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TP53 Arg72Pro Gene Polymorphism in Patients with Malignant Head and Neck Tumors

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Abstract

Several studies have shown that the Arg72Pro TP53 gene polymorphism is associated with an increased risk of malignancies. The aim of this study is to investigate the distribution of the genotypes of Arg72Pro TP53 gene polymorphism in patients with malignant tumors of the head and neck (MTHN). The study involved 117 patients including 90 males and 27 females, of mean age 54.3±8.6, having MTHN. The control group consisted of 70 Uzbek volunteers (mean age 51.0±6.2 years) without malignant tumors. The frequency of the distribution of the genotypes of Arg72Pro TP53 polymorphism in patients having MTHN included Arg/Arg - 0.1, Arg/Pro - 0.68, Pro/Pro - 0.20; χ^2=99.308, p=0.000. In healthy volunteers the ratio of Arg/Arg: Arg/Pro: Pro/Pro was found to be 0.27:0.52:0.20; χ^2=21.57, p=0.000. The heterozygous genotype was dominant in the patients with MTHN, as well as those in the control group. However, the findings were significantly higher in the study group, viz., 0.68 as against 0.52 in the control group (p=0.03). The homozygous genotype Arg/Arg was rarely encountered in the patients, viz., 0.11 in the study group compared with 0.27 in the control group (p=0.005). The risk of being an MTHN carrier was found to be associated with the polymorphic marker gene TP53, and particularly, with the accumulation of the Arg/Pro genotype [OR: 1.93 (95% CI: 1.05-3.55), while reducing the frequency of the Arg/Arg genotype [OR: 0.34 (95% CI: 0.15-0.73)]. IJBM 2011; 1(4):217-220.

Key words: *Arg72Pro TP53 gene polymorphism, Uzbek patients, malignant tumors of the head and neck.*

Introduction

Despite numerous studies showing a direct role in the mechanisms of TP53 tumor suppression and apoptosis, the nature of the relationship between gene expression and its mutant forms with tumor suppressor and tumor transforming functions are not clearly evidenced. At present there is no doubt that the process of carcinogenesis is run with the primary changes in the molecular genetic level, and predisposition to malignant tumors and tumor progression can be triggered not only by somatic mutations but also by allelic polymorphisms of genes. In recent years, dozens of polymorphic candidate genes have been identified, which are assigned specific roles in the development of cancer risk. In this series, TP53 gene has a special place. The human TP53 tumor suppressor gene is located on chromosome 17p13 and encodes a 53 kDa nuclear phosphoprotein, which plays a central role in many cellular processes, such as cell-cycle control, DNA repair and apoptosis [1-5]. Since TP53plays an important role in cell cycle regulation and in maintenance of genome stability by preventing mutations, it is often referred to as the guardian of the genome [6]. Loss or mutation of TP53 is probably the commonest single genetic change in cancer [7, 8]. The protein encoded by the gene, in normal conditions, acts as a tumor suppressor, but its mutant forms can promote tumor development. If in healthy, growing cells the protein p53 has a rapid turnover, and its concentration is quite low, then in response to stressful signals that could lead to DNA damage (hypoxia, viral infection, radiation exposure, etc.), the protein level begins to rise rapidly. DNA damage signalizes to the accumulation of p53, which, in turn, blocks cell cycle progression in G1 phase, thus preventing replication of damaged DNA. If the damage is not repaired, the protein activates cellular proteases and, above all caspases, causing apoptosis. Thus, mutation in the TP53 gene makes DNA sensitive to damaging agents. The predominant proportion of TP53 aberrations represented by missense

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mutations (75%) in the DNA-binding domain that lead to amino acid substitutions in the protein p53 and inhibits the binding of protein with targets. Other changes in TP53 include insertions and deletions (9%), nonsense and silent mutations (an average of 5%). Other rare aberrations can also lead to a shift of the reading frame and disrupt the functional properties of the p53 protein, as the tumor suppressor protein [13-16].

However, the question of the prognostic significance of multiple mutations of TP53 has not yet acquired sufficient significance for practical oncology and continues to be the subject of ongoing debate and discussion of geneticists and molecular biologists. An attempt to link indicators of carcinogenesis such as the degree of infestation, the nature of the metastasis, regional lymph violation (lymphatic drainage) with an increase in the number of mutations or the amino acid polymorphisms has proved that the problem is not easy to solve. The variability of gene expression may be associated with periods of disease progression or could be present at all stages. This includes the effects of treatment and, in particular, iatrogenic components, chemo methods, as well as different effects of stress, ethnic specificity [17-19].

