

## Expression of p53 Protein and Ki-67 Antigen in Phyllodes Tumor of The Breast

Yu-Jan Chan<sup>1</sup>Be-Fong Chen<sup>1</sup>Chin-Long Chang<sup>2</sup>Tsen-Long Yang<sup>3</sup>Chi-Chen Fan<sup>1</sup><sup>1</sup>Department of Pathology;<sup>2</sup>Department of Medical Research;<sup>3</sup>Department of Surgery; Mackay Memorial Hospital, Taipei, Taiwan, R.O.C.

---

### Key Words

breast;

Ki-67;

MIB-1;

p53;

phyllodes tumors

Phyllodes tumors (PT) are uncommon biphasic breast tumors that occur in adult females about 44-52 years old.<sup>1-4</sup> PT represent 0.3-1.5% of breast neoplasms and are composed of a benign epithelial component and a cellular, spindle cell stroma forming leaf-like structure.<sup>1,4,5</sup> No 1 morphologic finding is reliable in predicting the clinical behavior. Different investigators have subdivided PT into "benign and malignant" or "benign, borderline, and malignant" categories by evaluating several pathologic features including tumor size, tumor margin, stromal cellularity, mitotic count and degree of nuclear atypia. However, the criteria of grading are different among different authors in published reports.<sup>1,3,6,7</sup> Ward and Evans reported that stromal overgrowth was a significant histologic indicator of malignant behavior in cystosarcoma phyllodes.<sup>8</sup>

Mutation of p53 tumor suppressor gene is common in some human tumors such as breast carcinoma, soft tissue

**Background.** Phyllodes tumors (PT) of the breast are uncommon, and it is often difficult to predict their clinical behavior from histologic features in individual cases. In addition to routine morphology, the studies of p53 protein and Ki-67 antigen expression in PT may be useful to differentiate benign from malignant tumors.

**Methods.** Immunohistochemical analyses using monoclonal antibody to label p53 protein and another monoclonal antibody MIB-1 to label Ki-67 antigen were performed on the tissue sections of 63 PT from 56 patients. The percentages of positive staining tumor cells were compared with the tumor gradings and clinical outcomes.

**Results.** According to histologic criteria, this series contained 50 benign and 13 malignant tumors. The p53 protein expression showed a significant difference between benign and malignant lesions. Within the group of benign lesions, 5 out of 50 (10%) tumors had p53 expression > 10%, whereas nine out of 13 (69%) malignant tumors revealed p53 expression > 10% ( $p < 0.005$ ). The Ki-67 antigen was also well correlated with tumor grading. Eleven out of 13 (85%) malignant tumors but only 8 out of 50 (16%) benign tumors showed Ki-67 antigen increased > 10% ( $p < 0.005$ ). Three patients progressed from benign to malignant tumors. All the first and recurrent tumors in these 3 patient showed Ki-67 > 10%.

**Conclusions.** P53 protein and Ki-67 antigen expression are correlated with the histology grading. In tumors with benign morphology but having a Ki-67 antigen > 10%, it is necessary to treat the patient and follow up properly to avoid recurrence and malignant transformation.

sarcomas, osteosarcoma and brain tumors.<sup>9-13</sup> Altered p53 expression has been detected in the nuclei of tumor cells with immunostaining on paraffin-embedded tissue sections.<sup>14-17</sup>

Ki-67 antigen is a cell proliferation-related protein that can be labeled with monoclonal antibody MIB-1. MIB-1 immunostaining can be applied on tissue sections to assess proliferative activity in different types of tumor, including breast carcinoma.<sup>18,19</sup> The percentage of positive cells, the MIB-1 index, is usually low in benign lesion and increases in malignant tumors.

Because of the difficulty in predicting the clinical outcome, and because even morphologically benign tumors are capable of local recurrence and occasional metastases,<sup>2,4</sup> ancillary studies in addition to routine morphology for predicting clinical behavior are necessary. This was a retrospective study to assess the correlation of p53 protein and Ki-67 antigen with the histologic

grading and clinical outcome of PT.

**METHODS**

From 1986 to 2000, 63 surgical specimens, including 8 recurrent tumors from 56 patients were found in the files of Mackay Memorial Hospital. Clinical information including the age, type of operation and follow-up data were reviewed.

