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Role of *p53* Gene Mutation in Tumor Aggressiveness of Intracranial Meningiomas

The mutations that occur in the *p53* tumor suppressor gene have been studied in various human malignant tumors. However, little is known about this gene in meningiomas. To investigate the relationship and frequency of *p53* gene mutations, the p53 polymerase chain reaction-single stranded conformational polymorphism (PCR-SSCP) and immunohistochemical study were performed on the 41 intracranial meningiomas (21 benign, 11 atypical, and 9 malignant). The higher the p53 protein expression rate, the poorer the histologic grade (9.5%, 72.7%, and 88.9% in benign, atypical and malignant meningioma, respectively) (p=0.000). The *p53* protein expression rate was higher in recurrent meningioma (71.4%) than in nonrecurrent meningioma (10.5%) (p=0.002). PCR-SSCP method was performed in positive p53 protein immunoreactivity cases. *p53* gene mutation rate was higher in the atypical (62.5%) and malignant (25%) meningiomas than in the benign meningioma (0%) (p=0.232). Also, the rate was higher in recurrent menigioma (20%) than in nonrecurrent meningioma (0%) (o=0.495). Among five to eight exons of the *p53* gene, the mutation was observed on exon 7 more frequently. In conclusion, p53 immunoreactivity and *p53* gene mutation are closely correlated with histologic grade and histologic atypia of intracranial meningiomas. *p53* gene mutation would be considered as a useful marker to detect the progression of intracranial meningiomas.

Key Words : *Genes, p53; Meningioma, benign, atypical; Recurrence*

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INTRODUCTION

Meningiomas are predominantly benign tumors which arise from arachnoidal cells (1). Meningiomas may result from an adverse effect of cranial irradiation (2) and trauma (3) but the development mechanism is unknown.

Menigiomas are histopathologically classified into benign, atypical, and malignant types. There may be a discordance between histological and biological behavior of meningiomas and it makes difficult to define the malignancy of meningioma. Also, the effects of the histological prognostic factor in recurrent cases have been somewhat controversial. These led many other studies to identify the quantitative parameters involved in recurrence and prognostic factors.

p53 gene is located at the 13.1 short arm of chromosome 17 and composed of 11 exons. p53 protein, composed of 393 amino acids, is a nuclear phosphoprotein and involved in regulating the proliferation, differentiation and apoptosis of cells (4). These functions are activated by sequence-specific binding of p53 protein with DNA (5). When *p53* gene mutation occurs and then sequences of p53 protein changes, p53 protein loses its binding capacity with DNA and acts in a dominant negative fashion that blocks p53 binding capacity (6).

p53 gene mutation has been commonly found in human malignancies such as cancers involving colon, lung, breast and other organs (7). However, little is known about this in meningiomas (8).

In the present study, to figure out the relationship of the p53 protein immunoreactivity and the *p53* gene mutation to the progression to the malignancy and recurrency of meningioma, respectively, the p53 immunoreactivity in benign, atypical, and malignant meningiomas including recurrent one was analyzed. Then, as for the positive cases,

Table 1. Analysis of immunoreactivity of p53 protein and *p53* gene mutation in benign nonrecurrent meningiomas

Case no.	p53 protein immunoreactivity	*p53* gene mutation
1	−	ND
2	−	ND
3	−	ND
4	−	ND
5	−	ND
6	−	ND
7	−	ND
8	−	ND
9	−	ND
10	−	ND
11	−	ND
12	−	ND
13	−	ND
14	−	ND
15	−	ND
16	−	ND
17	−	ND
18	1+	−
19	1+	−

ND: not determined, −: negative for p53 protein immunoreactivity or *p53* gene mutation. Results of positive p53 immunostaining are graded as follows: 1+, <10%; 2+, 10-50%; 3+, >50%

Table 2. Analysis of immunoreactivity of p53 protein and *p53* mutation in recurrent, atypical and malignant meningiomas

Case no.	No. of recurrence	Histologic type	p53 protein immunoreactivity	*p53* gene mutation
20		A	1+	exon7
21		A	-	ND
22		A	-	ND
23		A	2+	exon 8
24		M	-	ND
25		M	1+	exon 7
26		M	1+	-
27		M	1+	exon 8
28		B	-	ND
	1st	B	-	ND
29		A	-	ND
	1st	ND	ND	ND
30		A	1+	-
	1st	ND	ND	ND
31		A	1+	-
	1st	A	1+	exon 7
32		M	2+	-
	1st	M	1+	-
33		M	2+	-
	1st	M	1+	-
34		A	1+	exon 5
	1st	A	2+	exon 5
	2 nd	A	1+	-
	3 rd	M	1+	-

B, benign; A, atypical; M, malignant

the PCR-SSCP was performed.