A common TP53 polymorphism at codon 72 of exon 4 (dbSNP ID: rs1042522), designated as Arg72Pro, has been reported to modify the risk and/or prognosis of many types of cancers [8, 20-22]. This polymorphism derives from a single-nucleotide substitution at codon 72, where either CCC encodes proline or CGC encodes arginine, resulting in a non-conservative change. This polymorphism is located in a proline-rich domain of p53, which is known to be important for the growth suppression and apoptotic functions (20 Evidence is emerging indicating that the Arg allele and the Pro allele of the TP53 codon 72 polymorphism are not equivalent in biochemical property and function (23). The two polymorphic variants of wild type TP53 have been shown to have different biochemical properties like differential binding to components of the transcriptional machinery, inducing cell death, cell-cycle arrest and besides these functions TP53 regulates the various DNA-repair processes.

Several studies have shown that the 72Arg p53 forms are much more efficient triggers in programmed cell death, compared with the 72Pro forms. Experimental studies have demonstrated that the 72Arg forms have greater capacity to cause failure at the level of mitochondrial respiratory chain, which is accompanied by inactivation and release into the cytoplasm of cytochrome C. In contrast, the 72Pro variants are able to induce higher levels of delay cells in the G1-phase of the cell cycle and ensures DNA repair without reducing it to apoptosis. Thus, based on the above experimentation, we have to conclude that the polymorphic marker Arg72Pro is associated with an increased risk of malignancies [24-26].

Review of the literature has revealed that thus far none of the polymorphisms of TP53 have been studied in the Uzbek population.

The aim of this study is to investigate the distribution of the genotypes of Arg72Pro TP53 gene polymorphism in patients with malignant tumors of the head and neck (MTHN).

Material and methods

The study involved 117 patients including 90 males and 27 females, of mean age 54.3±8.6, having MTHN. The control group consisted of 70 Uzbek volunteers (mean age 51.0±6.2 years) without malignant tumors.

All respondents were Uzbek nationals, representing three generations. Interestingly, significant relationships were found to exist between the polymorphisms of TP53 and the risk of MTHN. The risk of MTHN has been shown to be associated with the heterozygous state in the exon 4 polymorphic site, and with reduction the frequency of the "wild" Arg/Arg genotype of TP53.

Verification of the head and neck tumors was performed using cytomorphological methods. In these cases, the discrepancy between the clinical symptoms and morphological diagnosis not observed. The patients were mainly at the II and III stages in the TNM system of the disease.

Isolation of Genomic DNA was performed using a set of DIAtom™ DNA Prep200 (Laboratory IsoGene, Russia).

PCR amplification was performed using the set of genotyping at polymorphic marker gene TP53 Arg72ProPCR kit (Gene Test, Russia); thermocycler GeneAmp® PCR System 9700 (Applied Biosystems, USA).

The following sequence primers were used:
Forward primer:
5`-AGGAGCTGCTGGTGCAGGGGCCGCG-3`,
Reverse primer:
5`-TAAGGACAAGGGTTGGGCTGGGGACCTGGA-3`

Amplification conditions:

First denaturation	10 cycles	30 cycles
95°C – 5 min	94°C – 20 sec	94°C – 20 sec
	TD* – 50 sec	64°C – 50 sec
	72°C – 20 sec	72°C – 20 sec

Note: **TD – touchdown polymerase chain reaction. At the recommended annealing temperature of 56°C, the first annealing was conducted at 66°C and gradually decreased at the 10th annealing to 56°C.*

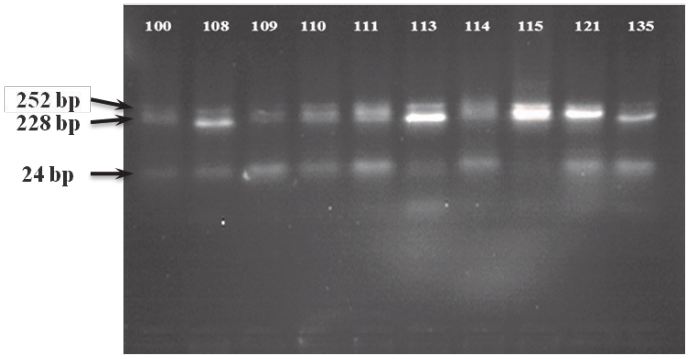
The results of amplification detection were confirmed with horizontal electrophoresis in 1% agarose gel at ~15V/cm for 30 minutes. As the electrophoretic buffer used was 1xSB buffer (Gene Test, Russia). Gels were stained with Ethidium bromide solution (0.01%), and viewed under ultraviolet light (λ=254 nm) using the Vilber Lourmat (France) Electronic Ballast Transilluminator ECX -15M. The results are documented using the photographing system Gel Imager-2 (Helicon, Russia).

Thus, using the electrophoretic detection, fragment with length 252 bp was determined. This corresponds to the amplified DNA segments containing the polymorphic

sequence of the gene Arg72Pro TP53.

To determine the genotype, subsequent restriction of the amplification products was done using the restriction endonuclease TP53-Arg72Pro. DNA fragment, containing the genotype Arg/Arg, was consistent with 252 bp. The fragment containing the genotype Pro/Pro, was split by the restriction endonuclease into fragments of size 228 and 24 bp. Fragments of size 252, 228, and 24 bp consistent heterozygote Arg/Pro (Fig 1).

