Genetic Status of p53 in Stomach Cancer: Somatic Mutations and Polymorphism of Codon 72

V. A. Belyavskaya*, V. K. Vardosanidze, O. Yu. Smirnova*, E. I. Karakin*, I. V. Savkin*, P. A. Gervas***, N. V. Cherdyntseva***, and M. I. Voevoda*,****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 2, pp. 205-209, February, 2006
Original article submitted December 20, 2004

The incidence of somatic mutagenesis of p53 oncosuppressor protein in malignant tumors of the stomach and genetic polymorphism of p53 were studied in patients with stomach cancer on DNA samples isolated from tumor tissues obtained during surgery. The incidence of Pro/Pro genotype increased in the patients, while the percentage of Arg/Pro heterozygotes was markedly lower compared to long-living persons without cancer. The incidence of p53 somatic mutations in exons 5, 7, 8 was 70.8%; multiple mutations were detected in half of the examined patients. The relationship between the intensity of p53 mutagenesis and histological structure of the tumor was detected. The contribution of p53 genetic status to the risk of stomach cancer can be more effectively evaluated on DNA samples isolated from not only tumor cells, but also from normal tissues. The effects of epigenetic factors determining the intensity of somatic mutagenesis of p53 in tumors should be taken into account.

Key Words: *stomach cancer; p53; gene polymorphism; somatic mutations*

Stomach cancer (SC), a highly incident pathology all over the world, is a multifactorial disease; the risk of SC in genetically predisposed individuals is modulated by environmental factors. Individual predisposition to the disease can be linked with polymorphism of genes involved in carcinogenesis, regulating cell cycle and apoptosis. Oncosuppressor protein p53, encoded by p53 gene is a key regulator of apoptosis of modified cells [8]. Polymorphism of p53 gene in coding region (codon 72 of exon 4) involves 2 allele of chromosome 17: different allelotypes determine expression of protein carrying Arg (CGC) or Pro (CCC), which manifests in different functional activity of p53 at the phenotypical level [15]. Some authors reported more effective induction of apoptosis by the Fas/FasL route involving T-cell components of the immune response in patients with Pro-allele homozygotic squamous-cell carcinomas of the head and neck [9]. Cell culture studies showed higher efficiency of Arg-allelotype as apoptosis inductor, but in the presence of extra somatic mutations of p53 gene its protein product can bind p73 and subsequently block this p53 analog, thus blocking of the apoptotic pathway [13]. The sensitivity of p53 Arg-allele to degradation increases after E6 protein binding of human papilloma virus oncogene types [12]. Hence, Arg- and Pro-containing p53 proteins are characterized by different interactions with transcription activation molecules of regulatory genes and with external factors, which determines specific features of apoptosis reaction and possible effects of its allele variants on predisposition to cancer.

The data on associative role of single-nucleotide polymorphism in codon 72 of p53 gene in

*Vector Institute of Virology and Biotechnology, Koltsovo; **Institute of Therapy, Siberian Division of Russian Academy of Medical Sciences, Novosibirsk; ***Institute of Oncology, Tomsk Research Center, Siberian Division of Russian Academy of Medical Sciences. ***Address for correspondence:*** oncology@info.tsu.ru. N. V. Cherdyntseva

0007-4888/06/1412-0243

malignancy of different location, including SC, are contradictory [15], which can be due to population characteristics and effects of environmental factors and to the somatic status of p53 in the tumor. Somatic mutations in p53 gene are most incident genetic changes in tumor cells in cancer of different genesis, including SC. For malignant tumors of barrier organs (lung, stomach, and skin cancer) the incidence of somatic mutations in p53 gene and their profiles depend on exposure to external carcinogens. According to WHO classification, the components of anthropogenic origin in foodstuffs, water, air, and *Helicobacter pylori*, an important etiologic agent of SC belong to these carcinogens [7]. Exogenous carcinogens as stress factors induce transcription of p53 gene, making it the most sensitive target for somatic mutagenesis [10], and increase the risk of DNA damage by endogenous factors with high mutagenic potential produced during inflammatory response to infectious agents.

