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1. Solovyeva N.I., Gureeva T.A., Timoshenko O.S., Moskvitina T.A., Kugaevskaya E.V.

Furin as proprotein convertase and its role in normal and pathological biological processes.

Furin belongs to serine intracellular Ca2+-dependent endopeptidases of the subtilisin family, also known as proprotein convertase (PC). Human furin is synthesized as zymogen with a molecular weight of 104 kDa, which is then activated by autocatalytic in two stages. This process can occur when zymogen migrates from the endoplasmic reticulum to the Golgi apparatus, where a large part of furin is accumulated. The molecular weigh t of the active furin is 98 kDa. Furin relates to enzymes with a narrow substrate specificity: it hydrolyzes peptide bonds at the site of paired basic amino acids and furin activity exhibits in a wide pH range 5-8. Its main biological function is activation of the functionally important protein precursors. It is accompanied by the launch of a cascade of reactions, which lead to appearance of biologically active molecules involved in realization of specific biological functions both in normal and in some patologicheskih processes. Furin substrates are biologically important proteins such as enzymes, hormones, growth factors and differentiation, receptors, adhesion proteins, proteins of blood plasma. Furin plays an important role in the development of processes such as proliferation, invasion, cell migration, survival, maintenance of homeostasis, embryogenesis, as well as the development of a number of pathologies, including cardiovascular, oncologic and neurodegenerative diseases. Furin and furin-like proprotein convertases participate as key factors in the realization of the regulatory functions of proteolytic enzymes, the value of which is currently being evaluated as most important in comparison with the degradative function of proteases.

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2. Gnatenko D.A., Kopantsev E.P., Sverdlov E.D.

Role of fibroblast growth factors in pancreatic cancer.

Fibroblast growth factors belong to a family of growth factors that are involved in various processes in organism and have a wide range of biological functions. Specifically for pancreas, FGFs are important during both organogenesis and carcinogenesis. One of the main characteristic of pancreatic cancer, is it close interaction between cancer and stromal cells via different factors, including FGF. Pathological changes in FGF/FGFR signaling pathway is a complex process. The remodeling effects and stimulation of tumor growth are mostly depend not only on types of receptors, but also from their isoforms. FGF/FGFR signaling pathway is a perspective specific marker for cancer progression, and a potential drug target, which can be used for treatment of pancreatic cancer.

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3. Varizhuk A.M., Sekridova A.V., Tankevich M.V., Podgorsky V.S., Smirnov I.P., Pozmogova G.E.

Conformational polymorphysm of G-rich fragments of DNA Alu-repeats. II. the putative role of G-quadruplex structures in genomic rearrangements.

Three evolutionary conserved sites of Alu repeats (PQS2, PQS3 and PQS4) were shown to form stable inter- and intramolecular G-quadruplexes (GQs) in vitro. Structures and topologies of these GQs were elucidated using spectral methods. Self-association of G-rich Alu fragments was studied. Dimeric GQ formation from two distal identical or different putative quadruplex sites â€“ (PQS2)2, (PQS3)2 or PQS2-PQS3 â€“ within one lengthy DNA strand was demonstrated by a FRET-based method. Oligomer PQS4 (folded into a parallel intramolecular GQ) was shown to form stacks of quadruplexes that are stabilized by stacking interactions of external G-tetrads (this was confirmed by DOSY NMR, AFM microscopy and differential CD spectroscopy). Comparative analysis of the properties of various GQs allowed us to put forward a hypothesis of two general mechanisms of intermolecular GQ-dependant genomic rearrangements: 1) formation of a dimeric GQs; 2) association of pre-folded intramolecular parallel GQs from different strands into GQ-stacks. Thus, the observed co-localization of G-rich motifs of Alu elements with double-strand break hotspots and rearrangement hotspots may be accounted for by the specific secondary structure of these motifs. At the same time, this is likely primarily due to high abundance of such G-rich Alu fragments in the genome.

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4. Brovkina O.I., Gordiev M.G., Toropovskiy A.N., Khodyrev D.S., Enikeev R.F., Gusev O.A., Shigapova L.H., Nikitin A.G.

Determination of EGFR gene somatic mutations in tissues and plasma of patients with advanced non-small cell lung cancer.

