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Aberrant Expression of p53 Gene Product in Malignant Melanoma

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According to the current concept of carcinogenesis, the alterations of p53 tumor suppressor gene have been the most frequently detected in both human cancer cell lines and cancer tissues freshly isolated. This study was conducted to investigate the p53 gene alteration in malignant melanoma. Nineteen tumor tissues were obtained from 19 patients with malignant melanoma and examined for the expression of p53 protein by immunohistochemical staining with mouse monoclonal anti-p53 antibody, NCL-p53-DO-7. Twelve out of 19 cases (63%) showed positive reactions for p53 protein : 26, 21 and 16% of which had low, intermediate and high reactivity, respectively. p53 alteration more frequently expressed in female (10/12) than male patients (2/7) with malignant melanoma($p<0.05$). The incidence of expression of p53 protein was compared according to the stages and the sites of tissue obtained. The positive rate for p53 protein was not significantly different between the stages. The positive rates for p53 protein were five out of five (100%), one out of two (50%) and six out of twelve (50%) in tissues obtained from the metastatic, lymph node, and primary sites, respectively. The difference in the positive rates, however, is not statistically significant. These results suggest that p53 gene is a frequent target for mutation in the development of malignant melanoma.

Key Words : *Malignant melanoma, p53 protein, Immunohistochemical staining.*

INTRODUCTION

Since genetic alterations were reported to be involved in the development and progress of various types of cancers, many attempts have been made to get the information on the incidence and role of the multistep genetic events in the genesis and progression of cancer. A series of previous experiments has revealed that the accumulation of abnormal oncogenes and tumor suppressor genes is clearly related to certain steps on the transformation to malignant cells. Recently, it was reported that alterations of the p53 gene are the most common genetic events related to carcinogenesis in a variety of organs, particularly the colon, lung, breast, stomach, ovary and brain, etc. (Iggo et al., 1990 ; Hong et al., 1994 ; Nigro et al., 1989 ; Baker et al., 1989).

Concerning malignant melanoma, however, only a

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few data on p53 alterations are available, because melanoma is not a common cancer in humans (Ro et al., 1993 ; McGregor et al., 1993 ; Stretch et al., 1991). Although malignant melanoma is an uncommonly developed malignancy, melanoma may serve as a useful model to investigate the role of p53 gene alteration in multistep carcinogenesis, because melanoma may be transformed from benign nevus and dysplastic nevus (Elder et al., 1980). In previous reports, p53 alteration was not found in the above mentioned preneoplastic lesions of melanoma, while it was not infrequently detected in malignant melanoma tissues (McGregor et al., 1993 ; Stretch et al., 1991). In this study, we examined the expression of altered p53 gene at the protein level by immunohistochemical staining with anti-p53 antibody in malignant melanoma tissues. We have also analyzed the expression of p53 protein by the stages and the sites of tissues obtained ; primary, lymph node or metastatic site.

MATERIALS AND METHODS

Tumor Specimens

Nineteen tumor specimens were obtained from 19 patients with malignant melanoma surgically treated at the Korea Cancer Center Hospital. The patients consisted of seven males and 12 females with a median age of 57 years (range, 38-83). Of the 19 specimens, 12 were obtained from the primary site, two were from lymph nodes and five were from the metastatic site other than lymph node. Histological diagnosis was made after staining of the formalin-fixed, paraffin-embedded sections with hematoxylin-eosin. Pathological staging was performed according to the TNM staging system approved by the American Joint Committee on Cancer (Beahrs et al., 1992).

Immunohistochemical Staining

Formalin-fixed, paraffin sections were stained immunohistochemically with mouse monoclonal anti-p53 antibody, NCL-p53-DO-7 (DO-7, Novocastra Lab. Ltd., UK) by avidin-biotin peroxidase method (Vector Lab. Burlingame CA), as described previously. 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. After quenching the endogenous peroxidase activity by adding 3% hydrogen peroxide, the slides were treated with 3% normal goat serum for 15 min to block the nonspecific binding. The slides were incubated with DO-7 at a dilution of 1 : 100 in a moisture chamber for 2 hr at room temperature. DAB was used as a substrate to determine the antibody binding in the immunohistochemical staining by the strep-ABC method (van den Berg et al., 1993). For negative controls, phosphate buffered saline was used. Brown nuclear staining was considered to be positive. In immunohistochemical staining, p53 protein in normal cells is not detectable, because its level is very low due to its short half-life and rapid disappearance. Therefore, the stained cells are judged to have the proteins of the altered p53 gene, because the proteins produced by altered p53 remain for a long period by the binding with other proteins (Finlay et al., 1988).