We studied the role of p53 genetic status in combination interactions between somatic mutations and polymorphism of codon 72 in the pathogenesis of SC.

MATERIALS AND METHODS

Forty-one patients (aged 39-70 years) with SC treated at Cancer Department of Municipal Hospital of Novosibirsk in 1997-1998 were examined. Samples of tumors with morphological characteristics in accordance with P. Lauren's classification [1] were provided by V. K. Vardosanidze.

Cancer is a prevalent disease shortening human life span, and hence, we examined long-living individuals without cancer for control [4]. Control group consisted of 125 long-living subjects aging 85-105 years (mean age 91.7±1.6) without cancer from the Novosibirsk and Tyumen regions.

DNA was isolated from resected tumors of patients and from peripheral blood of long-living subjects using proteinase K and phenol-chloroform extraction [3].

Sequences of p53 gene exons 5, 7, and 8 were amplified using oligonucleotide primers complementary to the terminal sequences of exons and containing additional restriction sites for XbaI and BlgII. Primer structure: for exon 5: 5'-AGTCTAG ATCTCCTTCCTCTTCCTACAG (13094-13113), 5'-AGAGATCTATCGCTATCTGAGCAGCGCTC (13276-13296); for exon 7: 5'-AGTCTAGAGGG CCTGTGTTATCTCCTAG (14039-14058), 5'-AGA GATCTGGAGTCTTCCAGTGTGAT (14146-14166); for exon 8: 5'-AGTCTAGATCTCTTTTCCTATC CTGAGT (14489-14507), 5'-AGAGATCTTCGCTT AGTGCTCCCTGGGG (14627-14644). DNA (5 μl solution, or 0.2 μg) was added to 45 μl solution containing 67 mM Tris-HCl (pH 8.8), 1.5 mM $MgCl_2$, 0.01% Twin-20, 0.2 mM each deoxynucleotide triphosphate, 2 U Taq polymerase, and 200 ng each primer. Forty amplification cycles were carried out, each cycle included annealing (1 min at 56°C), elongation (2 min at 72°C), and denaturation phases (1 min at 95°C).

PCR-SSCP (single strand conformation polymorphism) analysis was carried out as described previously [3]. Gel concentrations were modified depending on the size of the fragment: 6% for exon 5, 10% for exon 7, and 7% PAAG for exon 8. Electrophoresis was carried out in Tris-borate buffer at 12-14°C for 12-20 h at 7-9 mA current. The gel was then exposed for 10 min in ethidium bromide solution (10 μg/ml) and photographed.

Polymorphism of p53 gene codon 72 was studied by PCR-BstU1-PDRF analysis [11]. The results were statistically processed using precise Fisher's test, Mann—Whitney's test, and χ^2 test using Statistica for Windows software.

RESULTS

A significantly higher incidence of A1A1 (Pro/Pro) genotype was detected in patients with SC, while the incidence of heterozygotic variant was drastically lower than in elderly subjects without cancer (Table 1). A higher incidence of Pro allele was observed in patients with atypical SC more often than in tumors of intestinal and diffuse structure. The distribution of genotypes in long-living subjects without cancer was comparable to that in middle-aged donors, residents of West Siberia [2].

More than 80% mutations of p53 gene occur in exons 5, 7, and 8 encoding DNA-binding domain, which determined the choice of these exons for our study. The incidence and location of p53 somatic mutations in tumors of patients with SC and their clinical pathological phenotypes are presented in Table 2.

The majority of tumors (70.8%) have at least one mutation in any of exons. Mutations in only one exon were detected in 34.1% tumors, multiple mutations (in 2 and more exons) in 36.6% tumors. These data confirm high incidence of p53 somatic mutagenesis in tumors, with the maximum values in tumors of the intestinal and atypical histotypes; high incidence of multiple mutations is characteristic of these tumors. Total incidence of mutations was lower in diffuse SC and the incidence of multiple mutations was 3-fold lower than of single mutations. The incidence of single mutations did not

TABLE 1. Distribution of p53 Gene Genotypes and Alleles (Codon 72, Exon 4) in Elderly Subjects without Cancer and SC Patients