The presence of activating mutations in the EGFR gene influences cell proliferation, angiogenesis, and increases metastatic ability; it has a significant impact on the choice of medical therapy of non-small cell lung cancer (NSCLC). The use of targeted therapy with tyrosine kinase inhibitors requires performance of appropriate genetic tests. The aim of this study was to design a real-time PCR-based diagnostic kit for fast and cheap of EGFR mutations testing in paraffin blocks and plasma, and kit validation using samples from patients with NSCLC, and also comparative estimation of diagnostic features of real-time PCR with wild type blocking and digital PCR for mutation testing in blood plasma. The study included 156 patients with various types of adenocarcinoma differentiation. It was designed a simple and efficient real-time PCR-based method of detecting L858R activating mutation and del19 deletion in the EGFR gene for DNA isolated from paraffin blocks. Kit for EGFR mutations was validated using 411 samples of paraffin blocks. The proposed system showed high efficiency for DNA testing from paraffin blocks: a concordance with results of testing with therascreenÂ® EGFR RGQ PCR Kit (`Qiagen`, Germany) was 100%. It has been shown the possibility of using this test system for the detection of mutations in plasma

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5. Tapbergenov S.O., Sovetov B.S., Tapbergenov A.T.

Features of influence adenosine, AMP and hyperadrenalinemiya on the immune status, metabolic enzymes of purine nucleotides and the antioxidant defense system.

Administration of a large dose of adrenaline (4 mg/kg 60 min before analysis) increased blood levels of total leukocytes, lymphocytes, decreased T-cell suppressors, leukocyte migration inhibition reaction (LMIR) and NBT test, but increased the level of conjugated dienes (CD). Administration of AMPand adenosine increased levels of total leukocytes, lymphocytes, T- lymphocytes, T-helpers, decreased the level of malondialdehyde (MDA), LMIR, and T-cell suppressors. Sympathetic hyperactivation induced by administration of a large dose of adrenaline (4 mg/kg 60 min before analysis) was accompanied by an increase in heart and liver activities of glutathione peroxidase (GPx), catalase, AMP deaminase (AMPD), and adenosine deaminase (AD). Administration of AMP or adenosine caused a decrease in activities of glutathione reductase (GR), GPx, catalase, a decrease in the MDA level and an increase in activities of AMPD and AD in the heart. In the liver AMP and adenosine also caused a decrease in activities of glutathione reductase (GR), GPx, a decrease in the MDA level and an increase in activities of AMPD and AD. The data obtained suggest that administration of adrenaline, AMP, and adenosine influences activity of enzymes involved in purine nucleotide metabolism. However, in contrast to adrenaline, administration of AMP or adenosine does not provoke stress reaction.

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6. Sirota T.V.

Standardization and regulation of the rate of the superoxide-generating adrenaline autoxidation reaction used for evaluation of pro/antioxidant properties of various materials.

The superoxide-generating reaction of adrenaline autoxidation is widely used for determination of the activity of superoxide dismutase and pro/antioxidant properties of various materials. There are two variants of the spectrophotometric registration of the products of this reaction. The first is based on registration of adrenochrome, as adrenaline autooxidation product at 347 nm; the second employs nitro blue tetrazolium (NBT) and registration of diformazan, a product of NBT reduction at 560 nm. In the present work, recommendations for the standardization of the reaction rate in both variants have been proposed. The main approach consists in the use of the pharmaceutical form of 0.1% adrenaline hydrochloride solution. Although each of two adrenaline preparations available in the Russian market has some features in kinetic behavior of its autooxidation; they are applicable in the superoxide generating system based on adrenaline autooxidation. Performing measurements at 560 nm, the reaction rate can be regulated by lowering the concentration of added adrenaline, whereas during spectrophotometric registration at 347 nm, this cannot be done. These features of adrenaline autoxidation may be due to the fact that the intrinsic multistage process of the conversion of adrenaline to adrenochrome, which is recorded at 347 nm, is coupled with the transition of electrons from adrenaline and intermediate products of its oxidation to oxygen, carbon dioxide, and carbonate bicarbonate ions, which is detected in the presence of added NBT.

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7. Yakovlev A.A., Lyzhin A.A., Aleksandrova O.P., Khaspekov L.G., Gulyaeva N.V.

Trophic factors deprivation induces long-term protection of neurons against excitotoxic damage.

