10/12 5-HTTVNTR, 939C/T DRD2, 521C/T DRD4, 833C/T HTR2A and T-182C NET functional polymorphisms with psychological parameters.

Methods: We performed molecular genetic testing of 102 athletes (mean age – 22.7±0.49 yrs.) of high qualification. Genotyping was performed by real-time PCR (CFX 96 Bio-Rad Real-Time PCR System). Genomic DNA was extracted using the GeneJet Genomic DNA purification Kit (Thermo Scientific, USA). The psychophysiological parameters of athletes were studied using the hardware and software complex "NS-PsychoTest" and the psychoemotional ones with the Lüscher Color Psychology Test. High reaction rate, low attention concentration, high number of mistakes made during the test as well as high number of points on the stress scale and the degree of deviation from the autogenic norm indicate the low stress resistance of the subject.

Results: Comparison of genotyping and psychophysiological testing results revealed that the reaction rate was higher for athletes with the T/T genotype of *DRD2* gene ($p = 0.002$) and for human with C/T or C/C genotypes of *DRD4* gene than those with other genotypes ($p = 0.048$). In addition, for subjects with the C/C genotype of DRD4 gene the attention concentration level was significantly lower as compared with the ones with C/T or T/T genotypes (0.015). We have also defined that athletes with the 12/12 genotype of 5-HTT VNTR gene are characterized by high number of mistakes that were made during psychophysiological testing than those with 10/10 or 10/12 genotypes ($p = 0.032$).

Analysis of genotyping and psychoemotional testing results revealed that people with the 12/12 genotype of 5-HTT VNTR gene are characterized by high number of points on the stress scale than those with the 10/10 or 10/12 genotypes ($p = 0.034$). We have also established that athletes with the T/T genotype of NET gene have a higher degree of deviation from the autogenic norm as compared with athletes with C/T or C/C genotypes ($p = 0.035$).

Conclusion: Subjects with 12/12 of the 5-HTTVNTR gene, T/T DRD2, C/C DRD4, and T/T of NET are more likely to express emotional instability. These variants might be markers of low stress resistance.

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Novel lentiviral vectors for studies of glioblastoma

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Key words: lentiviral vectors, viral transfection, gene expression, glioblastoma

Motivation and Aim: Stable cell lines which express transgenes and have specified properties can significantly speed up experiments. This is particularly relevant for cancer research and rapidly dividing tumors such as glioblastomas. Moreover, some experiments (for example, evaluation of long-term effect of drugs using genetically encoded indicators or modulation of gene expression using RNA interference) are impossible without stable gene expression. Finally, glioblastoma cells which stably express transgenes are indispensable tools for the in vivo experiments with transplanted tumors. Lentiviral vectors (LVV) enable stable integration of transgenes into the host genome and can provide essentially permanent gene expression.
The aim of the work was generation, production in our home laboratory and testing of novel LVV vectors for making stable lines of human glioblastomas. We began by generation of two LVV which encode for the green fluorescent protein fused to a mitochondrial localization signal (MTS-GFP) and Cas9 endonuclease fused to a nuclear localization signal (FLAG-NLS-Cas9).
Methods: Lentiviral shuttle plasmids (pTYF) containing either EF1α-MTS-GFP or EF1α-FLAG-NLS-Cas9 inserts were generated using conventional cloning protocols. Lentiviral vectors were bulked up and titred using protocols established by Kasparov & Teschemacher laboratory [1]. Two primary human glioma cell lines were trasnfected at different MOI. Efficacy of transfection was estimated using confocal microscopy.
Results: Confocal microscopy of the glioma cell line transfected with LVV-EF1α-MTS-GFP confirmed the selective accumulation of GFP in the mitochondria. Immunocytochemical staining of the glioma cell line transfected with LVV-EF1α-FLAG-NLS-Cas9 confirmed predominantly intranuclear distribution of the protein. The intensity of the signals was proportional to the dose of LVV.
Conclusion: We have developed and tested lentiviral vectors suitable for generation of stable glioblastoma cell lines. Our next step is to use these cell lines to investigate the effect of the inactivation of different genes on the viability of tumor cells.
Acknowledgements: Supported by the “5-100 project”.

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Application of new bioinformatics approach for prediction of metabolic capabilities for carbohydrate fermentation in human gut microbiome

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Key words: human gut microbiome, carbohydrate metabolism by bacteria, 16S RNA bioinformatics analysis

Introduction: Human gut microbiota is the complex and dynamic community of commensal, symbiotic and pathogenic microorganisms that are present within the human body and has an enormous impact on human health. Gut microbiome participates in breakdown and fermentation of complex carbohydrates, which comprise a key natural source of carbon and energy for a variety of heterotrophic microbes. Short-chained fatty acids produced during fermentation of carbohydrates by gut microbiota are a crucial source of energy for colonocytes.

Results: We used the metabolic subsystem approach implemented in SEED genomic platform to reconstruct pathways of carbohydrate fermentation in bacteria that inhabit human gastrointestinal tract. We analyzed metabolic pathways for utilization of over 45 carbohydrates including 19 monosaccharides, 20 oligosaccharides and 7 sugar alcohols as well as pathways for synthesis of short-chain fatty acids (propionate, butyrate) in 2228 human gut bacteria with sequenced genomes. Analyzing presence or absence of sugar-specific transporters and glycosyl hydrolases, we have been able to distinct ability to utilize certain oligosaccharides (and polysaccharides) from the ability to only utilize corresponding monosaccharide. The absence of monosaccharide transporters in many cases can be explained by the presence of other catabolic/utilization pathways that can produce this monosaccharide intracellularly. We also studied non-orthologic displacements of enzymes in certain pathways and alternative pathway variants. The overall ability to utilize a variety of carbohydrates presents metabolic potential of microbiome community. Acquired results allowed us to build a pipeline for analysis of 16S RNA samples from human gut microbiota. We designed a collection of samples from scientific papers that includes 16S sequencing data and additional metadata information such as diet preferences, country of origin, disease status etc. We applied our algorithm to this collection and received data on metabolic potential of human gut microbiota from a vast variety of sources. We will use these results to improve the precision of our algorithm and prepare it for clinical practice. The anticipated results of our work can provide ability to rationally modulate microbial communities through the controlled dietary polysaccharide composition and use of prebiotics, and thus have far reaching potential for therapeutic applications in medicine.

Conclusions: We have conducted reconstruction of carbohydrate fermentation metabolic pathways in human gut microbiota on a large set of reference genomes. We used this data to develop an algorithm for automatic analysis of metabolic potential for new 16S samples of human gut microbiota. These results provide useful insights into human gut microbiota functioning and can be used later in clinical practice.

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Glucose variability in insulin-treated type 1 and type 2 diabetic subjects

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Key words: diabetes, glucose variability, continuous glucose monitoring, insulin

Motivation and Aim: A growing body of evidences indicates applicability of glucose variability (GV) analysis for estimation of glycemic control and the risk of complications in patients with diabetes [1–3]. Continuous glucose monitoring (CGM) provides excellent opportunity for glucose variability (GV) estimation. We aimed to compare CGM-derived GV parameters in day-time and nocturnal hours in insulin-treated subjects with type 1 and type 2 diabetes.
Methods and Algorithms: The CMG data from 130 type 1 diabetic and 117 type 2 diabetic patients were analyzed. Original software *Sakharok* was applied for time in range analysis. The GV parameters: Mean Amplitude of Glucose Excursions (MAGE), Lability Index (LI), Low Blood Glucose Index (LBGI), High Blood Glucose Index (HBGI), Continuous Overlapping Net Glycemic Action (CONGA), and Mean Absolute Glucose (MAG) were calculated with *EasyGV* software [4].
Results: Patients with type 1 diabetes, as compared to those with type 2 diabetes, had higher mean 24-hour GV parameters: MAGE ($p = 0.000002$), LI ($p < 0.00001$), LBGI ($p < 0.00001$), HBGI ($p = 0.008$) and MAG ($p = 0.0002$). Nocturnal MAGE, LI, MAG, HBGI, CONGA and time in hyperglycemic range were also higher in patients with type 1 diabetes (all $p \leq 0.02$). Nocturnal LBGI and the prevalence of hypoglycemia were similar in both groups. In day-time hours type 1 diabetic subjects, as compare to those with type 2 diabetes, demonstrate lower CONGA ($p = 0.04$) and higher prevalence of hypoglycemic episodes ($p = 0.000004$).
Conclusions: Insulin-treated patients with type 1 diabetes, as compared to type 2 diabetic subjects, have greater 24-hour CGM-derived GV parameters with more pronounced glucose fluctuations in the hyperglycemic range at night and more prevalent episodes of hypoglycemia in the daytime.

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Glucose variability in type 1 diabetic patients with different stages of chronic kidney disease

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Key words: diabetes, chronic kidney disease, glucose variability, continuous glucose monitoring, insulin

Motivation and Aim: A growing body of evidences indicates applicability of glucose variability (GV) analysis for estimation of glycemic control and the risk of complications in diabetes [1–3]. The patterns of GV changes at different stages of diabetic chronic kidney disease (CKD) are needed to be clarified. We aimed to assess the relationships between GV parameters and renal function in patients with type 1 diabetes (T1D) at different stages of CKD.
Methods and Algorithms: We observed 127 patients with T1D, 48 M/79 F, from 18 to 72 years of age. Patients were divided into 5 groups: 1) no signs of CKD (CKD0), $n = 27$; 2) CKD C1-C2, $n = 67$; 3) CKD C3-C4, $n = 26$; 5) CKD C5 (hemodialysis, $n = 7$). Time in ranges and GV parameters: Mean Amplitude of Glucose Excursions (MAGE), Lability Index (LI), Low Blood Glucose Index (LBGI), High Blood Glucose Index (HBGI), 2-hour Continuous Overlapping Net Glycemic Action (CONGA), and Mean Absolute Glucose (MAG) were derived from 72-hour continuous glucose monitoring.
Results: As compared to patients without CKD, the values of MAGE, LI, HBGI were increased significantly in patients with CKD C1-C2 (all $p < 0.05$). No differences were found in all GV parameters between CKD C3-C4 and CKD0 groups. In patients on hemodialysis time in hyperglycemic range, MAGE, LI, CONGA and HBGI were significantly higher as compared to other groups (all $p < 0.05$). In patients with CKD C3-C5 there were negative correlations between HBGI, CONGA and estimated glomerular filtration rate (eGFR): $r = –0.42$, $r = –0.34$ respectively. In patients with eGFR ≥ 60 ml/min/1.73 m^2 HbA1c levels correlated positively with mean monitored glucose ($r = 0.56$), time in hyperglycemic range ($r = 0.55$), HBGI ($r = 0.54$), CONGA ($r = 0.53$), MAGE ($r = 0.42$), LI ($r = 0.36$), and MAG ($r = 0.53$, all $p \leq 0.01$). On the contrary, in patients with lower eGFR none of GV parameters correlated with HbA1c.
Conclusions: The results demonstrate for the first time non-linear relationships between eGFR and GV parameters in patients with T1D. The assessment of GV should be applied for glycemic control assessment in addition to HbA1c in patients with eGFR < 60 ml/ min/1.73 m^2.

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Omics-based approach to profiling of human atherosclerotic plaques

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Key words: copy number variation, DNA methylation, gene expression, human atherosclerotic plaques, microarrays

Motivation and Aim: Genome-wide association studies (GWAS) of atherosclerosis and its complications have been very successful, but the level of knowledge about underlying mechanisms of these diseases remains inadequate. In the present study, the DNA copy number, DNA methylation and gene expression of vascular tissues of patients with advanced atherosclerosis were investigated together.
Methods and Algorithms: The Agilent SurePrint G3 Human CGH + SNP 2 × 400 K, Illumina Human Methylation27 BeadChip and Illumina HumanHT-12 microarrays were used to study the advanced atherosclerotic plaques of carotid and coronary arteries, and healthy internal thoracic arteries. The results for certain loci were verified by quantitative real time PCR and bisulfite pyrosequencing with blood and vascular tissues.
Results: We found that CNVs regions were enriched with genes previously associated with mainly atherosclerosis risk factors but not the disease as a whole. The identified CNV genes were associated with immune/inflammation responses, olfactory transduction and metabolic pathways. The gain in chromosomal region 10q24.31 (*ERLIN1*) was not listed in the Database of Genomic Variants. Furthermore, two patients contained the gain in 10q24.31 (*ERLIN1*) and one patient contained the gain in 12q24.11 (*UNG*, *ACACB*) that affected only the blood DNA. An additional two other patients harboured these CNVs in both the arteries and blood.
Overall, the disease-related DNA methylation effect size was relatively modest. The advanced atherosclerotic plaques in comparison with the healthy arteries were characterized by the predominant DNA hypermethylation changes. These genes were annotated with muscle system process and positive regulation of cytosolic calcium ion concentration in Gene Ontology terms. In contrast, hypomethylated genes encode molecules belonging to different biological processes such as development, immune/inflammation responses, lipid storage, and programmed cell death. In advanced atherosclerotic plaques the most pronounced hypomethylation was registered in 2q31.1 (*HOXD4/HOXD3/MIR10B*). Moreover, methylation changes at this locus in blood cells were consistently associated with smoke and ischemic stroke.
The majority of differentially expressed genes were down-regulated in advanced atherosclerotic plaques. “Cellular response to metal ion” and “extracellular matrix organization” were the most significant Gene ontology terms among the down- and up-regulated genes, respectively. Unexpectedly, genes involved in immune and inflammatory responses were down-regulated in advanced atherosclerotic plaques to compare with the healthy arteries.
After merging the DNA methylation with gene expression data, we identified four hypermethylated-downregulated genes (*LIPE*, *CIDEC*, *TMEM88*, *GATA2*) and one hypomethylated-upregulated gene (*S100A4*). Only a minority of CNVs (16 %), differentially methylated (19 %) and expression (11 %) genes have previously been linked to atherosclerosis-related diseases in GWAS and expression studies.
Conclusion: Analysis of DNA extracted from blood of patients with severe atherosclerosis indicated a possible somatic origin for CNV. The vast majority of CpG-sites were hypermethylated in the atherosclerotic plaques and connected with genes involved in smooth muscle cell function. Functional enrichment analysis of CNVs, differentially methylated and expression genes demonstrated that common Gene Ontology term was related to immune/inflammation responses. We suggested that *LIPE*, *CIDEC*, *TMEM88*, *GATA2* and *S100A4* genes in vascular tissues of patients can be a “drivers” in respect of atherosclerosis. In whole, our data support the hypothesis that CNVs, DNA methylation and gene expression could be a new biomarkers to uncover susceptibility loci for future atherosclerosis and its clinical complications studies with larger cohorts, paired tissues and more sensitive single cells methods.
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Development and optimization DNA platform for suppressing cancer cells

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Key words: deoxyribozymes, DNA nanomachines, cancer

Motivation and Aim: Worldwide cancer is the second leading cause of death [1]. Gene silencing therapeutics (GST) tend to be relevant approaches against cancer. The main aim of them is suppressing expression of genes responsible for cancer development. For example, deoxyribozymes (Dz) are widely used in GST of cancer due to their catalytic abilities to cleave phosphodiester bonds in RNA [2]. However, all known GST have low selectivity and efficiency. We propose to solve these problems by using DNA nanomachine (DNM-1) based on Dz, which capable to untwist and cleave secondary structure of housekeeping gene RNA only in cancer cells [2].
Methods and Algorithms: As a target for cleavage we chose messenger RNA (mRNA) of the housekeeping gene DAD1 (Defender Against Cell Death 1). Neuroblastoma cells were chosen as an object of study. The DNA segment of the gene N-Myc, which is overexpressed in neuroblastoma cells [3], was chosen as the biomarker sequence.
Experiments on the cleavage of DAD1 RNA fragments of 20 and 46 nucleotides (nt) were carried out with Dz, binary Dz (bDz) and DNM-1 in the presence or absence of the biomarker N-Myc sequence at a temperature of 37 °C in buffer with the physiological concentrations of K^+, Na^+ and Mg^{2+} ions (pH = 7.4). The degree of mRNA DAD1 cleavage was evaluated by denaturing polyacrylamide gel electrophoresis after 1, 5 and 24 hrs of incubation.
Results: It was found that Dz cleaved DAD1 RNA of 20 nt after 1 hr, while bDz and DNM-1 only after 5 hr in the presence of the N-Myc biomarker. Furthermore, the 46 nt DAD1 RNA was cleaved by the DNM-1 and Dz after 1 hrs; while using bDz cleavage was not observed. After 24 hr DNM-1 cleaved the 46 nt RNA in presence of the biomarker sequence more effective than Dz. DNM-1 without arms cleaved the 20 nt RNA with equal efficiency such as usual DNM-1, but it could not cleave 46 nt RNA.
Conclusion: This result indicates that the design of the DNM-1 allows untwisting the RNA secondary structure and efficiently and selectively cleave it only in the presence of the neuroblastoma cells-specific biomarker. Moreover construction of DNA platform can be changed for using different biomarkers. This opportunity opens broad horizons for using DNA nanomachines based on Dz in GST of the cancer.

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Heterogeneity of motility of chondrocyte population from minipig's knee-joint

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Key words: chondrocyte, cell migration, cell tracking, bioinformatics, mathematical modelling

Motivation and Aim: One of the technology of tissue engineering is a scaffold (natural or artificial extracellular matrix) colonization by appropriate cells. To implement this technology, it is necessary to learn how to control cell motility by the parameters of the medium. This, in turn, requires an experimental study (certification) of cell motility in the first stage of tissue engineering. Our aim was to study basic characteristics of chondrocyte motility in vitro by mathematics and bioinformatics methods.

Methods and Algorithms: Chondrocytes were isolated from the cartilage tissue of the knee joint of experimental female minipigs aging 1–1.5 weeks. The cell movement was photographed in Cell-IQ long-term observation system for 43 h, 6 times per hour (details in [1]). Cell tracking was performed by hand, aiming on center of the cell, and cell trajectories as series of coordinates ($x(t)$, $y(t)$) were obtained. Empirical distribution of displacements (l) between two successive observation moments was calculated. 10000 sets of trajectories with the lengths corresponding to the lengths of the experimental ones were generated based on empirical distribution. In generated trajectories the distribution average displacement on trajectory ($\langle l \rangle$) is narrower than in the experimental ones. We divided the trajectories into 3 groups: (1) $\langle l \rangle < \langle min(\langle l \rangle) \rangle$, (2) $\langle min(\langle l \rangle) \rangle < \langle l \rangle < \langle max(\langle l \rangle) \rangle$, and (3) $\langle l \rangle > \langle max(\langle l \rangle) \rangle$. We roughly classified shape of cell projections into "sticks" (cell with narrow lamellipodia) and "sails" (wide lamellipodia), and labeled trajectories containing only "sail" shapes as type-1, and the rest trajectories as type-2.

Results: Most chondrocytes from the 1st group demonstrate trajectory of type-2, chondrocytes from the 2nd group contain almost equal numbers type-1 and type-2 trajectories, and for chondrocytes from the 3rd group type-1 prevail over type-2 trajectories. Displacement of cells from its starting points is the smallest in the 1st group. The cells from the 2nd group demonstrate persistence of angles between two successive displacements.

Conclusion: Chondrocytes form minipigs' knee demonstrate different motility. For tissue engineering optimization it is worth to select "rapid" subpopulation of the cells. To perform this it is necessary to find markers correlated with cell motility to use for example flow cytometry method.

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Detection of the lymphatic capillaries in the optic nerve sheath

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Key words: lymphatic system, human eye, optic nerve sheath, lymphatic capillaries

Motivation and Aim: The idea about lymph outflow of intraocular fluid and presence of lymphatics in the human eye is interesting, but disputable. Participation of the lymphatic system in the outflow of intraocular fluid may explain the pathogenesis of glaucoma, inflammatory eye diseases and dissemination of tumors [1]. There are many studies in this theme, though we have not univocal proofs of existence of lymphatic structures in the eye. This study presents the evidence for lymphatic capillaries in the optic nerve sheath.

Methods and Algorithms: The object of study were fragments of enucleated eyes ($n = 10$), harvested from patients without ocular disease. For morphological study samples were treated according to standard procedures for light microscopy. Paraffin – embedded sections of human optic nerve and conjunctiva were immunostained with the lymphatic specific endothelial markers Podoplanin (Monosan, Netherlands) and LYVE-1 (Abcam, England) and the vascular specific endothelial marker CD31 (Abcam, England). We used the protocol for immunohistochemical staining, which has already been used in our previous studies [2].

Results: The presence of conjunctival lymphatics was verified and used as a control tissue.

In the optic nerve sheath were identified vessels with positive Podoplanin immunostaining. These structures did not show criteria of blood capillaries (absence of erythrocytes, negative immunostaining with vascular specific endothelial marker CD-31). However, LYVE-1 + staining were demonstrated in these lymphatic structures as well as in the blood vessels.

Conclusion: Lymphatic capillaries in the human optic nerve sheath were identified with immunostaining with the lymphatic specific endothelial marker Podoplanin.

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Regeneration of rat skeletal muscle induced by MSC-populated collagenous scaffolds

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Key words: collagenous scaffolds, mesenchymal stem cells, skeletal muscle, regeneration

Motivation and Aim: Application of mesenchymal stem cells (MSCs) for tissue regeneration is currently a well-developed practice performed on many types of tissues, including the skeletal muscle. To ensure survival of MSCs after implantation into the tissue the cells are planted on biocompatible scaffolds that provide adhesive surface and mechanical strength of the graft. A very popular material for such scaffolds is collagen, as it is non-immunogenic, biodegradable and can be used in various forms. In this work we aim to compare the influence of three collagen-based scaffolds on regeneration of rat skeletal muscle in the presence of adipose-derived MSCs.
Methods and Algorithms: The work was performed on Wistar rats. The scaffolds used were porous collagen sponge (PCS), swine intestinal submucosa (SIS) and Belkosin (BLK) – collagenous sausage capsule used in meat industry. Rat adipose-derived MSCs were cultivated on said scaffolds in 10^6 cells/ml concentration for 14 days with 20 % FBS. Each scaffold was placed into *musculus soleus* laceration site immediately after the injury and sealed with sutures. Histological examination was performed 14 days after the operation.
Results: All the examined scaffolds caused inflammation at the implantation site, as the areas around the scaffolds were filled with neutrophils, but the least inflammation was seen around the PCS scaffold. BLK showed almost no biodegradability and maintained its solid structure, and very few cells were able to migrate inside the scaffold, remaining mostly on its surface. PCS and SIS, on the other hand, having looser fibrous structure, were well interlaced with recipient cells and intermediate tissue. All grafts caused muscle regeneration: young myofibers with centralized nuclei were observed on the periphery of the inflammation zone, for BLK and SIS, and more deeply infiltrating it, for PCS. Moreover, PCS seemed to support angiogenesis better than SIS and BLK. Only single vessels were found around SIS and BLK, while small groups of vessels were found around PCS and permeating it.
Conclusion: To summarize, out of three examined collagenous scaffolds, BLK showed the least biocompatibility, as it barely disintegrated in the tissue. SIS and PCS allowed better permeation by recipient cells and tissue, but, evidently, PCS provides the best structural organization for tissue regeneration due to its soft porous structure. Further experiments with the same scaffolds will be carried out to compare the effect of adipose- and bone marrow-derived MSCs on muscle regeneration.
Acknowledgements: The work was performed within the framework of the state program of fundamental scientific research No. 0108-2018-0004.

