

Patterns of p53 Expression in Phyllodes Tumors of the Breast

— An Immunohistochemical Study —

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Authors performed an immunohistochemical analysis using monoclonal antibody to p53 protein on 15 cases of benign and malignant phyllodes tumor of the breast along with a review of other conventional clinicopathological parameters to investigate the meaning of p53 expression. The cases were composed of 8 benign and 7 malignant lesions. The pattern of p53 expression showed a statistically significant difference between these benign and malignant lesions ($p < 0.005$). None of the benign cases expressed p53 whereas 6 out of 7 malignant cases did. Among malignant phyllodes tumors, the pattern of expression was diffuse and strong in two cases while granular and relatively weak in the remaining 4 cases. p53 expression seemed to be a unique feature of malignant phyllodes tumors, thereby, one of the most significant parameters for the differentiation of benign and malignant phyllodes tumors of the breast.

Key Words: p53, Phyllodes tumor, Cystosarcoma phyllodes, Breast neoplasm

INTRODUCTION

Cystosarcoma phyllodes is a relatively rare neoplasm having partial similarity to fibroadenoma, occupying about 0.5% of tumors of the breast (Auger et al., 1989). The tumor shows a variable clinicopathological behaviour, ranging from an apparently benign course to malignancy, but predictable prognostic parameters have not been clearly established as yet, and the WHO categorized this tumor as 'Phyllodes Tumor of the Breast' (WHO, 1981).

Major efforts have been given to the evaluation of the clinicopathological significance of multiple histological parameters such as stromal cellularity, cellular pleomorphism, mitotic index or growth pattern (expansile vs invasive) in differentiating benignity and malignancy of the tumor without clear-cut results (Ward and Evans, 1986; Murad et al., 1988;

Auger et al., 1989).

Recently, aberrant expression of p53 protein has been seen in many kinds of malignant neoplasm, thus we conducted an immunohistochemical study on 15 cases of phyllodes tumors of the breast to compare the patterns of p53 expression in histologically benign and malignant phyllodes tumors.

MATERIALS AND METHODS

The clinicopathological aspects of a total of 15 cases, diagnosed as phyllodes tumors of the breast (cystosarcoma phyllodes) during the period of January 1986 to December 1991, were reviewed from the files of the Department of Pathology, Seoul National University Hospital. The cases were composed of 8 benign and 7 malignant cases. In each case, clinical course, therapeutic modalities, size of the tumor, and histological parameters such as mitotic count, cellular atypism, necrosis and hemorrhage were evaluated.

The age distribution of benign and malignant phyllodes tumor patients ranged from 24 to 45 years (mean 37.4 years) and 23 to 57 years (mean 34.1 years), respectively. For immunohistochemical analysis of p53 protein, 5 μ m thick sections of

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Table 1. Clinicopathological and Immunohistochemical Findings in Benign Phyllodes Tumors

No.	Age (cm)	Size Pattern	Grwoth (/10HPF)	Mitosis	Atypism Hemorrhage	Necrosis/ Hemorrhage	Treatment	p53 Expression
1	24	6.0	E	3	-	-	Excision	-
2	45	5.0	E	5	-	-	Excision	-
3*	44	10.0	E	4	-	-	Excision	-
4	36	5.0	E	4	+	-	Excision	-
5	38	1.5	E	1	-	-	Excision	-
6	42	5.0	E	4	-	-	Excision	-
7	29	3.0	E	7	+	-	Excision	-
8	41	5.0	E	3	+	-	Excision	-

* : The case with local recurrence

E : Expansile

Table 2. Clinicopathological and Immunohistochemical Findings in Malignant Phyllodes Tumors

No.	Age	Size (cm)	Growth Pattern	Mitosis (/10HPF)	Atypism	Necrosis/ Hemorrhage	Treatment	p53 Expression
1*	23	4.5	I	26	+	-	SM+RM	D
2	24	6.0	I	49	+	+	SM+AD	D
3*	32	3.0	I	10	+	+	SM	G
4	23	5.0	I	72	+	+	SM+AD	G
5*	57	3.5	E	28	+	-	SM+AD+CTX	G
6	37	11.0	E	35	+	-	SM+AD+RT	G
7	43	5.0	E	12	+	-	Excision	-

