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Over-Expression of p53 Protein in Squamous Cell Carcinoma of the Skin

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p53 gene mutations have been known to be highly related to the particular stage of transformation in various types of human cancers. This study was conducted to investigate the p53 mutations at the protein level by an immunohistochemical method using anti-p53 antibody, NCL-p53-DO-7. Twenty-five cancer specimens were obtained surgically from patients with squamous cell cancer of the skin at the Korea Cancer Center Hospital. The cancers were classified according to the possible etiology into two groups, burn scar originated and UV-related cancers. Overexpression of p53 protein was detected in ten (40%) out of 25 cases tested : six (40%) of 15 cases associated with burn scar and four (40%) of ten cases related to UV exposure. In all normal skin cells in specimens, p53 protein was not stained at all. The stages and histological grades were evaluated for their relationship with the overexpression of p53 protein. No significant difference was found between the overexpression of p53 protein and the stages or histological grades. These results demonstrating that 40% of skin cancers were positive for p53 overexpression suggest that the alterations of the p53 gene may play a role and the exact role of p53 gene in the development of squamous cell carcimoma of the skin will be studied.

Key Words : *p53 overexpression, Squamous cell carcinoma, Immunohistochemical detection.*

INTRODUCTION

The accumulating data on the mutations of the p53 gene reveal that the p53 gene is one of the most frequently involved in the genesis of various types of human cancers. Frequent mutations on the p53 gene have been reported in colon, lung, breast, stomach, ovary, brain cancer, and melanoma (Baker et al., 1989 ; Nigro et al., 1989 ; Takahashi et al., 1989 ; Iggo et al., 1990 ; Mashiyama et al., 1991 ; Tamura et al., 1991 ; McGregor et al., 1993 ; Milner et al., 1993 ; Wagat et al., 1993 ; Hong et al., 1994 ; Rhim et al., 1994).

In squamous cell cancer of the skin in Korea, however, only one datum (Ro et al., 1993) on p53 mutations is available at present, although more data have been reported in other countries (Brash et al., 1991 ; Moles et al., 1993).

The product of the p53 gene, located on the

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short arm of chromosome 17, band 13 (McBride et al.1986), is a protein containing 393 amino acids. p53 protein is reported to control the growth and differentiation of cells by regulating DNA replication and RNA transcription, although the precise function of the p53 gene in normal cells is not yet clearly delineated (Eliyahu et al., 1985 ; Fields and Jang, 1990 ; Bargonetti et al., 1991).

The mutant proteins produced by mutant p53 gene have been known to lose their ability to suppress the transformation induced by certain oncogenes, such as ras or myc, which means that the mutation of the p53 gene plays an important role in the carcinogenesis of human cancers (Eliyahu et al., 1985 ; Hinds et al., 1989). Thus, recently, correction of p53 mutation is one of the targets for genetherapy trials using wild type p53 gene transfection (Asai et al., 1994).

Recently, it has been suggested that immunohistochemical detection of p53 protein corresponds to detection of a mutated, but not wild type form of the protein (Baker et al., 1989 ; Iggo et al., 1990 ; Pavelic et al., 1992 ; Takashi et al., 1993 ; Hong et al., 1994). In this study, the mutation of the p53 gene product was studied at the protein level using an immunohistochemical method with anti-p53 antibody in 25 cancer specimens obtained from patients with squamous cell carcimoma. We have also analyzed the overexpression of p53 protein in relation to stages and histological grades.

MATERIALS & METHODS

Tumor specimens

Twenty-five cancer specimens were obtained surgically from patients with squamous cell cancer of the skin at the Korea Cancer Center Hospital. The patients consisted of 16 males and nine females with a median age of 46 years (range, 26-75). The specimens obtained were classified as burn scar originated or ultraviolet (UV)-related cancer. Of the 25 specimens, 15 were obtained from burn scar sites and ten were from sites strongly exposed to sunlight. Histological diagnosis and grading were made after staining the tissues with hematoxylin-eosin. Pathological staging was performed according to the TNM staging system (Beahrs et al., 1992).

Immunohistochemical detection

The sections of squamous cell carcinoma tissue, fixed in 10% phosphate buffered formalin and embedded in paraffin, were processed for the detection of p53 protein by immunohistochemical method using mouse monoclonal anti-p53 antibody, NCL-p53-DO-7 (DO-7, Novocastra Lab. Ltd., UK) and avidin-biotin peroxidase technique (Vector Lab. Burlingame CA, USA). The details have been described previously (Hong et al. 1994). In brief, sections deparaffinized on the silane-coated glass slides were heated in a water bath containing target unmasking fluid (TUF) solution (Kreatech Biotechnology B.V., Netherlands) at 90℃ for 10 min. Then, the slides were treated with 3% hydrogen peroxide to quench the endogeneous peroxidase activity, 3% normal goat serum for 15 min to block the nonspecific binding and DO-7 at a dilution of 1 : 100 for 2 hr at room temperature. DAB was used as a substrate for the immunohistochemical staining using the strep-ABC method(van den Berg et al., 1993). For negative controls, phosphate buffered saline was used.

