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НИИКЭЛ

Федеральное государственное бюджетное учреждение «Научно-исследовательский институт клинической и экспериментальной лимфологии»

Научно-исследовательский институт клинической и экспериментальной лимфологии (НИИКЭЛ) образован в 1991 году. Организатор и первый директор Института – академик РАН Бородин Юрий Иванович (в настоящее время главный научный сотрудник), с 2004 года директор – академик РАН Коненков Владимир Иосифович (в настоящее время - научный руководитель организации), с 2015 года обязанности директора исполняет профессор Летягин Андрей Юрьевич.

Основной целью исследований Института является получение и использование новой научной информации для реабилитации поврежденных структур организма, для создания новых высокоэффективных индивидуализированных технологий восстановления нарушенных функций лимфатической системы в комплексном лечении социально значимых патологий.

Поставленные задачи выполняются сотрудниками 9 научно-исследовательских лабораторий и клиники НИИКЭЛ. Общая численность сотрудников НИИКЭЛ – 214 человек, из них 2 действительных члена РАН, 1 профессор РАН, 16 докторов наук, 23 кандидата наук, 9 профессоров; 63,2% врачей высшей и первой категории; 35% среднего медицинского персонала имеют высшую и первую категории.

Клиника НИИКЭЛ на 115 коек оснащена современным высокотехнологичным оборудованием. Отделения эндокринологии, ревматологии, хирургии, гинекологии, анестезиологии и реанимации клиники являются базой для разработки и внедрения новых эффективных технологий профилактики, диагностики, лечения и реабилитации больных с патологическими изменениями лимфатической системы. Пациентам из различных регионов РФ оказывается специализированная, в том числе высокотехнологичная медицинская помощь по направлениям: «эндокринология», «ревматология», «сердечно-сосудистая хирургия», «травматология и ортопедия», «акушерство и гинекология». НИИКЭЛ включен в реестр организаций, работающих в системе ОМС. В клинике постоянно действует бесплатная школа для пациентов, страдающих сахарным диабетом и остеопорозом. **В 2015 году в клинике НИИКЭЛ было пролечено более 4,5 тыс. человек.**

Специалисты Института осуществляют экспертную работу в РАН, Минздраве России, ФАНО России, Российском научном фонде, Правительстве Новосибирской области. В проводимых исследованиях НИИКЭЛ активно сотрудничает со многими российскими и зарубежными научными центрами, университетами, компаниями, лечебными учреждениями. Сотрудники Института принимают участие в работе Международного общества лимфологов, Европейской федерации иммуногенетики, Европейской антиревматической лиги (EULAR), Европейской ассоциации по изучению сахарного диабета (EASD), Американской диабетической ассоциации (ADA), Европейской академии естественных наук и других научных сообществ. В НИИКЭЛ постоянно организуются и проводятся международные научные форумы по проблемам лимфологии, клеточной биологии, эндокринологии, ревматологии, эндоэкологии.

Ведущие и молодые ученые института получали премии Правительства РФ в области науки и техники, Президиума РАМН и Федерального ФОМС, премии им. Пирогова РАМН, удостоены Государственной научной стипендии для ведущих ученых России и для талантливых молодых ученых, неоднократно получали гранты и стипендии от Регионального общественного фонда содействия отечественной медицине, РФФИ, ГНТП «Национальные приоритеты в медицине и здравоохранении», Министерства образования и науки РФ, Российского научного фонда, администрации Новосибирской области, мэрии г. Новосибирска. Разработки НИИКЭЛ оценены 15 золотыми медалями Сибирской ярмарки, дипломами Межрегиональной ассоциации «Здравоохранение Сибири», дипломом «Лидер инноваций в академической науке».

Институтом получено 87 патентов на изобретения, вышло в свет более 5 тысяч публикаций, из них 112 монографий, 84 методических и учебных издания, 60 сборников научных трудов, около 2 000 статей в отечественных и зарубежных журналах. В рецензируемых журналах опубликовано 94,2% научных статей.

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CONTENTS

HEREDITARY SPASTIC PARAPLEGIAS IN SUDAN: RELATIVE FREQUENCIES ACCORDING TO THE MUTATED GENE AND IDENTIFICATION OF THE SECOND *SPG57* MUTATION AFFECTING TFG OLIGOMERIZATION

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Motivation and Aim: To estimate the relative frequencies of known HSP genes in Sudanese families with the disease and perform genotype-phenotype correlation to extend the clinical spectrum associated with HSP genes.

Methods and Algorithms: We used next generation sequencing to screen 74 HSP-related genes in 23 consanguineous families from Sudan and candidate gene sequencing in two other families (total of 25 families).

Results: We established a genetic diagnosis in six families with autosomal recessive HSP (*SPG11* in three families and *TFG*/*SPG57*, *SACS*, and *ALS2* in one family each). An autosomal dominant HSP (*ATL1*/*SPG3A*) was also identified in one additional family. Six out of seven identified variants were novel. The *TFG/SPG57* variant (p.Arg22Trp in the PB1 domain) is the second SPG57 HSP variant to be identified worldwide, and we demonstrated its impact on TFG oligomerization *in vitro*. Patients did not present with visual impairment as observed in a previously reported SPG57 family (p.Arg106Cys in coiled coil domain), suggesting unique contributions of the PB1 and coiled coil domains in TFG complex formation/function and a possible phenotype correlation to variant location. Some families manifested marked phenotypic variations implying the possibility of modifier factors complicated by high inbreeding.

Conclusion: We identified the first Sudanese families carrying novel variants in 6 HSP genes. The difficulty to reach a genetic diagnosis in the majority of studied families suggests the possibility of new genes, unusual models of inheritance or noncoding variations underlying spinocerebellar degeneration.

Acknowledgments: We thank the families for their contribution to this study and members of the DNA and cell bank of the ICM for their help as well as Federica Barreca, Emeline Mundwiller, Khalid El Hachimi and Delphine Bouteiller for their valuable contribution.

THE ROLE OF Q/N-RICH REGIONS IN THE INDUCTION OF AMYLOIDOGENESIS

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Key words: *amyloid, prion, yeast, Sup35, PSIA*

Motivation and aims: An important feature of many amyloid-forming proteins supposed to be crucial for their aggregation, is presence of compositionally biased regions, particularly glutamine (Q) and/or asparagine (N)-rich subsequences. Those regions are considered to play role not only in the formation of amyloids, but also in the interaction of different amyloids. So, overproduction of polyQ-peptide (103Q) in yeast induces aggregation of several preferably Q-rich proteins [1]. Therefore, the aim of the given research is to discover, if there exists a direct correlation between the composition of amyloid protein and the composition of proteins, which co-aggregate with amyloid.

Methods: To perform a search for candidates for novel amyloids, we used proteomic method, PSIA (Proteomic Screening and Identification of Amyloids) [1], recently developed by us, with some modifications including high-performance liquid chromatography for separation of tryptic peptides [2].

Results: We used two variants of prion-forming domain (PrD) of yeast protein Sup35 – the wild-type variant, which was Q-rich, and the variant with all Qs substituted with Ns, which was N-rich. As a result, the overproduction of wild-type Sup35 PrD induces formation of detergent-resistant aggregates of 4 proteins, while the N-substituted variant induces aggregation of 11 proteins, and only 2 proteins of this set are overlapped. The most important is that only 2 of all 13 found proteins are Q/N-rich.

Conclusion: Though the presence of compositionally different amyloids in the cell induces aggregation of dissimilar sets of proteins, there is no strong correlation between the composition of the corresponding amyloid-forming protein and the composition of proteins, whose aggregation is induced by him.

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STUDY OF OSTEOGENIC CELLS MOTILITY FOR TISSUE ENGINEERING PROTOCOLS DESIGN

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Key words: *3D tissue-scaffolds, osteogenesis, cells motility.*

Motivation and Aim: Tissue engineering for bone implant processing is of great importance for practice medicine. Basic properties required for 3D tissue-scaffols are: the ability to mimic the structure and biological functions, provide mechanical support, differentiation and proliferation of osteogenic cells. Eventually an implantation osteoconductive scaffolds seeded with the patient's autologous cells in vitro to accelerate the process of bone regeneration in defect sate in vivo. Main problem is unsuitable physico-chemical properties of the material for bone substitution. Despite that tissue engineering of bone implants is of

great importance, there is no protocol for this procedure. To develop such protocol for certain scaffold, the data about cell behaviour on the scaffold are needed. Especially, statistical parameters of cell motility are basic for modeling of the process and prediction of cell propagation into scaffolds.

Methods and Algorithms: Osteogenic cells were obtained from fragments of trabeculae tissue of the femoral head bones of the experimental mini-pig. Cells were cultured in RPMI and RPMI GlutoMAX media supplemented with 10% fetal bovine serum and maintained under standard conditions: 37˚C, 5% CO^2. Phase images were captured by automated cell culture and analysis system (Cell-IQ, CM Technologies). Imaging settings: objective – Nikon CFI Plan Fluorescence DL 10X, exposure time – 3 ms, Z-stack 20μM, cycle interval – 9 min, 12 positions per well of 6 well plate. After image contrasting, cell tracking was performed by hands through the images using time-lapse imaging Fiji program. Functions for cell motility evaluation were developed in Mathematica 10. Statistics for directed runs and angles of turns were calculated as well as cell motility coefficients for chondroblasts on cultural well surfaces with different physico-chemical parameters.

Results: Protocols for cell extraction from bone specimens were designed, and cell motility was evaluated. Coefficient of chondroblast motility was found larger on plastic surface than on gelatin, which is in consistent with published data.

Conclusion: The designed experiments and obtained data will allow developing a clinical protocol for settlement implants with osteogenic cells for bone tissue regeneration.

The work was supported by RFBR grant 15-29-04875.

ASSOCIATION OF RS505151 IN PCSK9 GENE WITH LIPID PROFILE IN RUSSIAN POPULATION

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Key words: *familial hypercholesterolemia, proprotein convertase subtilisin/kexin 9 gene, population*

Motivation and Aim: mutations in LDLR, APOB, PCSK9 genes determine the development of autosomal dominant forms of familial hypercholesterolemia. The PCSK9 gene encodes an enzyme involved in the metabolism of low density lipoprotein (LDL) by post-transcriptional regulation of the LDL receptors [1, 2, 3]. Purpose: to perform analysis of PCSK9 rs505151 in Russian population and the population sub-samples of persons with hypercholesterolemia.

Methods: genotyping rs505151 in the PCSK9 was carried out in a population and in the subgroup with hypercholesterolemia (total cholesterol level >300 mg/dl). Subgroups were included in the analyses in the HAPIEE project framework (9360 participants, 45-69 years old, 50% men). Blood lipid levels were determined using standard enzymatic assays. Genotyping of the PCSK9 rs505151 was performed using PCR-RFLP and then confirmed by direct sequencing.

Results: frequencies of AA, AG and GG genotypes were 89%, 11% and 0% in population. Frequencies of AA, AG and GG genotypes were 86%, 14% and 0% in the subgroup with hypercholesterolemia. The frequency of G allele was 5% in population and 7% in the subgroup with hypercholesterolemia ($p>0.05$). Analysis of rs505151 association with lipid profile and PCSK9 protein blood levels showed that this polymorphism does not significantly contribute to forming hypercholesterolemia in Caucasian populations of Western Siberia.

Conclusion: the PCSK9 rs505151 alleles and genotypes frequency in the population and in the population subgroup with hypercholesterolemia were determined for the first time in Russia. The Caucasian population of West Siberia does not significantly differ from populations of Europe by alleles and genotypes frequencies. The PCSK9 rs505151 SNP was not associated with serum lipid levels in Caucasian population of West Siberia.

Acknowledgements: reported study was supported by RFBR, research project №14-04-01594.

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GENOME-WIDE ASSOCIATION STUDIES OF COMPLEX HUMAN TRAITS: HISTORY AND PERSPECTIVES

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Key words: *genome-wide association studies, human complex traits, genetic analysis, genomics, functional genomics*

During the last decade, genome-wide association studies (GWAS) have transformed our understanding of genetic control of complex human traits. While in the beginning of 2000s only a handful of polymorphisms were known to be robustly associated with complex human traits, nowadays we know thousands of such loci [1]. To mention just a few examples, the number of loci identified for type 2 diabetes is now more than 60 [2], for breast cancer – almost 80 [3], and for height – close to 700 [4]. The progress in GWAS continues and increasing availability of large cohorts allows for identification of alleles with progressively smaller effects.

However, the large amount of results obtained in GWAS did not yet fully translate into new biological knowledge, in large part because the output of GWAS (a 'locus') does not allow directly and unequivocally implicate the biological function and mechanism through which genetic variation leads to phenotypic variation. Developments in high-throughput molecular biologic technologies together with initiatives, which establish collections of functional genomics resources, open an opportunity to generate targeted hypotheses concerning biological mechanisms underlying genetic associations detected by GWAS. These hypotheses could further be addressed by functional studies, ultimately leading to better understanding of processes underlying biological control of human complex traits.

In this talk, I will make a historical overview of genome-wide association studies and discuss latest trends and possible future directions in disentangling complex human traits via use of genetic and genomic approaches.

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EXPERIMENTAL MODEL OF OPISTHORCHIASIS AS A TOOL FOR BIOMEDICAL RESEARCHES

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Key words: *O. felineus, hamsters, mice, model of opisthorchiasis*

Motivation and Aims: Opisthorchiasis is widespread in Russia, especially in the Ob-Irtysh region. Disturbances in the hepatobiliary system under the opisthorchiasis conditions promote to the development of various liver pathologies, up to cholangiocarcinoma, and destabilize the work of other organs and body systems. Obviously, we have to apply a system approach to the study of pathological processes caused by opisthorchiasis. It is possible with use of experimental models.

Methods and Algorithms: Inbred C57BL/6 mice and Syrian hamsters (*Mesocricetus auratus*) were taken for the modeling of the experimental opisthorchiasis. After the introduction of *Opisthorchis felineus* (*O. felineus*) metacercariae to animals, we investigated the consequences of infection at the acute (in a 2-4 weeks) and chronic (in a 6 months) stages of opisthorchiasis. In the separate series of experiments in mice the *O. felineus* invasion was combined with the chronic social stress (20-30 days).

Results: It was found that infected hamsters consume more food and have an increased body weight in a month after *O. felineus* invasion, indicating the metabolic disturbances. Acute and chronic stages of opisthorchiasis in mice were accompanied by elevated alanine aminotransferase activity in blood, which is the main parameter of liver damage. The changes in the blood cells composition were found at the acute stage of opisthorchiasis, which was accompanied by activation of hematopoietic stem cells of myeloid and erythroid set. It was more expressed in hamsters than in mice. Increased eosinophilia and the reduced formation of hematopoietic stem cells were at the chronic stage of opisthorchiasis. No significant differences in blood corticosterone level were between control and infected mice after 2 and 4 weeks of *O. felineus* invasion, however this parameter increased significantly under the combination of infection with social stress. Furthermore, the interconnection of two factors significantly increased the blood levels of proinflammatory cytokine interleukin-6. Simultaneous action of both factors caused the increase of spleen cathepsins B and L activity. The major effect on activity of the cysteine proteases in hippocampus and hypothalamus had *O. felineus* invasions, which was a predominant factor in the simultaneous action of the two.

Conclusion: Thus, *O. felineus*-conditioned opisthorchiasis causes changes not only in the liver but also in the organs and systems distant from the direct location of parasite in the host organism. The proposed models allow of conducting the complex biomedical and pharmacological researches in the dynamics of pathological changes caused by opisthorchiasis.

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THE STUDY OF REGULATORY SNPS OF THE GENES SHARED BY ASTHMA AND TUBERCULOSIS

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Key words: *genetic predisposition, inversely comorbid, bronchial asthma, tuberculosis*

Motivation and Aim: Bronchial asthma (BA) and tuberculosis (TB) are dystropic (inversely comorbid) diseases, and their combination is a rare event. Therefore, BA and TB are interesting model systems for researching of the molecular causes of a dystropy. The research of genetic reasons of inversely comorbid diseases is an important for the identification of genetic markers of susceptibility and the search of new drug targets.

Methods and Algorithms: We carried out an association analysis of 16 SNPs in the promoter regions of candidate genes shared by BA and TB (*IL8*, *IL10*, *TNF*, *HLA-DRB1*, *IL1B*, *IL6*, *CXCL10*, *SLC11A1*, *TNFRSF1B*, *CD4*) using a total of 314 patients with TB, 121 with asthma, and 280 healthy controls. Statistical analysis was carried out using $\chi 2$, Odd's ratio (OR), and Bayes factor (BF); p-value $p \leq 0.05$ was considered significant.

Results: There were no statistically significant differences between BA and TB patients. However, we found statistically significant differences in the frequencies of both alleles and genotypes of the rs1800872 (*IL10*) and the rs2239704 (*TNF*) between control and TB ($p = 0.008$, $p = 0.024$ for *IL10* alleles and genotypes, respectively; and $p = 0.002$, $p = 0.011$ for *TNF*) and BA ($p = 0.023$, $p = 0.045$ for *IL10* alleles and genotypes, respectively; and $p = 0.084$ for *TNF* alleles). For the frequencies of genotypes significant differences from the control group were registered for BA for the rs1800629 (*TNF*) ($p = 0.036$) and for TB for the rs525891 (*TNFRSF1B*) ($p = 0.033$) and for the rs2069832 (*IL6*) ($p = 0.022$). Allele A of the rs1800872 (*IL10*) was associated with the increased risk of the development of BA (OR = 1.62) and TB (OR = 1.6), while CC genotype was protective against TB (OR = 0.59). The risk effect regarding TB for the C allele of the rs2239704 (*TNF*) (OR = 1.76) was confirmed by Bayesian statistics (BF = 12.2), and is incarnated primarily through the CC genotype (OR=1.83), while the AA genotype is protective (OR = 0.46; BF = 5.2). Also the TA genotype of the rs525891 (*TNFRSF1B*) (OR = 0.62) and AA genotype of the rs2069832 (*IL6*) (OR = 0.38; BF = 4.3) were protective against TB.

Conclusion: The results show the overlap between the loci of the susceptibility to BA and TB, thus confirming the idea that the development of these diseases is governed by shared molecular mechanisms.

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AN ASSOCIATION BETWEEN COMMON DISEASES AND THE POLYMORPHISMS OF THE CANDIDATE GENES FOR THE DISEASES OF THE CARDIOVASCULAR SYSTEM AND INFECTIOUS AND ALLERGIC DISEASE

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Key words: *common diseases, candidate genes, genetics polymorphism*

Motivation and Aim: Use of an integrated approach taking into account both possible the pleiotropic effects of genes, and phenotypical relationships at normal and pathological conditions is important for understanding of the genetic mechanisms underlying the formation of common diseases. The aim of this study was to determine pleiotropic and syntropic effects of candidate genes in the onset and development of common diseases on the example of residents of Tomsk.

Methods and Algorithms: We carried out the analysis of associations between coronary heart disease (CHD), bronchial asthma (BA), and tuberculosis (TB), from one hand, and the candidate genes for cardiovascular continuum disorders, infectious and allergic diseases, from the other hand. Overall, 24 polymorphisms in 17 genes were studied. Statistical analysis was carried out using $\chi 2$, Odd's ratio (OR), and Bayes factor (BF); p-value $p \leq 0.05$ was considered significant.

Results: Seven genes were found to be associated with the diseases; in some cases the risk effect was confirmed by Bayesian statistics. The following genes were found to be associated with CHD only: *ACE* (rs4291, OR=5.78, p=0.002, BF=57.13; rs4291/rs4343 haplotype, OR=5.94, p=0.002, BF=54.48); *GNB3* (rs5443, OR=0.55, p=0.039); and *TNFRSF1B* (rs1061622, OR=6.63, p=0.003, BF=56.94). The *IL12A* gene was found to be associated with BA alone (rs568408, OR=2.03, p=0.015). The *LTA* gene (rs909253) was associated with BA only if tested alone (OR=2.16, p=0.007, BF=5.8) and in combination with the *TNF* gene (rs909253/rs1800629) it was associated with all studied diseases (OR=2.37, p=0.015, BF=3.45 for CHD; OR=2.06, p=0.045 for BA, and OR=2.23, p=0.018 or OR=0.57, p=0.034 for TB).

A wide number of associations was observed for *NOS3* gene as it was associated with CHD (rs2070744/VNTR, OR=0.34, p=0.04), TB (rs1799983, OR=0.55, p=0.024; VNTR/rs1799983, OR=0.57, p=0.047) and BA (rs1799983, OR=1.97, p=0.02; rs2070744/rs1799983, OR=2.95, p=0.006, BF=9.25). The *IL12RB1* gene (rs3746190/rs11575926) was associated with CHD (OR=0.11, p=0.007, BF=13.23) and TB (OR=0.12, p=0.002, BF=19.71). Some loci were shown to explain a significant proportion of phenotypic variance of several quantitative endophenotypes: 14.19% for total cholesterol in CHD (*TNFRSF1B*); 9.11% for erythrocyte sedimentation rate in TB (*LTA*); 10.68% for LDL in CHD (*NOS3*); 2.98% to 6,55% for the blood counts in TB (*NOS3*).

Conclusion: The results show wide involvements of the studied genes into determination of both, pathological conditions and endophenotypes of the studied common diseases.

THE USE OF DISCIMINANT ANALYSIS AND ARTIFICIAL NEURONAL NETWORK IN BREAST CANCER DETECTION

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Key words: *artificial neuronal networks, discriminant analysis, diagnosis, breast cancer*

Motivation and Aim: Tumorigenesis is accompanied with the changes in different systems of the organism. There are many factors involved that affect each other. The combined effect from two factors often exceeds the sum of the effects therefore increasing the individual effect. Important requirement of tumor development is ability of cancer cells to cause death of the lymphocytes. Therefore, in establishing the algorithm of the diagnostics of the breast cancer, we used several parameters approach – specifically, activity of caspases and the ratio of lymphocytes subsets.

Methods and Algorithms: 108 peripheral blood samples were obtained from the Oncologic clinic of Republic of Karelia. 15 of them were collected from patients with breast benign disease, 20 – stage I of breast cancer, 30 – stage II breast cancer, 43 – stage III of breast cancer. As control samples, group of 30 healthy controls was similarly studied to establish normal ranges and means. Caspase-3, -6, -8, -9 activity assay was carried out in peripheral blood lymphocytes. The ratio of T-cell subsets such as CD3, CD4, CD8, CD16, CD20, CD25 и CD95 was estimated. Statistical analysis was performed using Statgraphics Plus 5.0 software. Artificial neural network (multilayer perceptron) was developed with a 3-layer design, with one hidden layer.

Results: The 11 criteria such as activity of caspase-3, -6, -8, -9 and rate of CD3, CD4, CD8, CD16, CD20, CD25 and CD95 T-cell subsets in peripheral blood were tested as a diagnostic biomarkers. Based on discriminant analysis for 138 cases, it was found that the best separation into groups should use all 11 biomarkers. Discriminant analysis allows for a correct attributing to the group 98.9% of patients. The second approach in classifying the cases was based on the artificial neural network which included the 11 neurons on the input layer, the 5 neurons on the output layer and 6 on the single hidden layer. The network was tested on 138 observations. All of them were correctly classified and error rate was zero. Based on both discriminant analysis and neural networks using free R-statistics, we developed software that automatically with certain probability (% - discriminant analysis) and in a binary system “yes / no” (1/0) differentiates the blood samples into groups of benign breast disease, stage I, stage II or stage III of breast cancer and group without pathology.

Conclusion: Using discriminant analysis and neural network allows the development of high-precision computational tool in noninvasive differential diagnosis of breast pathologies based on peripheral blood biomarkers. Some of them – T-cells – already widely used in laboratory and clinical practice, other one – the caspase activity – does not require laborious methods of analysis and significant costs, making them available for routine determination.

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ANTIBODIES-PROTEASES AS A NEW MARKER FOR DIAGNOSTICS OF HIV INFECTION

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Key words: *human blood antibodies; HIV infected patients; catalytic IgGs (abzymes); hydrolysis of human histones*

Motivation and Aim: An important problem of biochemistry and molecular medicine is the search of new markers of viral and autoimmune diseases. Now methods of enzyme immunoassay and PCR are used for the diagnostics of HIV infection. These approaches make it possible to establish the presence or absence of disease, but you can't use them to predict the dynamics of the disease (the transition from one stage to another) and its outcome. Therefore, the development of diagnostics new methods of the patient status with HIV infection, prediction of disease progression and determination of the effective therapy are important fundamental tasks. Histones and their post-translational modifications play a key role in chromatin remodeling and gene transcription. Besides intranuclear functions, histones act as damage-associated molecular pattern molecules when they are released into the extracellular space

Materials and methods: Equimolar mixture of homogeneous five human histones (H1, H2a, H2b, H3, H4) was from Sigma. The homogeneous IgGs were isolated electrophoretically and immunologically from sera of HIV-infected patients by chromatography on several affinity sorbents. Sera of 10 healthy volunteers and 32 HIV-infected patients (18–40 year old; men and women) including 13 at the stage of pre-AIDS and 19 at the stage of generalized lymphadenopathy according to the classification of the Center of Disease Control and Prevention were used to study proteolytic abzymes.

Results: It was shown that sera of HIV-infected patients and healthy donors contain autoantibodies against histones using ELISA. We have shown for the first time that 100 % of IgGs purified from the sera of 32 HIV-infected patients efficiently hydrolyze from one to five human histones. Several strict criteria check revealed that hydrolysis of histones was an intrinsic property of immunoglobulins. The relative efficiency of histones hydrolysis significantly varied for IgGs of different patients. IgGs from the sera of 40 % of healthy donors also hydrolyze histones but with the average efficiency approximately 16-fold lower than that of HIV-infected patients. Similar to proteolytic abzymes histones-hydrolyzing IgGs from HIV-infected patients were inhibited by specific inhibitors of serine and metal-dependent proteases as so specific inhibitor of thiol-like proteases.

Conclusion: According to our preliminary data, the titer of protease antibodies and the level of its activities (hydrolyzing myelin basic protein and histones) depend on the stage and characteristics of HIV infection. Detection of abzyme activity may serve as an additional criterion in assessing the rate of progression of HIV infection. A strict substrate specificity of antibodies of HIV-infected patients and the relative ease of their detection methods can afford to develop additional methods of differential diagnosis of HIV infection.

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PROGNOSTIC SIGNIFICANCE OF TUMOR-ASSOCIATED LYMPHANGIOGENESIS IN LOWER LIP SQUAMOUS CELL CARCINOMA

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Key words: *lower lip squamous cell carcinoma, lymphatic vessels, LYVE-1, Podoplanin*

Motivation and Aim: Lower lip squamous cell carcinoma (LLSCC) is a common oral malignancy, and it is responsible for over 30% of all oral squamous cell carcinomas, but in patients with LLSCC, occurrence of lymph node metastasis is more frequent than in other cutaneous head and neck SCCs. It is known that LLSCC without keratinization has a great metastatic potential than LLSCC with keratinization. Thus, identification of biological markers that could provide prognostic information about the invasive or metastatic potential of these lesions is important. It is known that lymphangiogenesis play an important role in tumor progression. Therefore, the goal of this study was to analyze the immunohistochemical expression of lymphatic markers LYVE-1 and Podoplanin in hyperkeratosis and in LLSCC.

Methods and Algorithms: Seventy-one cases of the lower lip lesions, obtained from the files Novosibirsk Regional Oncology Center were selected for this study. The specimens were divided into three groups: a lower lip hyperkeratosis group consisting of 22 cases; LLSCC with keratinization consisting of 34 cases and LLSCC without keratinization consisting of 15 cases. To analyze lymphangiogenesis we performed immunostains of the lower lip biopsy material for the lymphatic-specific markers LYVE-1 and Podoplanin. Samples of tissue were fixed in 10% neutral formalin, processed by standard histological techniques and embedded in paraffin. All steps of the immunohistochemical reaction were performed by using BENCHMARK / XT slide stainer (Ventana). The lymphatic vessels density were analyzed morphometrically in all groups and compared by the non-parametric Mann-Whitney test and the Wilcoxon signed rank test. A level of significance of 5% ($p<0.05$) was adopted for all tests.

