

## RESEARCH COMMUNICATION

# Prognostic Value of p53 Expression in Early Stage Cervical Carcinoma Treated by Surgery

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### Abstract

**Objective:** To evaluate the prognostic significance of p53 protein expression in patients with early stage cervical carcinoma treated by surgery alone in a well-controlled study. **Methods:** A matched case-control study was performed in patients with stage Ib-IIa cervical carcinoma who underwent radical hysterectomy with pelvic lymphadenectomy. Patients had neither lymph node metastasis nor involvement of the parametrium and surgical margins, and did not receive any adjuvant treatment. Cases included 30 patients who had tumor recurrence within 5 years after surgery; controls included 60 patients who were disease-free for at least 5 years after surgery. Cases and controls were within 10 years of age, had the same stage and tumor type, and underwent surgery on as close to the same date as possible. The tumor sizes of cases and controls were within 1 cm of each other. Expression of p53 protein was studied by immunohistochemistry. Expression was considered positive when at least 10% of tumor cells showed nuclear staining. **Results:** No significant difference of p53 expression was observed between the case group and the control group (33% versus 40%). High histologic grade of tumors and lymphovascular space invasion were significantly associated with tumor recurrence in multivariable analysis ( $p=0.012$  and  $0.014$ , respectively). **Conclusion:** In this study, expression of p53 did not correlate with tumor recurrence. Immunohistochemistry for p53 protein appears to provide no prognostic information in the patients with early stage cervical cancer treated by surgery.

**Key Words:** Uterine cervix - carcinoma - recurrence - p53 - radical hysterectomy

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### Introduction

Cervical cancer is one of the most common cancers in women worldwide (Pisani et al., 2002). Most cervical cancer patients in early stage (Ib-IIa) can be successfully treated by radical hysterectomy with pelvic lymphadenectomy, with an overall 5-year survival rate of 80-95 % (Burke et al., 1987; Larson et al., 1988; Aoki et al., 2001). In these surgically treated patients, several factors may be predictive of the risk for recurrence such as large tumor size, adenocarcinoma histology, high tumor grade, deep stromal invasion, lymphovascular space invasion, parametrial invasion, positive surgical margins, and lymph node metastasis, with the latter three being sufficiently adverse to justify adjuvant radiation therapy (Burke et al., 1987; Larson et al., 1988; Smiley et al., 1991; Schorge et al., 1997; Zreik et al., 1996; Manusirivithaya et al., 2001). However, approximately 10 % of the patients without significantly adverse factors still develop tumor recurrence (Burke et al., 1987; Larson et al., 1988;

Manusirivithaya et al., 2001). To date, there is no consensus about other criteria for identifying patients with high risk of recurrence among those without the involvement of lymph nodes, parametrial tissue, or a surgical margin (Koh et al., 2000). Biomolecular markers therefore deserve careful study.

The p53 gene on chromosome 17 encodes a 53-kDa protein that acts as a tumor suppressor gene and encodes a DNA-binding transcription factor that is responsible for cell cycle checkpoints that are activated after exposure to DNA damaging agents (Ashcroft et al., 2000). Immunohistochemistry is available for the evaluation of p53 protein expression. Since this protein is not normally detected in tissues due to its short half life, positive staining for p53 protein by immunohistochemistry is considered to be abnormal and may be caused by p53 mutation or abnormal accumulation of p53 protein in the absence of gene mutation (Hall and Lane, 1994; McCluggage et al., 2005). p53 expression is felt to be a poor prognostic predictor in many types of malignancies;

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however, studies on p53 expression in cervical carcinoma have reported conflicting results with respect to prognostic significance (Bremer et al., 1995; Tsuda et al., 1995; Hunt et al., 1996; Uchiyama et al., 1997; Rajkumar et al., 1998; ; Chen et al., 2000; Dimitrakakis et al., 2000; Tjalma et al., 2001; Horn et al., 2001; Huang et al., 2001; Brenna et al., 2002; Graflund et al., 2002; Jain et al., 2003; Wootipoom et al., 2004; Ikuta et al., 2005; Shiohara et al., 2005).

Most studies on p53 expression in cervical carcinoma had heterogeneous patient populations and non-uniform methods for p53 immunohistochemistry. Thus, a comparison of the previously reported data on the prognostic role of p53 in cervical cancer is difficult and it remains uncertain whether p53 expression is a useful prognostic predictor for tumor recurrence in early stage cervical cancers. A study of a more homogenous population may help clarify the prognostic significance. To this end, a matched case-control study was designed to control the known confounding factors affecting tumor recurrence such as patient age, tumor stage, histologic type, and tumor size. Other possible associated factors such as histologic grade, depth of invasion and lymphovascular space invasion were also evaluated. The objective of this study was to assess the value of immunohistochemical detected p53 as an additional prognostic indicator for guiding management of patients with surgically-treated, early stage cervical cancer.

