

p53 Protein Overexpression in Astrocytic Neoplasms

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Abnormalities of the p53 gene are the most common molecular change in human cancer. In the central nervous system, mutant p53 gene is frequently identified in the tumors with astrocytic differentiation. To investigate the relation between histologic subtypes and p53 protein overexpression, we examined 81 cases of astrocytic neoplasms (24 benign astrocytoma, 28 anaplastic astrocytoma and 29 glioblastoma multiforme) using the standard immunohistochemical method. All were formalin-fixed and paraffin-embedded tissue. The p53 immunoreactivity was found in 4/24 benign astrocytoma, 18/28 anaplastic astrocytoma, 22/29 glioblastoma multiforme. The degree of immunoreactivity closely correlated with histologic subtypes ($p < 0.001$). Overall p53 protein expression was most frequently detected in glioblastoma multiforme, but strong immunoreactivity (3+) was more frequently found in the anaplastic astrocytoma than in glioblastoma multiforme. Although the frequency of p53 protein expression is low, 4 benign astrocytoma showed distinct nuclear staining.

In conclusion the malignant progression of astrocytic neoplasms may be associated with increasing expression of p53 protein.

Key Words: Astrocytoma, anaplastic astrocytoma, glioblastoma multiforme, p53 protein, immunohistochemistry

The previous studies of the proliferative potential in benign and malignant astrocytic tumors using the ³H-thymidine labeling index (Hoshino and Wilson, 1979), Ag-NORs count (Han *et al.* 1992), flow cytometric analysis (Fitzgibbons *et al.* 1988), immunohistochemical stain for proliferating cell nuclear antigen (Barbareschi *et al.* 1992; Cho *et al.* 1994) reported the close correlation with histologic subtypes. On the other hand, the wild-type p53 gene involved in the regulation of cell cycle (Lambkin *et al.* 1994). In several reports of molecular genetic changes of the p53 gene in

astrocytic neoplasms, they are thought to result in the loss of normal growth inhibitory function which is a fundamental step in the development of tumors (McDonald and Dohrmann, 1988; Fufts *et al.* 1989; James *et al.* 1989; Mashiyama *et al.* 1991; Venter and Thomas, 1991; Sidransky *et al.* 1992). Abnormalities of the p53 gene can be detected by various methods. The role of mutant p53 gene in tumor progression of brain tumors was mostly investigated using the molecular genetic analysis. The mutant p53 gene produces an abnormal protein having increased stability, and can be detected by immunohistochemical method (Hall and Lane, 1994; Lambkin *et al.* 1994). A few previous studies of immunohistochemical analysis for p53 protein in the central nervous system tumors described more frequent expression in malignant astrocytic tumors than in benign tumors (Barbareschi *et al.* 1992; Ellison *et al.* 1992; Lee *et al.* 1994). However, they analyzed a relatively small

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number of cases or used a fresh frozen tissue and no extensive study using the immunohistochemical stain of paraffin sections exists in astrocytic neoplasm. In this report we retrospectively evaluate the immunohistochemical expression for p53 protein in 81 paraffin sections of astrocytic tumors according to the histologic subtypes.

MATERIALS AND METHODS

The routinely fixed and paraffin embedded specimens were obtained from the Yonsei University College of Medicine and Wonju College of Medicine. All paraffin blocks of the specimen were histologically reviewed and tumors were classified into three histologic subtypes by the WHO classification (Kleihues *et al.* 1993). Neoplasms composed of uniform astrocytes with mildly increased cellularity and a few mitoses were classified as benign astrocytoma (Fig. 1A). The tumors showing moderate to marked cellularity, nuclear atypia, neovascularization, endothelial cell proliferation, endothelial cell proliferation,

and frequent mitoses were defined as anaplastic astrocytoma (Fig. 1B). Glioblastoma multiforme had a pseudopalisading with central necrosis in addition to the histologic features of anaplastic astrocytoma (Fig. 1C).

