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copies with the large dynamic range and a high throughput. The paper reviews microRNA biogenesis, the origin of circulating microRNAs, and methods used for their quantification. The special technical features of ddPCR, which make it an attractive method both for studying microRNAs as biomarkers of human pathologies and for basic research devoted to aspects of gene regulation by microRNA molecules, are also discussed.
DOI: 10.18097/PBMC20166204403

6. Zamay G.S., Belayanina I.V., Zamay A.S., Komarova M.A., Krat A.V., Eremina E.N., Zukov R.A., Sokolov A.E., Zamay T.N.

DNA aptamers selection for breast cancer.

A method of selection of DNA aptamers to breast tumor tissue based on the use of postoperative material has been developed. Breast cancer tissues were used as the positive target; the negative targets included benign tumor tissue, adjacent healthy tissues, breast tissues from mastopathy patients, and also tissues of other types of malignant tumors. During selection a pool of DNA aptamers demonstrating selective binding to breast cancer cells and tissues and insignificant binding to breast benign tissues has been obtained. These DNA aptamers can be used for identification of protein markers, breast cancer diagnostics, and targeted delivery of anticancer drugs.
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7. Alessenko A.V., Bachurin S.O., Gurianova S.V., Karatasso Y.O., Shevtsova E.F., Shingarova L.N.

Tumor necrosis factor-alpha - potential target for neuroprotector dimebon.

Dimebon (Dimebolin) is an antihistamine drug which has been used in Russia since 1983. Recently Dimebolin has attracted renewed interest after being shown to have positive effects on persons suffering from Alzheimer's disease. Animal studies have shown that dimebon acts through multiple mechanisms, both blocking the action of neurotoxic beta-amyloid peptides and inhibiting L-type calcium channels, modulating the action of AMPA and NMDA glutamate receptors. Our experiments with cell culture L929 and mice have shown that dimebon may exert its neuroprotective effect by blocking cytotoxic signals induced by proinflammatory cytokines such as TNF-a which are believed to play a central role in Alzheimer's disease. Dimebon (10 mg/ml) protected mouse fibroblasts L929 against the toxic action of TNF-a. Our study included 65 male mice. TNF-a (10 mg per mouse), dimebon (0,2 mg/kg) and their combination were injected intraperitonealy. Changes in the level of molecular species of sphingomyelin and galactosyl ceramide in hippocampus, cerebellum and cerebral cortex within 30 min, 2 h, 4 h, and 24 h after injection were detected by chromato-mass-spectrometry. Maximal changes in sphingomyelin and galactosyl ceramides contents of different molecular species after single TNF-a administration were found in the hippocampus, and were less expressed in the cerebral cortex and cerebellum after 24 h. Dimebon itself did not induce changes in the sphingolipid spectrum in brain sections, but protected them against disorders induced by TNF-a in the brain. Modern strategies in the search of new therapeutic approaches are based on the multitarget properties of new drugs. According to our results TNF-a may serve as a new target for dimebon.
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8. Gureev A.P., Shmatkova M.L., Bashmakov V.Yu., Starkov A.A., Popov V.N.

The effect of fenofibrate on expression of genes involved in fatty acids beta-oxidation and associated free-radical processes.

Fenofibrate is a synthetic ligand for peroxisome proliferator-activated receptors subtype alpha (PPARa); it is used for the treatment of a wide range of metabolic diseases such as hypertriglyceridemia, dyslipidemia, diabetes and various neurodegenerative diseases. We have studied the effect of fenofibrate on b-oxidation of fatty acids and related free-radical processes. The most effective concentration of fenofibrate (0.3%) added to the chow caused a significant decrease of the body weight of mice. The data obtained by quantitative PCR demonstrated increased hepatic gene expression responsible for b-oxidation of fatty acids in peroxisomes and mitochondria. Enhancement of oxidative processes caused a 2-fold increase in the rate of reactive oxygen species (ROS) production, as evidenced by determination of the level of lipid peroxidation (LPO) products in the liver. Mitochondrial antioxidant systems are more sensitive to elevated ROS production, as they respond by increased expression of SOD2 and PRDX3 genes, than cytoplasmic and peroxisomal antioxidant systems, where expression of CAT1, SOD1, PRDX5 genes remained unaltered.
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9. Razygraev A.V., Taborskaya K.I., Petrosyan M.A., Tumasova Zh.N.

Thiol peroxidase activities in rat blood plasma determined with hydrogen peroxide and 5,5`-dithio-bis(2-nitrobenzoic acid).