MATERIALS AND METHODS

Subjects

H&E stained slides, clinical records, and pathologic reports from 174 cases of meningiomas from Korea University Hospital and Gachon Medical College Gil Medical Center from 1984 to 1995 were reviewed. According to the WHO classification (9), the 174 cases were classified into 157 cases of benign, 11 cases of atypical, and 6 cases of malignant meningiomas.

In the second consideration, the 7 cases showing recurrence within 5 years of follow-up after complete excision, were classified into 1 case of benign meningioma, 4 cases of atypical meningiomas, and 2 cases of malignant meningiomas. The one case of benign meningioma recurred one year after a complete excision. Among the four cases of recurrent atypical meningiomas, one of them involved brain parenchyma in third time recurrency and two of the other cases, which showed radiological evidence of recurrence, had no operation performed.

As the opposite group, the 19 cases showing no recurrence in 5 years of follow-up periods were abstracted, which were wholly benign meningiomas.

Each 4 cases of atypical and malignant meningiomas, which could not define recurrence and perform a complete excision, were added to the subjects.

Including debut and recurred tumors of recurrent meningiomas, a total sum of 41 tumors were studied : 21 benign, 11 atypical, and nine malignant meningiomas (Table 1, Table 2).

Methods

Four μm thickness sections which were obtained after formalin fixation and paraffin embedding were dewaxed with xylene for 3 to 5 min, and rehydrated with graded alcohols and distilled water. Then the sections were placed in a glass jar with phosphate buffered saline (PBS) and irradiated in a microwave oven for 5 min and then in 4℃ PBS for 5 min. The endogenous peroxidase activity was blocked with 3% hydrogen peroxide, then rinsed with PBS and reacted with normal gout serum containing immunoglobulin for 10 min to inhibit nonspecific bindings. The sections were incubated with monoclonal antibody, DO7, at a 1:50 dilution, for 2 hr, then reacted with secondary antibody for 20 min. After rinsing with PBS, applied peroxidase-labeled streptavidin for 30 min. The sections were developed in 3,3'-diaminoben-

