

1. Yunusova N.V., Tugutova E.A., Tamkovich S.N., Kondakova I.V.

The role of exosomal tetraspanins and proteases in tumor progression.

Major (CD9, CD63, CD81) and others (CD82, CD151, Tspan8) tetraspanins are widely represented in exosomes, where they interact with various proteins and form functional tetraspanin complexes. Tetraspanin complexes include proteases. Tetraspanin-associated exosomal proteases (ADAM proteases, MMPs, EMMPRIN) play an important role in the processes of cell motility, migration, invasion and formation of metastases. Also, a significant contribution to tumor progression is made by proteases that are not associated with tetraspanins. They destabilize intercellular contacts, promote migration and invasion of tumor cells, participate in the regulation of the expression IGF-I, VEGF and transcription factors activation/deactivation. The role of other proteases of exosomes in the processes of tumor progression is being clarified.

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2. Buneeva O.A., Medvedev A.E.

Ubiquitin-independent protein degradation in proteasomes.

Proteasomes are large supramolecular protein complexes present in all prokaryotic and eukaryotic cells, where they perform targeted degradation of intracellular proteins. Until recently, it was generally accepted that prior proteolytic degradation in proteasomes the proteins had to be targeted by ubiquitination: the ATP-dependent addition of (typically four sequential) residues of the low-molecular ubiquitin protein, involving the ubiquitin-activating enzyme, ubiquitin-conjugating enzyme and ubiquitin ligase. The cytoplasm and nucleoplasm proteins labeled in this way are then digested in 26S proteasomes. However, in recent years it has become increasingly clear that using this route the cell eliminates only a part of unwanted proteins. Many proteins can be cleaved by the 20S proteasome in an ATP-independent manner and without previous ubiquitination. Ubiquitin-independent protein degradation in proteasomes is a relatively new area of studies of the role of the ubiquitin-proteasome system. However, recent data obtained in this direction already correct existing concepts about proteasomal degradation of proteins and its regulation. Ubiquitin-independent proteasome degradation needs the main structural precondition in proteins: the presence of unstructured regions in the amino acid sequences that provide interaction with the proteasome. Taking into consideration that in humans almost half of all genes encode proteins that contain a certain proportion of intrinsically disordered regions, it appears that the list of proteins undergoing ubiquitin-independent degradation will demonstrate further increase. Since 26S of proteasomes account for only 30% of the total proteasome content in mammalian cells, most of the proteasomes exist in the form of 20S complexes. The latter suggests that ubiquitin-independent proteolysis performed by the 20S proteasome is a natural process of removing damaged proteins from the cell and maintaining a constant level of intrinsically disordered proteins. In this case, the functional overload of proteasomes in aging and/or other types of pathological processes, if it is not accompanied by triggering more radical mechanisms for the elimination of damaged proteins, organelles and whole cells, has the most serious consequences for the whole organism.

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3. Kuzikov A.V., Masamrekh R.A., Archakov A.I., Shumyantseva V.V.

Methods for determining of cytochrome P450 isozymes functional activity.

The review is dedicated to modern methods and technologies for determining of cytochrome P450 isozymes functional activity, such as absorbance and fluorescent spectroscopy, electron paramagnetic resonance (EPR), nuclear magnetic resonance (NMR), Raman, Mossbauer, and X-ray spectroscopy, surface plasmon resonance (SPR), atomic force microscopy (AFM). Methods of molecular genetic analysis were reviewed from personalized medicine point of view. The use of chromatate-mass-spectrometric methods for cytochrome P450-dependent catalytic reactions' products was discussed. The review covers modern electrochemical systems based on cytochrome P450 isozymes for their catalytic activity analysis, their use in practice and further development perspectives for experimental pharmacology, biotechnology and translational medicine.

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4. Florinskaya A.V., Ershov P.V., Mezentsev Y.V., Kaluzhskiy L.A., Yablokov E.O., Buneeva O.A., Zgoda V.G., Medvedev A.E., Ivanov A.S.

The analysis of participation of individual proteins in the protein interactome formation.

It becomes increasingly clear that most proteins of living systems exist as components of various protein complexes rather than individual molecules. The use of various proteomic techniques significantly extended our knowledge not only about functioning of individual complexes but also formed a basis for systemic analysis of protein-protein interactions. In this study gel-filtration chromatography accompanied by mass-spectrometry was used for the interactome analysis of human liver proteins. In six fractions (with average molecular masses of 45 kDa, 60 kDa, 85 kDa, 150 kDa, 250 kDa, and 440 kDa) 797 proteins were identified. In dependence of their distribution profiles in the fractions, these proteins could be subdivided into four groups: (1) single monomeric proteins that are not involved in formation of stable protein complexes; (2) proteins existing as homodimers or heterodimers with comparable partners; (3) proteins that are partially exist as monomers and partially as components of protein complexes; (4) proteins that do not exist in the monomolecular state, but also exist within protein complexes containing three or more subunits. Application of this approach to known isatin-binding proteins resulted in identification of proteins involved in formation of the homo- and heterodimers and mixed protein complexes.

