RESEARCH ARTICLE

Prognostic Value of Pathological Characteristics of Invasive Margins in Early-stage Squamous Cell Carcinomas of the Uterine Cervix

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Abstract

Background: To evaluate the pathological characteristics of invasive margins in early-stage cervical squamous cell carcinomas and their association with other clinicopathological features including clinical outcomes. Materials and Methods: Patients with FIGO stage IB-IIA cervical squamous cell carcinomas who received surgical treatment and had available follow-up information were identified. Their histological slides were reviewed for prognostic variables including tumor size, grade, extent of invasion, lymphovascular invasion, involvement of vaginal margin or parametrium, and lymph node metastasis. The characteristics of invasive margins including invasive pattern (closed, finger-like, or spray-like type), degree of stromal desmoplasia, and degree of peritumoral inflammatory reaction were evaluated along the entire invasive fronts of tumours. Associations between the characteristics of invasive margins and other clinicopathological variables and disease-free survival were assessed. Results: A total of 190 patients were included in the study with a median follow-up duration of 73 months. Tumour recurrence was observed in 18 patients (9%). Spray-like invasive pattern was significantly more associated as compared with closed or finger-like invasive pattern (p=0.005), whereas the degree of stromal desmoplasia or peritumoral inflammatory reaction was not. Low degree of peritumoral inflammatory reaction appeared linked with lymph node metastasis (p=0.021). In multivariate analysis, a spray-like invasive pattern was independently associated with marked stromal desmoplasia (p=0.013), whilst marked desmoplasia was also independently associated with low inflammatory reactions (p=0.009). Furthermore, low inflammatory reactions were independently associated with positive margins (p=0.022) and lymphovascular invasion (p=0.034). The patients with spray-like invasive pattern had a significantly lower disease-free survival compared with those with closed or finger-like pattern (p=0.004). Conclusions: There is a complex interaction between cancer tissue at the invasive margin and changes in surrounding stroma. A spray-like invasive pattern has a prognostic value in patients with early-stage cervical squamous cell carcinoma.

Keywords: Cervical cancer - squamous cell carcinoma - prognosis - invasive pattern - stromal reaction

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Introduction

Cerivical cancer is one of the most common cancers in women. Early-stage cervical cancer (FIGO stage IB-IIA) is treated by radial hysterectomy with pelvic lymph node dissection. Histopathological examination of the resection specimen provides important information used to justify adjuvant treatment. Potential histopathological prognostic features recognized in most studies include tumor grade, the presence of lymphovascular space invasion (LVSI), and the extent of tumor invasion or spread (e.g. deep stromal invasion, involvement of vaginal margin or parametrium, and lymph node metastasis) (Suprasert et al., 2010). The pathological characteristics of invasive margin or tumor front have been described as potential prognostic features in several types of cancers (Bryne et al., 1998). However, there is a limited number of studies focusing on these findings in cervical cancer (Kainz et al., 1994; Chao et al., 1999; Kristensen et al., 1999; Horn et al., 2006a; 2006b; 2012; Eggen et al., 2007; Fregnani et al., 2007).

The main histopathologic characteristics at the invasive margin of carcinoma include the invasive growth pattern of carcinoma and the associated changes in the surrounding stroma such as stromal desmoplasia and peritumoral inflammatory reaction (Horn et al., 2006a; Horn et al., 2006b). Invasive pattern may reflect the spreading capacity of cancer cells and may be associated with decreased survival in cervical cancer patients (Horn et al., 2006a). The stroma adjacent to invasive carcinoma could be induced to have an immature appearance (stromal desmoplasia), which may, in turn, promote the growth

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and metastatic potential of cancer cells (Tsujino et al., 2007; Polanska and Orimo, 2013). On the other hand, peritumoral inflammatory reaction may reflect a host immunologic response against cancer cells and has been reported to be a favorable prognostic feature (Kainz et al., 1994; Fregnani et al., 2007).

In this study, we aimed to evaluate the association of the histological characteristics of invasive margin (invasive pattern, stromal desmoplasia, and peritumoral inflammatory reaction) in early-stage cervical squamous cell carcinoma, and to assess the association between each characteristic and other clinicopathological features including clinical outcomes.

Materials and Methods

The study was approved by the Institutional Ethics Committee. Patients with FIGO stage IB-IIA squamous cell carcinoma of the uterine cervix treated by radical hysterectomy with pelvic lymph node dissection at Chiang Mai University Hospital between January 2003 and December 2006 were identified. The clinical data and follow-up results were obtained from medical records. Only the cases with follow-up information were included in this study. Regarding the treatment policy for cervical cancer patients, post-operative adjuvant radiation and/or chemotherapy was given when patients had any of the high-risk pathological factors (i.e. lymph node metastasis, parametrial involvement, or vaginal margin involvement) or when patients had a combination of extensive LVSI and residual uninvolved cervical stromal thickness <3 mm. Extensive LVSI was defined by a total count of more than 10 foci of LVSI in all tumor sections.