* : The case with local recurrence

E : Expansile, I : Infiltrative

SM : Simple mastectomy, RM : Radical mastectomy, AD : Axillary dissection,

CTX : Chemotherapy, RT : Radiation therapy

D : Positive with diffuse pattern, G : Positive with granular pattern

paraffin embedded tissue, fixed in 10 % buffered neutral formalin, were obtained from each case. The sections were deparaffinized and hydrated through xylene, graded alcohol, and phosphate buffered saline.

Immunohistochemical staining was performed using monoclonal antibody to p53 protein(Clonc DO7; Novocastra, U.S.A.) by avidin-biotin peroxidase complex method(ABC). The primary antibody was 1:50 diluted and incubated overnight at 4°C. Biotinylated polyclonal antimouse goat immunoglobulin was used as secondary antibody. Three cases of fibroadenoma were included as normal controls.

Statistical analysis was performed using chi-square test with Yates correction.

RESULTS

Clinicopathological Findings

The overall clinicopathological findings of benign and malignant phyllodes tumors are summarized in Table 1 and Table 2, respectively. All patients presented with a palpable breast mass, and the size of the tumors ranged from 1.5 to 10 cm (mean 5.1 cm) in benign cases and 3.5 to 11 cm (mean 6.3 cm) in malignant cases. Local recurrences were found in 3 malignant cases and 1 benign case. The growth patterns of benign lesions were all expansile with relatively fair circumscription, while 5 cases of malignant lesions had infiltrative margins. Mean mitotic counts were 3.9/10HPF and 33.1/10HPF in benign and malignant cases, respectively. The malignant cases revealed higher cellularity in general with atypism, and mild to moderate cellular atypism was observed in 3

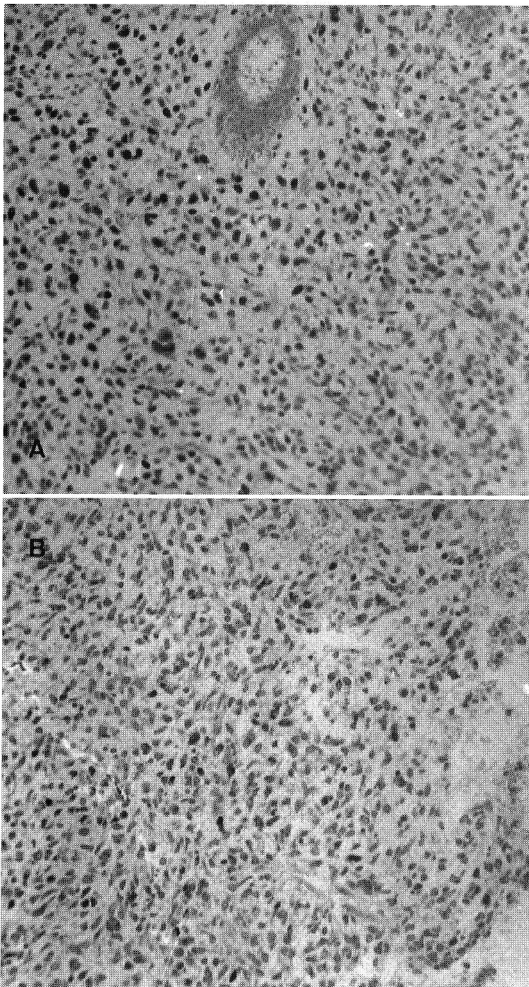


Fig. 1. (A) Diffuse positive reaction of p53 in the nuclei of malignant phyllodes tumor(x200), (B) Granular pattern of p53 expression in discrete stromal tumor cell nuclei of malignant phyllodes tumor(x200).

benign cases. Necrosis or hemorrhage were present in 3 malignant cases, but not in any of the benign cases.

