Biomeditsinskaya Khimiya

ISSN 2310-6905 (online), ISSN 2310-6972 (print)

2015 volume 61 issue 3

1. Kugaevskaya E.V., Timoshenko O.S., Solovyeva N.I.

Angiotensin converting enzyme: the antigenic properties of the domain, role in alzheimer's disease and tumor progression.

Angiotensin converting enzyme (ACE, EC 3.4.15.1) was discovered and characterized in the Laboratory of biochemistry and chemical pathology of proteins under the direction of academician V.N. Orekhovich, where its physiological function, associated with a key role in the regulation of the renin-angiotensin (RAS) and the kallikrein-kinin systems that control blood flow in the body and homeostasis was first deciphered. We carried out a search for structural differences between the two highly homologous domains (N- and C-domains) of somatic ACE (sACE); it was based on a comparative analysis of antigenic determinants (or B-epitopes) of both domains. The revealed epitopes were classified with variable and conserved regions and functionally important sites of the molecule ACE. Essential difference was demonstrated between locations of the epitopes in the N- and C-domains. These data indicate the existence of structural differences between the domains of sACE. We studied the role of the domains of ACE in the metabolism of human amyloid beta peptide (Ab) â€“ the main component of senile plaques, found in the brains of patients with Alzheimer's disease (AD). Our results demonstrated that only N-domain ACE cleaved the Ab between residues R5â€“H6, while, the C-domain of ACE failed to hydrolyze this region. In addition, the effect of post-translational modifications of Ab on its hydrolysis by the ACE was investigated. We show that isomerization of residue D7, a common non-enzymatic age-related modification found in AD-associated species, does not reduce the affinity of the peptide to the N-domain of ACE, and conversely, it increases. According to our data, the role of ACE in the metabolism of Ab becomes more significant in the development of AD. RAS is involved in malignant transformation and tumor progression. RAS components, including ACE and angiotensin II receptors type 1 (AT1R) are expressed in various human tumors. We found a significant increase in the level of ACE activity in the tumor tissue of squamous cell carcinoma of the cervix. In our viewpoint, the increase in ACE activity may be a marker of poor clinical prognosis.

DOI: 10.18097/PBMC20156103301

2. Sokolov N.N., Eldarov M.A., Pokrovskaya M.V., Aleksandrova S.S., Abakumova O.Yu., Podobed O.V., Melik-Nubarov N.S., Kudryashova E.V., Grishin D.V., Archakov A.I.

Bacterial recombinant L-asparaginases: properties, structure and anti-proliferative activity.

For more than 40 years L-asparaginases are used in combined therapy of acute lymphoblastic leukemia in children and the range of tumors sensitive to these enzymes constantly extends. This review summarizes results of studies aimed at creation of new systems for heterological expression of bacterial L-asparaginases as Erwinia carotovora (EwA), Helicobacter pylori (HpA), Yersinia pseudotuberculosis (YpA) and Rhodospirillum rubrum (RrA); special attention is paid to isolation of purified enzymes and their crystallization, modification by chitosan/polyethylene, physicochemical, kinetic and structural properties characterization, and the study of the cytotoxic or anti-proliferative activity of new recombinant L-asparaginases on cell cultures in vitro. The resultant recombinant L-asparaginases (EwA, YpA, HpA Ð¸ RrA) exhibit reasonable cytotoxic action on the human leukemia cells comparable to the pharmacologically available L-asparaginase EcA and represent practical interest in respect to creation, on their basis, new effective antineoplastic remedies. Further prospects of researches on bacterial L-asparaginases are associated with development of analogs of Rhodospirillum rubrum L-asparaginase (RrA) by means of directed changes of the protein structure using genetic engineering, development of chito-PEGylation for receiving L-asparaginase preparations with improved pharmacokinetic characteristics.

DOI: 10.18097/PBMC20156103312

3. Shumyantseva V.V., Bulko T.V., Baychorov I.Kh., Archakov A.I.

Molecularly imprinted polymers in electro analysis of proteins.

