

1. Ptitsyn K.G., Novikova S.E., Kiseleva Y.Y., Moysa A.A., Kurbatov L.K., Farafonova T.E., Radko S.P., Zgoda V.G., Archakov A.I.

Use of DNA-aptamers for enrichment of low abundant proteins in cellular extracts for quantitative detection by selected reaction monitoring.

The relationship between the amount of a target protein in a complex biological sample and its amount measured by selected reaction monitoring (SRM) mass spectrometry upon the affinity enrichment of target protein with aptamers immobilized on a solid phase was studied. Human thrombin added in known concentrations to cellular extracts derived from bacterial cells was used as model target protein. It has been demonstrated that the affinity enrichment of thrombin in cellular extracts by means of the thrombin-binding aptamer immobilized on the surface of magnetic microbeads results in an approximately 10-fold increase of the concentration of target protein and a 100-fold decrease of the low limit of a target protein concentration range where its quantitative detection by SRM is possible without an interference from other peptides present in a tryptic digest.

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2. Nakhod K.V., Rusanov A.L., Luzgina E.D., Druzhilovskiy D.S., Luzgina N.G., Lisitsa A.V.

Quality control study of engineered skin tissue.

OMERO service was used to annotate the cell line HaCaT microscope images by two independent expert groups. The images were obtained in the course of developing tissue-engineered epithelium which consisted of several layers of the keratinocytes. Evaluation of expert opinions was performed by calculation of specificity, sensitivity and accuracy. The best convergence of opinions (91%) was achieved for the confluence of the cell monolayers. Accuracy 70% was observed in determining the extent of cell differentiation after 10 days of incubation. The paper illustrates the usefulness of OMERO service for dynamic cross-validation of quality in the development and standardization of cell preparations.

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3. Grigorieva D.V., Gorudko I.V., Kostevich V.A., Vasilyev V.B., Cherenkevich S.N., Panasenko O.M., Sokolov A.V.

Exocytosis of myeloperoxidase from activated neutrophils in the presence of heparin.

Exocytosis of myeloperoxidase (MPO) from activated neutrophils in the presence of the anionic polysaccharide heparin was studied. It was determined that the optimal concentration of heparin (0.1 u/ml), at which there is no additional activation of cells (absence of amplification of exocytosis of lysozyme contained in specific and azurophilic granules). It was found that after preincubation of cells with heparin (0.1 u/ml) the exocytosis of MPO from neutrophils activated by various stimulants (fMLP, PMA, plant lectins CABA and PHA-L) increased compared to that under the action of activators alone. In addition, it was shown that heparin in the range of concentrations 0.1-50 u/ml did not affect on the peroxidase activity of the MPO isolated from leukocytes. Thus, the use of heparin at a concentration of 0.1 u/ml avoids the artifact caused by the loss of MPO in a result of its binding to neutrophils, and increases the accuracy of the method of registration the degranulation of azurophilic granules of neutrophils based on determination of the concentration or peroxidase activity of MPO in cell supernatants.

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4. Shtam T.A., Samsonov R.A., Volnitskiy A.V., Kamyshinsky R.A., Verlov N.A., Kniazeva M.S., Korobkina E.A., Orehov A.S., Vasiliev A.L., Konevega A.L., Malek A.V.

Isolation of extracellular micro-vesicles from cell culture medium: comparative evaluation of methods.