Figure 1
Results of gel electrophoresis of digested PCR products.



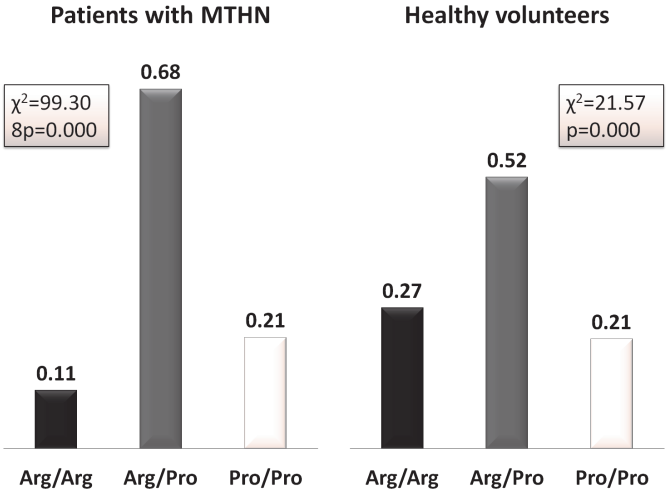
The genotype distributions in control subjects conformed to Hardy-Weinberg equilibrium. To compare the genotype frequencies, we used the standard test χ^2 Pearson. To reject the null hypothesis (no difference) the levels of statistical significance $p<0.05$ were taken. The relative risk (odd ratio, OR) of the disease at a particular genotype was calculated by the multiplicative model of inheritance. OR was specified with a 95% confidence interval.

The frequency of the distribution of the genotypes of Arg72Pro TP53 polymorphism in patients having MTHN included Arg/Arg - 0.1, Arg/Pro - 0.68, Pro/Pro - 0.20; χ^2=99.308, p=0.000 (Fig 2). In healthy volunteers the ratio of Arg/Arg: Arg/Pro: Pro/Pro was found to be 0.27:0.52:0.20; χ^2=21.57, p=0.000.

The heterozygous genotype was dominant in the patients with MTHN, as well as those in the control group. However, the findings were significantly higher in the study group, viz., 0.68 as against 0.52 in the control group (p=0.03). The homozygous genotype Arg/Arg was rarely encountered in the patients, viz., 0.11 in the study group compared with 0.27 in the control group (p=0.005). The risk of being an MTHN carrier was found to be associated with the polymorphic marker gene TP5, and particularly, with the accumulation of the Arg/Pro genotype [OR: 1.93 (95% CI: 1.05-3.55), while reducing the frequency of the Arg/Arg genotype [OR: 0.34 (95% CI: 0.15-0.73)].

During the study is not detected associations between the clinical status of the patients (dynamic indicators of the progression of tumor growth, disruption of the development of primary features and lymphogenous hematogenous metastasis and the dynamics of their changes) with the Arg72Pro TP53 polymorphism.

Figure 2
Distribution of genotypes of Arg72Pro TP53 polymorphism in patients with MTHN and in healthy volunteers



Conclusion

The results of this study suggest that patients with malignant maxillofacial tumors possess a heterogeneous polymorphism in the TP53 group providing the basis for the assumption of the presence of different mechanisms involved by the polymorphic markers in the pathogenesis of malignant maxillofacial tumors.

Low levels of "wild" genotype Arg/Arg in patient groups suggests the role of hereditary factors in the implementation of a painful program. However, recent studies on the impact of the adverse factors, (viz., smoking, alcohol, nasvay, betel, occupational exposures) on the oral mucosa, revealed their ability to disrupt the normal expression of TP53, leading to oral carcinoma. Particularly, the combination of nicotine and alcohol causes significant damage in their combined effect. The role of nicotine as a carcinogen, has long been recognized and is well known; however, it is now evident that alcohol as a co-carcinogen, greatly increases the deleterious effect of the nicotine [27-30]. Until recently, it was believed that the unique composition of saliva could protect the mouth from many diseases and including cancers; however, after exposure to nicotine, it was found that saliva itself becomes a corrosive medium, first attacking the own DNA. Tumor markers identified during the molecular genetic studies of carcinogenesis, can soon establish the metastatic behavior of cancer cells.

Unfortunately, now, the commonly accepted staging system of the disease using TNM is not in the spirit of the time. In the near future, more information is warranted for the identification of tumor cell populations, with the possible degree of metastasis. Molecular markers today can be widely used in clinical practice to identify risk groups enabling early diagnosis and prognosis of cancer. Further research in this area will facilitate the identification of a specific mapping of the clinical symptoms of the disease by linking them to changes in the chemical structure of the protein, and therefore, the ability

to quickly apply the methods of molecular gene correction to produce therapeutic effects.

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