We classified the tumors into benign and malignant groups. Tumors with 0-5 mitoses/10HPF and 1 or more of the following findings: mild or moderate increased cellularity, mild or moderate atypia and a predominantly pushing margin, were put into the benign category. Malignant tumors were defined as more than 5 mitoses/10 HPF and 1 or more of the following findings: moderately or mark increased cellularity, moderate or mark atypia, stromal overgrowth, a predominantly infiltrating margin. Stromal overgrowth was defined according to Ward and Evans.<sup>8</sup>

Representative formalin-fixed paraffin-embedded tissue sections showing the most mitotically active and cellular areas of each tumor were cut 5 um in thickness. Immunostaining using monoclonal antibodies DAKO-p53, DO7 and DAKO MIB-1 (DAKO, Holland), both with a dilution of 1:50 were performed, respectively. Positive and negative controls were included. Sections with squamous cell carcinoma that had been found previously stained positive were used for positive control of p53 and MIB-1. Negative controls were performed by

omitting the primary antibody. Nuclei with any detectable staining above background levels were scored as positive. The most active areas with maximal number of nuclei staining were chose to perform counting using a 10x10 square grid that had been placed in the eyepiece. The p53 expression and Ki-67 antigen (MIB-1 index) were defined as the percentage of positive nuclear staining after counting 1 thousand neoplastic stromal cells. We separate the percentage of p53 and Ki-67 into four groups as follows: 0-10%, 11-30%, 31-50% and 51-100%. Fisher’s exact probability test was performed to compare results of p53 and Ki-67 antigens with the morphological benign and malignant groups of PT. It was chosen as statistically significant if the *p*-value less than 0.05.

**RESULTS**

There were 50 benign and 13 malignant tumors. The patients ranged from 14 to 69 years old and the tumors ranged from 1.0 to 25 cm in size. The clinical findings are shown in Table 1.

The results of p53 and Ki-67 expression are listed in Table 2. The p53 expression in most of the benign tumors was low; negative to 10%. Five out of 50 (10%) benign tumors showed > 10% p53 expression. Nine out of 13 (69%) malignant tumors had p53 expression > 10% (Fig. 1). There were > 10% Ki-67 antigen in 11 out of 13 (85%) malignant tumors (Fig. 2). Only 8 out of 50 (16%) benign tumors revealed > 10% Ki-67. Three out of the 50 benign and 6 out of the 13 malignant cases reveal stromal over-

**Table 1. Clinical data of tumors of 56 patients**

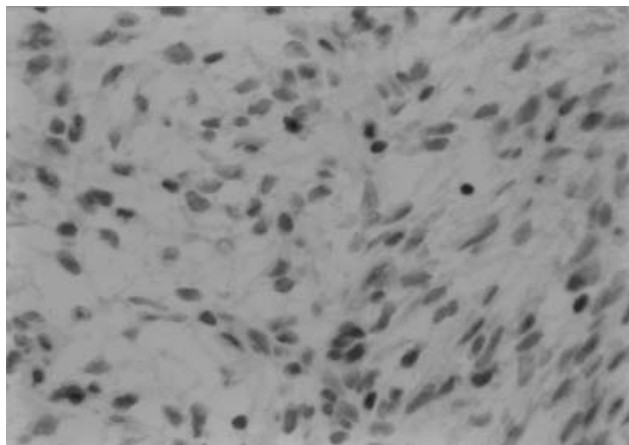
	No. of cases	Age of patients (years)		Size of tumor (cm)		Type of operation		
		Mean ± SD	Range	Mean ± SD	Range	EX	SM	MRM
Benign	50	42.4 ± 14.3	14 - 69	6.2 ± 5.4	1.0 - 25	30	20	0
Malignant	13	44.3 ± 9.9	27 - 62	8.2 ± 5.3	1.5 - 19	1	5	7

EX = excision; MRM = modified radical mastectomy; SM = simple mastectomy.

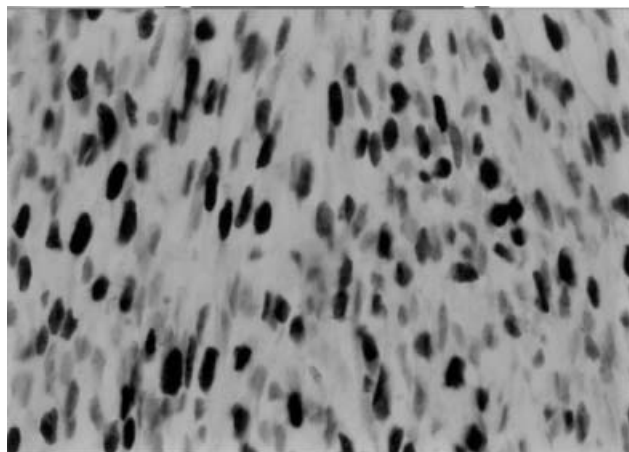
**Table 2. Expression of p53 and Ki-67 in phyllodes tumor of breast**