Enhancement of cytotoxic effects of temozolomide in glioblastoma cell lines by Tdp1 inhibitors

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Key words: glioblastoma, Tdp1 inhibitors, drug screening, combination therapy

Motivation and Aim: Glioblastoma (GBM) is the most common primary brain tumor in adults. Despite the multimodal standard of care (surgical resection, radiotherapy and adjuvant chemotherapy with temozolomide (TMZ)), the median survival of GBM patients (MS) remains approximately 12–15 months. There are the data that the chemotherapy adds only 2.4 months to MS [1]. Given the low efficacy of therapy, strategies aimed at overcoming resistance and enhancing the response to TMZ are being actively studied. It is well known that the efficacy of TMZ depends on the DNA repair systems activity. Accordingly, the key enzymes of repair systems are considered to be the most tempting targets for creating new chemotherapeutic drugs. One of the promising targets is the enzyme tyrosyl-DNA-phosphodiesterase 1 (Tdp1). Besides the ability to remove covalent adducts of Top1 and DNA, Tdp1 can hydrolyze AP sites in DNA and induce its repair. This capability is important to repair the damages inducing by the monofunctional alkylating agents, such as TMZ. Thus, the aim of our study is to determine whether the Tdp1 inhibitors (Tdp1 Inh) can enhance the cytotoxic effects of TMZ in GBM cell lines (GCLs).

Methods and Algorithms: Cytotoxic effects of TMZ (Calbiochem) alone and in combination with Tdp1 Inh (four Tdp1 Inhs were kindly provided by Prof. Salakhutdinov from N.N. Vorozhtsov Novosibirsk Institute of Organic Chemistry) were tested on established GCLs U87 and SNB19 by standard colorimetric MTT test.

Results: Sensitivity to TMZ was tested at the concentration of 2000 μM. Tdp1 Inhs were also tested at the maximum concentration of 100 μM. Applied separately and at given concentrations, TMZ had suboptimal and Tdp1 Inhs had no cytotoxic effects on the selected GCLs. Two of the Tdp1 Inhs potentiated TMZ therapy in both cell lines. Combination of Inh1 with TMZ resulted in a decrease of viability by 26.4 % in the case of U-87 (2000 μM TMZ+Tdp1 Inh 100 μM) and by 36.5 % in the case of SNB19 (2000 μM TMZ+Tdp1 Inh 75 μM), as compared to TMZ alone. Combination of Inh4 with TMZ decreased of viability only in SNB19 by 26.7 %. Other Tdp1 Inhs did not show any synergetic effects.

Conclusion: We believe that owing to their low cytotoxicity and synergetic effects with TMZ, Tdp1 Inhs could potentially improve the therapeutic efficacy of standard of care in GBM. Of note, there is the difference between the sensitivity of GCLs to the combination of TMZ and Tdp1 Inh. It is likely to be a consequence of the repair capacity in these cell lines. Thus, the activity of Tdp1, as well as the other DNA repair enzymes, should be assessed in each specific case.

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Complex analysis of cytokine concentrations using X-map technology in women with uterine myoma

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Key words: leiomyoma, cytokines, growth factors, Bio-Plex

Motivation and Aim: Uterine myoma (leiomyoma) is a widespread gynecological disease, with the highest incidence in women of the perimenopausal period. Among the factors contributing to the development of fibroids, there are: a violation of sex hormones production, a genetic predisposition, chronic inflammatory diseases of the female sexual sphere. It is assumed that cytokines are potential effectors of sex hormones on the growth of leiomyoma. The aim of our study was to analyze total concentration of the most significant cytokines that play an active role in pathogenesis of uterine myomatosis.
Methods and Algorithms: 36 patients of European origin with a diagnosis of uterine myoma were examined. The age of the patients ranged from 23 to 54 years. The sizes of myomatous nodes were from 5 to 180 mm. In blood serum, concentration of 27 cytokines was determined simultaneously by flow-through fluorimetry using a Bio-Plex 200 two-beam laser analyzer from Bio-Rad.
Results: Analysis of proinflammatory cytokines concentration in the serum of women with uterine myoma showed a statistically significant decrease in concentration of IFNγ 73.46 ± 22.53 pg/ml, relative to the healthy group 822.70 ± 139.84 pg/ml. Production level of IFNγ positively correlates with concentration of functionally associated IL-1β, IL-12 and TNF-α. In serum of women with uterine myoma, a reduced content of anti-inflammatory cytokine IL-4 was 4.66 ± 0.77 pg/ml, relative to the healthy group of 36.0 ± 2.27 pg/ml. Concentration of the serum IL-1 receptor antagonist (IL-1ra) was also significantly reduced in uterine myoma. Concentration of IL-1ra protein in serum of women with myoma was 85.49 ± 19.83 pg/ml, at a concentration of this factor in serum of healthy women 305.5 ± 45.76 pg/ml. At the same time, patients with fibroids have a higher concentration of IL-10, a cytokine with strong anti-inflammatory properties, which plays an important role in limiting the body's immune response to foreign antigens. Its content in blood of healthy women is 2.70 ± 0.64 pg/ml, while in women with uterine myoma 18.98 ± 10.7 pg/ml ($p = 0.13$).
Conclusion: The immune system, its cellular subpopulations and soluble factors play a decisive role in controlling tumor growth. Decreased production of pro-inflammatory cytokines in women with uterine myoma IFNγ, TNF-α and increased production of anti-inflammatory (IL-10) in response to hormonal dysregulation may be one of the reasons for the growth of myomatous nodes.

Verification of MODY diabetes: phenotypic and molecular-genetic characteristics

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Key words: MODY diabetes, molecular-genetic investigation, young patients, mutations

Purpose: To verify the mutations in the genes associated with the development of MODY 1-14 diabetes in individuals with phenotypic characteristics of these subtypes of diabetes mellitus (DM).
Materials and methods: We examined 161 patients aged 2 months to 35 years (62 males, 99 females) meeting the criteria: age of diagnosis of DM under 40 years, absence of antibodies to b-cells and GAD, normal body weight, absence of absolute need for insulin therapy for 2 years after the detection of hyperglycemia, preserved secretion of b-cells. All patients were underwent a clinical examination, biochemical blood test, determination of HbA1c, C-peptide, laboratory tests. Molecular genetic research was performed using the next-generation sequencing technology and direct automatic sequencing by Sanger.
Results: Mutations in MODY candidate genes were detected in 24 probands and 27 relatives (51 people). In the *GCK* gene (MODY2) 13 different mutations were detected (3 of them previously undescribed) in 13 probands and 17 relatives, in *HNF1A* (MODY3) – 7 different mutations in 7 probands and 6 relatives, in the proband and two relatives in the *HNF1B* gene (MODY5), the proband in the gene *NEUROD1* (MODY6), the proband in the gene *CEL* (MODY8), the proband and two relatives in the gene *ABCC8* (MODY12).
The age of probands in the diagnosis of GCK-MODY ranged from 4 to 34 years, the group comprised 5 males and 8 females. The median of glycated hemoglobin was 6.2 [4.5; 7.8] %, the C-peptide – 0.7 [0.2; 1.8] ng/ml (reference values 0.7–1.9).
The age of probands in the diagnosis of HNF1A-MODY ranged from 9 to 33 years, the group was 1 male and 6 female. Median glycated hemoglobin was 7.0 [5.0; 8.1] %, C-peptide – 0.6 [0.1; 1.6] ng/ml.
The age of probands in the diagnosis of rare MODY subtypes ranged from 23 to 35 years, the group comprised 2 males and 2 females. Median glycated hemoglobin was 7.2 [6.0; 9.1] %, C-peptide – 0.4 [0.1; 0.9] ng/ml. In 35 patients out of 51 (69 %) there were no clinical manifestations of carbohydrate metabolism disorders in diagnosing the disease and hyperglycemia was detected during routine examinations. Peripheral polyneuropathy was diagnosed in 9 patients (18 %) with MODY diabetes, 4 (8 %) had diabetic retinopathy and 5 (10 %) had nephropathy. Dyslipidemia prevailed among the concomitant pathologies (in 18 patients, 35%). Twenty-two patients (43 %) before the verification of diabetes type used diet, 12 (24 %) – insulin therapy, 16 (31 %) – oral hypoglycemic drugs, 1 (2 %) – combined therapy.
Conclusions: 1. In the city of Novosibirsk MODY diabetes was verified in 51 patients, MODY type 2 prevailed. 2. In most cases when diagnosing MODY diabetes there are no clinical manifestations of carbohydrate metabolism disorders, which indicates the need for careful examination of patients at risk for developing monogenic forms of DM.
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Effect of mesenchymal stromal cells from different sources on skin regeneration

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Key words: cutaneous wounds, mesenchymal stromal cells, bone marrow, adipose tissue, fetal skin, angiogenesis

Motivation and Aim: Mesenchymal stromal cells (MSC) are a promising resource for tissue regeneration. Their beneficial effect on skin wound healing was reported in both experimental animals [1] and humans [2]. However, successful clinical application of MSC requires selection of the optimal cell source and studying mechanisms of their action. In this work, the influence of MSC from different tissues on healing of skin defects in rats was evaluated.
Methods and Algorithms: MSC were isolated from adult bone marrow and adipose tissue or fetal skin of rat and characterized in terms of their phenotype and differentiation potential. Full-thickness skin wounds were made on the back of rats, and the cells or αMEM medium (as a control) were injected into the wound bed. Wound biopsy was performed 14 days after the injury.
Results: Most cells from bone marrow and adipose tissue expressed MCS markers CD73 and CD90, but their differentiation potentials were dissimilar: adipose-derived cells exceeded those from bone marrow in adipogenic potencies, but were not capable of terminal osteogenic differentiation. Fetal skin fibroblasts, judging by paucity of $CD73^+$ and $CD90^+$ cells and low ability for both osteogenesis and adipogenesis, included only few MSC. Injection of 1×10^6 bone marrow-derived or adipose-derived MSC into wound led to an increase in number of blood vessels in granulation tissue. Cells from fetal skin exerted a similar effect only when applied in the amount of 3×10^6. Injection of cells derived from bone marrow or fetal skin did not greatly affect inflammation or coverage of wound with epidermis, whereas adipose-derived MSC slightly enhanced inflammation and in some cases inhibited epithelialization.
Conclusion: The results obtained indicate that MSC may promote skin wound healing by stimulating angiogenesis in the granulation tissue. The best regenerative effect is provided by MSC derived from bone marrow.
Acknowledgements: The work was performed within the framework of the state program of fundamental scientific research No. 0108-2018-0004.

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Application of plant ROS1 5-methylcytosine-DNA glycosylase as tool for directed epigenetic demethylation in human cells

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Key words: epigenetic editing, protein and genomic engineering, DNA methylation/demethylation

Motivation and Aim: DNA methylation is a reversible epigenetic mark for transcriptional gene silencing in diverse organisms including plants and animals. In higher eukaryotes, two general modified DNA bases 5-methylcytosine (mCyt) and its oxidized derivative 5-hydroxymethylcytosine (hmCyt) play epigenetic roles. In mammals, active DNA demethylathion can occur by oxidation or deamination of mCyt, catalyzed by TET dioxygenases and AID/APOBEC deaminases respectively followed by base excision DNA repair. On the other hand, in plants mCyt can be directly removed by specialized bifunctional DNA glycosylases DEMETER and REPRESSOR OF SILENCING 1 (ROS1). However, the exact functions and substrate specificity of these plant DNA glycosylases remain unknown. Furthermore, ROS1 and DEMETER is a potential instrument for epigenome editing.
Methods and Algorithms: In this study, we cloned and purified a catalytically active fragment of ROS1 from *Nicotiana tabacum*. ROS1 activity was investigated on substrates with a CpG dinucleotide, in which cytosine was methylated, hemimethylated or hydroxymethylated. Besides, we transfected HEK293 cell line by a plasmid coding for wild-type *ROS1* or D1445N to detect of mCyt level variation.
Results: It was shown that ROS1 possessed an enzymatic activity to remove hmCyt and mCyt residues from DNA substrates. Moreover, substrates containing mCyt were digested more efficiently then substrates containing hmCyt in both CpG sites with only one modified strand and with fully modified site. ROS1 was expressed in human cells and caused global DNA demethylation.
Conclusion: The results obtained in the investigation suggest that plant ROS1 DNA glycosylase can contribute to change of mCyt level in human DNA and can be used as tool for quick and specific epigenome editing.
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Computer assessment of interaction between chemical comounds and human kinome

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Key words: kinome, kinases, (Q)SAR, PASS, web

Motivation and Aim: Human kinome is a large group of enzymes related by structure and function. Kinases play a major role in the regulation of almost all processes in a living cell, thus alterations of the kinases' activity can lead to or accompany with various diseases. Because of the such impact of kinases, they were earlier claimed the major drug targets of the XXI century. Nowadays, drug discovery needs to deal efficiently with the limited selectivity of the kinase inhibitors to detect their targets associated with the side effects at the early stages of development and select compounds that have acceptable kinases inhibition profile to be successful drugs or chemical probes. That was a reason for us to develop computational tool based on PASS software dedicated to the computer assessment of interaction between chemical compounds and human kinome and provide it as a freely available web-service.
Methods and Algorithms: We curated and utilized experimental data on inhibition of kinases by chemical compounds from ChEMBL database to train PASS. To access these data we used a local version of ChEMBL running under MySQL and PHP-scripts. R-packages were used to provide rigorous statistical validation of PASS predictions, including validation of the classification quality and early recognition. Web-service KinScreen was built to provide our results to the scientific community using PHP, JavaScript and Python programming languages.
Results: In our study we investigated the influence of the different strategies for training set creation on the quality of the classifiers. Implementing one of the strategies, we trained PASS and developed the web-service providing users with the computer assessment of inhibitory activity for chemical compounds against more than 300 of protein kinases (www.way2drug.com/kinscreen). The quality of prediction was assessed as ROC AUC calculated using leave-one-out cross-validation, and it exceeded 0.85. Besides the prediction, KinScreen provides a list of compounds from ChEMBL with the predicted profiles of inhibition of kinases, which are similar to one of the query molecule.
Conclusion: The computational tool for the assessment of interaction between small organic compounds and human kinome was developed and provided to the scientific community to accelerate process related to the kinase drug discovery and system biology studies.
Acknowledgements: This work was supported by Russian Science Foundation – Department of Science & Technology (India) grant No. 16-45-02012-INT/RUS/RSF/12.
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Circulating DNA-markers for diagnostics and monitoring of lung cancer: analysis of LINE-1 retrotransposons methylation in the blood

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Key words: lung cancer, diagnosis, prognosis, markers, methylation, retrotransposons, circulating DNA

Motivation and Aim: Malignant cell transformation is accompanied by two processes of DNA methylation changes: hypermethylation in CpG islands of tumor suppressor genes and global hypomethylation in repetitive DNA sequences (retrotransposons). The study aim was the estimation of the changes of LINE-1 retrotransposons methylation level in the blood from healthy subjects and lung cancer (LC) patients before and during antitumor therapy, assessment of its value as a tumor marker.

Methods and Algorithms: Blood samples were taken from healthy subjects (n = 32) and LC patients (n = 35), and also from LC patients during the post-treatment follow-up period. CirDNA was isolated from plasma and blood cell-surface-bound eluates. Concentrations of methylated LINE-1 region 1 (LINE-1met) were assayed by real-time methylation-specific PCR. In order to normalize the LINE-1 methylation level, the LINE-1 region 2 concentration was evaluated, which was independent of the methylation status (LINE-1Ind).

Results: The LINE-1 methylation level, determined as the ratio LINE met/LINE Ind, in csb-cirDNA from LC patients was significantly lower than in csb-cirDNA from healthy subjects (P = 0.005). In the total group of LC patients LINE-1 methylation level was shown significantly increased during the follow-up after chemotherapy ($P < 0.05$) and after surgery comparable to the methylation level before treatment ($P < 0.05$, paired t-test). The revealed association between LINE-1 methylation level and effect of antitumor therapy was more pronounced in squamous cell lung cancer compared with adenocarcinoma ($P < 0.05$ and $P > 0.05$, respectively). All relapse-free patients within the follow-up period (n = 25) were characterized by an increase of LINE-1 methylation level, and patients who experienced disease recurrence (n = 10) showed a decrease to values corresponding to the level before treatment.

Conclusion: Our data provide evidence that LINE-1 methylation level represents a valuable tool for diagnostics, evaluation of cancer treatment efficiency and post-treatment monitoring.

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Analysis of variance in the development of new drugs

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Key words: mathematical modeling, sorbents, modifiers, silver

Motivation and Purpose: The modern technology for the development of new drugs is closely connected with the formation and study of virtual mathematical constructions, created on the basis of experimental data and possessing all the properties of a real object, and also allows to regulate the parameters of physicochemical and technological processes in production [1].

Methods and Algorithms:The construction of a new silver-containing drug based on a porous sorbent carrier [2, 3] and the assessment of technological and physicochemical parameters of production was carried out with a mathematical apparatus of Statistica 6.0 programs. using multivariate analysis of variance developed by R. Fisher (ANOVA from Analysis of variance) [4].

Results: The composition of the silver-containing preparation "AlSi/Ag" was developed, the optimal concentration of auxiliary substances (AS) was determined, a direct proportional relationship was established between the sorption activity of the new silver-containing new drugs based on the porous sorbent carrier, the amount of auxiliary substances (AS) and the stages of the technological process.

Conclusion: The use of the mathematical apparatus in the development of a new drug made it possible to identify the optimal composition and technological parameters of the process of manufacturing the silver-containing preparation "AlSi/Ag".

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Genetic adaptation of human populations to climatic conditions of Northern Eurasia

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Key words: SNP, human populations, genetic diversity, natural selection, adaptation

Motivation and Aim: A modern human appeared in East Africa and was adapted to live in hot climatic conditions. After leaving Africa in the process of migration, the human populations settled other regions including regions with severe climatic conditions, such as Northern Eurasia. But the role of adaptive evolution in the formation of genetic characteristics of populations living in the territory of Northern Eurasia has been poorly studied. Long-term adaptive evolution of the gene pool of the indigenous population of this territory could lead to the formation of stable adaptive complexes. Thus, the purpose of the study is to search for genetic markers of adaptation of human populations to climatic conditions of Northern Eurasia.
Methods and Algorithms: 17 population samples from Northern Eurasia and data on 43 populations from the "1,000 genomes" and HGDP projects were studied within the present work. Polymorphic genetic markers included in the analysis showed the natural selection effect, according to literature data, and/or they belong to genes associated with cold tolerance, namely involved in such processes as muscle contraction regulation, thermoregulation, regulation of arterial pressure, lipid metabolism, etc. [1–6]. Thus, 25 SNPs were selected. The distribution of frequencies of the SNPs studied in the populations was analyzed using a test based on the degree of the genetic differentiation of populations (FDIST).
Results: As suggested by FDIST, significantly higher differentiation of populations of these markers was shown. Directional selection signals were recorded by us for the markers of the *LEPR*, *LRP5*, *UCP2* and *MAPK1* genes. The *LEPR* gene associated with the obesity development has been confirmed in various ethnic groups [7]. Mutations of the *LRP5* gene were responsible for the occurrence of pseudogliomatic osteoporosis and increased bone resorption syndrome [8]. The product of the *UCP2* gene takes part in thermogenesis, regulation of fat metabolism and energy consumptions and protection from oxygen reactive forms [9]. The *MAPK1* gene is involved in a variety of cellular processes including proliferation, growth and differentiation, and it is associated with the cancer development [10].
Conclusion: As a result of the presented study, we revealed the effect of directional selection in connection with the adaptation of human populations to the cold climatic conditions of Northern Eurasia in the process of human settling around the globe.
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MicroRNA in lymph in experimental breast cancer

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Key words: breast cancer, lymph, N-methyl-N-nitrosourea

Motivation and Aim: Breast cancer (BC) is the leading cause of cancer death in women worldwide due to the complicated etiology involving both genetic and environmental factors. Therefore, experimental animal models of BC are used for study development and treatment of BC. So, experimental BC model in rat often used for researcher the development of BC because the main steps of pathogenesis and progression, and also, the histology are similar to BC in humans. N-methyl-N-nitrosourea (MNU) is a DNA alkylating agent which can induce a mammary tumor, and does not require metabolic activation to exert mutagenic effects, and also, the tumors had a more aggressive potential. MicroRNAs (miRNAs) are approximately 22–25 nucleotide small non-coding RNAs. miRNAs took part in cell proliferation, development, differentiation, tumorigenesis, and are widely dysregulated in various cancers. The expression levels of miRNA-21, miRNA-221, miRNA-222 and miRNA-429 in lymph of chemically induced of BC in rats, not sufficiently studied.
Materials and Methods: In the experiment were used rats-female Wistar 10–12 week-old, weighted 250–300 g. MNU (Sigma) was administered into second mammary gland on right side to 100 Wistar rats at 30 mg/kg body weight dissolved in warm physiological saline and acidified to pH 5.0 with acetic acid, once per week 5 times. In control group animal was given an identical volume of physiological saline. 24-weeks after MNU administration in experimental group of animal the second mammary gland with tumor were excised under anesthesia with a dose of Nembutal 40 mg/kg body weight intraperitoneally injection (Sigma). Animals from control group and BC-control group don't receive any manipulations, whereas part of MNU-induced BC animal received chemotherapy alone (CMF group). Among operated animals was randomly divided next groups: Resection group (excised mammary gland with tumor), Resection/CMF group (excised mammary gland with tumor and done chemotherapy). 26-weeks after administration MNU and treatment lymph specimens (thoracic duct lymph) were collected. The gene expression (miRNA-21, miRNA-221, miRNA-222, miRNA-429, and U6 (small RNA)) was determined by absolute nucleic acid quantification method with 4.0 Light Cycler software (Roche).
Results: In BC group in thorax duct lymph were detected significantly increased expression levels of miRNA-21 compare with control group ($p < 0.05$). After chemotherapy in thorax duct lymph observed significantly decreased levels of the miRNA-21 expression compare with BC group. In animal from BC group were detected significantly increased levels of miRNA-221 expression compare with control group ($p < 0.05$). After chemotherapy in thorax duct lymph observed significantly decreased levels of the miRNA-221 expression compare with BC group ($p < 0.05$). In BC group in thorax duct lymph were detected significantly decreased expression levels of miRNA-429 compare with control group ($p < 0.05$). After chemotherapy in thorax duct lymph observed significantly increased levels of the miRNA-429 expression compare with BC group.
Conclusion: In summary, in lymph miRNA-21 and miRNA-221 are diagnostic-relevant tumor markers. MicroRNA-429 is diagnostic-relevant marker of the effectiveness of chemotherary.

Lymph cytokines – markers of oncogenesis

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Key words: lymph, cytokines, breast cancer, chemotherapy, panagen, Wistar rats

Motivation and Aim: Breast cancer (breast cancer) maintains a leading position among oncological pathologies in women all over the world. Cytokines as factors of the lymphatic and lymphoid systems play an important role in the pathogenesis of socially significant pathologies to which cancer belongs. Of particular relevance is the study of the cytokine profile of lymph in tumor growth, since the lymphatic system plays an important role in the pathogenesis and spread of the tumor process and metastasis is predominantly lymphogenous. The purpose of this work is to examine the levels of cytokines in the lymph involved in the pathogenesis of breast cancer.

Materials and Methods: In the experiment were used rats-female Wistar. Breast cancer was modeled by introduction of N-methyl-N- nitrosourea 5 times, every 7 days subcutaneously in area 2 th mammary gland on the right. After 6 months, formed a breast cancer (adenocarcinoma). Performed resection of breast cancer, chemotherapy according to the scheme of CMF after 6 months from the start of the experiment. So some of the animals subjected to surgery alone or chemotherapy alone (cyclophosphamide, methotrexate, 5-fluorouracil). Some animals combine both types of therapy, as well as a separate group to the administration of chemotherapy added Panagene drag presenting a fragmented DNA. To investigate the concentration of cytokines used in lymph test system Bio-Plex Pro Rat Cytokoness 24-Plex Assay (Bio-Rad, USA).