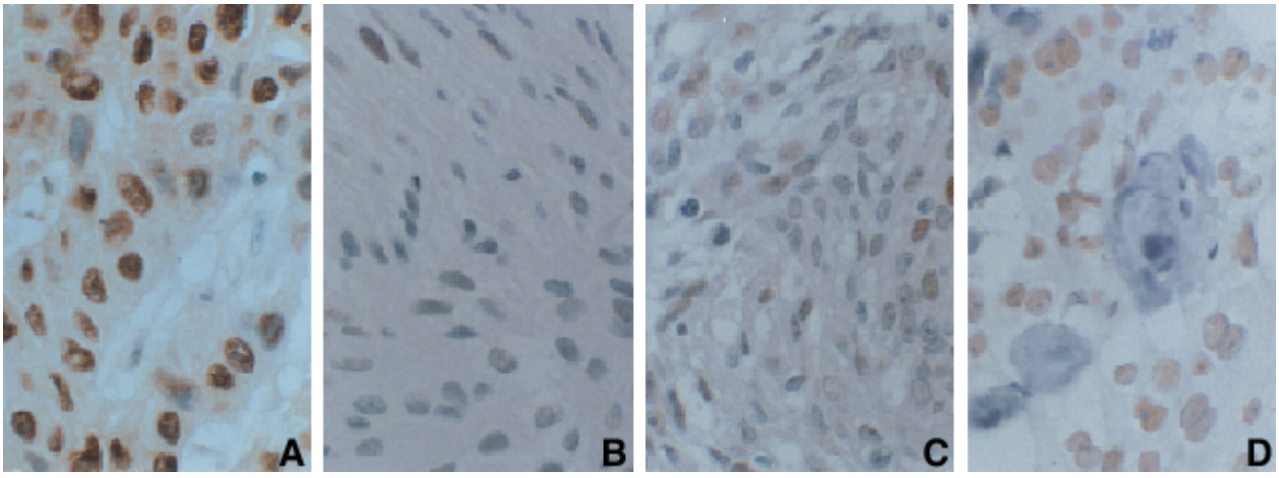

Fig. 1. Immunohistochemical stains of p53 protein. A: Positive control, colonic adenocarcinoma. B: Benign meningioma, case 18. C: Atypical meningioma, case 34, first recurrence. D: Malignant meningioma, case 33, debut tumor.

zidine (DAB) solution and counter-stained with hematoxylin.

The entire sections were systemically examined on high-power fields (400 ×) for p53 immunoreactivity. The extent of positivity, referring to an approximation of the number of positively immunostained tumor cell nuclei, was graded as follows; 0, no staining; 1+, staining of less than 10%; 2+, staining of 10-50%; 3+, staining of more than 51% (Fig. 1).

In order to identify *p53* gene mutation, PCR-SSCP was performed. 10-20 μm thickness sections from paraffin blocks were dewaxed in xylene followed by rinses in ethanol to remove xylene, then left at room temperature to remove ethanol. G'nome DNA Isolation Kit (Bio101) was used to isolate DNA. 0.9 mL cell suspension, 25 μL RNase, 50 μL cell lysis denaturing solution were reacted at 55℃ for 15 min then added 12.5 μL protease mix. The tissues digested at 55℃ for 24 hr and 250 mL saltout mixture from a G'nome Kit was added, then left at on ice for 10 min. After centrifugation, 15 mL supernatant was moved to test tube. Then, 2 mL TE buffer, 8 mL ethanol were added at -20℃ for 1 hr and centrifuged at 1500 rpm for 15 min. The tube were dried at room temperature to obtain DNA pellet, added 100 μL TE buffer, stored at -20℃ and used for template DNA.

PCR was done at exon 5, 6, 7, and 8 of *p53* gene. 2 μL DNA (aprox. 200-400 ng), 0.2 mM dNTP, H_2O, 10 × buffer, 0.25 U *Taq* polymerase were added, then repeated 35 times using a PCR thermal cycler (Perkin Elmer 2400) at 94℃ for 30 sec, 60℃ for 25-45 sec, and at 72℃ for 30 sec. Using 1.2% agarose gel electrophoresis, amplified PCR products were confirmed and for PCR positive control group, PCR was done on β-globin from DNA. The DNA sequences from each primer were as follow;

p53	exon 5	sense : 5'	TACTCCCCTGCCCTCAACAA 3'
		antisense : 5'	CATCGCTATCTGAGCAGCGC 3'
	exon 6	sense : 5'	GCTTGGCCCCTCCTCAGCAT 3'
		antisense : 5'	CTCAGGCGGCTCATAGGGCA 3'
	exon 7	sense : 5'	TCTGACTGTACCACCATCCA 3'
		antisense : 5'	CTGGAGTCTTCCAGTGTGAT 3'
	exon 8	sense : 5'	TGGTAATCTACTGGGACGGA 3'
		antisense : 5'	CGGAGATTCTCTTCCTCTGT 3'
β-globin		sense : 5'	TGACGGGGTCACCCACACTG TGCC 3'
		antisense : 5'	CTAGAACCATTGGGGTGGA CGATG 3'

Ten μL PCR product was boiled, and cooled with ice, then observed in electrophoresis using 12.5% polyacrylamide gel at 200 V for 5 hr. Then, the gel fixed with EtOH for 10 min and observed DNA single band pattern using silver staining method. First, the gel was added to 1% nitric acid for 3 min and washed with 100 mL distilled water, then reacted with 100 mL 0.2% silver nitrate solution for 20 min until the appearance of the positive band. PCR was done at *p53* DNA from normal blood cells for positive control group. By comparing the SSCP band, the gene mutation was analyzed (Fig. 2).