	No. of tumors	p53 (%)				Ki67 (MIB-1 index) (%)			
		0-10	11-30	31-50	51-100	0-10	11-30	31-50	51-100
Benign	50	45 (90%)	2 (4%)	3 (6%)	0 (0%)	42 (84%)	5 (10%)	2 (4%)	1 (2%)
Malignant	13	4 (31%)	3 (23%)	5 (38%)	1 (8%)	2 (15%)	5 (38%)	2 (15%)	4 (31%)

growth in routine sections. However, the 3 benign cases without recurrent showing stromal overgrowth had low



**Fig. 1.** Immunohistochemical staining shows slight increase in expression of p53 protein to 13% in this malignant tumor. (original magnification ×400).



**Fig. 2.** Immunohistochemical staining for Ki-67 is positive in many stromal cells in this malignant tumor. (original magnification ×400).

mitotic count and < 10% p53 and Ki-67.

The follow-up period ranged from 1 month to 15 years (mean 3 years). The period of the 13 malignant cases were 2 to 11 years (mean 7 years). No patient died of PT in the follow-up period. Three patients having > 10% p53 and 4 patients having > 10% Ki-67 had benign morphologic features and courses.

Seven patients had recurrent tumors, 2 to 7 years after the first operation. All 7 patients received tumor excision in first operation. In second operation, patients 1 and 2 received modified radical mastectomy; patients 3, 4, and 6 had simple mastectomy, patient 5 and 7 had repeat excision. Patient 7 got simple mastectomy in the third operation. Five patients having < 10% positivity of p53 and 3 patient having < 10% Ki-67 had recurrence.

The p53 and Ki-67 results of the 7 patients with recurrent tumors were listed in Table 3. Three patients (cases 1-3) progressed from benign to malignant tumors and all showed increased p53 expression in the recurrent tumors. The Ki-67 antigen was high, > 10% in both first and recurrent tumors in these three patients. Only 5 out of 47 benign tumors without or with benign recurrence showed increased Ki-67 expression > 10% ( $p < 0.005$ ). Four patients had recurrent benign tumors. Patient 7 had recurrence twice but benign morphology.

**DISCUSSION**

PT are rare neoplasms that may cause difficulty in predicting the clinical outcome by evaluating histologic features. Many authors classified PT into benign, borderline and malignant groups.<sup>1,5,6,15,17,20,21</sup> Other investiga-

**Table 3. P53 and Ki-67 in the first and recurrent phyllodes tumor**

Diagnosis	Interval of recurrence (years)	p53			Ki-67		
		1st	2nd	3rd	1st	2nd	3rd
1. Be to M	2	35%	71%		34%	25%	
2. Be to M	4	1%	16%		22%	23%	
3. Be to M	7	0%	33%		35%	28%	
4. Be to Be	2	0%	0%		0%	7%	
5. Be to Be	2	12%	19%		25%	60%	
6. Be to Be	5	0%	0%		0%	14%	
7. Be to Be	3 & 1	0%	0%	2%	0%	0%	8%

Be = benign, M = malignant. 1st = first specimen, 2nd = recurrent specimen, 3rd = second recurrence.

**Table 4. Summary of published reports about p53 and/or Ki-67 compared with grading of phylloid tumor**

	% of positive cases (positive cases/total cases)			
	% of positive cells	Benign	Borderline	Malignant
Feakins <sup>15</sup>	p53 > 30%	0% (0/27)	18% (3/17)	39% (5/13)
Gatalica <sup>23</sup>	p53 > 5%	0% (0/13)	NA	25% (3/12)
	Ki-67 mean%	7.73	NA	23.42
Kim <sup>16</sup>	p53	Negative (0/8)	NA	86% positive (6/7)
Kleer <sup>21</sup>	p53 positive	29% (2/7)	57% (4/7)	50%(3/6)
	Ki-67	3.6%	16%	50%
Kocova <sup>24</sup>	Ki-67 mean%	4.7	NA	15.4
Kuenen <sup>17</sup>	p53 > 10%	0% (0/10)	12.5%(1/8)	100%(1/1)
	Ki-67 > 20%	10% (1/10)	37.5%(3/8)	100%(1/1)
Millar <sup>22</sup>	p53 > 34%	0% (0/9)	NA	100% (6/6)
Niezabitowski <sup>20</sup>	p53 > 9%	4% (2/52)	0% (0/23)	14% (6/42)
	Ki-67 > 11.2%	4% (2/52)	17% (4/23)	52% (22/42)