Results: In rats with breast cancer content of most studied cytokines such as, IL-1β, IL-2, IL-4, IL-6, IL-7, IL-12, IL-13, IL-17A, MIP-1α, MIP-3α , RANTES, TNF-α, MCP-1 was significantly higher than in intact animals. Surgical removal of the tumor resulted in a significant decrease in the content in the lymph as a pro-inflammatory cytokine. Comparative performance study cytokine content in the lymph after tumor removal from intact animals showed that the content of cytokines such as IL-10, IL-18, GRO/KC, RANTES were significantly higher in the control animals group. Conducting chemotherapy has led to a significant decrease in the content of IL-1β, IL-4, IL-6, IL-7, IL-10, MIP-1α, MIP-3α, RANTES in rat breast cancer lymph. Comparative study of cytokine content in the lymph operated animals after the administration of chemotherapy and Panagene revealed that most of the content indicators cytokines such as IL-5, IL-6, IL-7, IL-10, IL-13, IL-17A, IL-18, GRO/KC, IFNγ, MIP-3α in the lymph was higher after administration of the drug Panagene.

Conclusion: In a comparative study of the cytokine profile of Wistar rats, it was found that the quantitative content of cytokines in breast cancer depends on the type of therapy for the disease. The breast cancer stimulates the production of both pro-inflammatory and anti-inflammatory cytokines, the main producers of which are immunocompetent cells, cells of the tumor microenvironment and the tumor itself. The production of cytokines can change in the course of progression, suppression of tumor development or metastasis. Levels of lymph cytokines can serve as a diagnostic criterion for tumor growth, as well as a prognostic criterion for the effectiveness of therapy and the risk of metastatic breast cancer.

Effect of cytokines on functional properties of fibroblasts

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Key words: fibroblasts, proliferation, migration, conditioned media

Motivation and Aim: In the process of skin regeneration/reparation for various lesions, a number of cells participate, including fibroblasts (Fb), whose functional activity is regulated by a whole spectrum of cytokines and growth factors, including those secreted by other cells populations. On the other hand, these factors are currently considered as potential therapeutic agents that increase the efficiency of regeneration [1].

Methods and Algorithms: The proliferation of primary Fb under the influence of erythropoietin (Epo, 33 IU/ml, Recormon, Germany), VEGF (10 ng/ml, BioVision, USA) and conditioned medium(CM) from multipotent stem cells (MSC) and endothelial cells EA.hy926 was studied in vitro in MTT test. To obtain CM, the cells were cultured in DMEM/F12 for 72 hours. The cell proliferation index and the migration of fibroblasts were evaluated in cell or double-chamber cells by changing the cellular impedance on xCELLigence (Roche Applies Science, Germany). Migration of Fb during wound healing with the application of culture method with desquamation of the monolayer was analyzed using Axio Observer z1 (Carl Zeiss).

Results: In comparison with control level, proliferation of the fibroblasts significantly increased under the influence Epo, VEGF, CM-EA.hy926, CM-MSC of as early as post-addition (experimental) hour 8. Starting from experimental hour 32, the proliferative potential of fibroblasts aproximated to the plateau level for VEGF (cells idex, CI = 1.63) and EPO (CI = 2.1), and the cellular index grows to 24 h (cells index = 2.6) for CM-EA.hy926, CM-MSC. The impedance data revealed stimulating effect of VEGF, CM-EA.hy926, CM-MSC and slightly EPO on fibrblast migration. On experimental hour 8, fibroblast migration along VEGF, EA.hy926, CM-MSC concentration gradient significantly exceeded the corresponding control data, and this difference persisted to the end of experiment with CI = 1.57; 1.55; 1.57, respectively (7.5-fold greater than in control). It was revealed that fibroblasts close the acellular surface to 70 % of the initial values by 48 hours of the experiment, and the area of closure of the cell-free surface in the presence of CM-EA.hy926, CM-MSC is about 90 %.

Conclusion: Our results suggest that cytokines exert a stimulating effect on the functional state of fibroblasts, which allows the activation of fibroblasts in vitro for subsequent therapeutic effects in the treatment of various types of tissue damage.

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Polymorphic variants of signaling pathway Akt1/GSK-3β gene and tardive dyskinesia in schizophrenic patients

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Key words: tardive dyskinesia, schizophrenia, gene, polymorphism, signaling pathway, side effect

Motivation and Aim: Schizophrenia is a severe mental disorder. Its pathophysiological and genetic causes are ongoing subject of study [1]. Mainstays in the treatment of schizophrenia are antipsychotic medications. However, apart from having beneficial effects, antipsychotic drugs also cause tardive dyskinesia (TD) – a drug-induced extrapyramidal movement disorder which occurs about 20–30 % in patients with severe mental illness [1, 2]. The Akt1 gene, also known as PKB (protein kinase B), is involved in regulation of neuronal plasticity and located at 14q32.32, has been associated with schizophrenia [3, 4]. GSK3β gene (glycogen synthase kinase 3 beta) encodes a protein which is negative regulator of glucose homeostasis, involved in a variety of neurochemical processes, and polymorphic variants of the gene have been found associated with neurodegenerative disorders [5]. Our objective is to investigate the role of one polymorphic variant of *GSK3β* (rs334558) and two polymorphic variants of *Akt1* (rs3730358, rs1130214) genes in development of tardive dyskinesia in patients with schizophrenia in Russian population.
Methods and Algorithms: We were examined 459 patients with schizophrenia. Mean age was 42.1 ± 12.4 years. Mean duration of disease was 13 years for schizophrenic patients. The main criteria for including the patients in the study were clinically verified diagnosis of schizophrenia, according to the International Statistical Classification of Diseases and Related Health Problems, 10th Revision (ICD-10: F20) and absence of organic or neurological disorders. The severity of TD was evaluated by AIMS scale. For DNA extraction we were used standard phenol-chloroform protocol. Genotyping was carried out on 3 SNP's (rs334558, rs3730358, rs1130214) and used the fluorogenic 5′-exonuclease TaqMan technology and an amplifier real-time polymerase chain reaction system "StepOne Plus" (Applied Biosystems, USA). Statistical analysis was carried out with SPSS software, release 17. Statistical significance of tested associations was considered for significance at a P-value less than 0.05. Hardy-Weinberg equilibrium for distribution of genotypes was tested by χ^2-test. The frequency of genotypes and alleles were compared using χ^2-test. Investigating certain genotypes (and their combinations) with TD made with odds ratio and 95 % confidence interval.
Results: This study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki 1975, revised in Fortaleza, Brazil, 2013), established for experiments involving humans. We recruited patients from three psychiatric hospitals located in the Tomsk, Kemerovo, and Chita oblasts (regions) of Siberia, Russia. In total 459 white patients with verified diagnosis of schizophrenia were recruited. The distribution of genotypes of studied genes corresponded to the Hardy-Weinberg equilibrium. Before starting statistical analysis we divided patients into 4 partially overlapping groups: patients without TD, patients with general TD diagnosis, patients with limb-truncal TD type and patients with orofaciolingual TD type. We didn't get statistically significant results for genotypes and alleles of polymorphic variant rs334558 ($\chi^2 = 0.367$, $p = 0.832$) of *GSK3β* gene and for *Akt1* rs3730358 ($\chi^2 = 1.178$, $p = 0.555$), rs1130214 ($\chi^2 = 3.136$, $p = 0.208$) for general diagnosis of TD. Also we doesn't reach significant results with comparison between groups of patients with different types of TD and without it. Odds ratio metrics does not allow us to conclude about the possible effects for genotypes and alleles.
Conclusion: We have received negative results for all types of statistical analysis for different types of TD, and our results aren't support hypothesis about involvement this polymorphic variants of signal pathways genes. Nowadays were conducted a lot of studies about tardive dyskinesia's pathology, and they were prove that is complexed side-effect. It needs further prospective association-like studies to discover the nature of TD and it helps increase level of life for schizophrenic patients.
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Investigation of biological effects of cluster complexes of metals and their conjugates with cyclodextrins *in vivo* and *in vitro*

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Key words: cluster complexes, photodynamic therapy

Motivation and Aim: Due to their x-ray contrast and luminescent properties, cluster complexes of heavy metals are promising agents in such areas as radiation diagnostics, orthopedics and traumatology, fundamental biological research [1, 2]. In order to understand the possibilities of introducing these compounds into clinical practice, it is necessary to thoroughly study their effect on living cells and the animal organism.
Methods and Algorithms: Our group synthesized cluster complexes with the chemical formula $[\{Nb_6Cl_{12}\}\ (H_2O)_6]Cl_2$, which showed high x-ray contrast. The conjugate of this cluster with cyclodextrin molecules was also obtained. The next stage was the study of biocompatibility of the obtained complexes.
On cell cultures cytotoxicity of solutions of the studied complexes was evaluated using MTT-test and double coloring with Hoechst and Propidium iodide dyes.
We compared the acute toxicity of the test substances on mice Balb/C. To assess the impact of cluster complexes in the organs of animals and identify the target organs morphological analysis of liver, kidney, spleen, heart and lungs of animals 2 weeks after injection of solutions was carried out.
Results: In cell cultures it was shown that conjugation with cyclodextrin reduces the cytotoxicity of these complexes. On the mouse model cluster complexes showed the opposite effect-toxicity of the cluster with cyclodextrin was higher than without cyclodextrin. The analysis of organs showed that intravenous injection of cluster complex with cyclodextrin causes stasis of blood in many organs.
Conclusion: The results obtained in the experiment suggest that the conjugation of the niobium cluster complex $[\{Nb_6Cl_{12}\}\ (H_2O)_6]Cl_2$ with cyclodextrin molecules increases the biocompatibility of this compound *in vitro*, but does not allow its injection into the body intravenously in high dosage.

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Interactome network topology in diabetic retinopathy

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Key words: diabetic retinopathy, interactome network, cytokine genes, glycated hemoglobin

Motivation and Aim: The diabetic retinopathy (DR) is one of the heaviest vascular complications of type 2 diabetes (DM). There are known, that DR are formed as a result of complex interaction genetic, metabolic and hemodynamic factors [1, 2] which may form Interactome network, in our opinion. The studying of topology of given Interactome is actual for understanding of mechanisms of DR development from positions of the theory of complex networks. The realization of computer modelling of connections of cytokines genotypes, which are involved in regulation of inflammation and angiogenesis, and HbA1c levels – the integrated marker of hyperglycemia is the purpose of this research.
Methods and Algorithms: 201 Caucasoid females with DM 2, including 90 patients with DR and 111 patients without other are included in research. There were studied 13 SNPs in the promoter regions of following genes: *IL1B:-31 C/T* (rs1143627), *IL4:-590 C/T* (rs2243250), *IL6:-174 C/G* (rs1800795), *IL10:-592 C/A* (rs1800872) and *-1082 A/G* (rs1800896), *TNFA:-238 A/G* (rs361525), *-308 A/G* (rs1800629) and *-863 C/A* (rs1800630), *VEGF:-2578 C/A* (rs699947) and *+936 C/T* (rs3025039), *MMP2:-1306 C/T* (rs243865), *MMP3:-1171 5A/6A* (rs3025058), *MMP9:-1562 C/T* (rs3918242). The connection of genetic factors and the HbA1c level carried out by quantile analysis. The visualization of gen-gen and gen – protein interaction at groups with and without DR as Interactome network carried out in program Cytoscape v.3.6.0.
Results: The ability to self-control of promotion activity of regulators genes of an immune inflammation, angiogenesis and HbA1c level for the account of closed regulatory contours submitted by two closed triplets with general hub as protein tops HbA1c-LL is the prominent feature of topology of the gene-proteins network, which is associated with protection to DR development. Whereas for interactome network topology by ДR development, the low clusterization level for the account only two open triplets without general protein (HbA1c-HH) hub is characteristic.
Conclusion: Results of our research show, that construction of interactome biological networks of transcription regulation both metabolic ways and their topological analysis on the basis of bioinformation platform Cytoscape allows to build and study intergenic and gene – proteins interactions with reference to research of DM pathogenesis complications.

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Reperfusion activates AP-1 and heat shock response in donor kidney parenchyma after warm ischemia

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Key words: ischemia, kidney transplant, network analysis, AP-1 transcription factors, heat shock proteins

Motivation and Aim: Utilization of kidneys from extended criteria donors lead to an increase in average Warm Ischemia Time (WIT), which is associated with larger degrees of ischemia-reperfusion injury (IRI). Kidney resuscitation by extracorporeal perfusion *in situ* allows up to 60 minutes of asystole after the cardiac death. Molecular studies of kidney grafts from human donors with critically expanded WIT are warranted.
Methods and Algorithms: Transcriptomes of two human kidneys from two different donors were profiled after 35–45 minutes of WIT and after 120 minutes of normothermic perfusion and compared.
Results: Baseline gene expression patterns in ischemic grafts display substantial intrinsic differences. IRI does not lead to substantial change in overall transcription landscape, but activates a highly connected protein network with hubs centered on Jun/Fos/ATF transcription factors and HSP1A/HSPA5 heat shock proteins. This response is regulated by positive feedback. IRI networks are enriched in soluble proteins and biofluids assayable substances, thus, indicating feasibility of its longitudinal, minimally invasive assessment *in vivo*.
Conclusion: Mapping of IRI related molecules in ischemic and reperfused kidneys provides a rationale for possible organ conditioning during machine assisted *ex vivo* normothermic perfusion. Studies of natural diversity of the transcriptional landscapes in presumably normal, transplantation-suitable human organs are warranted. As transplantation outcomes may be influenced by summarily outputs of the networks formed both by protective and by injury-promoting molecules, larger transcriptome-based studies of donors organs should be performed, and the resultant networks correlated with short and long-term clinical outcomes.
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Light as a therapeutic resource

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Key words: circadian system, zeitgeber, bright light

Motivation and Aim: The circadian system (CS) aids survival by predicting the geophysical daily and seasonal cycles and by maintaining the organism's internal temporal organization. Nevertheless, the CS may be impaired by: 1) degenerative changes in the Suprachiasmatic Nucleus (SCN), the master clock of the organism, 2) incorrect exposure to Zeitgebers, the ambient factors that entrain the SCN with the geophysical cycles, 3) a deficient response of target tissues to the SCN control. Altogether, these disturbances may cause cognitive, emotional, behavioral and clinical problems that often end in a temporal disruption and in the metabolic syndrome, which in turn is associated, among other conditions, to high arterial pressure, obesity, cardio- and cerebrovascular accidents and cancer.

This report will show some consequences of incorrect light exposure (the main human Zeitgeber) as well as the effects of bright light therapy on age-related CS disturbances, but also on hospitalized depressed patients and on the academic results of schoolchildren.

Methods: Using multisensory data holders (Kronowise KW6, Cronolab, Universidad de Murcia, Spain), we recorded: 1) the light levels in the spaces occupied by subjects, 2) the actual light levels perceived by the same subjects, 3) their general motor activity and 4) their peripheral temperature. Their cognitive, health and emotional status as well as their sleep time and quality were recorded by using appropriate psycho-physiological tests. Further, a group of institutionalized elders were exposed, for 1.5 h daily, 7 days, to 10,000 lux of artificial bright light. Their psychophysiological status was recorded along three successive weeks: the first one to obtain basal data; the artificial bright light therapy was administered during the second week and the third one was used to check the eventual post-effects. Regarding depressed patients and schoolchildren, the time of symptoms remission and the academic qualifications, respectively, served to evaluate the consequences of different illumination in the psychiatric wards and in the classrooms.

The circadian results were analyzed off-line by the seller of the data holders using specific software for circadian analysis (Kronowizard, http://www.um.es/cronobiologia).

Results: High levels of natural light improved the health and cognitive status, the sleep quality and the mood in elder subjects, a result that was also obtained when exposed to artificial bright light. Increasing the exposure to natural light shortened the hospitalization time in depressive patients. Schoolchildren studying in well illuminated classrooms obtained better academic qualifications. Most light-related improvements were statistically significant in elder, in depressive patients and in schoolchildren.

Conclusion: Light is an affordable, effective and fast-acting therapeutic resource that causes significant improvements in general health, cognitive functions, sleep and emotional status of institutionalized elder and depressive patients. In addition, improves the academic results of schoolchildren.

MetaTox – web application for generation metabolic pathways and toxicity estimation

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Key words: LMNA, PASS, site of metabolism, biotransformation, xenobiotics metabolism, acute toxicity, carcinogenicity, adverse effect

Motivation and Aim: Many xenobiotics, including drugs, are metabolized in the human organism by multiple enzyme systems. The metabolite of the drug can significantly differ from the parent compound on bioactivity profiles or adverse and toxic effects [1].
Methods and Algorithms: The generation of the metabolites is based on a database of fragments, which describe a transformation of a substrate structure to a metabolite structure for 15 most common classes of xenobiotic's biotransformation reactions. The estimation of probability of the generated metabolite formation is performed on the basis of integrated assessment of probabilities of biotransformation reactions and their positions using PASS (http://way2drug.com/passonline), which is based on the training set consisting of more than 3.500 substrates.
Results: We created the web-application MetaTox (http://way2drug.com/mg) for the generation of xenobiotics metabolic pathways in the human organism. The MetaTox user can manually select parameters of a metabolic tree generation, such as its depth and the metabolite formation probability threshold. The prediction accuracy estimated by the leave-one-out cross-validation procedure calculated separately for the probabilities of biotransformation reactions and their position is about 0.9 on the average for all reactions. The estimation of acute toxicity, based on GUSAR software [2], and estimations of the organ-specific carcinogenicity [3] and adverse effects, based on PASS software [4], are integrated in the MetaTox.
Conclusion: MetaTox [5] is a freely available web application for the evaluation of metabolic pathways and toxic effects of xenobiotics, which is useful in the early stages of the drug discovery cycle.
Acknowledgements: Supported by the Russian Science Foundation (No. 14-15-00449).

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Study of mtDNA copy number and LINE-1 in atherosclerosis

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Key words: mtDNA copy number, LINE-1 methylation, atherosclerosis

Motivation and Aim: It is known that the mtDNA copy number per cell decreases with age and in some diseases. In the nuclear genome, mechanisms that support tissue-specific DNA methylation profiles are disrupted with age, leading to decline of methylation level, known as "epigenetic drift" [1]. Overall genome methylation level can be estimated with a surrogate marker retrotransposon LINE1, occupying about 18 % of human genome. Methylation level in this locus decreases with age [2]. The aim of the study was to estimate mtDNA copy number in atherosclerosis and its correlation with LINE-1 methylation level.

Methods and Algorithms: The study included 67 patients with carotid atherosclerosis and 26 asymptomatic volunteers as control group. In both samples, DNA was extracted from leukocytes. In the patients, fragments of carotid atherosclerotic plaques obtained during endarterectomy were studied as well. MtDNA copy number was estimated by multiplex quantitative PCR [3]. LINE-1 methylation level in the same samples was studied by pyrosequencing in the previous study [4]. Statistical processing was performed using Spearman correlation, Mann-Whitney and Wilcoxon tests.

Results: MtDNA copy number in leukocytes of patients was lower than in the atherosclerotic plaques: an average 1303 copies per cell in leukocytes, and 3.369 copies per cell in plaques ($p < 0.001$). The analysis revealed negative correlation of mtDNA copy number with the LINE-1 methylation in patients, both in plaques ($r_s = –0.52$, $p < 0.05$) and in the blood ($r_s = –0.41$, $p < 0.05$). In the control group, mtDNA copy number was positively correlated with age ($r_s = 0.62$; $p < 0.05$).

Conclusion: Correlation of the mtDNA copy number in atherosclerotic plaques with LINE-1 methylation level suggests existence of epigenetic regulation of mitochondrial genome replication. The negative character of the correlation may as well indicate a compensatory amplification of mtDNA in response to the mitochondrial dysfunction and increased oxidative stress in atherosclerotic plaques.

Acknowledgements: The study was supported by the RFBR (16-04-01481-A).

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Natural bispecific antibodies – biochemical markers of autoimmune pathology

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Key words: antibodies, bispecific antibodies, immunoglobulins, autoimmune diseases, systemic lupus erythematosus, multiple sclerosis

Motivation: Bispecific antibodies contain simultaneously two distinct antigen-binding centers against two different antigens. We have shown that as the result of Fab-arms exchange human milk and placenta contains bispecific antibodies. Molecules, containing both types of light chains (κλ-IgG) were presented by subclasses (IgG1-4). Interestingly, bispecific human milk κλ-IgG are presented mostly by IgG1 (74 %), IgG2-IgG4 (5–16 %) and placenta κλ-IgGs consisted of 43.5 % IgG1, 41.0 % IgG2, 5.6 % IgG3 and 7.9 % IgG4. Moreover, human milk contains up to 54 % of chimeric κλ-IgG, 17 % κλ-sIgA and placenta in average contains up to 15.0 % κλ-IgG. Here we show the content of bispecific IgG in the blood serum of autoimmune patients.
Methods: The affinity chromatography, SDS PAGE, Western blotting and ELISA were used to obtain bispecific IgG fractions from blood of healthy donors, systemic lupus erythematosus and multiple sclerosis patients.
Results: Here we show that the serum of patients with rheumatic pathology contains significantly higher concentrations of bispecific IgG molecules than in healthy donors. Since the formation of bispecific antibodies may be due to unknown processes occurring in immune system during autoimmune pathology, the presence of such antibodies in the serum of patients with systemic lupus erythematosus and multiple sclerosis may be a new biochemical marker of autoimmune disorders.
Acknowledgements: The reported study was funded by RFBR, according to the research projects 16-34-60066 mol_a_dk, 16-04-00603 a, 16-04-00604 a and by the President Grant for young scientists MK-410.2017.4.

Natural bispecific antibodies: generation, isolation, biological functions

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Key words: bispecific antibodies, bispecific immunoglobulins, IgG, autoimmune diseases, human milk, human blood, human placenta

Motivation and Aim: Natural IgG presented in biological fluids are considered as stable molecules with two identical antigen-binding sites. The Fab arms exchange was first described only for the IgG4 subclass. We have shown that human milk contains up to 54 % of bispecific κλ-IgG molecules and up to 17 % κλ-sIgA. Interestingly, bispecific human milk κλ-IgG are represented by all subclasses, of which the most significant is IgG1 (74 %) [1]. Later we have shown the existence of bispecific κλ-IgG molecules of all IgG1-4 subclasses in human blood and placenta [2, 3].
Methods: Here we show using the affinity chromatography, SDS PAGE, Western blot and ELISA that the Fab arms exchange occurs in healthy donors and patients with systemic lupus erythematosus and multiple sclerosis, as well as spondylitis and system scleroderma.
Results and Conclusion: The presence of natural bispecific antibodies in the human serum earlier has been shown in Hashimoto thyroiditis [4], myasthenia gravis [5] and rheumatoid arthritis [6]. In the listed papers the only presence of several clinically relevant bispecific molecules of IgG4 subclass was described. Here we show that the serum of autoimmune and rheumatic patients contains significant amounts of κλ-IgG, presented with all IgG1-4 subclasses. Since chimeric κλ-IgG molecules are overrepresented in the blood of autoimmune patients comparing to healthy donors, it could be considered that bispecific antibodies are biochemical markers of autoimmune and rheumatic disorders.
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FastPrep: full solution for sample homogenization and extration of DNA, RNA and proteins

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Key words: sample homogenization, FastPrep, DNA, RNA, proteins

Motivation and Aim: MP Biomedicals presents the FastPrep-24 5G Instrument, a new high throughput model of the FastPrep homogenizer offering a unique means by which virtually any type of sample, no matter how difficult, can be quickly and consistently lysed within 40 seconds. The FastPrep-24 5G Instrument uses a unique, optimized motion to disrupt cells through the multidirectional, simultaneous beating of specialized Lysing Matrix beads on the sample material and is designed to homogenize up to 24 samples in 2ml tubes. Developed for difficult and resistant samples, the FastPrep-24 5G Instrument lyses thoroughly and quickly any tissues and cells and thus allows easy and reproducible isolation of stable RNA, active proteins and full-length genomic DNA.