Extracellular vesicles (EV) are secreted by cells of multicellular organisms. EV mediate specific mode of intercellular communication by horizontal exchange of substances and information. This phenomenon seems to have an essential biological significance and became a subject of intensive research. Biogenesis, structural and functional features of the EV is being commonly studied in in vitro condition. Several methods of EV isolation from cell culture medium are established, however selection of method might influence on obtained results. The choice of the optimal method depends usually from the amount of medium and the aims of the research while is still challenging issue. We performed a comparative analysis of four different methods of EV isolation from cell culture medium: differential ultracentrifugation, ultracentrifugation with a 30% sucrose/D2O cushion, precipitation with plant proteins and immune-affinity capturing. EV isolated by different approaches were compared in terms of following parameters: size, concentration, morphology of EV, contamination by non-vesicular particles, content of exosomal tetraspanins on the EV surface, content of total proteins, RNA, and several glioma-associated miRNAs. Applied methods included nano-particle tracking analysis (NTA), dynamic light scattering (DLS), cryo-electron microscopy, flow cytometry and RT-qPCR. On the base of obtained results, we developed practical recommendations that may help researchers to make a best choice of EV isolation method.

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5. Popova T.N., Safonova O.A., Stolyarova A.O., Verevkin A.N.

The effect of the biologically active additive epiphamine on antioxidant and NADPH-generating enzymes activity under experimental cerebral ischemia/reperfusion in rats.

The effect of biologically active additive with immunomodulator properties epiphamine on the activity of antioxidant (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione transferase) and NADPH-generating (glucose-6-phosphate dehydrogenase, NADP-isocitrate dehydrogenase) enzymes has been investigated at experimental cerebral ischemia/reperfusion in rats. The results obtained indicate

epiphamine-induced changes of these enzymes activities towards control values. Changes in the content of lactate, a marker of the pathology development, have also been found in experimental animals under ischemia and epiphamine administration caused changes similar to those observed in the case of enzyme activities studied. In most cases, the changes were dose-dependent. Thus, epiphamine can be of considerable interest from the point of view of metabolic changes pharmacological correction at the development of the pathology accompanied by oxidative stress.

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6. Zaporozhchenko I.A., Bryzgunova O.E., Lekchnov E.A., Osipov I.D., Zaripov M.M., Yurchenko Yu.B., Yarmoschuk S.V., Pashkovskaya O.A., Rykova E.Yu., Zheravin A.A., Laktionov P.P.

Representation analysis of miRNA from clarified urine and urine microvesicles in prostate malignancies and non-malignant neoplasms.

Urine of prostate cancer patients contains tumor-specific biopolymers, including protein- and microvesicles-associated miRNAs that can potentially be used as oncomarkers. Previously we have characterized urine extracellular vesicles and demonstrated diagnostic potential of their miRNA cargo. In this study, we have performed a comparative analysis of the expression of 84 miRNA in paired samples of urine microvesicles and clarified urine from healthy men, patients with benign hyperplasia and cancer of the prostate using miRCURY LNA miRNA qPCR Panels. Subsets of miRNAs with differences in expression between the fractions of the urine were found in all three groups. Two groups of miRNA were identified based on the patterns of their differential expression. They regulate several key signaling pathways associated with prostate cancer development.

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7. Bozhenko V.K., Stanojevic U.S., Trotsenko I.D., Zakharenko M.V., Kiseleva Y.Y., Solodkiy V.A.

Comparison of matrix proteinase mRNA expression in morphologically normal, neoplastic, and metastatic colon tissue and colon biopsies from healthy donors.

Matrix metalloproteinases (MMPs) responsible for the extracellular matrix remodeling, the activation of various growth factors, and angiogenesis play an important role in the colorectal cancer (CRC) development. In the present work the comparative analysis of MMP-7, -8, -9, and -11 mRNA as well mRNA of the Ki-67 proliferation marker in tissue samples obtained from CRC patients and healthy individuals. Employing the real time PCR method the expression levels of several MMPs (MMP-7, -8, -9, and -11) and cell proliferation marker, Ki-67, were simultaneously measured in 256 tissue samples obtained from 112 patients with CRC: 112 samples of the primary tumor (CRC), 112 samples of the most distant border of morphologically normal colonic mucosa (MNT), 16 samples of liver metastases and from 16 healthy volunteers who underwent colonoscopy and biopsy. The expression of both MMPs studied and Ki-67 was found to be elevated in CRC primary tumors and liver metastases compared with the normal mucosa. CRC tumor and metastatic cells exhibited similar proliferative activity. The metastases are characterized by the highest cross-correlation of MMPs among tissue types tested. For the first time it was shown that normal mucosa from healthy individuals and CRC patients varied in the MMP-8 expression level. They also had dissimilar MMP correlation patterns thus suggesting that epithelial cells adjusted to CRC tumor differ from mucosal epithelial cells of healthy individuals.

