RESEARCH COMMUNICATION

# Cyclooxygenase-2 Expression in Cervical Squamous Cell Carcinoma: the Significance of Expression in Neoplastic Cells within the Lymphovascular Space

**Jongkolnee Settakorn[1], Surapan Khunamornpong[1]*, Kornkanok Sukpan[1], Jatupol Srisomboon[2], Sumalee Siriaungkul[1]**

## Abstract

**Background: Cyclooxygenase-2 (COX-2) activity is related to the development and progression of cervical cancer. Previous studies have shown that COX-2 expression in early stage (stage IB-IIA) cervical squamous cell carcinoma is associated with lymph node metastasis in tumors with lymphovascular space invasion (LVSI), and that COX-2 expression may facilitate lymph node metastasis after LVSI occurs. In this study, we evaluated whether COX-2 expression of neoplastic cells within lymphovascular spaces (tumor emboli) would provide additional prognostic information. Methods: Immunohistochemical stained slides for COX-2 on 150 cases of stage IB-IIA cervical squamous cell carcinoma with LVSI were evaluated for expression of COX-2 in tumor emboli. Results were correlated with overall COX-2 expression of tumor and clinicopathologic features using statistical analysis. Results: Expression of COX-2 was detected in 49.3% of cases. Expression of COX-2 in tumor emboli (LV-COX-2 expression) was identified in 61 cases (40.7%). LV-COX-2 expression was associated with high LVSI count ($p<0.001$) and had a marginal association with tumor COX-2 expression ($p=0.050$) and lymph node metastasis ($p=0.063$). In tumors showing high LVSI count, LV-COX-2 expression was an independent predictor for lymph node metastasis ($p=0.038$, 95% CI=1.030-2.725) whereas tumor COX-2 expression ($p=0.550$) was not. Conclusion: Evaluation of COX-2 expression in tumor emboli may provide additional prognostic value for lymph node metastasis in cervical squamous cell carcinomas with a high LVSI count.**

**Key Words:** Cervical squamous cell carcinoma - COX-2 - prognosis - lymphovascular space invasion - tumor emboli

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## Introduction

Cyclooxygenase-2 (COX-2), an isoform of cyclooxygenase, is a key enzyme in the synthesis of prostaglandins. COX-2 expression is normally induced by inflammatory stimuli but is detectable in precancerous lesions and cancers of several sites including gynecologic organs (Munkarah and Ali-Fehmi, 2005). Clinical studies have documented a correlation of COX-2 expression with increased metastatic potential, higher tumor stage, and poor response to treatment (Kakiuchi et al., 2002; Munkarah and Ali-Fehmi, 2005; Liu et al., 2006; Haffty et al., 2008). There is great interest in COX-2 expression as a poor prognostic indicator as well as the potential therapeutic benefit for cancer treatment with COX-2 inhibitors (Young et al., 2008).

In our previous study, we demonstrated that COX-2 expression of cervical squamous cell carcinoma may provide additional prognostic information in tumors showing lymphovascular space invasion (LVSI) (Khunamornpong et al., 2009). We have postulated that COX-2 expression may facilitate lymph node metastasis after LVSI occurs. This postulation raises a further question of whether COX-2 expression in the tumor emboli would provide prognostic information in addition to the COX-2 status of the tumor (tumor COX-2 expression). To our knowledge, the significance of COX-2 expression in neoplastic cells within the lymphovascular spaces (LV-COX-2 expression) of cervical squamous cell carcinoma has not been evaluated.

## Materials and Methods

This study was approved by the Ethics Committee of the Faculty of Medicine, Chiang Mai University. The cases of stage IB-IIA squamous cell carcinoma of the uterine cervix treated by radical hysterectomy with lymph node dissection between January 2003 and December 2006 were retrieved from the surgical pathology files of the Department of Pathology, Faculty of Medicine, Chiang Mai University. Clinical data were obtained by chart review. The slides of each case were reviewed for

*[1]Department of Pathology, and [2]Obstetric and Gynecology, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand *For Correspondence: skhunamo@med.cmu.ac.th*

histological findings (histological type, size, grade, fraction of cervical stromal invasion, thickness of residual uninvolved stroma, parametrial and vaginal involvement, LVSI, and lymph node metastasis).

