POLYMORPHISM OF BIOTRANSFORMATION GENES OF XENOBIOTICS GSTM1 AND GSTT1 OF PROBABLE MARKERS OF RISK OF LUNG ONCOLOGY, IN THE YAKUT POPULATION

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It was first established that the risk of developing lung cancer in the Yakut population is associated with the zero genotype of the GSTM1 gene, which may indicate a higher risk of cancer for carriers of this genotype. Polymorphism in the genes GSTM1, GSTT1, which encodes the isoforms μ, τ of the glutathione-S-transferase enzyme characterized by deletion in both alleles leads to complete absence of synthesis of the protein product and resulting in a deep suppression of the enzyme function. According to the reports, there is conflicting data on the association of deletion-genotype GSTM1 with the risk of developing lung cancer. [4] The aim of the research was to study polymorphism of the GSTT and GSTM1 genes patients with lung cancer.

Materials and Methods. Molecular genetic studies were conducted in 36 people of Yakut nationality with lung cancer. The control group was selected taking age and ethnicity into account. It included 65 people. The main criteria for selecting a control group were the absence of cancer. A standard phenolic-chloroform extraction method was used to isolate DNA. The analysis of polymorphic variants of specific sections of the genes GSTM1, GSTT1 was carried out using polymerase chain reaction methods and while using the primer structure described in the works [Gronau et al., 2003]. When comparing genotype frequencies, the standard χ² criterion was used. The relative risk (OR) of the development of the disease with a specific genotype was calculated using the standard formula \[ \text{OR} = \frac{a/d}{c/b} \], where \( a \) and \( b \) are the number of patients with and without a mutant genotype, respectively, and \( d \), \( c \) is the number of people in the control group who have and don’t have a mutant genotype. OR is indicated with a 95% confidence range.

Results. According to our data in the selection studies of patients with lung cancer and control it is revealed that the frequency of occurrence of the “zero” genotype GSTM1 (0/0) in the group of patients was higher (66.6%) than in healthy individuals (43.0%). Where differences has reached a level of statistical significance - \( \chi^2 = 5.16, p = 0.023, \text{OR} = 0.67 (0.26-1.69) \). The analysis of deletion polymorphism of the GSTT1 gene did not reveal any significant differences in the frequency distribution of genotypes depending on oncopathology. The frequency of occurrence of deletion polymorphism in the group of patients was 41.7%, and in the healthy group 32.3% (\( \chi^2 = 0.347, p = 0.561 \)). Obviously, the GST genes, especially GSTM1, are involved in the pathogenesis of various types of cancer and act as modifiers and risk factors for a variety of diseases associated with adverse environmental factors. According to published data of other authors, the influence of polymorphism of GST-genes is associated with the development of numerous malignant tumors: lung cancer [6], bladder, rectum, stomach, esophagus, breast, ovaries and skin [1,2,3,5,7,9]. There are works showing the connection of polymorphism of genes encoding the enzymes of the second phase of detoxification, with chronic and hereditary diseases: chronic bronchitis, bronchial asthma [8]. In addition, it has been shown that the deletion of the GSTM1 and GSTT1 genes leads to increased sensitivity to xenobiotics (in particular, to thermal decomposition products of tobacco). In our work with a high degree of reliability it was shown that the increased risk of developing lung cancer in the Yakut population can be related to the zero genotype of the GSTM1 gene.

Prospects for further research. It was found that the risk of developing lung cancer in the Yakut population is associated with the zero genotype of the GSTM1 gene, which may indicate a higher risk of cancer for carriers of this genotype. This can cause a severe tolerance of chemotherapy treatment of Yakut patients with lung cancer. Studies in this direction can be continued. This could be a study not only of polymorphism of the genes GSTM1 and GSTT1 but also of other polymorphisms of xenobiotic detoxification genes in Yakut population.