The immunoreactivity was graded as low, intermediate and high according to the intensity of staining and the percentage of positive cells. The intensity was scored into three levels : 1, weak staining ; 2, moderate staining ; 3, strong staining. Percentage of the staining was assessed by the proportion of positively stained cells and classified into 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 immunoreactivity was determined by adding the score of both intensity level and staining level. The immunoreactivity was expressed as low, intermediate, and high : low if the sum of the levels was 1-2, intermediate if 3-4, and high if 5-6. According to these grading criteria, two independent pathologists made the grading in order to obtain objective results.

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 immunohistochemical staining for p53 proteins was performed in 19 malignant melanoma tissues using anti-p53 antibody, DO-7 (Table 1). Twelve out of 19 cases (63%) showed positive reactions for the p53 protein : 26, 21 and 16% had low, intermediate and high immunoreactivity, re-

Table 1. Immunohistochemical Analysis for p53 proteins in 19 malignant melanomas

Patients	Stage	Age/Sex	Site of Tissues	Immunoreactivity for p53 protein
1	I	75/F	primary	low
2	I	45/F	primary	low
3	II	60/M	primary	—
5	II	59/F	primary	—
4	II	59/F	primary	low
6	III	60/M	primary	—
7	III	48/M	primary	—
8	III	51/M	primary	—
9	III	58/M	primary	—
10	III	83/M	primary	intermediate
11	III	57/F	primary	high
12	III	74/F	primary	high
13	III	52/F	lymphnode	—
14	III	45/F	lymphnode	high
15	III	46/F	metastatic	intermediate
16	III	52/F	metastatic	intermediate
17	III	38/F	metastatic	intermediate
18	IV	60/M	metastatic	low
19	IV	38/F	metastatic	low

Immunoreactivity was defined as low, intermediate and high according to the intensity of staining and the percentage of positive cells, as described in Materials and Methods.

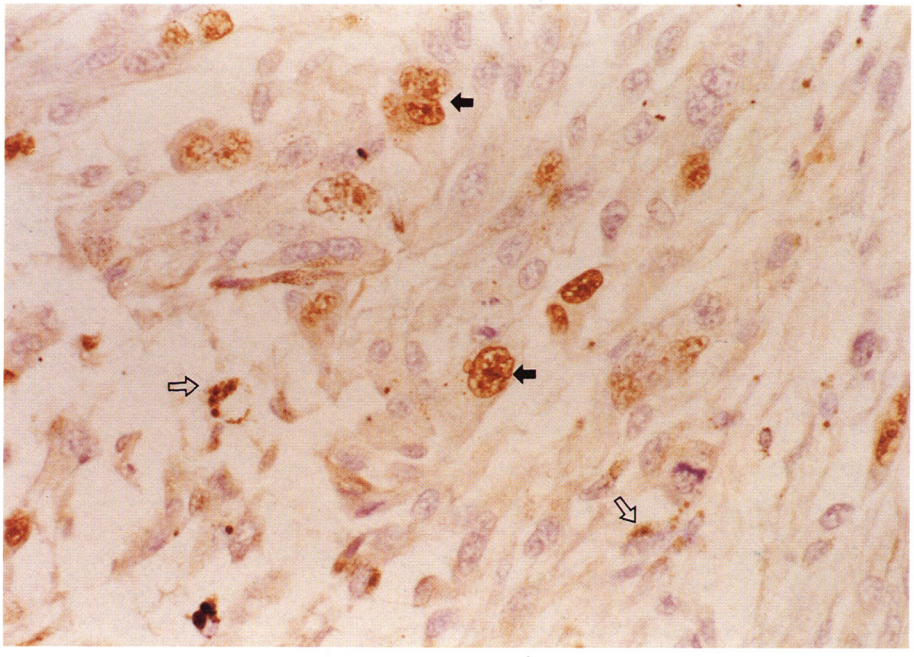

Fig. 1. Immunohistochemical staining for p53 protein in a formalin-fixed, paraffin-embedded section of malignant melanoma. Immunoreactivity is localized in the nucleus of the tumor cells(solid arrows). Melanin pigment is localized in the cytoplasm(open arrows).

spectively (Fig. 1). The incidence of expression of p53 protein was compared with the stages and the sites of tissue obtained, primary or metastatic site. The positive rate for p53 protein was not significantly different between the stages(Table 2). Of the five tissues obtained from metastatic sites, all(100%) were positive for p53 protein, which tended to be higher than that of the primary site (50%) or lymph node (50%), however, the difference was not statistically significant(Table 3). The positive rate for p53 protein was significantly higher in female patients(10/12) than in male (2/7)(Table 4).