The immunoreactivity was graded as low, intermediate or high according to the intensity of staining and percentage of positive cells. The intensity was scored into three levels : 1, weakly staining ; 2, moderately staining ; 3, strongly staining. Percentage of staining was assessed by the proportion of positively stained cells and classified into

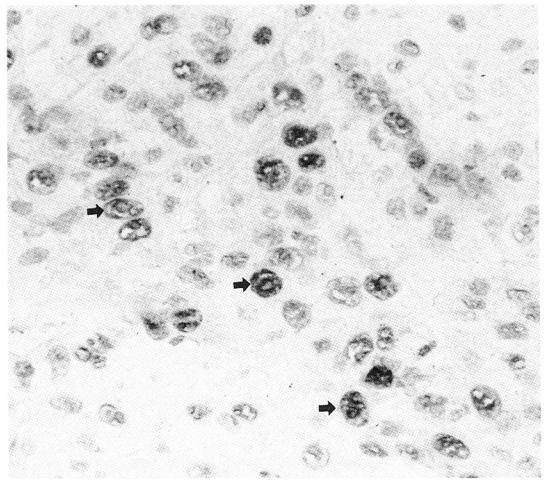

Fig. 1. Immunohistochemical staining for p53 protein in a formalin-fixed, paraffin-embedded section of squamous cell cancer of the human skin. Immunostaining is observed in the nucleus of the cancer cells(arrows).

three levels : 1, less than 10% of cells were stained ; 2, 10-50% of cells were stained ; 3, more than 50% of cells were stained. Overall evaluation of the detection of p53 mutant cells was determined by adding the intensity level to the staining level. The immunodetection for p53 mutation was expressed as low, intermediate or high : low if the sum of the levels was 1-2, intermediate if 3-4, and high if 5-6. In order to obtain objective results, two independent pathologists made the grading.

Statistical analysis

Statistical significance was evaluated by means of the chi-square test, with $p<0.05$ as the criterion of statistical significance.

RESULTS

The results of p53 mutant cells detected immunohistochemically and clinical characteristics are listed in Table 1. The overexpression of p53 protein was observed in ten (40%) out of 25 cases of squamous cell carcinoma. The cancers were classified according to the possible etiology into two groups, burn scar originated and UV-related cancers. In the 15 cases where the cancers originated from burn scar sites, six (40%) were positive. In the ten cases, suspected to be related to UV exposure, four (40%) were positive. In all normal skin cells of the specimens used in this study, p53 protein was not stained at all (data not shown). The stages and histological grade were evaluated for their relationship with the overexpression of p53 protein. No significant relationship was found between the overexpression of p53 protein and the stages or histological grades.

DISCUSSION

The p53 gene, initially reported to be a dominant

Table 1. Overexpression of p53 proteins and histological grade in 25 squamous cell cancers of human skin

Patient No.	Age/ Sex	Etiology	Stage	Histological grade	Immunodetection of p53 proteins
1	39/M	burn	II	G1	low
2	43/F	burn	IV	G2	low
3	41/M	burn	II	G1	intermediate
4	73/F	burn	II	G2	intermediate
5	29/M	burn	III	G2	high
6	38/M	burn	III	G2	high
7	64/M	burn	II	G1	—
8	58/F	burn	II	G1	—
9	30/M	burn	II	G2	—
10	41/M	burn	II	G1	—
11	56/F	burn	II	G1	—
12	75/M	burn	II	G1	—
13	26/M	burn	III	G1	—
14	45/M	burn	III	G1	—
15	42/M	burn	IV	G2	—
16	49/F	UV	I	G3	low
17	64/F	UV	III	G3	low
18	58/M	UV	II	G2	intermediate
19	31/M	UV	II	G2	high
20	60/M	UV	II	G2	—
21	72/F	UV	II	G2	—
22	31/F	UV	III	G1	—
23	46/M	UV	III	G3	—
24	68/M	UV	III	G2	—
25	57/M	UV	IV	G2	—

The immunodetection of p53 proteins was defined as low, intermediate or high according to the intensity of staining and the percentage of positive cells, as described in Materials and Methods.