Results: All cases of the lower lip hyperkeratosis and LLSCC were positive for LYVE-1 and Podoplanin. With respect to the pattern of staining, specimens exhibited a predominantly peripheral staining for LYVE-1 and Podoplanin in inflammatory infiltrates and tumor sites. The greatest maintenance of Podoplanin+ lymphatic vessels, in comparison with LYVE-1 +-vessels was revealed. Comparison of the volume density of lymphatic vessels showed that Podoplanin + - lymphatic vessels volume density in hyperkeratosis was by 50% less than in LLSCC without keratinization and 24% smaller than in LLSCC with keratinization. Whereas in LLSCC without keratinization the lymphatic vessels volume density was 51% higher than in the LLSCC with keratinization.

Conclusion: This study has shown the greater development of lymphatic vessels in LLSCC without keratinization in comparison with hyperkeratosis and LLSCC with keratinization, thus contributing to the development of metastasis.

MICRO RNA EXPRESSION IN MATERNAL AND FETAL TISSUES IN NORMAL AND COMPLICATED HUMAN PREGNANCY IN THE FIRST TRIMESTER

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Key words: *micro RNA, gene expression, pregnancy, chorion, embryo, decidua, early pregnancy loss*

Motivation and aim: Coordinated work of gene cascades in maternal and fetal cells is crucial for motherplacenta-fetus system formation and functioning. Reasons for up to 60% of spontaneous abortions remain unknown. Micro RNAs act as a post-transcriptional gene regulators, and affect gene expression directly in the cells of their origin or take part in intercellular signaling through vesicular transport. In current work we investigated the expression of three micro RNAs in embryonic, chorionic and decidual tissues after spontaneous abortion compared to normal first trimester pregnancies.

Methods and Algorithms: MiRTarBase search using Perl script was conducted and four mature micro RNAs were chosen for further investigation. Samples of chorionic, embryonic tissue and decidua were taken after surgical termination of normally progressing pregnancies (n = 14) and spontaneous abortion (n = 10) in 5-9 week of gestation. Total RNA was extracted by the acid guanidinium thiocyanate phenol method. The quantity of RNA was assessed spectrophotometrically. Micro RNA and gene expression was analyzed using real-time PCR method. MiR-92a-1-5p was used as a reference gene. All experiments were conducted in triplicates. Data were analyzed using the ΔCT method and Spearman correlation analysis.

Results: We found four micro RNA which are putative predicted multigene regulators in group of metalloproteinases and cytokine genes. Hsa-miR-335-5p, hsa-miR-203a, hsa-miR-204-5p and hsa-miR-338 were chosen for real-time PCR expression study. The expression of all four micro RNAs investigated was equal in chorionic, embryonic and decidual tissues and there were no differences found between normal pregnancy and early pregnancy loss.

Conclusion: The results demonstrated that miR-203a-3p, miR-335-5p, miR-204-5p and miR-338 expression level was equal in chorionic, decidual and embryonic tissues and in spontaneous abortion compared to normal gestation.

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POLYURETHANE-BASED FIBER MATERIALS FOR TISSUE ENGINEERING: FABRICATION AND GENERAL PROPERTIES

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Key words: *electrospinning, polyurethane, tissue engineering*

Motivation and Aim: Polyurethanes (PUs) have good stiffness and elasticity as well as biocompatibility and are widely used in medicine. At the same time low biological stability of most PUs and high propensity of electrospun PU matrices shrink the limits of their engineering application.

Methods and Algorithms: Matrices were produced from 3-7% PU (Tecoflex EG-80A or Pellethane 2363-80A, Lubrizol Co, USA) blended with 5÷20% gelatin (protein:PU, wt: wt %) in hexafluoro-2-propanol using the NF-103 electrospinning system (MECC, Japan). Matrix structure was studied with the JSM-6460 LV (Jeol, Japan) scanning electron microscope. Dynamic stress load of 3D matrices was investigated using Zwick/Roell Z100 testing machine. Primary human fibroblasts (HF) and human umbilical vein endotheliocytes (HUVEC) were seeded in culture dishes and at the surface of 3D matrices, cell viability and morphology were studied using Almar Blue kit and fluorescence microscopy with Cell Tracker Orange and SYBR Green I.

Results: All studied 3D matrices consisted of 1.12±0.44÷1.39±0.6 μm fibers with pore size of 3.6±1.3÷5.8±2.7 μm. Introduction of the protein in PU matrices decreased the shrinkage of 3D matrices up to 10% depending from protein content and increased the stiffness of the material. Tensile strength varied from 10.8 ± 0.3 MPa to 15.6 ± 1.8 MPa and from 8.1±1.2 MPa to 8.4±0.5 MPa for Tecoflex-gelatin matrices and Pellethane-gelatin matrices, respectively. Scanning electron microscopy demonstrated that incubation of matrices in PBS at 37° C for 30 days induced changes in the structure of the fibers, but had no effect on the tensile strength of the matrices.

Cell culture studies with HF and HUVEC showed that 40 to 75% of cells are retained on PU-based 3D matrices depending on the cell type and composition of the fibers. Fluorescence microscopy did not demonstrate any alterations of morphology of cells grown on 3D matrices as compared with cells on the culture plastic.

Conclusion: The data demonstrate high potential of PU-based protein enriched fibrous 3D matrices for manufacturing of small diameter vascular prostheses, patches or valves and other implants that require high strength and elasticity of the material.

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INTRATUMOR MORPHOLOGICAL HETEROGENEITY IN BREAST CANCER: TOWARDS PERSONALIZED PROGNOSIS AND THERAPY

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Keywords: *breast cancer, tumor heterogeneity, prognosis, therapy*

Motivation and Aim: Although intratumor heterogeneity represents a main obstacle towards successful cancer prognosis and treatment, it may be a powerful model to develop new methods of personalized cancer care. In this study, we focused on the study of intratumor morphological heterogeneity in breast cancer and its role in cancer prognosis and therapy efficiency.

Methods and Algorithms: This study involved 434 patients underwent neoadjuvant chemotherapy (NAC) ($T_{1-4}N_{0-3}M_{0-1}$) and 249 naïve ($T_{1-4}N_{0-3}M_{0-1}$) patients with breast cancer. The association of different morphological structures with metastasis and therapy efficiency was assessed by Kaplan-Meier analysis and Chi-square test. Confocal microscopy was used for 3D imaging of structures and analysis of cancer stem cells (CSCs). Array CGH and gene expression microarrays were applied for whole genome and transcriptome profiling of laser-microdissected structures.

Results: Breast tumors with 3-5 types of structures demonstrated chemoresistance and increased metastasis than cases with 1-2 types of structures. Alveolar and trabecular structures were found to be associated with poor response to NAC, increased lymph node involvement, and decreased metastasis-free survival. Simultaneous calculation of alveolar and trabecular structures increased the prognostic value significantly. The association of these structures with hematogenic metastasis was observed only in NAC-treated patients. Alveolar structures correlated with high frequency of distant metastasis only in patients with poor response to NAC, whereas trabecular structures – in chemosensitive cases. 3D imaging showed that different structures represent specific architectural arrangements of tumor cells. Structures were found to be not associated with chromosome instability, but related to gene expression changes. Gene expression results, as well as analysis of CSCs, suggested that morphological structures reflect stages of epithelial-mesenchymal transition and are associated with stemness.

Conclusion: Intratumor morphological heterogeneity actually reflecting tumor invasiveness status may serve as a novel predictive and prognostic histological criterion in breast cancer.

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NEUROPEPTIDE RECEPTOR GENE POLYMORPHISMS AND SLEEP DISORDERS

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Objective: To study the association between candidate gene polymorphisms NPSR1 rs324981 and sleep disorders in the open population of men of 45-64 years of Novosibirsk.

Methods: The study of the association between candidate gene polymorphisms and sleep disorders was carried out during the examination of a random representative sample of men of 45-69 years (n = 1770). The response rate was 61%. The median age is 56.5 year. Every 12 subject was selected for genotyping (n = 147). The questionnaire filled by self-test was used to assess the level of sleep. Statistical analysis was performed using SPSS-11.5.

Results: The level of sleep disorders in the male population of 45-64 years was 79.9%. The frequency of homozygous C / C genotype of neuropeptide S (gene NPSR1 rs324981) was 19.4%, T / T genotype occurs in 27.8%, C / T genotype - 52.8%. The prevalence of T allele was 54.2%, and the C allele was 45.8% which were dominated in men. It was associated with growing trend in dissatisfaction of sleep quality in those men. Men T- allele carriers, most evaluated their sleep as “satisfactory” in 69% of cases, ($\chi 2 = 15{,}713$ df = 8, $p < 0.05$).

Conclusion: T-allele of neuropeptide S (gene NPSR1 rs324981) is associated with sleep disorders in men.

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POLYMORPHISM-G308A GENE TNF-A AND SLEEP DISTURBANCE IN OPEN MALE POPULATION AGED 25-64 YEARS IN SIBERIA (PROGRAM WHO "MONICA-PSYCHOSOCIAL")

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Purpose: to study the association between polymorphism- G308A gene tumor necrosis factor TNF-a and sleep disorders in the open population of men aged 25-64 in metropolis of Western Siberia (Novosibirsk) (epidemiological study WHO «MONICA-PSYCHOSOCIAL»).

Methods: This work is made using a material III screening as part of the WHO "MONICA-psychosocial" representative sample of men of 25-64 years of open population of Novosibirsk, made in 1994 (n = 657 men - 44, 3 ± 0,4 years, The response - 82.1%). The questionnaire filled by the subjects themselves was used to assess the level of sleep. Techniques were standardized and strictly complied with the requirements of the protocol of the "MONICA". Genotyping of the studied polymorphism-G308A gene tumor necrosis factor TNF-a was conducted in the laboratory of molecular genetic studies.

Results: The level of sleep disorders in the male population aged 25-64 was as follows: 48.3%: assessment of sleep "satisfactory" - 39.6%, "bad" - 7.6%, "very bad" - 1.1%. Genotype G / G gene TNF-α occurs in 79.1% of individuals, genotype A / G - in 19% of cases and genotype A / A in 1.9% of men. Estimation of sleep as «good» (98.3%) was more likely met in those carriers with genotype G / G TNF-α gene in comparison with other genotypes. In contrast, carriers of heterozygous genotype A / G gene TNF-α more likely had «satisfactory» (30%) and less likely «good» (15.2%) sleep compared to those with other genotypes.

Conclusions: The significant association between the level of sleep disorders and certain polymorphism- G308A gene tumor necrosis factor TNF-a was found.

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"LIQUID BIOPSY" DEVELOPMENT IN LUNG CANCER: CIRCULATING LINE-1 RETROTRANSPOSONS HYPOMETHYLATION STUDY

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Key words: *Lung cancer, Circulating DNA, DNA methylation, LINE-1, L1Hs, MIRA*

Motivation and Aim: Circulating DNA has recently gained attention as a fast and non-invasive way to assess tumor biomarkers. Since hypomethylation of LINE-1 repetitive elements was described as one of the key hallmarks of tumorigenesis, we aimed to establish whether the methylation level of LINE-1 retrotransposons changes in cell-surface-bound fraction of circulating DNA (csbDNA) of lung cancer patients.

Methods and Algorithms: LINE-1 specific products were amplified using csbDNA from lung cancer patients (n=3) and healthy controls (n=3). NEBNext DNA Library Prep Master Mix Set for Illumina (NEB, USA) was used to generate libraries from amplicons for deep sequencing. Libraries were further sequenced on Illumina HiSeq 2000 platform. Methylated CpG Island Recovery Assay (MIRA) coupled to qPCR-based quantitation was performed to assess integral methylation level of LINE-1 promoters in csbDNA of non-small cell of lung cancer patients before treatment (n=59) and healthy controls (n=47).

Results: Deep sequencing of amplicons revealed that hypomethylation of LINE-1 promoters in csbDNA of lung cancer patients is more pronounced for the human-specific L1Hs family. L1PA2-4 families have not demonstrated any statistically significant differences. MIRA followed by qPCR-based quantitation was performed, and statistical analysis demonstrated significant difference in L1Hs promoter methylation index between cancer patients and healthy individuals (p-value = 0.0012), i.e. L1Hs promoters are hypomethylated in csbDNA of lung cancer patients. Since this finding could be used as a novel non-invasive biomarker, we performed ROC-curve analysis to assess its feasibility to distinguish lung cancer samples from controls. The results for the cohort from this study (n = 100, AUC = 0.69, p = 0.0012) confirm that use of MIRA to detect hypomethylation in csbDNA is a promising non-invasive approach.

Conclusion: Further in-depth investigation is promising to evaluate whether these changes could be used for pretreatment assessment of lung cancer patients as the novel non-invasive biomarker, alone or in combination with other established epigenetic markers.

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EFFECTS OF LITHIUM SALTS ON HEPATOCELLULAR CARCINOMA-29

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Key words: *lithium salts, nanosized forms of lithium, hepatocellular carcinoma-29, target cells*

Motivation and Aim: One of the most important trends in contemporary biomedical research is study of tumor growth and cancer metastasis as well as ways of their treating. The various nanosized structures including lithium compounds are actively used for this purpose. The aim of our study was to identify the anti-tumor effects of various forms of lithium salts.

Methods and Algoritms: Experiments were performed in vitro with hepatocellular carcinoma-29 cell culture (HCC-29). Lithium salts (citrate and carbonate) and their nanosized forms (10 nm) were introduced into the cell medium at concentrations ranging from 0.00001 mM to 20 mM. Cell viability was determined by culturing HCC-29 with lithium (MTT test); cell phenotyping was performed through morphological criteria developed previously [1] at concentration of 5 and 10 mM (light, electron microscopy) and identification of the target cells of different forms of lithium salts was carried out.

Results: Dose-dependent decrease in HCC-29 cell viability was found with the introduction of original and nanosized forms of carbonate and lithium citrate; 5 stages of cell differentiation and the target cells of lithium salts were also identified. It was shown that proliferating cells of I and II differentiation stage were the target cells of original and nanosized forms of lithium citrate, and differentiated cells of IV and V differentiation stages were the ones for nanosized forms of lithium carbonate.

Conclusion: Nanosized forms of lithium salts in comparison with the original forms had the more pronounced antitumor effect in lower concentrations allowing more efficient use of lower doses of drugs.

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LINAGLIPTIN ALLEVIATES PODOCYTE INJURE AND INCREASES GLOMERULAR NEPHRIN EXPRESSION IN A MODEL OF TYPE 2 DIABETIC NEPHROPATHY

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Key words: *diabetes, diabetic nephropathy, linagliptin, nephrin*

Motivation and Aim: A growing body of evidences indicates the potency of dipeptidyl peptidase-4 (DPP-4) inhibitors for reducing of albuminuria in diabetes [1]. It has been demonstrated that the disturbances of podocyte function can be responsible for elevation of albumin excretion rate at initial stages of diabetic nephropathy [2]. Expression of nephrin and other key podocyte-specific proteins is downregulated in diabetes [3, 4]. Therefore, the aim of our study was to assess the effect of DPP-4 inhibitor linagliptin on podocyte structure and glomerular nephrin expression in *db/db* diabetic mice.

Methods: Eight-week-old male diabetic *db/db* mice (BKS.Cg-Dock7^{m}+/+Lepr^{db}/J) were treated with linagliptin (10 mg/kg per day by gavage) or saline for 8 weeks. Renal structural changes were analyzed quantitatively through the light and electron microscopic images. Nephrin staining in glomeruli was assessed by immunohistochemistry. The morphometric analysis was performed using *ImageJ* program.

Results: At the end of the experiment linagliptin-treated mice, as compared to vehicle-treated animals, demonstrated significant reduction in glomerular basement membrane and podocyte foot process width (both $p<0.01$). The number of podocyte foot processes was increased ($p=0.007$), and the number of endothelial fenestrae in glomerular capillaries tended to be increased ($p=0.1$) on linagliptin treatment. The volumetric density of nephrin-expressing glomeruli was increased markedly in linagliptin-treated group ($p<0.05$).

Conclusions: The results demonstrate that DPP-4 inhibitor linagliptin ameliorates podocyte injury and increases glomerular nephrin expression in a model of type 2 diabetic nephropathy. The data provide further explanation for the mechanism of antialbuminuric effect of DPP4 inhibitors in diabetes.

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MUTATIONAL LANDSCAPE OF PROSTATE TUMORS BASED ON WHOLE EXOME SEQUENCING

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Key words: *prostate cancer, candidate genes, tumor tissue, variants, functional significance, rare alleles.*

Motivation and Aim: Prostate cancer (PC) is one of the most common malignancies of the male population worldwide. Extremely rapid increase in the incidence of prostate cancer and the high prevalence of the disease in the world testify to the need to study the mechanisms underlying the development of prostate cancer.

Methods and Algorithms: New possibilities in molecular genetics investigations using NGS technology can expand the possibilities of tumor heterogeneity investigation, which is especially important for the genetic heterogeneity of pathologies with a large number of candidate genes. To find new genes involved in the pathogenesis of prostate cancer, we conducted a whole exome sequencing in samples of normal and tumor tissue for 8 patients with prostate cancer. The variants revealed were annotated using ANNOVAR software tool [1]. To identify the events associated with the tumor, the variants with frequencies more than 0.03 in any of the databases 1000 Genomes Project (European, East Asian, All), ExAC, ESP were excluded. The functional significance of the observed changes was carried out using following databases: SIFT, PolyPhen (using annotations for rare alleles), MutationTaster, MutationAssessor [2], FATHMM, CADD.

Results: We detected 41542 variants in normal tissue sample, 45948 - in tumor, in average. All the samples contained mutations in the *ATM*, and *TP53* genes. After all stages of bioinformatic analysis 35 candidate genes, involved in cell cycle control, apoptosis signaling, androgen processes of cell growth and differentiation, transcription repair were selected - *MUC16, MUC6, MTCH2, ZNF844, PRSS3, SSTR1, PDE11A, L2HGDH*, *etc.*

Conclusion: The study revealed a number of genes which role in prostate cancer has not been described previously. The analysis is to be continued to determine the involvement of the identified genes in the pathogenesis of prostate cancer.

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GENETIC FACTORS AFFECTING FIBROGENESIS AND ENDOTHELIAL FUNCTION IN CHRONIC HEPATITIS C

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Key words: *chronic hepatitis C, single nucleotide polymorphisms, association with the quantitative traits*

Motivation and Aim: HCV- infection leads to chronic inflammation, steatosis, fibrosis, oxidative stress, DNA damage and genomic instability. The study of genes involved in these processes, will identify targets for therapy that helps prevent chronicity of HCV infection, complications and oncological transformation.

Methods and Algorithms: We performed a case-control study to detect associations of 58 SNPs in genes involved in fibrogenesis, endothelial function, intercellular interactions and reparation with chronic hepatitis C (HCV). We also analyzed associations of these SNPs with the levels of total bilirubin, cholesterol, alanine aminotransferase, aspartate aminotransferase, collagenase, elastase and alpha-2-macroglobulin among patients. We studied cases of HCV infection (n=184) and population control (n=285). Genotyping was performed with mass spectrometry on Sequenom MassARRAY. Statistical analysis was carried out in the software environment R.

Results: Single nucleotide polymorphisms of genes taking part in fibrogenesis (*ADAMDEC1*- rs3765124, rs10087305; *MMP3* - rs679620) and endothelial function (*KIAA1462* - rs3739998) showed association with chronic hepatitis C. Patients with chronic hepatitis C had higher frequency of genotypes AA - rs3765124 (36,5% instead of 24,3% in the control group; p=0.0080; OR=1.79), CC - rs10087305 (7,5% instead of 2,4%; p=0.0242; OR=3,34), AA - rs679620 (36,2% instead of 19,6%; p=0.0018; OR=2.32) and CC - rs3739998 (26.8% instead of 15.9%; p=0.0074; OR=1.95). The following genes were associated with the quantitative traits: *ADAMDEC1* (rs3765124) with the cholesterol level (p=0,0020); *MMP3* (rs679620) with the bilirubin level (p=0.0320); *ITGB5* (rs1007856) with the elastase level (p=0.0300); *TGFB1* (rs1800469) with the macroglobulin level (p=0.0340). No associations were revealed with the alanine aminotransferase, aspartate aminotransferase and collagenase levels.

Conclusion: Matrix metallopeptidase (*MMP3*), its activity regulator (*ADAMDEC1*), integrin (*ITGB5*), transformative growth factor (*TGFB1*) and *KIAA1462* genes are important genetic risk factors for HCV infection and its progression.

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REGENERATION OF LYMPH NODES IN CONDITIONS OF AGE-INDUCED IMMUNODEFICIENCY AFTER PHYTOTHERAPY

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Key words*: regeneration, lymph node, lymphoid follicle, gerontology, phytotherapy*

Motivation and aim. The solution of one of actual problems of modern gerontology and lymphology is search of the means strengthening neolymphogenesis. This problem may be decided by overcoming affirmation of modern medicine that «new lymphoid organs are not formed at a late stage of ontogenesis» [1, 2]. The aim of our study is to estimate effect of phytotherapy on regeneration of aged lymph nodes.

Methods. The experiment was conducted on 80 white male rats of Wistar breed aged 1.5-2 years (old animals).

Animals were given bioactive phytocomposition in which the active components were flavonoids (rutin), arbutin, dietary fibers [3]. Daily dose was 0.1-0.2 g/kg. The histological method was used for the study of lymph nodes in the late stage of ontogenesis and after phytocorrection.

Results. With age, anatomic and morphometric parameters of lymph nodes have unidirectional changes, which are expressed in increase of the connective tissue, reducing the size of functional compartments and, particularly, the area of lymphoid nodules with germinative centers and also in the reduction of lymphoproliferative activity and development of immune deficiency. Overcoming age-induced immune deficiency of lymph nodes is achieved by using lymphotropic phytotherapy. The use of phytocomposition causes intra- and extranodulary changes of lymphoid tissue. There is an increase of numerical density of lymphocytes, plasmocytes, lymphoblasts that is followed by hypertrophy (or a hyperplasia) of some structural zones in lymph node. The lymphoproliferation after phytotherapy increases the regenerative capacity of lymphoid tissue.

We revealed the ectopic formation of lymphoid follicles in the subcapsular zone and in the medullary substance of lymph node. Evidence of the formation of lymphoid follicles is the absence of CD38+cells [4]. We also noted the formation of lymphoid follicles outside the main lymph node. Regeneration of lymphoid tissue outside the lymph node is a sign of compensation for age-induced changes. We observed structures resembling the architecture of the accumulation of lymphocytes (infiltrate), lymphoid follicles after phytotherapy. Ectopic accumulations of lymphoid cells are called «tertiary lymphoid organs» and they are described usually in pathological situations associated with varying degrees of immunodeficiency [1, 2, 4]. We consider neolymphogenesis as manifestation of regeneration, resulting in increased immunodeficiency at the late stage of ontogenesis after phytotherapy.

Conclusion. The lymphotropic phytotechnology is positioned as the possibility of optimizing the functions of the lymph node through neolymphogenesis at the late stage of ontogenesis to increase nonspecific resistance of the organism.

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MITOCHONDRIAL DNA HETEROPLASMY IN THE BLOOD AND CAROTID ATHEROSCLEROTIC LESIONS

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Key words: *(mitochondrial DNA, heteroplasmy, atherosclerosis)*

Motivation and Aim: Atherosclerosis is accompanied by increased oxidative stress. MtDNA is largely exposed to oxidative damage, since it is located near the ROS production sites. Oxidative mtDNA damage can lead to somatic mutations that should exist presumably in heteroplasmic state. So, heteroplasmic mtDNA variation could serve as an indicator of oxidative stress. Recently, considerable tissue-dependent mtDNA heteroplasmy was found in humans. However, most of these studies were performed on postmortem material and did not analyze blood cells and atherosclerotic lesions. To estimate heteroplasmic mtDNA variation in atherogenesis, we have described heteroplasmic positions in the blood and ablated carotid plaques of living patients.

Methods and Algorithms: 10 paired samples of DNA extracted from the ablated carotid plaques and from the blood of the patients were analyzed. MtDNA was sequenced and analyzed on the MiSeq platform (Illumina), using 0.9% threshold for variant calling. Heteroplasmic variations at different levels were evaluated. Criteria for heteroplasmic state calling were: minimum 200x coverage; at least 10 reads with the variant; strand bias value < -20; genotype quality value >25. CA-repeats in positions 514-523 and cytosine stretches in positions 303-315 and 16183-16193 were not considered for the analysis. Contamination was excluded after comparing candidate heteroplasmies with phylogenetic mtDNA data.

Results: Mean number of heteroplasmic positions (>1%) per sample was 6.0 in the blood and 7.4 in the plaques. Transitions/transversions ratio was 8.5:1, comparing to 20:1 for population polymorphism. Most of the heteroplasmies were registered at the level less than 3%. Several deletions in poly-A stretches were revealed in the protein-coding genes (i.e. frameshift mutations). All these deletions had high genotype quality and no strand bias. 27% of the registered heteroplasmies (not including deletions) were located in the major noncoding region which encompasses 7% of the total mtDNA sequence. That is consistent with high D-loop mutation rate. Percentage of the mutations in the RNA genes roughly corresponded to the cumulative length of the RNA genes in the human mitochondrial genome (20-25%). 52% of the heteroplasmies were revealed in the protein-coding genes (69% of the mtDNA length), and half of them were missense mutations.

Conclusion: There is considerable low-level heteroplasmic mtDNA variation in atherosclerosis. The heteroplasmic variability characteristics differ from those of inherited polymorphisms by greater number of transversions, single nucleoide deletions and missense mutations.

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ANALYSIS OF NUCLEAR PORE COMPLEX GENES IN GLIOBLASTOMA BY TRANSCRIPTOME PROFILING

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Key words: *glioma, RNA-seq, cell culture, human brain, gene expression*

Motivation and Aim: The nuclear pore complex (NPC) is essential not only to regulating nuclear-cytoplasm transport but also to controlling genome organization and expression. Therefore the NPC is linked with many diseases including cancers [1, 2]. Nucleoporins have been directly implicated in cancers via three routes: chromosomal translocations generating fusion proteins; changes in protein expression levels; and single point mutations. The aim of present study was comparison of NPC genes expression in the cells of normal brain cultures and secondary glioblastoma (GBM).

Methods and Algorithms: Primary cell cultures from surgical samples of GBM and normal brain have been isolated and propagated in F12/DMEM medium supplemented by fetal bovine serum under standard conditions. The cell culture samples were processed for RNA extraction. This was followed by RNA-sequencing and filtration of reads. Cufflinks were used for assessment of gene expression level and finding differently expressed genes.

Results: We revealed set of differently expressed NPCs gene in normal brain and GBM cell cultures. Most of them are expressed higher in the GBM cells. The antisense RNA transcript of nucleoporin Nup50 (Nup50-AS1) is the only one among NPC-related genes that is expressed in tumor less than in normal brain. At the same time expression of Nup50 gene is not distinguished in tumor and normal brain cell cultures. It has been suggested that there is a higher level of Nup50 protein in tumor cells and it is regulated by antisense RNA transcript.

Conclusion: The RNA-seq analysis of the cells cultures of normal brain and GBM confirmed association of some NPCs genes with tumor progression. The protein Nup50 is a member of the FG-repeat containing nucleoporins and functions as a soluble cofactor in importin-alpha:beta-mediated nuclear protein import. It has been recently reported that Nup50 is required for cell differentiation and exhibits nuclear dynamics which is dependent on active transcription by RNA polymerase II. The expression of gene Nup50-As1 increases in the human neocortex during development period spanning infancy to adulthood [2]. Our finding demonstrates the complicated role of nuclear pore complex proteins in tumor progression.