A completely self-contained system, the FastPrep-24 5G Instrument eliminates the risk of cross-contamination and time-consuming clean-up associated with manual lysis methods.

Samples and buffers are simply added to a Lysing Matrix tube containing specialized Lysing Matrix beads. The ergonomic design of the instrument ensures easy loading of the sample tubes that remain securely sealed during the processing. The homogenization speed and duration times are digitally controlled.

The vertical angular motion of the FastPrep-24 5G Instrument causes the lysing matrix particles to impact the sample from all directions simultaneously, releasing nucleic acids and proteins into the protective buffer. After centrifugation, the supernatant is collected for further purification process.

When compared to traditional homogenization methods such as vortexing, syringe shearing, grinding with a mortar and pestle or hammering samples that have been frozen in liquid nitrogen, the FastPrep-24 5G homogenizer will save hours of work during the sample preparation stage and will provide higher yields of intact DNA, RNA and proteins.

A wide variety of specialized Lysing Matrix tubes containing beads of different materials, sizes and shapes have been tailored to guarantee a thorough homogenization of samples from diverse sources including bacteria, yeast, fungi, botanical samples, insects, mammalian tissues and cultured cells.

High performance FastPrep purification kits used in conjunction with the FastPrep-24 Instrument provide ready-to-use methods for the release and subsequent purification of intact DNA, RNA, and proteins from virtually any source.

FastDNA Kits quickly and efficiently isolate genomic DNA with a silica-based Geneclean procedure. Eluted DNA is ready for digestion, electrophoresis, PCR and any other desired application.

The single-reagent extraction method of the FastRNA Pro Kits safely releases total RNA into the proprietary RNApro Solution where it is instantly stabilized. RNA in RNApro Solution is extracted with chloroform and precipitated with ethanol. The resulting high-quality RNA is ready for downstream applications including RT-PCR and Northern analysis.

Conclusion: MP Biomedicals presents the full solution for sample homogenization, DNA, RNA, protein extraction. The FastPrep-24 5G can simultaneously process for up to 24 samples, FastPrep-96 may be used for high throughput homogenization, SuperFastPrep-2 is an ideal model for expedition use. The variety of 16 Lyzing Matrixes allows perfect homogenization of virtually any type of sample within 40 seconds.

Cellular technologies based on dendritic cells

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Key words: dendritic cells, cell vaccine, DNA-constructions, oncology, transplantation

Motivation and Aim: Cell technologies are a new and promising direction in modern biology and medicine. One of the directions of cellular technologies based on autologous dendritic cells is the use of in vitro induced dendritic cells transfected with DNA constructs encoding epitopes of tumor-associated antigens (with cellular cancer immunotherapy) or epitopes of the main antigens involved in the development of post-transplant complications specific cell prophylaxis of post-transplant complications). The purpose of this work is to evaluate the effectiveness of the use of dendritic cells transfected with antigen-specific DNA constructs to induce a cytotoxic antitumor immune response in vitro in cancer (breast cancer, colorectal cancer, non-small cell lung cancer) and to suppress the "graft rejection" reaction in experimental model in linear mice.

Methods and Algorithms: The original DNA constructs encoding epitopes of the main tumor-associated antigens of epithelial neoplasms (breast cancer, colorectal cancer, non-small cell lung cancer), which were transfected into dendritic cells of cancer patients, were used to induce an antigen-specific antitumor immune response. The obtained antigen-specific dendritic cells were cultured with autologous mononuclear cells to induce a cytotoxic antitumor immune response, the efficacy was assessed by direct cytotoxic assay. An experimental model of "transplant rejection" was placed on female C57Bl/6 mice. Antigen-specific suppression of transplant rejection was accomplished by 2-fold administration of a co-culture of dendritic cells transfected with a DNA construct encoding the major epitopes of histocompatibility antigens involved in the development of the transplant rejection reaction and splenocytes. The effectiveness of suppression of the "graft rejection" reaction was evaluated by the increase in the graft rejection time.

Results: The effectiveness of cellular preparations obtained using dendritic cells and antigen-specific DNA constructs was demonstrated in experiments on induction of an antitumor immune response in vitro and in in vivo experiments on a mouse model of "graft rejection".

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Exome analysis of patients with maturity onset diabetes of the young in Russia

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Key words: maturity-onset diabetes of the young, MODY, exome sequencing, genetic diagnosis

Motivation and Aim: Maturity onset diabetes of the young (MODY) is a hereditary form of diabetes with an autosomal dominant type of inheritance, onset at a young age, and a primary defect in pancreatic β-cells [1]. The aim of the study was to identify the spectrum of mutations in Russian patients with MODY phenotypes using whole-exome sequencing.

Methods and Algorithms: We diagnosed MODY using a combination of clinical signs and next generation sequencing. The mutations that we identified were then verified by Sanger sequencing.

Results: Among the 19 patients who had the clinical signs of MODY, the likely causative mutations were detected in the known MODY genes (*GCK*, *HNF1A*, and *ABCC8*) in 9 patients. We have identified a number of pathogenic mutations in MODY-associated genes (rs762263694, chr7:44190602 c.439 C>G, rs193922297, rs144723656 in *GCK*; rs483353044 in *HNF1A*; rs72559717 in *ABCC8* [2]). Most of the detected substitutions were missense mutations, and only one of them was located in noncoding regions (chr7:44189459 c.580-1G>A in *GCK* gene).

Conclusion: In the present study, we diagnosed MODY using a combination of clinical criteria and next-generation sequencing (NGS). The NGS method was effective as a first-line screening test for the detection of MODY-associated mutations.

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Defining the genetic control of human blood plasma glycome using genome-wide association study

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Key words: glycomics, glycans, genome-wide association study

Motivation and Aim: Glycosylation is the most common post-translational modification of proteins. Glycans are directly involved in the pathophysiology of every major disease. Defining genetic factors, altering glycosylation, provides a basis for novel approaches to diagnostic and pharmaceutical applications. Here, we aimed to conduct genome-wide association study (GWAS) of human blood plasma glycome measured by the recently developed Ultra Performance Liquid Chromatography (UPLC) technique.

Methods and Algorithms: We conducted GWAS of human plasma glycome in four cohorts: TwinsUK ($N = 2763$, discovery stage), PainOR, COGS and QMDiab (replication stage, total $N = 1048$). As the number of quantified glycan traits measured by UPLC varied from 36 to 42 across cohorts, we developed, validated and applied a protocol that resulted in the harmonized set of 36 glycan traits. Based on the biochemical structure of 36 original glycans, we computed additional 77 derived traits leading to total of 113 glycan traits.

Results: We found 14 loci that were significantly associated (P-value $< 1.66 \times 10^{-9}$) with at least one glycan trait. Of the 14 loci, 4 were previously reported to be associated with plasma glycome composition [1]. For the other 10 loci the significant association with plasma glycome was demonstrated for the first time. Seven out of ten loci were replicated independent cohorts with P-value < 0.005. Additionally, we replicated five of six previously published loci.

Conclusion: Our efforts brought the total number of loci significantly associated with total plasma N-glycome from 6 to 16, and the number of replicated loci from zero to 12. Improvements in measurement technology in combination with extended genome reference panel provided additional power to our analysis

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Association of intracellular proteolytic systems and locomotor proteins in tissues of primary tumor and lymphogenous metastases of breast cancer

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Key words: breast cancer, metastases, proteasomes, calpains

Motivation and Aim: The capability for active movement in an extracellular matrix, where in remodeling of the cytoskeleton by actin binding proteins plays a significant role in metastases formation. The proteasomes and calpains modify the biologically important molecules involved in the pathogenesis and progression of a variety of malignancies, including breast cancer (BC).The aim of the work was to study the association of actin binding proteins (cofilin, gelsolin, arp-3, thymosin-4-β, Ser45 β-catenin) and proteasomes and calpains activities in tissues of primary tumors and lymphogenous metastases of BC.
Methods and Algorithms: In our study 40 patients were included with primary luminal and triple negative (TNC) breast cancer in stage $T_{1-3}N_{0-2}M_0$. Patients had not received neoadjuvant treatment. The material for the investigation were samples of tumor, adjacent tissues and lymphogenous metastases. Proteasome chimotrypsin-like (ChTL) and caspase-like (CL) activities, calpain activity (CA) were determined by hydrolysis of fluorogenic oligopeptides. The levels of p45 Serβ-catenin, Arp3 protein, and gelsolin in cytokeratin 18 positive cells were studied by flow cytometry. The levels of cofilin and thymosin-4-β were determined by Western blotting.
Results: ChTL, CL activity of proteasomes and CA increased in luminal and TNC breast cancer and lymphogenous metastases tissue compared to adjacent tissues. The thymosin-4-β level was significantly higher in luminal A and TNC metastases compared to the primary BC tissue. The cofilin level was significantly higher in metastases of luminal A and B molecular subtype compared to the primary BC tissue. There were shown the association of actin binding proteins (cofilin, gelsolin, arp-3, thymosin-4-β, Ser45 β-catenin) and intracellular systems of proteolysis in primary tumors and lymphogenous metastases, which was the same for different molecular subtypes of BC. The level of p45-Ser-β-catenin correlated to increasing of ChTL activity of proteasomes in primary tumors. The levels of p45-Ser-β-catenin and gelsolin in lymphogenous metastases correlated to increasing of ChTL and CL proteasomal activities in primary tumors. The cofilin level in lymphogenous metastases correlated to CA of cancer tissue. This may be associated to destruction of adhesion intercellular contacts and promote the exit of cells into regional lymph nodes of calpaines.
Conclusion: Probably, the remodeling of actin cytoskeleton by actin binding proteins plays an important role in tumor dissemination of breast cancers and they are regulated by proteasomes and calpains.

Orthopoxviral TNF-binding protein

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Key words: TNF-binding protein, immunomodulator, variola virus

Motivation and Aim: An unusually high production of cytokines or chemokines as well as increased complement activation can drive development of chronic inflammatory autoimmune diseases. State-of-the-art biological therapies, recombinant receptors, or specific antibodies that target immune and inflammatory mediators are now effectively used. However, these newer drugs are not equally effective for all patients and can cause adverse effects, making the search for new immunomodulatory proteins of great importance. The discovery and investigation into immune modulators from variola virus (VARV) and other orthopoxviruses has great potential for guiding new and effective drugs for autoimmune diseases.
Methods and Algorithms: Recombinant baculoviruses have been produced that express two-domain TNF-binding and chemokine-binding CrmB proteins of VARV and cowpox virus (CPXV). Interaction between human (h) or mouse (m) TNFs and the N-terminal TNF-binding domain of the CrmB protein (TNFBD) of CPXV or VARV produced in *E. coli* cells had been analyzed by surface plasmon resonance (SPR) measurements.
Results: In spite of their significant sequence similarity between species (82–96 %), their physicochemical and biological properties are notably different [1]. Removal of the C-terminal chemokine-binding domain from the VARV-CrmB protein had no effect on its efficiency to inhibit TNF-induced cytotoxicity [2]. The binding affinity of the recombinant viral receptors VARV-CrmB and CPXV-CrmB for the corresponding ligands was analyzed by SPR. The results showed that the K_D for the viral receptors affinity of the interaction with TNF are differed depending on the species. Thus, K_D for the VARV-CrmB/hTNF complex was 2.48×10^{-9} M; for VARV-CrmB/mTNF – 3.62×10^{-10}; for CPXV-CrmB/hTNF – 4.10×10^{-9}; for CPXV-CrmB/mTNF – 8.52×10^{-10} [3].
Conclusion: VARV CrmB has substantially higher TNF-neutralizing activity in experimental systems *in vitro* and *in vivo* compared with analogous protein of CPXV. The results obtained in this study indicate the prospects of further study of the recombinant protein TNFBD synthesized in bacterial cells as a therapeutic TNF antagonist. A fruitful direction of research may be the use of the gene encoding the TNFBD protein in hybrid plasmids or viral vectors for gene therapy of several chronic inflammatory diseases.
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Polymorphism of genes of endothelial dysfunction, mitochondrial biogenesis coactivators and plasminogen-plasmin system in development of cardiovascular complications in rheumatoid arthritis

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Key words: rheumatoid arthritis, cardiovascular risks, *NOS3* polymorphism, *PPAR* polymorphism, *PPARGC1A* polymorphism, *PPARGC1B* polymorphism, *PAI1* polymorphism

Motivation and Aim: The cardiovascular accidents are most often reason of death at patients with rheumatoid arthritis (RA). Thus the mechanism of progressing of atherosclerotic vascular lesions at RA, including genetic factors, remains under discussion. Analysis of the association between *NOS3*, *PPARG γ*, *PPARGC1A*, *PPARGC1B* and *PAI-1* genes polymorphism and presence of high/low cardiovascular risk at patients with RA.
Methods and Algorithms: 73 RA patients are included in research. 67.1 % of patients had high risk of development of cardiovascular complications. The method of ultrasonic examination of brachiocephalic arteries with definition of Intima-media thickness (IMT) was used as substitute marker of cardiovascular risk. Comparison was carried out in view of appropriate age-sex norms. Exceeding specified parameters of IMT value was regarded as presence high cardiovascular risk. NOS3 (rs2070744), *PPARG2* (rs1801282), *PPARGC1A* (rs8192678), *PPARGC1B* (rs7732671), *PAI1* (rs1799889) genes polymorphism was investigated by SYBR Green I Real-Time PCR (Litex, Russia).
Results: Frequencies of *NOS3-786TT* genotypes significantly differed between patients with different risk of development of cardiovascular complications. Complex genotypes which frequency prevails in group with low risk of development of cardiovascular events are revealed. There are homozygous *NOS3-786TT* genotype and the genes, having ability to adjust it expression, be on the structure of all genotypes. The greatest distinctions between analyzed groups are submitted in complexes *NOS3-786 TT:PPARGC1B203AlaAla* and *NOS3-786TT:PPARGC1B 203AlaAla:PPARG 12 ProPro.*
Conclusions: The polymorphic genes which analyzed by us, can jointly influence by change of risk of vascular complications at RA patients.

Virtual screening of MEK1 inhibitors among natural compounds based on ChEMBL data and (Q)SAR models

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Key words: (Q)SAR, MEK1 inhibitors, signal transduction, GUSAR, ChEMBL

Motivation and Aim: Protein kinases, including MEK kinases, are the ones of the main elements of these cascades, which involve into regulation of many intracellular activities, including apoptosis, differentiation and proliferation of cells, as well as other metabolic processes in cells [1]. Therefore, it is necessary to search for new MEK inhibitors. The aim of study is identification of possible MEK1 inhibitors using virtual screening based on quantitative and qualitative structure-activity relationships models and data from ChEMBL database.
Methods and Algorithms: The structures of low molecular organic compounds, which were tested on MEK1 inhibition, with experimental data were extracted from ChEMBL database. The set of natural compounds (67553 structures) from InterBioScreen were used as a source of potential MEK1 inhibitors. The accuracy of prediction (Q)SAR models was estimated by the 5-fold cross-validation procedure. The creation of SAR and QSAR models was carried out by GUSAR software that uses the Quantitative Neighborhoods of Atoms (QNA) and Multilevel Neighborhoods of Atoms (MNA) descriptors and radial basis functions with self-consistent regression [2].
Results: Two sets of compounds tested for inhibition of MEK1 were created based on data from ChEMBL: for qualitative models (1.474 structures with qualitative data on active and inactive compounds divided by 1000 nM threshold for IC50 values and 50 % inhibition) and quantitative models (706 structures with exact data of IC50 values in nM). Five-fold cross-validation (Q)SAR models showed acceptable accuracy of models for virtual screening. The average values of specificity, sensitivity, balanced accuracy for qualitative models were 0.897, 0.820, 0.858, and 0.763, 0.899, 0.831 for quantitative models, respectively. The average values of R2 and RMSE were 0.539 and 0.671, respectively. Two consensus models (SAR and QSAR) were created based on the initial sets of compounds. These (Q)SAR models were used for virtual screening. At the first stage of virtual screening, a qualitative model was used to select the most probable inhibitors of the MEK1 kinase. As a result, 978 the most probable inhibitors of MEK1 were selected. IC50 value was predicted for the selected compounds using the QSAR model, and 100 of the most active inhibitors of MEK1 kinase were selected. The possibility of their binding to MEK1 was evaluated by docking (AutoDock).
Conclusion: The effectiveness of the 2-step method of virtual screening was shown. The selected compounds can be used for experimental testing of the MEK1 inhibition.

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Radiofrequency ablation of varicose veins in obese patients

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Key words: varicose veins, radiofrequency ablation, obese patients

Motivation and Aim: Taking into account some disadvantages of traditional surgical intervention and steadily growing interest in minimally invasive methods of treatment, new methods have been developed, implemented and successfully used: obliteration of the vein by endovenous laser ablation, radiofrequency ablation [1]. Minimally invasive methods play a special role in obese patients [2].
Methods and Algorithms: The paper analyzes the results of examination and treatment of 68 patients with primary varicose veins of the lower limbs with CVI class: C2 – (56.0 %) patients, C3 – (37.5 %), C4 – (6.5 %) patients (section C of the classification CEAP) has been introduced. The patients were divided into two groups. The first group of patients (16 people) were obesity 2 degrees (BMI 37.8 kg/m^2 [35.3; 39.5].) The second group with BMI not more 25 kg/m^2 [20.4; 24.8] were comparison group. According to clinical and ultrasound parameters, the groups of patients were comparable. The diameter of the great saphenous vein (GSV) ranged from 7 mm to 24 mm, the diameter of the smalt saphenous vein While the diameter of individual Varisov great saphenous vein up to 28–32 mm. All patients had undergone radiofrequency ablation obliteration of the trunk of GSV or SSV under tumescent anesthesia. Varicose tributaries of the GSV or SSV is removed by miniphlebectomy. The average duration of the main stage of the RF on one limb was 20 min [17.2; 22.4] and stage of miniphlebectomy 28 min [22.3; 36.8]. depending on the severity of varicose transformation of the tributaries.
Results: Follow up examination was performed 8 weeks and 1 year after surgery. The clinical result and ultrasound data were evaluated. According to ultrasound in the target trunk of the LSV and SSV, there was no blood flow in both groups. Recurrence of varicose veins in the examined patients was not revealed, as well as increasing symptoms of chronic venous insufficiency. During the ultrasound examination of pathology of the deep vein not locked. The left portion of the GSV on the lower leg was not dilated vein walls fell down during compression, indicating the absence of thrombotic masses. In one case (6.2 %), an overweight patient in the postoperative period formed a portion of indurative cellulite with relapses of acute inflammation during the year.
Conclusion: 1. The results of radiofrequency obliteration in obese and normal-weight patients are comparable; 2. Tthe preliminary results of the study allow us to recommend the implementation of radiofrequency obliteration in patients with obesity 2 degree.

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Role of DNA methylation in incomplete penetrance of copy number variations

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Key words: copy number variations, DNA methylation, incomplete penetrance

Motivation and Aim: Most of copy number variations (CNVs) in the human genome exhibit incomplete penetrance with unknown underlined mechanisms. One of such mechanisms may be epigenetic modifications of DNA, in particular, DNA methylation. Here, we report about differential methylation of intragenic CpGs of *IMMP2L* gene in a proband with maternal 7q31.1 microdeletion and de novo 15q11-q13 microdeletion.
Methods and Algorithms: The proband with Prader-Willi phenotype with signs of autism spectrum disorders was analyzed using aCGH. The presence of 7q31.1 and 15q11-q13 microdeletions in his parents and healthy sibling was investigated by real-time PCR. DNA methylation level in intragenic CpGs of *IMMP2L* gene was measured by bisulfite amplicon next generation sequencing. Genome-wide expression profile was assessed by microarray analysis (Agilent Technologies).
Results: De novo 15q11-q13 microdeletion consistent with Prader-Willi phenotype (OMIM: 176270) in our patient as well as 7q31.1 microdeletion of maternal origin were detected in a proband. The 7q31.1 microdeletion affected included only exons 1-2 of the *IMMP2L* gene. *IMMP2L* gene is located in the critical region for the autistic disorder locus on chromosome 7q (AUTS1). The bisulfite sequencing of 87 intragenic CpG-sites revealed the comparable level of methylation in the proband, healthy sibling without microdeletion and father. Whereas a reduced methylation level and increased *IMMP2L* expression were observed in healthy mother's peripheral blood lymphocytes in comparison with a child.
Conclusion: Obtained results provide evidence for a possible compensation of *IMMP2L* haploinsuffiency in a healthy mother with 7q31.1 microdeletion due to epigenetic mechanisms.
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Copy number calling from Infinium MethylationEPIC array in human carotid atherosclerotic plaques

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Key words: copy number variation, EPIC, methylation, human atherosclerotic plaques

Motivation and Aim: Atherosclerosis usually affects the carotid arteries and the plaque instability responsible for the majority of acute events such as transient ischemic attacks and strokes. It was shown that high-density methylation arrays provide a robust and economic platform for detecting copy number and methylation changes in a single experiment [1]. The aim of this study was to use the DNA methylation data to identify the copy number alterations (CNAs) in carotid atherosclerosis plaques with different stability classified by histopathological analysis.

Methods and Algorithms: Carotid endarterectomy specimens were obtained from 16 patients. The carotid atherosclerotic plaques were subsequently sectioned and processed for morphological and histological examination. Bisulfite-converted DNA was processed using the Infinium MethylationEPIC BeadChip (Illumina). We performed estimation of CNAs based on the methylation arrays, using the R package conumee [2]. The copy number profiles were normalized by stable samples (CNV.fit). CNAs were annotated by BiomaRt package. Gene Ontology annotation was performed by WebGestalt (GO:ID, count of genes; pfdr < 0.05).

Results: CNAs analysis identified 64 CNAs in the atherosclerotic plaques where 30 CNAs were shared by unstable and stable plaques. Average size of CNAs was 3.2 Mb. There were from 2 to 15 CNAs per sample in unstable plaques and 1–7 CNAs in stable plaques. The CNA genes of unstable atherosclerotic plaques were enriched in Gene ontology terms associated with regulation of metabolic process (0030162, 11; 0051346, 20; 0050790, 30; 2001280, 2) and other terms (0045744, 5; 0006952; 24). CNAs found in stable plaque were significantly enriched for genes involved in translation (0006412, 11) and negative regulation of smooth muscle cell proliferation (0048662, 3).

Conclusion: In summary, CNAs can be generated from DNA methylation data of carotid atherosclerotic plaques. The results of this study suggest a possible link between CNAs of carotid atherosclerotic plaques with different stability and several biological processes.

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PRP in treatment of non-healing ulcers

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Key words: PRP, non-healing ulcers, trophic ulcers platelet-rich plasma

Motivation and Aim: Trophic ulcers occur in 1–2 % of persons of working age and in 4–5 % of persons of elderly and senile age. Platelet-rich plasma (PRP) helps in enhancing the wound healing. The aim was to evaluate the effectiveness of PRP in the treatment of chronic non-healing ulcers of lower limbs [1, 2, 3].

Methods and Algorithms: 19 (46.4 %) males and 22 (53.6 %) females of the total 41 patients with leg ulcers has been analyzed. The average age of patients was 57.8 [50; 68] years. Patients mainly had diabetes 39 % (16 patients), chronic venous insufficiency 41 % (17 patients) and other causative 20 % (9 patients). All patients were divided randomly into 2 study groups: comparison group (15 patients) and study group (26 patients). In the comparison group, patients received standard leg ulcer therapy. In the study group, injections of platelet-riched plasma (PRP) under ulcers were made twice. The statistical processing of data was carried out using standard approaches on a personal computer using statistical software Statistica 10.0. Differences were considered statistically significant at $p < 0.05$.