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8. Grishin D.V., Zhdanov D.D., Gladilina Ju.A., Pokrovsky V.S., Podobed O.V., Pokrovskaya M.V., Aleksandrova S.S., Milyushkina A.L., Vigovskiy M.A., Sokolov N.N.

Construction and characterization of a recombinant mutant homolog of the CheW protein from *Thermotoga petrophila* RKU-1.

In the work a recombinant chemotaxis protein CheW from *Thermotoga petrophila* RKU-1 (TpeCheW) and its mutant homolog (TpeCheW-mut) were created. It was shown that, despite the low homology with CheW prototypes from intestinal bacteria, these proteins didn't cause metabolic overload and were well expressed by cells of *E. coli* laboratory strains. We have discovered a broad spectrum of industrial valuable properties of the TpeCheW-mut protein such as stability in a wide range of temperatures and pH, high expression level, solubility and possibility of the application of a simple low-stage purification methodology with the use of preliminary heat treatment. Possible directions of the scientific and industrial application of this protein were claimed.

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9. Ershov P.V., Mezentsev Yu.V., Yablokov E.O., Kalushskiy L.A., Florinskaya A.V., Svirid A.V., Gilep A.A., Usanov S.A., Medvedev A.E., Ivanov A.S.

Study specificity of isatin interactions with P450 cytochromes.

Cytochrome P450-dependent monooxygenase systems exist basically in all living organisms, where they perform various important functions. The coordinated functioning of these systems involves many proteins participating in different protein-protein interactions (PPI). Previously, we have found that the endogenous non-peptide bioregulator isatin (indole-2,3-dione), synthesized from indole by means of certain cytochromes P450 (e.g. P450 2E1, P450 2C19, P450 2A6) regulates affinity of some PPI. In this work, an attempt has been undertaken to register a direct interaction of isatin with a set of different proteins related to the functioning of cytochrome P450-dependent monooxygenase: five isoforms of cytochromes P450, two isoforms of cytochrome b5, cytochrome P450 reductase, adrenodoxin, adrenodoxin reductase and ferrochelatase. The study has shown that isatin binds specifically only to cytochromes P450 with high affinity (the equilibrium dissociation constant (Kd) is about 10⁻⁸ M).

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10. Kudinov V.A., Zakharova T.S., Torkhovskaya T.I., Ipatova O.M., Archakov A.I.

Pharmacological targets for dyslipidemias correction. Opportunities and prospects of therapeutic usage.

Literature data on influence of existing and new groups of drug preparations for dyslipidemias correction are systemized, and molecular mechanisms of their effects are reviewed. The results of experimental and clinical investigations aimed at revealing of new pharmacological targets of dyslipidemias correction were analyzed. The approaches for activation of high density lipoproteins functionality are described. The implementation of alternative preparations with new alternative mechanisms of action may be suggested to improve the effectiveness of traditional treatment in the future.

11. *Ramenskaia G.V., Melnik E.V., Petukhov A.E.*

Phospholipase D: its role in metabolism processes and disease development.

Phospholipase D (PLD) is one of the key enzymes that catalyzes the hydrolysis of cell membrane phospholipids. In this review current knowledge about six human PLD isoforms, their structure and role in physiological and pathological processes is summarized. Comparative analysis of PLD isoforms structure is presented. The mechanism of the hydrolysis and transphosphatidylation performed by PLD is described. The PLD1 and PLD2 role in the pathogenesis of some cancer, infectious, thrombotic and neurodegenerative diseases is analyzed. The prospects of PLD isoform-selective inhibitors development are shown in the context of the clinical usage and the already-existing inhibitors are characterized. Moreover, the formation of phosphatidylethanol (PEth), the alcohol abuse biomarker, as the result of PLD-catalyzed phospholipid transphosphatidylation is considered.

