



Prognostic Significance of the Polymorphism of the Tp-53 Oncosuppressor Gene and Mdn-2 Protooncogene in the Development of Chronic Myeloid Leukemia

1. Khomidchonova Shakhzoda
Khasanzoda

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¹ Assistant, Fergana Institute of Public
Health Medicine

Abstract: The deep involvement of the TP53 gene abnormalities in the development of different types of tumors is due to its performance of a wide range of functions that prevent and/or inhibit tumor growth.

In this article highlights of prognostic significance of the polymorphism of the tp-53 oncosuppressor gene and mdn-2 protooncogene in the development of chronic myeloid leukemia.

Key words: polymorphism, TP-53, oncosuppressor gene, protooncogene, chronic myeloid leukemia, medicine.

Unlike other cells of the body that are subject to cell cycle arrest, p53-independent apoptosis or necrosis under stress, B lymphocytes are prone to p53-mediated apoptosis. For this reason, the disruption of the processes of programmed cell death as a result of a violation of the function of TP53 is fundamental for the development and progression of lymphoproliferative diseases. Thus, in mice, when the TP53 function is turned off, malignant lymphomas are the dominant form of neoplasia.

In addition, inactivation of TP53 in B-lymphocytes leads to less effective functioning of intracellular signaling systems that stop the cell cycle in phases G1 and G2 when damaged, disruption of DNA repair, more effective adaptation of cells to hypoxia and stimulation of angiogenesis, weakening of control over the structure of telomeres and inhibition of differentiation. Since TP53 plays a central role in the mediation of the action of alkylating agents and other chemotherapeutics, the deficiency of its function inevitably leads to the formation of a phenotype of multiple drug resistance of lymphoma cells. The TP53 gene is located at 17p13.1 and consists of 19,144 pairs of nucleotides (bp).

Aberrations of the TP53 gene play a key role in the pathogenesis of many human oncological diseases, including the occurrence, development and progression of DVCCL, however, to date, knowledge about the specific mechanisms of formation and significance of disorders in the TP53 gene in this variant of non-Hodgkin's lymphomas (NHL) Possible molecular deficiencies are of a limited nature. Considering that the resultant effect of TP53 anomalies largely depends on the tissue-specific context and the specific type of cells in which its activity manifests itself, it is impossible to mechanically transfer general knowledge about the participation of TP53 in oncogenesis to DVCCL. Being the most complete database of mutations in the TP53 gene in human tumors, the IARC TP53 mutation database

is designed to collect, structure and annotate data that can subsequently accurately interpret the significance of the TP53 mutation in a specific pathology and use them in standard clinical practice.

Medulloblastoma is a neuroepithelial embryonic tumor of the cerebellum, it is most often detected in childhood, although according to statistics, up to 24-30% of such neoplasms are found in patients over the age of 18 and even the elderly. Modern technologies for the treatment of medulloblastoma, including surgical treatment in combination with radio and chemotherapy, have significantly improved the outcome and increased the life expectancy of patients. Thus, the five-year survival rate is 50-70%, depending on the histological features of the tumor and the age of the patient, the therapy regimens used. In patients with medulloblastoma who are at risk (and this is the age of up to 3 years), early metastasis, non-radical removal of the tumor, a 5-year relapse-free period is observed in less than 25%. Significant progress has been made in the treatment of tumors of this type, further progress in therapy will depend both on an in-depth study of their biology, in particular, molecular genetic characteristics and the development of refined prognostic indicators on this basis, and the improvement of combined treatment. The clarification of the histological structure of such tumors and the search for histological prognostic criteria has been carried out continuously over the past century, whereas cytogenetic and especially molecular genetic studies in neuro-oncology have recently been used.

Until now, when sequencing TP53 in the tumor tissue of patients with DVCCL, almost all studies have focused on finding mutations in exons 5-8. However, according to e1 a1. Taking into account mutations in untranslated regions and introns of the gene, TR53 is one of the most frequently mutated in DVCCL, which may indicate an underestimation of the frequency and functional effect of aberrations in TR53 in this disease. Variants of damage to the TR53 gene in DVCCL, such as allelic imbalance and abnormal methylation of the promoter, are less covered in the literature. The few published studies devoted to the study of polymorphisms of the germ line of TR53 in DVCCL were limited to the analysis of individual markers, however, taking into account modern ideas about the structural organization of TR53, it is obvious that knowledge about the haplotype is necessary to clarify the effect of markers of this gene on its function.

In this regard, the aim of this study was to determine its role in the tumor progression of DVCCL on the basis of a comprehensive study of the variability of the TP53 gene due to somatic mutations, promoter methylation, allelic imbalance, functionally significant polymorphisms and haplotype structure.

The analysis of the biological significance of the detected mutations was carried out according to the literature, using databases and tools IARC TP53 mutation Database, The TP53 UMD mutation database in human cancer and Human Gene Mutation Database. Additionally, bioinformatic analysis of missense mutations was performed using the online program Polyphen-2.

Evaluation of the methylation of the promoter of the TP53 gene required preliminary bisulfite conversion of 300-500 ng of DNA from each sample using the EZ DNA Methylation Kit (Zymo research, USA), according to the manufacturer's protocol. Next, methyl-specific PCR was performed in two test tubes with primers specific to the methylated and unmethylated allele.