Immunohistochemical stain for p53 protein

For immunohistochemical staining, we used monoclonal antibody DO7 (Novocastra Laboratories, Newcastle, UK) which was diluted 1:50. The immunostaining technique was accomplished by the three-step avidin-biotin complex (ABC) method using the LSAB kit (Dako, Carpinteria, CA, USA). Sections, 4 μ m thick, were taken onto silanized slides and dried at 60°C for more than 1 hour. Sections were deparaffinized in xylene and rehydrated: applied 3% hydrogen peroxide to cover the entire specimen and incubated for 5 minutes at room temperature: applied blocking serum and incubated 20 minutes: applied primary antibody and incubated 20 minutes: applied link antibody and incubated 20 minutes: applied peroxidase-labelled streptavidin and incubated 20 minutes: applied substrate-chromogen rea-

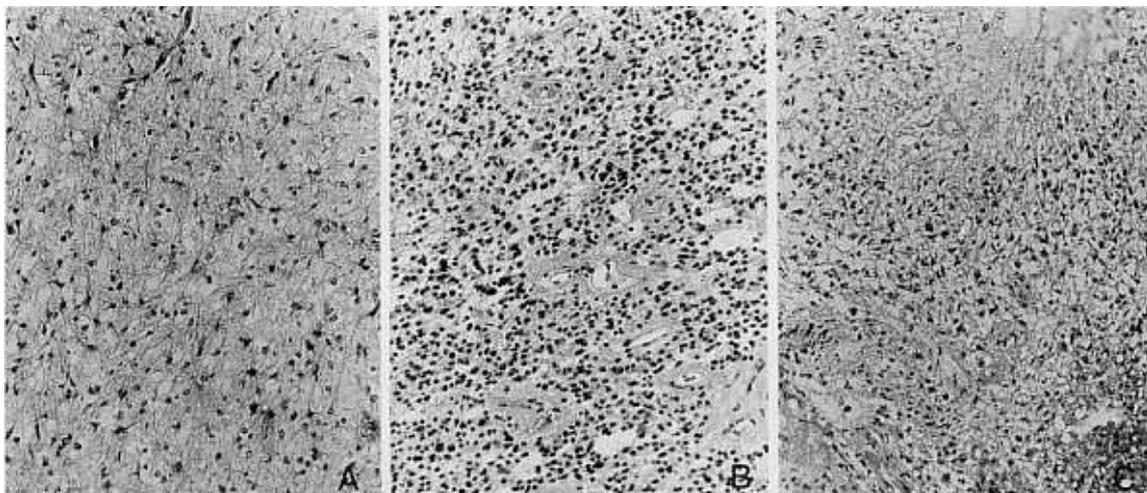


Fig. 1. Histologic subtypes of astrocytic tumors. A: Benign astrocytoma is composed of uniform astrocytes with mildly increased cellularity and rare mitosis (H & E \times 200). B: Anaplastic astrocytoma shows moderately to markedly increased cellularity, nuclear atypia, neovascularization, endothelial cell proliferation and frequent mitoses (H & E \times 200). C: Glioblastoma multiforme has a pseudopalisading with central necrosis in the background of anaplastic astrocytoma (H & E \times 200).

gent (AEC, Biomed, Foster city, CA, USA) and incubated 30 minutes; counterstained by Mayer's hematoxylin; covered the slides using aqueous-based mounting medium. Sections of the infiltrative mammary carcinoma were used as positive control. Negative control included the mammary carcinoma where the primary antibody was omitted.

Scoring of the immunohistochemical stain

The immunostaining results were scored as 0; negative, 1⁺; less than 10% of the p53 protein positive tumor cells (Fig. 2A), 2⁺; from 10% to 30% of positive cells (Fig. 2B), 3⁺; more than 30% of positive cells (Fig. 2C).