Identification of LVSI was based on the criteria of Roman et al (1998), with the exclusion of equivocal results. A semiquantitative stratification of LVSI was determined from a total count of the lymphovascular spaces in all tumor slides; cases with 10 spaces or less involved were designated as 'low' LVSI and cases with >10 spaces involved as 'high.' A representative formalin-fixed, paraffin-embedded tissue block containing invasive carcinoma was selected for immunohistochemistry in each case. The immunostains were performed using anti-COX-2 polyclonal antibody (Diagnostic Biosystem, CA, dilution 1:300) as previously described (Khunamornpong et al., 2009). Human colonic adenocarcinoma was used as positive control and omission of the primary antibody as a negative control. Overall expression of COX-2 (tumor COX-2 expression) was scored 'positive' when at least 10% of neoplastic cells showed cytoplasmic staining of at least moderate intensity. The clinical and histopathologic data of these cases were previously reported in another study on COX-2 expression by immunohistochemical stain (Khunamornpong et al., 2009).

Only cases with the presence of LVSI were included in this study, as the sole presence of LVSI is an important predictive factor for lymph node metastasis (Khunamornpong et al., 2009), After the evaluation of tumor COX-2 expression was completed, the immunostained slides of these cases were re-evaluated to identify positivity of COX-2 in the neoplastic cells within the lymphovascular spaces. Due to the relatively small number of neoplastic cells present in the lymphovascular spaces, expression of COX-2 in these cells (LV-COX-2 expression) was considered when there was positive cytoplasmic staining of at least moderate degree in at least one neoplastic cell. The histologic features and the follow-up status of the patients in cases with positive LV-COX-2 expression and those without were compared, with an emphasis on the previously reported predictive factors for lymph node metastasis, including parametrial involvement, high LVSI count, and COX-2 expression (Khunamornpong et al., 2009).

The correlation of variables was evaluated by the Chi squared test or Fisher's exact test, as appropriate. Multivariate analyses were performed by logistic regression and Cox regression models in a forward stepwise fashion. A result was considered to be statistically significant when the p value was less than 0.05.

## Results

From 196 cases of stage IB-IIA cervical squamous cell carcinoma, 46 cases without LVSI were excluded; there were 150 cases included in the current study. Tumor COX-2 expression was observed in 74 cases (49.3%). LV-COX-2 expression (Figure 1) was identified in 61 cases (40.7%). The comparison between the status of LV-COX-2 expression and other histologic variables including follow-up status is shown in Table 1. LV-COX-2 expression was associated with high LVSI count (p<0.001) and had a marginal association with tumor COX-2 expression (p=0.050) and lymph node metastasis (p=0.063). The rate of recurrence was not different in either group, with or without LV-COX-2 expression.

**Table 1. Comparison of Clinicopathological Parameters between LV-COX-2 Positive and LV-COX-2 Negative Tumors**

| Parameter | LV-COX-2 expression | | p value |
|---|---|---|---|
| | Positive (n=61) | Negative (n=89) | |
| Histology | | | |
| Histologic grade 2-3 | 53 (86.9%) | 70 (78.7%) | 0.197 |
| Parametrial involvement | 19 (31.2%) | 29 (32.6%) | 0.853 |
| Vaginal involvement | 4 (6.6%) | 15 (16.9%) | 0.063 |
| Invasion of outer third of stroma | 51 (83.6%) | 81 (91.0%) | 0.170 |
| Residual stroma < 3mm | 39 (63.9%) | 64 (71.9%) | 0.301 |
| High LVSI count | 45 (73.8%) | 35 (39.3%) | <0.001 |
| Overall COX-2 | 36 (59.0%) | 38 (42.7%) | 0.050 |
| Lymph node metastasis | 27 (44.3%) | 27 (30.3%) | 0.081 |
| Follow-up status | | | |
| Overall recurrence | 6/58 (10.3%) | 10/85 (11.8%) | 0.791 |
| Recurrence in cases without adjuvant therapy | 2/16 (12.5%) | 3/31 (9.7%) | 1.000 |