NA = not applicable (the authors did not apply borderline category in their studies).

tors preferred benign and malignant without borderline category.<sup>7,16,22-24</sup> One report suggested that no difference could be identified between borderline and malignant lesions in terms of local and distant relapse.<sup>25</sup> Local recurrences may occur in all categories of PT.<sup>3,5,26</sup> Tumors with only mild atypia and low mitotic count such as 3/10HPF may have distant metastases after a period of follow-up.<sup>2</sup>

Several published series showed that expression of proliferative antigen Ki-67 and/or p53 protein were correlated with the tumor grading (Table 4).<sup>15-17,20-24</sup> In our study, 5 out of 50 benign and 9 out of 13 malignant cases revealed > 10% p53 Expression, which was significant ( $p < 0.005$  Fisher exact test). As for Ki-67 antigen, 11 out of 13 malignant but only 8 out of 50 benign tumors were > 10% ( $p < 0.005$  Fisher exact test).

Table 4 is a summary of the published series of p53 and Ki-67 expression.<sup>15-17,20-24</sup> There is no generalized accepted standard % to define "high expression". Different authors applied different cut-off levels, from 5-34% for p53 and 11.2-20% for Ki-67. However, all the authors concluded that expression of p53 and/or Ki-67 correlated with the morphologic grading, as they did our study.

We identified increased expression of p53 in recurrent tumors that progress from benign to malignant, patients 1-3 in Table 3. Over-expression of p53 is supported by a report about detecting a mutation in exon 7 of the p53 gene in a case that progressed from benign to malignant. The p53 expression progressed from 2 to > 50% in that case.<sup>23</sup> The Ki-67 antigen was high, 22-35%, in both

the first benign and recurrent malignant tumors of patients 1-3. Compared with the benign tumors without or with benign recurrence, only 5 out of 47 tumor reveal increased Ki-67 expression > 10% ( $p < 0.005$ ).

In Table 3, 2 patients with increased p53 value and four patients with increased Ki-67 got recurrence; beside, all 3 cases with malignant change in recurrent tumor showed increased Ki-67 in the first morphologically benign tumor. Only 1 case showed increase of p53% in the first benign tumor. As far as recurrence and malignant change are concerned, Ki-67 is the better indicator.

The recurrent tumor of case 5 in Table 3 revealed higher Ki-67, to 60%. However, the mitotic count was low, less than < 5 mitoses per 10 HPF. There is possibility that sampling error of mitotic count on routine sections might be misleading for tumor grading. The Ki-67 antigen reflects the true proliferation activity and is worrisome in regard to morphologically undergrading of tumors.

In conclusion, p53 and Ki-67 expression correlate well with the morphologic gradings of PT. Although there are some cases having < 10% positivity appear malignant and some cases > 10% who appear benign morphologically. The  $p$  value was < 0.005 (Fisher exact test) and is significant in comparing the cases with 0-10% to those with 11-100%. In tumors with benign morphology, additional study showing increased Ki-67 expression > 10% needs to be treated and followed up properly to avoid recurrence and malignant transformation.

## ACKNOWLEDGMENTS

This study was supported by grant 9147 from the Medical Research Department of Mackay Memorial Hospital, Taipei, Taiwan, R.O.C.