Statistical analysis

The data was analyzed using the SPSS PC⁺. Correlation between the histologic subtypes and p53 overexpression was tested by Spearman's rank correlation. The chi-square test was used to investigate the statistical significance in association between the histologic subtypes and p53 overexpression.

RESULTS

The examined tumors were classified as 24 benign astrocytoma, 28 anaplastic astrocytoma

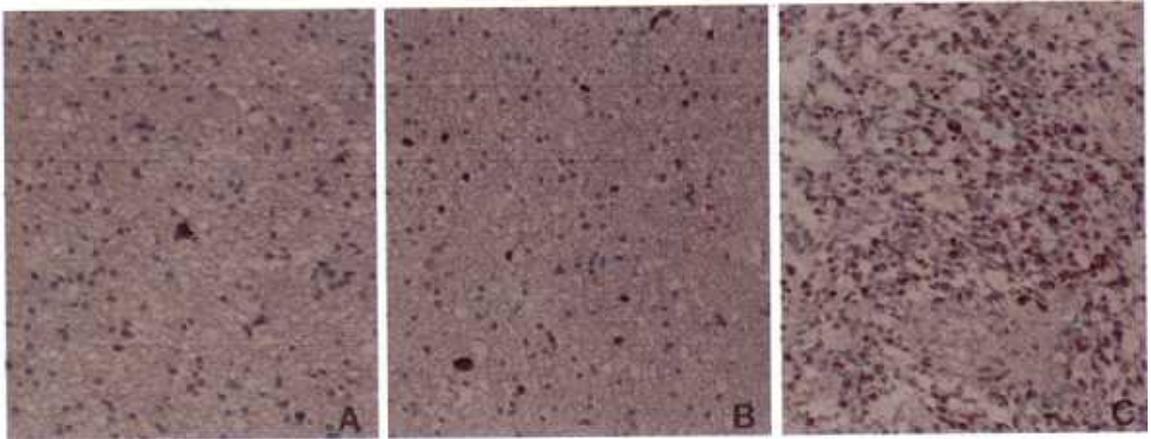


Fig. 2. The immunostaining results are scored as 0: negative, 1⁺: less than 10% of p53 protein positive tumor cells (A, $\times 400$), 2⁺: 10~30% of positive cells (B, $\times 200$), 3⁺: more than 30% of positive cells (C, $\times 200$).

Table 1. Number of cases according to the scores of p53 protein overexpression and the histologic subtype of astrocytic neoplasms

Histologic subtypes	Score of p53 protein overexpression(%)				Total
	0	1 ⁺	2 ⁺	3 ⁺	
Benign astrocytoma	20(83.3)	3(12.5)	1(4.2)	9(0)	24(100)
Anaplastic astrocytoma	10(35.7)	10(35.7)	2(7.1)	6(21.4)	28(100)
Glioblastoma multiforme	7(24.1)	10(34.5)	9(31.0)	3(10.3)	29(100)
Total	37(45.7)	23(28.4)	12(14.8)	9(11.1)	81(100)

$\chi^2=27.50973$, $p<0.001$

and 29 glioblastoma multiforme. The results of immunohistochemical staining are summarized in Table 1. The p53 immunoreactivity was clearly observed in the nuclei of the neoplastic astrocytes. Intra and intertumoral heterogeneity of staining intensity and positive cell distribution were found. Every stained nucleus in whole section was interpreted as positive regardless of intensity. p53 Expression was found in 44 cases (54.3%) of all tumors. Immunoreactivity was found in 4 out of 24 benign astrocytoma (16.7%), 18 out of 28 anaplastic astrocytoma (64.3%), and 21 out of 29 glioblastoma multiforme (75.9%). There was a statistically significant correlation between p53 overexpression and histologic subtypes ($p < 0.001$). The difference between benign and anaplastic astrocytoma was the most distinct while the difference between anaplastic astrocytoma and glioblastoma multiforme was not significant. Although overall p53 expression was most frequently detected in the glioblastoma multiforme, tumors showing 3⁺ immunoreactivity were more frequently found in the anaplastic astrocytoma than in glioblastoma multiforme. Four immunostained benign astrocytoma showed 1⁺ in 3 cases and 2⁺ in one case. The p53 expression was not noted in reactive gliosis or non-neoplastic astrocytes.