Earlier it has been shown that extracellular glutathione peroxidase (GPx3) from human plasma is able to use cysteine (Cys-SH) instead of glutathione (GSH) as a thiol substrate. In the present study, the ability of rat plasma to utilize not only GSH, but also Cys-SH and homocysteine (Hcy-SH), in the thiol peroxidase reaction has been confirmed. The molar ratio between thiol and H_2O_2 in the catalyzed reaction was 2:1. The specific activity increased with fractionation of proteins. At a fixed thiol concentration of 0.23 mM, the saturation by H_2O_2 with vmax app of 100, 128, and 132 nmol H_2O_2 / s per 1 ml of plasma was found for DL-Cys-SH, L-GSH, and DL-Hcy-SH, respectively. Rank distributions of activities towards all three thiol substrates within plasma protein fractions are fully identical (the probability of random full coincidence was less than 0.01). The statistical analysis confirms that Cys-SH peroxidase, Hcy-SH peroxidase, and GSH peroxidase activities are closely associated with each other. The most probable outcome of this result is the ability of rat GPx3 to utilize all three thiols as substrates for oxidation. Probably, thiol peroxidase is a participant of formation of plasma cystine (Cys-SS-Cys) from Cys-SH in plasma. If the forms of Hcy exhibit different toxic effects, it can be suggested that thiol peroxidase regulates Hcy toxicity in hyperhomocysteinemia through Hcy-SH oxidation to homocystine (Hcy-SS-Hcy).
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10. Ivanov Yu.D., Pleshakova T.O., Malsagova K.A., Kaysheva A.L., Kopylov A.T., Izotov A.A., Tatur V.Yu., Vesnin S.G., Ivanova N.D., Ziborov V.S., Archakov A.I.

AFM fishing of proteins under impulse electric field.

A combination of (atomic force microscopy)-based fishing (AFM-fishing) and mass spectrometry allows to capture protein molecules from solutions, concentrate and visualize them on an atomically flat surface of the AFM chip and identify by subsequent mass spectrometric analysis. In order to

