IFN-DCs. Migration index in response to concentration gradient of primary glioma line-derived soluble factors (6 cell lines) ranged from 32.6 ± 9 to 48.4 ± 15.5. To assess the effect of supernatants of primary lines on the cytotoxic activity of IFN-DCs four primary high-grade glioma lines were used. The cytotoxicity of intact LPS-stimulated donor IFN-DCs against HEP-2 cells was an average of 34.8 ± 7.4%. Supernatant pretreatment of IFN-DCs reduced the cytotoxic activity of DCs in 8 out of 16 experiments (with an average of 43%). In other cases, supernatants of high-grade glioma lines, in contrast, possessed a mild stimulating activity and enhanced the cytotoxicity of DCs on average about of 34%. Thus, tumor cells in glioma patients produce chemoattractants capable of providing migration of DCs to tumor. At the same time glioma-derived soluble factors change the cytotoxic activity both ways. One can suppose thereupon that impairment of DC cytotoxic activity in glioma patients occurs not only due to tumor cell impact but because of unknown mechanisms.

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P52a

Multiplex analysis of 27 cytokines produced by low-grade and high-grade glioma cells

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Glioma is the most common type of primary brain tumor. Despite aggressive therapy, glioma virtually always recurs. Soluble factors produced by glioma cells play an important role in progression of tumor growth. Glioma-derived soluble factors can stimulate tumor cell proliferation in autocrine and paracrine manners, affect the tumor microenvironment and promote angiogenesis and metastasis. Besides, glioma-derived cytokines are able to disturb antitumor immune response by inhibiting the functions of effector lymphocytes and inducing immunosuppressive processes. The aim of the present study was to determine the profile of cytokines produced by primary low-grade and high-grade glioma cell cultures. The study was held in 21 patients with brain tumors after receiving a written informed consent. There were 6 patients with histologically verified low-grade glioma (Grade II) and 15 patients – with high-grade glioma (Grade III–IV). Tumor tissues from patients were obtained during surgical resection. Cell suspensions were prepared by mechanical and enzymatic disaggregation followed by culturing in DMEM/F12 medium containing 10% FCS. The levels of 27 cytokines were measured using multiplex analysis (Bio-Rad, USA) in 7-days supernatants collected upon reaching the cellular subconfluence. Low-grade glioma cells produced low level (Me <25 pg/ml) of pro-inflammatory cytokines IL-1β, TNFα and anti-inflammatory cytokines IL-1ra, IL-10, IL-13. The level of these cytokines in supernatants of high-grade glioma cultures were in the middle range (25–500 pg/ml), excepting IL-1β (Me 5.9 pg/ml). Concerning cytokines with immunoregulatory activity in supernatants of low-grade glioma cultures, only IL-12 level was in the middle range (Me 77 pg/ml), whereas the level of other immunoregulatory cytokines (IL-2, IFNγ, IL-4, IL-5, IL-15, IL-17) was low (Me <25 pg/ml). High-grade glioma cells actively produced not only IL-12 (Me 336 pg/ml), but also IFNγ and IL-15 (Me 355 pg/ml and Me 57 pg/ml, respectively). The level of growth factors and cytokines, which act as regulators of hemo- and immunopoesis, was low in supernatants of low-grade glioma cultures. Median concentration of G-CSF, GM-CSF, IL-7, FGFb and PDGF was less than 25 pg/ml, but IL-9 concentration was in the middle range (Me 74 pg/ml). High-grade glioma cultures produced, besides IL-9, also G-CSF (Me 263 pg/ml), FGFb (Me 60 pg/ml) and PDGF (Me 63 pg/ml). The level of FGFb and G-CSF correlated with the glioma grade (R = 0.87, p = 0.002, and R = 0.66, p = 0.002, respectively). The IL-7 and GM-CSF level in high-grade culture supernatant was low (Me <25 pg/ml). IL-6 and VEGF play important role in the maintenance of tumor cell proliferation and the stimulation of tumor angiogenesis. Both low-grade and high-grade glioma cultures produced high level (>500 pg/ml) of IL-6 (Me 1933 pg/ml and 3551 pg/ml, respectively) and VEGF (Me 1004 pg/ml and 14887 pg/ml, respectively). VEGF level correlated with glioma grade (R = 0.86, p = 0.002). Concerning CC-chemokines the level of Eotaxin was low in low-grade glioma cultures (Me 11 pg/ml). The mild-level production was found for MIP-1α (Me 71 pg/ml) and RANTES (231 pg/ml), high-level – for MCP-1 (Me 9468 pg/ml) and MIP-1β (Me 990 pg/ml). High-grade glioma cells produced not only MIP-1α (Me 208 pg/ml) and RANTES (Me 311 pg/ml) in the middle range but also Eotaxin (Me 107 pg/ml). Eotaxin level correlated with the glioma grade (R = 0.87, p = 0.002). High-grade glioma cells produced high-level of MCP-1 (Me 9868 pg/ml) and MIP-1β (Me 1923 pg/ml). Both low-grade and high-grade glioma cells produced high level of CXC-chemokines IL-8 and IP-10 (Me >1000 pg/ml). The level of IL-8, which acts as chemoattractant for immune cells as well as for endothelial cells involved in angiogenesis, correlated with glioma grades (R = 0.72, p = 0.002). Thus, the grade of gliomas associated with broadening the spectrum of cytokines and enhanced level of cytokines production by tumor cells.