Group	Allele, %		Genotype, %		
	A1	A2	A1A1	A1A2	A2A2
Elderly donors (*n*=125)	84/250 (33.6)	166/250 (66.4)	19 (15.2)	46 (36.8)	60 (48.0)
SC patients (*n*=30)	23/60 (38.3)	37/60 (61.6)	11 (36.6) *p*1(Y)=0.0157 OR=3.23 (95% CI)	1 (3.3) *p*1(Y)=0.0434	18 (60.0)
Intestinal SC (*n*=11)	8/22 (36.3)	14/22 (63.6)	4 (36.4)	0	7 (63.4)6
Diffuse SC (*n*=10)	4/20 (20.0)	16/20 (80.0)	2 (20.0)	0	8 (80.0)
Atypical SC (*n*=9)	11/18 (61) *p*1(Y)=0.0356 *p*2(Y)=0.037	7/18 (39)	5 (55.56)	1 (11.1)	3 (33.3)

Note. A1: Pro; A2: Arg; p1, p2(Y): significance of differences with Yates' correction compared to elderly donors, intestinal SC, and diffuse SC, respectively.

depend on clinical morphological phenotype of SC. No association between p53 Pro/Arg polymorphism and the presence and type of p53 gene somatic mutations in tumor cells of patients with SC was detected.

Mutations in p53 gene exons 5, 7, and 8 are the most prevalent genetic changes in stomach tumors, with a pronounced trend to an increase in the incidence of mutations in high risk zones for the disease development [6]. Published data on the relationship between the incidence of p53 gene mutations and tumor histotype are contradictory, but regularities were detected indicating involvement of mutations in different SC histotypes in the carcinogenesis at its different stages: at the initiation stage in intestinal and at the progressive stage in diffuse SC histotype [9]. Analysis of our findings with consideration for the disease stage revealed a similar trend: the incidence of mutations in patients with stage II intestinal SC was 2-fold higher (87.5%) than in diffuse SC (46%). The incidence of multiple mutations was also appreciably higher in intestinal and atypical histotypes than in diffuse one, but the absence of statistically significant differences in the incidence of solitary mutations does not permit us draw an unambiguous conclusion about the relationship between mutagenesis intensity in SC patients and type of tumor structure.

It was hypothesized on the basis of data on multiple intensive mutagenesis of p53 in patients with lung cancer [10] that exposure to exogenous mutagens is an important factor of mutation processes induction through negative effects on the protein components of reparation system, stress activation of transcription activity of p53 gene with its transformation into actively mutating gene [10]. Intensive and multiple mutagenesis of p53 gene detected in our study in SC patients can be due to the effects of a wide spectrum of exomutagens, *H. pylori* vital activity products, among other things, as this bacterium is highly prevalent in the population of this region, and due to the effects of endogenous mutagens of inflammatory genesis, induced by damaging agents [7].

TABLE 2. Incidence of p53 Somatic Mutations in Tumors of Different Histological Structure in SC Patients (%)

Clinical pathological phenotype	Number of tumors with somatic mutations		
	one and/or more exons	one exon	2 and more exons
Total group (*n*=41)	29 (70.8)	14 (34)	15(36.6)
Intestinal SC (*n*=13)	11 (84.6)	6 (46.0)	5 (38.4)
Diffuse SC (*n*=17)	8 (47.0)*+	6 (35.3)	2 (11.8)*+
Atypical SC (*n*=11)	10 (90.9)	2(18.0)	8 (72.7)

Note. $p<0.05$ compared to: *intestinal SC, +atypical SC.