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5. *Vakhrusheva T.V., Sokolov A.V., Kostevich V.A., Vasilyev V.B., Panasenko O.M.*

Enzymatic and bactericidal activity of monomeric and dimeric forms of myeloperoxidase.

This study was carried out to compare the enzymatic and bactericidal activity of mature, dimeric myeloperoxidase (MPO) and its monomeric form. Dimeric MPO was isolated from HL-60 cells. Hemi-MPO obtained from dimeric MPO by reductive cleavage of a disulfide bond between protomeric subunits was used as the monomeric form. Both peroxidase and halogenating (chlorinating) activities of MPO were assayed, each of them by two methods. Bactericidal activity of the MPO/•2DŽ2/Cl- system was tested using the Escherichia coli laboratory strain DH5a. No difference in the enzymatic and bactericidal activity between dimeric MPO and hemi-MPO was found. Both forms of the enzyme also did not differ in the resistance to HOCl, the main product of MPO. HOCl caused a dose-dependent decrease in peroxidase and chlorinating activity, and the pattern of this decrease was identical for dimeric MPO and hemi-MPO. At equal heme concentration, a somewhat higher bactericidal effect was observed for the hemi-MPO/•2DŽ2/Cl- system compared with the dimeric MPO/•2DŽ2/Cl- system. However, this is most likely not related to some specific property of hemi-MPO and can be accounted for by the higher probability of contacting between bacterial surface and hemi-MPO molecules due to their two-fold greater number relative to that of dimeric MPO molecules at the same heme concentration. By using Western-blotting with antibodies to MPO, we showed, for the first time, that the dimeric molecule of MPO could be cleaved into two monomeric subunits by HOCl, most probably due to oxidation of the disulfide bond between these subunits. This finding suggests that appearance in blood of MPO corresponding in mass to its monomer may result from the damage of dimeric MPO by reactive halogen species, especially upon their overproduction underlying oxidative/halogenative stress in inflammatory diseases.

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6. *Yakimovskii A.F., Shantyr I.I., Vlasenko M.A., Yakovleva M.V., Kryzanovskaia S.Yu.*

The influence of acizolum to bioelements content in rat's blood plasma, parenchimal organs and brain.

Zinc content in blood plasma and brain tissue of rats was studied by analytic mass-spectrometry with inductively coupled plasma. In control (saline-treated animal) zinc content in plasma was 3.6 ± 1.4 mg/ml, in the liver 12.5 ± 2.5 mg/mg, in the spleen 10.9 ± 4.1 mg/mg, in the brain 8.7 ± 3.0 mg/mg. After a single intraperitoneal injection of zinc donator acizolum (24 mg/kg) zinc content decreased in all examined tissues, especially in brain. After a course of sequential acizolum injections (seven administrations during two weeks) essential elevation of zinc content in blood plasma and tissues investigated was detected. The maximal increase zinc concentration in blood plasma and liver was detected in 15 h after the last acizolum injections. Selen, calcium, copper and iron contents demonstrated a more complex behaviour. The obtained data suggest that prolonged acizolum administration has a significant impact on the bioelements content, and this should be taken into consideration when this zinc donator is used as a drug.

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7. *Kalatanova A.V., Makarov V.G., Faustova N.M., Gushchin Ya.I., Makarova M.N.*

Evaluation of the cardioprotective effect of ubiquinol on the model of reperfusion injury of rat myocardium.

The cardioprotective effect of ubiquinol on the model of myocardium reperfusion injury in rats was investigated. The study was carried out using mature males of outbred rats. Myocardial ischemia-reperfusion injury was performed after 30-minute ligation of the left coronary artery followed by reperfusion. The main criteria for assessing the development of pathology included the results of electrocardiography, biochemical analysis of blood plasma, histological and histochemical study of the myocardium. Development of the reperfusion damage of the myocardium caused specific changes in non-treated animals. The best therapeutic effect on biochemical indices was provided by a drug with the known cardioprotective activity – Mexidol and the tested object ubiquinol at doses of 2-6 mg/kg. Evaluation of the results of electrocardiography allowed to confirm the development of ischemic myocardial damage in all groups. The results of histochemical and histological examination of the myocardium suggest a high cardioprotective activity of ubiquinol at a dose of 3 mg/kg and a potential cardioprotective effect of ubiquinol in doses closest to the therapeutic doses of 2 and 6 mg/kg. Ubiquinol is a dose 9 mg/kg showed signs of prooxidant activity, manifested in the form of aggravation of reperfusion injury of the myocardium. The most effective in the conditions of experimental pathology is 1% solution of ubiquinol, at a dose of 3 mg/kg, whose cardioprotective effect is comparable or higher than that for the reference drug Mexidol at the therapeutic dose. In doses that are greater than therapeutic ubiquinol is able to act as a pro-oxidant.