Statistical analysis

The tumors were divided into groups of benign, atypical, malignant meningiomas, and as groups of nonrecurrent and recurrent meningiomas, respectively. The chi-square test was used to investigate the statistical significance in association between immunoreactivity of p53 protein and *p53* gene mutation.

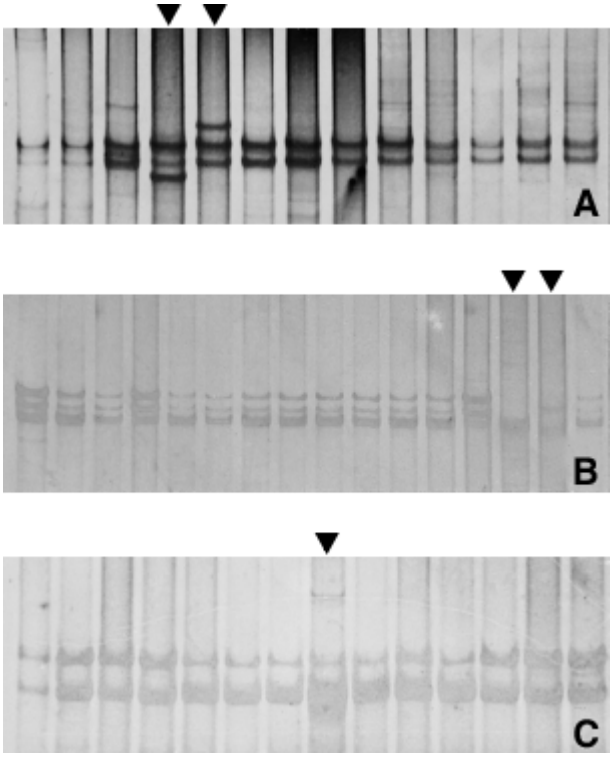

Fig. 2. PCR-SSCP analysis of *p53* exon 5(A), 7(B), 8(C). Left two lanes are controls (blood) and the other lanes are meningiomas. Arrow heads indicate lanes showing bands with mobility shift.

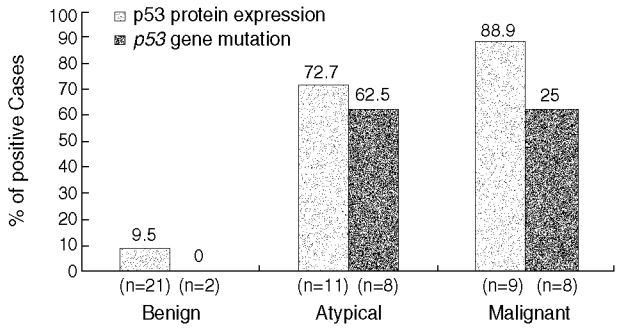

Fig. 3. p53 protein expression rate increased according to the poorer histologic grade (o=0.000). Especially, the difference between the benign meningioma and atypical and malignant meningiomas was marked. *p53* gene mutation rate was higher in the atypical and malignant meningiomas than in the benign meningioma (o=0.232).

RESULTS

Immunoreactivity of p53 protein

The positive reactions observed were weaker than colon cancer which used as a positive control group and (1+) positive cell count in 14 tumors, (2+) in four tumors, (3+) was not observed in any tumor (Table 1, 2, Fig. 1).

Positive immunoreactivity was observed in two of 21 benign meningiomas (9.5%), eight of 11 atypical meningiomas (72.7%), and eight of nine malignant meningiomas (88.9%). This showed that immunoreactivity of p53 protein was significantly higher as histological malignancy gets higher (p=0.000) (Fig. 3).

Positive immunoreactivity of p53 protein was expressed in five of seven cases of recurrent meningiomas (71.4%) but only in two of 19 cases in nonrecurrent meningiomas (10.5%). This showed that immunoreactivity of p53 protein was significantly higher in recurrent meningiomas than in nonrecurrent meningiomas (p=0.002) (Fig. 4). Two cases of recurrent malignant meningiomas showed (2+) immunoreactivity but three of four cases of atypical recurrent meningiomas showed (1+) and benign recurrent meningioma showed negative reaction (Table 2).

p53 gene mutation

PCR-SSCP was done in 12 of 18 tumors, which showed positive immunoreactivity of p53 protein. Seven cases of *p53* gene mutation resulted (38.9%). The mutation was observed in two cases on exon 5, three cases on exon 7, two cases on exon 8, and none on exon 6 (Table 1, 2, Fig. 2).