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KATIS: INTEGRATIVE INFORMATION SYSTEM FOR COMPLEMENTARY MEDICINE

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Key words: *Information system, complementary medicine*

Motivation and Aim: The Internet represents a lot of databases, information systems and portals specialized in complementary medicine. Most of these systems are specialized and represent only information about one category of alternative medicine. In addition, these systems are not able to support the computerized detection of suitable therapies based on patient specific indications. Furthermore, based on these systems various apps are available today. To develop and implement an information system which represents the main knowledge of complementary medicine is the goal of our work.

Methods: Using information fusion methods it was our idea to integrate the most relevant knowledge of alternative medicine. Therefore, we developed the warehouse based web-based information system KATIS. Furthermore, based on KATIS we developed the a new app called ALMEKO, which was implemented for mobile usage of the KATIS.

Results: We developed and implemented a web based information system which represents the knowledge of complementary medicine. This system is called KATIS. Based on KATIS we developed the app

Conclusion: We implemented an integrative information system for complementary medicine which allows the user to search for individual therapies based on patient-specific indications.

Availability: KATIS: http://www.komplementäre-medizin.de/
ALMEKO: https://play.google.com/store/apps/details?id=de.icancode.almeko&hl=de

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THE ASSOCIATION OF THE MUTATION D4301N IN GENE *TTN* WITH SUDDEN CARDIAC DEATH

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Key words: *sudden cardiac death, TTN, rs767084399, D4301N-TTN*

Motivation and Aim: Sudden cardiac death (SCD) is more than 50% of all cardiovascular deaths [1]. In some cases the causes of sudden death remain unexplained after comprehensive medico-legal autopsy. In foreign study the missense mutation D4301N in gene *TTN* was detected in cases of autopsy-negative sudden unexplained death by whole exome next-generation DNA sequencing. This ultra-rare possibly pathogenic mutation is absent in publically available exome databases [2]. Gene *TTN* (2q31) encodes titin, a large protein of striated muscle. Mutations in this gene are associated with hypertrophic and dilated cardiomyopathy [3]. The aim of the study is investigation of the association between mutation D4301N-TTN (rs767084399) and sudden cardiac death.

Methods and Algorithms: The SCD group was formed using WHO criteria for sudden cardiac death (n = 381, mean age 53.3±8.8 years, men - 70.9%, women - 29.1%), the control group was selected according to sex and age from the DNA bank of project HAPIEE, MONICA (n = 377, mean age 53.1 ± 8.3 years, men - 68.3%, women - 31.7%). DNA was isolated by phenol-chloroform extraction of the myocardial tissue in SCD group, and venous blood in the control group. Genotyping was done by PCR followed by analysis of restriction fragment length polymorphism.

Results: The carriers of the rare allele A of the mutation D4301N-TTN were not found in the SCD and control groups.

Conclusion: The mutation D4301N in gene *TTN* is not associated with the SCD in the samples of suddenly deceased residents of Novosibirsk.

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FEATURES OF miRNA INTERACTION WITH mRNA GENES IN CORONARY HEART DISEASE

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Key words: *miRNA, miR-619-5p, mRNA, binding sites, ortholog genes*

Motivation and Aim: In total, 268 binding sites for 2578 miRNAs were found in mRNA of 187 genes involved in the development of coronary heart disease. Of these, 52 were located in the coding domain sequence (CDSs), 23 were located in the 5′-untranslated region (5′UTRs), and 193 were located in the 3′-untranslated region (3′UTRs). From the database of miRNAs involved in the development of coronary heart disease, none of the miRNA had binding sites on the mRNA of 187 genes that play a role in coronary heart disease. It is possible that these miRNAs act on mRNA genes that are not included in the database of genes involved in the development of coronary heart disease.

Methods and Algorithms: The target genes for miR-619-5p were revealed using the MirTarget program. This program defines: a) the origin of the initiation of miRNA binding to mRNAs; b) the localisation of miRNA binding sites in the 5′UTR; the CDS and the 3′UTR of the mRNA; c) the free energy of hybridisation (ΔG, kJ/mole); and d) the schemes for nucleotide interactions between the miRNAs and the mRNAs. The ratio ΔG/ΔGm (%) was determined for each site (ΔGm equals the free energy of an miRNA binding with its perfect complementary nucleotide sequence).

Results: Features of the interaction of miRNA with the mRNA of 85 target genes are described below. mRNA of some genes can bind five or more miRNAs. Five miRNAs bind to the mRNA of the *MTHFR, PLA2G7* genes. Six miRNAs bind to the mRNA of the *AS3MT, F2RL3, MLXIPL, PPP1R3B*, and *TGFB1* genes. Seven miRNAs bind to the mRNA of the *IL6R, LDLR, MLXIPL,* and *NPC1L1* genes. These data indicate a strong dependence of the expression of these genes on miRNA. The mRNA for the *CD36* and *PLA2G7* genes has multiple binding sites for miR-466, which belongs to a class of unique miRNAs. The mRNA for the *IGF1, NOS1*, and *PPARA* genes has multiple binding sites for miR-574-5r, which also belongs to a class of unique miRNAs. We have previously shown that unique miRNAs are encoded in the human genome, and they have more than 300 binding sites. These miRNAs include miR-619, miR-5095, miR-5096, miR-3960, miR-1322, and some of the miRNAs of the miR-1273 family. Expression of a significant part of the genes involved in the development of coronary heart disease may depend upon these unique miRNAs. For example, miR-619-5p has 14 target genes, whereas miR-5095 and miR-5096 have 10 target genes each. miR-1273a,c,d,e,f,g,h family miRNAs have 38 binding sites, including 19 binding sites for miR-1273g-3p in mRNA for 17 genes. Some miRNAs have a high free energy of binding to the mRNA of several genes. miR-1273d, consisting of 25 nt, binds to the mRNA of the *SMARCA4* gene with a binding free energy of -125 kJ/mole, which is 92% of the maximum binding free energy. miR-762, consisting of 22 nt, binds to the mRNA of the *CSMD1* gene with a binding free energy of -127 kJ/mole, which is 94% of the maximum binding free energy. miR-6789-5p, consisting of 24 nt, binds to mRNA of the *HIF1A* and *FADS3* genes with a binding free energy of -129 kJ/mole and 132 kJ/mole, which is 88% and 90% of the maximum binding free energy, respectively. miR-6089-5p, consisting of 24 nt, binds to the mRNA of the *TGFB1* gene at two sites with free energies of binding of -132 kJ/mole and -136 kJ/mole, which is 89% and 91% of the maximum binding free energy, respectively. The same miRNA binds to the mRNA of the *IL6R* gene with a binding free energy of -138 kJ/mole, which is 93% of the maximum binding free energy.

Conclusion: These data show that the interactions between the examined miRNA and mRNA can serve as a basis for selecting associations between miRNA and mRNA for diagnostic evaluation of coronary heart disease. Association means the connection of one miRNA with mRNA of one or more genes, or one or more miRNAs with the mRNA of a single gene.

OPTIMIZATION OF METHODS OF ANALGESIA WITH UTERINE ARTERY EMBOLIZATION BY ANALYZING STRESS MARKERS – REACTION

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key words: *uterine artery embolization, pain, analgesia*

Motivation and Aim: Uterine artery embolization (UAE) is a highly organ-modern method of treatment of uterine fibroids. Treatment of acute pain after UAE is the most important objective of modern anesthesiology and resuscitation. Purposes of the study: 1. to determine the level of severity of the stress response in patients with UAE; 2. to evaluate proposed methods of analgesia (including the first proposed method of regional analgesic depot injections) and to determine the most appropriate and adequate method.

Materials: Our study includes three groups of patients: 1. analgesia with non opioid analgesic of central action; 2. preoperative subcutaneous administration of complex drugs in the lumbar interspinous area - depot injection (nonopioid analgesic central action (Nefopam), ropivacaine, corticosteroid); 3. intraoperative administration of opioids at the time of uterine artery embolization + nonsteroidal anti-inflammatory drug. Evaluation of methods of analgesia has been performed using analysis of hemodynamic parameters (heart rate, blood pressure, mean arterial pressure(MAP), electrocardoigraphy (ECG), markers of stress response (cortisol, adrenocorticotropic hormone (ACTH), glucose), Pain Quality Assessment Scale (PQAS).

Results: Comparable data of ACTH (pg/ml) have been shown in Groups №1 and №2: at 24 hours after the UAE - 7,04 + 2,40 and 7,25 + 3,14, at 48 hours after UAE – 12,90 + 4,64 and 11,14+ 4,27. Worse data of ACTH have been shown in Groups №3: at 24 hours after the UAE - 10,52 + 1,02, at 48 hours after UAE - 31,10 + 5,40. Significantly better values of cortisol have been shown in Groups №2: at 24 hours after the UAE - 4,51 + 2,83, at 48 hours after UAE – 12,81 + 1,77. Significantly worse values of cortisol (mcg/dl) have been shown in Groups №3: 24 hours after the UAE - 13,34 + 2,97, at 48 hours after UAE - 15,95 + 1,25. Values of cortisol have been shown in Groups №2: at 24 hours after the UAE - 10,36 + 3,07, at 48 hours after UAE - 12,61 + 3,55. The levels of glucose in the study groups have comparable values, but the trend towards higher rates was in the group №3. Most stable hemodynamics (mmHg) have been shown in Groups №2: immediately after UAE the MAP – 85,0 $\pm$ 8,77; at 24 hours after the UAE the MAP – 79,85 $\pm$ 5,17 at 48 hours after the UAE the MAP – 80,09 $\pm$ 6,81. Less stable hemodynamic parameters have been shown in Groups №1 and №3: immediately after UAE the MAP – 99,5 $\pm$ 9,83 and 87,0 $\pm$ 7,0; at 24 hours after the UAE the MAP – 79,17 $\pm$ 8,22 and 84,50 $\pm$ 10,50 at 48 hours after the UAE the MAP – 73,83 $\pm$ 7,44 and 79,50 $\pm$ 9,50. The PQAS showed that 35% of patients of groups №1 and №2 had the 0-1 score immediately after UAE, whereas 20% patients of group №3 had the 0-1 score. The PQAS showed that 70% of patients of group №2 had the 0-1 score at 48 after UAE, whereas 60% patients of group №1 and 50% patients of group №1 had the 0-1 score.

Conclusoins: Group №2 showed more appropriate results of analgesia for UAE in comparison with groups of №1 and №3. At the same time, data of groups №1 and №3 allow to achieve necessary analgesia under certain conditions.

THE PROLIFERATION OF MONONUCLEAR CELLS FROM HEALTHY DONORS UNDER THE INFLUENCE OF PLATELET-RICH PLASMA AND ITS DERIVATIVES

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Motivation and Aim: At the present time there is a problem of regenerative medicine associated with stem cells without culturing animal growth factors, such as fetal bovine serum. In this regard, platelet-rich plasma obtained from the blood of patients is a good equivalent. Thus the aim of this study was to investigate the effect of platelet-rich plasma and its derivatives on the proliferative activity of mononuclear cells (MNCs) of peripheral blood from healthy donors

Methods and Algorithms: Peripheral MNC from healthy donors were isolated by density gradient on Ficoll / verografin (ρ = 1,078 g / L).

Platelet rich plasma was prepared from peripheral blood (10 ml) of healthy donors. Peripheral blood was centrifuged at 3800 RPM (EBA20, Hettich, Germany) in special tubes (Plasmolifting ™), containing sodium heparin with a specialized thixotropic gel to obtain platelet-rich plasma. Further in the obtained plasma platelets were counted and concentrated in 1 ml plasma. To obtain platelet lysate platelet rich plasma was frozen in liquid nitrogen and thawed twice. Also prepared platelet rich plasma was activated by 10% calcium solution.

Proliferative capacity of MNCs was tested in the spontaneous and mitogen-stimulated (Concanavalin A (ConA) is 5 ug / ml) MTT-test with addition of 10% FBS, 10% platelet rich plasma or 10% lysates of platelets or 10% platelet rich plasma, activated calcium. The obtained data are expressed in optical density units (OD).

The study results are statistically processed using program Statistica 10.0 for Windows software (StatSoft, USA).

Results: During the study of proliferative activity it has been revealed that all kinds of platelet-rich plasma enhance both spontaneous proliferation of MNC and stimulated ConA proliferation of MNC compared with the same effect of FBS.

It was also shown that the Con A-stimulated proliferation of MNC with the addition of all types of platelet-rich plasma was higher compared with their effect on the spontaneous proliferation of MNCs.

Conclusion: Thus, autologous platelet-rich plasma can be used instead of FBS in cells culture of patients for further cell transplantation into target organs.

COMBINATIONS OF POLYMORPHISMS IN CYTOKINE AND MATRIX METALLOPROTEINASE GENES ARE ASSOCIATED WITH CHRONIC KIDNEY DISEASE IN TYPE 2 DIABETIC SUBJECTS

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Key words: *diabetes, inflammation, acute-phase proteins, single-nucleotide polymorphism, cytokine*

Motivation and aim: The activation of inflammatory pathways and disturbances in the collagen turnover are involved in development of chronic kidney disease (CKD) in diabetes [1-3]. The combinations of cytokine gene polymorphisms associated with type 2 diabetes were reported recently [4]. We aimed to identify combinations of polymorphisms in the genes of cytokines (*TNFA, IL1B, IL4, IL6* and *IL10)* and matrix metalloproteinases *(MMP3, MMP2, MMP9)* associated with CKD in type 2 diabetic patients.

Materials and methods: A total of 222 Caucasoid patients with type 2 diabetes and glomerular filtration rate (GFR) ≥30 ml/min/1.73 m^2 were enrolled in the study. Analysis of polymorphisms *C238A, G308A, C863A* in *TNFA, IL1B T31C, IL4 C590T, IL6 G174C, IL10 C592A, IL10 G1082A, VEGFA A2578C, VEGFA C936T, MMP2 T1306C, MMP3 -1171 5A/6A, MMP9 C1562T* was performed using TaqMan. The combinations of polymorphisms, associated with stage 3 CKD, were identified by bioinformatics analysis.

Results: Thirty two polymorphism combinations associated with reduced GFR (CKD 3) were identified ($p<0.005$). These combinations included homozygous genotype *TNFA -308GG* and *-238GG* (21 and 11 combinations respectively), *IL1B -31TT* (n=17), *IL4 -590CC* (n=9), *IL6* -174*GG* (n=8), *+936CC* (n=14), *MMP3 5A/5A* (n=16) and *MMP9* -1562*CC* (n=13). The associations of gene combinations with CKD demonstrated high OR (in the range of 2.1-15.7) and specificity (75.6-99.4%).

Conclusion: The combinations of polymorphisms in the gene promoters of cytokines (*TNFA, IL1B, IL4, IL6, IL10*) and matrix metalloproteinases *(MMP3, MMP2, MMP9)* can modify the risk of CKD in type 2 diabetic subjects.

Acknowledgements: the study was funded by Russian Science Foundation (grant 14-15-00082).

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IMMUNOGENETIC FACTORS ASSOCIATED WITH CHRONIC LOW-GRADE INFLAMMATION IN TYPE 2 DIABETIC SUBJECTS

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Key words: *diabetes, inflammation, acute-phase proteins, single-nucleotide polymorphism, cytokine.*

Motivation and aim: The growing body of evidence indicates that chronic low-grade inflammation is involved in development of type 2 diabetes and diabetic vascular complications [1-4]. In this work we aimed to study the associations between serum levels of acute-phase proteins and single-nucleotide polymorphisms (SNPs) in promoters of *TNFA, IL1B, IL4, IL6* and *IL10* in type 2 diabetic subjects.

Materials and methods: A total of 148 Caucasoid patients with type 2 diabetes were examined. The levels of high-sensitivity C-reactive protein (hsCRP) and α1-acid glycoprotein (α1-AGP) were measured by ELISA and compared to control (30 healthy subjects). The study of SNPs in the gene promoters of *TNFA* -308 G/A (rs1800629*), TNFA* -863 C/A *(*rs1800630*), IL1B* -31 T/C (rs1143627)*, IL4* -590 C/T (rs 2243250)*, IL6* -174 G/C (rs1800795*), IL10* -592 C/A (rs1800872) and *IL10* -1082 A/G (rs1800896) was performed using TaqMan SNP Genotyping Assays. The combinations of SNPs, associated with high hsCRP and α1-AGP levels, were identified by bioinformatics analysis.

Results: The increase in serum levels of hsCRP and α1-AG was found in diabetic patients when compared to control (both $p<0.0001$). Nine combinations of SNPs, associated with high (≥75 percentile) hsCRP levels, were identified. Genotype *IL10 -1082AA* and *TNFA -308GG* was found in 9 and 7 of these combinations respectively. Genotype *IL10 -1082AA* was associated with high levels of α1-AGP as a single trait (OR=3.38, $p=0.03$), as well as a component of four combinations of SNPs.

Conclusion: The combinations of SNPs in promoters of proinflammatory and anti-inflammatory cytokines (*TNFA, IL1B, IL4, IL6, IL10*) can modify the intensity of chronic low-grade inflammation in type 2 diabetic subjects.

Acknowledgements: the study was funded by Russian Science Foundation (grant 14-15-00082).

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SINGLE-NUCLEOTIDE POLYMORPHISMS IN PROMOTERS OF CYTOKINE GENES ARE ASSOCIATED WITH SERUM CYTOKINE LEVELS IN TYPE 2 DIABETIC PATIENTS

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Key words: *diabetes, inflammation, single-nucleotide polymorphism, cytokine*

Motivation and aim: A growing body of evidence indicates that chronic low-grade inflammation is involved in the pathogenesis of diabetic vascular complications [1, 2]. Although the mechanisms of enhanced inflammatory response in diabetes have not been precisely defined yet, the role of immunogenetic factors could be supposed [3]. The aim of the study was to assess the associations of single nucleotide polymorphisms (SNPs) in the gene promoters of *IL4*, *IL6, IL10* and *TNFA* with serum levels of cytokines in type 2 diabetic subjects.

Materials and methods: We studied 145 Caucasoid patients with type 2 diabetes. Seven SNPs: *IL4* -590 *C/T* (rs2243250), *IL6* -174 *G/C* (rs1800795), *IL10* -592 *A/C* (rs1800872) and -1082 *A/G* (rs1800896), *TNFA* -238 *A/G* (rs361525), -308 *G/A* (rs1800629) and -863 *C/A* (rs1800630), were revealed by real-time PCR. The levels of IL-6 and TNF-α were assessed by Multiplex analysis, concentrations of IL-4 and IL-10 were determined by ELISA and compared to control (20 healthy subjects matched by sex and age).

Results: Serum level of IL-6 was elevated and IL-4 level was decreased in diabetic patients compared to controls (p=0.04 and p=0.005 respectively). Concentrations of TNF-α and IL-10 demonstrated a tendency to increase (p=0.07; p=0.09). The *IL6* (-174) *GG* genotype was associated with higher IL-6 levels as compared to *CC* and *CG* genotype (p=0.01 and p=0.004). The *TNFA* (-863) *CC* and *CA* carries had higher TNF-α levels compared to those with *AA* genotype (p=0.04). The concentration of IL-10 was lower in patients with *IL10* (-592) *CC* compared to *AA* genotype (p=0.01). The presence of *CC* at *IL4* -590 position was associated with lower IL-4 concentration (p=0.02).

Conclusion: The SNPs in the promoters of *IL4* (rs1800795)*, IL6* (rs1800795)*, IL10* (rs1800872) and *TNFA* (rs1800630) can affect the levels of pro-inflammatory and anti-inflammatory cytokines in type 2 diabetic subjects.

Acknowledgements: the study was funded by the Russian Science Foundation (grant 14-15-00082).

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INVERSE CHANGES IN SERUM CONCENTRATIONS OF INFLAMMATORY AND ANGIOGENIC MEDIATORS IN PATIENTS WITH TYPE 2 DIABETES

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Key words: *diabetes, inflammation, angiogenesis, cytokine, acute-phase proteins.*

Motivation and aim: Systemic inflammation and impaired angiogenesis are considered as the cornerstones in pathogenesis of diabetic vascular complications [1-3]. The aim of the study was to assess the relationships between serum levels of inflammatory and angiogenic markers in type 2 diabetic patients.

Materials and methods: We observed 210 patients, 43-70 years of age. Thirty healthy patients matched by sex and age were acted as control. The levels of IL-6, IL-18, TNF-α, VEGF-A, VEGF-C, VEGF-D, placental growth factor (PLGF), angiopoietin-2, epidermal growth factor (EGF), heparin-bound EGF-like growth factor (HB-EGF), endoglin, soluble Fas ligand (sFASL), insulin-like growth factor-binding protein-1 (IGF-BP1), transforming growth factor α (TGF-α), and urokinase plasminogen activator (uPA), were assessed by Multiplex assay. The levels of high-sensitivity C-reactive protein (hsCRP), α1-acid glycoprotein (α1-AGP) were measured by ELISA.

Results: The increased serum levels of IL-6, IL-18, TNF-α, CRP and α1-AGP were found in diabetic patients when compared to control (all $p<0.05$). Diabetic subjects had decreased levels of VEGF-A ($p=0.03$), VEGF-C ($p=0.006$), PLGF ($p=0.04$), endoglin ($p=0.01$), IGF-BP1 ($p=0.03$), and uPA ($p=0.02$). Concentrations of other regulators demonstrated no significant differences between two groups. The elevation of NT-proBNP levels was associated with increment of sFASL ($p=0.02$) and VEGF-A ($p=0.03$). Patients with highest (≥75 percentile) hsCRP levels, as compared to those with lowest one (≥25 percentile), had decreased concentrations of VEGF-A ($p=0.007$), VEGF-C ($p=0.02$), VEGF-D ($p=0.004$) and PLGF ($p=0.04$).

Conclusion: The elevation of serum levels of inflammatory markers (hsCRP, α1-AGP, IL-6, IL-18, TNF-α) combined with depletion of regulators of angiogenesis (VEGF-A, VEGF-C, PLGF, endoglin, IGF-BP1, and uPA) in type 2 diabetic subjects.

Acknowledgements: the study was funded by the Russian Science Foundation (grant 14-15-00082).

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MUTATION DIVERSITY OF PAH GENE IN MOSCOW PATIENTS WITH PHENILKETONURIA

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Key words: *phenylketonuria, phenylalanine hydroxylase gene, PAH, mutation analysis*

Motivation and aim: Phenylketonuria (PKU) is an inherited disorder of phenylalanine metabolism. Mostly PKU caused by mutations of a phenylalanine hydroxylase (*PAH*) gene. In Europe, including European part of Russia, the most prevalent pathogenic mutation is R408W. Another mutation types (IVS12+1G>A, IVS10-11G>A, P281L, R261Q, R252W, R158Q, I65T) are more or less common. In fact, the range and frequency of pathogenic alleles vary between different countries and regions. Historically Moscow region is characterized by the high genetic heterogeneity, consequently the analysis of *PAH*-mutations between PKU-affected people from Moscow population may help to detect the whole spectrum of mutations, in particular rare variants.

Methods and algorithms: In total, 75 unrelated PKU probands from the Moscow region were analyzed. SNP-detection approach based on Real-Time PCR was used for searching of 16 prevailing mutations of the PAH gene. 24 patients, whose genotypes weren't identified by the PCR, were researched by the NGS. All 13 exons with flanking intronic regions of the *PAH* gene were amplified and sequenced by Ion Torrent's technology. Primary NGS data analysis was performed with Torrent server 4.4.3 software. For SNP calling was used Torrent Variant Caller 4.4 plugin. Variant annotation was performed using in house developed software and dbSNP Build 147.

Results: The overall efficiency of testing amounts to 96%. The complex approach allowed to identify 26 types of single-nucleotide or indels PKU-associated mutations: R408W (counted allele's frequency, 50%), R261Q (8,67%), IVS10nt546 (4,67%), R158Q (4%), IVS12+1G>A (4%), Y414C(2,67), IVS4+5G>T (2,67%), R252W (2%), L48S (2%), c.664_665delGA (2%), R111X (2%), R261X(1,33), IVS11+1G(1,33%), G188D (0,67%), E280K (0,67%), P281L (0,67%), F161S (0,67%), c.60+5G>T (0,67%), E390G (0,67%), A300S (0,67%), F55L (0,67%), c.913-7A>G(0,67%), F55Leufs (0,67%), R176X (0,67%), L311P (0,67%), R270K (0,67%). Pathogenic status of IVS8-7A>G/c.913-7A>G and F161S/c.482T>C ("Untested"on dbSNP) have been specified in current studies [1]. R408W and R261Q amount to 73,3% and 16% of genotypes respectively. ½ of variants accounted for less than 1%.

Conclusion: The present results confirm heterogeneity of the *PAH* locus among Moscow region's PKU patients. The NGS approach allow us to refill list of rare *PAH* pathogenic variants.

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ASSOCIATION OF POLYMORPHISMS IN THE MATRIX METALLOPROTEINASE GENES WITH EXTRA-ARTICULAR SYMPTOMS OF RHEUMATOID ARTHRITIS

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Key words: *rheumatoid nodules, MMP2-1306, MMP3-1171, MMP9-1562, SNP*

Motivation and Aim: Rheumatoid nodule is considered specific extra-articular symptom of rheumatoid arthritis (RA) and one of the diagnostic criteria associated with severe disease. Matrix metalloproteinases (MMPs) are seen as major proteolysis enzymes involved in the implementation of destructive processes in RA and in a number of data correlate with disease activity. MMPs can play a key role in the development of extra-articular symptoms of RA. The aim of this study was to investigate MMP gene polymorphism in Caucasoid RA patients with and without rheumatoid nodules. The control group included healthy women.

Methods and Algorithms: 162 women with RA satisfied (ACR, 1987) criteria were included in this study, rheumatoid nodules were detected in 38 of them (23,5%). We have studied single nucleotide polymorphisms of the promoter region of genes MMP2-1306 (rs243865), MMP3-1171 (rs3025058), MMP9-1562 (rs3918242). Genotyping was performed by method of restriction fragment length polymorphism. In the statistical analysis of the results of the studies we used indicators such as the frequency of genes, genotypes and their combinations, specificity, odds ratio (OR) with the calculation of 95% confidence interval (OR 95% CI). Calculation of the OR was performed by the method of Woolf-Haldane. Significant difference in the distribution of the studied traits in alternative groups was determined by criterion $\chi 2$ with Yates correction.

Results: Differences in the frequencies of MMP genes between groups of patients with and without rheumatoid nodule were not detected, may be due to the small group of patients with nodules. However, the relatively healthy women RA patients had a reduction in the frequency of the homozygous genotype *MMP3 6A6A* (OR=0.29, P=0.0062 in group with the presence of rheumatoid nodules and OR=0.52, P=0.0295 in group without nodules). Also in both groups of relatively healthy patients we observed increased incidence of *MMP2-1306* TT homozygous gene variant (OR=2.94, P=0.0498 in group with rheumatoid nodules and OR=3.08, P=0.003 in group without nodules).

Conclusions: Polymorphism at promoter region of MMP genes may determine the development of the disease and the presence of extra-articular symptoms of RA.

DYSFUNCTION OF AUTISTIC GENES EXPRESSION IN THE HIPPOCAMPUS OF MALE MICE WITH THE DISTURBANCES OF SOCIAL BEHAVIOR INDUCED BY CHRONIC SOCIAL DEFEAT STRESS

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Key words: *social defeats, depression, abnormal social interactions, low communication, repetitive behaviors, autistic genes*

Motivation and Aim: Ability of people to communicate with each other is a necessary component of social behavior and normal development of individuals living in community. Apparent decline in sociability can be the result of negative social environment, development of affective and neurological disorders including depression and autism. In this work we studied expression of genes that are involved into autistic spectrum disorders in the hippocampus of male mice with long chronic social defeat stress (CSDS) which accompanied by disturbances in social behaviors.