One of the strategies to induce tolerance of neurons to toxic injury is preconditioning. Preconditioning is caused by a weak damage of cells, which become more resistant to subsequent, more severe damage. We found that preconditioning by deprivation of trophic factors, or deprivation of trophic factor and glucose effectively protects neurons against subsequent toxic effects of glutamate. Deprivation of trophic factors plays a decisive role in the development of resistance, regardless of whether it has been combined with glucose deprivation or not. Neuronal protection is achieved when the deprivation lasts from 30 min to two hours and is kept for a period of from one to five days. Preconditioning is accompanied neuronal secretion of cathepsin B occurs. We suggest that this phenomenon is associated with a more general process of exocytosis of lysosomes triggered by deprivation of trophic factors.

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8. Ruzaeva V.A., Morgun A.V., Khilazheva E.D., Kuvacheva N.V., Pozhilenkova E.A., Boitsova E.B., Martynova G.P., Taranushenko T.E., Salmina A.B.

Development of blood-brain barrier under the modulation of HIF activity in astroglial and neuronal cells in vitro.

Barriergenesis is the process of maturation of the primary vascular network of the brain responsible for the establishment of the blood-brain barrier. It represents a combination of factors that, on the one hand, contribute to the process of migration and tubulogenesis of endothelial cells (angiogenesis), on the other hand, contribute to the formation of new connections between endothelial cells and other elements of the neurovascular unit. Astrocytes play a key role in barriergenesis, however, mechanisms of their action are still poorly examined. We have studied the effects of HIF-1 modulators acting on the cells of non-endothelial origin (neurons and astrocytes) on the development of the blood-brain barrier in vitro. Application of FM19G11 regulating expression of HIF-1 activity and GSI-1 suppressing gamma-secretase and/or proteasomal activity resulted in the elevated expression of thrombospondins and matrix metalloproteinases in the developing blood-brain barrier. However, it caused the opposite effect on VEGF expression thus promoting barrier maturation in vitro.

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9. Nosareva O.L., Ryazantseva N.V., Stepovaya E.A., Shakhristova E.V., Stepanova E.A., Gulaya V.S.

The role of heat shock proteins 27 and 70 in redox-dependent regulation of apoptosis in Jurkat tumor cells.

Heat shock proteins Hsp) act as molecular chaperones, protecting enzymes and other proteins against reactive oxygen species. The objective of the study was to investigate the role of Hsp27 in maintaining the balance of the glutathione system and Hsp70 concentrations as well as in implementing Jurkat tumor cell apoptosis. Addition of the Hsp27 inhibitor KRIBB3 (5-(5-ethyl-2-hydroxy-4-methoxyphenyl)-4-(4-methoxyphenyl)-isoxazol) to Jurkat

cells resulted in glutathione redox imbalance (increased GSSG and increased glutathione reductase activity), a decrease in Hsp70 concentrations, and also increased cell apoptosis as compared with to the intact cell culture. The proposed selective regulation of chaperone activity is a promising direction in regulating apoptosis at the cellular level.
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10. Kholodenko I.V., Kholodenko R.V., Manukyan G.V., Yarygin K.N.

The hepatic differentiation of adult and fetal liver stromal cells in vitro.

The liver has a marked capacity for regeneration. In most cases the liver regeneration is determined by hepatocytes. The regenerative capacity of hepatocytes is significantly reduced in acute or chronic damage. In particular, repair mechanisms are not activated in patients with alcoholic cirrhosis. Organ transplantation or advanced methods of regenerative medicine can help such patients. The promising results were obtained in clinical trials involving patients with various forms of liver disease who received transplantation of autologous bone marrow stem cells. However, to improve the effectiveness of such treatment it is necessary to search for more optimal sources of progenitor cells, as well as to evaluate the possibility of using descendants of these cells differentiated in vitro. In this study we isolated stromal cells from the liver biopsies of three patients with alcoholic cirrhosis, conducted their morphological and phenotypic analysis, and evaluated the hepatic potential of these cells in vitro. The stromal cells isolated from fetal liver were used for comparison. The results of this can serve as a basis for the development of a new method for the treatment of end-stage liver disease. The stromal cells isolated from the liver biopsies for a long time proliferate in a culture and this which makes it possible to expand them to large amounts for subsequent differentiation into hepatocyte-like cells and autologous transplantation.
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11. Zernii E.Yu., Gancharova O.S., Ishutina I.E., Baksheeva V.E., Golovastova M.O., Kabanova E.I., Savchenko M.S., Serebryakova M.V., Sotnikova L.F., Zamyatnin Jr. A.A., Philippov P.P., Senin I.I.

Mechanisms of perioperative corneal abrasions: alterations in tear film proteome.