According to the histologic grade, five of eight atypical meningiomas (62.5%), two of eight malignant meningiomas (25%), and none of the benign menigiomas showed mutation. The atypical and malignant meningiomas showed higher rates of *p53* gene mutation than benign meningioma (Fig. 3).

The mutation was not observed in two cases of nonrecurrent meningiomas, which showed overexpression of p53 protein in immunohistochemical stain and one of five debut tumor from recurrent meningiomas exhibited the mutation. But, the statistical significance of difference was low because of the smaller number of samples (p=0.495) (Fig. 4). In one recurrent meningioma, which recurred three times in sequence, its debut tumor showed a mutation on exon 5. The mutation on exon 5 was observed in first time recurrence, too, but was not observed in second and third times recurrence (Table 2).

DISCUSSION

The prognosis of meningiomas depends on the location of tumor, resection margin, and radiological and surgical findings than histopathological parameters (10).

Mirimanoff et al. (10) reported overall survival rates of five years, ten years, 15 years were at 83%, 77%, and 69%,

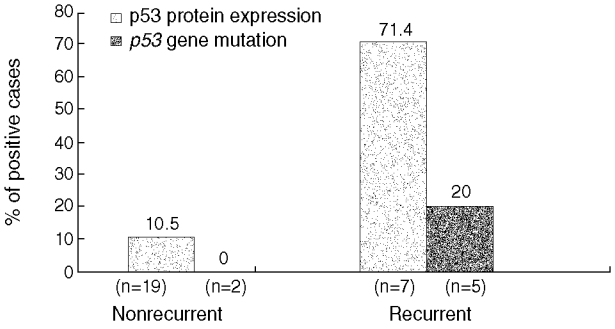

Fig. 4. p53 protein expression (p=0.02) and *p53* gene mutation (p=0.495) rates were higher in recurrent meningioma than in nonrecurrent meningioma.

respectively. The recurrence-free rates of the total resection were 93%, 80%, and 68%, but those of partial resection were 63%, 45%, 9%, respectively. This showed that recurrence-free rate depends upon the extent of tumor removal. But, even after total resection, meningiomas may recur and as the recurrences repeat (11), the tumors tend to be atypical or malignant (12).

Jaaskelainen et al. (11) reported recurrence rates within 5 years of atypical and malignant meningiomas were 38% and 78%. However, in some cases, there was no evidence of pathologically atypical findings in the recurrent cases after total resection (10).

Genes associated with predisposition of brain tumors are *NF-1*, *NF-2*, and *p53*. The alteration of the *NF-1* gene was associated with development of neurofibroma and astrocytoma (13), whereas the inactivation of *NF-2* gene was associated with development of schwannoma of VIII cranial nerve and meningioma (14). Both *NF-1* and *NF-2* are located at chromosome 22. Also, deletion of the short arm of chromosome 10 and 1 were observed in meningiomas (15).

Considering complex clinical, histopathological, biological features of meningiomas, and the possible involvement of multiple genes from chromosome 22 and other chromosomes, meningiomas may have been caused by several selected genetic pathways at the molecular level. In colon cancer, the gene mutation associated with malignant transformation and development of tumor were studied (16). There is increasing evidence that indicates the development and malignant progression of human astrocytomas may occur from sequential genetic event (17, 18).

In human brain tumors, the most common genetic abnormalities were the loss of heterozygosity in chromosome 17p where *p53* gene located (19). p53 protein, a product of *p53* gene, acts as a negative regulator to suppress the cell proliferation at the G1 phase (20). However, the mutant p53 protein from mutated *p53* gene loses its normal function and activates cell proliferation and malignant transformation (21). The mechanisms to inactivate p53 protein functions in human tumors are as follow; most commonly through a mutation, formation of complex body association with tumor protein such as mdm2, and inhibition of intake of p53 protein into the nucleus of the cell (22).