Methods: Chronic social defeat stress was generated in male mice by exposure to CSDS in daily agonistic interactions [1]. The hippocampus was dissected according to the map presented in the Allen Mouse Brain Atlas. The collected samples were sequenced at JSC Genoanalytica (http://genoanalytica.ru/, Moscow, Russia). The Cufflinks program was used to estimate the gene expression levels in FPKM. Genes were considered to be differentially expressed at the level of statistical significance $p < 0.05$ and $q < 0.05$.

Results: Male mice with experience of social defeats displayed avoidance of social contacts with conspecific, immobility and low communication. Exploratory activity (rearing) and approaching behavior time towards partner were decreased and number of episodes of repetitive self-grooming behavior was increased in the defeated mice in comparison with the control. These symptoms were similar to those that are used in animal models of autistic spectrum disorders. Analyzing of RNA-seq database of whole transcriptome of the hippocampus we found in the defeated mice increased expression of genes which are associated with autistic spectrum disorders in humans: *Shank2, Reln, Nlgn2, Pcdh10, Arx, Vegfa, Ep300.*

Conclusion: Chronic social defeat stress induces the development of depression-like state in male mice under CSDS which is accompanied by disturbances in social behaviors and changes in the expression of genes that are involved in autistic spectrum disorders.

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ROLE OF EPITHELIAL TO MESENCHYMAL TRANSITION IN LIVER ASSOCIATED WITH OPISTHORCHIS FELINEUS INFECTION

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Key words: *liver, fibrosis, epithelial mesenchymal transition, Opistorchis felineus*

Motivation and Aim: Periductal fibrosis is the main complication of chronic liver opisthorchiasis, associated with *Opistorchis felineus*. There are a few opinions about mechanisms of fibrosis up to date. One of such mechanisms could be epithelial to mesenchymal transition (EMT). EMT is characterized by loss epithelial (E-cadherin) and acquire mesenchymal phenotype (α-SMA) (Pinzani M., 2011). Opisthorchiasis is a widespread disease with serious complications, so the study of mechanisms of fibrosis seems to be urgent task of both applied and fundamental character.

Methods and Algorithms: The study has been conducted on 60 6-8-week-old male Syrian golden hamsters, divided into 2 groups: control and infected with 50 metacercariae of *O. felineus*. The infection in the hamsters was verified by the ether-acetic coproovoscopy. Animals were housed in standard conditions with free access to food and water. Liver samples were obtained on week 10, 14, 18, 22, 26, 30, 34 and 52 after infection. To further study the sections were treated by standard histological methods (Kovner A.V. et al., 2016).

Results: Fibrotic complications in the liver may be the cause of several implication: formation of pathological changes in the parenchyma, due to damage to their own vasculature, and toxic and mechanical effects of the parasite. Immunohistochemical analysis of the liver samples in the dynamics of the infection revealed that participation of cholangiocytes in the implementation of EMT was unlikely. Starting from 22 weeks of the experiment, processes of EMT were intensified in hepatocytes. This was reflected in increased expression of markers of SMAD-signaling pathway and enhanced expression of α-SMA. Moreover, starting from 18 weeks of the experiment, the amount of collagen-forming hepatocytes (stellate hepatocytes) was increased. At all periods of the experiment, it was revealed that Kupffer cells made the largest contribution to the development of EMT.

Conclusion: Thus, EMT is one of the mechanisms of fibrogenesis in chronic opisthorchiasis. The main contributions to the implementation of this process are associated with Kupffer cells and hepatocytes.

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APPLICATION OF THE OPTICAL TECHNIQUES FOR STUDYING BLOOD IN COLORECTAL CANCER DIAGNOSING

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Key words: *optical methods, red blood cells, blood serum, diagnosing, colorectal cancer*

Introduction. The aim of this work was to assess the potential of the optical methods for studying red blood cells (RBC) and the serum (BS) of patients with colorectal cancer (CRC) in diagnosing of the disease.

Materials and Methods. A total of 48 persons aged from 49 to 72 with CRC, 12 of them with a tumor in the terminal stage after combination therapy were examined. The control group consisted of 12 healthy people. Electric, viscoelastic parameters of RBC were investigated by dielectrophoresis, their optical parameters by the terahertz spectroscopy. The optical properties of BS were studied by ellipsometry, the FTIR- and Raman-spectroscopy. The reaction of the monoclonal antibodies with Tumor Type M2-Pyruvate Kinase (Tu M2-PK) in BS was examined by ProteOn XPR36 (BioRad).

Results. Rigidity, viscosity, electrical conductivity of RBC, the indexes of aggregation and destruction were significantly higher in patients with CRC and the average diameter of the cell, polarizability, capacitance of membranes, the dipole moment, the amplitude of RBC deformation being lower than those in the controls ($p<0,001$-$0,05$). A correlations between electrical conductivity and the presence of metastases ($r=-0,68$, $p=0,02$), between polarizability and the lymph nodes damage ($r=-0,62$, $p=0,04$) were identified. The study of RBC by terahertz spectroscopy revealed the low levels of amplitude transmittance over the whole frequency range in patients with CRC compared to the control group ($p<0,001$-$0,05$). A tendency for increasing the refractive index of thin films obtained from the serum in patients with CRC was observed in considering the ellipsometric parameters ($p = 0,057$). The degree of heterogeneity, the thickness of the films were significantly higher in patients with CRC compared to the controls ($p=0,02$-$0,05$) in correlation with the last stage of the disease ($r = 0,448$, $p<0,05$). The intensities of the peaks of the IR spectra regions amide III (1250, 1310 cm^{-1}) and amide I (1685cm^{-1}) were greater in patients with CRC compared to the controls ($p<0,001$-$0,05$). The area of the intermediate peak of 1418 cm^{-1} was, however, lower in CRC ($p<0,05$). The areas of peaks (1005-1520 cm^{-1}) in Raman spectra were significantly lower in patients with CRC compared to the healthy ones ($p = 0,006$–$0,045$), correlating with the stage of the process ($r = –0,84$, $p = 0,002$), anti-tumor therapy ($r = 0,91$, $p<0,01$). Tu M2-PK levels in CRC terminal stages was above than those in initial ones and the controls ($p<0,0001$-$0,02$), correlating with the presence of metastases ($r = 0,76$,$p<0,01$).

Conclusion. There were significant differences in the optical parameters of RBC and BS of patients with CRC compared to the healthy ones, establishing the correlation of these parameters with disease stage, lymph node involvement, presence of metastases.

ASSOCIATION OF T-WAVE AXIS AND QTC INTERVAL WITH CSF1R AND CCR2 GENES POLYMORPHISM AND THE COMPONENTS OF METABOLIC SYNDROME IN THE GENERAL MALE POPULATION OF NOVOSIBIRSK

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Key words: *T-wave axis, QT_c interval, gene polymorphism, metabolic syndrome, general population*

Motivation and Aim: T-wave axis deviation and QT_c interval prolongation have predictive significance in relation to fatal cardiovascular events, including sudden cardiac death. Adverse electrophysiological changes in myocardium occur not only as a result of the rare congenital membrane channelopathies but mostly are secondary, due to the various pathological processes, including metabolic. They, in turn, may also be genetically determined. Therefore, electrical instability of the myocardium, obviously, has a multigenetic and multifactorial basis. It's interesting to study T-wave axis and QT_c interval association with CSF1R and CCR2 genes 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). In all patients' electrocardiograms T-wave axis in the frontal plane and QTc interval were measured. 393 people (after excluding cases with missing values in at least one controlled variable the data from 373 people was used) were random selected for defining 34293TC/CA (rs386693509) - CSF1R gene polymorphism and 419 people (after excluding cases with the absence of values of at least one controlled variable data from 393 persons was used) were random selected to determine 64V/64I (rs1799864) - CCR2 gene polymorphism. The components of the metabolic syndrome (obesity, hypertension, hypertriglyceridemia and hypo-α-lipoprotenemia) were evaluated according to the WHO criteria. Data analysis was performed in a multivariate linear regression model: T-wave axis or QTc interval were included as the dependent variable; age, CSF1R or CCR2 gene polymorphism and the components of the metabolic syndrome were simultaneously included as the independent variables.

Results: T-wave axis was independently associated with 34293TC/CA (rs386693509) - CSF1R gene polymorphism (p=0.034), obesity (p=0.008) and hypertriglyceridemia (p=0.018). QT_c interval was independently associated with a 64V/64I (rs1799864) – CCR2 gene polymorphism (p=0.04) and hypertension (p=0.0003).

Conclusion: The results indicate the prospects of the integral approach to the analysis of genetic, hemodynamic, metabolic and electrophysiological potential pro-arrhythmic factors.

THE SUPERFICIAL STEM CELLS CONTRIBUTE TO THE RENEWAL AND RESHAPING OF ARTICULAR CARTILAGE

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Key words: *articular cartilage/chondrocytes/stem cells*

Since it is neither innervated nor vascularized, articular cartilage has extremely little regenerative capacity. Recently, genetic tracing has revealed chondrocyte progenitors at the cartilage surface.

Here, we characterized these progenitors utilizing *in vivo* genetic approaches. H2B-GFP retention revealed that the superficial cells divide more slowly than underlying articular chondrocytes. Clonal genetic tracing demonstrated that superficial cells renew their number by symmetric division, express the mesenchymal stem cell marker CD73, and generate chondrocytes via both asymmetric division and symmetric differentiation, the classical behavior of adult stem cells referred to as population asymmetry.

Quantitative analysis of cellular kinetics in combination with PTA-enhanced micro-CT showed that superficial cells generate an excess number of chondrocytes, which are utilized for growth of the underlying epiphyseal bone, facilitating cartilage renewal.

We conclude that superficial cells are postnatal stem cells capable of maintaining their own population as well as generating chondrocytes. These cells both facilitate renewal of juvenile articular cartilage and provide chondrocytes for endochondral growth of underlying epiphyseal bone.

HEMOZOIN IS A POTENTIAL TARGET FOR DEVELOPING NEW OPISTHORCHIASIS/CLONORCHIASIS DRUGS

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Key words: *Opisthorchis felineus, Clonorchis sinensis, hemozoin*

Motivation and Aim: Many species such as *Plasmodium* spp., *Schistosoma* spp., *Fasciola hepatica* and *Echinostoma trivolvis* are blood-feeding parasites. The key physiological process for them is heme detoxification. The most prevalent pathway involves formation of iron-containing black crystals named "malaria pigment" or hemozoin (Hz). In addition Hz and its synthesis pathway are promising targets for new antiparasitic drug discovery. Our comparative histopathological studies of the hepatobiliary system during experimental opisthorchiasis led us to hypothesize that formation of Hz is likely a part of the nutrition process of *O. felineus* [1]. A comprehensive analysis of the gut contents in some members of the family Opisthorchiidae (*O. felineus* and *C. sinensis*) was the main aim of our study.

Methods and Algorithms: In this study we used a complex analytical approach combining different types of electron microscopy, spectroscopy, mass spectrometry (MS) and Fourier-transformed infrared (FTIR) spectroscopy to reveal the nature and the origin of the gut contents of members of the family Opisthorchiidae.

Results and Conclusion: Using transmission electron microscopy, we demonstrated for the first time the presence of disintegrating blood cells in the gut of *O. felineus* as well as electron-dense crystals in the gut of *O. felineus* and *C. sinensis.* Electron energy loss spectroscopy revealed iron atoms in these crystals, and MS of the purified pigment demonstrated the presence of heme. FTIR spectroscopy identified the signature peaks of the common iron-carboxylate bond characteristic in crystals isolated from *O. felineus* and *C. sinensis.* Scanning electron microscopy showed layered ovoid crystals of various sizes from 50 nm to 2 μm. Morphological, chemical and paramagnetic properties of these crystals and its biocrystallization were similar to those of hemozoin from *Schistosoma mansoni*. Our results show that the diet of *O. felineus* and *C. sinensis* includes blood and detoxification of the free heme produced during the digestion proceeds via formation of crystals of hemozoin on the surface of the lipid droplets, similar to *S. mansoni*. These data advance the understanding of the processes taking place in the host-parasite system during infection caused by *O. felineus* or *C. sinensis*. Because formation of Hz is specific to blood-feeding parasites, our findings offer new avenues for the control and treatment of human opisthorchiasis/clonorchiasis. Moreover these data may reveal potential new targets enabling rational drug-design approaches directed not only against formation of Hz but also against other mechanisms involved in heme detoxification in *O. felineus* and *C. sinensis*.

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CLINICAL EFFICACY OF BIOMEDICAL CELLULAR PRODUCTS IN EXPERIMENTAL BOWEL INFLAMATION DESIASE

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Key words: *bone marrow-multipotent mesenchymal stromal/stem cells, bowel inflammation, multipotent mesenchymal stromal/stem cells-conditioned media*

Motivation and Aim: Therapy by multipotent mesenchymal stromal/stem cells (MMSC) is positioned as a new and promising method for the treatment of several inflammatory and degenerative processes (1).

Methods and Algorithms: Experimental inflammatory process in the intestine of male mice C57Bl6 induced a 3.5% solution of dextran sulfate, added to drinking water (2). $2x10^5$ BM-MMSC derived from GFP-B6 mice or 200 µl of the conditioned media from the MMSC (CM-MMSC) was injected to some animal.

Results: On day 21 of the experiment in the control group of mice with inflammatory process in the intestines, induced by a solution of dextran sulfate in the lamina propria of the mucosa of the base of the villi of the small intestine remained the areas with lymphocytic infiltration, with an increased content of intraepithelial lymphocytes. In rare cases administration of BM-MMSC lead to accumulation of neutrophils in the lamina propria of mucosa and lymphocytes infiltration in intestinal villi. In the group of mice that received the therapy of the CM-MMSC, identified areas with inflammatory infiltration and marked the high content of intraepithelial lymphocytes. Therapy by BM-MMSC or by CM-MMSC leads to a greater extent with the introduction of the CM-CMMS, at the base of intestinal crypts observed an increased content of Panetta cells and more mitoses in the crypts, in comparison with the small intestine of control animals. The results of the morphological analysis indicate that the structure of the small intestine in the group of mice that received CM-MMSC is more consistent with the structure of the intact intestine. In addition, the administration of BM-MMSC or the CM-MSCS showed restoration of homeostasis of the small intestine.

Conclusion: Thus, the therapeutic efficacy of BM-MMSC or CM-MSCS in inflammatory processes in the gut has been shown.

Availability: So, BM-MMSC or CM-MMSC may be used for therapy in animals and humans with gastroenterology pathology.

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EXPERIMENTAL MODEL OF BILE DUCT CANCER: EXPRESSION OF CHOLANGIOCARCINOMA GENES-MARKERS

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Key word: *Opisthorchis felineus, cholangiocarcinoma, biomarkers, RT-PCR*

Motivation and Aim: It is established to be a strong association between bile duct cancer and parasitic infestation of the liver caused by trematodes of the family Opisthorchiidae. We have recently demonstrated that cholangiocarcinoma can develop in Syrian hamsters (*Mesocricetus auratus*) infected by *Opisthorchis felineus* and administered with dimethylnitrosamine (DMN). The aim of this work was to study the expression genes – potential markers of cholangiocarcinoma on our experimental model.

Methods: Our study showed that hamsters, which underwent the complex influence of *O. felineus* and DMN, demonstrated development of mass-forming cholangiocarcinoma in thirtieth week [1]. Type of tumor was determined using histological techniques. After RNA extraction from all specimens, real-time PCR was carried out for measurement of expression levels of following mRNA: Tert, Tp53, Tgfb1, Osbpl8, Anxa1, Ext1, Krt7. Additionally, mRNA expression levels of Tgfb1, Anxa1, Ext1, Krt7 were measured in hamster livers from all experimental groups [1]. To compare the data between groups, statistical significance was determined by Mann-Whitney U test ($P < 0,05$).

Results: It was shown that the expression of all investigated mRNA was increased in cholangiocarcinoma tissue. Besides, some statistical significant data of mRNA expression levels were found for experimental groups [1]. The increased expression levels of *Anxa1* and *Krt7* in liver of DMN-treated hamster were observed. The up-regulated expression of *Tgfb1*, *Anxa1*, *Ext1*, *Krt7* in liver of *O. felineus*-infected hamster was registered. The expression levels of *Tgfb1*, *Anxa1*, *Krt7* in liver of *O. felineus*+DMN-treated hamster were also increased.

Conclusion: The findings indicate that increased levels of mRNA considered as cholangiocarcinoma markers for humans have been revealed in our experimental model [1]. Thereby, the model can be used for translational studies of mechanisms of bile duct cancer development.

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REGULATORY SINGLE NUCLEOTIDE POLYMORPHISMS (RSNPS) AT THE PROMOTERS 1A AND 1B OF THE HUMAN APC GENE

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Key words: *regulatory SNPs, APC, promoters, EMSA, luciferase reporter, oncopathology*

Background: Germline mutations in the coding sequence of the tumour suppressor APC gene give rise to familial adenomatous polyposis (which leads to colorectal cancer) and are associated with many other oncopathologies. The loss of APC function because of deletion of putative promoter 1A or 1B also results in the development of colorectal cancer. Since the regions of promoters 1A and 1B contain many single nucleotide polymorphisms (SNPs), the aim of this study was to perform functional analysis of some of these SNPs by means of an electrophoretic mobility shift assay (EMSA) and a luciferase reporter assay.

Results: First, it was shown that both putative promoters of APC (1A and 1B) drive transcription in an in vitro reporter experiment. From eleven randomly selected SNPs of promoter 1A and four SNPs of promoter 1B, nine and two respectively showed differential patterns of binding of nuclear proteins to oligonucleotide probes corresponding to alternative alleles. The luciferase reporter assay showed that among the six SNPs tested, the rs75612255 C allele and rs113017087 C allele in promoter 1A as well as the rs138386816 T allele and rs115658307 T allele in promoter 1B significantly increased luciferase activity in the human erythromyeloblastoid leukaemia cell line K562. In human colorectal cancer HCT-116 cells, none of the substitutions under study had any effect, with the exception of minor allele G of rs79896135 in promoter 1B. This allele significantly decreased the luciferase reporter's activity

Conclusion: Our results indicate that many SNPs in APC promoters 1A and 1B are functionally relevant and that allele G of rs79896135 may be associated with the predisposition to colorectal cancer.

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EFFECTS OF THE COMPLEX OF MELATONIN, ALUMINIUM OXIDE AND POLYMETHYLSILOXANE ON THE LIVER STRUCTURE IN TYPE 2 DIABETIC MICE

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Key words: *diabetes, liver, apoptosis*

Motivation and aim. A growing body of evidences gives support the notion that pineal hormone melatonin may mitigate the dysfunction of β-cells and delay the development of diabetes and its complications [1]. The ability of melatonin to attenuate progression of non-alcoholic fatty liver disease was suggested recently [2]. In this study we examined the long-term effects of melatonin, aluminium oxide and polymethylsiloxane (complex M) [3] on structural changes in the liver in *db/db* mice, a model of type 2 diabetes.

Methods. Eight-week-old female diabetic *db/db* mice *(BKS.Cg-Dock7^{m}+/+Lepr^{db}/J)* were treated with complex M (0,665 mg/kg per day by gavage) or distilled water for 8 weeks. Structural changes in the liver were analyzed quantitatively from the light and electron microscopic images. Immunohistochemical evaluation of the apoptosis markers (Bcl-2 and Bad) was performed on paraffin sections using streptavidin-biotin indirect method using a kit *Novostain 500, NCL-RTU-D*; staining for LYVE-1 was detected with antibodies *Isotype: Rabbit polyclonal, bs-1311R; Bioss*.

Results. Complex M-treated mice as compared to placebo-treated animals demonstrated attenuated lipid accumulation and retarded structural changes in the liver. The areas of immunohistochemical staining for LIVE-1 in liver sinusoidal endothelial cells increased significantly in mice received the complex M. The ratio of apoptosis regulating proteins shifted toward the prevalence of anti-apoptotic protein Bcl-2 upon pro-apoptotic protein Bad.

Conclusions. Complex of melatonin, aluminum oxide and polymethylsiloxane alleviates structural changes in the liver in type 2 db/db diabetic mice. The effect of the complex could be mediated by suppression of apoptosis and improvement of lymphatic draining in the liver.

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CIRCULATING DNA AS A SOURCE OF NOVEL TYPE OF CANCER BIOMARKERS

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Key words: *circulating DNA, epigenetics, fragmentomics, 8-oxo-dG*

Motivation and Aim: An assesement of cell-free circulating DNA (cfDNA) fragments, or Liquid biopsy, is indeispensable for early diagnosi and non-invasing monitoring of cancer. However, the majority of the cfDNA studies aim at relatively simple search for cancer-driving mutations or particular variants associated with susceptibility and resistance to targeted therapy. Important, understudied properties of cfDNA may become either a treasure trove for mining of novel cancer biomarkers or even indicate that cfDNA, by itself, could be a therapeutical target.

Results: cfDNA fragments are biologically active as they are enriched in 8-oxo-dG. The oxidized DNA is a stress signal released in response to oxidative stress. It might contribute to systemic abscopal effects of localized irradiation treatments. The mass release of oxidized DNA that accompanies apoptotic and necrotic processes in radio- and chemotherapy treated tumors may aid survival of residual cancer cells and even instigate their resistance to further treatment. The selective removal of oxidized DNA from the bloodstream or the block of respective oxidized DNA-dependent signaling may be developed as an adjuvant treatment. Moreover, the fragmentation patterns of cfDNA are non-random. They reflect fragmentation of DNA during apoptosis that in turn, may associate with epigenetic landscapes. Circulating nucleotide fragments copy number depends on the nucleosomal positioning in given DNA locus. PCR primer systems may be tuned to the regions that would produce higher DNA amplification outcomes. Sensitivity of detection can be increased by simultanious isolation of "free" DNA molecules and these adsorbed to cells' surface.

Conclusion: The prevalence of certain DNA fragments may directly reflect nucleosome positioning within certain loci and serve as a proxy for gene expression levels. This opens a novel field in biomarker research, tentatively called "fragmentomics".

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BIOMARKERS OF AGE IN THE "STATIONARY PHASE AGING" MODEL

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Key words: *biomarkers of aging, senescence-associated β-galactosidase, cytogerontology, stationary phase aging, cell senescence, 8-oxo-2'-deoxyguanosine*

Motivation and Aim: Currently gerontologists search for biomarkers of aging (including cell aging) which can allow determining age of organism/cell quickly and easily. Senescence-associated beta-galactosidase (SA-β-Gal) remains the most popular biomarker of cell aging. Nowadays, another biomarker, 8-oxo-2'-deoxyguanosine (8-oxo-dG), is becoming popular in gerontology. We have investigated applicability of these markers to our "stationary phase aging" model, i.e. increase in the probability of dying for cultured cells upon retardation and subsequent complete cessation of their proliferation within one passage.

Methods and Algorithms: Experiments were performed on transformed Chinese hamster cells (B11-dii FAF28 line, clone 237). The cells were cultivated for 14-15 days at 37°C in Carrel glass flasks using DMEM supplemented with 10% bovine serum and antibiotics. In the first series of experiments on the 4th, 8th, and 15th day contents of 8-oxo-dG and dG in DNA hydrolyzate were analyzed chromatographically using Beckman-Gold chromatograph (USA) at a wavelength of 254 nm. In the second series of experiments on the 7th and 14th day the cells were fixed for 3-5 minutes in 2% formaldehyde and 0.2% glutaraldehyde and incubated with X-Gal for 12-16 hours at 37°C.

Results: It was found that the ratio of 8-oxo-dG/dG increased with the "age" of cell culture. On the 15th day the cells became to die and the ratio had significantly increased ($22.40 \cdot 10^{-5}$) compared to this index on the 4th ($6.26 \cdot 10^{-5}$) and on the 8th ($4.42 \cdot 10^{-5}$) days when the cells had reached monolayer and gone into the stationary phase of growth. It was also found that 14-day-old culture had much higher percentage of cells staining for SA-β-Gal than the «young» (7-day-old) cells.

Conclusion: Thus, 8-oxo-dG accumulates in the stationary phase aging culture of Chinese hamster cells as evidenced by a significant increase in the ratio of 8-oxo-dG/dG in DNA of the cells on the 15th day. Consequently, it is possible to predict an increase in the probability of death in the cell culture evaluating expression of this biomarker. Furthermore, stationary phase aged cells express SA-β-Gal demonstrating a good correlation of this parameter with "age" of cell culture. We believe that both methods can be used to determine the biological age of cells in testing of new potential geroprotectors.

PREIMPLANTATION GENETIC SCREENING USING NGS

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Key words: *preimplantation genetic screening (PGS), preimplantation genetic diagnostics, aneuploidy, assisted reproductive technologies, IVF, comparative genomic hybridization (CGH), next-generation sequencing (NGS)*

Motivation and Aim: Chromosomal abnormalities are the main reason of spontaneous abortion of embryos with normal morphology. PGS allowed to augment the efficiency of IVF by increasing implantation rates and reducing the frequency of early pregnancy loss. The purpose of this study was to determine whether NGS could accurately detect aneuploidy in human embryos concomitantly with array-CGH.

Methods and Algorithms: Trophectoderm biopsy embryo cells of 38 embryos were obtained at 5-6 days of development. Whole genome amplification was performed using the WGA-PCR PicoPlex SingleCell WGA Kit (Rubicon Genomics, USA) and the MDA GenetiSure Pre-Screen Amplification and Labeling Kit (Agilent, USA).

aCGH: Labeling was carried out with the SureTag DNA labeling Kit Agilent (USA). Biochip Sure Print G3 8x60 aCGH Agilent (USA) and SureScan Microarray Scanner were used for aCGH analysis. Array analysis and interpretation of results were made with Agilent CytoGenomics software.

For NGS: WGA and MDA products were enzymatically fragmented. Sequencing libraries were prepared by the Life Technologies Ion Xpress Plus Fragment Library according to preparation guide. Samples were barcoded and pooled into 30 sample multiplexes. The sequencing runs were performed with 200 bp chemistry using the Ion PI™ Sequencing 200 Kit v3, Ion PI™ Chip v2 and Ion Proton sequencer. Bioinformatics analysis was carried out by in house developed protocol, which included filtering non-unique genome regions, GC correction and coverage normalization. 441 776 – 6 025 611 reeds per sample (median 4 112 701) was obtained for each sample. The presence of aneuploidy was determined by the value of the Z-score (> 3 < - 3) and the normalized chromosome coverage. The presence of Y was determined by Y-chromosome coverage.

Results: The results of 36 (95%) embryos match exactly for NGS and array-CGH.

Conclusion: PGS using NGS can also be used for aneuploidy analysis along with array-CGH.

RANDOM FOREST ALGORITHM PROVIDES ACCURATE PREDICTION OF GLUCOSE TRENDS IN DIABETIC SUBJECTS

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Key words: *diabetes, continuous glucose monitoring, data mining, Random Forest*

Motivation and aim: Continuous glucose monitoring (CGM) and new mathematical approaches for analysis of glucose fluctuations open up new possibility for prediction of glucose trends. The aim of the study was to test the application of Random Forest [1] algorithm for predicting of interstitial glucose values in diabetic subjects.

Materials and methods: A CGM database, obtained from patients with both type 1 and type 2 diabetes, via the Medtronic CGM System Gold device, was used. To develop glucose prediction algorithm 1080 records were chosen randomly from the database. After primary processing selected files underwent the procedure of secondary signs generation by a method which can be called "ladder" [2]. Further processing of the data array was performed using the universal Random Forest method. The original algorithm based on the Random Forest was developed in the R environment via the RSTURIO software, and then it was embedded in an application running on the Java language and the R Apache platform. The algorithm was trained individually for each case on a set of randomly selected segments of the CGM record that represents 50% of the entire recordings. Subsequently, the algorithm was verified on the data not included in the training set. Model adequacy was evaluated by the mean square error of all the predicted values. The application allows you to upload CGM data and to get the prediction, so the time between file uploading and prediction appearance was measured.