Analysis of p53 codon 72 polymorphism using DNA isolated from tumor samples showed a 2-fold increase in the incidence of Pro-allele homozygotic variant paralleled by a drastic decrease in the heterozygote incidence in the group of SC patients compared to long-living subjects. This can indicate the presence of genetic changes in tumor cell DNA involving codon 72 region and leading to loss of heterozygosity with subsequent selection of tumor cells homozygotic by Pro-allele. This phenomenon of heterozygote decrease by this polymorphism was detected in studies of tumors from patients with breast, lung, and bladder cancer [5], which can indicate common mechanisms of carcinogenesis for heterozygote carriers irrespective of tumor type and location. On the other hand, selection of tumor cells towards the Pro-allele homozygosity indicates specific features in the pathogenesis of SC. It seems to contradict the data indicating that Pro-allele more effectively induces FasL/Fas-mediated apoptosis of tumor cells by cytotoxic T-lymphocytes [11], but the positive role of Pro-allele does not manifest under conditions of impaired apoptotic signaling in tumor cells because of mutations in p53 gene. In this case the cell not only fails to undergo apoptosis, but it can "readdress" the apoptosis-inducing signal to cytotoxic T-lymphocyte contacting with it, thus causing its death. The "risk significance" of Pro-homozygosis in combination with somatic mutagenesis of p53 detected in our study can indicate that SC develops according to this scheme in these homozygote carriers. Presumably, p53 heterozygosis is a defense factor, because the number of heterozygotes in the group of SC patients was 11-fold lower than in the control, which is in line with the findings in lung, ovarian, renal, and bladder cancer [14].

Thus, the data on high incidence and multiple mutagenesis of p53 gene and involvement of homozygotic Pro variant in the absence of heterozygotes in SC patients indicate that SC emerges in the Novosibirsk region under conditions of pleiotropic exposure to many exogenous factors and its development is mediated by different molecular mechanisms because of functional difference in polymorphic p53 variants. Presumably, coordinated functioning of both p53 allelotypes ensures more effective protection from combined effects of transforming signals on the epithelium of the gastric mucosa. Further studies with the analysis of genetic identity of DNA isolated from normal and tumor cells of the same patients are needed for more complete understanding of the role of genetic status in the process of tumor emergence and progress in SC.

The authors are grateful to O. N. Kungurtsev and N. A. Smetannikov for assistance in the study and preparation of the paper.

REFERENCES

1. V. I. Chissov and S. L. Dar'yalova, *Selected Lectures in Clinical Oncology* [in Russian], Moscow (2000).
2. V. V. Lyakhovich, V. A. Vavilin, N. I. Gutkina, *et al.*, *Vopr. Med. Khim.*, No. 5, 330-338 (1997).
3. S. Harrington and J. Macgui, *Molecular Clinical Diagnosis. Methods* [in Russian], Moscow (1999).
4. N. A. Smetannikova, M. A. Smetannikova, V. A. Belyavskaya, *et al.*, *Sibirsk. Onkol. Zh.*, Nos. 2-3, 124-128 (2004).
5. M. Bonafe, C. Ceccarelli, F. Farabegoli, *et al.*, *Clin. Cancer Res.*, **9**, No. 13, 4860-4864 (2003).
6. J. Breton, F. Sichel, A. Abbas, *et al.*, *Mutagenesis*, **18**, No. 3, 299-306 (2003).
7. Paula P. Magalhaes, *Carcinogenesis*, **24**, 145 (2003).
8. C. Prives, *Cell*, **78**, No. 4, 543-546 (1994).
9. G. N. Ranzani, O. Luinetti, L. S. Paolovan, *et al.*, *Cancer Epidemiol. Biomarkers Prev.*, **4**, No. 3, 223-231 (1995).
10. S. N. Rodin and A. S. Rodin, *Proc. Natl. Acad. Sci. USA*, **95**, 11,927-11,932 (2001).
11. R. Schneider-Stock, *Am. J. Pathol.*, **164**, No. 4, 1233-1241 (2004).
12. A. Storey, M. Thomas, A. Kalita, *et al.*, *Nature*, **393**, 229-234 (1998).
13. M. Thomas and A. Kalita, *Mol. Cell. Biol.*, **19**, 1092-110 (1999).
14. Y. C. Wang, C. Y. Chen, S. K. Chen, *et al.*, *Clin. Cancer Res.*, **5**, No. 1, 129-134 (1999).
15. Z.-W. Zhang, N. J. Laurence, A. Hollowood, *et al.*, *Clin. Cancer Res.*, **10**, No. 1, Pt. 1, 131-135 (2004).