Immunohistochemical Findings

The epithelial components of both benign and malignant cases showed completely negative staining to p53 protein. The stromal cells of control cases (fibroadenoma) and all benign lesions also showed a negative reaction. In 7 malignant phyllodes tumors, a positive reaction was observed in the stromal tumor cell nuclei of 6 cases. The major pattern of positive p53 nuclear staining was of two

types; diffuse and granular. In diffuse pattern which was present in 2 cases, most tumor cells showed intense nuclear staining (Fig. 1A) while granular pattern which was present in 4 cases revealed spotty and comparatively weak staining in only discrete and small proportions of cells (Fig. 1B).

DISCUSSION

Phyllodes tumor (Cystosarcoma phyllodes) of the breast is an uncommon neoplasm of the breast, occupying 0.5 % of all breast tumors and 2.5 % of fibroepithelial tumors. It is histologically characterized by ductal component and stromal overgrowth (Auger et al., 1989), and has benign and malignant forms, and possibly a borderline category. About 30 % of phyllodes tumors are histologically diagnosed as malignant (Vorherr H, 1985), but the differentiation of benign and malignant forms based on histopathological ground alone is sometimes difficult, thus many authors performed clinicopathological analyses on phyllodes tumors to document the criteria for malignancy (Ward and Evans, 1986; Murad et al., 1988; Coho-Cedermark G et al., 1991). Among these are cellular atypism, mitotic count, infiltrative margins, and necrosis etc, but none of these seem to represent a unique or characteristic feature of malignant phyllodes tumor. Even the evidence of metastasis, which is a unique characteristic of malignancy, because therapeutic modalities such as range of excision are usually different. Thus as diagnostic adjunct, DNA ploidy pattern on flow cytometric analysis has been studied by El-Naggar et al (1990) with positive results, however flow cytometry is not an ideal procedure in routine surgical process.

p53 is a 53-kd nuclear phosphoprotein, coded by the p53 gene located on the short arm of chromosome 17, and though its precise function is not clearly understood as yet, it has been implicated in control of the cell cycle (Purdie et al., 1991). The wild-type p53 gene behaves as a tumor suppressor while its mutated forms have tumorigenic potential, and it has been reported that the p53 gene is a site of frequent mutation or deletion in various kinds of human neoplasm including tumors of the breast, lung, colon etc and through this mutation or overexpression, p53 is believed to play an important role in the tumor progression. Recently, along with detection of gene level abnormalities, immunohistochemical detection of p53 expression in the paraffin-embedded tissues using monoclon-

al antibodies to p53 has been made possible (Porter et al., 1992). Normal p53 is usually expressed at low levels and has an extremely short half-life (Rogel et al., 1985) and thus its expression cannot be detected by usual immunohistochemical methods. But in the presence of mutated forms with their prolonged half-life or overexpression, p53 can be detected. In current data on immunohistochemical surveys of various human tumors, aberrant p53 expression seems to be exclusively found in varying proportions of malignant tumors though exceptionally rare cases of benign neoplasm showed expression of p53 (Porter et al., 1992; Purdie et al., 1991).

Based on the above information, we performed an immunohistochemical analysis of phyllodes tumors to investigate the relationship between hither to described criteria of malignancy and p53 expression in this type of tumor. As previously described, p53 expression was not observed in histologically benign cases while most of the malignant cases (6 out of 7) showed positive staining. Thus p53 expressions seem to be unique feature of malignancy in the phyllodes tumor of the breast and can be regarded as one of the most powerful adjuncts for the differentiation of the benign and malignant phyllodes tumors. As far as we know, the expression pattern of p53 in phyllodes tumors has not been reported in the literature, and we believe that p53 expression can be added to the conventional histopathological criteria in the differentiation of benign and malignant phyllodes tumors. It is not certain whether the pattern of p53 expression such as diffuse or granular reaction is related to the degree of malignancy or other poor prognostic parameters as in carcinoma of the breast (Cattoretti et al., 1988) because of the limited number of cases included in this study. Further evaluation of accumulated cases will confirm or deny this possibility.

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