The histologic slides of each case were reviewed by one pathologist (S.S.). The pathologic characteristics were recorded as follows: tumor size, histologic grade, depth of cervical wall invasion (fractional thirds), residual uninvolved cervical stromal thickness, LVSI, and lymph node metastasis. The slides were subsequently reviewed by another pathologist (S.K.) for the characteristics of invasive margin (invasive pattern, stromal desmoplasia, and peritumoral inflammatory reaction), without knowledge of the clinical data or the results of previous pathological review.

In all tumor sections from each case, the characteristics of invasive margin were evaluated within a zone of 1 low-power field width (5.0 mm diameter, ×40 magnification) along the invasive margin of carcinoma by placing the deepest point of invasion at the center of field. Invasive pattern was classified as closed (or pushing margin), finger-like, and spray-like pattern, based on previous descriptions (Horn et al., 2006a) (Figure 1), with additional quantitative criteria. The closed pattern was defined by a circumscribed tumor border composed of closely packed nests or trabeculae of carcinoma cells. The finger-like pattern was defined by infiltration of nests or trabecular groups neoplastic cells with rounded edges. The spray-like pattern was defined by infiltration of small irregular cell groups with sharpened edges or the presence of single cells. When there was a mixture of invasive patterns, the classification was based on the predominant pattern that involved more than 50% of tumor margin areas, except for the diagnosis of closed pattern which required the presence of circumscribed border in more than 90% of margin areas. When there was a mixture between the finger-like pattern and the spray-like pattern, the classification was based on the predominant pattern that involved more than 50% of tumor margin areas.

The degree of stromal desmoplasia was scored into 3 grades: grade 1, absent or minimal, grade 2, moderate, and grade 3, marked. Marked desmoplasia was characterized by the presence of thick zone of loose immature stroma composed of fibroblastic proliferation that was evidently observed at the low-power magnification in more than 50% of tumor margin areas (Figure 2A).

The degree of peritumoral inflammatory infiltration was scored into 3 grades; grade 1, absent or minimal, grade 2, moderate, and grade 3, marked. Marked inflammatory reaction was defined by the presence of a continuous rim of leukocytic infiltration along the invasive margin in all tumor slides with at least a focal thick zone of dense cellular infiltration (Figure 2B). Type of inflammatory infiltration was classified by the predominant population (more than 50%) of infiltrating leukocytes such as lymphocytes, plasma cells, and eosinophils.

The clinicopathologic data were analyzed using STATA software version 11. The association between variables or between each variable and clinical outcome was evaluated by Fisher Exact test. Multivariate analyses were performed by logistic regression using the variables

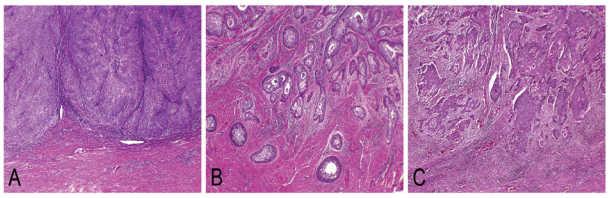

Figure 1. Invasive Patterns in Cervical Squamous Cell Carcinoma (Original Magnification ×40). A) Closed pattern, **B)** Finger-like pattern, and **C)** Spray-like pattern

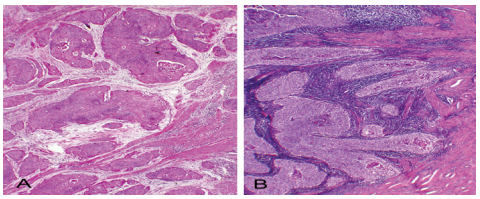

Figure 2. Changes in the Surrounding Stroma of Cervical Squamous Cell Carcinoma (original Magnification ×40). A) Marked stromal desmoplasia, and **B)** Marked inflammatory reaction

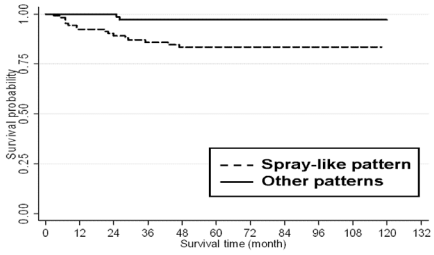

Figure 3. Kaplan-Meier Plot of Spray-Like Invasive Pattern and Disease-Free Survival (p=0.004)

with p value <0.1 in univariate analysis. Disease-free survival was estimated by Kaplan-Meier method and log-rank test for equality of survivor function. A p value <0.05 was considered as statistically significant.