Results: At the predictive window of 50-120 min an adequate prediction was obtained for all CGM fragments. The maximum running time was 1.83 seconds. The mean prediction error was 8.8±1.4 mg/dL.

Conclusion: Random Forest algorithm can be employed successfully to predict CGM values at the predictive window of 50-120 min. Server-based application like this could be used in close-loop insulin-delivery systems.

Availability: server part of the program that handles client requests: https://github.com/tabakovKonstantin/DiabetesRestServ; server for calculating:

https://github.com/tabakovKonstantin/DiabetesRApacheS; the client application: https://github.com/tabakovKonstantin/DiabetesClient?f; details of the program (in Russian): https://www.dropbox.com/s/we3ryf0t3v4lgb4/Text_Tabako.

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ALGORITHMIC ADJUSTMENT OF INSULIN REGIMEN REDUCES GLUCOSE VARIABILITY IN PATIENTS WITH TYPE 1 DIABETES IN HOSPITAL SETTINGS

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Key words: *diabetes, glucose variability, insulin, continuous subcutaneous insulin infusion*

Motivation and aim: High glucose variability (GV) has been identified recently as a risk factor for cardiovascular disease in diabetic subjects [1, 2]. Besides, increased GV is associated with hypoglycemic events [3]. The aim of the study was to evaluate the possibility of reduction of GV in hospitalized patients with type 1 diabetes using stepwise adjustment of insulin regimen.

Materials and methods: We observed 93 patients with type 1 diabetes (38 M/55F) aged 19-76 years, on multiple daily injections (MDI) or continuous subcutaneous insulin infusion (CSII), in hospital settings. The average hospital stay was 12-14 days. Using the 7-point glucose monitoring data, the mean blood glucose, Mean Amplitude of Glycemic Excursions (MAGE), Mean Absolute Glucose (MAG) and Lability Index (LI) was calculated for every 3 days. Regular adjustment of insulin regimen was based on these data and included (among other options) switching from MDI to CSII for patients with high GV.

Results: At the moment of hospital admission 8 patients were on CSII, while others were on MDI insulin dose varied in the range of 0.3-1.2 U/kg,. Diabetes duration was 1-50 years. At the discharge, 34 patients were on CSII and 59 patients remained on MDI. Baseline LI, MAGE and MAG correlated with diabetes duration ($r=0.31$, $r=0.36$, $r=0.3$, resp.) and with HbA1c level ($r=0.46$, $r=0.41$, $r=0.37$, resp.), all $p<0.001$. At days 9-12 the mean glucose dropped by 1.16 mmol/L, MAGE by 1.21 mmol/L, LI by 1.19 $(mmol/L)^2$/hour and MAG by 0.23 mmol/L/hour as compared to days 1-3 (all $p<0.0001$). In patients with baseline MAGE >5 mmol/L decrease in mean glucose and GV parameters was much more (7-8-fold) pronounced than in others (all $p<0.0001$). However, GV at day 9-12 did not depend from insulin regimen, diabetes duration or HbA1c level.

Conclusion: In most patients with type 1 diabetes a significant reduction of GV can be achieved by stepwise adjustment of insulin regimen in hospital settings, with the greatest effect observed in those with initially high GV.

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SOMATIC DNA METHYLATION LANDSCAPE OF CORONARY ARTERY DISEASE PATIENTS

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Key words: *DNA methylation, copy number variation, coronary atherosclerosis, vascular tissues, microarrays*

Motivation and Aim: Epigenetic mechanisms of gene regulation in context of cardiovascular diseases are of considerable interest. We hypothesize that epigenetic heterogeneity of arteries can contribute to regional vascular bed differences in susceptibility to atherosclerosis. So far, our current knowledge of the DNA methylation profiles for atherosclerosis affected and healthy human vascular tissues is still limited.

Methods and Algorithms: The Illumina HumanMethylation27 BeadChip microarray was used for DNA testing from right coronary arteries in the area of advanced atherosclerotic plaques (CAP) and healthy internal mammary arteries (IMA) of six patients with coronary artery disease. The DNA methylation of CpG-sites located within *MIR10B, PNPLA2* and *GNAS (NESP55)* genes was further quantified by bisulfite pyrosequencing in paired vascular tissue samples of fifty patients. Statistical analysis was carried out using WEB-based GEne SeT AnaLysis Toolkit and R Software.

Results: The first step included DNA methylation screening of more than 27000 CpG-sites in twelve samples from six patients (CAP and IMA from the same patient in order to avoid the effect of interindividual variation). The resulting DNA methylation patterns were markedly different between atherosclerosis affected and healthy vascular tissues. A total 358 CpG sites showed significant differences in DNA methylation (FDR adjusted $P<0.05$), defined as a mean methylation difference of at least 20% between CAP and IMA samples. The most representative terms in KEGG included neuroactive ligand-receptor interaction (16 genes; FDR adjusted $P=0.02$). Forty differentially methylated genes have previously been linked to atherosclerosis related diseases in candidate-gene based association studies, but most of the loci were not previously connected to the disease. The next step was to validate and replicate results concerning DNA methylation of some relevant loci from received list by bisulfite pyrosequencing in paired vascular tissue samples of fifty patients. We confirmed DNA methylation pattern of three genes: *MIR10B* was markedly hypomethylated in CAP, and *PNPLA2* and *GNAS (NESP55)* were hypermethylated CAP in comparison with IMA.

Conclusion: These results highlight the importance of DNA methylation changes in the pathogenesis of atherosclerosis. Novel candidate genes and biological pathways for future study have been identified. The study was supported by the Russian Science Foundation (№16-15-10150).

STRATEGIES FOR MATURATION AND ANTIGEN LOADING OF DENDRITIC CELLS FOR ANTI-CANCER IMMUNOTHERAPY

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Key words: *dendritic cells, DNA delivery, anti-cancer immunotherapy*

Motivation and Aim: Dendritic cell (DC) immunotherapy is supposed to be a perspective method for anti-cancer therapy. Efficient DC induction *in vitro* demands efficient delivery and expression of tumor antigens as well as efficient maturation strategies to obtain mature DC [reviewed in 1]. We performed comparison of four maturation strategies and different methods of DC transfection in order to induce anti-tumor T cell response.

Methods: Immature DC (iDC) were generated from monocytes using GM-CSF and IL-4. iDC were loaded with MCF-7 lysate and then matured with one of four selected maturation strategies: (1) TNF-a; (2) LPS and IFN-g; (3) TNF-a, IL-1², IFN-g, R848, PGE2; 4) TNF-a, IL-1², IFNa, IFN-g, poly I:C. Thus, four types of DC were generated: DC1, DC2, DC3 and DC4, respectively. Expression of CD83, CD86, CD14, HLA-DR during DC maturation was confirmed by flow cytometry (Cytomics FC 500, "Beckman Coulter Inc."). T cells were obtained from peripheral blood mononuclear cells using EasySepkit (StemCell). Cytokine production was evaluated by ELISA (Vector-Best). The number of antigen specific T cells was analyzed by IFN-γ ELISPOT assay. Efficacy of GFP encoding DNA delivery into DC by Lipofectamine 2000, Metafectene Pro, MATra reagent and using alphavirus vectors SFV and SIN also were studied.

Results: DC3 and DC4 were shown to express higher levels of CD83 and CD86 as well as they produced maximal levels of IL-6 and minimal levels of immunosuppressive IL-10. DC3 and DC4 stimulated proliferation and Th1-polarisation of T cells. T cells activated by DC4 showed a larger number of IFN-γ-producing T cells against MCF-7 breast cancer cells. Maximal rate of GFP-positive viable DC was observed for MATra reagents. Virus vectors were shown to possess a lover efficacy in DC transfection inducing cell death.

Conclusion: DC maturation with TNF-a, IL-1², IFNa, IFN-g, poly I:C is the most attractive strategy for future clinical trials in cancer immunotherapy. Magnetic transfection with MATra was shown to be the more preferential approach for loading of DC with DNA encoding tumor antigens.

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MATHEMATICAL MODEL OF INTERSTITIAL FLUID DYNAMICS WITH CONSIDERING OF TRANSCAPILLARY TRANSPORT AND LYMPHATIC DRAINAGE

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Key words: *mathematical modeling, edema, interstitial fluid pressure, protein concentration*

Motivation and Aim: Microcirculation is one of the key elements of the human cardiovascular system, since most of the exchange of nutrients and decay products is carried out at the level of the smallest vessels.

The individual processes involved in interstitial fluid volume and protein regulation (microvascular filtration, lymphatic return, and interstitial storage) are relatively simple, whereas their interactions are exceedingly complex. There is a notable lack of the first-order method that relates interstitial fluid pressure and protein to critical parameters commonly used to characterize the movement of interstitial fluid and protein [1]. Therefore, the purpose of the present study is to develop a simple, transparent, and general algebraic approach that predicts interstitial fluid pressure (Pi) and protein concentrations (Ci) and takes into consideration all three processes.

Methods and Algorithms: In this paper we consider for a non-Newtonian fluid through the blood capillary and the processes occurring in the surrounding capillary tissue modeled as proper environment. These two tasks are connected using boundary conditions based on the hypothesis of transcapillary transport by Starling. We discussed one-dimensional unsteady problem of fluid flow in tissue with consideration of transcapillary metabolism and lymph fluid drainage from tissue. It is proposed that the new method of lymphatic drainage of fluid from the tissue based on physiological data lymph fluid.

Results: The present work provides a predictive characterization of interstitial fluid balance resulting from the interaction of microvascular, interstitial, and lymphatic function. This approach has made advances to conventional fluid balance models because does not neglect lymphatic function and its effect on interstitial fluid pressure; does not assume that interstitial fluid pressure or protein concentration is constant; characterizes fluid balance in terms of resistances to fluid transport; provides a general algebraic solution in terms of microvascular and lymphatic parameters.

Conclusion: It is possible that the three parameters: the microvascular filtration coefficient, effective lymphatic resistance, and interstitial compliance interact with each other, resulting in nonlinear behavior.

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TREATMENT OF PATIENTS WITH LYMPHEDEMA OF SCROTUM AND PENIS

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Key words: *lymphedema scrotum penis*

Motivation and Aim: Primary lymphoedema scrotum and penis may develop at different ages and has approximately the same distribution frequency for men. According to the literature, the number of observations of these patients by different authors is usually small, ranging from isolated cases to 10-30. The urgency of this problem is caused by several factors. Recurrent erysipelas may cause a marked increase in the volume of the affected tissues accompanied by limphorrhea. Patients showed a significant decrease in quality of life limiting among others, and sexual function. Surgical treatment is often associated with intraoperative blood loss and postoperative infectious complications, especially for a large volume of the affected tissues. Lack of adequate compression therapy leading to a further increase is the cause of relapse. The aim of the study was to investigate the immediate and long-term results of treatment of patients with primary lymphedema of the external genitalia.

Methods and Algorithms: 8 patients with primary lymphedema of the external genitalia aged 14 to 52 years have been observed from 2001 to 2013. Recurrent erysipelas and balanoposthitis occurred in 4 patients (2 men with lymphorrhea, one patient with papillomatosis of the scrotal skin). Three patients with primary lymphedema of scrotum and penis was undergone surgical debalking of scrotum and circumcision. surgical treatment was limited by circumcision alone in one patient.

Results: One man had the penis skin necrosis in the postoperative period, he also underwent the re-resection operation on the scrotum, resection of soft tissue of the penis in year after surgery. The plastic surgery with excision of the scar and the penis formation, the displaced flap of the right iliac region has been performed in connection with the formation of rough scar on the penis after 2 years.

Conclusion: Surgical treatment of primary lymphedema of external genitalia requires surgery with the significant increase of the affected tissues, recurrent erysipelas in remission. Complex decongestive treatment is useful in case of huge volume of affected tissues.

It is necessary to continue the affected organ bandaging in the early postoperative period. Medical compression hosiery with distributed pressure in the perineum must be applied during outpatient treatment stage.

PROTEOMIC SCREENING FOR AMYLOID-FORMING PROTEINS IN BACTERIA *ESCHERICHIA COLI*

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Key words: *amyloid, E.coli, HPLC, MALDI, protein identification*

Motivation and aims: Amyloids represent protein fibers exhibiting cross-beta structure. They are found in different organisms, from bacteria to human, and can be pathogenic, useless, and functional. Functions of amyloids in bacteria spread from biofilm formation to parasite-host interaction. Importantly, bacterial amyloids identified to date were found accidentally, and screenings for amyloid-forming proteins in the proteomes of these organisms wцere never carried out before. The goal of this study was to implement a proteome-wide screen for candidates for amyloid-forming proteins in well-known prokaryote *Escherichia coli*.

Methods: Screening for candidates for novel amyloid-forming proteins was performed with previously developed PSIA (Proteomic Screening and Identification of Amyloids) approach [1] improved by HPLC-separation of the tryptic peptides [2].

Results: We identified 61 detergent-resistant proteins. This protein set was 3-5 fold enriched with potentially amyloidogenic regions predicting by different bioinformatics algorithms (WALTZ, SARP) in comparison with the entire *E. coli* proteome. 56 of 61 proteins contain potentially amyloidogenic regions, and four (BcsC, MukB, YfbK, and YghJ) carry low-complexity N- and Q-rich regions that is the hallmark of a number of known amyloid-forming proteins [2].

Conclusion: There is unexpected diversity of *E.coli* proteins forming detergent-resistant aggregates *in vivo* at the physiological level of expression. These proteins are rich in potentially amyloidogenic low-complexity regions.

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EXPRESSION OF THE LYMPHATIC MARKERS (LYVE-1, PROX-1, PODOPLANIN) IN HUMAN EYES

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Key words: *lymphatic system, human eye, ciliary body, choroid, lymphatic capillaries, lymphatic channels*

Motivation and Aim Until recently, the presence of lymphatic outflow of intraocular fluid was controversial. After the discovery of molecular markers of lymphatic endothelial cells it became possible to identify the lymphatic capillaries and vessels in the various structures of the human eye in normal and pathological conditions. Participation of the lymphatic system in the outflow of intraocular fluid is interesting, in particular, for the understanding of the pathogenesis of glaucoma and treatment development. The aim of this work was to identify the structural components of the lymphatic drainage of the human eye in normal conditions.

Methods and Algorithms The object of study were enucleated fragments of medical patients' eyes (n = 5) without pathology of the vision. For morphological study of eye the samples were treated according to standard procedures for light and electron microscopy. Paraffin sections were stained with monoclonal antibodies against vascular endothelial markers CD34 (Novocastra, Germany), lymphatic lymphatic endothelial markers LYVE -1 (Abcam, England), Podoplanin (Monosan, Netherlands) and Prox-1 (Covance, Germany), a marker for fibroblast growth factor receptors FGFR (Abcam, England) and examined under a light microscope "Leica DME» (Germany). Ultrathin sections were examined in an electron microscope «JEM 1010» (Japan).

Results Using immunohistochemistry and electron microscopy studies were revealed organ-specific lymphatic capillaries (LYVE-1 +, Prox-1 +, Podoplanin +) in the ciliary body and the optic nerve sheath of the human eye in normal condition and showed the presence of structured interstitial spaces, limited by collagen fibers and fibroblasts. The lymphatic channels were shown in the choroid of human eyes. The channels are formed by fibroblast-like cells and pigment cells, and spaces bounded by elongated cells - so-called "lacunae", which presumably are considered as the lymphatic structures. At the same time revealed capillaries and lacunae did not show positive CD34 immunohistochemical staining.

Conclusion and Availability Organ-specific lymphatic capillaries were revealed in the ciliary body and the optic nerve sheath. In the choroid of normal human eyes were identified lymphatic channels and lacunae by using lymphatic endothelial markers LYVE-1, Podoplanin and Prox-1.

POLYMORPHISMS IN THE GENES OF VASCULAR ENDOTHELIAL GROWTH FACTOR ARE ASSOCIATED WITH THE EARLIER ONSET OF RHEUMATOID ARTHRITIS

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Key words: *rheumatoid arthritis, SNP, VEGF A, rs3025039, rs699947*

Motivation and Aim: Genetic factors are involved in the developing of rheumatoid arthritis (RA). In RA pathogenesis a pannus formation play a crucial role. One of the factors contributing to this process is the vascular endothelial growth factor (VEGF). The existence of an association between the VEGF SNP and RA progress is supposed. The aim of this study was to investigate the involvement of VEGF+C936T (rs3025039) and VEGF-C2578A (rs699947) SNPs in developing of RA.

Methods and Algorithms: 229 Europeoid women with RA were included in our study. Patients had American College of Rheumatology (ACR)-defined RA (1987 classification criteria). The genotyping was performed by restriction fragment length polymorphism analysis of PCR-amplified fragments (PCR-RFLP), furthermore the age of disease onset was determined. Description analysis and Mann—Whitney U-test were employed for statistical processing the results.

Results: No differences were found between groups with VEGF-C2578A (rs699947) SNPs. The age of disease onset was significantly different between group with various genotypes for VEGF+C936T SNPs: 42,5 years for VEGF+936CC compare with 47,8 years for VEGF+936CT (p-value=0,015). 168 (73,36%) and 59 (25,76%) patients had VEGF+936CC and VEGF+936CT genotype, respectively. The last mentioned are consistent with other investigator's results. Two patients (0.88%) had VEGF+936TT genotype and this small group wasn't included in analysis.

Conclusion: Analysis of vascular endothelial growth factor polymorphisms (rs3025039) may be useful in clinical practice for evaluation of predisposition to earlier RA.

NUCLEOTIDE SEQUENCE COMPLEXITY MEASURES AND SNP CONTAINING SITES IN HUMAN GENOME

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Key words: *genome, text complexity, Hurst exponent, nucleotide polymorphisms*

Motivation and Aim: Here we consider applications of text complexity measures to genomic sequences and SNP containing sites. The study of genomic context of single nucleotide polymorphisms (SNPs) represented in the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/) is of greater interest. Association of SNP position in human genome with mononucleotide repeats was shown earlier. We updated context analysis of SNP containing sites using novel software based on text complexity estimates and extended applications of SNP analysis from human "1000 genomes" data to rat and mouse genomes.

Methods and Algorithms: Pre-processing and data filtration is important for effective detection of single nucleotide polymorphisms, detection of transcription factor binding sites. It was earlier shown that low complexity sequence regions, poly-tracks and simple repeats are related to systematic errors in genome sequence reads mapping and interpretation of sequencing results. The sequence complexity measures could be roughly dived to entropy estimates, linguistic complexity and algorithmic estimates including Lempel-Ziv compression method [1]. The measures of data quality are especially important for variant calling: in the particular case of SNP calling, a great number of false-positive SNPs may be obtained.

Results: We have developed several measures to distinguish between sequence errors and candidate SNPs, based on a base call's nucleotide context and its mismatch type. The project aim is to realize and apply algorithms of DNA sequence complexity estimates to analysis of sequencing in human genome and in model organisms.

Conclusion: Low complexity profiles keep more information extending just measures of mononucleotide patches. The irregularities of mutation hot-spots in genome have been shown earlier on a limited data. The molecular mechanism of the observed effect of lowering the text complexity on flanks of SNP genome position can be explained by the increased frequency of double-helix DNA breaks in flanking positions [2].

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DIOXIN-MEDIATED UPREGULATION OF ONCOSTATIN M IN U937 MACROPHAGES

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Key words: *Oncostatin M, macrophage, dioxin, AhR, cytokines*

Motivation and Aim: The environmental pollutant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin) is the most toxic among the dioxin xenobiotics and induces a broad spectrum of biological effects, including immunotoxicity and cancer [1]. Macrophages are key regulators of the innate immune response, as well as one of the first types of cells responding to stress, so the study of dioxin action in these cells is important. It is known that TCDD exposure effects some cytokines expression [2] but our analysis [3] showed that the list of such cytokines is not yet completed. We have investigated an effect of TCDD on Oncostatin M (OSM) expression in U937 macrophages.

Methods and Algorithms: Real-time PCR experiments were performed to investigate OSM mRNA expression dynamics at 6 and 24 hours after TCDD exposure in U937 macrophage-like cells.

Results: The data obtained demonstrate that OSM is upregulated after 6h of TCDD exposure, and maintains its overexpression after 24 hours. Transcription factor AP-1 is known to be the activator of OSM expression in macrophages [4]. We have shown that *FOSB* and *FOSL2* genes, coding AP-1 subunits, are upregulated simultaneously with OSM in U937.

Conclusion: Oncostatin M is supposed to play fundamental roles in mechanisms of inflammation in pathology [5]. Predicted activation of Oncostatin M expression in monocytes/macrophages, which are a primary source of OSM, can explain a spectrum of biological effects of AhR ligands exposure, including immunotoxicity and cancer.

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TO THE PROBLEM OF AT-ENRICHMENT OF GENOME REGULATION AREAS

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Key words: *DNA physical properties, electrostatics, transcription regulation, genome evolution*

Motivation and Aim: Here we offer an approach to the subject based on the DNA physical properties that provide its transcription regulation functionality.

Methods and Algorithms: DEPPDB and its tools [1,2] were used to carry out the analysis.

Results: Genome DNA physical properties define its shape in the functional space and influence its interactions with proteins, esp. for transcription regulation. DNA is highly charged and electrostatics contributes greatly to the subject, as well as other nucleotide content-based DNA physical properties such as curvature, bendability and thermal stability. E. potential (EP) is distributed non-uniformly along DNA molecule and correlates with GC content, strongly depending on sequence composition. Measured binding frequency of RNA polymerase to DNA correlates to the calculated EP.

Binding sites of transcription factors are located in long areas of high EP. EP distribution on transcription factors protein molecule surface reflects that of their binding sites. Promoters in average have high value and heterogeneity of EP profile. The transcription starting sites of prokaryotic genomes are characterized by extensive (hundreds of bp) zone of high EP and some peculiarities directly around TSS. This is associated with protein binding and formation of physical properties due to transcription machinery. Specific details of the TSS EP architecture are similar in related taxa. Promoters up-element demonstrates electrostatic nature. E. effects on genome functioning interact with other physical properties of DNA, in particular - bending, thermal stability, supercoiling – in both, formation, and transcription regulation. The distribution of curved DNA in promoter regions is evolutionarily preserved. Strongly curved DNA fragments have high AT content.

Conclusion: E. plays important and universal role in transcription regulation in prokaryotes, affecting proteins binding probability and positioning accuracy. It may influence horizontal gene transfer, TR systems evolution and contribute to genome regulatory regions high AT content in such diverse domains as Bacteria and Archea. Physical properties formation principles affect such fundamental problems as Chargaff's II rule, redundancy of the genetic code, neutrality of synonymous substitutions.

Availability: DEPPDB is available at http://deppdb.psn.ru or http://electrodna.psn.ru

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2. A.A. Osypov et al (2012) DEPPDB – DNA Electrostatic Potential Properties Database. Electrostatic Properties of Genome DNA elements, *JBCB*, 10(2) 1241004

TO THE QUESTION OF NONSYNONYMY OF SYNONYMOUS SUBSTITUTIONS

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Key words: *DNA physical properties, synonymous substitutions, multifunctionality, genome evolution*

Motivation and Aim: Here we offer a fundamentally new approach to the subject based on the DNA properties that provide its multifunctionality.

Methods and Algorithms: DEPPDB and its tools [1,2] were used to carry out the analysis.

Results: Genome DNA physical properties define its shape in the functional space and influence its interactions with proteins, esp. for transcription regulation. DNA is highly charged and electrostatics contributes greatly to the subject, as well as other nucleotide content-based DNA physical properties such as curvature, bendability and thermal stability. We have shown earlier several common properties of electrostatic profiles around transcription factors binding sites, promoters, and transcription start sites overall. All of them possess increase in electrostatic potential value that helps to locate and connect to binding sites for transcription regulation proteins - transcription factors and RNA polymerase. Resulting profile around transcription start sites has common architecture among all prokaryotic taxa with variations in specific proportions between its elements. Notably electrostatic profile, exhibiting common features of the transcription regulation regions, spans over the beginning of the coding area and therefore the precise nucleotide composition needed to support this (and some others) physical properties interfere with the protein-coding program. A part of this interference may manifest itself in the form of prevalence of specific codons among the available synonymous ones, or the biases between synonymous aminoacids, thus introducing the nonsynonymy of synonymous substitutions under the press of natural selection. Also there is a need for ensuring compliance with the consensus matrix for transcription factors binding sites, that are known to often occupy the beginning of coding regions.

Conclusion: The apparent nonsynonymy of synonymous substitutions may lead to different wrong estimations of the sequences fate, including misuse of the molecular clock and mistaken evaluations of specific mutations biomedical importance.

Availability: DEPPDB is available at http://deppdb.psn.ru or http://electrodna.psn.ru

Acknowledgements: RFBR grants 14-44-03683 and 16-04-01865.

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CYTOCHROMES P450 AS NEW DRUG TARGETS IN PLATYHELMINTHES

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Key words: *opisthorchiasis, cytochrome P450, parasitic diseases, drug development*

Introduction. The heme-monoxygenase system CYP450 is essential for biotransformation of sterols and xenobiotics, for synthesis of signaling molecules in all living organisms. Most eukaryotes including free-living flatworms evolved numerous paralogues of the CYP gene. Notably, by contrast, parasitic flatworms have only one gene. Expression of CYP mRNA in adult worms is higher than in other life stages, probably CYP plays a critical role in the pathogen's metabolism that contributes to cell survival and drug resistance. The function of this unique enzyme in parasites is unknown. The studies of P450 will help to reveal the biochemical function of P450 in Platyhelminthes, and to evaluate its potential as a molecular therapeutic target in the treatment of parasitic diseases.

Results. To study the biochemical and catalytic characteristics of the unique parasitic hemoprotein P450 (CYP), recombinant his-tagged CYP from *O. felineus* and *C. sinensis* flukes have been investigated. Cs CYP and OF CYP were expressed in DH5alfa *E. coli* strain. CYPs were purified using a two-step liquid chromatography. The heme iron ion (Fe II) in CYPs could produce high spin state (the peak at 440 nm), so CYP proteins were active during and after the purification. To assess the potential ligands the Cs CYP was titrated by different substances and its binding characteristics were evaluated using UV-visible spectroscopy. UV-visible type II binding spectra (with an increase in absorbance at 420 nm (Soret peak) and a decrease at 390 nm) were determined for the interaction of purified Cs CYP with the universal azole inhibitors (ketoconazole, clotrimazole, miconazole, econazole, triademinol, 4phenyl imidazole) as well as with *T. cruzi and M. tuberculosis* CYP51 inhibitors (LP 10, CYP I-181, 44841). The KD values for the ligands were calculated (GraphPad Prism) from the spectral titration curves and they were in the micromolar range. The most effective inhibitor was 44841 compound, (KD=1.3 mkM), the top compound from *T. cruzi* CYP51 inhibitors. To validate lead compounds for inhibitory effect we studied the action of them *in vitro*, and we found that some of azole and other CYP inhibitors have killing effects on juvenile *O. felineus* worms in the micromolar range.

Conclusion. This report presents the first biochemical and catalytic study of the CYP enzyme of any of the parasitic flatworms. We have been characterized its biophysical properties and found its micromolar inhibitors, that have killing effects on worms. The results of the study demonstrate that cytochromes p450 is promising new drug targets in Platyhelminthes.

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MAIN SECRETORY PROTEINS OF THE EUROPEAN LIVER FLUKE: *OPISTHORCHIS FELINEUS* THIOREDOXIN PEROXIDASE IN HOST TISSUES

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Keywords: o*pisthorchiasis, thioredoxin peroxidase, parasitic diseases, bile ducts*

Introduction. Opisthorchiasis leads to the liver and the pancreas disorders, causing biliary dyskinesia, the stomach and intestine diseases, vascular dystonia, an allergy, cholangiofibrosis, liver abscesses. The excretory-secretory product (ESP) of parasite is been considered to determine the "host-parasite" interaction and to be the key way of the trematodes pathogenicity. Regulation of ESP product synthesis and excretion is important and emerging issue in parasitology, molecular biology and immunology.