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8. *Usynin I.F., Poteryaeva O.N., Russkikh G.S., Zubova A.V., Boiko K.Yu., Polyakov L.M.*

Apolipoprotein A-I stimulates secretion of insulin and matrix metalloproteinases by islets of Langerhans.

The development of type 2 diabetes mellitus (DM2) is accompanied by disturbances in lipid metabolism. These include the increase in serum levels of atherogenic fractions of very low-density (VLDL) and low-density lipoproteins (LDL), total cholesterol, triglycerides and apo B. In contrast, the level of antiatherogenic high density lipoproteins (HDL) and the content of apolipoprotein A-I (apoA-I) decreased. To study the effect of the observed metabolic changes on insulin secretion in vitro, we used the islets of Langerhans isolated from the rat pancreas. It has been found that incubation of the islets in the presence of serum of the obese patients and patients with decompensated DM2 leads to a decrease in insulin secretion by 2.4 and 5.0 times, respectively. On the contrary, the addition of HDL to the incubation medium increased the insulin secretion by 3.4 times. A similar effect was observed in the presence of apoA-I, the main protein component of HDL. In the presence of apoA-I, the extracellular activity of matrix metalloproteinases (MMPs) demonstrated a 10-fold increase. The addition of LDL and VLDL to the islets did not change the secretion of insulin and activity of MMP. Our results testify to the important role of HDL and apoA-I in regulation of the insulin secretion by b-cells and the activity of MMPs in the islets of Langerhans.

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9. *Taraskina A.E., Zobotina A.M., Nasyrova R.F., Sosin D.N., Sosina K.A., Ershov E.E., Grunina M.N., Krupitsky E.M.*

The effect of antipsychotic drug on monoamine receptors in peripheral blood mononuclear cells: affinity linked mechanism.

Schizophrenia is one of the most serious and common mental disorders, which is characterized by high levels of pathogenic heterogeneity as well as neuroimmune abnormalities, which require treatment with antipsychotic drugs. Monoamines are one of the key neurotransmitters which play an important role in neuroimmune interactions of the human organism. We suggest that the quantity of the monoamine receptors on mononuclear cells of the peripheral blood (PBMCs) can be associated with the cytokine profile of patients. With this quantity being a key component of the mental status correction mechanism in antipsychotic therapy. In this study we measured cytokine levels (IL-6, IL-1b and TGF-b) in blood serum, the protein expression status of the serotonin receptor 5HTR2A and the dopamine receptors D1 (DRD1), DRD2, DRD3 in PBMCs of drug-naive, first episode schizophrenia patients before and after the treatment with olanzapine and haloperidol. This study has shown for the first time that the antipsychotic therapy leads to a decrease in protein levels of monoamine receptors in PBMCs associated with the affinity of the drug used. Blood cytokine levels were significantly higher in serum from studied patients as compared with the reference values. The antipsychotic-linked change of the TGF-b production caused by the therapy is probably associated with the reduction of various monoamine receptors. The relationship between the psychopathological status and the protein level of 5HTR2A suggests that the amount of 5HTR2A may serve as a potential biomarker for the personalized appointment of the antipsychotic therapy.

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10. Skuratovskaia D.A., Vulf M.A., Kirienkova E.V., Mironyuk N.I., Zatolokin P.A., Litvinova L.S.

The role of single nucleotide polymorphisms in GIPR gene in the changes of secretion in hormones and adipokines in patients with obesity with type 2 diabetes.

The relationship between the rs2302382, rs8111428 and Glu354Gln (rs1800437) polymorphisms in GIPR (glucosedependent insulinotropic polypeptide receptor) gene and plasma levels of mediators involved in the regulation of carbohydrate metabolism in obese patients with type 2 diabetes (before and after a test breakfast) was investigated. The contribution of polymorphic variants of rs2302382, rs8111428 in GIPR gene in the predisposition to type 2 diabetes in individuals belonging to the Slavic population of Russia was found. Polymorphisms rs2302382 and rs8111428 in the GIPR gene were characterized by the nonequilibrium cohesion. The decrease in the level of expression of the GIPR gene in adipose tissue of the small intestine mesentery in the carriers of the CC genotype rs2302382 and AA rs8111428 was associated with the increase in the plasma leptin level, whereas during normal expression, the plasma content of insulin, and GIP (in persons with the genotype of the polymorphism rs2302382 and AG polymorphism rs8111428), resistin and ghrelin (in individuals with the genotype of the polymorphism rs2302382) increased. We propose the stimulating effect of GIP on the secretion of resistin, leptin and ghrelin, with an increase in insulin production in obese patients with type 2 diabetes.

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