Results

There were 190 cases of FIGO stage IB1-2 to IIA cervical squamous cell carcinoma with available follow-up included in this study. The mean patient age was 45.1 ±SD 8.9 years. Pelvic lymph node metastasis was present at surgery in 53 patients (28%). One hundred and nine patients (57%) received post-operative adjuvant therapy. Tumor recurrence was observed in 18 patients (9%). The follow-up period ranged from 3 to 120 months (mean 66.3 ±SD 33.4 months, median 73 months).

Regarding the invasive pattern, tumors with spray-like pattern accounted for the majority of cases (n=111, 58%), followed by those with finger-like and closed patterns (n=65, 34%, and n=14, 7%, respectively). In 15 of 111 cases with spray-like pattern, the invasive fronts of tumors also showed areas of closely packed irregular infiltrative groups mimicking the closed pattern. In 5 of 65 tumors with finger-like pattern, a component of spray-like pattern involving between 10 and 50% of margin areas was observed.

Table 1. Association between the Histologic Characteristics of Invasive Margin and the Rates of Lymph Node Metastasis and Tumor Recurrence

Histologic characteristics (n=190)	No. of cases with lymph node metastasis (%)	p value	No. of cases with recurrence (%)	p value
Predominant invasive pattern				
Closed, n=14	6 (43)	0.087*	0 (0)	0.005*
Finger-like, n=65	17 (26)		2 (3)	
Spray-like, n=111	30 (27)		16 (14)	
Degree of stromal desmoplasia				
Grade 3, n=43	15 (35)	0.252†	5 (12)	0.562†
Grade 2, n=89	23 (26)		9 (10)	
Grade 1, n=58	15 (26)		4 (7)	
Degree of peritumoral inflammatory reaction				
Grade 3, n=56	9 (16)	0.021†	4 (7)	0.594†
Grade 2, n=113	35 (31)		12 (11)	
Grade 1, n=21	9 (43)		2 (10)	

*Spray-like pattern vs other patterns, †Grade 3 (marked) vs grade 1-2 (low)

Table 2. Univariate Analysis for the Association between the Histologic Characteristics of Invasive Margin and other Clinical and Pathologic Variables

Variable	Invasive pattern* p value	Degree of desmoplasia† p value	Degree of inflammation‡ p value
Spray-like invasive pattern	-	0.008	NS
Marked desmoplasia	0.008	-	<0.001
Low inflammatory reaction	NS	<0.001	-
Age >45 years	NS	NS	0.01
Stage IB2-IIA	NS	NS	NS
Tumor size >4 cm	NS	NS	NS
Tumor grade 2-3	NS	NS	NS
Deep invasion (outer third)	NS	0.048	0.025
Residual stroma <3 mm	NS	0.001	<0.001
LVSI	NS	NS	<0.001
Extensive LVSI	0.036	0.078	<0.001
Parametrial involvement	NS	0.009	<0.001
Positive vaginal margin	NS	NS	0.003

*Spray-like pattern vs other patterns, †Marked degree vs low degree, ‡Low degree vs marked degree LVSI: lymphovascular space invasion, NS: p value>0.1

Table 3. Multivariate Analysis for the Assoication of Histologic Characteristics of Invasive Margin with Other Pathologic Features (Variables without correlation not shown)

Characteristic of invasive margin	p value	Odds ratio	95% confidence interval
Spray-like invasive pattern			
Marked desmoplasia	0.013	2.71	1.23-5.94
Extensive LVSI	0.058	1.81	0.98-3.35
Marked desmoplasia			
Spray-like invasive pattern	0.006	3.22	1.39-7.45
Low inflammatory reaction	0.009	5.57	1.54-20.08
Low inflammatory reaction			
Marked desmoplasia	0.018	4.87	1.31-18.13
Positive vaginal margin	0.022	12.99	1.44-117.34
Lymphovascular space invasion	0.034	2.65	1.08-6.50
Parametrial involvement	0.063	3.11	0.94-10.28

Stromal desmoplasia grade 3 was observed in 43 patients (23%), grade 2 in 89 (47%), and grade 1 in 58 (31%). Peritumoral inflammatory reaction grade 3 was observed in 56 patients (29%), grade 2 in 113 (59%), and grade 1 in 21 (11%). The inflammatory cells were predominantly lymphocytes and plasma cells in the large majority of cases (n=175, 92%). Eosinophils were predominant in only 15 cases (8%), and accounted for 10-50% of inflammatory cell population in another 40 cases (21%).