Results: According to preliminary calculations, a combination of traditional treatment with the introduction of PRP leads to an acceleration of healing of ulcers by 31.4 % compared to the control group. Six months after discharge in the main group, 85 % of patients have had full healing of leg ulcers. In the omparison group, 76.28 % of patients have had complete healing. In PRP group the dynamics of epithelialization was correlated with the level of anti-inflammatory cytokines, particularly interleukin-4, and the immunomodulatory effect determined by interleukin-6.

Conclusion: Under ulcers injections of platelet-riched plasma (PRP), accelerates the epithelization period of skin lesions of the lower limbs in patients with non-healing ulcers. When PRP is used, the dynamics of epithelialization is associated with the level of anti-inflammatory cytokines, in particular interleukin-4, and the immunomodulatory effect determined by interleukin-6.

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Circulating adiponectin concentrations are sex-dependently associated with specific SNPs of *ADIPOQ* and *LEP* genes

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Key words: adiponectin, leptin, single nucleotide polymorphism

Motivation and Aim: Atherogenesis is known to involve abnormal regulation of metabolism in adipose tissue. Recently we have shown that ratios of serum concentrations of leptin to insulin and adiponectin to endothelin are sex-dependently associated with extent of coronary atherosclerosis [1]. The aim of the study was to explore the association of circulating adiponectin and leptin concentrations with the specific *ADIPOQ* and *LEP* genes polymorphisms.

Methods and Algorithms: Three single nucleotide polymorphisms (SNPs) of *ADIPOQ* gene (rs17300539, rs182052, rs266729) and two SNPs of *LEP* gene (rs2167270, rs7799039) were screened for their association with adiponectin and leptin concentrations in the study that enrolled 319 men and 132 women aged 61.2±9.4 years with coronary heart disease. Serum adiponectin and leptin were measured by ELISA kits. Five SNPs of *ADIPOQ* and *LEP* genes were genotyped by real-time PCR (7500 Real Time PCR System, Applied Biosystems, USA). Data were analyzed using Statistica software package version 7.0 (StatSoft Inc, USA). Data were shown as median and 25 and 75 percentile values.

Results: Among five eligible SNPs analyzed in this study, two SNPs of *ADIPOQ* gene and one SNP of *LEP* gene were significantly correlated with adiponectin concentrations in men, but not in women. Adiponectin levels were significantly higher in men with the homozygous genotype (GG) compared to men with the heterozygote mutant and mutation type genotypes (GA+AA) in rs182052 polymorphism of *ADIPOQ* gene (7.75 (5.57; 12.61) μg/mL *vs* 6.75 (5.09; 10.23) μg/mL, correspondingly, $P = 0.026$).

Adiponectin levels were significantly lower in men with the homozygous mutation genotype (GG) in rs266729 of *ADIPOQ* gene compared to men with the heterozygote mutant and wild type genotypes (CG+CC) (5.80 (4.59; 8.87) μg/mL *vs* 7.39 (5.28; 11.48) μg/mL, $P = 0.028$). Homozygous mutation genotype (GG) in rs2167270 in the 5′ region of *LEP* gene was associated with lower serum adiponectin concentrations (5.41 (4.67; 7.18) μg/mL *vs* 7.67 (5.35; 11.31) μg/mL, $P = 0.0003$).

Conclusion: Three common SNPs were associated with plasma adiponectin levels in men, but not in women. This study provides evidence for a role of variation in adipokine genes in endogenous adiponectine concentrations.

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Genes of cytokines in controlled and uncontrolled asthma in children (East Siberia)

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Key words: asthma, cytokines, gene polymorphism, disease control

Motivation and Aim: Atopic bronchial asthma (ABA) is a multifactorial disease; its development is dependent on many environmental and genetic factors. Genetic risk factors can affect the clinical phenotype of ABA and the level of therapeutic control over the disease. It was suggested that the therapeutic control of the disease is genetically mediated and depends on the presence of one or another allele in genes of mediators, participating in ABA pathogenesis. The knowledge about genetic markers will allow to forecast the clinical course of ABA in children. We carried out the analysis of association between genes of pro-inflammatory and anti-inflammatory cytokines with the level of therapeutic control of ABA.
Methods and Algorithms: In children with controlled and uncontrolled ABA (CABA and UABA, respectively; $n = 110$) and in a population sample ($n = 138$), we analysed 11 polymorphisms: *IL2* (rs2069762), *IL4* (rs2070874, rs2243250), *IL5* (rs2069812), *IL10* (rs1800872, rs1800896), *IL12B* (rs3212227), *TNFA* (rs1800629, rs1800630), *TGFB1* (rs1800469), and *IFNG* (rs2069705), encoding cytokines taking an active part in the development of allergic inflammation.DNA extracted from blood by standard salting-out method. Genotyping of SNPs was performed by restriction fragment length polymorphism assay.
Results: According to the results of the study, the prevalence of alleles and genotypes of the analysed loci in the East Siberia Europeans is consistent with the data in other world European populations. We observed statistically significant differences between UABA and control groups for the prevalence of *IL2* (rs2069762) polymorphism: GG genotype was more common in control group (14.1 % compared to 5.9 %, $p = 0.03$). It was shown that the *IL2*T* allele and TT genotype of the rs2069762 are associated with the increased risk of uncontrolled ABA. A comparison of the haplotypes of *IL4* (rs2070874 and rs2243250) gene with correction for sex and age in framework of additive model revealed that the most common haplotype CC (prevalence in ABA/CABA/UABA groups is 0.75/0.76/0.74, respectively) is protective against the development of ABA (RR 0.53 ± 0.32; $p = 0.044$). A comparative analysis of *TNFA* (rs1800629 and rs1800630) haplotypes established that the GC haplotype is also protective against the risk of ABA (RR 0.59 ± 0.17; $p = 0.003$) while the GA haplotype is positively associated with the disease (RR 2.07 ± 0.25; $p = 0.003$). This was true for BA regardless of the control over the disease (CABA GA: RR 1.93 ± 1.2; $p=0.041$; UABA GA: RR 2.43 ± 0.31; $p = 0.005$).
Conclusion: Thus, it was established that the studied cytokine genes are important for the development of ABA in children. These data were obtained for the first time for Europeans of East Siberia. They are of interest in terms of the accumulation of the data about the impact of cytokine genes polymorphism on the development of ABA and its therapeutic control in children.

Carnitine Palmitoyltransferase1A (CPT1A) P479L allele prevalence in the Arctic native populations of East Siberia

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Key words: Carnitine Palmitoyltransferase 1A, gene polymorphism, Arctic populations, infants

Motivation and Aim: A common variant of CPT1A deficiency (P479L, c.1436 C/T.), first identified in the Canadian Inuit population, has also been found in various regions of Alaska, Canada, and Greenland [1, 2]. The prevalence of P479L genotypes in Russian Arctic regions has not been studied so far. The aim was to determine the allele prevalence in two populations of the Taymyr Peninsula: Dolgans-Nganasans and Nenets.
Methods and Algorithms: Newborn dried blood spots (DBS) were collected for infants born in 2010-13 including 108 Dolgan-Nganasans (Syndassko, Kataryk, Novaya, Levinskiepeski villages) and 105 Nenets (Nosok village). Genotyping of the P479L mutation was performed by restriction fragment length polymorphism assay. DNA extracted from BDS was first amplified by PCR using the following primers: 5′-CTGGCCAGGTTTGGATTTT-3′ and 5′-TCCAGGATGAAGCAGAGAGG-3′. The amplification products were digested with BstMC I restriction endonuclease ("Sibenzyme", Russia) resulting in 252 bp fragment for T-allele and 169 and 83 bp fragments for C-allele. Chi-square test was used to determine the frequencies difference.
Results: The P479L genotype frequencies have clear distinction between two populations. Seven newborns were heterozygous in Dolgan-Nganasans (7/108; 0.07; 95 % CI: 0.03–0.13) and none was heterozygous in Nenets (0/105) (p = 0.006). No homozygotes for rare allele have been detected in both groups. The prevalence of rare T-allele in Dolgan-Nganasans was 0.03.
Conclusion: The prevalence of the 479L allele of Carnitine Palmitoyltransferase 1A gene in Russian Arctic native populations is low and corresponds to the frequencies reported for non-aboriginal Alaskan and Canadian populations. Probably, it is due to differences in diet patterns. In this context, our study supports the hypothesis of the traditional diet impact and historical benefit for those with the P479L variant in some Arctic regions. We suppose that slightly increased prevalence of P479L variant in Dolgans may be explained by proximity to the sea coast.

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Platelet-rich plasma coated PCL nanofibers boost viability and proliferation of human mesenchymal stem cells

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Key words: Polycaprolactone (PCL), nanofibers, COOH plasma, Platelet-rich plasma, cell viability

Motivation and Aim: The problem of reconstruction of tissues is one of actual problems of fundamental and applied biomedical science. Tissue engineering requires the use of new materials including bio-degraded nanofibers. Most of the available polymer nanofibers are superhydrophobic and biologically inert, while the structure of nanofibers has a similar structure with extracellular matrix and therefore this material has great potential for tissue engineering. Surface modification of nanofibers needs to be effective for immobilization of biological active molecules. The aim of this project is to study the deposition of plasma polymer films containing functional COOH groups and the immobilization of PRP (platelet reach plasma) on adhesion, proliferation and apoptosis of derived bone marrow mesenchymal stromal cells (MSCs).
Methods and Algorithms: Nanofibrous used as substrates were prepared by electrospinning of PCL solution. The deposition of plasma polymers was carried out using a vacuum system UVN-2M. Ar, CO_2 and C_2H_4 gases were used as precursors. The nanostructure of the nanofibers was studied by scanning electron microscopy (SEM). Covalent bonding of PRP to the PCL scaffolds was performed by subsequent immersing of PCL-COOH in N,N'-Dicyclohexylcarbodiimide solution in water. The chemical composition of the sample surfaces was characterized by X-ray photoelectron spectroscopy. Cell attachment were analyzed by stained cytoskeleton with Phalloidin. Cell Proliferation were investigated using the Click-iT™ EdUAlexa Fluor™ 488 Imaging Kit. The Hoechst 33342 was employed to assess the morphological changes in apoptosis.
Results: The immersion of plasma coated PCL-COOH in PRP solution led to significant changes in the layer chemistry. It was found that new peaks located at 1652 and 1598 cm^{-1} attributed to C=O stretching and NH_2 bending of amides, respectively. These new peaks confirm the incorporation of the molecules with peptide bonds. It was shown that the adhesion of MSCs to the modified surfaces (PCL-COOH-PRP) resulted in the formation of the significant actin-rich cytoskeleton. The percentage of proliferating cells on PCL-COOH-PRP nanofibers was equal to 44±2.7 % after 24 hours incubation. At the same time the percentage of proliferating cells on PCL-COOH was only 20±3.4 %. On unmodified nanofibers the percentage of proliferating cells was 6±0.8 %. The cell proliferation slows down after 72 h incubation on PCL-COOH-PRP due to contact inhibition (reach confluence). It was found that after 24 h 23±5 % nucleus of adhered cells to unmodified PCL scaffold showed some features of apoptosis, while the modified PCL nanofibers exhibited low percentages of apoptotic/death cells (1±0.06 %).
Conclusion: Our results have shown the bonding of PRP will influence the cell proliferation level and cell viability and this material is highly promising for tissue engineering.

Personalization treatment of the bladder leukoplakia

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Key words: bladder leukoplakia, transurethral resection

Motivation and Aim: According to different authors, leukoplakia with the location in the neck of the bladder and in the trigone of bladder is found during the cystoscopy among 63.6–100 % of patients with persistent dysuria, chronic pelvic pain [1, 2].
Methods and Algorithms: We analyzed the results of therapy of 55 women with bladder leukoplakia and chronic recurrent cystitis, who were underwent treatment at the RICEL–Branch of IC&G SB RAS, from 2015 to 2017. Patients underwent transurethral resection of leukoplakia sites with Karl Storz resectoscope, biopolar energy, with passive irrigation of 0.9 % sodium chloride solution. At the end of the operation, after evacuation of the resected mucosal areas, the drug Collegel ADL (Coletex Ltd, Russia) was injected into the bladder. After the injection of the gel, a Foley catheter No. 14 was placed in the bladder and was pinched for 40 minutes. The next day, the catheter was removed. The instillations of the drug were carried out for 4 weeks after the operation, one injection per week. There were no complications during surgical intervention and in the early postoperative period among all the patients.
Results: There were no active complaints among patients 40 (72.7 %) in 7 days after the operation. The urination was free, painless. The remaining 15 (27.3 %) women reported moderately painful urination and a slight pulling pain in the lower abdomen. At the control test, 49 patients (89.09 %) reported clinical improvement 1 month after the treatment. At the control urethrocystoscopy 1 month after the operation, all patient's bladder mucosa in the transurethral resection area was pale pink. Fragments of leukoplakia were not determined. A urethrocystoscopy made 6 months after the treatment did not reveal a focus of the leukoplakia of the bladder mucosa among 50 (90.9%) patients. In 9.1 % (5 patients) with the area of the bladder wall that underwent resection was moderately oedematous and hyperemic. The revealed changes in the bladder wall indicated a persistent chronic inflammatory process. The control endoscopic examination of the bladder 12 months after the treatment revealed areas of leukoplakia in the neck section in 12.7 % (7 patients).
Conclusion: The results of combined treatment of bladder leukoplakia indicate the high effectiveness of this method, which allows to achieve a positive effect in a comparatively short period of time. Minimal surgical trauma, satisfactory hemostasis, the absence of intraoperative complications, more rapid recovery of the bladder mucosa and, as a result, a short period of rehabilitation prove the effectiveness of the proposed method of treatment.

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The advantage of the multidimensional data visualization for the characterizing of the immune system state

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Key words: breast cancer, immune system, multidimensional data visualization

Motivation and Aim: According the complex structure and multidimensional functioning, the immune system is considered to need in the application of systems biology approach for its adequate characterizing [1]. The multidimensional data visualization approach of the NovoSpark Corporation (Canada), presenting the immune system state as unified characteristic, allows to reflect complexity of the immune system and to provide available data interpretation [2]. The aim is to demonstrate the advantages of using the multidimensional visualization for the characterizing of the immune system state in breast cancer (BC) patients with different disease outcome in comparison of the estimation of separate immunological parameters.
Methods and Algorithms: 65 BC patients (31 without and 34 with tumor progression) with stages T1-4N0-3M0 were enrolled into the study. 52 parameters of the innate and adaptive arms of the immune system were estimated. The multidimensional data visualization approach of the NovoSpark Corporation (Canada) was used to characterize the immune system state as an integral unit [3].
Results: Basing on well-known involvement of the immune system in cancer pathogenesis, we could expect significant differences between its states in BC patients with and without tumor progression. But really, when we estimated separate immunological parameters, we found, that only IL-1β secretion by immune cells was distinguished in the women with and without tumor progression: 241 (77–718) pg/ml and 606 (177–1846) pg/ml respectively, $p < 0.05$. When we used the multidimensional data visualization approach, we found that visual images of immune system states in BC patients with and without tumor progression were located in different, not overlapping areas of multidimensional data space. Wilks' lambda for the discriminant functions that describes the states of the immune system in BC patients was 0.059.
Conclusion: The multidimensional data visualization of the immune system state identifies differences in the cases, when separate parameters estimation has no efficiency.
Acknowledgements: Supported by the RFBR (17-29-06037).

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Effectiveness of cytocine antihypoxic cerebroproteciton

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Key words: experimental hemorrhagic stroke, interleukin-1, interleukin-1 receptor antagonist, interleukin-2

Motivation and Aim: The search for new drugs for correction of hemorrhagic stroke is still actual. Ischemic damage of brain tissue leads to the formation of energetic deficit, development of glutamate-calcium and cytokine cascade. The purpose of work was studying of effect of cytokine drugs (IL-1Ra and IL–2) on the dynamics of index of carbohydrate-energetic balance, oxidant stress, expression of genes of the early reaction and the intensity of postinsult neurological and cognitive disorder in experimental hemorrhagic stroke (administration of autoblood in internal capsule of brain in rats) in administration in dose of IL-1Ra 7.5 mg/kg and of IL–2 0.01 mg/kg during 18 days.
Methods and Algorithms: In homogenate of brain in acute period of stroke and in the phase of restoration (4 and 18 days) using the biochemical methods the ATP, ADP, AMP, the content of products of oxidative modification of protein (AFG and KFG), peroxidation of lipids (DK, TK, MDA). Antioxidant protection was evaluated by the activity in brain tissue SOD, catalase, glutathionperoxidase. Expression of c-Fos protein in sensor-motor zone of cortex was founded by the indirect immunofluorescence. Using the standard methods oriental-studying habits, neurological deficit (by scale of Stroke – index McGrow), the conditioned reflex of passive avoidance.
Results: Results of our experiment proves that in rats experimental hemorrhagic stroke was accompanied with typical pathophysiological indication – formation of mitochondrial dysfunction with following energetic deficit, activation of nitrogen oxide system and formation of free-radical process of damages of proteins and lipids on the background of apoptosis activity processes leads to suppression. We set that administration of IL-1Ra (mostly) and IL–2 optimizes all indexes – decreases degree of oppression of oxidative processes in Kreb's cycle, increases the intracellular stock of ATP, stabilizes the activity of pro- and antioxidant results when the synthesis of c-Fos protein was inducted, activation of apoptosis, improves the index of movement activity, psychoneurological status using the McGrow's Stroke-index and the procreation of reflex of passive avoidance. Effects more expressed in recovery period that testifies the necessity of application of cytokine drugs as cerebroprotective in hemorrhagic stroke. On the studied model experimental hemorrhagic stroke activity of IL-1Ra and IL–2 to prevent neurological complication more that in Thiocetam.
Conclusion: Cytokine drugs of interleukin row – recombinant IL-1Ra and IL-2 cause effective of stabilization action on mitochondrial dysfunction and connected to it phatological changes in energetic metabolism, free-radicalprocesses of apoptosis. This proves that IL-1Ra and Ronkoleukin can be used as effective chain in complex therapy of afterischemic state, also for effective protection of brain tissue in hemorrhagic stroke.

Oxidative stress protection of the bone marrow-derived mesenchymal stem cells by erythropoietin

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Key words: bone marrow – derived mesenchymal stem cells, hydrogen peroxide, proliferation, apoptosis

Motivation and Aim: Bone marrow-derived mesenchymal cells (BM-MSCs) have been proposed as a prominent candidate for the development of tissue engineering products. BM-MSCs are able to migrate into injured tissues, engraft and differentiate into many cell types, to produce cytokines, chemokines and growth factors, participating thus directly in tissue repair and regeneration [1]. It has been shown that transplanted MSCs are exposed to oxidative stress at the site of injury, which leads to a decrease in their viability and an increase in apoptosis. Erythropoietin (EPO) is an anti-apoptotic, anti-oxidative, anti-inflammatory and proangiogenic cytokine. Inhibicion of apoptosis in ischemic/hypoxic tissue is the major cytoprotective effect of EPO [2]. The purpose of research is to study the impact of EPO on the resistance of BM-MSCs to oxidative stress.
Methods and Algorithms: BM-MSCs were isolated from human and cultured under standard conditions. BM-MSCs was treated with 33.4 U/ml recombinant human erythropoietin (Recormon, Roche Diagnostics, Germany) 24 h. Oxidative stress was induced by the addition of 2 mmol L^{-1} H_2O_2. The proliferative activity BM-MSCs was measured by the MTT colorimetric assay. Expression of EPOR on BM-MSCs was confirmed to flow cytometry analysis. The percentage of apoptotic cells was detected by flow cytometry with Annexin V-FITC Apoptosis Detection Kit.
Results: After treatment with erythropoietin, the number of EPOR on BM-MSCs increased 1.8-fold ($p < 0.032$). It was shown that the treatment of cells with erythropoietin before the induction of oxidative stress cancels the effect of hydrogen peroxide, the level of cell proliferation corresponds to control (0 mM H_2O_2). While the treatment of cells with erythropoietin after the addition H_2O_2 did not abrogate the effect of oxidative stress. The average percentages of Annexin+/PI– (early apoptotic cells) were highest in H_2O_2-induced BM-MSCs and it was much lower when processing with EPO before the induction of oxidative stress. Significant differences observed between necrotic cells H_2O_2-induced BM-MSC and BM-MSCs treated with EPO before adding H_2O_2 ($p < 0.05$).
Conclusion: The results obtained in this study indicate that the treatment with erythropoietin of BM-MSCs leads to an increase in the number of EPOR on their surface. The oxidative stress does not affect the proliferation of BM-MSCs treated with erythropoietin. The erythropoietin reduces the number of cells in early apoptosis and necrosis after addition of H_2O_2. Thus, the treatment with erythropoietin of BM-MSCs protects from the influence of oxidative stress.

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The efficiency of applying mathematical methods to analyze the occurrence of genotypes and alleles G-308A TNF-α among atopic dermatitis patients with manifestations of connective tissue dysplasia

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Key words: atopic dermatitis, connective tissue dysplasia, genotypes and alleles G-308A TNF-α, statistical research methods

Motivation and Aim: 96 % of 255 patients have revealed the connective tissue dysplasia of different manifestation degrees. Considering the hypothesis on the polygenic nature of the susceptibility which is formed to atopic dermatitis and connective tissue dysplasia, the peculiarities of distributing allelic variants of TNF-α genes in multilocus haplotypes have been analyzed. The adhesion between HLA II DRB1, DQB1, DQA1 genes and gene TNFα HLA class III made us study the issue of multilocus haplotype frequency allocation in groups of atopic dermatitis patients and healthy people.
Methods and Algorithms: Clinical and standard genetic-statistical methods were used to evaluate the course of atopic dermatitis and manifestations of connective tissue dysplasia.
Results: No dysplastic changes of the connective tissue have been revealed when studying the occurrence frequency differences of homozygous and heterozygous genotypes TNF-α in position G-308A for atopic dermatitis patients with and without systematic connective tissue dysplasia manifestations. Therefore, we have supposed the appearance of connective tissue dysplasia with atopic dermatitis patients is connected with the considerable influence of external factors.
The occurrence frequency analysis of DRB1-DQA11-DQB1- TNFα four-locus haplotypes has shown that the frequency of DRB1*03-DQA1*0501-DQB1*0201-TNFα-308A and DRB1*15-DQA1*0102-DQB1*0602-8-TNFα-308G haplotypes is higher in the reference group compared with the group of atopic dermatitis patients, which indicates the protective importance of the given haplotypes in the development of atopic dermatitis.
Conclusion: The application of mathematical analysis for gene groups remarkably raises the efficiency of searching additive genetic complexes playing a significant role in creating an individual's resistance or predisposition to the disease. Basing on the values of diagnostic coefficients and the pattern recognition procedure, it is possible to perform early diagnostics and anticipating prognosis of atopic dermatitis development yet at the preclinical stage of the disease.
Multilocus allele combinations can serve as the genetic basis of clinical polymorphism and pathogenetic heterogeneity of atopic dermatitis.