## Materials and Methods

### *Patients and variables*

To study the association between p53 expression and tumor recurrence in cervical cancer patients, we used a matched case-control study design. The medical records including follow-up information of all patients with stage Ib-IIa cervical carcinoma, who were primarily treated by radical hysterectomy with pelvic lymphadenectomy between January 1992 and June 1998 at Chiang Mai University Hospital were reviewed. Exclusion criteria were: 1) neuroendocrine carcinoma histology, 2) lymph node metastasis or parametrial or surgical margin involvement, 3) pre- or post-operative adjuvant treatment, and 4) death from other causes. Postoperatively, patients were followed under the standard surveillance program and were investigated for recurrence if there was any suspicious symptom and sign from clinical history and physical examination. All patients who had tumor recurrence within 5 years after surgery were defined as 'cases'. There were 30 recurrent cases identified in this study. Presence of tumor recurrence was detected by physical examination and/or radiologic investigations, and confirmed in 17 cases by biopsy.

Controls were those who had no recurrence after the follow-up period of at least 5 years. Controls were selected to be within 10 years of age from cases with recurrence and having the same stage of disease. The tumors of recurrent cases and controls were matched for tumor histology (squamous or non-squamous) and size (within one centimeter difference in maximum dimension). Two controls, who were operated on nearest to the date of

**Table 1. Summary of Recurrent Cases and Controls**

Parameter	Recurrent Cases (n = 30)	Controls (n = 60)
Mean age (year)	41.1 (± 7.1)	40.8 (± 6.1)
Mean tumor size (cm)	2.7 (± 1.5)	2.6 (± 1.4)
Stage		
Ib	28 (93.3%)	56 (93.3%)
IIa	2 (6.7%)	4 (6.7%)
Histology		
Squamous CA	19 (63.3%)	38 (63.3%)
Adenocarcinoma	8 (26.7%)	18 (30.0%)
Adenosquamous CA	3 (10.0%)	4 (6.7%)

surgery for each recurrent case, were chosen according to the eligibility criteria. Sixty controls were identified, over 80% of which were operated on within 2 years of the date of surgery of the matching recurrent cases.

Histologically, all tumors of cases and controls were classified as squamous cell carcinoma or non-squamous carcinoma (adenocarcinoma and adenosquamous carcinoma). Histologic variables also assessed included: histologic grade (grade 1 versus 2-3), depth of invasion ( $\leq$  versus  $>$  inner half of cervical wall thickness), and lymphovascular space invasion (presence versus absence). All dissected lymph nodes were reviewed (mean 29.8 nodes for each case) and confirmed to be negative.

### *p53 expression*

A formalin-fixed, paraffin-embedded tissue block containing carcinoma was cut at 3 microns for each recurrent case and for each control. The tissue sections were deparaffinized, hydrated in graded alcohols ending in distilled water, and blocked for endogenous peroxidase with 3% hydrogen peroxide. For antigen retrieval, sections were boiled in TE-buffer (10 mM Tris, 1 mM EDTA, pH 9.0) for 7 minutes in a pressure cooker. Then, monoclonal mouse anti-human p53 protein (clone DO-7, DAKO, dilution 1:2000) was applied to the sections and left at room temperature for 1 hour. The reaction was detected with streptavidin-biotin-peroxidase complex and stained with diaminobenzidine (DAB). The sections were then counterstained with hematoxylin. Positivity of p53 protein was evaluated in tumor nuclei. In each section, at least 1,000 tumor nuclei were counted at x1000 magnification, excluding those in necrotic area. The p53 expression was scored based on the percentage of positive cells to the total number of tumor cells counted. A minimum of 10% positive cells was used as the cut-off value for positive p53 expression. Variable cut-off percentages of cells other than 10% (0, 5, 20, 30, and 50%) were also applied to evaluate the effect of the cut-off value on the prognostic significance of p53 expression. Positive control sections for p53 expression included high-grade serous adenocarcinoma and colorectal cancer, both showing diffuse staining ( $> 95\%$  cells). Negative control sections included normal proliferative endometrium and immature metaplastic squamous epithelium, both showing staining in less than 10% cells.

### *Statistical analysis*

For statistical analysis, the association between p53

protein expression and clinicopathological factors for case and control groups was evaluated by simple conditional logistic regression analysis for univariable analysis, and by conditional multiple logistic regression analysis for multivariable analysis. A p value of  $\leq 0.05$  was considered as statistically significant. Statistical software used in this study was STATA version 7.0.