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A89

P450 1A subfamily as component of exosomes derived from HepG2 cells

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Exosomes are small vesicles formed in vesicular bodies in the endosomal network many different types of cells. Exosomes are abundantly released by tumor cells. Current knowledge of exosomes suggests that they can play an important role in the development and progression of cancer through modulation of intercellular communication within the tumour microenvironment by the transfer of protein, lipid, and RNA cargo.
Hepatocellular carcinoma is the most common primary malignancy of the liver. HepG2 represents a pure cell line of human liver carcinoma. The purpose of the current study was characterization of exosomes derived from HepG2 cells line.

Exosomes were isolated from HepG2 cell culture supernatant by a series of subsequent centrifugation steps. Morphology of exosomes was determined by electron microscopy. To characterize HepG2 cell derived exosomes we also examined the presence of the ER-residing protein Calnexin by Western blot. Data showed that Calnexin was absent in exosomes. Taken together, these results indicate that vesicles obtained from cell-free supernatants of HepG2 cells exhibit properties of exosomes.

Proteome analysis was performed for proteins commonly expressed in HepG2 cells such as cytochromes P450 that serve important roles in the cellular detoxification process and drug metabolism. We identified members of protein families cytochromes P450 CYP1A1 and CYP1A2 in both HepG2 cells and exosomes.

Thus, hepatocyte-derived exosome population should be useful in our further understanding of the hepatic function and in the identification of components that may serve as biomarkers for hepatic alterations. These tumor-derived extracellular vesicles represent a mediator of the tumor microenvironment, and their presence in the peripheral circulation may serve as a surrogate for tumor biopsies, enabling real-time diagnosis and disease monitoring.

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The TP53 mutations in the Russian patients with de novo DLBCL


Background: TP53 dysfunction is implicated in lymphomagenesis and disease progression. Information about the frequency and spectrum of TP53 mutations in the Russian patients with diffuse large B-cell lymphoma (DLBCL) in the current version of the IARC TP53 Mutation Database R17 is not represented. The goal of this work was to study the frequency, spectrum and functional significance of TP53 mutations in Russian patients with DLBCL.

Material and methods: At the present time the pilot group of 14 patients were included in the study. Diagnosis was assessed according to the criteria of the WHO classification system. Genomic DNA was isolated from formalin-fixed, paraffin embedded tissue blocks. Direct sequence analysis of gene TP53 was performed according to the IARC protocol, 2010 update.

Results: In two patients were identified single nucleotide substitutions that are not described in the current version of the PubMed database. All of mutations occurred in the DNA-binding domain of p53. The nonsense mutation Arg196Ter was detected in one patient. Previously it was shown that formation of this premature stop codon might activate the nonsense-mediated RNA decay pathway. The second patient had two missense mutations – Leu130Phe and Arg156Cys. The first of them leads to p53 inactivation according to the analysis of the functional importance of amino acid substitutions using service PolyPhen-2.

Conclusion: We detected TP53 mutation in 14% cases. The mutational rate in our study is in good agreement with other studies where the frequency of the TP53 mutations in patients with DLBCL ranged mostly from 13% to 23%.

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T140

Epigenetically active xenobiotics in cancer prevention and therapy optimization


Nowadays the term “epigenetics” is described as regulation of gene expression persisting from one cell division to the next, despite a lack of changes in the underlying DNA sequence. The “epigenome” refers to different epigenetic states of a cell recognized as heritable environment influence on genome. The main epigenetic phenomena in mammals are DNA methylation and histone modifications, which are tightly interdependent. Many authors classify microRNA regulation as a third epigenetic phenomenon. Moreover, recently the discussion has been open that many different factors modifying DNA conformation represent a new class of epigenetic agents. In response to various environmental stimuli, cells produce different epigenetic changes that determine either an active or a repressed chromatin state.

Epigenetic perturbations have been shown to associate with exposure to a range of drugs and toxicants, including non-genotoxic carcinogens. Consequently, on one hand, potential impact of epigenomics on drug development is under consideration as even well-known pharmacological drugs were shown to cause epigenetic changes that may be beneficial or hazardous. In particular, epigenetic effects were described for synthetic estrogens and contraceptives, beta-blockers and fluoroquinolone antibiotics, neuroleptics and anesthetics, chemotherapeutics and statins. Drug influence on gene silencing might have some therapeutic advantage in addition to the unfavourable effects. In particular, valproate, hydralazine and procainamide might be utilized to induce gene expression in cancers, where activation of a methylated gene might be of benefit. Screening of xenobiotics for epigenetic activity might identify new potential drugs for some specific diseases.

On the other hand, epigenetic aspects of drug safety are investigated intensively. Many recent reviews in this field of research were devoted to the test elaboration for revealing different short-term and longer-lasting epigenetic changes modifying gene expression. Most of the short-term epigenetic screening tests are