Metabolic syndrome associated *FTO* gene polymorphism in populations of Buryats and Russians of Eastern Siberia

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Key words: Buryats, metabolic syndrome, obesity, single-nucleotide polymorphisms, *FTO* (*rs8050136*)

Motivation and Aim: Metabolic syndrome (MetS) is an actual problem of modern medicine. The MetS is directly linked to abdominal obesity, which reflects insulin resistance and hypertension. The *FTO C83401A* (*rs8050136*) single-nucleotide polymorphism is associated with obesity [1, 2]. The aim of this work is to study *FTO* gene polymorphism in different ethnic groups.
Methods and Algorithms: This study was performed on Eastern (N = 132) and Western (N = 278) Buryats, Russians of East Siberia (N = 122) and Metis, the progeny of mixed marriages of Buryats with Russians (N = 54). Genotyping was performed using real-time PCR with competitive TaqMan allele-specific probes.
Results: The frequency of the *FTO 83401A* allele was 26.5 % in the Eastern Buryat, 24.1 % in the Western Buryat and 38.5 % in the Russians of Eastern Siberia. The occurrence of the *FTO 83401A* allele in Metis is 38.0 %.
Conclusion: The frequency of metabolic syndrome associated *FTO 83401A* allele in the Russians of Eastern Siberia corresponds to the frequency range found in European populations [3]. A low-frequency occurrence of *FTO 83401A* among Buryats may be indicative of a lower population-wide risk of abdominal obesity and diseases associated with it. However, metisation shifts gene frequency and changes the degree of risk.
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Lithium and autophagy in hepatocellular carcinoma

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Key words: hepatocellular carcinoma, lithium carbonate, autophagy

Motivation and Aim: Hepatocellular carcinoma (HCC) is one of the most malignant tumors, with high heterogeneity of tumor cells and frequent resistance to chemotherapy. Autophagy is a process of intracellular degradation, and its role in the development and progression of HCC is poorly understood and ambiguous [1]. Earlier, we investigated the antitumor properties of lithium [2]. There are data showing the ability of lithium to induce autophagy in tumor cells by modulation of the phosphatidyl inositol signalling pathway [3]. This study evaluated the ability of lithium to induce autophagy in HCC cells *in vivo*.
Methods and Algorithms: Hepatocellular carcinoma-29 (HCC-29) cells were injected to intact CBA mice in the right thigh muscle. The control group included mice with an intact tumor; a group of NS mice obtained 0.9 % normal saline, and a group of LC mice received 20 μM lithium carbonate (Li_2CO_3). The autopsy material was processed by the standard technique of electron microscopy. Morphometric analysis was performed using Image J software (USA). The volumetric (Vv) and numerical (N_A) densities of initial autophagic vacuoles (AVi), degradative autophagic vacuoles (AVd) and lysosomes were determined in accordance with the guidelines for monitoring autophagy [4]. Significant difference was estimated with the Mann–Whitney U-test, $p < 0.05$ was considered statistically significant.
Results: The number of cells with induced autophagy (containing autophagic vacuoles) was 59 % in the LC group, Control – 48 %, NS – 35 %. When lithium carbonate was injected, a significant increase in the number of AVd, as well as Vv and NA autophagic vacuoles (AVi+AVd) was detected. There were no significant differences in the evaluation Vv and N_A of AVi and lysosomes.
Conclusion: Autophagy can be a survival mechanism for cancer cells under stress, but autophagy can also contribute to their autophagic death. Lithium carbonate induced autophagy in HCC-29 cells, favoring the accumulation of predominantly late autophagic structures (AVd). Thus, stimulation of autophagy with lithium may represent a new therapeutic approach to the therapy of hepatocellular carcinoma.

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Role of the dominant placental protein pregnancy specific *β-1*-glycoprotein in the regulation of T-cell immune memory

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Key words: pregnancy specific *β-1*-glycoprotein, memory T cells, immune tolerance

Motivation and Aim: Pregnancy specific glycoprotein (PSG) is a placental protein, that's level in the dynamics of pregnancy is steadily growing (up to 200–400 μg/ml, III trimester). It is known that PSG contributes to the formation of immune tolerance to the semi-allogeneic fetus, but its role in the regulation of T-cell immune memory remains undeveloped. It is obvious that directed regulation of immune memory opens up wide clinical possibilities. The aim of the work was to study the role of PSG in the regulation of molecular genetic processes of immune memory T cells differentiation.

Methods and Algorithms: Authentic native human PSG was obtained by the proprietary method [1]. Monocultures of memory T cells ($CD45R0^+$) were obtained using MACS® technology (Miltenyi Biotec, Germany) from the peripheral blood mononuclears of healthy donors, (n = 12, ♀). $CD45R0^+$ (10^6 cells/ml) cells were cultured in complete medium (48 h, 37 °C, 5 % CO_2) with TCR activator and IL-2 (10 ng/ml). After the 48-hours cultivation expression of phenotypic (CD25, CD28, CD71) and genetic (*U2af1l4*, *Gfi1*, *hnRNPLL*, *hTERT*) markers determining T cell differentiation was evaluated using flow cytometry and qRT-PCR [2]. The statistical analysis was performed using the W-Wilcoxon test.

Results: It was found that PSG (10, 100 μg/ml) suppressed the expression of CD25 and CD71 on memory T cells. When evaluating the expression of the *U2afl4*, *Gfi1*, *hnRNPLL* genes regulating the alternative splicing of the *Ptprc* gene coding for CD45, it was found that PSG reduced the expression of the *Gfi1*and *hnRNPLL* genes, but increased the expression of the *U2af1l4* gene, thus preventing the formation of the mature CD45R0 isoform. At the same time, PSG reduced the replicative potential of memory T cells, estimated by the expression of *hTERT*. Thus, it has been established that PSG suppresses the functional activity and decrease the replicative potential of memory T cells.

Conclusion: In general, the data obtained open the possibility of pharmacological application of PSG for directed suppression of the immune response against autoantigens and grafts and immunotherapy for recurrent abortions.

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Molecular bioidentification as the key point on the way of solving problems in biomedicine and biotechnology

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Key words: molecular bioidentification, biology theory, biotechnology, biomedicine

Identification as an action to identify certain signs (features) with a unique object of nature is used in many branches of scientific knowledge: chemical, biological, medical, engineering, forensic. Therefore, the methodology of procedure for establishing the relationship of the identity of certain biological characteristics inherent in some living organism (in whole or in part) can be called biological identification or briefly – bioidentification. An integral initial step of any bioidentification is a description (exposing) parameters of biological characteristics (bioidentifiers) – individualization of their bearer (individual bioidentification) or biological typing of group of bearers (taxonomic bioidentification). Later on, based on this, comparison patterns are registered, which play an important role in the bioidentification procedure. Signs in bioidentification can be a variety of parameters of the characteristics of biological objects (morphobiometric data, physiological and biochemical traits of organisms or their groups, the structure of the DNA molecule, etc.). Hence, the bioidentification itself, depending on the type of bioidentifier, can be either specific, i. e. belong to a system of features (e. g. morphological, genetic) or complete (complex – taking into account heterogeneous bioidentifiers). Currently bioidentification passes mainly to the level of molecular bioidentification, since a significant polymorphism in the widely used molecular bioidentifiers provides a possibility with high probability to identify the organism and to reveal biological kinship [1, 2]. Molecular bioidentifiers have significant advantages: codominance of inheritance, high differentiating ability, interlaboratory reproducibility of results, and, consequently, they integratively correspond more to the criteria of evidence and are more reliable [3]. Databases on organisms – DNA registers – are created everywhere for such various applied purposes of biology and medicine as: biological systematics, medical and veterinary diagnostics, personalized approaches to the treatment of diseases, accurate genotyping of useful microorganisms for biotechnology, conservation of species and biodiversity [3]. DNA registers contain information about concrete bioidentification characteristics or so-called individual genetic profiles. In the consequent, with databases containing bioidentification options are almost all modern tools of systems biology. Thus, the development and creation of methods of molecular bioidentification, the quality of their implementation, as well as the development of tools for verification of all stages of bioidentification serves as a crucial node in the subsequent solution of all modern biomedical and biotechnological problems. In conclusion, it should be noted the exceptional importance of observance of bioethical principles in matters of molecular bioidentification.

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The interaction effect of angiogenesis and endothelial dysfunction-related gene variants increases the susceptibility of recurrent pregnancy loss

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Key words: recurrent miscarriage, single nucleotide polymorphism, gene-gene interactions, endothelial dysfunction

Motivation and Aim: One of the most important problems in obstetrics is recurrent pregnancy loss (RPL). The frequency of this complication makes up 1 to 5 % of all pregnancies [1, 2]. RPL is one of the most troublesome areas in reproductive medicine since the etiology of this disease is often unknown, and modern diagnostics and known evidence-based treatment strategies are not effective enough [2, 3]. The role of genetic polymorphisms in the pathogenesis of RPL has been studied intensively. However, the findings of these studies are often contradictory even when studying the same ethnic group and are rarely replicable in other populations. Complex diseases, including miscarriage, are believed to have a polygenic basis and gene-gene interactions can play a significant role in the etiology of the disease. This study was conducted to investigate the association of gene-gene interactions of angiogenesis, endothelial dysfunction-related gene polymorphisms, and RPL.
Methods and Algorithms: A case–control study was conducted with 253 RPL-unrelated patients with 2 or more spontaneous pregnancy loses and 339 healthy women with no history of pregnancy complications. Seven functional SNPs were selected from *ACE*, *MTHFR*, *SERPINE-1*, *NOS3*, *TP53* and *VEGF* genes based on our previous studies [4]. Genotyping of single nucleotide polymorphisms (SNPs) was performed using real-time polymerase chain reaction (Real-Time PCR), restriction fragment length polymorphism (RFLP) or allele-specific polymerase chain reaction techniques methods.
Results: It is shown that genotypes 677TT of the *MTHFR* gene, 894GT of the *NOS3* gene, 936CT and 936TT of the *VEGF* gene are associated with a predisposition to RPL in the Russian population. By Multifactor dimensionality reduction analysis, a two-locus model (C936T and G634C of *VEGF*) of gene-gene interaction was the best for predicting RM risk, and its maximum testing accuracy was 67.4 % and maximum cross-validation consistency was 10/10. The significant role of additive and epistatic effects in the gene-gene interactions of the SNPs of the *SERPINE-1*, *ACE*, *NOS3*, *MTHFR* and *VEGF* genes to RPL has been demonstrated.
Conclusion: The results show that gene-gene interactions are of great importance to RPL susceptibility. It was also found out that the analysis of genotype combinations of several allelic variants provides more information on RPL risk as compared with the analysis of different polymorphic markers.
Acknowledgements: Supported by the RFBR (No. 18-44-700007).

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Large genome-wide association study provides insight into the genetic architecture of low back pain and its risk factors

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Motivation and Aim: Back pain (BP) is a common debilitating condition with poorly understood pathogenesis. In the majority of cases BP is transient; however, in 10 % of cases it develops into a chronic condition, which places a great socioeconomic burden on society. BP shares underlying genetic predisposition with a number of its risk factors including intervertebral disk degeneration, depression and anxiety, educational attainment, obesity and other pain conditions such as chronic widespread pain. The precise genes underlying risk of BP are unknown and large-scale genetic studies of BP and closely related traits are currently limited. We set out to determine genetic loci associated with back pain using a large sample of individuals from the UK Biobank (UKBB) dataset and the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium drawn from cohorts in Europe and the USA.
Methods and Algorithms: A total of 350.000 individuals of European ancestry from the UKBB were used in the discovery phase (91.100 cases and 258.900 controls). For replication, we used a combination of the UK Biobank participants of European, African and Asian ancestry not included in the discovery set, and data from the CHARGE (total $N = 159.070$). Conditional and joint analysis was used to find SNPs independently associated with the phenotype. Post-GWAS analyses included LD-score regression, heritability estimates, the analysis of pleiotropy and genetic correlations.
Results: Five genome-wide significant loci were identified after adjusting for genomic control, of which four replicated: rs12310519 ($p = 3.5e{-}14$), rs7814941 ($p = 1.8e{-}11$), rs3180 ($p = 1.7e{-}11$), and rs1865442 ($p = 3.8e{-}13$). Two loci have been reported previously (rs12310519 near *SOX5*; rs7814941 near *GSDMC*/*CCDC26*) and two were novel associations with BP (rs1865442 in *C8orf34* and rs3180 in between *SPOCK2* and *CHST3*). All the loci had significant pleiotropic effects on intervertebral disc degeneration and rs3180 had a pleiotropic effect on height. Strong genetic correlation with BP was observed for traits related to demographic factors, smoking and education, obesity-related traits and depression. Independent genetic correlations with BP were revealed for depression, neuroticism, sleep disturbance, overweight, and smoking by partial correlation analysis. We also demonstrated significant enrichment of neurological pathways.
Conclusion: This is the largest GWAS for BP thus far, involving more than 500,000 individuals in total. Apart from identifying two new loci, we provide evidence for pleiotropic effects of genetic factors underlying BP, height, and intervertebral disc issues. We also identified independent genetic correlations between BP and depression symptoms, neuroticism, sleep disturbance, overweight, and smoking. Overall, the results demonstrate extremely complex genetic architecture of back pain that overlaps with genetic composition of psychiatric, anthropometric and socio-demographic risk factors.
Acknowledgements: The research has been conducted using the UK Biobank Resource (project No. 18219). The authors thank the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Musculoskeletal Working Group for the replication.

A search for potential anthelminthic drugs using the model of *Opisthorchis felineus*-induced Opisthorchiasis

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Key words: *Opisthorchis felineus*, anthelmintic drugs, hamsters, mice

Motivation and Aim: Opisthorchiasis is a dangerous parasitic disease of the hepatobiliary system, which is caused by flatworms of the *Opisthorchis* genus. *O. felineus* helminths are common in Europe and especially in Western Siberia, where the population's infection rate can reach more than 70 %. The drug of choice of opisthorchiasis treatment is praziquantel (PrzQ); however, it does not result in 100 % ejection of parasites from the host and has many side effects. Therefore, it is relevant to search for new anthelminthic drugs that are no less or even more effective than PrzQ and do not have severe adverse effects.

Methods and Algorithms: Four types of potential anthelmintics were investigated: a micellar complex of PrzQ with sodium glycyrrhizinate (PrzQ-Na_2GA) in the 1:10 ratio, natural antioxidant curcumin, an extract of chanterelle mushrooms (*Cantharellus cibarius*), and a chemically synthesized compound from the class of salicylanilides (encipher MST-02). Mobility and survival of adults and larvae of *O. felineus* were analyzed *in vitro*. The number of parasites in the liver and *O. felineus*-infected hosts' health condition (*Mesocricetus auratus* hamsters or C57BL/6 inbred mice) after intragastric administration of the test substances was evaluated after experiments conducted *in vivo*. The physiological state of the experimental animals was evaluated by analyzing weight dynamics, biochemical blood indices, behavior, and relative weights of organs (liver, spleen, and thymus). Disturbances in the hepatobiliary system were evaluated by histological examination of liver slices.

Results: *In vitro* experiments: MST-02 and curcumin had a significant immobilizing effect on the adult parasite specimens, whereas the extract of *C. cibarius* and MST-02 were effective against juvenile *O. felineus*. *In vivo* experiments: PrzQ-Na_2GA and curcumin significantly reduced the number of adult parasites in the hamster liver. The extract of *C. cibarius* and MST-02 had a preventive anthelmintic effect on the first day of administration to mice. There were no pronounced deviations in body weight, relative weights of organs, or behavior of the animals after administration of the test compounds. The PrzQ-Na_2GA complex lowered cholesterol and total protein levels in blood to control values as effectively as pure PrzQ. Curcumin and PrzQ-Na_2GA normalized microcirculatory processes in the liver and yielded a significant improvement in the structural and functional characteristics of this organ.

Conclusion: The test substances have anthelminthic effects comparable with those of PrzQ, without noticeable adverse effects on the host.

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Variability in Gibbs energy of tRNA molecules in mitochondrial genomes of Chordates: neutral selection or evolution towards optimization of translation?

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Key words: mtDNA, Gibbs energy, stability, mitochondrial tRNA, ancestral state reconstruction.

Motivation and Aim: It is known that translation of frequent codons in prokaryotes and some eukaryotes is optimized by increasing the copy number of corresponding tRNA gene. However, highly streamlined mitochondrial genomes of Chordata mostly hold only one tRNA gene for each amino acid. So how is mitochondrial translation optimized? We hypothesized that stability of tRNA molecules might be an important variable, correlating with codon usage (CU). It is known that translation of frequent codons in prokaryotes and some eukaryotes is optimized by increasing the copy number of corresponding tRNA gene. However, highly streamlined mitochondrial genomes of Chordata mostly hold only one tRNA gene for each amino acid. So how is mitochondrial translation optimized? We hypothesized that stability of tRNA molecules might be an important variable, correlating with codon usage (CU).
Methods and Algorithms: To test this hypothesis we reconstructed secondary structures and Gibbs energy of each tRNA from almost 4000 Chordata mito-genomes, as well as deriving various genomic features for every species. Ecological data was downloaded from the AnAge database. We also conducted reconstruction of ancestral tRNA states, using the CAT evolutionary model, at each internal node of phylogenetic tree to observe the evolutionary trend in stability.
Results: We observed that (i) In different classes of Chordata tRNA stabilities are highly variable: tend to be more stable in Aves versus Mammalia and in Actinopterygii versus Amphibia and Reptilia. GC content of the whole mitochondrial genome demonstrates the same relationship, suggesting that tRNA stability, might be just a neutral consequence of the whole genome GC content. However, comparing tRNA GC content with whole genome – we observed that warm-blooded opposed to cold-blooded Chordata have increased tRNA GC content versus background – it is possibility that tRNA stability might be under stronger selection in species with high basal metabolic rate. (ii) Comparing different species within each class, we observed positive correlations between tRNA stability and whole genome GC content. (iii) Comparing different tRNA molecules within the same genome of each species, we observed a positive correlation between tRNA stability and CU, especially in warm-blooded species. We concluded that tRNA stabilities of warm-blooded Chordata species, tend to be under stronger selection constraints of translation efficiency than those of cold-blooded Chordates.
Acknowledgements: The study was supported by the 5 Top 100 Russian Academic Excellence Project at the Immanuel Kant Baltic Federal University.

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Analysis of clinical forms of leg lymphedema. Single-center experience

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Key words: leg lymphedema, clinical forms, genes, AndSystem, associated network

Motivation and Aim: Causes of leg lymphedema are different [1]. One of them is lymphatic dysplasia as a result of a defect in the genes involved in lymphangiogenesis. Mutations in these genes lead to familial lymphedema.The first mutations that lead to lymphatic dysplasia were found in the FLT4 gene, which is a growth factor of lymphatic vessels [2].The purpose of the research: clinical analysis of different forms of leg lymphedema in the single clinical center as well as reconstruction of molecular genetic network in order to find new genes responsible for appearance of lymphedema.

Methods and Algorithms: The first step was the creation of associative molecular genetic network between the genes of VEGFR-3/VEGF-C signaling pathway using the AndSystem program, in order to see what genes and proteins they interact with. The second step was a clinical analysis of 404 medical reports of patients suffering from leg lymphedema and observed in our clinical center.

Results: The filtration of the associative network made it possible to identify 17 genes and 16 proteins which are closely associated with the main genes of the VEGFR-3/VEGF-C signal pathway. According to the analysis of 404 patients, there were 84.9 % women and 15.1 % men. Out of 404 patients with leg lymphedema, 76.2 % of the patients suffer from primary lymphedema, 23.8 % from secondary lymphedema.

Along with this 3.95 % (16) had multisegmental forms of lymphedema (leg lymphedema with face and/or hand lymphedema), 1.2 % (5) had lymphedema-associated syndromes (distichiasis or haemangiomatosis), and 3.7 % (15) had familial lymphedema. Out of 155 patients with primary lymphedema, 37 % suffer from unilateral lesions and 73.5 % from bilateral lesions, including lymphedema-associated syndromes and multisegmental forms.

Conclusion: Also according to the research, it was revealed that the female sex is more vulnerable to this pathology. Causes of primary (idiopathic) leg lymphedema are not clear. Analysis of molecular genetic associative network has identified 16 genes most closely associated with genes of VEGFR-3/VEGF-C signaling pathway. Their disquisition can help to identify some of them as new candidate genes of lymphedema.

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Weighted interaction SNP network for samples of individuals with high and low cognitive abilities

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Key words: cognitive abilities, genetic markers, exome

Motivation and Aim: One of the most modern searching tools for the genetic basis of multifactorial traits, including cognitive abilities, is the whole genome association study. However, almost always within the framework of such projects, each marker is considered separately. Due to this significant amount of information may be lost. We presume that the network method of weighted genetic interaction of genotypes will make it possible to increase the informative value of the association studies. We present the results of detecting the interaction patterns between SNPs of samples of individuals with high and low cognitive abilities.
Methods and Algorithms: The analysis was carried out using the data of whole exome sequencing of a sample of elderly people from the Russian population of Tomsk, healthy in relation to neurological and neurodegenerative pathology with high and low cognitive function scores (according to the Montreal Cognitive Assessment, MoCA). The total sample volume was 82 samples, 44 samples with high, and 38 with low cognitive abilities. The exome data was processed in a statistical environment R using the WGCNA package [1]. For each sample, we calculated the Weighted Convergence network for genotype. In each group, clusters were selected that did not significantly overlap with the clusters of the second group. As hub markers are selected, the correlation coefficient between the genotype and the module eigengenes of the modules is greater than 0.9. Those with emissions were subsequently excluded.
Results: We identified 7 clusters characteristic for group with high cognitive abilities and 5 clusters for group with low cognitive abilities. For five clusters we found highly enriched (with Benjamini–Hochberg correction) Gene Ontology (GO) terms associated with biological adhesion ($p = 1.14 \times 10^{-8}$), regulation of GTPase activity ($p = 8.7 \times 10^{-5}$), cytoskeleton organization ($p = 2.9 \times 10^{-4}$), vesicle-mediated transport ($p = 4.5 \times 10^{-4}$), negative regulation of nervous system development ($p = 2.4 \times 10^{-2}$), cognition ($p = 3.5 \times 10^{-2}$) etc. Also we detected hub markers which localized in 5 genes (*GLRA4*, *OR56A3*, *PNLIPRP2*, *C2orf54*, *TIAM2*). Comparison of samples with Fisher exact test showed differences for two markers: rs4907817 (*GLRA4*), $p = 0.02$; rs6708304 (*C2orf54*), $p = 0.01$.
Conclusion: The obvious influence of the shown biological processes on cognitive abilities and the received level of significance suggest that the genes revealed by this approach are candidates for more detailed consideration in the works devoted to the analysis of the genetic variability of cognitive functions.
The work was supported by the Russian Science Foundation (grant No. 16-15-00020).

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Up-to-date digital infrared thermography in biomedicine

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Key words: quantitative infrared thermography (IRT), sorption-enhanced IRT (SEIRT), external loads to the organism, organism systemic response, synchronous combined examination, breathing dynamics, pulse wave, computer analysis, humans, experimental animals

Motivation and Aim: In the classical sense, infrared thermography (IRT) is still associated with no more than with the obtaining of object thermal images invisible to the eye. The expansion of the optical range of perception towards the infrared brings, of course, new information about the studied objects. However, this information is incommensurably poorer than that which can now be obtained by processing IRT thermograms quantitatively [1]. We also justified that biomedical information becomes richer if it is received in real time as an organism response to external interventions. In addition, we convincingly demonstrated [2] that data representing only a temperature map of the body surface is able to give some diagnostic information short in comparison with the information obtained in the regime of a synchronous extraction of diagnostic data from different independent physical channels reflecting the systemic response of the body to external functional tests. The aim of this talk is to present all these modern trends of the IRT in biomedicine.
Methods and Algorithms: The investigated subjects were humans and animals (mainly rats and minipigs). Focal plain array-based infrared camera TKVr-IFP/SVIT allowing investigation with a temperature sensitivity of 0.03 °C and a recording rate of 100 frames per second was used for dynamic measurements of both skin temperature and breathing characteristics (with the original SEIRT method [3]). The heart rate was determined from the pulse waves measured synchronously with the IRT data using the original device connected to the Biopac MP 100 standard system. As provoking functional tests, various interventional (thermal, etc.) impacts on the organism were realized. The diagnostic data were subjected to a joint computer processing.
Results: It is demonstrated that the complex multimodal approach to diagnostics, where a main information component is the modern IRT, gives new unique information in the studies of biomedical objects.
Conclusion: Up-to-date digital IRT combined into a single complex with other synchronously applied physical methods of investigation is, when implementing the principle of interventional provoking loads on the body, a powerful tool of biomedical diagnostics and goes far ahead of the classical thermal imaging methodologies.
Acknowledgements: Supported by the RFBR (grant No. 18-08-00956).