Table 2. Immunodetection of p53 proteins in 25 squamous cell carcinomas of human skin

Etiology of cancer	Immunodetection of p53 proteins			
	total cases	Low	Intermediate	High
UV-related	4/10(40%)*	2/10(20%)	1/10(10%)	1/10(10%)
Burn-related	6/15(40%)	2/15(13%)	2/15(13%)	2/15(13%)
Total	10/25(40%)	4/25(16%)	3/25(12%)	3/25(12%)

The immunodetection of p53 proteins defined as low, intermediate or high according to the intensity of staining and the percentage of positive cells, as described in Materials and Methods. *, No. positive/no. total cases(%)

Table 3. Overexpression of p53 proteins and pathologic grading in 25 squamous cell carcinomas of human skin

Pathologic grading	Immunodetection of p53 proteins		Total
	Positive(%)	Negative(%)	
G1	2(20%)	8(80%)	10
G2	6(50%)	6(50%)	12
G3	2(67%)	1(33%)	3
Total	10(40%)	15(60%)	25

oncogene, is now proven to be a suppressor gene, those typical alterations are characterized by the loss of heterozygosity. The typical type of p53 aberration was reported to occur in both alleles, loss of one allele and mutation in the remaining allele. Approximately 80% of p53 alterations are single-base substitutions and 90% of them are missense mutations(Baker et al., 1989 ; Nigro et al., 1989).

A number of previous reports have demonstrated that p53 mutation is evidently related to particular stages in the multistep carcinogenic process in a variety of types of human cancers, although the mutation rates are shown in a wide range according to the cells transformed. The proteins produced by mutant p53 gene have been reported to transform the cells activated by other oncogenes by losing their ability to suppress the transformation(Eliyahu et al., 1985 ; Hinds et al., 1989).

Anti-p53 monoclonal antibody, DO-7, used in this study, is able to stain both normal and altered p53 gene products. Therefore, the immunodetection of p53 protein depends upon the amount of p53 proteins in cells. The p53 protein encoded by normal p53 gene has a short half life, approximately 5-30 min, and consequently rapid disappearance, resulting in levels below detection by the staining. In contrast to normal p53 protein, altered p53 proteins are relatively stable by binding to certain intracelluar proteins related to hsp70 or adenovirus protein E1b, not by the increased synthesis (Zantema et al., 1985 ; Finlay et al., 1988 ; Wang el al., 1989 ; Iggo et al., 1990). Accordingly, cells with altered p53 gene are exclusively stained by the immunohistochemical method with DO-7, because of the high level of p53 protein in cells with mutant p53 gene (Baker et al., 1989 ; Iggo et al., 1990 ; Pavelic et al., 1992 ; Takashi et al., 1993).

In previous reports, the treatment of the paraffin-embedded sections with TUF solution at 90℃ may have resulted in an increase of frequency of binding with anti-p53 antibodies (van den Berg et al., 1993). We have compared the antibody bindings in paraffin embedded cancer specimens treated with or without TUF solution. With treatment with TUF solution, the positive rate and stain intensity considerably increased but normal cells were not stained despite the treatment with TUF solution. Therefore, we treated the specimens with TUF solution in this study.

In this study, we have demonstrated that the incidence of overexpression of p53 protein was 40% in 25 squamous cell carcinomas of the skin. The incidence of overexpression of p53 protein is similar to the previous data reported by Brash et al. (1991), McGregor et al. (1993), and Ro et al. (1993). However, in contrast to our results, about 15% of p53 mutations were reported by Pierceall et al. (1991), and Moles et al. (1993) . The exact mechanisms of difference in the frequency of p53 mutations

are uncertain, but they may be explained by the size of the specimens and the heterogeneity of the cancer cells.

Recently, p53 protein has been suggested to participate in the inhibition of replicative DNA synthesis which follows DNA damage, such as UV-induced damage (Maltzman et al., 1984). The reasons for the enhanced response to the DNA damage may be due to the replicative arrest for the optimal repair of the damaged DNAs and the prevention of propagation of potentially harmful mutations. The loss of the function of the p53 gene by mutation is thought to be highly associated with the transformation of cells activated by some oncogenes due to the failure of cancer cells to enter the arrest phase. The double base substitutions of CC to TT are known to be pathognomonic forms of the UV-induced mutations and to be found uniquely in squamous cell carcinoma of the skin (Brash et al., 1991). These results give us evidence for the possible role of UV-exposure in the direct mutation of the p53 gene, which is also suggested by the epidemiologic data. In this study, the same incidence, 40%, of p53 gene mutation was observed in the UV-related group and in the non-UV-related group. Whether the absence of difference in p53 gene mutation between the UV-related group and the non-UV-related group is true or due to other factors, such as the small size of specimens is a subject which remains to be determined. We have also compared the incidence of p53 mutations with the stages and histological grades of cancer cells, no significant relationship being observed between them. Our data also showed the tendency to a higher positive rate in poorly differentiated tumors, (G1, 20% ; G2, 50% ; G3, 67%), however, the difference was not statistically significant. These results demonstrating that 40% of p53 mutant proteins were detected in squamous cell cancer of the skin enable us to understand the carcinogenesis process more precisely.

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