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Measuring of the level of oxidized and reduced glutathione and other thiols and disulfides in tissues and blood is a sensitive marker of disturbances of the redox balance. Nevertheless, methodical approaches to their estimation require careful observation of the storage and sample preparation conditions for biological material for analysis, the choice of an optimal, specific, sensitive analytical method giving well-reproducible results[1, 2].

Despite the fact that the glutathione system is one of the most important systems in maintaining the redox balance in tissues, an accurate measurement of the GSH / GSSG ratio represents a certain methodological problem until now. The levels of both GSH and GSSG in tissues fall within a range easily measured by most currently used methods (1-5 mM and 0.01-0.05 mM, respectively). However, the measurement of GSH in blood plasma may present certain problems due to the sensitivity required for quantification (1-10 μM) and the easy transition of GSH to GSSG during blood sampling and sample preparation[3, 4, 5].

Glutathione is normally found in the form of GSH, and the GSSG content does not exceed 5% of the level of its reduced form, but given the rapid transition ability of the reduced form of glutathione to oxidized during manipulation of tissue sampling, careful attention should be paid to sample preparation for analysis. Potential errors in such measurements are often not fully recognized or evaluated.

To exclude postmortem oxidation of GSH in blood samples, the following conditions must be met. Blood is recommended to be taken to vacutainers (vacuum tubes for blood sampling) with EDTA to stabilize thiols or EDTA + NEM – in the measuring of oxidized glutathione[6, 7, 8]. It is allowed to store samples at -80°C for several months. To prevent the formation of disulfides in the storage of samples, an acidic extract is prepared on a cooled sulfosalicylic[7] or metaphosphoric acid[9], or on perchloric acid with the addition of EDTA[10]. Acidic extract should be prepared immediately after blood sampling or after centrifugation of blood in case of working with plasma.

One of the most popular methods for measuring GSH in the literature is Ellman’s method, proposed in 1959 to estimate common tissue sulfhydryl groups[11]. Unfortunately, the extinction coefficient of the resulting product for dilute solutions was underestimated in the original publication of 1959 (13,600 M-1 · cm-1), and, as noted in[12], this error has been preserved in the literature. The determination of GSH with Ellman’s reagent usually gives excessive amounts of this compound, since in the reaction with Ellman’s reagent the total content of sulfhydryl groups of not only GSH but also other minor non-protein thiols (Cys, γ-GCys, etc.) is determined. Based on the fact that the cysteine content, as a rule, is more than two orders of magnitude lower than that of glutathione, they are usually neglected in the routine determinations of glutathione. At the same time, the definition of GSH by the Ellman method is incorrectly used to evaluate its content in blood and tissues when thiol-containing drugs are introduced (N-acetylcysteine, lipoic acid, unitiol, etc.), since these compounds themselves can react with Ellman’s reagent.

For the specific measuring of GSH, enzymatic analysis methods exist that allow a high level of accuracy in assessing the actual level of GSH provided that the correct procedure for sample preparation of biological material for analysis is performed. The enzymatic method, the most common and popular now, is the recycling enzymatic method with glutathione reductase (GR). This method allows to estimate the content of both total glutathione (GSH + 2GSSG) and oxidized glutathione (GSSG). The content of the latter is measured in the presence of a masking reagent to eliminate free sulfhydryl GSH groups[4, 6, 7, 9, 10]. The GSH content is calculated from the difference between the total glutathione content (GSH + 2GSSG) and the GSSG.

To measure the GSSG content, a masking alkylating reagent – N-ethylmaleimide (NEM) or 2-vinylpyridine (VP) is added to the sample to bind the free sulfhydryl GSH groups. In the case of NEM, there is a need to remove its excess, since NEM is an inhibitor of GR[6, 10]. For this purpose, the extraction of excess NEM with dichloromethane[6] or diethyl ether or purification on a Sephadex-10 chromatographic column, QAE-25 Sephadex[10] is used, which complicates the analysis procedure, especially with its routine using. If VP is used as a masking agent for sulfhydryl