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12. *Selivanova O.M., Rogachevsky V.V., Syrin A.K., Galzitskaya O.V.*

Molecular mechanism of amyloid formation by Ab peptide: review of own works.

A characteristic feature of amyloid structures is polymorphism. The study of amyloid structures and their formation process was carried out for synthetic and recombinant Ab(1-40) and Ab(1-42) peptide preparations. In the study of these peptides, we recognized fibrils of different morphologies. We observed fibrillar formations in the form of single fibrils, ribbons, bundles, bunches, and clusters. Polymorphism of fibrils was observed not only when the environmental conditions changed, but under the same conditions and this was a common characteristic of all amyloid formations. Fibrils of Ab(1-40) peptides tended to form aggregates of fibrils in the form of ribbons, while Ab(1-42) peptide under the same conditions polymerized in the form of rough fibrils of different diameters and tends to branch. We assume that the formation of fibrils of Ab(1-40) and Ab(1-42) peptides occurs according to a simplified scheme: a destabilized monomer → a ring oligomer → a mature fibril consisting of ring oligomers. Proceeding from the proposition that the ring oligomer is the main building block of amyloid fibril (similar to the cell in the body), it is easy to explain fibril polymorphism, as well as fragmentation of mature fibrils under various external influences, branching and irregularity of diameter (surface roughness) of fibrils. One aspect of the study of amyloidogenesis is the determination of the regions of the protein chain forming the core of the amyloid fibril. We theoretically predicted amyloidogenic regions for two isoforms of Ab peptides capable of forming an amyloid structure: 16-21 and 32-36 residues. Using the method of tandem mass spectrometry, these regions were determined experimentally. It was shown that the regions of Ab(1-40) peptide from 16 to 22 and from 28 to 40 residues were resistant to the action of proteases, i.e. its formed the core of the amyloid fibril. For Ab(1-42) peptide the whole sequence is not available for the action of proteases, which indicates a different way of associating ring oligomers in the formation of fibrils. Based on electron microscopy and mass spectrometry data we proposed a molecular model of the fibril formed by Ab(1-40) and Ab(1-42) peptides.

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13. *Tamkovich S.N., Yunusova N.V., Somov A.K., Kakurina G.V., Kolegova E.S., Tugurova E.A., Laktionov P.P., Kondakova I.V.*

Comparative sub-population analysis of exosomes from blood plasma of cancer patients.

To increase the sensitivity and specificity of the developed methods for diagnosis of oncological diseases using exosomes of blood, a stage of pre-selection of tumor exosomes from a common pool of circulating microvesicles is required. In the present work, universal proteins have been identified, their expression has been increased in the exosomes of patients with colorectal cancer, head and neck squamous cell carcinomas, and lung cancer. The use of antibodies against major exosomal proteins will further develop a simple and high-performance method of affinity isolation of tumor exosomes.

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14. *Osmolovskiy A.A., Orekhova A.V., Kreyer V.G., Baranova N.A., Egorov N.S.*

Possibility of application of extracellular protease of micromycet *Aspergillus ochraceus* VKM F-4104D for determination of protein C content in human blood plasma.

It was shown that the activator activity of protein C, determined in normal plasma using *Aspergillus ochraceus* protease, is comparable with the activity of commercial protease analogue from the South American copperhead venom (Protac[®]). It was found that protease of *A. ochraceus* can be used to determine protein C in plasma with its reduced content similar to Protac[®]. Comparison of the activator protein C activity of *A. ochraceus* protease and the commercial analogue showed some excess of the activator activity of the fungal preparation, which may make it a promising substitute for the snake activator in diagnostic kits for determining the protein C content in clinical laboratories.

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