Journal of Korean Medical Science
Vol. 9, No. 4, 299-303 AUG. 1994

p53 Protein Overexpression and Allele Loss of p53 Gene in Gastric Adenocarcinoma

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Gene alterations of p53 tumor suppressor gene such as point mutations, deletions or insertions occur in various human cancers. p53 protein overexpression was studied immunohistochemically in 80 gastric adenocarcinomas using an anti-human p53 antibody (Pab 1801) and the avidin-biotin-peroxidase technique. We have also analyzed allele loss of the human p53 gene in 54 cases of gastric adenocarcinoma using polymerase chain reaction and restriction fragment length polymorphism. p53 immunostaining was also demonstrated in 48 of 80 carcinomas (60%).
Normal mucosa was always negative. No relation could be found between p53 immunostaining and the degree of differentiation. 21 of the 54 patients(39%) were informative for the p53 exon 4. In ten of these informative cases(47.6%), tumor DNAs showed allele loss when compared with nonmetastatic lymph node DNAs. Seven of the ten(70%) showed p53 immunoreactivity. These findings suggest that mutations of the p53 gene may play a role in the development of gastric adenocarcinoma and that allele loss of p53 frequently occurs in p53 immunoreactive gastric adenocarcinoma

Key Words : *Gastric adenocarcinoma, p53 gene, LOH, Immunohistochemistry.*

INTRODUCTION

The cellular p53 gene was so named because of its original identification as an overexpressed 53 kilodalton protein in malignantly transformed cell lines(Crawford et al., 1981). During its early years of study, it was classified as an oncogene because of its ability to transform cells(Eliyahu et al., 1984). However, in 1989 the Vogelstein group showed that p53 was actually a tumor suppressor gene(Nigro et al., 1989). The wild-type gene was capable of arresting growth in transfection experiments(Baker et al., 1990) or in some cases, send a cell into a programmed spiral of death(Culotta and Loshland, 1993). Mutant forms not only deprive cells of the wild type's beneficial effects but can spur abnormal cell growth(Culotta and Loshland, 1993). This mechanism enabled mutant p53 to transform cells. In colon cancer, one copy of p53 was inactivated by point mutation ; the other copy was removed by allelic deletion(Baker et al., 1990). These findings exactly matched the two-hit model proposed by Knudson(1971) and observed with the retinoblastoma gene, establishing p53 as a true tumor suppressor gene. Mutation and allele loss of the p53 gene

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This work was supported in part by St. Paul's Hospital clinical research funds.

have been associated with tumors from a wide variety of human organs and hematopoietic tissue(Hollstein et al., 1991 ; Nigro et al., 1989). The spectrum of mutations in p53 induced in variable human cancer can identify particular carcinogens and define the biochemical mechanisms responsible for the genetic lesions in DNA that cause human cancer (Harris, 1993).

In Korea, gastric adenocarcinoma is a common malignant neoplasm and it accounts for 28.2 percent of male cancer and 17.5 percent of female cancer according to the report of the Ministry of Health and Social Affairs of Korea(Ministry of Health and Social Affairs, 1989).

Understanding the mechanism by which p53 mutations lead to gastric tumorigenesis has substantial practical and theoretical importance. Therefore, we made a immunohistochemical study and examined the frequency of allele loss of p53 using polymerase chain reaction(PCR) and restriction fragment length polymorphism(RFLP).

PATIENTS AND METHOD

Immunohistochemistry for p53

We carried out a complete one-hour immunohistochemical study(Reed et al., 1992) to evaluate p53 overexpression. Sections from paraffin-embedded material were immunostained using an anti-human p53 antibody detecting both wild-type and mutant p53(PAb 1801-PharMingen, San Diego, CA). Incubation with the primary antibody was performed at a dilution of 1 : 100(1 ug IgG/ml) for 20min at 50℃. The avidin-biotin-peroxidase complex method was used (Hsu et al., 1981). The streptavidin-horseradish peroxidase (Research Genetics, Huntsville, AL) or strepavidin-alkaline phosphatase (Research Genetics, Huntsville, AL) detection system was then applied to all capillary channels, followed by a 10-min incubation at 50℃. After drainage, the tissue sections were ready for the chromogen reaction with stable DAB (Research Genetics, Huntsville, AL). Positive and negative(incubation with mouse myeloma protein-IgG 1ug/ml) controls were also included.