Perioperative corneal abrasion is an ophthalmic complication commonly found in patients underwent general anesthesia. In this study, correlations between development of corneal injury and proteomic changes in tear film during general anesthesia were examined using an animal (rabbit) model. Being started after 1-h anesthesia, the process of accumulation of pathological changes in the cornea unequivocally led clinically significant abrasions following 3-6 h of the narcosis. The corneal damage was associated with alterations in profiles of major proteins of the tear film. Analysis of the tear proteome pointed to depression of lachrymal glands function, and suggested serotransferrin, serum albumin and annexin A1 as potential tear markers of the complication. The tear film alterations included fast drop of total antioxidant activity and activity of superoxide dismutase, and decrease in interleukin-4 and increase in interleukin-6 content indicating development of oxidative and pro-inflammatory responses. These findings suggest antioxidant and anti-inflammatory therapy as prospective approach for prevention/treatment of perioperative corneal abrasions. The observed anesthesia-induced effects should be considered in any study of ocular surface diseases employing anesthetized animals.
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12. Mikurova A.V., Rybina A.V., Skvortsov V.S.

Prediction of selective inhibition of neuraminidase from various influenza virus strains by potential inhibitors.

A universal model of inhibition of neuraminidases from various influenza virus strains by a particular has been developed. It is based on known 3D data for neuraminidases from three influenza virus strains (A/Tokyo/3/67, A/tern/Australia/G70C/75, B/Lee/40) and modeling of 3D structure of neuraminidases from other strains (A/PR/8/34 and A/Aichi/2/68). Using docking and molecular dynamics, we have modeled 235 enzyme-ligand complexes for 185 compounds with known IC50 values. Selection of final variants among three results obtained for each enzyme-ligand pair and calculation of independent variables for generation of linear regression equations was performed using MM-PBSA/MM-GBSA. This resulted in the set of equations individual strains and the equations pooling all the data. Thus using this approach it is possible to predict inhibition for neuraminidase from each of the considered strains by a particular inhibitor and to predict the range of its action on neuraminidases from various influenza virus strains.
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13. Kudinov V.A., Ipatova O.M., Fyodorov I.G., Totolyan G.G., Merzlikina N.N., Kovalyov O.B., Torkhovskaya T.I., Uchaikin V.F., Starovoitova I.E., Milyutin D.V., Nikonova S.M.

Investigation of efficacy of phospholipovit for correction of the hepatic encephalopathy.

This paper presents the results of clinical studies on the efficacy and safety of the drug Phospholipovit in different groups of patients, in particular with hepatic encephalopathy and with a high risk of its development (chronic alcohol intoxication). Efficacy of treatmernt was evaluated by the LNT test (link numbers test), and standard liver plasma markers (ALT, AST, GGT, AP). The LNT test in patients with encephalopathy showed better improvement after 5 days course of Phos-pholipovit than after standard therapy. In both clinical trials liver enzyme activities, assayed in pa-tients declined more rapidly in the group of patients treated with Phospholipovit, as compared in patients received standard therapy alone. The highest clinical effect of the drug on the liver function tests was observed at a daily dose of 6.4 g of phospholipids (infusional 2 times a day) for 5-10 days. At the end of treatment a two-fold decrease in the activity of AST was observed in patients receiv-ing Phospholipovit compared to the control. This results of clinical results can be regarded as a manifestation of the expressed membrane repairing action of essential phospholipids, reinforced by their introduction into the body in the form of nanoparticles.
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14. Skorodumova L.O., Babalyan K.A., Sultanov R., Vasiliev A.O., Govorov A.V., Pushkar D.Y., Prilepskaya E.A., Danilenko S.A., Generozov E.V., Larin A.K., Kostryukova E.S., Sharova E.I.

GSTP1, APC and RASSF1 gene methylation in prostate cancer samples: comparative analysis of MS-HRM method and Infinium

HumanMethylation450 BeadChip beadchiparray diagnostic value.

There is a clear need in molecular markers for prostate cancer (PC) risk stratification. Alteration of DNA methylation is one of processes that occur during Ã•Ã‘ progression. Methylation-sensitive PCR with high resolution melting curve analysis (MS-HRM) can be used for gene methylation analysis in routine laboratory practice. This method requires very small amounts of DNA for analysis. Numerous results have been accumulated on DNA methylation in PC samples analyzed by the Infinium HumanMethylation450 BeadChip (HM450). However, the consistency of MS-HRM results with chip hybridization results has not been examined yet. The aim of this study was to assess the consistency of results of GSTP1, APC and RASSF1 gene methylation analysis in Ã•Ã‘ biopsy samples obtained by MS-HRM and chip hybridization. The methylation levels of each gene determined by MS-HRM were statistically different in the group of PC tissue samples and the samples without signs of tumor growth. Chip hybridization data analysis confirmed the results obtained with the MS-HRM. Differences in methylation levels between tumor tissue and histologically intact tissue of each sample determined by MS-HRM and chip hybridization, were consistent with each other. Thus, we showed that the assessment of GSTP1, APC and RASSF1 gene methylation analysis using MS-HRM is suitable for the design of laboratory assays that will differentiate the PC tissue from the tissue without signs of tumor growth.