## Results

### Patients

A summary of the clinical and pathologic details of the recurrent cases (n = 30) and controls (n = 60) is presented in Table 1. The mean age and the mean tumor size of both groups were similar (41.1 versus 40.8 years and 2.7 versus 2.6 cm, respectively). The majority of the patients had stage Ib tumors (93.3% in each group) and had squamous cell carcinoma (63.3% in each group).

### p53 expression

The mean percentage of positive cells was 7.73% in recurrent cases (range 0 to 28.12%) and 8.9% in controls (range 0 to 65.65%). No positive staining in benign cervical mucosa was observed. p53 protein was more frequently expressed in squamous cell carcinoma compared to adenocarcinoma ( $p < 0.001$ ). The proportion of squamous cell carcinoma with positive expression was 47% in recurrent cases and 55% in controls, whereas that

**Table 2. Association between Microscopic Findings and Tumor Recurrence by Univariable Analysis**

	Recurrent cases (n=30)		Controls (n=60)		Crude odds ratio	95% CI of odds ratio	p value
p53 expression							
Negative	20	66.7	36	60.0	1 <sup>a</sup>		
Positive	10	33.3	24	40.0	0.7	0.3-1.9	0.518
Tumor grade							
1	6	20.0	29	48.3	1 <sup>a</sup>		
2-3	24	80.0	31	51.7	8.1	1.8-36.9	0.007
Lymphovascular space invasion							
Absent	8	26.7	33	55.0	1 <sup>a</sup>		
Present	22	73.3	27	45.0	3.8	1.3-10.6	0.013
Depth of invasion							
$\leq$ inner half	8	26.7	25	41.7	1 <sup>a</sup>		
$>$ inner half	22	73.3	35	58.3	2.0	0.8-5.3	0.160

<sup>a</sup> reference group

**Table 3. Association between p53 Expression and other Microscopic Findings with Tumor Recurrence by Multivariable Analysis**

	Adjusted odds ratio <sup>a</sup>	95% CI of odds ratio <sup>a</sup>	p value
p53 expression (negative vs. positive)	0.7	0.2-2.2	0.520
Tumor grade (1 vs. 2-3)	7.9	1.5-40.6	0.014
Lymphovascular space invasion (absent vs. present)	4.8	1.4-16.1	0.012
Depth of invasion ( $\leq$ inner half vs. $>$ inner half)	1.8	0.6-5.7	0.306

<sup>a</sup>adjusted for the remaining variables in the table

of non-squamous carcinoma was 9% and 14%, respectively. Tumors with positive expression ( $\geq 10\%$  positive cells) were slightly less frequent in the recurrent case group (33%) compared to controls (40%). The difference did not reach the significance level ( $p = 0.518$ ). There was no significant difference in the proportion of p53 expression between cases and controls at different cut-off values (0 to 50 %) for positive expression (results not shown).

### Other histologic variables

Other histologic parameters were assessed for recurrent cases versus controls and the results are presented in Table 2. High histologic grade (grade 2-3) and the presence of lymphovascular space invasion were significantly more frequent in the recurrent case group compared to the control group (80.0 % versus 51.7 % for grade,  $p = 0.007$ ; and 73.3 versus 45.0 % for lymphovascular space invasion,  $p = 0.013$ , respectively). The proportion of cases with deep stromal invasion ( $>$  the inner half) was higher in cases than in controls (73.3 % versus 58.3 %), but the difference was not statistically significant ( $p = 0.160$ ).

### Multivariable analysis

The conditional multiple logistic regression analysis showed that high histologic grade and lymphovascular space invasion were significantly associated with tumor recurrence ( $p = 0.014$  and  $0.012$ , respectively). The results are presented in Table 3. There was no significant difference in p53 expression between recurrent cases and controls. No correlation between p53 expression and other prognostic histologic variables (tumor grade, depth, lymphovascular space invasion) was observed (results not shown).