In the review the main approaches to creation of recognition materials capable of competing with biological specific receptors, (polymeric analogs of antibodies or molecularly imprinted polymers, MIP) for the electro analysis of functionally significant proteins such as a myoglobin, troponin T, albumin, human ferritin, calmodulin are considered. The main types of monomers for MIP fabrication, and methods for MIP/protein interactions, such as a surface plasmon resonance (SPR), nanogravimetry with use of the quartz crystal resonator (QCM), spectral and electrochemical methods are discussed. Experimental data on electrochemical registration of a myoglobin using MIP/electrode are presented. For a development of electrochemical sensor systems based on MIPs, o-phenylenediamine (1,2-diaminobenzene was used as a monomer. It was shown that the imprinting factor Imax(MIP)/Imax(NIP), calculated as a myoglobin signal ratio when embedding in MIP to a myoglobin signal when embedding in the polymer received without molecular template (NIP) corresponds 2-4.

DOI: 10.18097/PBMC20156103325

4. Cherepanov S.A., Baklaushev V.P., Gabashvili A.N., Shepeleva I.I., Chekhonin V.P.

Hedgehog signaling in the pathogenesis of neuro-oncology diseases.

The review summarizes current knowledge on the Hedgehog signaling pathway, its role in normal embryogenesis and/or initiation and progression of neuro-oncological diseases, especially of high-grade gliomas, the most malignant neuroepithelial tumors. The main proteins forming the Hedgehog signaling pathway include Shh, PTCH1, SMO, HHIP, SUFU and GLI1 isoforms. Effects of other signaling pathways on the family of transcription factors GLI and other proteins are described. The review summarizes modern data about the impact of the Hedgehog signaling pathway on proliferation, migration activity and invasiveness, and also on tumor neoangiogenesis and tumor cell chemoresistance. The role of the Hedgehog signaling pathway in

origin of cancer stem cells and epithelial-mesenchymal transition is also analyzed. Some prospects for new anticancer drugs acting on components of the Hedgehog signaling pathway inhibitors are demonstrated.
DOI: 10.18097/PBMC20156103332

5. Kuznetzova K.G., Kazlas E.V., Torkhovskaya T.I., Karalkin P.A., Vachrushev I.V., Zakharova T.S., Sanzhakov M.A., Moshkovskiy S.A., Ipatova O.M.

The influence of doxorubicin incorporated in phospholipid drug delivery nanosystem on HEPG2 cells proteome.

A phospholipid drug delivery nanosystem with particle size up to 30 nm elaborated at the Institute of Biomedical Chemistry has been used earlier for incorporation of doxorubicin (Doxolip). This system demonstrated higher antitumor effect in vivo as compared with free doxorubicin. In this study the effect of this nanosystem containing doxorubicin on HepG2 cell proteome has been investigated. Cells were incubated in a medium containing phospholipid nanoparticles (0.5 mg/ml doxorubicin, 10 mg/mL phosphatidylcholine). After incubation for 48 h their survival represented 10% as compared with untreated cells. Cell proteins were analyzed by quantitative two-dimensional gel electrophoresis followed by identification of differentially expressed proteins with MALDI-TOF mass spectrometry. The phospholipid transport nanosystem itself insignificantly influenced the cell proteome thus confirming previous data on its safety. Doxorubicin, as both free substance and Doxolip (i.e. included into phospholipid nanoparticles) induced changes in expression of 28 proteins. Among these proteins only four of them demonstrated different in response to the effect of the free drug substance and Doxolip. Doxolip exhibited a more pronounced effect on expression of certain proteins; the latter indirectly implies increased penetration of the drug substance (included into nanoparticles) into the tumor cells. Increased antitumor activity of doxorubicin included into phospholipid nanoparticles may be associated with more active increase of specific protein expression.
DOI: 10.18097/PBMC20156103343

6. Samenkova N.F., Kisrieva Y.S., Petushkova N.A., Kuznetsova G.P., Larina O.V., Trifonova O.P., Karuzina I.I., Ipatova O.M., Lisitsa A.V.