DISCUSSION

We present a close relationship between p53 protein overexpression and histologic subtypes of astrocytic neoplasms. Gliomas are the most common tumors of the central nervous system. They often recur and evolve into malignancy with time (Venter and Thomas, 1991). Various molecular genetic abnormalities supporting the development and progression of the brain tumor have been investigated. There are cytogenetic studies of polymorphic loci on chromosomes 1p and 7p and additional loci on chromosomes 10 and 17, mutation of p53 gene, amplification of epidermal growth factor receptor (EGFR), *N-myc* and *c-erbB-2* proto-oncogenes (McDonald and Dohrmann,

1988; Venter and Thomas, 1991). The p53 gene locates chromosome 17p13.1 which is the single most common target for genetic alterations in human cancer (Chang *et al.* 1993). The role of the p53 gene in brain tumors has been investigated in a few cases. Most of them have used molecular biology techniques. The genetic abnormalities include point mutations, deletions, rearrangements, or allelic loss (Hollstein *et al.* 1991). p53 Mutation is frequently identified in brain tumors showing astrocytic differentiation. The phenotypically transformed p53 gene, or the inactivation of wild-type p53 protein, may play a role in tumorigenesis (Hall and Lane, 1994). The stabilization of the wild-type protein becomes nonfunctional. The stabilizing mechanisms of the p53 protein have been investigated. The role of large protein T antigen of SV40 and product of the *mdm2* gene were discussed (Hall and Lane, 1994). Functional and structural changes in the p53 gene results in the accumulation of protein, which can be detected by immunohistochemical stain (Hall and Lane, 1994). Immunohistochemical demonstration of p53 protein overexpression in a few astrocytic tumors has been reported (Barbareschi *et al.* 1992; Lee *et al.* 1994). Although we evaluated 81 cases, the results of immunohistochemical findings are similar to those of other studies which analyzed less than 40 cases. They reported benign astrocytomas revealed a low p53 labeling index (1.3) or negative reaction for p53 protein (Barbareschi *et al.* 1992; Lee *et al.* 1994). Our data showed that p53 protein expressed both in benign and malignant tumors, although the expression rate was very low (4 out of 24) in benign astrocytoma.

Sidransky *et al.* (1992) described the histological progression of brain tumors as being associated with a clonal expansion of cells that have previously acquired a mutation in the p53 gene. An induction of human wild-type p53 expression in a glioblastoma tumor cell line had a negative growth regulation (Mercer *et al.* 1990). In contrast to the reports which described the loss of genetic material on chromosome 17, with equal frequency in both benign and malignant glioma (Fults *et al.* 1989; Venter and Thomas, 1991), our results demon-

strate a significant difference between benign and malignant tumors. However, the difference between anaplastic astrocytoma and glioblastoma multiforme is not significant. A strong immunoreactivity (3+) is more frequently found in anaplastic astrocytoma than in glioblastoma. These results indicate that different molecular or genetic events, other than p53 gene abnormality, may be involved in the malignant progression of astrocytoma.

Benign astrocytomas can progress to malignancy and show a different histologic subtype even in the same tumor (Schiffer *et al.* 1988). When a small piece of tumor is obtained, frequent immunoreactivity for p53 protein should be considered as the possibility of malignant progression of the tumor.

In conclusion, the immunohistochemical stain is a useful in detection of the altered p53 protein and identification of malignant astrocytic neoplasms. However, not all anaplastic astrocytoma and glioblastoma multiforme show the overexpression of p53 protein.

Further investigation of the other genetic changes in tumor progression using practical, more simple, and useful method will be necessary to predict the prognosis of biopsied tumors and understand the tumorigenesis of the astrocytic neoplasm.

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