Table 2. Relationship between stages and p53 Expression in Malignant Melanoma

stage	Immunoreactivity for p53 protein positive/total(%)
I	2/2(100%)
II	1/3(33%)
III	7/12(58%)
IV	2/2(100%)

Table 3. Relationship of p53 Expression between Primary and Metastatic Sites in Malignant Melanoma

Site of tissues	Immunoreactivity for p53 protein positive/total(%)
Primary	6/12(50%)
Lymphnode	1/2(50%)
Metastatic	5/5(100%)

Table 4. Relationship between Sex and p53 Expression in Malignant Melanoma

Sex	Immunoreactivity for p53 protein	
	positive(%)	negative(%)
male	2(29%)	5(71%)
female	10(83%)*	2(17%)

* : $p<0.05$

DISCUSSION

The human p53 gene is located on the short arm of chromosome 17, band 13 (McBride et al., 1986). The product of the p53 gene is a protein containing 393 aminoacids which has some role in regulating cell growth negatively by affecting DNA replication and RNA transcription. The p53 protein is also reported to suppress the transformation of cells induced by oncogenes, such as ras gene (Finlay et al., 1988). To date, however, the precise function of the p53 gene in normal cells has not yet been clearly defined.

On the other hand, the aberrant proteins produced by altered p53 gene have been reported to play an important role in the genesis of diverse types of human cancers by losing its ability to suppress the transformation (Eliyahu et al., 1985 ; Hinds et al., 1989). The mechanisms by which the cells with altered p53 genes are frequently detected in cancer cells may be related to the prolonged effects of p53 protein on DNA replication (Bargonetti et al., 1991).

Anti-p53 monoclonal antibody used in this study, DO-7, is able to stain both normal and altered p53 gene products. On the other hand, the detectability of immunoreactivity for p53 protein is dependent 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 below the level of detection by the staining. In contrast to normal p53 protein, altered p53 proteins are relatively stable because of binding to certain intracellular proteins related to hsp70 or adenovirus protein E1b, not by increased synthesis (Zantema et al., 1985 ; Wang et al., 1989 ; Iggo et al., 1990). Therefore, 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 altered p53 gene.

In the detection of p53 gene alterations, immunohistochemical staining with anti-p53 antibody and polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) analysis have been frequently used. However, a minute discrepancy has been observed between the two methods. Recently we (Hong et al., 1994) have examined the p53 gene mutations in 25 primary gastric cancer tissues by using both immunohistochemical staining with DO-7 and PCR-SSCP for exon 4-8. Immunohistochemical staining was more simple and sensitive in screening of p53 mutation than PCR-SSCP.

It has been previously reported that the treatment with TUF solution of the paraffin-embedded sections at 90℃ may result in the binding frequency, with anti-p53 antibodies, similar to that observed in frozen specimens (van den Berg et al., 1993). We

thus initially studied the antibody binding in paraffin embedded cancer specimens with or without treatment with TUF solution. By the treatment with TUF solution, the positive rate and stain intensity increased in tumor cells while no positive stain was observed in the normal part of the samples (data not shown). From these results, we treated the tissues with TUF solution in immunohistochemical staining.

Malignant melanoma may develop through a multistep process and its sequence is thought to be common acquired nevus followed by dysplastic nevus and followed by neoplastic transformation (Elder et al., 1980). In the previous reports, the alterations of p53 gene were not observed in common acquired nevi and dysplastic nevi (Lassam et al., 1993 ; McGregor et al., 1993), while the alterations of p53 gene were observed at various incidences in malignant melanoma. The positive rates for p53 alteration in malignant melanoma were reported to be various, ranging from 4 to 62.5% (McGregor et al., 1993 ; Lassam et al., 1993 ; Ro et al., 1993). In our results, the positive rate was 63%.

It has also been reported that the immunoreactivity for p53 gene may be a marker for an aggressive phenotype of malignant melanoma (Gannon et al., 1990). Our data also showed the tendency to be higher in positive rate in metastatic tissues (100%) compared with the primary tissues (50%), however, the difference was not statistically significant. Expression of p53 alteration was more frequent in female(83%) rather than in male patients (28%) with malignant melanoma($p<0.05$). At the present time, the meaning is uncertain. The results presented in this paper suggest that the alterations of p53 gene may be one of the important gene changes during the development of malignant melanoma and p53 gene may be a possible target for gene therapy by restoring the normal function of p53 gene(Asai et al., 1994).

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