## Discussion

The present study aimed to resolve whether p53 expression is a useful prognostic predictor in a surgically treated cervical cancer patients using a matched case-control study design. Previous studies have shown discordant results with respect to p53 expression in cervical carcinoma. Most were based on patient populations with variable proportions of mixed tumor stages (ranging from Ia to IV) or mixed treatment modalities (surgery and/or radiation therapy). Even in series confined to stage Ib-IIa patients, there were still mixed population of patients with and without lymph node metastasis, parametrial or surgical margin involvement, resulting in variable proportions of patients receiving adjuvant therapy (Hunt et al., 1996; Uchiyama et al., 1997; Chen et al., 2000; Dimitrakakis et al., 2000; Horn et al., 2001; Graflund et al., 2002; Ikuta et al., 2005). Furthermore, follow-up duration of the patients was not always uniform, resulting in the inclusion of cases with a relatively short follow-up in some studies (Bremer et al., 1995; Chen et al., 2000; Tjalma et al., 2001; Ikuta et al., 2005). All patients in our study had stage Ib-IIa tumors and were treated only by radical hysterectomy with pelvic lymphadenectomy under the same surgical techniques and

follow-up protocol. In all patients, there was no pelvic lymph node metastasis and no tumor involvement of parametrial tissue and surgical margin. To our knowledge this is the only well-controlled study on p53 expression in early stage cervical cancer.

Although p53 mutations are rare in cervical carcinomas (Tommasino et al., 2003), the frequency of p53 expression in previous reports has ranged from 9% to as high as 75% of cervical cancers (Graflund et al., 2002; Wootipoom et al., 2004), suggesting p53 may be playing an important role in this cancer, and possibly on the basis of abnormal accumulation of non-mutant p53 protein due to altered p53 homeostasis in tumor cells rather than p53 mutation (Ikuta et al., 2005; McCluggage et al., 2005). The results for p53 expression in our study are in keeping with the range of 30-40% in recent studies on early stage cervical carcinomas (Chen et al., 2000; Dimitrakakis et al., 2000; Ikuta et al., 2005). Expression of p53 protein was much more common in squamous cell carcinoma than in adenocarcinoma similar to previously reported data (Hunt et al., 1996; Dimitrakakis et al., 2000; Horn et al., 2001; Tjalma et al., 2001). The proportion of cells expressing p53 in our recurrent cases was generally low (<30% of cells positive) and was slightly lower than observed in the control group.

There was no correlation of p53 expression with tumor size, histologic grade, depth of invasion, and lymphovascular space invasion in early stage cervical carcinomas. In some studies that included cases with advanced tumor stages, p53 expression was reported to correlate with stage, tumor size, and grade (Bremer et al., 1995; Tjalma et al., 2001; Huang et al., 2001; Shiohara et al., 2005). It is possible that p53 expression might increase in the more advanced stages of cervical carcinoma due to increased abnormalities in control of p53 expression or degradation or an increase incidence of p53 mutation (Hall and Lane, 1994; McCluggage et al., 2005). Histologic grade of tumor and lymphovascular space invasion remained histologic variables that in our study were significantly associated with tumor recurrence by both uni- and multivariable analysis, similar to previous observations (Smiley et al., 1991; Schorge et al., 1997). Depth of invasion of more than the inner half of cervical wall thickness also tended to correlate with a risk of recurrence (73.3 % versus 58.3 %).

Variation in the immunohistochemical methods with respect to specific antibody clones, dilutions, antigen retrieval methods and the cut-off criteria for positive expression can also affect results and contribute to the controversy over the prognostic value of p53 detection (McCluggage et al., 2005). In an endometrial neoplasia model by McCluggage et al (McCluggage et al., 2005), expression of p53 protein was observed in normal proliferative endometrium at low dilutions (1:10 to 1:1000) of the most commonly used p53 antibody (DO-7) (McCluggage et al., 2005). This finding suggested that p53 expression detected by low antibody dilution could sometimes be non-specific and unreliable. It should be noted that dilutions in the range of 1:50-1:100 have been used in many of the studies that showed a lack of prognostic significance of p53 expression in cervical

cancer (Hunt et al., 1996; Dimitrakakis et al., 2000; Tjalma et al., 2001; Graflund et al., 2002; Brenna et al., 2002; Wootipoom et al., 2004). This raises the question as to whether the absence of prognostic significance of p53 expression might be related to a loss of specificity of p53 immunostaining. The dilution of p53 antibody used in our setting (1:2000) was based on recent suggestions for p53 immunostaining (McCluggage et al., 2005). The 10% cut-off level used in this study is the most commonly used value. Nevertheless, using different cut-off levels did not seem to significantly affect the association between p53 expression and prognosis.

In conclusion, using a carefully controlled patient population, we were able to show that p53 expression failed to predict tumor recurrence in early stage cervical cancer. p53 expression did not correlate with tumor recurrence in surgically treated cervical cancer patients without involvement of pelvic lymph nodes, parametrium, or surgical margins. On the other hand, the histologic parameters of tumor grade and lymphovascular space invasion were predictive of tumor recurrence. Expression of p53 protein by immunohistochemistry does not appear to provide prognostic information to guide further management in this group of patients.

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