Allele loss of p53

1) DNA extraction

We also analyzed allele loss of p53 exon 4 in 54 cases where it was possible to extract chromosomal DNAs in tumor and non-metastatic lymph nodes. All of them were surgically resected specimens. Paraffin-embedded blocks of each tissue sample were microdissected and stained with hematoxylin-eosin ; subsequent sections were then cut from the properly faced block, and the circumscribed tumor areas and the surrounding nonneoplastic mucosae were separated by light microscope for scraping of the slides. Chromosomal DNA was extracted from the selected areas of unstained deparaffinized sections. Scraped paraffin sections were incubated overnight at 52℃ in the lytic solution(10 mM Tric-HCl, 1 mM EDTA, 1.25 ug/ul of proteninase K, 0.15% of NP-40 and 0.15% of tween 20) by using a modification of the method of Goelz(Goelz et al., 1985). DNAs were successively extracted by phenol-chloroform-isoamylalcohol method and ethanol precipitation. DNA concentration was determined by measuring the optical density at 260 nm and adjusted at 200-500ng/ul. Purity was assayed by ratio of optical density at 260 nm and 280 nm.

2) PCR and restriction endonuclease treatment

A BstU I polymorphic site in p53 exon 4 has been previously reported(Meltzer et al., 1991). For p53 exon 4, some studies in which primers were used for the PCR amplification were published-(Greenwald et al., 1992). The upstream primer was 5'-CAGATGAAGCTCCCAGAA-3' ; the downstream primer was 5'-GTGTAGGAGCTGCTGGTG-3'. Each PCR amplication was carried out using 2 ul of genomic DNA(about 200-500 ng) in a final volume of 20 ul, which was mixed with 20 pmol of each primer, 2 ul of 10x reaction buffer, 2 ul of 1.25 mM deoxynucleotide triphosphate, 0.5 unit of Taq DNA polymerase and distilled water. Amplification was reached to plateau phase after 35 cycles with a Thermocycler(Ericomp, San Diego, California) at 95℃ for 1 min, 58℃ for 1 min and 72℃ for 2 min. Then, the entire PCR reaction product of p53 was digested with 8 units of BstU I restriction endonuclease at 59℃ for 3 h. The digestion products were electrophoresed on a 8℃ nondenaturing polyacrylamide gel at 100 V for 1 hour. Controls for complete digestion were also performed.

RESULTS

Immunohistochemistry of p53 in gastric adenocarcinoma

In immunohistochemical reactivity using PAb

Table 1. Results of immunoreactivity analysis and the degree of differentiation in gastric adenocarcinomas

	+	−	total
WD	9(56.3%)	7(43.7%)	16
MD	22(64.7%)	12(35.3%)	34
PD	17(56.7%)	13(43.3%)	30
total	48(60%)	32(40%)	80

WD : well differentiated, MD : moderately differentiated, PD : poorly differentiated adenocarcinoma.

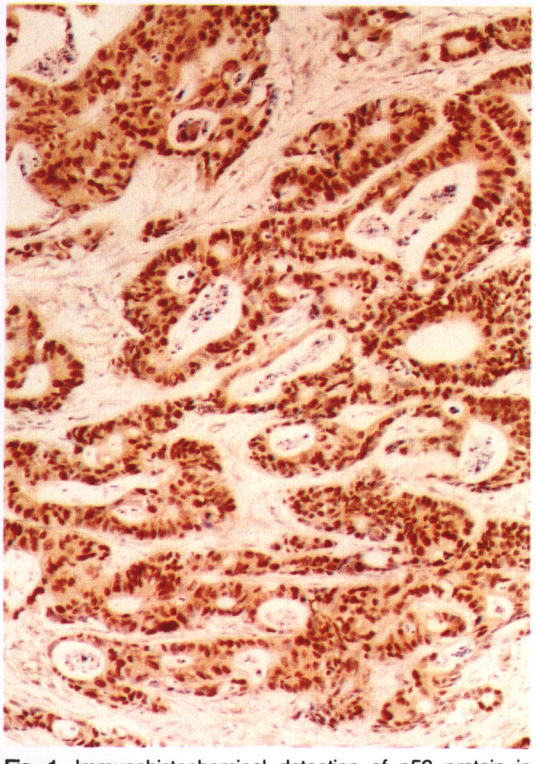

Fig. 1. Immunohistochemical detection of p53 protein in paraffin section of gastric adenocarcinoma with anti-p53 antibody(Pab 1801) and avidin-biotin complex detection(× 400).