increase the AFM-fishing efficiency we have applied pulsed voltage with the rise time of the front of about 1 ns to the AFM chip. The AFM-chip was made using a conductive material, highly oriented pyrolytic graphite (HOPG). The increased efficiency of AFM-fishing has been demonstrated using detection of cytochrome b5 protein. Selection of the stimulating pulse with a rise time of 1 ns, corresponding to the GHz frequency range, by the effect of intrinsic emission from water observed in this frequency range during water injection into the cell.
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11. Fattakhov N.S., Vasilenko M.A., Skuratovskaia D.A., Kulikov D.I., Kirienkova E.V., Zatolokin P.A., Beletskaya M.A., Litvinova L.S.
Pathogenetic significance of C774T single nucleotide polymorphism of the endothelial NO synthase gene in the development of metabolic syndrome.
The relationship between nitric oxide production and metabolic disorders and the role of endothelial nitric oxide synthase (eNOS or NOS3) in metabolic syndrome (MS) remain poorly understood and need deeper investigation. In this context the role of the NOS3 gene in pathogenesis of MS is of special interest. The aim of the study was to investigate association of NOS3 single nucleotide polymorphism C774T with risk of MS in the Slavic population of the Kaliningrad region and the relationship of this polymorphic variant with some parameters of endothelial dysfunction. The study included 128 patients (48 men and 80 women aged from 36 to 52 years) with MS. The control group consisted of 126 healthy volunteers (60 men and 66 women aged from 30 to 40 years). Genotyping was performed by real-time PCR. Serum nitrite levels were determined spectrophotometrically by the Griess method. Serum levels of endothelin-1 and eNOS were evaluated by ELISA. The study has shown association of T allele (OR=2.06; p=0.0004; CI: 1.38-3.08) and CT genotype (OR=1.97; p=0.014; CI: 1.14-3.40) C774T polymorphism of the NOS3 gene with risk of MS in the Slavic population of the Kaliningrad region. Allele C (OR=0.48; p=0.0004; CI: 0.32-0.72) and homozygous CC genotype (OR=0.41; p=0.001; CI: 0.24-0,69) C774T polymorphism of the NOS3 gene were associated with reduced risk of the development of MS. Significant differences in serum levels of eNOS and endothelin-1 depended on the CT and TT genotypes of C774T polymorphism of the NOS3 gene in MS.
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12. Bozhenko V.K., Kharchenko N.V., Vaskevich E.F., Kudinova E.A., Oorzhak A.V., Rozhkova N.I., Trotsenko I.D.
Mammaglobin in peripheral blood and tumor in breast cancer patients.
Currently, no molecular biological markers do exist for early diagnosis of breast cancer. One of the possible candidates for the marker of early breast cancer is mammaglobin (MGB1) or SCGB2A2 (secretoglobin, family 2A, member 2), characterized by the maximal expression level in early breast cancer. Using the RT-PCR method MGB1 mRNA expression was examined in 57 tumor tissue samples and 57 samples of morphologically non-malignant tissue (MNT) of breast cancer (BC) patients. Specificity and sensitivity of the MGB1 mRNA assay in peripheral blood of BC patients was evaluated by nested PCR. 169 blood samples (from 95 BC patients, 22 from patients with benign breast tumors, 28 from patients with tumors of other localizations, and 24 samples from healthy donors) have been analyzed. MGB1 expression was significantly higher in BC tissue samples compared to MNT (p=0.0019). The maximal expression level was in the samples T1 (p=0.013), stage I BC (p=0.037), GI (p=0.0019). The MGB1 expression positively correlated with expression of estrogen (p = 0,034) and progesterone (p=0.0004) receptors. Sensitivity and specificity of the MGB1 mRNA assay in peripheral blood were 60.6% and 92.3%, respectively. Expression of MGB1 was higher in BC than MNT and it decreased during BC progression. The sensitivity and specificity of the MGB1 mRNA assay may be used as an additional diagnostic method.
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13. Biktagirova E.M., Sattarova L.I., Vagapova G.R., Skibo Y.V., Chuhlovina E.N., Kravtsova O.A., Abramova Z.I.
Biochemical and immunological markers of autoimmune thyroiditis.
Correlations between biochemical and immunological markers of programmed cell death (apoptosis), and the functional state of the thyroid gland (hyperthyroidism, euthyroidism, hypothyroidism) have been investigated in autoimmune thyroiditis (AT) (also known as chronic autoimmune thyroiditis). Annexin V, TRAIL and TNF-a, as well as DNA-hydrolyzing antibodies were used as the main markers. Increased levels of TRAIL were found in the serum of AT patients (hyperthyroidism>hypothyroidism>euthyroidism) compared with healthy individuals. The highest frequency of antibodies to denatured DNA (Abs-dDNA) had the highest frequency in AT patients (97%) compared with healthy controls. Among these patients, 75% had hyperthyroidism, 85% had hypothyroidism, and 84.7% had euthyroidism. Abs hydrolyzing activity demonstrated correlation dependence with symptoms of the thyroid dysfunction.
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14. Porokhovnik L.N., Kostyuk S.V., Ershova E.S., Stukalov S.M., Veiko N.N., Korovina N.Yu., Gorbachevskaya N.L., Sorokin A.B., Lyapunova N.A.
The maternal effect in infantile autism: elevated DNA damage degree in patients and their mothers.
Infantile autism is a common disorder of mental development, which is characterized by impairments in the communicative, cognitive and speech spheres and obsessional stereotyped behaviour. Although in most cases, pathogenic factors remain unclear, infantile autism has a significant hereditary component, however, its etiology is also under the influence of environmental factors, including the condition of the mother's body during pregnancy (â€œmaternal effectâ€•). Oxidative stress is assumed to play a key role in the pathogenesis of infantile autism. It is known that oxidative stress has a prominent genotoxic effect, which is realized through inducing single and double strand breaks of the nuclear DNA. We evaluated the degree of DNA damage in patients with infantile autism and their mothers using DNA comet assay. The comet tail moment and DNA per cent ratio in the tail were assessed for each individual. The two parameters appeared to be strongly correlated (r=0.90). Mean and median values of both parameters were considerably higher in the sample of autistic children, than in age-matching healthy controls. Interestingly, these parameters were also elevated in healthy mothers of autistic children, with no difference from the values in the group of autistic children. The control group of healthy women of reproductive age, who had no children with autism, differed by the DNA comet tail moment from the group of mothers of autistic children, but did not differ significantly from the control group of healthy children. The results suggest that there are genotoxic factors in mentally healthy mothers of autistic children, which can determine the pathological process in the foeti via environmental â€œmaternal effectâ€• during gestation.

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15. Belik I.V., Ivantsova A.A., Mamedova Z.E., Denisenko A.D.

Antibodies against modified low-density lipoproteins and their complexes in blood of patients with various manifestations of atherosclerosis.

The study included 79 patients with coronary artery disease, 25 individuals with preclinical atherosclerosis and 59 healthy controls. Key lipid parameters were examined in all the participants. Levels of antibodies (Abs) against (IgG and IgM) LDL modified by malondialdehyde (MDA), acetic anhydride and hypochlorite, were determined by the enzyme-linked immunosorbent assay (ELISA). Abs specificity was tested by competitive ELISA. Circulating immune complexes (CIC) were isolated by precipitation in polyethylene glycol. Abs to hypochlorite-modified low density lipoprotein (hypochlorite-LDL) were detected in the serum samples. These Abs did not demonstrate cross-reactivity with MDA-modified LDL (MDA-LDL) and acetylated LDL (acetyl-LDL). Patients with coronary artery disease had increased levels of CIC ($p<0.0001$) and decreased levels of Abs (IgM) to hypochlorite-LDL, compared with healthy controls and patients with preclinical atherosclerosis ($p=0.006$). A correlation between the levels of Abs (IgG) to the hypochlorite-LDL and Abs to MDA- and acetyl-LDL was found. There was a correlation between the content of the Abs (IgM) to MDA- and acetyl-LDL and the concentration of CIC-cholesterol. Lipid parameters did not correlate with Abs levels.

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