Analysis of proteomic profile changes of zebrafish embryos during exposure to doxorubicin, built-in the phospholipid transport nanosystem.

The proteome profile of Danio rerio embryos grown in the medium containing doxorubicin, included in the phospholipid transport nanosystem (doxolip) has been investigated using combination of 1D-electrophoresis with subsequent MALDI-TOF-PMF mass spectrometry. Cultivation of growing of D. rerio embryos in the medium with doxolip caused a substantial increase in expression of the cytoskeletal proteins, a decrease in the number of nuclear proteins involved in DNA and RNA synthesis and disappearance of vitellogenin 2 in comparison with control (the cultivation medium containing the phospholipid transport nanosystem). Analysis of the proteomic profiles of doxolip-treated embryos suggests lower toxicity of doxorubicin incorporated in the phospholipid nanosystem.
DOI: 10.18097/PBMC20156103350

7. Bogdanov K.V., Nikitin M.M., Slyadnev M.N.

Allele polymorphism analysis in coagulation factors F2, F5 and folate metabolism gene MTHFR by using microchip-based multiplex real time PCR.

Single nucleotide polymorphism (SNP) genotyping methods are widely used for the detection of hereditary thrombophilias caused by genetic defects in the coagulation system. The hereditary thrombophilias are frequently associated with higher incidences of point mutations in hemostasis (F2 20210G>A, F5 1691G>A) and folate metabolism (MTHFR 677C>Đ¢, MTHFR 1298A>C) genes. Moreover, the combination of gene abnormalities in F2 or/and MTHFR with F5 Leiden mutation leads to increased risk of developing thrombosis. Thus, simultaneous detection of the multiple gene mutations in a sample has important clinical relevance. The microchip-based multiplex real time PCR for estimation of allele specific polymorphism in hemostatic and folate metabolism genes presented here has a high efficiency and may be used for laboratory diagnosis. The optimized protocol for estimation of 4 different types of genetic polymorphisms allowed PCR to be performed with minimal quantity of DNA template and PCR reagents including Taq polymerase and a short-term thermocycling.
DOI: 10.18097/PBMC20156103357

8. Bukharina N.S., Ivanov Yu.D., Pleshakova T.O., Frantsuzov P.A., Andreeva E.Yu., Kaysheva A.L., Izotov A.A., Pavlova T.I., Ziborov V.S., Radko S.P., Archakov A.I.

Atomic force microscopy fishing of gp120 on immobilized aptamer and its mass spectrometry identification.

A method of atomic force microscopy-based fishing (AFM fishing) has been developed for protein detection in the analyte solution using a chip with an immobilized aptamer. This method is based on the biospecific fishing of a target protein from a bulk solution onto the small AFM chip area with the immobilized aptamer to this protein used as the molecular probe. Such aptamer-based approach allows to increase an AFM image contrast compared to the antibody-based approach. Mass spectrometry analysis used after the biospecific fishing to identify the target protein on the AFM chip has proved complex formation. Use of the AFM chip with the immobilized aptamer avoids interference of the antibody and target protein peaks in a mass spectrum.
DOI: 10.18097/PBMC20156103363

9. Sokolik V.V., Maltsev A.V.

Cytokines neuroinflammatory reaction to the action of homoaggregatic and liposomal forms of b-amyloid 1-40 in rats.