The types of *p53* gene mutations are deletion, point mutation, frame shift mutation, and insertion etc. The point mutations, the most common type, occur in 4 hot spot regions from exon 5 to 8, these regions determine the sequences of p53 protein (23). The mutant p53 protein has a stable structure and longer half-life than normal p53 protein, making it easier to detect by immunohistochemical stains (24). Using PCR-SSCP, the detection rate of *p53* gene mutation was somewhat lower than p53 protein immunoreactivity (25, 26). However, some tumors that showed negative reaction in immunohistochemical stain showed mutations on the molecular biology level (27).

The overexpression of normal p53 protein by p53 protein stimulants and the stabilization of normal p53 protein from the binding of cellular or viral oncoprotein result in a false positivity on immunohistochemical stains. The nonsense mutation, deletion of gene, and frame shift mutation may result in a false negativity (28). Also, the sensitivity of PCR-SSCP is somewhat lower that it can not detect the mutation of few cells (26). However, many reported that there is a significant correlation between the overexpression of p53 protein and *p53* gene mutation at a genetic level (27).

The present study did not perform PCR on tumors showing negative immunoreactivity of p53 protein but did PCR-SSCP on 18 tumors showing positive immunoreactivity of p53 protein. From PCR-SSCP, seven out of 18 positive tumors showed *p53* gene mutation (38.9%). This is thought to be because the mutation is not from exon 5, 6, 7, and 8; the false-positivity on the immunohistochemical stain due to the stabilization of wild-type p53 protein, and low sensitivity of PCR-SSCP used in the present study were all included in these cases.

The studies on p53 of meningiomas were extremely scarce compared to other human tumors. Mashiyama et al. (8) analyzed the DNA sequence of eight cases of benign meningiomas and discovered a change of the CGC->GGC at codon 175 in one case. Karamitopoulou et al. (29) identified the intranuclear positivity in 11 cases of 57 benign meningiomas using an immunohistochemical stain with the DO-seven antibody. In most positive cases, the positivity was observed in a few scattered cells and DNA sequencing to confirm the mutation did not occur.

Ohgaki et al. (18) performed PCR-SSCP and direct DNA sequencing on 15 cases of benign meningiomas and seven cases of atypical meningiomas. The *p53* gene mutation was not observed. This suggested that *p53* gene may not play a role in the development of meningiomas. The inactivation

of *p53* tumor suppressor gene from other mechanisms such as the formation of the complexes of p53 protein with viral or mammarian (mdm2) oncoprotein were suggested (30). Lindblom et al. (15) observed deletion of chromosome 22 as well as on the short arm of chromosome 17 in malignant meningiomas. These findings suggested that the changes of *p53* gene in chromosome 17 might relate to malignant transformation in meningiomas.

Wang et al. (26) observed negative nuclear p53 staining in 19 cases of benign meningiomas but four of five cases of atypical meningiomas and two cases of malignant meningiomas showed positive nuclear p53 staining. After PCR-SSCP, one case of malignant meningioma showed an intense nuclear staining with a band shift on exon 5 and direct DNA sequencing revealed an alteration of alanine to threonine in codon 161 (GCC->ACC). These results indicated that the *p53* gene mutation should be considered as a marker for malignant transformation in meningiomas. Also, even in the absence of detectable gene mutation, the p53 protein immunoreactivity was associated with histologic atypia.

The results of this study showed significant differences in p53 immunoreactivity between benign, atypical, and malignant meningiomas. The *p53* gene mutation was observed in seven of 16 atypical and malignant meningiomas but was not observed in two benign meningiomas. These indicate that p53 protein immunoreactivity and *p53* gene mutation are associated with histological atypia and considered as a marker for the malignant transformation.

The p53 protein immunoreactivity of recurrent meningiomas (71.4%) was higher than nonrecurrent meningiomas (10.5%) and the difference was statistically significant (p=0.002). The mutation was not observed in two cases of nonrecurrent meningiomas but observed in one of five cases of recurrent meningiomas and the difference was statistically insignificant. These results show the possibility that the recurrence of meningioma is related to histologic atypia rather than *p53* gene mutation. It is thought that more samples, with clinical follow-up and molecular biological studies in many cases of totally resected benign, atypical, and malignant meningiomas, are needed to find out the direct relationship between recurrence of meningioma and p53.

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