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 CYPIA1 and CYPIA2 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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A36

The TP53 mutations in the Russian patients with de novo DLBCL

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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