Table 1 shows the rate of lymph node metastasis and tumor recurrence in each type or degree of the 3 characteristics of invasive margin (invasive pattern, stromal desmoplasia, and peritumoral inflammatory reaction). Cases with spray-like invasive pattern had the highest recurrence rate compared with those with closed pattern or finger-like pattern (p=0.005). Among tumors with spray-like pattern, there was no difference in the recurrence rate between tumors with usual spray-like pattern and those with areas mimicking the closed pattern (14% vs 20%, p=0.451). Among tumors with finger-like pattern, 5 cases with a minor component of spray-like pattern had no recurrence.

Stromal desmoplasia was not associated with nodal metastasis or recurrence. The degree of peritumoral inflammatory reaction was inversely associated with the risk of lymph node metastasis (p=0.021). The predominant type of inflammatory cells was not associated with lymph node metastasis (p=0.241) and recurrence (p>0.999).

Table 2 shows a summary of the association between the characteristics of invasive margin and other clinicopathological variables. In this table, the degree of desmoplasia and inflammatory reaction was dichotomized for comparison as marked (grade 3) and low (grade 1-2). The spray-like invasive pattern was associated with marked desmoplasia and extensive LVSI. Although the LVSI rate was similar between the cases with spray-like pattern and those with the other invasive patterns (77% vs 75%, p=0.864), the rate of extensive LVSI was significantly higher in the spray-like pattern group (48% vs 31%, p=0.036). Marked desmoplasia was associated

with spray-like pattern, low inflammatory reaction, deep stromal invasion, residual cervical stroma <3 mm, and parametrial involvement. Low inflammatory reaction was associated with marked desmoplasia, age >45 years, LVSI (including extensive LVSI), and the invasive extent of tumors (deep stromal invasion and residual stroma <3 mm), parametrial involvement, and vaginal margin involvement.

Multivariate analysis showed an independent correlation between spray-like invasive pattern and marked desmoplasia (p=0.013) and a marginal correlation with extensive LVSI (Table 3). Marked desmoplasia was independently correlated with spray-like pattern (p=0.006) and low inflammatory reaction (p=0.009). Low inflammatory reaction had independent correlations with marked desmoplasia (p=0.018), LVSI (p=0.034), and positive vaginal margin (p=0.022), with a marginal correlation with parametrial involvement.

In univariate analysis, lymph node metastasis was significantly associated with the following variables: parametrial involvement (p<0.001), LVSI (p<0.001), residual stroma <3 mm (p=0.003), tumor grade 2-3 (p=0.026), and low inflammatory reaction (p=0.021). Upon multivariate analysis, lymph node metastasis was independently correlated with parametrial involvement (p=0.001, odds ratio 3.64, 95% confident interval [CI] 1.65-8.00), and LVSI (p=0.011, odds ratio 5.22, 95% CI 1.47-18.56).

Tumor recurrence was significantly associated with the spray-like invasive pattern (p=0.005) and marginally associated with LVSI (p=0.079). There was no association between tumor recurrence and the other variables including lymph node metastasis (p>0.999) and adjuvant therapy (p=0.461). In multivariate analysis, the spray-like pattern was the only variable independently correlated with recurrence (p=0.015, odds ratio 6.53, 95% CI 1.45-29.45). By Kaplan-Meier survival analysis, patients with spray-like invasive pattern had a significantly lower disease-free survival compared with those with closed or finger-like pattern (p=0.004) (Figure 3).

Discussion

To our knowledge, the complex relationship of each histopathological characteristic of the invasive margin has not been well evaluated in previous studies of cervical squamous cell carcinoma. In this study, the relationship between each characteristic of invasive margins and the association of these characteristics with other pathological features as well as clinical outcomes in cervical squamous cell carcinoma were identified. The evaluation of invasive margin characteristics is based on hematoxylin and eosin-stained sections and could be done in a routine surgical pathology practice, without additional expense for special staining or advanced technique.

The spray-like pattern of invasive margin of cervical carcinoma was associated with decreased disease-free survival in this study, similar to the findings in previous reports (Horn et al., 2006a; 2012). The poor prognosis of spray-like pattern could not be explained by an extensive tumor growth as the type of invasive patterns was not associated with the extent of cervical stromal invasion in our study. The spray-like pattern represents the higher degree of cell dissociation compared with the other invasive patterns, hence it is more likely to be associated with dissemination of neoplastic cells (Horn et al., 2006a).