An injection model of preclinical stages of Alzheimer's disease has been reproduced in rats. It was accompanied by the decrease in the latent period of conditioned reflex avoidance, increasing levels of endogenous b-amyloid peptide 1-40 and activation of inflammatory cytokines (IL-1b, TNF-a, IL-6, IL-10) in the cerebral cortex, hippocampus and blood serum of experimental animals. We belive that changes identified at the biochemical level are prerequisite to modulate neuronal functions in rats induced by Ab40_Human administration. The toxic effect of exogenous b-amyloid 1-40 homoaggregates caused intense response of the cytokine system, while its liposomal form caused the soft information signal to the activation of innate immunity.
DOI: 10.18097/PBMC20156103373

10. Zverinsky I.V., Zverinskaya H.G., Sutsko I.P., Telegin P.G., Shlyahtun A.G.

Effects of berberine on the recovery of rat liver xenobiotic-metabolizing enzymes after partial hepatectomy.

We have studied the effect of berberine on the recovery processes of liver xenobiotic-metabolizing function during its compensatory growth after 70% partial hepatectomy. It was found the hepatic ability to metabolize foreign substances are not restored up to day 8. Administration of berberine (10 mg/kg intraperitoneally) for 6 days led to normalization of both cytochrome P450-dependent and flavin-containing monooxygenases. It is suggested that in the biotransformation of berberine involved not only cytochrome P450, but also flavin-containing monooxygenases.

DOI: 10.18097/PBMC20156103381

11. Vazhnichaya Ye.M., Mokliak Ye.V., Kurapov Yu.A., Zabozlaev A.A.

Role of mexidol (2-ethyl-6-methyl-3-hydroxypyridine succinate) in the obtaining of stabilized magnetite nanoparticles for biomedical application.

Magnetite nanoparticles (NPs) are studied as agents for magnetic resonance imaging, hyperthermia of malignant tumors, targeted drug delivery as well as anti-anemic action. One of the main problems of such NPs is their aggregation that requires creation of methods for magnetite NPs stabilization during preparation of liquid medicinal forms on their basis. The present work is devoted to the possibility of mexidol (2-ethyl-6-methyl-3-hydroxypyridine succinate) use for solubilization of magnetite NPs in hydrophilic medium. For this purpose, the condensate produced by electron-beam evaporation and condensation, with magnetite particles of size 5-8 nm deposited into the crystals of sodium chloride were used in conjunction with substance of mexidol (2-ethyl-6-methyl-3-hydroxypyridine succinate), and low molecular weight polyvinylpyrrolidone (PVP). The NP condensate was dispersed in distilled water or PVP or mexidol solutions. NPs size distribution in the liquid phase of the systems was determined by photon correlation spectroscopy, iron (Fe) concentration was evaluated by atomic emission spectrometry. It is shown that in the dispersion prepared in distilled water, the major amount of NPs was of 13-120 nm in size, in mexidol solution - 270-1700 nm, in PVP solution - 30-900 nm. In the fluid containing magnetite NPs together with mexidol and PVP, the main fraction (99.9%) was characterized by the NPs size of 14-75 nm with maximum of 25 nm. This system had the highest iron concentration: it was similar to that in the sample with mexidol solution and 6.6-7.3 times higher than the concentration in the samples with distilled water or PVP. Thus, in the preparation of aqueous dispersions based on magnetite NPs condensate, mexidol provides a transition of Fe to the liquid phase in amount necessary to achieve its biological activity, and PVP stabilizes such modified NPs.

DOI: 10.18097/PBMC20156103384

12. Gorodetskaya I.V., Gusakova E.A.

Effect of the thyroid status on the proteinases/inhibitors system under stress.

Ð¢he alarm-stage of stress reaction (Ð°n hour after the stress of swimming of rats in a cage during an hour) is characterized by the stimulation of trypsinÐµ-like activity (TLA) in the liver, and especially in the blood. At the resistance stage (48 hours after the stress) there is normalization of TLA in the blood and limitation of its growth in the liver. At the stage of exhaustion (an hour of stress during 10 days) the most significant increase of TLA in the liver and blood develops. Experimental hypothyroidism (25 mg/kg merkazolil within 20 days) per se causes a reduction of TLA, defines more pronounced stimulation of proteolysis in the alarm-stage, prevents its normalization at the resistance-stage, and promotes its excessive activation at the stage of exhaustion. Introduction of small doses of L-thyroxine (1.5-3.0 g/kg during 28 days) does not affect the system of proteolysis, limitis the increase of TLA at the alarm- and exhaustion stages, prevents its stimulation at the resistance-stage. The dependence of the changes in the proteases/inhibitors system under stress from the level of iodine-containing thyroid hormones in the blood is due to their influence on the activity of endogenous proteinase inhibitors (a1-antitrypsin and a2-macroglobulin) and on the permeability of lysosomes membranes.