Results. In order to study the mechanisms of synthesis and excretion of the main protein from the *Opisthorchis felineus* secretome - thioredoxin peroxidase, which has immunomodulatory effect on host immune cells, the thioredoxin peroxidase recombinant protein was expressed and purified in a bacterial system. Rabbit polyclonal antibodies against this protein were obtained.

Using these antibodies the thioredoxin peroxidase accumulation was measured in the worm culture medium, and its temporal dynamics was assessed. Thioredoxin peroxidase accumulation was also measured during the simulation of inflammation *in vitro* using prooxidative agents (hydrogen peroxide, paraquat). It was shown that after the treatment of worms with hydrogen peroxide the excreted thioredoxin peroxidase was significantly increased in culture medium. In contrast, paraquat, an intracellular oxidative agent, did not change the level of the protein excretion. Immunohistochemistry was performed on tissue samples from the golden hamsters *Mesocricetus auratus* with chronic opisthorchiasis (3 months after the infection). We found that parasite's thioredoxin peroxidase was accumulated in the bile duct epithelial cells and in fibrous tissue surrounding the bile ducts.

Conclusion. The obtained results confirm the role of the thioredoxin peroxidase as one of the key parasite's defense molecules. The data expand our understanding of the molecular and molecular-genetic processes underlying the host-parasite interaction, and are of great importance for the development of ideas concerning the mechanisms of opisthorchiasis pathogenicity and the opisthorchiasis-associated diseases in humans.

Acknowledgements. This work was supported by the Russian Foundation for Basic Research [grant numbers 16-04-00356a, 15-04-03551a, 15-54-45132 Ind_a] and the state project of the Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences [#0324-2015-0004].

GUT MICROBIOTA IN CASE OF PARKINSON'S DISEASE AND OTHER NEUROLOGICAL PATHOLOGIES: COMPARATIVE STUDY

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Key words: *gut microbiota, metagenome, Parkinson's disease, 16S sequencing*

Motivation and Aim: Recently it is shown that nervous system of host organism interact with gut microbiota. In case of neurological disorders, especially for Parkinson's disease gut microbiota altered[1]. But little known about differences in microbiota composition among different neurological pathologies.

Methods and Algorithms: The study was conducted in a three groups of patients (60 control subjects, 60 subjects with Parkinson's disease and 32 subjects with other neurological pathologies, including multiple sclerosis, idiopathic dystonia and essential tremor). After the total DNA isolation and library preparation sequencing of the variable V3–V4 16S rRNA gene regions was performed by using MiSeq Reagent Kit v2 and MiSDefault device according to the manufacturer's recommendations. Taxonomic classification, alpha- and beta-diversity performed using QIIME Software 1.9.0. Determination of operation taxonomic units (OTU) performed with the usage of Greengenes v. 13.5 database (OTU's representative set picking) and HITdb (taxonomy assigning using RDP Classifier). Statistical comparison of the groups of samples was performed using Galaxy-based LefSe algorithm.

Results: The overall composition of fecal microbiota was affected by disease status both in terms of α- (chao1, Shannon and observed OTUs indices) and β- (weighted UniFrac) diversity. Gut microbiota of patients with Parkinson's disease and other neurological pathologies is characterized by lower taxonomic diversity in comparison with healthy control without significant difference between Parkinson's disease and other neurological pathologies. In addition, there were significant differences in compositional dissimilarity between groups based upon ANOSIM and MRPP statistical algorithms. According to LefSe algorithm, there were 44 species and 22 genera of bacteria and archaea with difference in abundance within groups. The top bacterial markers with highest LDA scores among the groups were *Christensenella minuta* on the species level and *Bifidobacterium* on the genus level for Parkinson's disease group, *Bacteroides massiliensis* on the species level and *Bacteroides* on the genus level for control group, *Anoxystipes fissicatena* and *Blautia* for other neurological pathologies group.

Conclusion: Gut microbiota composition is specifically altered in case of different neurological disorders.

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WORKFLOW FOR EXOME SEQUENCING IN IDENTIFICATION OF DE NOVO MUTATION IN THE *NCL6* GENE

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Key words: *Next-generation sequencing, whole-exome sequencing, de novo mutation, neuronal ceroid lipofuscinosis type 6 (NCL6), trio family analysis*

Motivation and Aim: Whole-exome sequencing using next-generation sequencing (NGS) technologies is gaining popularity in clinical practice. Associations between undescribed mutation with the clinical picture of disease is the true difficulty in analyzing of NGS data [1]. Trio family analysis (father, mother and affected child) is a very powerful approach to identify potentially pathogenic 'de novo' mutations in the proband. We sequenced the exome (~552 genes) of affected child with suspected of leukodystrophy and both unaffected parents by using the TruSight Inherited Disease sequencing panel (Illumina inc., San Diego, CA, USA) on a Miseq sequencing system (Illumina inc., San Diego, CA, USA). In total, we obtained 7 Gb of sequence data with 2091 variations from the human reference genome sequence that were subjected to several filtering steps.

Methods and Algorithms: The MiSeq system provides fully integrated on-instrument data analysis software. Basespace software performs secondary analyses on the base calls during the sequencing run. SNP's and short INDEL's are identified using the Genome Analysis Toolkit (GATK) by default. The number of candidate variants is reduced using a three-step filtration strategy to generate a short candidate mutation list. For the variant filtering and annotation we used Variant Studio version 2.2 (Illumina inc., San Diego, CA, USA) data analysis software. Initial quality filter removed less reliable variant calls and resulted in the identification of 1499 genetic variants. In the second step given the rare incidence of autosomal – recessive disease, we excluded known dbSNP variants from our variants database, reducing the number of candidate by more than 98% to a total of 37 variants. For further analysis, we applied an autosomal-recessive disease model and assumed that the mutation was inherited from both parents [2,3,4]. Therefore, we have customized trio family data filtering and have found a common homozygous mutation c.396dupT (p.Val133fs) in exon 4 of *CLN6*. Validation by Sanger sequencing also confirmed that the c.396dupT (p.Val133fs) mutation was indeed present in a homozygous state in the affected child and in a heterozygous state in the both parents.

Conclusion: We identified a pathogenic de novo mutation c.396dupT (p.Val133fs) in the *CLN6* gene, and made a diagnosis of neuronal ceroid lipofuscinosis type 6, suggesting that relatively high number of patients with neuronal ceroid lipofuscinosis type 6 may be hidden under the guise of leukodystrophy in Yakut population.

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ANALYSIS OF MIRNA-MRNA INTERACTOME IN HUMAN: GENERAL CHARACTERISTICS AND PREDICTIONS EVALUATIONS

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Key words: *miRNA-mRNA interactions, CLASH, miRNA target predictions*

Motivation and Aim: MicroRNAs are the short RNA molecules (~ 22nt long), which play a key role in the regulation of many biological networks. Nowadays more than 2'500 human's miRNAs are known, but the question about the mechanism of interaction between mRNA and miRNA is still open. It's considered, that miRNA has a special region - 'seed', which is crucial for a miRNA-mRNA binding. There are five widely used predictive tools: TargetScan, Pictar2, PITA, RNA22 and miRanda. They have different approaches to prediction. It was impossible to understand, which one is better and if we could trust these predictions.

Methods and Algorithms: However recently the new high-throughput experimental method «CLASH» was developed to identify all miRNA-mRNA interactions. It allows finding out all miRNA- mRNA cases in HEK293 cell line. We created an algorithm to analyse and compare data from "CLASH" experiment with predicted miRNA sites by all five algorithms. Expression data for mRNA and miRNA were used from FANTOM5 and GEO DataSets.

Results: For the comparative analysis we obtained 16'190 miRNA-mRNA interactions (according to CLASH data) and 19'398 predicted miRNA-mRNA interactions. We estimated working of miRNA-mRNA prediction programs by the following criteria: sensitivity, positive predicted value, predictions in different mRNA regions (3'UTR, CDS, 5'UTR), predictions for different types of interactions (5 classes), predictions of "canonical" and "nocanonical" interactions, and testing by using random data for miRNA binding sites. Expression analyze of miRNA revealed several interesting groups: highly expressed miRNAs without any interactions, highly expressed miRNAs with small number of interactions and lowly expressed miRNAs, which take part in a lot of interactions with mRNAs. Deep expression analysis found out mRNA formed the most interactions.

Conclusion: For all five miRNA prediction softwares we demonstrated a low sensitivity and positive predictive value that doesn't allow using them for experimental researching. Also we found group of interesting miRNAs and mRNAs, which could play a key role in cell regulation processes.

CANDIDATE SNP MARKERS OF AGGRESSIVENESS-RELATED COMPLICATIONS AND COMORBIDITIES OF HEREDITARY DISEASES PREDICTED BY A SIGNIFICANT ALTERATION IN THE AFFINITY OF TATA-BINDING PROTEIN FOR HUMAN GENE PROMOTERS

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Key words: *TATA box, candidate SNP marker, aggressiveness, gene expression change*

Motivation and Aim: Single nucleotide polymorphisms (SNPs) may mark hereditary diseases, their complications, comorbidities, and drug responses.

Methods and Algorithms: Using Web service SNP_TATA_Comparator developed earlier, here we analyzed unannotated SNPs near known SNP markers of hereditary diseases and found candidate SNP markers that can significantly alter the affinity of TATA-binding protein for human gene promoters, with aggressiveness-related consequences.

Results: Biomedical SNP marker rs1143627 in the human *IL1B* gene promoter may mark aggressive traits in patients who receive cytokine immunotherapy according to the clinical retrospective review [1]. Another biomedical SNP marker, substitution -51T→C, in the human *NOS2* gene promoter may be tested as a candidate SNP marker of gender-biased complications of lead (Pb) excess as low search behavior in females and high aggressiveness in males (murine model [2]). One more biomedical SNP marker rs10895068 causing an additional "+270" transcription start site (TSS) of the human *PGR* gene can be studied as candidate SNP marker of attractiveness and increase in aggression/rejection of pairing in female behavior (murine [3] and rabbit models).

Conclusion: After validation of these candidate markers by clinical protocols, these SNPs may be useful for physicians to improve treatment for patients as well as for each of us to choose a lifestyle preventing aggressiveness-related comorbidities and complications.

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EPIGENOMIC CHANGES IN POSTMORTEM BRAINS OF HUMAN ALCOHOLICS

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Key words: *Alcoholism, Neuroscience, Genomics, Epigenetics, Chromatin*

Motivation: Chronic alcohol abuse is associated with epigenetic changes including DNA methylation and histone modifications that ultimately control long-term changes in gene expression and behavior. Histone H3 lysine 4 trimethylation (H3K4me3), a promoter-enriched chromatin (epigenetic) mark of actively transcribed genes, has been implicated in psychiatric disorders including drug addiction. The effects of chronic alcohol on genome-wide distribution of the H3K4me3 mark and its relationship with alcohol-induced changes in gene expression are not well understood.

Methods: We used chromatin immunoprecipitation followed by next generation sequencing (ChIP-Seq) to obtain genome-wide distributions of this mark in superior frontal cortex from postmortem brains of 24 human alcoholics and 24 matched control cases. An antibody against H3K4me3 was used for the ChIP step to isolate specifically bound DNA and non-immunoprecipitated DNA was used as input control. Sequencing was carried out using Illumina HiSeq paired-end sequencing (2x100 base pairs), yielding 20-30 million reads per sample.

Results: We identified multiple H3K4me3 peaks differentially regulated in gene promoters between the alcoholic and control groups. Our gene network approach highlighted genes involved in synaptic transmission and myelination, as two functional groups potentially regulated by alcohol-induced changes in H3K4me3. The network approach identified subsets of functionally related transcripts that are regulated in agreement with H3K4me3 changes, suggesting cause and effect relationships between this epigenetic mark and gene expression. In addition, we identified H3K4me3 peaks differentially affected by alcohol in males and females.

Conclusions: These data provide support for our previous findings showing global epigenetic changes caused by alcohol in the human cortex. Taken together, our results point to an important role of the H3K4me3 modification in the regulation of alcohol-induced changes in gene expression and downstream neuroadaptations and pathologies associated with alcohol use disorders.

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CIRCULATING MICRORNA DINAMICS IN LUNG CANCER PATIENTS DURING THERAPY

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Key words: *lung cancer, circulating nucleic acids, microRNA, antitumor therapy*

Motivation and Aim: Expression levels of cancer-associated microRNAs were reported to be altered in serum/plasma samples from lung cancer patients compared with healthy subjects. The study aim was the estimation of the changes in expression level of miRNAs (miR-19b, -25, -125b, -126, -205) in blood plasma from lung cancer patients during combined therapy and assessment of their value as disease monitoring markers.

Methods and Algorithms: Blood samples were taken from patients (n=23) with non-small cell lung cancer (NSCLC) under the care of the Tomsk Cancer Research Institute. These samples were stabilized and fractionated into plasma and blood cells. MicroRNA was isolated from blood plasma using single-phase phenol-free extraction protocol and purified on silica-based spin columns. Concentration of miRNAs was measured by quantitative RT-PCR and normalized to miR-16 using dCt method.

Results: In this study we analyzed the dynamic expression changes of circulating DNA in blood plasma from lung cancer patients during the combined therapy. Circulating miRNAs were isolated from plasma samples of NSCLC patients before treatment, during 30 days after completing chemotherapy and after 15 days after surgery, by using developed methodological approach. In case of miR-19b and miR-125b analysis revealed that the miRNA expression level correlates with clinical response to chemotherapy and surgery. Increasing level of miR-19b and decreasing level of miR-125b were associated with therapeutic response. Using Repeated measures ANOVA analysis we demonstrated that the miR-19b and miR-125b expression levels changes throughout three check-up points during the combined treatment are characterized by the significant cubic trend (P=0.00284 and P=0.029). Changes of miRNA-126 expression level during post-treatment follow-up were not characterized by the definite trend and not correlated with changes of other miRNAs expression level. The significant correlation between miRNA-25 and miRNA-205 expression levels was shown. *Conclusions:* The clinical utility of the circulating miR-19b and miR-125b expression analysis from this study remains to be validated in large cohorts of patients with different histological types of tumors, at the different stage of disease and outcomes. One of the criteria for inclusion in the group must be susceptibility and/or resistance to therapy.

Acknowledgements: The research has been carried out with support of the grant from the Russian Science Support Foundation 14-04-01881, Post-doctorate program in TPU.

FOUNDER EFFECT IN SIBERIAN INDIGENOUS POPULATIONS THROUGH THE PRISM OF HEREDITARY DEAFNESS

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Key words: *founder effect; genetic deafness; gene GJB2; Siberian populations*

Motivation and Aim: Prevalence of many monogenic diseases can be determined by a number of factors forming the specifics of population structure (ethnic composition, migration, isolation, founder and bottleneck effects, proportion of consanguineous and assortative marriages). Nonsyndromic deafness is one of the most common sensorineural genetic disorders and several dozen genes contribute to its pathogenesis. Efforts to identify genes responsible for this monogenic disease have been challenged by its high genetic heterogeneity and different prevalence worldwide. Mutations in gene *GJB2* (MIM 121011, 13q11-q12) encoding connexin 26 (Cx26) account for a significant portion (up to 50%) of hereditary deafness. Spectrum of *GJB2* mutations and their prevalence are highly specific for various populations. Identification of major *GJB2* mutations and estimation of their frequency are important both for medical and genetic studies as well as for understanding of evolutionary history of populations of different ethnic origins. This study aims to evaluate the role of founder effect in prevalence of major *GJB2* mutations among indigenous populations of Siberia.

Methods: Data on genotyping of 7 STRs flanking *GJB2* gene (by GeneScan) and 12 intragenic and flanking *GJB2* SNPs (by restriction analysis and Sanger sequencing) were used for the reconstruction of common haplotypes for major *GJB2* mutations (p.W172C, IVS1+1G>A, c.235delC) (by using software package "Arlequin", algorithm EM).

Results: We investigated the molecular basis of deafness by screening of the *GJB2* mutations in the Tyva Republic and the Altai Republic and evaluated the mutational spectrum and contribution of the *GJB2* gene to hearing loss in deaf patients belonging to indigenous peoples of these regions (the Tuvinians and the Altaians). High frequencies of three *GJB2* mutations (p.W172C, IVS1+1G>A, c.235delC) allow us to suggest that these mutations are major for Turkic-speaking Tuvinians and Altaians having some common ethnogenetic milestones in the past. The specific conserved haplotypes were found for each of these mutations suggesting their single origin from a common ancestor.

Conclusion: Our findings imply the sufficient founder effect contribution to prevalence of major mutations p.W172C, IVS1+1G>A, and c.235delC in the *GJB2* gene among studied Siberian indigenous populations.

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MIGRATION OF BONE MARROW CELLS: RESEACH TECHNOLOGY

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The work is dedicated to the development of an experimental sample of a biochip for the study of migration and distribution of bone marrow cells and to evaluate the effectiveness of the biochip as the comparative study of migration in vivo activity of bone marrow cells by the comparative analysis of data obtained by microarray with data of electrophoretogram after PCR amplification and real time PCR data. As the cell marker used sry gene-Y-chromosome of male donor. The migration and distribution of Y-positive cells of the bone marrow of males donor have been studied at various periods after intravenous transplantation of organs of syngeneic females recipient.

The Y-chromosome marker (sry gene) was used for detection of donor cells in organs of origin syngeneic recipients (C57Bl female), which was determined by polymerase chain reaction (PCR). Semi-quantitative determination of the marker in the organs has been conducted using the software Quantity One in densitometer Geldok (Bio-Rad) in units of optical density of amplicons electropherograms. The polymerase chain reaction in real time on Authorized Termal Cycler - Light Cycler 480 II / 96 (Roche) has been used for the quantitative determination of the marker in the organs. The workstation for fluorescent microarray analysis comprising spotter «Microarray Printing SpotArray 24 System» device and contact printing microarrays and confocal fluorescent scanner «ScanArray Gx» (Perkin Elmer) have been used to create the microchip techniques quantifying specific marker of Y-chromosome. The workstation was used for quantitative analysis of the intensity of the luminescence spots microchip carried by software ScanArray Express (Perkin Elmer).

Thus, satisfactory qualitative agreement shows microarray analysis results, the analysis results and data of electropherograms obtained by real time PCR. Data obtained using the microchip techniques consistent with the experimental results obtained by other methods. The advantage of the proposed method is the biochip simultaneously and a plurality of analysis that allows analyzing the incomparably larger number of samples, reducing the level of experimental error due to multiple repetition of the analysis.

GENEQUERY: GLOBALLY CONNECTED NETWORKS OF GEO TRANSCRIPTIONAL PROFILES SHOW HYPOTHESIS GENERATION POTENTIAL AND REVEAL THAT TOCOPHEROLS RESCUE TREM2-ASSOCIATED MICROGLIAL DYSFUNCTION

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Key words: *GeneQuery, GEO, transcriptional networks, phenotype search, hypothesis generation, TREM2 deficiencies, Nasu-Hakola disease*

Motivation and Aim: Modern collections of transcriptional profiling experiments contain enormous wealth of information, which is severely underutilized due to inconsistent annotation, cross-platform differences and wide spectrum of conditions and tissues profiled. On the other hand, most of the modern pathway analysis tools rely on curated gene sets that quickly become outdated and often fail to capture true diversity of transcriptional responses in real biological systems. To reveal hypothesis generation potential of transcriptional profiling databases, we developed *GeneQuery*, new geneset-based global phenotype searching tool that makes use of gene expression data in GEO database.

Methods and Algorithms: GeneQuery circumvents aforementioned difficulties by introducing digital definition of phenotypes through gene modules co-expressed in a given dataset. Since there is an established connection between co-expression and co-regulation of groups of genes, we used co-expressed modules as a representative of particular phenotype in the transcriptional universe. Careful application of WGCNA approach [1] allowed us to automatically and unbiasedly obtain co-expressed modules of genes that are subsequently compared to the geneset in question. Using regular Fisher's exact test with Bonferroni correction, we were able to establish a phenotype search engine that finds biologically similar experiments based on the transcriptional signatures. Furthermore, using network methods we have analyzed the cross-connectivity of the overall "transcriptional universe" graphs of humans, mice, and rat, and have found that both conserved and species-specific clusters are present for each species. Overall, nearly half of all available transcriptional experiments (spanning over 400,000 samples) are included in the database, which is dynamic and easily expandable.

Results: We first used *GeneQuery* to unbiasedly characterize the "microarray expression universe" and then explored its hypothesis generation potential in various biological settings. GeneQuery revealed an unexpected connection between transcriptional signatures of patients with Nasu-Hakola disease, a rare neurodegenerative disease caused by TREM2 and DAP12 mutations, and a portion of the aging-signature in mouse brain consisting of genes responsive to α/γ-tocopherol treatment. Utilizing a mouse model of TREM2-associated microglial deficiency, we demonstrated that α/γ-tocopherol treatment rescued microglial function in *Trem2*-deficient mice but did not affect WT microglia.

Conclusions: These results validate a powerful new computational approach, highlight the critical role of TREM2 in microglial function, and suggest new therapeutic approaches for treating TREM2-associated neurodegeneration.

Availability: GeneQuery is available free of charge at https://artyomovlab.wustl.edu/genequery-alpha/searcher/.

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USING THE TECHNIQUES OF STOCHASTIC MODELLING AND INHOMOGENEOUS SEQUENTIAL PATTERN RECOGNITION PROCEDURE FOR THE PREDICTION OF POLYGENIC DISEASES

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Key words: *Polygenic diseases, prognosis of development, mathematical modeling.*

Motivation and Aim: Genetic architecture of polygenic diseases is stochastic and highly complex. For research one, at present, much attention is paid to the identification of composite markers, including the simultaneous carriage of multiple polymorphic variants of genes with the evaluation of interactions between them. In this regard, one of the important current trends in the field of high-precision forecasting models of complex diseases is to provide a combination of models and methods. The aim of this study was to investigate the possibility of combined use of formalized and non-formalized forecasting methods to build the model with the greatest potential of prediction.

Methods and Algorithms: A total of 995 people were examined, this group included healthy individuals and patients with various diseases of polygenic nature. Studied 13 points polymorphism localized in the promoter regions of 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 treatment results were evaluated on the basis of an original methodological approach involving a complex computer analysis of gene circuits of different dimensions. Intergenic interaction was assessed by MDR (Multifactor Dimensionality Reduction). The simulation modelling based on computational algorithm embedded in inhomogeneous sequential pattern recognition procedure was used to build models with the greatest potential predictions.

Results: Multi-genetic analysis revealed a highly informative gene ensembles associated with the development of specific polygenic diseases. With these genetic complexes and combined methods of mathematical simulation the bioinformatic matrix were created. On this basis for the four variants of the multifactorial diseases studied in our investigation the predictive algorithms with an optimal ratio of sensitivity and specificity to ensure maximum accuracy of the method (up to 89%) with minimum number of prognostic predictors were developed.

Conclusion: Comparative genetic studies with the analysis of complex genotypes in combination with different variants of mathematical modeling can be the basis for the development of fundamentally new ways to forecast development of early and differential diagnosis of polygenic human diseases.

COULD SINGLE-NUCLEOTIDE POLYMORPHIMS C(-1)T OF CD40 GENE BE USED FOR PREDICTING OF EUTHYROSIS DEVELOPMENT FOR PATIENTS WITH AUTOIMMUNE THYROID DISEASE?

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Key words: *family cases of AITD, CD40 C(-1)T, SNP for predicting of euthyrosis*

Motivation: Autoimmune thyroid disease (ATD), including diffuse toxic goiter (DTG) and chronic autoimmune thyroiditis with the outcome of hypothyrosis (CAT), are widespread and occur in 2 - 5% of people in the general population. Despite the availability of treatment, the proportion of patients with decompensated hypothyroidism ranges from 33 to 50%. Current recommendations for the management of patients with DTG and CAT are paying attention to some behavioral and environmental factors that are associated with likelihood degrease of disease remission or compensation. However, pathogenesis of ATD is based on the interaction of both environmental and genetic factors. CD40 gene plays a key role in the development of current diseases. Carriage of CC genotype of C (-1) T CD40 gene is associated with increased risk of DTG. Naturally the question arises, what is the contribution of the SNPs in the achievement of compensation / remission ATD.

Aim: To estimate the connection between single-nucleotide polymorphisms (SNP) C (-1) of CD40 gene and achievement of compensation during the treatment for persons with AITD and family history of DTG and/or CAT.

Methods and Algorithms: 70 patients (35 families) with ATD were included to the study. The genetic test was conducted for all participants. Euthyrosis on the top of already administered medications is reached, if the measured TSH (thyroid stimulating hormone) level is 0,167-4,05 mU / l. SNP C(-1)T of CD40 gene was tested by PCR.

Results: The average age of patients was 42 [27; 58] years old. 7 male (10%) and 63 (90%) female subjects were included in the study. The number of patients with diagnosed CAT was 46 (66%) and DTG - 24 (34%) subjects. Euthyrosis on the top of already administered medications was determined for 20 (28%) patients in total, 7 (10%) of them experienced DTG, and 13 (18%) patients had CAT. The CC genotype of C(-1) T CD40 gene was prevailed for patients who do not achieve compensation or remission of ATD on the top of already administrated medications comparing with the group of patients who achieve euthyrosis (68% and 50%, respectively, p = 0,029).

Conclusion: Measuring of SNP C(-1)T of CD40 gene can be used as a predictor for identification of risk group of ATD patients who could fail euthyrosys on the top of administered therapy.

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GENETIC FITNESS OF DEAF PEOPLE IN THE SAKHA REPUBLIC

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Key words: *GJB2, deafness; genetic fitness; Eastern Siberia, Russia*

Motivation and Aim: Introduction of a sign language in schools for deaf people led to growth of their genetic fitness, which has doubled the *GJB2* gene, associated deafness in the USA over the past 200 years [1]. High prevalence of the *GJB2*-deafness [2] and relatively recent (~ 60 years ago) introduction of sign language among deaf people were recorded among indigenous Yakut population (Eastern Siberia, Russia).

Methods and Algorithms: We have performed study of fertility of deaf people compared to their hearing siblings in Eastern Siberia. Fertility was determined as mean number of children born to specific group. Genetic fitness of deaf people was calculated as the ratio of their fertility to fertility of their hearing siblings [3].

Results: Data on 83 deaf people and 185 hearing siblings, aged 35-69 years was collected. 143 children accounted for 83 deaf individuals, whereas 185 hearing siblings had 422 children. Fertility of deaf people was estimated as 1.72 vs 2.28 of their hearing siblings. Overall the genetic fitness for deaf individuals is 0.75. There was no difference between genders. Our results are comparable with fitness of deaf women in Sweden – 0.76 [4], and lower than in USA – 0.88 [3] and higher than in Mongolia – 0.62 [5].

Conclusion: Thus, genetic fitness of deaf in Eastern Siberia is slightly reduced, which is still could possibly increase frequency of *GJB2*-deafness.