## REFERENCES

- Azzopardi JG, Ahmed A, Millis RR. Sarcoma of the breast. In: Bennington JL, ed. *Problems in breast and pathology*. Philadelphia: Saunders, 1979:346-65.
- Norris HL, Taylor HB. Relationship of histologic features to behavior of cystosarcoma phyllodes. *Cancer* 1967;20:2090-9.
- Pietruszka M, Barnes L. Cystosarcoma phyllodes: a clinicopathologic analysis of 42 cases. *Cancer* 1978;41:1974-83.
- Tavassoli FA. Phyllodes tumor. In: Tavassoli FA. *Pathology of the breast*. 1<sup>st</sup> ed. Stamford, Connecticut: Appleton & Lange, 1999:598-613.
- Moffat CJC, Pinder SE, Dixon AR, Elston CW, Blamey RW, Ellis IO. Phyllodes tumours of the breast: a clinical pathological review of thirty-two cases. *Histopathology* 1995;27:205-18.
- Rosen PP. Cystosarcoma phyllodes. In: Rosen PP. *Rosen's Breast Pathology* 1<sup>st</sup> ed. Philadelphia: Lippincott – Raven, 1997:155-72.
- Hart WR, Bauer RC, Oberman HA. Cystosarcoma phyllodes: a clinicopathologic study of twenty-six hypercellular periductal stromal tumors of the breast. *Am J Clin Pathol* 1978;70:211-6.
- Ward RM, Evans HL. Cystosarcoma phyllodes: a clinicopathologic study of 26 cases. *Cancer* 1986;58:2282-9.
- Barbareschi M, Leonardi E, Mauri FA, Serio G, Dalla Palma P. p53 and c-erbB-2 protein expression in breast carcinomas. An immunohistochemical study including correlations with receptor status, proliferation markers, and clinical stage in human breast cancer. *Am J Clin Pathol* 1991;98:408-18.
- Turner BC, Gumbs AA, Carbone CJ, Carter D, Glazer PM, Haffty BG. Mutant p53 protein overexpression in women with ipsilateral breast tumor recurrence following lumpectomy and radiation therapy. *Cancer* 2000;88:1091-8.
- Andreassen A, Oyjord T, Hovig E, Holm R, Florenes VA, Nesland JM, et al. P53 abnormalities in different subtypes of human sarcomas. *Cancer Res* 1993;53:468-71.
- Bodey B, Groger AM, Bodey B Jr, Siegel SE, Kaiser HE. Immunohistochemical detection of p53 protein overexpression in primary human osteosarcomas. *Anticancer Res* 1997;17:493-8.
- Yaziji H, Massarani-Wafai R, Gujrati M, Kuhns JG, Martin AW, Parker JC Jr. *Am J Surg Pathol* 1996;20:1086-90.
- Bruner JM, Connelly JH, Saya H. p53 protein immunostaining in routinely processed paraffin-embedded sections. *Mod Pathol* 1993;6:189-94.
- Feakins R, Mulcahy HE, Nickols CD, Wells CA. p53 expression in phyllodes tumours is associated with histological features of malignancy but does not predict outcome. *Histopathology* 1999;35:162-9.
- Kim CJ, Kim WH. Patterns of p53 expression in phyllodes tumors of the breast. *J Korean Med Sci* 1993;8:325-8.
- Kuenen-Boumeester V, Henzen-Logmans SC, Timmermans MM, Van Staveren IL, Geel AV, Peeterse HJ, et al. Altered expression of p53 and its regulated proteins in phyllodes tumors of the breast. *J Pathol* 1999;189:169-75.
- Weidner N, Moore DH II, Vartanian R. Correlation of Ki-67 antigen expression with mitotic figure index and tumor grade in breast carcinomas using the novel "paraffin" reactive MIB1 antibody. *Hum Pathol* 1994;25:337-42.
- Biesterfeld S, Kluppel D, Koch R, Schneider S, Steinhagen G, Mihalcea AM, et al. Rapid and prognostically valid quantification of immunohistochemical reactions by immunohistometry of the most positive tumour focus: a prospective follow-up study on breast cancer using antibodies against MIB-I, PCNA, ER, and PR. *J Pathol* 1998;185:25-31.
- Niezabitowski A, Lackowska B, Rys J, Kruczak A, Kowalska T, Mitus J, et al. Prognostic evaluation of proliferative activity and DNA content in the phyllodes tumor of the breast: immunohistochemical and flow cytometric study of 118 cases. *Breast Cancer Res Treat* 2001;65:77-85.
- Kleer CG, Giordano TJ, Braun T, Oberman HA. Pathologic, immunohistochemical, and molecular features of benign and malignant phyllodes tumors of the breast. *Mod Pathol* 2001;14:185-90.
- Millar EKA, Beretov J, Marr P, Sarris M, Clarke RA, Kearsley, Lee CS. Malignant phyllodes tumours of the breast display increased stromal p53 protein expression. *Histopathology* 1999;34:491-6.
- Gatalica Z, Finkelstein S, Lucio E, Tawfik O, Palazzo J, Hightower B, Eyzaguirre E. p53 protein expression and gene mutation in phyllodes tumors of the breast. *Pathol Res Pract* 2001;197:183-7.
- Kocova L, Skalova A, Fakan F, Rousarova M. Phyllodes tu-

- mour of the breast: immunohistochemical study of 37 tumours using MIB1 antibody. *Pathol Res Pract* 1998;194: 97-104.
25. Salvadori B, Cusumano F, Del Bo R, Delledonne V, Grassi M, Rovini D, *et al.* Surgical treatment of phyllodes tumors of the breast. *Cancer* 1989;63:2532-6.
26. Hawkins RE, Schofield JB, Fisher C, Wiltshaw E, McKinna JA. The clinical and histologic criteria that predict metastases from cystosarcoma phyllodes. *Cancer* 1992;69: 141-7.