1801, an intense immunostaining was observed in 48(60%) out of 80 gastric adenocarcinomas(Table 1). The distribution of staining was predominantly nuclear, although some cells showed slight positivity in cytoplasm(Fig. 1). No reactivity was seen in normal gastric mucosae and stromal cells. In immunoreactive cases, well, moderately, and poorly differentiated adenocarcinoma were 9(56.3%), 22(64.7%), and 17(56.7%), respectively(Table 1).

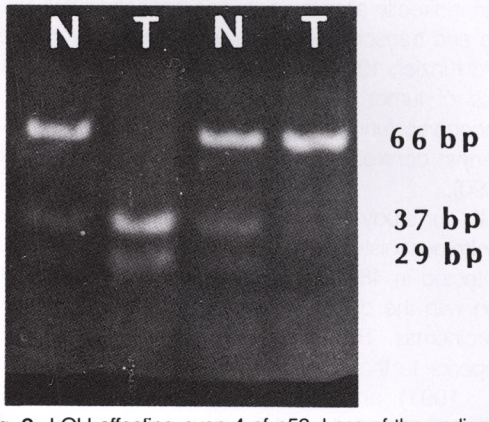

Fig. 2. LOH affecting exon 4 of p53. Loss of the undigested allele(left) and digested allele(right) are seen in DNAs extracted from gastric adenocarcinomas(T : adenocarcinoma, N : non-metastatic lymph node).

Allele loss of p53 exon 4

At the p53 exon 4 locus, 21(39%) out of 54 individuals assayed were heterozygous(informative). And 26(46%) out of the 54 were homozygote containing the restriction site, while 7(13%) patients were lacking the restriction site. Allele loss of p53 was demonstrated when either the uncut or the cut band was lost in heterozygous patients (Fig. 2). Allele loss was judged by 3 persons, and the cases in which allele loss was ambiguous were excluded. 10(47.6%) out of the 21 heterozygous patients demonstrated allele loss of p53 in the carcinoma tissue. In the subset of 21 informative patients in our series, four patients showed neither immunohistochemical evidence of p53 nor allele loss.

DISCUSSION

Discovering the mechanism of specific chromosomal regions has been an important step in the

understanding of various human malignancies. Mutations in p53 can reveal that an individual has an increased susceptibility to cancer owing to inheritance of a germline mutation, a concept first proposed for the Rb tumor suppressor gene(Knudson, 1971). In p53, more than 90 percent of the mutations are missense mutations that change the identifying characteristics of amino acid. This phenomenon can alter the conformation and increase the stability of the p53 protein ; these mutations also can indirectly alter the sequence-specific DNA binding and transcription factor activity of p53(Vogelstein and Kinzler, 1992). p53 mutations can cause both a loss of tumor suppressor function and a gain of oncogenic function by alteration of the repertoire of genes controlled by p53(Lane and Benchimol, 1990).

In our study analyzing the overexpression of p53 by immunohistochemistry, p53 immunoreactivity was detected in 48 of 80(60%) cases and had no relation with the degree of differentiation of the adenocarcinomas. This prevalence of p53 expression is superior to that in the report by Tamura(Tamura et al., 1991) and Seruca (Seruca et al., 1992). Although it was impossible to verify whether the p53 that was immunohistochemically detected in our study is a wild or mutant form, immunoreactive p53 may be a stable mutated protein. According to the report of Rodrigues(Rodrigues et al., 1990), when p53 is detected immunohistochemically in human colorectal cancer cell lines, it is actually a mutated form. This finding suggests that the mutation of the p53 gene may provide the cells with a certain growth advantage allowing their clonal expansion and that may be closely associated with the malignant transformation of gastric mucosa, but not the degree of differentiation of carcinomas.

We also observed a relation between the expression of p53 and the allele loss of p53. 10 out of 21 informative cases(47.6%) showed allele loss and 7 of these 10 cases(70%) demonstrated overexpression of p53. The frequency of allele loss of p53 is relatively high(47.6%) and superior to that in the report of Seruca (Seruca et al., 1992) and similar to a report which analyzed the esophagus(Rodrigues et al., 1990). This data suggests that allele loss frequently occurs in immunoreactive carcinomas and that the remaining allele may have another mutation.

Investigations of the p53 tumor suppressor gene are an example of the recent progress in molecular aspects of cancer research. And p53 may help suppress the uncontrolled growth that can lead to a tumor by putting the brakes on cell growth and division, pushing cells into a programmed death, and preventing the unruly amplification of DNA. Therefore, determination of the type and number of mutations of p53 in gastric adenocarcinomas may allow earlier detection of those at increased susceptibility to gastric adenocarcinoma and improve therapeutic methods for cancers.

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