A significant association between spray-like invasive pattern and the presence of LVSI has been demonstrated in previous study (Horn et al., 2006a). Although there was no difference in the rate of LVSI between tumors with spray-like pattern and those with the other patterns in the present study, spray-like pattern showed a significantly higher rate of extensive LVSI in univariate analysis and a trend toward a correlation with extensive LVSI in multivariate analysis. This finding is in keeping with the high capacity of cancer cell dissociation in spray-like pattern which could promote an extensive spread of cells along the vessels after LVSI occurs.

Although, the division of invasive pattern in cervical carcinoma into 3 types appears to be simplified in previous study (Horn et al., 2006a), a mixture of different invasive patterns in the same slide or across different slides of the same case was not uncommon in our study. We therefore added a quantitative criterion to classify the invasive pattern based on the predominant one. The proportion of cases in each invasive pattern in our study was comparable to that previously reported (Horn et al., 2006a).

Desmoplastic stroma surrounding cancer cells is an immature-type stroma which is similar to granulation tissue in wound-healing process (Tsujino et al., 2007). Desmoplastic stroma is composed of myofibroblasts and fibroblasts in extracellular matrix, accompanied by neoangiogenesis. Myofibroblast or fibroblasts may produce cytokines and growth factors that promote cancer progression and neoangiogenesis, and may also produce proteinases that facilitate invasive growth of tumor (Polanska and Orimo, 2013). The extent of desmoplasia or the quantity of fibroblasts in peritumoral stroma was found to be a prognostic predictor in cancers of several sites such head and neck, breast, and large intestine (Tsujino et al., 2007). An independent association between spray-like invasive pattern and marked desmoplasia suggests that carcinoma cells with this invasive pattern have a high capacity to induce stromal desmoplasia (Horn et al., 2006b). Although there was no significant association of stromal desmoplasia with tumor recurrence in our study, the association of marked desmoplasia with deep cervical wall invasion and parametrial involvement in univariate analysis is in keeping with the facilitating role of desmoplastic stroma in tumor invasion. It is noteworthy that marked stromal desmoplasia was also independently correlated with low inflammatory reaction in our study. In a previous study (Horn et al., 2012), no association between desmoplasia and inflammatory reaction was observed among 88 cases of cervical squamous cell carcinoma of mixed FIGO stages (IB-IV). The finding in our study may be more reflective of the early stage cases in which the entire cervical tissues were removed and sampled for histopathologic examination. Our finding also suggests that inflammatory reaction may have some inhibitory effect against the formation of stromal desmoplasia.

The criteria to classify the degree of peritumoral inflammatory reaction were not specifically described in details in previous studies (Chao et al., 1999; Kristensen et al., 1999; Horn et al., 2006a; Fregnani et al., 2007). In our study, a variation in the degree of inflammatory reaction from slide to slide in each case was not uncommon. Thus, we added the requirement of a continuous rim of inflammatory infiltration around the tumor in cases scored as 'marked' degree to reduce the subjectivity of grading. In this study, low inflammatory reaction was independently correlated with positive vaginal margin and LVSI. This finding suggests a possible role of inflammatory reaction to limit direct extension or lymphovascular invasion of cancer cells and is in keeping with an anti-tumor role of immune reaction (Fridman et al., 2012). The higher rate of lymph node metastasis in the cases with low inflammatory reaction may be related to the higher LVSI rate in this group.

Although peritumoral inflammatory reaction did not have a significant prognostic value in our study, its favorable prognosis has been previously observed in patients with cervical carcinoma (Kainz et al., 1994; Chao et al., 1999; Fregnani et al., 2007) as well as other cancer types (Jass et al., 2007). Recent studies focusing on anti-tumor role of T-cell subpopulations at the invasive margin or within the tumor, as identified by immunohistochemical stains, have shown a strong prognostic value in several types of cancer (Fridman et al., 2012). In our study, eosinophils were also found to be a major population of inflammatory cells in some cases. Eosinophils are known to have a tumor-killing capacity and they could be recruited by the cytokines produced by T-lymphocytes, which comprise an important part of tumor immunity (Benatar et al., 2010).

The effective current treatment policy contributes to a reduction in recurrence rate and an improvement in survival of early-stage cervical cancer patients (Suprasert et al., 2010). The improved clinical outcomes in the patients who received adjuvant therapy may result in the disappearance of prognostic significance of several well-established pathological variables (e.g. parametrial involvement, lymph node metastasis, etc) which were used to indicate adjuvant therapy, as observed in the present study.

In conclusion, there is a complex interaction between cancer tissue at the invasive margin and changes in surrounding stroma. Spray-like invasive pattern is the most important characteristic of invasive margin and has a prognostic value in patients with early-stage cervical squamous cell carcinoma.

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