Genotyping of rs78378222, rs1042522, rs17878362, rs1625895 and haplotyping of rs17878362-rs1042522 were carried out by PCR and analysis of the polymorphism of the lengths of the friction fragments (PDRF). The haplotyping of rs1042522-rs1625895 and rs1625895-rs17878362 was performed by the method of nested PCR with an allele-specific primer, which ensures the production of one of the rare alleles in the analyzed pair of markers. Subsequently, the PDRF analysis was performed to determine the allele of the second marker linked to it.

To assess the association between the studied parameters and the risk of the analyzed event, the odds ratio (OR) with a 95% confidence interval (CI) was calculated. When comparing the frequencies of features, the standard Pearson χ^2 criterion and the exact Fisher criterion were used.

According to the dbSNP database, about 750 polymorphisms are known in the TP53 gene, 50 of which are cited in the pubmed database. Most of them are rare in the population, about two dozen single-nucleotide polymorphisms have a frequency of occurrence of a rare allele in the population of more than 5%, and three — rs1042522, rs17878362 and rs1625895 — are the most well studied in terms of functional characteristics, prevalence in the population and association with the risk of tumors.

Over the past 15 years, the association of rs1042522, rs17878362 and rs1625895 with a predisposition to various human tumors has been widely studied, but the data differ and are not final. Combinations of genetic features (intergenic interactions) and endo-exogenous factors differ in individuals in populations with different ethnic origins, which may explain differences in predisposition. It is also extremely important to take into account that the final effect of polymorphism on the function of p53 may vary depending on the type of tumor cells, which indicates the relevance of further research and the need for new information expanding the understanding of the role of genetic polymorphism of TP53 in predisposition to cancer.

At the first stage of this work, the frequency distribution of alleles and genotypes of the three studied polymorphisms of the TR53 gene was calculated in a group of patients with DVCCL and a control sample. There were no statistically significant differences in the frequency distribution of alleles and genotypes gb1042522, gb1625895 and gb17878362 in the group of patients with DVCCL and control samples, as well as data on the association of the studied markers with the risk of DVCCL development.

However, since predisposition or resistance to multifactorial diseases is determined by a combination of a large number of small effects of individual factors, potentially the most productive strategy for identifying genetic variants underlying predisposition to DVCCL is to analyze the structure of disequilibrium by coupling markers of the TP53 gene and the detection of disease-related haplotypes.

Analysis of world literature data shows that the question of the significance of the polymorphism of the TR53 gene in predisposition to non-Hodgkin's lymphomas (NHL) remains unresolved at present. Weng et al. In the meta-analysis of the literature, we tried to summarize the data on the association of rs1042522 of the TR53 gene with a predisposition to NHL. His data are quite consistent with the results we obtained in a pilot study on a group of 100 patients with NHL that it is the Pro allele of this marker that may be "risky" in relation to lymphomas. However, in addition to the fact that, unlike rs1042522, the association of rs17878362 and rs1625895 with the risk of NHL has not been practically studied, the high diversity of NHL requires the analysis of these markers for individual histological variants of the disease, and the small effect of each of the polymorphisms individually on the risk of developing the disease is to assess them as part of haplotype groups.

Thus, the effect of polymorphisms of the TR53 gene on the formation of a predisposition to tumors, apparently, has an influence, the result of which is determined both by ethnicity, interactions with the environment and other genetic factors, histological affiliation of the tumor, and the level of heterozygosity for the studied polymorphisms and haplotype structure of the TR53 gene.

Strict evolutionary fixation of the polyadenylation signal of the TR53 gene was shown in the study of the genome of 63 mammalian species, as well as the ancient DNA of Neanderthals and Denisov samples.

The following fact draws attention to itself. In none of the currently published studies described in the literature, the rare allele C rs78378222 in healthy tissue was not found in a homozygous state. At the

same time, some of the samples of tumor tissue analyzed by us had the genotype C/C rs78378222. All of them represent cases of loss of heterozygosity in the TR53 gene in carriers of the heterozygous genotype A/C rs78378222 in the tumor tissue of the DVCCL.

The biological meaning of the loss of heterozygosity in the TR53 gene, which has a rare allele with rs78378222, in DVCCL, may be as follows. In cells with rs78378222, a decrease in the level of mRNA was confirmed in comparison with cells without this polymorphism and the presence of another nearby marker rs114831472. In case of detection of a rare allele from this marker, the level of cellular apoptosis was lower, compared with cells with the wild type of the TR53 gene. These data show that TP53 polymorphism rs78378222 disrupts p53 expression and inhibits apoptosis.

Bioinformatic analysis of the exome sequencing data of tumor tissue showed that in glioblastomas, unlike lung adenocarcinoma, there is a loss of the haplotype carrying the protective allele A. The authors suggested that the loss of the region of the genome carrying the frequent protective allele A occurs during tumor initiation or glioma progression.

A comprehensive analysis of the variability of the gene shows that the insufficiency of the TR53 function in DVCCL can be formed according to the "two-stroke" principle. According to him, two consecutive events may be necessary for the transition of a normal B cell to a tumor cell when at least part of the cases of DVCCL occur. The first event is a mutation or methylation of the TP53 promoter, leading to the formation of a cell with an increased risk of malignant transformation. To realize the tumor potential, a second event must occur in the cell — the loss of an intact allele of the gene.

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