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Features of spontaneous and induced mutagenesis in somatic cells depends on DNA repair foci

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Key words: DNA repair foci, chromosome aberrations, micronuclei, gene expression, CRISPR/Cas9

Motivation and Aim: Spontaneous DNA double-strand break repair foci and mainly γH2AX foci may be a result of unrepaired DNA double-strand breaks, erosion of telomeres or specific conformation of chromatin. The aim of this study was to analyze the relationship between the spontaneous level of γH2AX foci and the effectiveness of DNA double-strand break repair in human somatic cells.

Methods and Algorithms: γH2AX and 53BP1 foci and micronucleus frequency as a marker of DNA repair effectiveness were analyzed in lymphocytes of 54 healthy individuals after irradiation with 2 Gy γ-rays *in vitro*. Then, gene expression was analyzed by microarrays in individuals with various levels of γH2AX foci and micronuclei. Relationship between the expression of several identified genes, DNA repair effectiveness and cell survival was confirmed in 14 primary lines of placental fibroblasts and knockout HeLa cell lines, generated by CRISPR/Cas9.

Results: Spontaneous γH2AX foci level negatively correlated with the frequency of radiation-induced centromere-negative micronuclei ($R = –0.37$, $p = 0.025$). Gene expression microarrays indicated that differentially expressed genes were mostly involved not in DNA repair, but in the signaling pathways, including the TGFβ pathway and calcium signaling. Expression of *ADAMTS1*, *WHSC1* and *RBFOX2* genes in placental fibroblasts was correlated with the spontaneous level of γH2AX foci ($R = –0.66$, $p = 0.012$, $R = –0.73$, $p = 0.005$ and $R = –0.58$, $p=0.037$, respectively), and the expression of the *ADAMTS1* and *WHSC1* genes correlated with the frequency of radiation-induced micronuclei ($R = –0.63$, $p = 0.016$ and $R = –0.56$, $p = 0.037$, respectively). This indicates that the role of these genes in the mechanisms of genome stability is common for various cell types.

ADAMTS1, RBFOX2, and *WHSC1* knockout led to decrease in clonogenic survival (1.4–1.9-fold). *WHSC1* knockout increased the level of γH2AX and 53BP1 foci (2.1–2.3-fold), and *ADAMTS1* knockout increased the micronucleus frequency (4.3-fold). Finally, enriched functional groups of genes were identified in knockout cell lines by gene expression microarrays.

Conclusion: The obtained results indicate that the spontaneous level of DNA repair foci through the regulation of the expression of certain genes can influence spontaneous and induced mutagenesis in human somatic cells.

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Analysis of relationships between putative genetic markers and immune response in patients with uterine myoma

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Key words: uterine myoma, gene polymorphism, cytokines, non-invasive markers, immune response

Motivation and Aim: Among gynaecological pathologies, uterine myoma is most frequent non-inflammatory disease, and cytokines are known as one of the important its regulators. Gene polymorphisms and structural features of respective proteins are known to lead to different quality of immune response, and, as a consequence, to different disease outcome. So far, there are many gaps in understanding of relationships between genetic background affecting immune response and development of uterine myoma. Prediction of the disease development is quite actual task, and search of non-invasive molecular-genetic markers is one of the main modern subtasks. The main goal of this work was to find and characterize relationships between the levels of selected cytokines and growth factors in physiological liquids and single nucleotide variants in promoters of genes – important regulators of immune response.

Methods and Algorithms: For creation of the classification models, different methods of the machine learning approach were used (e. g., support vector machine, Fisher's discriminant analysis, random forest, and neural networks). These methods, in comparison to classical quartile analysis, represent the more modern approach to analysis of relationships between single nucleotide variants (SNV) identified in genes of patients – the prospective non-invasive markers of human pathologies – and the levels of cytokines and growth factors from physiological liquids. For identification of transcription factors whose binding sites could be potentially affected by the considered SNVs, the data from the GTRD database (http://gtrd.biouml.org/; [1]) was applied.

Results: As the initial data for analysis, 13 SNVs located in promoter regions of nine genes participating in immune response and the levels of 27 cytokines and growth factors measured in patients with myoma were analyzed. For expression of the relationships between SNVs and protein levels, the classification models were created. SNVs characterized by the strong association with the analyzed cytokines and growth factors were selected as the putative non-invasive markers of myoma. Application of the GTRD data to the selected significant SNVs allowed to highlight possible changes in biological pathways in patients with myoma. These results extend understanding of myoma development.

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Synthesis of caged NO and epinephrine compounds for optically controlled platelets activation

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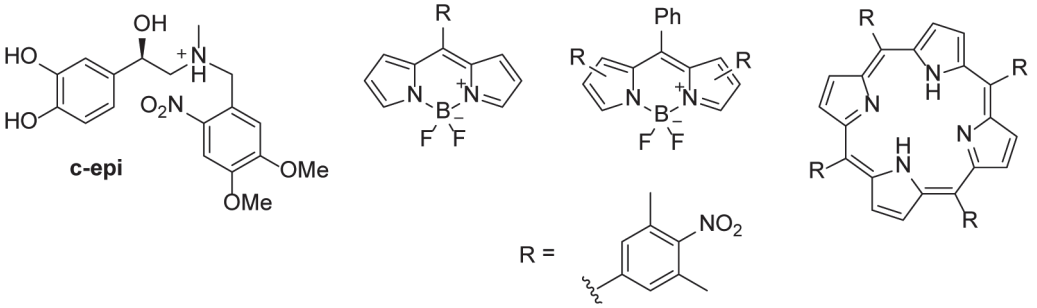
Key words: caged compounds, nitric oxide donors, blood platelets, platelet activation

Motivation and Aim: Platelets play an important role in blood coagulation and other normal and pathological processes in living organisms. Platelets reactions to different stimuli can be investigated *in vitro*, which helps to understand their function. In the last years photoinduced platelets activation attracted some attention [1]. It allows one to trigger cellular response by light using photolabile ("caged") analogs of bioactive molecules.

Methods and Algorithms: we synthesized caged epinephrine (**c-epi**) and several 2,6-dimethyl-1-nitrophen-4-yl substituted BODIPYs and *meso*-substituted porphyrin.



Results: We monitored activation of individual platelets with fluorescent microscope using Fluo-4 calcium indicator. Obtained **c-epi** didn't cause any calcium spikes without light at ~10^{-4} M concentration level. The activation proceeded efficiently only under UV (λ ~360 nm) irradiation.

Conclusion: Caged epinephrine can be used to trigger platelet activation optically and study the process at single cell level. Other synthesized compounds are promising for visible light induced generation of nitric oxide (NO).

Acknowledgements: This work was supported by Russian Science Foundation (grant No. 18-15-00049).

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Mechanisms of inactivation of the *TP53* gene in diffuse large B-cell lymphoma

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Key words: gene *TP53*, diffuse large B-cell lymphoma, promoter methylation, loss of heterozygosity, mutations, sequencing

Motivation and Aim: There is no complex description of the variability of *TP53* gene in diffuse large B-cell lymphoma (DLBCL) [1]. The purpose of current study was to comprehensive study of the *TP53* aberrations due to somatic mutations, promoter methylation and allelic imbalance in tumor tissue of DLBCL.
Methods and Algorithms: In the course of the study the frequency, spectrum and functional significance of aberrations in the *TP53* gene of 74 patients with DLBCL in Novosibirsk was described. Genomic DNA was isolated from formalin-embedded paraffin blocks of lymph nodes and extranodal tumor lesions biopsies by phenol-chloroform extraction method using guanidine. The tissue sections containing at least 70–80 % of the tumor cells were taken. The lloss of heterozygosity in *TP53* gene was performed by D17S796 microsatellite analysis. To screening of *TP53* mutations was performed Sanger's direct sequencing. Methylation analysis was carried out by methyl-specific PCR.
Results: 95 % of the mutations prevailed in the *TP53* gene sites encoding the DNA-binding domain. It is shown that localization of mutations "hot spots" in the studied sample of patients with DLBCL differ from the data presented in IARC *TP53* mutation database [2]. In the analyzed sample of patients, codons 275, 155, 272 and 212 were the "hot spots" of mutations. Presence of DLBCL with pathogenetic intron (IVS6-36G > C и IVS5+43G > T) and synonymous (p.A307A) mutations was revealed. The frequency of *TP53* promoter methylation in the study group was 5.8 %. Loss of heterozygosity in the gene was observed in 25 % of cases and was observed only in a subset of patients with modified (mutation or promoter methylation) status of *TP53*.
Conclusion: The results indicated the selection of functionally significant mutations in the *TP53* DNA-binding region in DLBCL. It is shown, that the lack of function of the gene in DLBCL can be formed on the two-hit principle.

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Bone mineral density changes in patients with Hodgkin's lymphoma

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Key words: Hodgkin's lymphoma, bone mineral density, BMD, osteoporosis

Motivation and Aim: Currently, Hodgkin's lymphoma (HL) is considered a potentially curable disease. The polychemotherapy including various combinations of cytostatic drugs and glucocorticoids (GCs) appear the standard of treatment in patients with HL. Patients with early stage disease (stage I to II) receive several cycles of chemotherapy followed by the radiotherapy. The possibility of achieving persistent disease-free survival poses a problem of ensuring a satisfactory quality of life and preventing the long-term consequences of antitumor therapy. One of those consequences may be a change in bone mineral density (BMD) [1]. The aim of the study was to assess the BMD in patients with HL.

Methods and Algorithms: The study included 38 patients, from 19 to 70 years of age (median 49 years). The disease was staged according to the Ann Arbor staging system: 16 patients had early stage disease and 22 patients had advanced stage disease (stage III to IV). Thirty four subjects received first- and second-line regimens (ABVD, BEACOPP, DHAP). Four patients received other regimens, including DAL-HD-2002, ProMACE-CytaBOM, COPDIC, and COPP. Radiotherapy was performed in 28 individuals. High dose therapy with autologous stem cells rescue (HDCT-ASCT) was conducted in one patient. The BMD was assessed by dual-energy X-ray absorptiometry (DXA).

Results: The decreased BMD was revealed in 20 observed HL subjects (52.6 %). The decline in BMD was more frequent in patients aged ≥ 50 years as compared to younger ones ($\chi^2 = 5.75$; $p = 0.02$). More patients with radiotherapy in anamnesis had decreased BMD compared to patients who received chemotherapy only ($\chi^2 = 8.82$; $p = 0.003$). Osteopenia was found in patient with HDCT-ASCT. Nineteen patients received regimens containing GCs had decrease in BMD. Based on FRAX assessment, the 10-year risk of major low-energy fractures and the hip fractures was expectedly higher in patients with osteoporosis and osteopenia as compared to subjects with normal BMD ($p < 0.05$).

Conclusion: The decrease in BMD is associated with radiotherapy and chemotherapy with GCs in patients with HL.

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Metabolomic studies of human cataract

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Key words: quantitative metabolomics, mass spectrometry, NMR spectroscopy, biochemical processes, cataracts

Motivation and Aim: A cataract (clouding of the lens) is the most common cause of vision declining of older people. Unlike most other human tissues, the lens has specific structure to be transparent: firstly, the lens consists mainly of fiber cells without organelles, and secondly, it lacks blood vessels. The protection of the lens is mainly provided by metabolites; most of them are synthesized in the lens epithelium or enter the lens through the epithelial layer from the surrounding aqueous humor (AH). Comparison of the metabolomic compositions of lenses and AH taken from patients with age-related nuclear cataract with lenses and AH taken from human cadavers without cataract can shed light onto molecular mechanisms underlying onset of cataract.
Methods and Algorithms: Quantitative metabolomic profiles of eye tissue extracts (lens, AH) were obtained with the combination of three methods – high-frequency 1H nuclear magnetic resonance (NMR) and ion-pairing high-performance liquid chromatography with optical (LC-OD) and high-resolution ESI-q-TOF mass-spectrometric detection (LC-MS) methods. NMR-based quantification was achieved with the use of one internal standard (DSS) for all metabolites under study, while LC-MS quantification required the construction of the calibration curves for each metabolite under study using the commercially available chemical standards.
Results: The concentrations of more than 80 metabolites were determined for four groups of samples: lenses and AH from cataract patients and lenses and AH from human cadavers. In cataractous lens the most abundant metabolites are (in descending order): myo-inositol, lactate, acetate, glutamate, glutathione; in AH – lactate, glucose, glutamine, alanine, valine. The concentrations of nucleotides, UV filters, antioxidants and some other metabolites in the lens are much higher than that in AH, while the levels of glucose and hydroxybutyrates are lower. The concentrations of the majority of metabolites in non-cataractous post-mortem samples of both lens and AH are higher than that in samples from the cataract patients.
Conclusion: Our metabolomic data confirm the hypothesis that although the age-related cataract usually manifests itself as the opacification of the lens nucleus, the initial site of the cataract onset might be the lens epithelial layer. The most important for the lens protection metabolites – antioxidants, UV filters, osmolytes – are synthesized in the lens epithelial cells. The reduced levels of these metabolites were found in the cataractous lenses; that indicates that the cataract development may originate from the dysfunction of the lens epithelial cells. The increase in the concentrations in non-cataractous post-mortem tissues for other metabolites corresponds to the post-mortem processes.
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Quantitative metabolomic analysis of *Sander lucioperca* and *Rutilus rutilus lacustris* gills and lenses

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Key words: lens metabolome, gill, mass spectrometry, NMR spectroscopy, fish, season

Motivation and Aim: The life processes of cells, tissues and whole organs are based on reactions involving a great variety of biological molecules. The whole complex of all metabolites is called "metabolome". Some metabolites enter the body from outside, the rest are the products or intermediates of metabolic reactions. Complex analysis of metabolome is the key moment necessary for understanding the mechanisms of molecular processes occurring in cells, tissues or organs. The changes in the composition of metabolites or changes in their content helps to elucidate the molecular basis for pathogenesis or the influence of ecological factors. Earlier we developed a method for quantitative metabolomic analysis of different tissues and successfully analyzed the metabolite concentrations in lenses, corneas, aqueous humors and serums of control and pathological patients. In this work we applied our metabolomic approach to the analysis of fish lenses and gills metabolome.
Methods and Algorithms: In this study we analyzed lenses and gills of two different types of fish – pike and roach, inhabiting the Novosibirsk region. The quantitative metabolomic profiling of samples of the lenses and gills has been performed with the combined use of high frequency ^{1}H nuclear magnetic resonance (NMR) and high-resolution LC–MS methods. Special attention was paid to the lipids removal method optimization.
Results: The concentrations of 60–80 most abundant metabolites in lenses and gills have been measured. It has been found that the concentrations of the majority of metabolites in the lenses of autumn fishes are noticeably higher than in winter ones. The most abundant metabolites in roach and pike lenses are *N*-acetyl-histidine, *N*-acetyl-aspartate, serine-phosphoethanolamine and threonine-phosphoethanolamine. These metabolites were attributed to the osmolytes in the fish lenses. The novel molecule 1-methyl-5-sulfanyl-histidine disulfide – was found in roach and pike lenses in rather high concentrations, which was presumably attributed to the antioxidants as its analogue ovothiol A.
Conclusion: The results obtained in the work show rather high dependence of osmolyte concentration on the season – *N*-acetyl-Histidine predominates in autumn fish lenses, while *myo*-inositole – in winter lenses. This difference may be attributed to the nutrition changes of fishes and lack of oxygen in winter season. The comparison of the lenses and gills of one specie shows that the levels of major osmolytes and antioxidants in lenses from tens to hundreds times higher than in gills. This speaks in favor their synthesis inside fish lenses.
Acknowledgements: Supported by the RFBR (projects No. 18-34-00137, 17-03-00656).

Exome-wide search and functional annotation of genes associated with severe forms of tick-borne encephalitis in Russians

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Key words: tick-borne encephalitis, whole-exome sequencing, Russian population, genetic predisposition, biological pathways

Motivation and Aim: Tick-borne encephalitis (TBE) may have a variety of clinical manifestations ranging from slight fever to severe neurological illness. The severity of TBE may be determined by both genetic factors related to the virus subtypes and the host's genetic susceptibility. To understand the genetic susceptibility to TBE virus-induced disease, we performed an exome-wide search for single-nucleotide variants (SNVs) and indels associated with severe forms of TBE in Russian population. Using functional analyses of genes, containing these variants, we revealed biological pathways through which they may influence the severity of the disease.
Methods and Algorithms: Blood samples were collected from 22 unrelated symptomatic Russian patients with severe forms of TBE and 17 Russian individuals from the control cohort. The preparation of libraries and exome enrichment were performed with an Agilent SureSelect Human All exon V5 Kit. Sequencing was carried out on a HiSeq Illumina 4000 platform. The reads that passed the quality assessment were aligned to the reference Hg19 genome with the BWA (bwa mem) program. SNVs and indels were identified using GATK pipeline. The pathogenic variants were revealed using the ANNOVAR database. Selection of variants that were not common (MAF < 0.05) in European population were performed using Exac Database. GO and pathway analyses were performed by DAVID tool.
Results: A total of 4869 harmful variants (4858 SNVs and 11 indels) that were not common in Europeans were identified in the target regions of the Agilent SureSelect V5 kit for the control samples. These variants were found within the bodies or in vicinity of 3684 genes. In the samples of TBE patients we identified 6141 harmful variants (4858 SNVs and 17 indels), specifying 4509 genes. 2407 out of these 4509 genes were found only in the samples of TBE patients. According to DAVID tool, the list of 2407 genes was enriched (FDR < 0.05) with genes located in plasma membrane and at the cell periphery and genes involved in extracellular matrix proteoglycans pathway. 19 out of 2407 genes revealed in TBE patients were annotated in the TBEVHostDB [1] as genes that may be probably involved in response to TBEV infection.
Conclusion: Our results provide new knowledge on genetic susceptibility to severe forms of TBE and could potentially be used for the design of personalized pharmacological strategies for the treatment of TBEV infection.
Acknowledgements: This study was supported by the grant from the Russian Science Foundation (project No. 16-15-00127).

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Hypovitaminosis D as a biomarker of cardiovascular risk in patients with rheumatoid arthritis

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Key words: vitamin D, hypovitaminosis, rheumatoid arthritis, cardiovascular risk, atherogenesis

Motivation and Aim: Hypovitaminosis of vitamin D (Vit. D) is currently a worldwide pandemic. Vit. D is a multifunctional biologically active agent in our body. Hypovitaminosis D associated with connective tissue disorders, autoimmune disorders, addictions to occurrence of a cancer etc. 52 % of patients with rheumatoid arthritis (RA) have Vit. D deficiency[1]. Cardiovascular events are the main cause of death of these patients. The risk of incident cardiovascular disease is increased by 48 % in patients with RA compared to the general population [2]. The aim – to investigate the lipid profile of blood serum in patients with RA with concomitant hypovitaminosis D and without it.
Material and methods: 11 patients with RA and hypovitaminosis D (main group) and 10 patients with RA and normal level of Vit. D (comparison group) were examined. All patients had 1–2 degree of inflammation. Hypovitaminosis D was diagnosed with a decrease in the serum level of Calcifediol below 20 ng/ml by ELISA test. The blood level of calcifediol is considered the best indicator of Vit. D status. Total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides were determined in the blood serum by colorimetric analysis. Methods of descriptive statistics were used for processing of the results.
Results: The increase in total cholesterol was observed in both groups of patients (6.3 ± 0.32 mmol/l – in the main group, 6.1 ± 0.30 mmol/l – in the comparison group). Differences in the values of this parameters were unreliable ($p > 0.05$). Also, there were no significant differences in the level of HDL. At the same time, the level of LDL in patients of main group (3.9 ± 0.14 mmol/l) was significantly ($p = 0.03$) higher than in patients of comparison group (3.5 ± 0.11 mmol/l). Also, reliable differences ($p = 0.02$) were observed in triglyceride levels between groups of patients (2.5 ± 0.09 mmol/l and 2.2 ± 0.08 mmol/l, respectively). As an axiom taken the position that atherosclerosis of the vessels is the basis of most of cardiovascular events, including patients with RA. Atherogenesis in patients with RA is a multifactorial process, including autoimmune, genetic and metabolic mechanisms. Hypovitaminosis D is one of many pathogenetic mechanisms of atherogenesis in the patients. However, these changes (hypovitaminosis D) can be eliminated and this fact is an important component in managing the disease and reducing cardiovascular morbidity and mortality in patients with RA.
C*onclusion*: Changes in the serum lipid spectrum in RA patients with concomitant hypovitaminosis D have an atherogenic orientation. Elimination of vitamin D deficiency helps to reduce cardiovascular risk in these patients.

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Metabolomic biomarkers for the estimation of post-mortem interval

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Key words: metabolomics, post-mortem interval, serum, aqueous humor, vitreous humor

Motivation and Aim: The analysis of post-mortem metabolomic changes in biological fluids opens the way to develop new methods for the estimation of post-mortem interval (PMI). It may also help in the analysis of disease-induced metabolomic changes in human tissues when the postoperational samples are compared to the post-mortem samples from healthy donors.
The goals of this study are to observe and classify the post-mortem changes occurring in human and rabbit (as a model animal [1]) blood, aqueous and vitreous humors (AH and VH), to identify the potential PMI markers among a wide range of metabolites, and also to determine which biological fluid – blood, AH or VH – is more suitable for the PMI estimation.
Methods and Algorithms: The quantitative metabolomic profiling of samples of human and rabbit serum, AH and VH taken at different PMIs has been performed with the combined use of high-resolution LC-MS and high-frequency NMR methods.
Results: The quantitative levels of fifty metabolites in human and rabbit serum, AH and VH at different PMIs have been measured. It has been found that the post-mortem metabolomic changes in AH and VH proceed slower than in the blood, and the data scattering is lower. Among the metabolites whose concentrations increase with time, the most significant and linear growth is found for hypoxanthine, choline and glycerol.
Conclusion: The obtained results suggest that the ocular fluids AH and VH may have some advantages over blood serum for the search of potential biochemical markers for the PMI estimation. Among the compounds studied in the present work, hypoxanthine, choline and glycerol give the biggest promise as the potential PMI biomarkers.
Acknowledgements: This work was supported by RFBR (project No. 18-34-00137).

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A synonymous variant in *GCK* gene as a cause of gestational diabetes mellitus

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Key words: gestational diabetes, splicing mutations, GCK

Motivation and Aim: MODY encompasses several inherited forms of diabetes caused by genetic defects resulting in β-cell dysfunction. Mutations in GCK gene (MODY2) are reported as a frequent cause gestational diabetes mellitus (GDM), with frequencies ranging from 0.1 % to 80 %. According to our results, based on the Next Generation Sequencing (NGS), GCK gene mutations are found in 17.8 % (39/219) of subjects with GDM. Here we report a case of GDM due to a synonymous GCK gene variant, pathogenicity of which was determined by in vitro studies.
Case report: A rare synonymous *GCK* variant (NM_000162: c.666C>G p.V222V; ExAC MAF, 0.0001) was detected in one of the subjects included in our "GDM-NGS" study. The variant was initially ranked as "Likely benign" according to the ACMG guidelines. The patient, a 22-year-old woman, was diagnosed with GDM at 29th week of pregnancy. She showed family history of diabetes (MODY phenotype) in three generations. Her pre-gestational BMI was normal (21.8 kg/m2). All autoantibodies (ICA, IA2, GADA, IAA) were negative. She showed mild fasting (6.1-6.4 mmol/l) and postprandial (8.0 mmol/l) hyperglycemia values since the 7th week of pregnancy without any therapy. At week 30 basal/bolus insulin therapy was started. At 39 weeks of gestation she gave birth to a healthy girl with normal birth weight (3.28 kg) and length (51 cm).
Affected members of the family were shown to have the same *GCK* variant by Sanger sequencing. MODY phenotype with strong family history prompted us to question "benign" nature of the synonymous variant and perform in vitro studies. Minigene studies demonstrated that c.666C>G substitution created a novel donor splice site in exon 6, which lead to formation of defective *GCK* mRNA with deletion of 16 nucleotides of exon 6 [1].
Conclusion: Our report emphasizes importance of careful clinical evaluation of all cases studied by NGS. In the case of strong clinical evidence rare apparently benign variants in MODY-related genes should be re-evaluated using additional methods.
Acknowledgements: Supported by the RSF (No. 16-15-10408).