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DEVELOPMENT OF CATARACT AS THE BASIC SELECTION TRAIT IN THE ONTOGENY OF SENESCENCE-ACCELERATED *OXYS* RATS

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Key words: *accelerated aging, cataract, lens, crystallins, molecular chaperone, small heat-shock protein, Alzheimer's disease, OXYS rats*

A non-transgenic model of accelerated senescence and associated age-related disorders in OXYS rats was established by selection and inbreeding of Wistar rats, which were sensitive to the cataractogenic effects of galactose-enriched diet. The development of cataracts was induced by galactose overconsumption at the start, but, after five generations, the early spontaneous cataract became the selection trait against the background of a normal diet. To date, as a result of carefully controlled selection, OXYS rats stably and spontaneously develop the characteristic accelerated-senescence phenotype, including early cataract (similar to human senile cataract), AMD-like retinopathy, osteoporosis, arterial hypertension, and – according to the recent findings - brain neurodegenerative pathology with the features specific for Alzheimer's disease. These led to the use of OXYS strain in the fundamental research and in the study of drug therapeutic effectiveness. It is widely known, that age-related cataracts are associated with degenerative changes in the ocular lens including the aggregation of proteins, mainly molecular chaperones - crystallins, but also amyloids. This biochemical aspect might be a fascinating hypothesis for a cataract as a "biomarker" of systemic changes, including neurological processes, in the selection of OXYS rats. We recently reported the downregulation of a-crystallin gene expression during retinopathy progression in OXYS rats. So, *the aim* of the present study was to analyze the dynamics of morphological changes in the OXYS lens and to compare it with lens mRNA levels for αA- and αB-crystallins in order to search for potential systemic commonalities between cataract and retinopathy. We examined OXYS rats' lens by means of light microscopy at 20 days (no clinical signs of cataract), 3 months (cataract prevalence is 100 %), and at 12 months (at the pronounced stages of disease) in comparison with age-matched Wistar rats (control group). In the lens of 20-day-old OXYS rats the minor aberrations in the packing of cortical fibers, and the signs of alterations in the transport activity and/or cell-to-cell contacts were detected. The likely-compensatory increase in the density of the lens epithelium was accompanied by upregulation of the αA- and αB-crystallin genes. At the age of 3 months, there were noticeable aberrations (and at 12 months, significantly enhanced aberrations) in the structure of the lens capsule and in organization of the cortical fibers in OXYS rats, whereas a-crystallin expression dipped below than in the Wistar rats. In addition a comparative SNP analysis was carried out to test the hypothesis that OXYS rats carry characteristic mutations in some among 57 genes and loci for which the associations with risk of cataract development are reported in human or in animal models. Summarizing, we showed that systemic changes in the expression and function of crystallins may underlie cataract and retinopathy progression in OXYS rats. Thus the selection for "cataract" trait might led to inheritable systemic changes including AMD-like retinopathy and brain Alzheimer's disease-like pathology in OXYS rats. *This study was supported by the Russian Foundation for Basic Research (Grant 14-04-00376* A*).*

SEROLOGICAL MARKERS IN RHEUMATOID ARTHRITIS: CIRCULATING DNA AND AUTOREACTIVE ANTIBODIES

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Key words: *rheumatoid arthritis, diagnostics, circulating nuclear DNA, mitochondrial DNA, real-time PCR, antibodies to citrullinated protein antigens, rheumatoid factor, C-reactive protein*

Motivation and Aim: Early diagnostics of rheumatoid arthritis (RA) is of importance for the effective recovery, which is hampered due to the unspecific clinical manifestations. Clinically used RA markers are the increased level of rheumatoid factor (RF), C-reactive protein (CRP), but they are found in other disorders. The presence of antibodies to citrullinated protein antigens (ACPA) was recently discovered to be useful diagnostically: it was more specific, although less sensitive, than the presence of RF. Elevated level of circulating extracellular DNA (cirDNA) concentration was earlier found in the blood plasma of systemic lupus erythematosis (SLE) patients and was associated with the disease pathogenesis. The aim of study was to reveal the association of cirDNA concentration changes with rheumatoid arthritis development and progression.

Methods and Algorithms: Blood samples were taken from 63 healthy subjects (HS) and 74 RA patients (ACR, 1987). Blood was fractionated, cirDNA was extracted from plasma and cell-surface-bound cirDNA fraction (csb-DNA). CirDNA concentration was measured by quantitative real-time PCR specific for LINE-1 repetitive elements from nuclear DNA (nDNA) and a fragment from mitochondrial DNA (mtDNA). Rheumatoid factor titers were estimated using immunonephelometric assay, C-reactive protein, ACPA, anti-ssDNA, anti-dsDNA antibodies were estimated using ELISA Kits.

Results: Reliable increase of the cir-nDNA from plasma was found for RA patients compared with healthy subjects (12.0 versus 8.4 ng/ml, Mann-Whitney U test, $p<0,01$). Cell-surface-bound nuclear DNA (csb-nDNA) concentration in blood from RA patients was significantly decreased (24 versus 50.8 ng/ml, $p<0.01$). Cell-surface-bound mitochondrial DNA (csb-mtDNA) concentration was increased (1.44×10^6 copies/ml versus 0.58×10^6 copies/ml $p<0.05$) in RA. According to ANOVA test the most valid for the discrimination of RA patients from controls were ACPA, csb-mtDNA levels, while csb-nDNA, RF, and CRP possessed lower power. Combination of 3 variables [csb-nDNA, csb-mtDNA, and ACPA] allowed the high accuracy of RA patient discrimination from healthy donors (97% sensitivity and 98 % specificity). Csb-nDNA and csb-mtDNA level was positively associated with development of anti-ssDNA and/or anti-dsDNA antibodies, while ACPA demonstrated negative association with anti-DNA response in RA patients.

Conclusion: Rheumatoid arthritis development is accompanied by significant changes of the circulating nuclear DNA and mitochondrial DNA levels found both in blood plasma and at the surface of blood cells. Data obtained indicate the further study of the cirDNA value as the potential factor, applied for diagnostics, and possibly participating in the rheumatoid arthritis pathogenesis.

THE GENETIC CHARACTERISTICS OF DIFFERENT SUBTYPES OF MODY DIABETES IN NOVOSIBIRSK

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Key words: *MODY, genes, diabetes mellitus*

Motivation and Aim. It is not always possible to verify the diagnosis based on phenotype and laboratory parameters of the type of diabetes mellitus, in such cases it is necessary to consider the use of genetic research to identify the hereditary forms of diabetes. The aim of research was to verify the different subtypes of MODY diabetes by molecular genetic research in patients with clinical symptoms of this nosology.

Methods and Algorithms. 17 patients with a clinical diagnosis of MODY were conducted examination, biochemical and hormonal blood tests, high-targeted sequencing of genes, mutations in which lead to the development of diabetes MODY 1-13. The results of molecular genetic research have verified the direct automatic sequencing Sanger.

Results. This group consisted of 7 males (41.2%) and 10 female (58.8%) (p> 0.05). The age of patients at the time of diagnosis of hyperglycemia ranged from 3 months to 38 years. The median duration of MODY diabetes was 3 [0; 23] years. We verified 4 subtypes of MODY diabetes in 17 patients after the molecular genetic research. 12 patients from 7 families had variations in gene glucokinase (GCK) (MODY 2): promoter (g.44189633T> C), exon 1 (g.44189037G> C), rs193922297, heterozygous variant (T> A), p.Arg37Trp, p.Leu147Val, p.Gly258Cys, p.Trp257Term. Two patients had MODY 3, options have been identified in the gene HNF1A: p.Asn62Ser, p.Met412Val. One patient had changes in CEL gene (MODY 8) variant p.Leu247Pro. Two patients from the same family had MODY 12. Mutation Ala1457Thr (rs72559717) identified in the heterozygous variant in a gene AVSS8 (MODY 12) is rare. This substitution was found at position 1457 and led to the replacement of alanine at amino acid tryptophan. Both patients with MODY 12 had disorders of lipid metabolism with youth, we researched genes APOA1, APOA2, APOA4, APOA5, APOB, APOC3, APOD, LDLR, LDLRAP1, LPL, PCSK9, SCARB1 and SREBF2. Contribution to the development of this disease in a patient can make Gly2Ser polymorphism in exon 1 of the gene and SCARB1 Ser474Ter nonsense mutation in exon 9 LPL gene.

Conclusions. 1. We verified 2, 3, 8 and 12 subtypes of MODY among patients with a clinical diagnosis of MODY diabetes by molecular genetic research in Novosibirsk. 2. MODY 2 prevailed: 12 patients had MODY 2 among the 17 patients with verified diagnosis of MODY diabetes in Novosibirsk. 3. Patients with MODY 12 diabetes also had violations in the genes that lead to violations of the lipid spectrum which requires further study.

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A MESENCHYMAL STEM CELL THERAPY CAN RESULT IN THE DIFFERENT OUTCOMES OF INFECTIOUS INFLAMMATORY PROCESS

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Key words: Mesenchymal stem cells, cell therapy, mycobacterial infection

Motivation and Aim: Mesenchymal stem cells (MSC) transplantation is an actively studied therapeutic approach used in regenerative medicine and in the field of regulation of immunoinflammatory response. A cell therapy with MSC produces sometimes adverse results or side effects, which demands for more personalized approach. Particularly, a "sharp-shooting" preconditioning of the cells, even autologous, with due regard for MSC phenotype and the target of cell therapy is needed. Depending on cultural conditioning MSC can be polarized to the phenotypes with predominantly pro- or anti-infectious properties. We studied the effects of transplantation of bone marrow-derived non-conditioned/naïve or poly(A:U)-conditioned MSC on the course of BCG infection *in vivo*.

Methods and Algorithms: Autologous bone marrow derived primary MSC of BALB/c mice were cultured for 4 weeks and conditioned with 1.0 μg/ml poly(A:U) for 24 hours. Mice were infected i.p. with 1×10^7 BCG and then in 11 and 12,5 weeks treated with two i.v. injections of naïve MSC (nMSC) or poly(A:U)-conditioned MSC (cMSC). The results were evaluated at the week 14. Samples of the liver, lungs and spleen were analysed in histological study: sections were stained by hematoxylin-eosin/Ziehl-Neelsen stains and the quantity of granulomas and bacilli were counted. Samples of infected organs were used for preparing the smears stained for luminescent microscopy. The numbers of mycobacteria-positive samples and CFU count in the organs were determined correspondingly after incubation in liquid medium MGIT and on solid Lowenstein-Jensen medium.

Results: Histological study revealed that nMSC induced 3-fold increase of bacterial quantity in the spleen granulomas, while cMSC induced significant decrease of number of bacteria in BCG-positive spleen granulomas. Analysis of the smears and growth on MGIT medium have shown that the samples of "BCG" and "BCG+nMSC" groups were positive correspondingly in 43 and 50% (luminescent microscopy) and 29 and 25% (MGIT) respectively while all "BCG+cMSC" samples were negative. Growth on Lowenstein-Jensen medium have shown that nMSC increased the number of bacteria in the organs 1,5-3-fold, but did not influence the number of samples/animals with viable BCG. In contrast administration of poly(A:U)-conditioned MSC decreased at least 4-fold the number of samples/animals with viable mycobacteria in the tested organs.

Conclusion: We concluded that MSC therapy can be effective in mycobacterial infection, but only in case of an appropriate conditioning of the cells.

NATURAL BISPECIFIC ANTIBODIES: NEW BIOCHEMICAL MARKERS OF AUTOIMMUNE DISEASES

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Key words: *systemic lupus erythematosus, antibodies, bispecific antibodies, human blood*

Motivation. There is a common belief that IgG molecules presented in biological fluids are monovalent molecules with stable structures and two identical antigen-binding sites. The Fab arm exchange was first described for IgG4 subclass in 2007, the reaction mechanism was described in 2011. In 2012 we have shown that human milk IgG contains up to 54% of bispecific molecules comprising κ- and λ- light chains simultaneously, and up to 17% of human milk sIgA are bispecific. Interestingly, bispecific human milk κλ-IgG are presented mostly by IgG1 (74%) and lower amounts of IgG2–IgG4 (5–16%).

Background. In 2015 we have shown that similar to human milk placenta antibodies undergo extensive Fab arms exchange and IgG preparations in average consists up to 15.0% of the IgGs containing both κ- and λ-light chains. Chimeric placenta κλ-IgGs consisted of: 43.5% IgG1, 41.0% IgG2, 5.6% IgG3, and 7.9% IgG4 there by, the relative content of chimeric IgGs in placenta is significantly lower than that of the milk. One can suppose that the observed phenomenon may appear due to the lower content of factor(s) stimulating the exchange in placenta comparing with milk.

Results & Conclusion. Here we show the content of chimeric κλ-IgGs in the blood of systemic lupus erythematosus. Since the serum of autoimmune patients contains significantly higher concentrations of bispecific IgG molecules than in healthy donors, the presence of bispecific antibodies in the serum of systemic lupus erythematosus and multiple sclerosis is a new biochemical marker of autoimmune disorders.

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THE ROLE OF REGULATION OF DIFFERENTIALLY EXPRESSED GENES IN PLACENTAL TISSUE IN THE DEVELOPMENT OF PREECLAMPSIA IN RUSSIAN AND YAKUT POPULATIONS

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Key words: *preeclampsia, rSNP, association study, placenta, transcriptome*

Motivation and Aim: Preeclampsia (PE) is one of the most serious pregnancy complications and the leading cause of maternal and perinatal morbidity and mortality. Currently the research of differentially expressed genes in placental tissue and their regulation is considered as a promising approach to the characterization of the PE molecular mechanisms. The aim of this work was to characterize the genetic components of PE by analysis of regulatory single-nucleotide polymorphisms (rSNPs) of new candidate genes detected in the research of the transcriptome in our previous study [1].

Methods and Algorithms: In this report, we present the results of study of 48 rSNPs in 23 new candidate genes, associated with PE according to transcriptome analysis [1] in 378 patients with preeclampsia and 513 women with uncomplicated pregnancies from Russian and Yakut populations using MassArray iPLEX (Sequenom).

Results: We have detected significant associations of PE with 11 rSNPs in 9 genes (*BHLHE40*, *PLIN2*, *CORO2A*, *SYDE1*, *LHB*, *HK2*, *INHA*, *NDRG1*, *SASH1*). It is noteworthy that rs10985257 in *CORO2A* gene was associated with PE in both ethnic populations. This gene encodes a member of the WD repeat protein family. Members of this family are involved in a variety of cellular processes including cell cycle progression, signal transduction, apoptosis, and gene regulation. Categories of "Gene Ontology" (GO) of this gene are protein binding and actin filament binding. For other genes, associated with PE in this work categories of GO are protein binding, DNA binding and hormone activity. These categories can characterize biological processes that apparently are involved in the molecular mechanisms of PE development.

Conclusions: Our results demonstrate that approach based on differential expression analysis with subsequent rSNP search can contribute to understanding PE genetics factors. This work was supported by the Russian Foundation for Basic Research (grant №14-04-01467).

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PROSPECTS OF OCTAHEDRAL METAL CLUSTERS FOR BIOMEDICAL APPLICATIONS

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Octahedral metal cluster complexes of molybdenum and rhenium are characterized by high chemical stability of the cluster core as well as by modifiability of an outer ligand environment, by bright long-lived luminescence in the red and near-infrared regions of the spectrum, by ability to generate singlet oxygen, and by high X-ray contrast. As a consequence they have a high potential for use in photodynamic therapy and in bioimaging systems as radiocontrast and fluorescent agents. For increase of biocompatibility, the metal cluster complex can be encapsulated within an inert biocompatible carrier matrix, such as polystyrene (PS) micro-beads or SiO_2. The aim of our research was to study radiopacity, the ability to generate singlet oxygen, cytotoxicity and photo activity, intracellular localization in vitro, biodistribution, pharmacokinetics, acute intravenous toxicity in vivo of octahedral rhenium and molybdenum cluster compounds with different inner/outer ligands.

To determine cytotoxicity and photo activity we used MTT assay. We studied intracellular localization of clusters using the fluorescent confocal microscopy and transmission electron microscopy (TEM).

We showed that cluster complexes of rhenium with the general formula $[\{Re_6Q_8\}L_6]n$ (Q = S, Se or Te; L = apical organic orinorganicligands) have low cyto and acute intravenous toxicity, did not penetrate cells and have high radiopacity. Whereas molybdenum cluster complexes, encapsulated in SiO_2 nanoparticles rapidly enter cells and stay there for a long time, showed photoinduced cellular toxicity, comparable with the commercially available photosesitysers.

Based on our results of the carried out studies of physicochemical, photophysical and biological properties of the most active compounds we selected photosensitizers and agents for the bioimaging and X-ray contrast agents. Thus nanoparticles with {Mo6X8}4+-cluster core have the highest rate of penetration into cells and photodynamic activity. While octahedral chalcogenide rhenium cluster complexes with the general formula [{Re6Q8}L6]n (Q = S, Se or Te; L = apical organic orinorganicligands), namely, Na4[{Re6Q8}(CN)6] and Na2H8[{Re6Se8}(P(CH2CH2CONH2)(CH2CH2COO)2)6] are more promising for the application as X-raycontrast agent.

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COMMONABILITY OF POLYMORPHIC REGULATORY SITES COMBINATIONS OF CYTOKINE GENES IN PATHOGENETICALLY DIVERSE DISEASES IN WOMEN

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Motivation and Aim: The results of recent genetic researches allow for revealing the polymorphic markers associated with the most frequent multifactorial diseases. Each individual gene polymorphism can be associated with several various pathologies [1]. Among other factors, this association can result from the pleiotropic or polygenic effect, epistatic interaction between genes [2]. The purpose of our research was to estimate associativity of some multifactorial diseases with a complex of genes of inflammation, destruction and angiogenesis.

Methods and Algorithms: The research covered 316 women with diabetes mellitus type 2 (DM2), 395 women with breast cancer (BC), 162 women with rheumatoid arthritis (RA) and 410 healthy controls matched by age. Polymorphisms in the regulation region of *TNF-A863C; TNF-A308G; TNF-A238G; IL1B C-31T; IL4-C590T; IL6-C174G; IL10A-1082G, IL10-*A*592C, MMP2-* C*1306T*, *MMP9* -C*1562T, VEGF-*A*2578*C, *VEGF+*T*936*C genes were detected by polymerase chain reaction (PCR) followed by the Restriction Fragment Length Polymorphism (RFLP) method. The differences in the genotypic frequencies of polymorphisms were determined by chi-square test with Yates correct or two- tailed Fisher exact test for group ≤ 5. Odds ratios (OR) were calculated with a 95 % confidence interval (CI).

Results: The study revealed 101 complex genotypes, which are positively associated with simultaneously three diseases, in women groups. Their specificity is 83.85–99.71 % for all the diseases. Genotypes with the maximal specificity of 99.71 % for the three diseases have high OR for all the diseases. These are: *TNF-863 CC-:IL4-590 CC:IL10-592 AA* - RA *(OR = 15.55)*, DM2 *(OR = 12.21)* and BC *(OR = 14.37)*; *TNF-863 CC:TNF-308 GG:IL4-590 CC:IL10-592AA* - RA (OR = 10.96), DM2 *(OR = 12.21)* and BC *(OR = 11.40)*. Pro- and - anti-inflammatory cytokines in complexes of homozygous genotypes are associated with a low level of production. *MMP9* homozygote in these complexes is associated with low production as well. The polymorphic position of *VEGF-2578* appears mainly heterozygous or homozygous with a low gene expression. On the contrary, *VEGF +936* polymorphic position occurred in the homozygous state associated with increased production. It is significant to note that relative ranking results of OR for one of three analyzed diseases from the greater to smaller are analogous to other diseases. It is remarkable that there is no complex genotype which would be positively associated with one disease and protective of another, and vice versa.

Conclusion: Common combinations of gene networks of regulatory factors are associated with certain character of infringements related to pathogenetically diverse pathological processes such as autoimmunity, endocrinology derangements and carcinogenesis.

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FREQUENCY OF GERMLINE MUTATIONS GENES CHEK, FANCL AND FANCI IN PATIENTS WITH BREAST CANCER IN THE REPUBLIC OF TATARSTAN

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Key words: *hereditary breast cancer, mutation, next-generation sequencing* (*NGS*)

Motivation and Aim: Breast cancer is the most common form of cancer and the second most common cause of cancer death among women in worldwide. In 2014 1639 breast cancer cases were diagnosed in the Republic of Tatarstan. Approximately 5–10% of breast cancer cases might be inheritable, up to 30% of which are due to BRCA1/2 mutations. Recently, new data showing the predisposition to Breast and ovarian cancer due to mutation in other reparation genes obtained. Among these genes are CHEK1/2, FANCL, FANCI. To understand frequency of occurrence of these genes mutation in tatar women population we screened 40 patients with hereditary breast cancer by NGS.

Methods: Targeted gene enrichment was performed using NimbleGen SeqCap EZ Choice (Roche) according to the manufacturer's instructions with further sequencing using an Illumina MiSeq instrument with read length 249 bp from each end of the fragment.

Results: Two FANCL patogenetic mutations c.1099_1100 insATTA and c.C112T were observed in 4 patients. Free mutations FANCI c.A1111G, c.G286A, G3541A, c.C3673T, c.A2604C and 2 mutation CHEK2 c.1100delC, c.A38G were detected in 6 and 2 women respectively.

Conclusions: The study demonstrated that breast cancer individuals in Tatar ethnos possess pathogenetic founder mutations in reparation genes with high frequency.

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ENDOVASCULAR SURGERY OF THE LOW EXTREMITIES IN DIABETIC PATIENTS WITH CRITICAL ISCHEMIA

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Key words: *diabetes mellitus, critical ischemia, endovascular surgery*

Motivation and Aim: The features of the diabetic liaisons of the lower limbs are some problems with angioplasty due to its multilevel and distal location. Percutaneous transluminal balloon angioplasty (PTBA) of the tibia arteries more often indicated as an alternative to primary amputation in patients with critical lower limb ischemia (CLLI), including diabetes mellitus (DM) in the past few years. According to the some data effectiveness of PTBA on shin arteries of TASC C/D class could be 86-91% and level of the major complications is low (8-12%) [1]. However, there is a data that compared with lesions of shin arteries TASC A class, TASC D stratification at risk of developing restenosis increases 2.4 times higher risk of amputation 3.8 times [2]. Therefore the aim of investigation was estimation of the effectiveness of the endovascular manipulations in correction of the shin arteries of the TASC D class in patients with diabetes mellitus.

Methods and Algorithms: Retrospective investigation of 65 patients with critical ischemia of lower limbs (CLLI) and diabetes mellitus complicated with liaisons of shin arteries of TASC D class (occlusions length of tibia and fibula arteries are more than 2 cm, diffuse liaisons of tibia and fibula arteries) was done. All patients were treated with PTBA.

Results: Through comprising of the current investigation results with previously obtained data on amputations frequency in patients with diabetes mellitus and shin arteries liaisons (without revascularization) was found decreasing of amputations from 14,5% to 8%. Therefore odds ratio (OR) of the high amputation in CLLI through the year after revascularization and without it was 0,55. In our work the PTBA on the shin level was successful in 93% of the patients. Despite the high immediate success of PTBA of the shin arteries primary patency of the shin arteries (including primary induced) after 12 months of PTBA performing was 75%. Therefore providing revascularization on the shin level leads to decrease amputation 1.8 times in patients with CLLI and diabetes mellitus.

Conclusion: Percutaneous transluminal balloon angioplasty could be an operation of the choice to save the limb

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LONG-TERM FOLLOW-UP RESULTS OF MOBILIZED AUTOLOGOUS MONONUCLEAR CELLS IN PATIENT WITH SYMPTOMATIC LOWER LIMB CHRONIC ISCHEMIA

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Key words: *chronic claudication, cell therapy, follow-up results*

Motivation and aim. About 9-20 % of patients with atherosclerosis have extended leg artery occlusion. Due to problems of surgical revascularization of this patient's group we get started investigation of the indirect angiogenesis various methods. For the last 20 years different methods of angiogenesis therapy have been studied. Methods of transplantations of the small fraction of the mononuclear cells from the bone marrow, peripheral blood or its combination with intravenous infusions or intramuscular injections are considered interesting. Different data had corresponded that there was small clinical effect in the nearest future, long-term outcomes studies were solitary. Therefore the aim of this investigation was the estimation of long-term results of the therapy with application of immobilized autologic mononuclears in patients with chronic ischemia and atherosclerosis obliterans of lower limb arteries.

Methods and algorithm: The retrospective selection (clinical trial protocol approved on 10.10.2007 by local EC) was performed to estimate the long-term results of the therapy with intramuscular injections of mobilized autologous mononuclear cells in patients with symptomatic lower limb chronic ischemia IIB–III level (Fontaine-Pokrovsky) treated in the Surgery Department of the Institute from 2011 to 2014. The therapy safety and effectiveness were estimated before treatment, three weeks and 3-5 years after the therapy. The study of follow-up results was conducted in 2015-2016 by telephone contacts with patients treated with of mobilized autologous mononuclear cells or their relatives. Estimation of compliance, number of amputations and death outcomes were included to survey. The complained patients were invited for the physical examination in clinic. The values of the ankle-brachial index (ABI), pain-free walking distance and maximum distance for Gardner test (speed of 3.2 km / h) were used to check the efficiency of angiogenesis therapy. The distance of painless walk averaged 62 m, the maximum distance was 127 meters. The average value of ankle-brachial index was 0.53.

Results: The painless walk distance was 138 m (2.2 times greater) and the maximum distance was 236 m (1.85 times more) in three weeks after the therapy. Average value of ABI was 0.49 and was not significantly different from the original value. Long-term results were followed up in 81.25% of patients in terms of 3-5 years after the therapy. 68.75% of patients had positive dynamics of disease course, 12.5% of patients underwent amputation of lower limb, 18.75% of the patients died. Painless walking distance was 283 meters (4.6 times more than the original), the maximum distance was 431 m (3.4 times more than before treatment). The changes of ABI before treatment, after treatment and in long-term period were not statistically significant. Conclusion: In the long-term period results of the application of the immobilized autologic mononuclears in patients with symptomatic lower limb chronic ischemia have showed effectiveness of the method. Therefore it could be used as method of choice in patients with comorbidity and multilevel lesions when surgical revascularization is technically impossible.

FUNCTIONAL ANALYSIS OF MUTATIONS REVEALED BY NGS DIAGNOSTICS

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Key words: *NGS, medical genetics, functional analysis of mutations.*

Motivation and Aim: NGS in medical genetics not only improved the discovery disease causative genetic variants, but also greatly promoted the identification of new genes responsible for monogenic diseases. However, NGS with subsequent bioinformatic analysis alone is not sufficient to make a decision about pathogenic role of candidate variants in a vast majority of cases. It requires a very important additional step - functional analysis of identified variants. Here we present several cases of functional analysis of SNV revealed by NGS.

Methods and Algorithms: Whole exome sequencing was used to search for causative variants in patients with different hereditary disorders. The raw sequencing data had been processed with a custom pipeline based on popular open-source bioinformatics tools BWA, Samtools, Vcftools, and in-house Perl scripts, using hg19 assembly as a reference sequence. Functional analysis included the following steps: cloning the gene with and without sequence variant in expression vector, transfection of appropriate cell culture, analysis at the RNA or protein level.

Results: Whole exome sequencing of patients with autosomal-recessive hypotrichosis revealed a homozygous missense mutation c.712G>T (p.Val238Leu) in a highly conserved position of type I keratin KRT25 (K25). Haplotype analysis indicated a founder effect. An expression study in the HaCaT cell line demonstrated a deleterious effect of the p.Val238Leu mutation on the formation of keratin intermediate filaments. Hence, we have identified a previously unreported missense mutation in the KRT25 gene causing ARH with woolly hair.

Whole exome sequencing of patients with neural amyotrophy revealed heterozygous sequence variant c.1079T>C (p.V360A) in *DHTKD1* gene. Analysis of enzymatic activity of patient's primary cell culture lysates of showed sequence variant has no impact on the DHTKD1 activity and, therefore, should be classified as benign.

COPY NUMBER ALTERATION OF *ERLIN1*, *ABCC9* AND *ACACB* GENES IN CORONARY ARTERY DISEASE

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Key words:*Copy number variation; atherosclerosis; coronary artery disease.*

Motivation and Aim: Oxidized fatty acid and cholesterol are a component of atherosclerotic lesions. Fatty acid oxidation regulated by several genes, including *ACACB* and *ABCC9*, and may have important role in the development of atherosclerosis. Cellular cholesterol levels are controlled by endoplasmic reticulum sterol sensing proteins, including *ERLIN1* [1]. Copy number variations (CNVs) have influence on phenotypes by changing transcription levels of genes and may have impacts on protein sequence, structure and function. The contribution of CNV in the development of proliferative diseases such as atherosclerosis is an important issue, which is the purpose of our study.