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15. Kurbatov L.K., Zgoda V.G.

A possibility to use the DNA-based probes as internal standards for Agilent Technologies microarray transcriptomic analysis.

Accuracy of the microarray technology results is raised by using the multi-stage normalization of results. One of the principal requirements of such normalization is usage of internal standards. The routine Agilent microarray-based gene expression analysis protocol utilizes a Spike-In Kit during preparation of the samples representing a mixture of RNA fragments in different ratios. RNA probes which were synthesized in vitro conditions could be also used to establish how the magnitude of the fluorescent signal reflects the presence of RNA in the sample. A significant disadvantage of this type of standards is a difficulty of their production and the low RNA stability. In accordance with the Agilent protocol, the presence of the T7 promoter is necessary for the synthesis of labeled cRNA during sample preparation procedure. We hypothesized that we can successfully synthesize any RNA sequence having such type of promoter in its start position. Moreover, DNA sequence would serve as a matrix in this case. Using a set of different genes attached downstream of the T7-promoter in the plasmid DNA we have demonstrated in this study that such system can serve as a reliable template for the fluorescent labeled RNA sequence synthesis. In comparison with the routinely used internal RNA based controls, this template is stable, easy to manufacture and can be easily obtained in large quantities.

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16. Buneeva O.A., Gnedenko O.V., Medvedeva M.V., Ivanov A.S., Medvedev A.E.

The effect of neuroprotector isatin on binding of some model proteins with beta-amyloid peptide: a biosensor study.

The amyloid-beta peptide 1-42 formed during proteolytic processing of the amyloid precursor protein (APP) plays a key role in the development or progression of Alzheimer's disease (AD) and other pathologies associated with formation of protein aggregates in the central nervous system. Recent proteomic profiling of mouse and rat brain preparations by means of beta-amyloid peptide immobilized on Affigel-10 revealed a large group of amyloid-binding proteins (n>80). Many (about 25%) of these proteins were previously identified as isatin-binding proteins. The aim of this study was to validate direct interaction between beta-amyloid peptide and highly purified intact and oxidized peroxiredoxin, M-type pyruvate kinase, alpha-enolase, and the effect of isatin on this interaction. The study performed using SPR-based Biacore 3000 and Biacore X100 biosensors has shown that all the proteins form molecular complexes with immobilized beta-amyloid peptide. The Kd values for these complexes varied from 8.36Ñ…10⁻⁸ M (peroxiredoxin) to 1.97Ñ…10⁻⁶ M (alpha-enolase). Oxidative modification of investigated proteins caused opposite effects on complexes of these peptides with beta-amyloid. The endogenous neuroprotector isatin increased dissociation of complexes formed by beta-amyloid peptide with both intact proteins (peroxiredoxin, glyceraldehyde-3-phosphate dehydrogenase) and/or oxidized proteins (peroxiredoxin, pyruvate kinase) used in this study.

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17. Korolev S.P., Pustovarova M.A., Starosotnikov A.M., Bastrakov M.A., Agapkina Yu.Yu., Shevelev S.A., Gottikh M.B.

Nitrobenzofuroxane derivatives as dual action HIV-1 inhibitors.

Human immunodeficiency virus first type (HIV-1) is a main cause of one of the most dangerous diseases, AIDS. The search for new inhibitors of the virus still remains an urgent task. One approach to suppress the HIV infection is to use a double-acting inhibitors, i.e. inhibitors directed to two stages of the viral life cycle. The catalytic domain of HIV-1 integrase has a similar spatial organization with ribonuclease (RNase H) domain of HIV-1 reverse transcriptase, and approach aimed to create HIV-1 integrase and RNase H double-acting is very promising. In this work we synthesized a series of 6-nitrobenzofuroxane derivatives and studied their ability to inhibit two viral enzymes â€“ integrase and RNase H HIV-1.

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