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qGEPS: the tool for relative quantification of gene expression in paired samples

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Key words: quantitative PCR, gene expression, data analysis, PCR efficiency, relative quantification

Motivation and Aim: Quantitative PCR (qPCR) remains the most widely used technique for gene expression evaluation, and the relative quantification (ΔΔCt method), when expression of target gene is assessed in target sample in comparison with control sample, is the most frequently used analysis type within qPCR. A number of tools were developed for qPCR data analysis, however, none of them is well enough for expression analysis with ΔΔCt method in a representative set of paired (target/control) samples. Here we present qGEPS (Quantification of Gene Expression in Paired Samples) – convenient tool for qPCR data analysis including relative quantification of gene expression in paired samples.
Methods and Algorithms: qGEPS represents an Excel workbook with VBA macros. This format is convenient even for novice users with no knowledge in programming. As input data, qGEPS uses fluorescent signals from dyes in each well at each PCR cycle as well as sample and gene names in each well. In our case, qPCR is performed on Applied Biosystems 7500 Real-Time PCR System, and the needed data can be exported from 7500 Software as Excel workbooks. To develop qGEPS, we used the huge experience that was obtained through operation of previously created ATG tool [1].
Results: The qGEPS tool allows estimation of threshold cycles (Ct) through analysis of fluorescent curves obtained from up to dozens of 96-well PCR plates and further versatile analysis of estimated Ct values. PCR efficiency is calculated for each pair of primers on the basis of fluorescent curve at the phase of power growth. This type of efficiency assessment showed good concordance with traditionally used "standard curve" in case of a statistically significant number of curves analyzed (50 and more) and allows saving the reagents. Compared to our previous ATG tool, two approximation models for efficiency calculation were excluded because they showed long calculation time and no benefit in accuracy of efficiency assessment. The main purpose of qGEPS is evaluation of gene expression with ΔΔCt method, when target samples are compared to control ones. This is especially necessary in cancer studies, as there is often need to compare each primary tumor with matched histologically normal tissue for a representative set of samples. The possibility of using several reference genes and performing statistical tests are also implemented in qGEPS.
Conclusion: We have developed qGEPS – user-friendly tool for the analysis of huge amounts of qPCR data. The tool is especially useful when paired samples are analyzed, for example tumors and matched normal tissues.
Acknowledgements: This work was financially supported by the Russian Science Foundation, grant No. 17-74-20064.

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Author index

Компания Huawei является ведущим мировым поставщиком ИКТ-решений. Благодаря установлению взаимовыгодных отношений с нашими партнерами и заказчиками компании Huawei удалось добиться существенных преимуществ в сфере операторских сетей, корпоративного и потребительского бизнеса, а также в сфере облачных технологий. Мы стремимся создавать максимальные преимущества для операторов связи, предприятий и потребителей путем разработки конкурентных ИКТ-решений и услуг. Оборудование и решения Huawei используются в более чем 170 странах мира. Компания обслуживает более трети населения земного шара.

Имея богатый опыт и технические знания в области НИОКР, Huawei придерживается стратегии тесного сотрудничества и интеграции с корпоративными заказчиками и предоставляет им широкий спектр высокоэффективных клиентоориентированных ИКТ-решений и услуг, на базе глубокого понимания их потребностей. Согласно этой стратегии Huawei предлагает широкий выбор передовых ИКТ-решений в сфере государственного управления, общественного сектора, финансов, транспорта, электроэнергетики, крупных предприятий, а также малых и средних предприятий (SME). Эти решения охватывают корпоративные сети, универсальные системы связи и взаимодействия (UC&C), системы облачных вычислений и центры данных, системы корпоративной беспроводной связи, сетевого электропитания, а также инфраструктурные услуги.

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Huawei is a leading global ICT solutions provider. Through our dedication to customer-centric innovation and strong partnerships, we have established end-to-end capabilities and strengths across the carrier networks, enterprise, consumer, and cloud computing fields. We are committed to creating maximum value for telecom carriers, enterprises and consumers by providing competitive ICT solutions and services. Our products and solutions have been deployed in over 170 countries, serving more than one third of the world's population.

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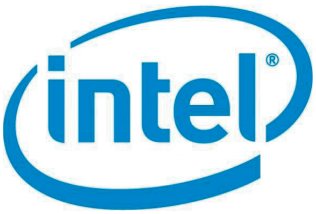

Корпорация Intel

Корпорация Intel была основана в 1968 году Робертом Нойсом и Гордоном Муром. На протяжении 50 лет Intel создает инновационные технологии, открывающие новые возможности для людей.

Корпорация Intel является мировым лидером в области микроэлектроники и информационных технологий. Intel создает технологии для умного мира эпохи больших данных. Основное внимание корпорация уделяет созданию интеллектуальных решений для умного мира, от устройств Интернета вещей и пользовательских ПК до коммуникационной инфраструктуры, технологий для центров обработки данных и суперкомпьютеров.

Штаб-квартира корпорации расположена в г. Санта-Клара, шт. Калифорния. Общий штат Intel насчитывает более 100 тыс. сотрудников в более, чем 60 странах по всему миру. Главным исполнительным директором корпорации является Роберт Свон (Robert Swan).

Intel в России

Первое представительство Intel в России было открыто в 1991 году в Москве. Сегодня в российских офисах Intel в Москве и Нижнем Новгороде работают более 800 человек.

В московском офисе компании представлены отделы маркетинга и развития бизнеса, группы по разработке программного обеспечения, юридический отдел.

В НИОКР центре Intel в Нижнем Новгороде создаются новые и инновационные продукты для разработки ПО. Сегодня он является одним из крупнейших центров исследований и разработок Intel в Европе. Более **700 специалистов и инженеров** разрабатывают программные инструменты и приложения для архитектур Intel. В Нижнем Новгороде также размещаются различные группы поддержки бизнеса (например, административно-хозяйственная часть, финансовый отдел, отдел ИТ, отдел кадров).

Центр исследований и разработок Intel в Нижнем Новгороде

Нижегородский офис Intel был является центром экспертизы корпорации в области высокопроизводительных вычислений, разработки программного обеспечения в области численных методов и беспроводной связи.

✉ MP Biomedicals
(ООО «МПБА диагностика»)
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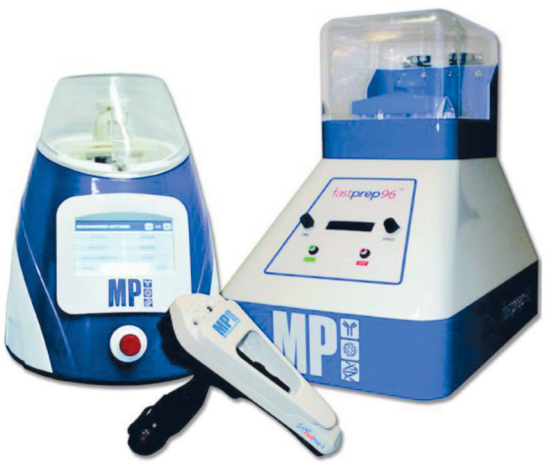
Компания ООО «МПБА диагностика» является дочерней компанией MP Biomedicals, ранее известной как ICN Biomedicals, основанной в 1959 году, признанного лидера в области производства широкого спектра химических реактивов, оборудования для пробоподготовки (система для гомогенизации FastPrep) и наборов реагентов. Каталог продукции компании MP Biomedicals включает более 55000 наименований высококачественных продуктов для проведения биохимических исследований, фармацевтического и биотехнологического производства, для различных отраслей иммунологии и генетики.



ООО «Рош Диагностика Рус» – официальный импортер продукции Roche в России и лицензиат компании F.Hoffmann–La Roche Ltd.

Roche Sequencing Solutions, подразделение Roche, ориентированное на решения для NGS, а в частности на пробоподготовку к NGS, предлагает:

-**Наборы KAPA Biosystems для приготовления библиотек ДНК** (включают баркодированные адаптеры, частицы для очистки, наборы для оценки концентраций ДНК и библиотек методом ПЦР в реальном времени).

-**Наборы для направленного отбора генов перед NGS:**

NimbleGen SeqCap EZ –гибридизационное обогащение панелей генов, экзомов, транскриптомов и метиломов;

HEAT-seq - амплификационное обогащение панелей генов, в том числе и панелей онкогенов;

AVENIO –гибридизационное обогащение панелей онкогенов из внеклеточной опухолевой ДНК и анализ данных.

ООО «Рош Диагностика Рус» предлагает комплексные решения, включающие в себя не только оборудование и реагенты, но и технический сервис, обучение персонала и постоянную методическую поддержку.

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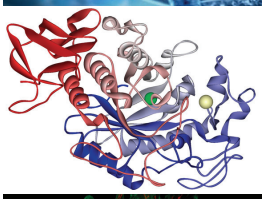
ООО «БиоЛайн» является официальным дистрибьютором компаний **BD Biosciences, Leica Microsystems, PerkinElmer, BioTek,** ведущих мировых производителей приборов и реагентов для биомедицинских исследований.

Благодаря уникальному портфолио продукции и опыту наших специалистов мы выполняем поставки и внедрение комплексных решений для разнообразных задач в области молекулярной и клеточной биологии.

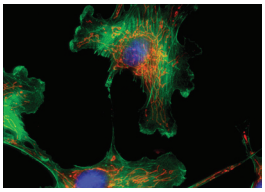
Молекулярно-генетические исследования

- Системы для выделения и молекулярного анализа одиночных клеток Becton Dickinson
- Станции для выделения ДНК, оборудование PerkinElmer для подготовки и контроля библиотек для NGS
- Наборы Nextflex для подготовки библиотек NGS PerkinElmer: полногеномное и таргетное секвенирование, транскриптомика, эпигенетика, метагеномика



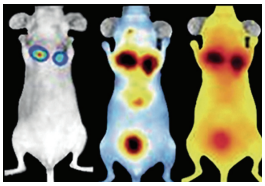
Протеомные исследования

- Передовые оптические технологии компании BioTek Instruments для биохимических исследований, идентификации и количественной оценки аналитов, исследования взаимодействия биомолекул
- Реагенты и расходные материалы PerkinElmer для протеомных исследований



Клеточные исследования

- Системы для проточной цитометрии и сортировки клеток компании BD Biosciences
- Оптическая визуализация клеток для моделирования процессов в клеточных культурах и на 3D сфероидах: решения PerkinElmer и BioTek Instruments
- Системы для конфокальной микроскопии Leica Microsystems



Исследования на животных

- Приборы для оптической визуализации ***in vivo*** Spectrum и Lumina, системы для КТ и ПЭТ компании PerkinElmer
- Оборудование для исследований на животных Leica Biosystems

Официальные дистрибьюторы BD Biosciences, Leica Microsystems, PerkinElmer, BioTek в России — компания «БиоЛайн»

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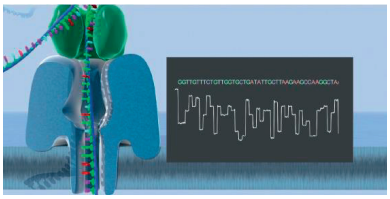
В каталоге компании представлена продукция ведущих мировых производителей, как: Abcam, Applied Biosystems, Binder, Bio-Rad, Corning, Eppendorf, Illumina, Ion Torrent, Lexogen, Oxford Nanopore Technologies, Panasonic (Sanyo), Sage Sciences, Sigma-Aldrich, Thermo Fisher Scientific, Qiagen:

- Наборы для подготовки библиотек, для высокопроизводительного секвенирования NGS, для исследовательских работ и, в онкологии, репродуктивной медицине, в изучении наследственных заболеваний, реагенты и наборы для капиллярного секвенирования.
- Секвенаторы капиллярные и высокопроизводительные NGS, оборудование для анализа качества НК для NGS, роботизированные станции для подготовки библиотек и секвенирования.
- Все для ПЦР, реагенты,наборы, пластик, амплификаторы.
- Нанопоровые секвенаторы Oxford Nanopore Technologies, наборы для секвенирования ДНК и РНК.


Секвенирование теперь доступно каждому!

Диаэм сегодня представляет продукцию Oxford Nanopore Technologies – это секвенаторы третьего поколения – MinION, GridION, PromethION.
Технология секвенирования Oxford Nanopore Technologies позволяет делать прямое прочтение цепей ДНК или РНК в режиме онлайн, длина рида ограничена только длиной фрагмента, а портативность оборудования и быстрая подготовка библиотек дает возможность секвенировать даже в полевых условиях с минимальными требованиями к генетической лаборатории. С Oxford Nanopore Technologies секвенировать теперь может каждый, даже тот, кто ранее и не задумывался о секвенировании - это просто и доступно.
Секвенирование третьего поколения не заменяет и не отменяет применение капиллярных секвенаторов по Сэнгеру или платформ NGS второго поколения, наоборот, сочетание трех поколений генетического анализа открывает новые возможности получения ранее неизвестных данных.
Специалисты Диаэм прошли обучение в Oxford Nanopore Technologies, осуществляют профессиональное консультирование и техническую поддержку, помогут спланировать эксперимент и подобрать необходимые наборы реагентов для решения конкретной задачи независимо от бюджета лаборатории.
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Компания АЛЬБИОГЕН — официальный дистрибьютор illumina и Lucigen

Компания ООО «АЛЬБИОГЕН» с 2015 года является эксклюзивным (единственным) официальным торговым представителем и дистрибьютором компании illumina на территории Российской Федерации, Республики Беларусь, Республики Казахстан и Республики Узбекистан.

Нашей задачей является обеспечение полного доступа клиентов к передовым технологиям и сервисам illumina, включая современные системы NGS и анализа ДНК-биочипов, программное обеспечение для биоинформатики и весь спектр реактивов.

ООО «АЛЬБИОГЕН» предоставляет полный комплекс услуг, связанных с продажей, технической поддержкой и сервисным (гарантийным и постгарантийным) обслуживанием продукции компании Illumina, а также обучением пользователей работе на данном оборудовании.

Инновационная и стремительно развивающаяся компания illumina Inc., являющаяся мировым лидером в области геномных технологий, заключила соглашение с компанией АЛЬБИОГЕН, специализирующейся на поставках оборудования и расходных материалов для секвенирования нового поколения (NGS) и анализа на ДНК-биочипах.

Новейшие продукты компании illumina, создаваемые совместно с ведущими мировыми учеными, позволяют изучать геном на очень глубоком уровне и дают возможность для новаторских достижений в науке, медицине, сельском хозяйстве и потребительской геномике. Более 90% научных статей, связанных с технологиями секвенирования нового поколения, сделаны при помощи оборудования Illumina.

Сотрудничество с компанией АЛЬБИОГЕН направлено на то, чтобы сделать технологии NGS и анализа ДНК-биочипов более доступными на территории Российской Федерации и в странах СНГ.

Компания АЛЬБИОГЕН использует свой обширный опыт в области продаж и продвижения продукции, знания передовых технологий и сеть региональных представителей для обеспечения быстрой, эффективной и бесперебойной работы лабораторий клиентов illumina.

Компания АЛЬБИОГЕН также является официальным дистрибьютором компании Lucigen, основными продуктами которой являются ферменты и реагенты для секвенирования нового пколения и молекулярной диагностики.

Компания СкайДжин предлагает к поставке со склада в Москве и под заказ наборы реагентов, оборудование, расходные материалы, реактивы, а также специализируется на сервисном обслуживании и поверке дозаторов, лабораторных весов различных производителей. Мы предлагаем гибкие условия работы и очень большой ассортимент продукции.

Поставляемая нашей компанией продукция широко используется в научно-исследовательских лабораториях и R&D центрах, лабораториях секвенирования, при решении практически любых молекулярно-биологических задач.

Большая часть производителей в нашем портфолио - это прямые, эксклюзивные поставки. Мы являемся первым звеном в поставках для таких компаний как New England Biolabs, Agilent Technologies, Oxford Nanopore Technologies, QIAGEN, 10x Genomics, NIMAGEN, Integrated DNA Technologies, Thermo Fisher Scientific, SIGMA-ALDRICH, BioSan, Gilson.

К флагманским продуктам наших линеек относятся:

- Набор для пробоподготовки образцов от New England Biolabs ULTRA II FS с интегрированной системой фрагментации и другие наборы серии ULTRA для образцов ДНК, РНК и микроРНК;
- Digital NGS: готовые панели и наборы для обогащения на основе ПЦР от QIAGEN с мономолекулярным баркодированием;
- Специализированные наборы для работы с микроРНК и анализа экспрессии от QIAGEN-Exiqon;
- Нанопоровые секвенаторы третьего поколения: портативный секвенатор MinION, высокопроизводительный секвенатор GridION;
- Уникальная система Chromium производства 10x Genomics для автоматической пробоподготовки геномов и транскриптомов единичных клеток.

За дополнительной информацией о производителях, товарах, ценах и условиях поставки обращайтесь к нашим квалифицированным специалистам.

Будем рады ответить на Ваши вопросы и помочь выбрать качественное и недорогое решение для Ваших задач!

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Тел: 8 (495) 215 02 22
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www.skygen.com

ХИМЭКСПЕРТ

Агентство Химэксперт

Информация о компании:

Компания Химэксперт существует 16 лет и давно зарекомендовала себя, как надежный поставщик приборов, реактивов и расходных материалов для молекулярной биологии. Мы собрали для своих клиентов самые интересные и перспективные бренды, большинство из которых в России можно приобрести только у нас.

Химэксперт предлагает оборудование для анализа ДНК и РНК, в том числе и методами NGS, фундаментальных протеомных и цитологических исследований, фармацевтики и биотехнологий, прикладного тестирования, включая идентификацию личности и установление родства в криминалистике и судебно-медицинской экспертизе.

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- Химэксперт осуществляет полную техническую и методическую поддержку наших клиентов: обратившись к нам, вы получаете помощь квалифицированных сотрудников в подборе оборудования и реагентов под поставленные задачи и их последующем использовании
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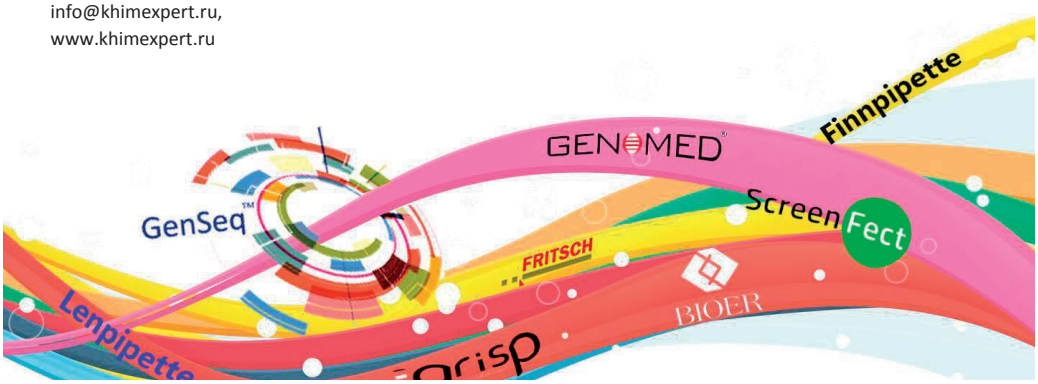

www.genexplain.com

The geneXplain GmbH is glad to welcome you at the BGRS/SB'2018 conference and is proud to introduce you the following software and database solutions for the needs of bioinformatics, systems biology and systems medicine:

geneXplain platform – is a high-performance tool for multi-omics data analysis, which allows identification of new therapeutic targets and biomarkers. A unique feature of the geneXplain platform is its Upstream Analysis. You can register and immediately receive access to a free account.

TRANSFAC database – is a unique collection of transcription factors, their experimentally validated binding sites (TFBS) and a widely known library of positional weight matrices (PWMs). The database has its own integrated methods for TFBS search. It can also be used as an integral part of the geneXplain platform. TRANSFAC is available online or can be downloaded as a set of flat files.

TRANSPATH database – is one of the biggest and most famous collections of signaling and metabolic pathways, which counts over 489000 reactions. The database can be applied for master-regulators search within the geneXplain platform. TRANSPATH is also available online in one package with HumanPSD database or can be downloaded as a set of flat files.

HumanPSD database – is a collection of genes, proteins and micro-RNAs, which includes information about disease biomarkers and clinical trials for various diseases. Besides the detailed biomarkers data, the database contains information about drugs.

BRENDA database – is a comprehensive enzyme and enzyme-ligand information system. Its manually derived core contains over 3 million data points about 77,000 enzymes annotated from 135,000 literature references.

PASS – is a software tool for evaluating the general biological potential of organic compounds based on their structural formula. This program predicts main and side pharmacological effects, molecular mechanisms of action, specific toxicities, and antitargets, actions associated with the metabolism and transport of pharmaceutical substances and their influence on gene expression.

PharmaExpert – is a software tool for analysis of the biological activity spectra of substances predicted by PASS and selecting compounds with the desirable set of biological activity, for analyzing the relationships between biological activities, drug-drug interactions and for multiple targeting of chemical compounds.

GUSAR – is a software tool for analysis of quantitative structure-activity/structure-property relationships (QSAR/QSPR) based on the structural formulas of the compounds and data on their activity/property, and for prediction of activity/property for new compounds. GUSAR can be easily applied to different routine QSAR/QSPR tasks, for building multiple models, and for prediction of the different quantitative values simultaneously.

If you got interested in any of the products, provided by GeneXplain, or you have any questions, please contact us by e-mail info@genexplain.com. We will be glad to help you!

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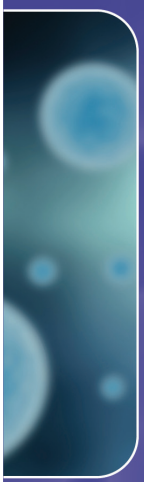
In Silico Biology

Journal of Biological Systems Modeling and Multi-Scale Simulation



```
function [ t, x ] = firstReactionMethod( ...
    stoich_matrix, propensity_fcn, tspan, x0,...
    rate_params, output_fcn, max_out)

if ~exist('rate_params', 'var')
    rate_params = [];
end

num_rxns = size(stoich_matrix, 1);
num_species = size(stoich_matrix, 2);

%Simulation loop
while t(rxnCount) <= max(span)
    % Step 1: calculate propensities
    a  = propensity_fcn(X(rxnCount,:), rate_params);
    % Step 2: identify the reaction that will occur
    r = rand(1,num_rxns);
    taus = -log(r)./a;
    [tau, mu] = min(taus);
    % Update time and execute reaction mu
    rxnCount = rxnCount + 1;
    T(rxnCount)    = T(rxnCount-1)    + tau;
    X(rxnCount,:) = X(rxnCount-1,:) + stoich_matrix(mu,:);

    if rxnCount > max_out
        warning('SSA:ExceededCapacity','');
        return;
    end
end

% Simulation completed
t = T(1:rxnCount-1);
x = X(1:rxnCount-1,:);
end
```

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AND STRUCTURE\SYSTEMS BIOLOGY

The Eleventh International Conference, BGRS\SB-2018

SYSTEMS BIOLOGY
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Symposium

Abstracts

Printed without editing

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ГЕНОМА\СИСТЕМНАЯ БИОЛОГИЯ

Одиннадцатая международная конференция

СИСТЕМНАЯ БИОЛОГИЯ
И БИОМЕДИЦИНА (SBioMed-2018)

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