DOI: 10.18097/PBMC20156103389

13. Pertsov S.S., Kalinichenko L.S., Koplik E.V., Nagler L.G., Alinkina E.S., Kozachenko A.I.

Effect of melatonin on antioxidant enzyme activities in blood erythrocytes of rats during acute emotional stress.

The effect of the epiphyseal hormone melatonin on the activity of antioxidant enzymes, glutathione peroxidase (GPx), glutathione reductase (GR), and Cu/Zn-superoxide dismutase (Cu/Zn-SOD) was studied in peripheral blood erythrocytes of behaviorally passive and active Wistar rats. Acute emotional stress was modeled by immobilization of animals for1 h with simultaneous electrocutaneous stimulation. Basal activity of antioxidant glutathione enzymes in erythrocytes of behaviorally passive rats was higher than that in active animals. Administration of melatonin (2 mg/kg, intraperitoneally) was accompanied by a decrease in the activity of GPx and GR in erythrocytes from non-stressed passive animals. After experimental stress, passive rats demonstrated a significant increase in the activity of Cu/Zn-SOD and GPx in peripheral blood erythrocytes. The absence of stress-induced changes in functional activity of antioxidant defense enzymes in the blood of behaviorally active animals suggests a relatively constant oxidative status of tissues in these animals under stress conditions. Melatonin administration had little effect on stress-induced changes in functional activity of the erythrocyte antioxidant system in passive rats. Active specimens pretreated with melatonin before stress exposure were characterized by activation of study antioxidant enzymes. Quantitative parameters of the erythrocyte antioxidant defense enzymes did not differ in behaviorally active and passive rats subjected to experimental stress after melatonin injection. Thus, exogenous melatonin abolishes differences in the activity of study antioxidant enzymes in erythrocytes of animals with different behavioral parameters under basal conditions and after experimental stress. In passive rats melatonin mainly reduced the initial tension of oxidative processes. By contrast, administration of this hormone to active specimens is followed by an increase in functional activity of the antioxidant enzyme system under conditions of acute stress.

DOI: 10.18097/PBMC20156103394

14. Popov S.S., Pashkov A.N., Agarkov A.A., Shulgin K.K.

Intensity of apoptotic processes, aconitate hydratase activity and citrate level in patients with type 2 diabetes mellitus complicated steatohepatitis under application of epifamin at basic therapy.

DNA fragmentation, caspase-1 and caspase-3, aconitate hydratase (AH) activities, and citrate content have been investigated in the blood of patients

with type 2 diabetes mellitus complicated by steatohepatitis. These indicators of apoptotic processes intensity and oxidative stress development were estimated after basic treatment and a combined therapy including epifamin. Before treatment DNA fragmentation blood leukocytes, decrease of AH activity and increase of caspases activities in the serum of patients were detected. Treatment with epifamin provided more pronounced changes in the investigated parameters towards control values as compared to basis therapy. Epifamin caused a positive effect on the citrate content in the serum of patients. Epifamin inclusion to the basic therapy was accompanied by a more pronounced changes towards the normal values of such biochemical parameters as ALT, AST, b-lipoproteins, cholesterol, fasting glucose and postprandial glucose levels. All these changes may be obviously attributed to epifamin-induced correction of the melatonin level and manifestation of adaptogenic properties and antioxidant effects of the hormone.
DOI: 10.18097/PBMC20156103400