Methods and Algorithms: We obtained 110 DNA samples of blood and 33 DNA samples of atherosclerotic plaques of coronary arteries from patients with coronary artery disease (CAD), and 100 DNA samples of blood from healthy individuals. Detection of CNVs was performed by qPCR using TaqMan® Assays. Statistical analysis was carried out using standard curve by Pfaffl method [2].

Results: We detected gain in the 10q24.31 region (*ERLIN1*) in 3 DNA samples of blood from patients and only one of patients had this CNV in DNA samples of atherosclerotic plaque of coronary arteries. On the other hand, loss in the 10q24.31 region (*ERLIN1*) was found in one DNA sample of blood from control group. In the 12q24.11 region (*ACACB*) we found gain in 3 patients and two of which had this CNV only in DNA samples of blood. As in the case mentioned above, loss of the 12q24.11 region (*ACACB*) was presented in one case in the control group. Gain in the 12p12.1 region (*ABCC9*) was presented in 5 DNA samples of blood and 2 DNA samples of atherosclerotic plaque from the affected group. This CNV was presented in 2 individuals of the control group – a gain in one person, a loss in the other.

Conclusion: We discovered CNVs of the 10q24.31 (*ERLIN1*), 12q24.11 (*ACACB*) and 12p12.1 (*ABCC9*) in arteries and blood of patients with coronary artery disease. Analysis of the DNA extracted from blood indicates a potential somatic origin for these CNVs. It is possible that the rate of cell proliferation in arteries is lower than that of blood that continuously renews, providing more opportunities for genomic alterations over time in blood cells.

Acknowledgements: The study was supported by the Russian Science Foundation (№14-15-00305).

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TREATMENT OF TROPHIC ULCERS USING PLATELET-RICH PLASMA

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Key words: *Platelet-rich plasma (PRP), trophic ulcers, diabetic foot syndrome*

Motivation and Aim: To develop treatment technology for torpid trophic ulcers including patients with diabetic foot syndrome with the use of platelet-rich plasma (PRP). Stimulation of angiogenesis is a key aspect in the healing of ulcers. So the impact of the platelet on pathogenetic mechanism of ulcer formation allows us to consider cell therapy in combination with etiotropic treatment as an effective modern alternative to traditional methods.

Methods and Algoritm: Patients have been randomized (ratio 1:1) into 2 groups (control group and main group). Patients of control group received standard treatment of trophic ulcers. Patients of main group received standard therapy and injections of platelet-rich plasma (doubly, intervals of 3-5 days). Evaluation of treatment methods has been performed using analysis of medical history, basic physiological parameters (blood pressure, heart rate, BH, body temperature), measurement of height and weight, ulcer planimetry, determination of the phase of wound healing process (smear-imprint of the wounds), transcutaneous oximetry. Platelet-rich plasma (PRP) is the plasma, in which the platelet count exceeds the normal (150,0—350,0 x 109/л) and is about 1000,0 x 109/l. PRP is an autologous source of growth factors, which is obtained by separation of whole blood by density gradient. Growth factors have a local activity and attract the undifferentiated cells into the damaged area, launching the process of mitosis of these cells. Platelet derived growth factors attract stem cells to the damaged area and induce their proliferation when they reach the area of injury. The more growth factors will be delivered to the wound the greater potential for wound healing will be reached. Injections of platelet have been performed in the margins of ulcer.

Results: The healing of venous ulcers in main group occurs on average by 20% faster than that of the control group. Necrolysis in the main group accelerated by 2.1 days, the appearance of granulation tissue has accelerated in the main group by 2.4 days, the emergence of regional and focal epithelialization - 3.9 days compared to the control group. The average speed of epithelialization has increased by 23.3% in the main group. Rate of decrease in ulcer area is an important parameter of evaluating of the proposed treatment method effectiveness. Significantly higher rate of reduction of ulcer area was observed in the main group (4.68 + 0.78%/day), than in the control group (3.51 + 0.45%/day). The cytology of wounds smear-imprints was carried out for prediction of wound healing process. Cytograms of regenerative type have been registered in 58% cases of the control group after 18 days of treatment. While cytograms of regenerative type were in 85% cases of the main group after 18 days of treatment.

Conclusion: Usage of plasma, platelet-rich (PRP) leads to more rapid healing of trophic ulcers. PRP efficacy determines the local activity of platelet growth factors that attracts stem cells to the damaged area and induces their proliferation after they reach the area of injury.

DEVELOPMENT OF TISSUE-ENGINEERING CELL-SEEDED CHITOSAN-POLYCAPROLACTONE BLENDS FOR VASCULAR SURGERY

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Key words: *tissue engineering, endothelial cells, mural cells, polycaprolactone, chitosan, vascular surgery*

Nowadays, there is a necessity of obtaining small-diameter vascular substitutes in vascular surgery. Tissue engineering provides the opportunity to overcome the long-term outcomes of synthetic vascular grafts. The choice of the optimal scaffold and cell source for seeding are key conditions to bring properties of vessel substitute to physiological. Previous publications have shown that a chitosan-polycaprolactone blend is a suitable biodegradable material for tissue engineering [1], [2].

In this study, for the first time, we suggest an efficient method to generate of tissue-engineered chitosan-polycaprolactone blends, cellularized by endothelial and mural cells of human cardiac explants. Cultured on the blended membranes cells demonstrate high levels of proliferation, adhesion and viability; retain their functional properties (taking up ac-LDL, forming tube-like structure in matrigel); maintain specific endothelial (CD 31, vWF) and mural markers (SMMHC, alpha-sma) and antigens and synthesis of extracellular matrix (collagen IV, fibronectin and elastin). In addition, we have found that proliferative properties of cells of human cardiac explants depend on blend ratio and neutralization conditions.

These results suggest that tissue-engineered chitosan-polycaprolactone blends seeded by endothelial and mural cells of human cardiac explants may be potential for development of substitutes for small diameter blood vessels with properties maximally close to the physiological.

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VIROME ANALYSIS FOR IDENTIFICATION OF VIRUSES IN BAT SPECIES FROM MOSCOW REGION

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The majority of infectious diseases that were discovered during the last few decades are actually zooantroponosis. Bats are widely distributed in the world and recognized reservoirs of many emerging human infection viruses. Analysis of the virome of bats that are distributed in different geographical regions is an actual approach for identifying the new species of viruses that potentially cause human infection disease. In addition, periodic monitoring of virome in bats populations may provide important information for zooantroponosis control. Numerous studies have described viruses in different bat species from countries of Europe, Asia, Africa but not Russia.

We characterized the fecal virome of 29 wild bats. Fecal samples were collected during 2015 in Moscow Region (Zvenigorod Biological Station, ZBS) from six species: *Myotis dasycneme*, *Myotis daubentonii*, *Myotis brandtii*, *Nyctalus noctula*, *Pippistrellus nathusii*, *Plecotus auritus*. Ectoparasite analysis of animals resulted in mites (24 samples) or mites and fleas (2 samples). The eight bats were healthy and the three animals were not examinated. We used PCR assay targeted on *Astroviridae*, *Coronaviridae*, *Herpesvirus*, *Lyssavirus I*, *Lyssavirus II*, *Caliciviridae* (nairovirus), *Filoviridae*, arenavirus, rotavirus, paramyxovirus. High throughput sequencing analysis was performed using Illumina MiSeq. Data analysis was conducted as described in study Dedkov et al., 2016.

The results revealed that 13 of 29 analyzed bats (45%) contained coronaviruses. After the SARS epidemic (a few years ago) some studies enabled hypotheses of bats as reservoir hosts of coronaviruses. It was demonstrated that 6 of the 15 recognized coronavirus species were only found in bats. In this work, we found that five of six investigated bat species are hosts of different strains similar to known coronaviruses (including porcine epidemic diarrhea virus). The only *P. auritu* (single sample) was free from coronaviruses. Our results demonstrated that ZBS populations of bats are abundant reservoir of coronaviruses.

We also confirmed other viruses that had previously been reported in different bat species from other regions: astroviruses were found in *M. dasycneme* and *M. dasycneme*. The members of herpesvirus and cypovirus genera were detected in *P. nathusii* and *M. brandtii* respectively. The twelve animals were healthy.

Our result revealed the partial genome sequences of two new novel mammalian viruses. The few sequence reads of virome from *M. daubentonii* showed similarities to Ippy mammarenavirus (51% protein identity with high e-value). In other sample several reads demonstrated ~71-78% protein identity with credible e-value to polymerase of different Rhabdoviridae family representatives .

Our work provides the first report about the bat viromes in Russia. It should enhance understanding of the viruses communities present in bat species found near human habitats.

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METHODS FOR DETECTING SITES OF 2'-O-METHYLATION IN RNA FOR APPLICATIONS IN BIOMEDICAL RESEARCH

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Key words: *RNA modification, detection of 2'-O-methylated nucleotides, diagnostic approaches*

Motivation. Mature forms of various classes of eukaryotic RNA contain numerous non-canonical nucleotides that are modified at bases or ribose. Post-transcriptional modifications of non-coding RNAs are essential for formation of a "proper" spatial structure of RNA molecules, intermolecular interactions and for further function of sophisticated supramolecular complexes. Recent studies have shown that modification levels of nucleotides in both mRNA and ncRNA can exhibit a dynamic nature. One of the most abundant types of modification in ncRNA is ribose 2'-O-methylation. In particular, human ribosomal RNAs include about 100 conserved 2'-O-methylated nucleotides.

Methods. The method of reverse transcription termination is one of the most widely used approaches for detection of 2'-O-methylated nucleotides in long RNAs. The conventional technique assumes the reaction of reverse transcription with radioactively labeled primer and separation of cDNA products in a polyacrylamide gel with further autoradiography.

Results. In the current study, two modified variations of this method have been proposed for quantitative evaluation of the level of 2'-O-methylation of individual nucleotides in long RNAs. First of all, an adaptation of the method for the usage of 5'-fluorescently labeled primers with further analysis of reverse transcription termination products on an automated DNA analyzer has been performed. The proposed approach allows not only to detect 2'-O-methylation sites but also to perform relative assessment of modification level, and therefore to obtain more complete information on the distribution of modified monomers in extended regions (up to 300-500 b) of an RNA-template. Secondly, a two-step real-time RT-PCR approach with heightened (>1.0 mM) dNTP concentration on the first step has been proposed, which allows quantification of the level of 2'-O-methylation of individual nucleotides in RNA-template. Using this approach, we have evaluated the modification level of several native sites of 2'-O-methylation in human 28S and 18S rRNAs isolated from cultured breast adenocarcinoma cells MCF-7 and MDA-MB-231 and primary breast cancer cells.

Conclusions. Both of the developed approaches eliminate the necessity of routine stages of sequencing polyacrylamide gel-electrophoresis and autoradiography, which extends the possibilities of using the methods for detection of 2'-O-methylation sites in RNA in biomedical research. The possibility to quantify the level of RNA post-transcriptional modification opens up new perspectives for characterization of cellular pathology models and diagnosis of human diseases.

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ENDOTHELIALIZATION OF SMALL DIAMETER ELECTROSPUN VASCULAR GRAFTS

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Key words: *vascular grafts, electrospinning, endothelialization, polycaprolactone*

Motivation and Aim: Absence of stenosis, inflammation, and induction of clotting in the implanted in bloodstream artificial vascular grafts (VG), imply endothelialization of the inner surface of VG [1]. This process is largely dependent on the efficient adhesion and proliferation of endothelial cells as well as their precursors on the surface of VG. To produce VG covered with endothelium a bioreactor and reliable protocols for culturing of endothelial cells on the inner VG surface are required.

Methods: We have optimized the composition of 3D matrices produced by electrospinning and destined for cultivation of endothelial cells and produce vascular grafts. Bioreactor for endothelialization of VG was designed and produced together with protocols of cell seeding and culturing.

Results: Human primary endothelial cells from umbilical cord vein (HUVEC) were used as a model cells. 3D matrices and vascular grafts with inner diameter of 1.8 mm were prepared from the solution of polycaprolactone (PCL) with gelatin in hexafluoroisopropanol using electrospinning set-up MECC NF-103. Estimation of adhesion efficacy and cell viability on the surface of matrices with different concentrations of gelatin demonstrated that optimal substrate for endothelial cells adhesion is PCL scaffolds with 10% gelatin treated with glutaraldehyde. Sedimentation and adhesion rates of endothelial cells (10^6 cells/ml, 6 mm column height) on the surface of culture plastic and 3D scaffolds were determined by microscopy. The sedimentation rate was 50 cells/mm^2·min, HUVEC started to form contacts with the support 10 min after precipitation and complete the adhesion in 45 min. Bioreactor is installed in a Petri dish of 100×100×16 mm (Sarstedt), and supplied with membrane pump and rotating assembly driven by electromagnets. The bioreactor and electromagnets unit are placed in a CO_2 incubator, electromagnets are controlled by an external programmable controller. To provide efficient and universal HUVEC adhesion the rotation regime was selected as rotation by ¼ of full turn every 30 seconds for the first hour and ¼ turn every 5 minutes for next 5 hours. After seeding the cells were cultured in a culture medium flow (60 µl/0.3 s, 1 cycle (2 min) includes 3 pumps and rotation per a quarter turn). It was shown that the cells cultured under these conditions evenly occupy wall of the prosthesis. In rotation mode, the long axis of the cell is oriented perpendicular, while in pumping mode it oriented along the flow direction.

Conclusion: Thus, 3D matrices and VG apt to endothelialization as well as a custom designed and manufactured bioreactor for production of cell-populated VG together with protocols of cell seeding and cultivation were elaborated for production of cell-populated VG.

This work was supported by a Russian Science Foundation grant (№ 14-15-00493).

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X-CHROMOSOME EPIGENETICS OF HUMAN PLURIPOTENT STEM CELLS

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Key words: *pluripotent stem cells, X-chromosome inactivation*

Motivation and Aim: Human pluripotent stem cells (hPSC) 46, XX have unstable epigenetic states of X-chromosomes, which may change in cell culture.

Methods, Algorithms and Results: In our work, we have shown that cell culture condition may influence the epigenetic status of X-chromosomes in human pluripotent stem cells. In particular, we demonstrated that the culture condition affects trimethylation of histone H3 at lysine K9 (H3K9me3). We found that one of the two X-chromosomes is enriched of H3K9me3 in hPSCs grown on matrigel. The H3K9me3 enrichment is associated with histone methyltransferase SETDB1 and protein KAP1, which is responsible for SETDB1 recruitment to chromatin. Pluripotent clones marked with green fluorescent protein (GFP) were derived from embryonic stem cell line HuEs9. We established that X-chromosomes in the clones expressing GFP demonstrate the whole spectrum of epigenetic states typical for the initial cell culture, and the percentage of cells with X-chromosome enriched with H3K9me3 does not significantly differ from that in the initial cell culture.

Conclusion: Thus, we concluded that heterogeneity of epigenetic states in X-chromosomes of the HuES9 culture could be associated with ongoing epigenetic processes affecting one of the two X-chromosomes.

PLATELET-RICH PLASMA INCREASE ON PROLIFERATION AND MIGRATION OF MESENCHYMAL STEM CELLS OF BONE MARROW

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Key words: *bone marrow-multipotent mesenchymal stromal stem cells, platelet-rich plasma, proliferation, migration*

Motivation and Aim: Platelet-rich plasma (PRP) is an autologous source of growth factors: PDGF, IGF, EGF, TGF, HGF, etc. These of growth factors may influence on the proliferation and migration activity of multipotent mesenchymal stem cells (MMSCs), the ability of MMSC to differentiate into osteoblasts, hondro- and adipocytes. MMSC are the attractive source of cells for regenerative medicine because these immature cells are present in many organs and tissues of body. The purpose of research was to assess the impact of PRP on proliferative and migratory activity of rat bone marrow MMSC (BM-MMSC).

Methods and Algorithms: BM-MMSCs were isolated from Wistar rats and cultured under standard conditions. PRP was prepared from peripheral blood of healthy donors after centrifugation in special tubes (Plasmolifting ™) under the conditions of 3800 rpm/min 6 min. The proliferative activity of BM-MMSCs was studied using xCELLigence System and results are presented in the form of a cellular index (CI). Migration activity of BM-MMSCs was investigated using In Cell Analyzer 2200 by desquamation of the monolayer, the result shows the percentage of the area of the closure of desquamation. These devices enable real-time to assess the functional activity of cells.

Results: It has been established that PRP influences on BM-MMSCs that begin to adhere to the tablet surface after 3 hour and proliferate in the first hours of the experiment (6 hours). We have shown that the spontaneous proliferative activity BM-MMSCs was higher (CI = 0.16, p = 0.03) compared to cells proliferation in presence of PRP (CI = 0.09) after 12 hours of the experiment. The spontaneous proliferation and proliferation in the presence of PRP became almost identical (CI = 0.17 and 0.18, respectively) after 18 hours of the experiment. Importantly, to the end of the experiment the proliferative activity of BM-MMSCs significantly increased by the action of PRP (CI = 0.2, p = 0.03) compared to the spontaneous proliferation (CI = 0.14).

The horizontal migration model revealed that the area closing surface of rat BM-MMSCs was 83% in case of PRP adding after 24 hours from the start of the experiment that was significantly higher than the area closing the spontaneous migration of the wound - 48% (p = 0.003). The area of closing of BM-MMCK surface was 94% and spontaneous migration closing area was 78% under PRP addition at the 36th hours of experiment. It should be noted that the area of closing of a surface of BM-MMCK in spontaneous migration and in the presence of PRP has reached 100% after 44 hours from the beginning of experiment, though the speed of wound closing was significantly higher (p=0,006) under PRP addition on time intervals of supervision (4 hours).

Conclusion: It has been revealed that PRP has the great stimulatory effect on the proliferative and migration activity of BM-MMSC.

Availability: The PRP is the source of growth factors to increase the regenerative potential of transplanted multipotent mesenchymal stem cells.

MUTATIONS SPECTRA OF MAJOR ONCOGENES IN PATIENTS WITH MULTIPLE PRIMARY NEOPLASIA

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Motivation and Aim: The study of genetic predisposition to cancer has a high predictive value for early detection and proper treatment. The occurrence of multiple non-metastatic primary malignancy in one patient reveal a genetic predisposition to the development of cancer.

Methods: A total of 8 patients of Novosibirsk Regional Clinical Oncology Hospital with multiple primary neoplasia were included in this study. Patient information, including sex, age, tumor type and family history were recorded. DNA was extracted from 5 ml of whole human blood. The next generation sequencing Ion AmpliSeq™ Cancer Hotspot Panel v2 for Ion Torrent PGM was used to investigate generative mutations spectrum in the samples from all patients. The panel used targeted 207 amplicons encompassing 2800 known cancer-relevant variants across 50 cancer-related genes. Each sample was individually barcoded, all 8 samples were pooled prior E-PCR, loaded on a 316v2 Chip and sequenced according to the Ion PGM 200 Sequencing protocol. The average depth of total coverage was >200, each nucleotide coverage was >50. Sequencing reads were analyzed using Torrent Suite software program with the 'variant caller v4.0.2' plugin and aligned to the human reference genome, hg19, which was uploaded on the Ion Reporter software v4.2 to perform variant calling and mapping.

Results: A total of 94 polymorphic variants in 207 regions covering "mutation hotspots" in 50 tumor-related susceptibility genes were found for 8 patients. Number of mutations per patient varies from 9 to 17 homo- or heterozygous SNP. Among the 50 genes included in panel, 17 genes were found mutated. All 8 patients had variations in *FGFR3* gene. Most of patients had mutations in *TP53, EGFR, PDGFRA* genes. Six patients in our cohort had at least two hotspot mutations associated with cancer according with COSMIC database.

Conclusion: Patients with multiple primary neoplasia revealed a large number of polymorphic variants in the major oncogenes. This data confirms the assumption of strong genetic predisposition to the development of cancer for patients with multiple primary neoplasia.

FROM PHARMACOGENETICS TO MODERN PHARMACOTHERAPY

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Key words: *drug, receptor, ligand, pharmacogenetics, pharmacokinetics, networks*

Pharmacotherapy is the key modality for many socially important diseases. Their substantial prevalence in the population and the growing knowledge about their pathogenesis form the objective basis for drug discovery and rational drug design [1]. Therapeutic and side effects are integral parts of a drug's action. Therefore, the efforts of clinicians, pharmacologists, pharmacists, and other relevant specialists are directed at increasing the therapeutic index through improvement of a drug's properties and its administration protocols and via development of new pharmaceuticals.

In the middle of the 20th century, first evidence appeared that individual therapeutic responses to a drug can vary widely. These examples laid the foundation for pharmacogenetics: the science concerned with the role of genetic factors in the response to a pharmacological agent. The term *pharmacogenetics* proper was proposed in 1959 by F. Vogel; by that time, some data had been already available on the disposition of a drug (within the human body) as a combination of the sequential stages of absorption, distribution, metabolism, interaction with the molecular target, and excretion. In addition, a mathematical platform had been developed for calculation of quantitative parameters that describe the above processes: pharmacokinetic estimates. Unification of these two approaches played a positive role in the advances of personalized pharmacotherapy in particular and personalized medicine in general. Genetic testing and therapeutic monitoring of drugs opened up the opportunities for real-life applications to clinical practice.

Theoretically speaking, pharmacogenetics fitted P. Erlich's theory well regarding the interaction of a receptor and a ligand as the basis for a pharmacological effect. The history of the development of pharmacology and pharmacotherapy along this path features many breakthroughs. New technological possibilities—'omics methods—ensured highly productive work on the design of new drugs in accordance with the above paradigm. Nonetheless, in recent years, some fundamental problems have appeared that follow from this theory, namely, the substantial failure rate among new promising pharmaceutical agents that have reached the stage of clinical trials. As much as 30% of this failure can be attributed to insufficient efficacy, and another 30% to toxicological problems [2]. These challenges and ways to overcome them were reviewed by a panel of experts in October 2011, who drafted the "White Paper" [3]. In the opinion of those authors, further development of pharmacology would be best ensured via implementation of the concepts of systems biology, which will allow modern pharmacologists to apply systems level ideas to practical problems associated with drug discovery and to study the therapeutic response in the context of increasing knowledge about the complicated relations among signaling, transcriptional, and metabolic networks and about individual differences (among patients), which result from genetic differences and environmental factors.

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SEX DIFFERENCES IN BRAIN METABOLITES AND BEHAVIOR IN HAMSTERS EXPERIMENTALLY INFECTED WITH *OPISTHORCHIS FELINEUS*

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Key words: *O.felineus infection, brain ^{1}H MRS metabolites, behavior*

Motivation and Aim: To determine the influence of *O. felineus* infection on brain ^{1}H MRS metabolites and behavior of the hamsters with chronic opisthorchiasis.

Methods and Algorithms: 44 eight-week-old male and female of Syrian (Golden) hamsters (*Mesocricetus auratus*) were obtained from SPF Vivarium, Department of Experimental Animal Genetic Resources of Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia. Each hamster from experimental group was administered with 50 *O. felineus* metacercariae by oral intragastric intubation. During 6 months, body weight, food and water consumption were measured regularly. 6 months after the infection animals have been tested in the open field (OF), T-maze alternation, social dominance tests, and subjected to ^{1}H MRS brain investigation (medial PFC, HPC, HPT). Spearman Rank Order correlations were used to examine the relationship between different types of male and female behavior, and choline containing compounds (Cho), GABA concentrations in medial PFC.

Results: Chronic *O. felineus* infection influences the behavior of male and female hamsters in different ways. We observed significant change in behavior using OF test and moderate increase in social dominance behavior in males. Females started to increase the water consumption 4 weeks after *O. felineus* invasion, but they had no significant changes in the most of behavioral tests compared to control animals. Sex differences were found in the brain response to the infection. In females, GABA level in the medial PFC was found to be significantly decreased, and Cho level was increased; Cho level in the hippocampus was decreased; we saw no changes in metabolites levels in hypothalamus. In males there were no significant changes in all three brain regions. Cho level in medial PFC was found to be significantly correlated with the water consumption in the control females. In the infected females significant negative correlations between GABA level and activity in the OF were found. In the control but not the infected males, GABA level was found to be positively correlated with social dominance.

Conclusion: O. felineus infection changes the hamster behavior and brain metabolite levels. At the same time there are significant sex differences.

HOCOMOCO COMPREHENSIVE MODEL COLLECTION AS A PRACTICAL GATEWAY TO REGULATORY MOTIF-OME OF HUMAN AND MOUSE TRANSCRIPTION FACTORS

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Key words: *transcription factor binding motifs, human, mouse, ChIP-Seq, HT-SELEX*

Motivation and Aim: Knowledge of sequence motifs resembling transcription factor binding sites is beneficial for a vast array of studies in regulatory genomics.

Methods and Algorithms: Using ChIPMunk [1] motif discovery tools we performed *de novo* motif discovery in more than two thousands data sets for human and mouse transcription factors studied by ChIP-Seq (in vivo, obtained from GTRD [2]) and HT-SELEX (in vitro [3]). The newly created binding models were benchmarked against known binding patterns for mammalian transcription factors.

Results: We present the latest release of the HOCOMOCO COmprehensive MOdel COllection [4] that provides binding models for 6 hundreds of human and almost 4 hundreds of mouse transcription factors. The primary collection provides classic mononucleotide position weight matrices (PWMs) which are linked with the hierarchical classification of transcription factors [5]. In addition, new release of HOCOMOCO includes dinucleotide position weight matrices based on ChIP-Seq data and a set of command-line java tools to facilitate motif finding with HOCOMOCO models.

Conclusion: We present a complete workflow used to build HOCOMOCO and discuss practical applications of the HOCOMOCO *motif*-ome in regulatory genomics.

Availability: HOCOMOCO and all the supporting tools are freely available online: http://hocomoco.autosome.ru and http://opera.autosome.ru.

Acknowledgements: This study was supported by RFBR grants 15-34-20423 and, partly, 14-04-01838.

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A VARIATION APPROACH TO SOLVING OF A PARAMETER IDENTIFICATION PROBLEM FOR THE MATHEMATICAL MODEL OF HIV DYNAMICS

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Key words: *mathematical model of HIV dynamics, ordinary differential equations, parameter identification, optimal treatment control, inverse problem, genetic algorithm.*

Motivation and Aim: Mathematical models in immunology are described by systems of nonlinear ordinary differential equations. It is important to determine parameters of these systems that characterize features of immunity and disease for constructing an individual treatment plan. The purpose of this work is the construction and investigation of the numerical algorithm for determining of coefficients in nonlinear system that describes HIV dynamics with treatment [2, 3] using additional information about given concentrations at fixed times.

Methods and Algorithms: The parameter identification problem (inverse problem) for mathematical model of HIV dynamics (*i.e.* dynamics of infected and uninfected CD4 T-lymphocytes, infected and uninfected macrophages, free virus, immune effectors (CD8 - cells)) with treatment using additional measurements of some concentrations in fixed times is numerically investigated. In this paper two inverse problems are considered [1]: firstly, the inverse problem for mathematical model without treatment and then optimal control problem for identify effective treatment function. The first inverse problem is reduced to minimization problem of least square function that describes the deviation between model and measured data. Then the problem of optimal treatment control is solved by minimizing another misfit function that characterizes combination of viral load and treatment costs. The numerical algorithm for solving inverse problems is based on stochastic approach (genetic algorithm).

Results: Individual parameters that characterize the human immune system have been identified. The optimal treatment control for an individual patient has been received.

Conclusion: It is shown that optimal treatment plan is better determined if previously individual parameters for patient are identified well. The results of the numerical calculations are presented and discussed.

Availability: Using the results of this paper one can make an individual patient's treatment plan. It will extend the duration of the patient's life.

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