**Table 2. Correlation of LV-COX-2 Expression and Risk Factors Associated with Lymph Node Metastasis**

| Parameter | LV-COX-2 expression | | | | p value |
|---|---|---|---|---|---|
| | Positive (n=61) | | Negative (n=89) | | |
| | Total | Metastatic | Total | Metastatic | |
| Parametrial involvement | | | | | |
| Positive (n=48) | 19 | 13 (68.4%) | 29 | 17 (58.6%) | 0.493 |
| Negative (n=102) | 42 | 14 (33.3%) | 60 | 10 (16.7%) | 0.051 |
| LVSI count | | | | | |
| High (n=80) | 45 | 25 (55.6%) | 35 | 12 (34.3%) | 0.058 |
| Low (n=70) | 16 | 2 (12.5%) | 54 | 15 (27.8%) | 0.323 |
| Tumor COX-2 expression | | | | | |
| Positive (n=74) | 36 | 19 (52.8%) | 38 | 14 (36.8%) | 0.168 |
| Negative (n=76) | 25 | 8 (32.0%) | 51 | 12 (23.5%) | 0.431 |

Univariate analysis of the predictive histologic features for lymph node metastasis showed significant association of nodal metastasis with parametrial involvement

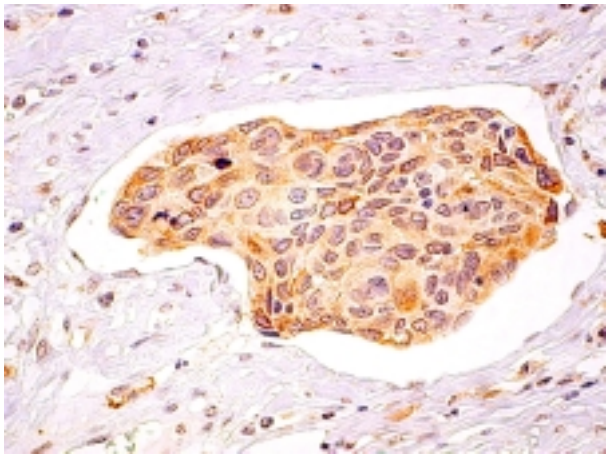

**Figure 1. COX-2 Expression in Neoplastic Squamous Cells within an Endothelium-bound Lymphovascular Space (x 400)**

($p<0.001$), residual stroma <3 mm ($p=0.030$), tumor COX-2 expression ($p=0.030$), and histologic grade ($p=0.037$). The association between LV-COX-2 expression cells and lymph node metastasis was not significant ($p=0.081$). Multivariate analysis showed that parametrial involvement was the only independent predictor for lymph node involvement ($p<0.001$, 95% CI=1.912-10.136).

With respect to the previously reported predictive factors for lymph node metastasis, the cases were stratified based on the following features: LVSI count (high vs. low), tumor COX-2 expression (positive vs. negative), or parametrial involvement (positive vs. negative), to examine whether there was any significance of LV-COX-2 expression in each subgroup (Table 2). Cases with LV-COX-2 expression showed a higher rate of lymph node metastasis in each of the three following subgroups: tumors with high LVSI count, tumors with COX-2 expression, and tumors with negative parametrium. However, the difference was not statistically significant. Marginal association between LV-COX-2 expression and lymph node metastasis was observed in the cases with high LVSI count ($p=0.058$) and those with negative parametrium ($p=0.051$).

As marginal association was observed in the group of tumors with high LVSI count and the group with negative parametrium, multivariate analysis was further examined in each group. In the group with high LVSI count, independent predictors for lymph node metastasis included parametrial involvement ($p=0.012$, 95% CI=1.314-9.100) and LV-COX-2 expression ($p=0.038$, 95% CI=1.030-2.725) but not tumor COX-2 expression ($p=0.550$). In the group with negative parametrium, only high LVSI count had a marginal association with lymph node involvement in multivariate analysis ($p=0.058$, 95% CI=0.967-7.277).

## Discussion

COX-2 expression in cervical carcinoma has been linked to the extent of tumor growth, local invasion, the presence of LVSI, lymph node metastasis, and poor clinical outcome (Ryu et al., 2000Gaffney et al., 2001; Ferrandina et al., 2002; Kim et al., 2002; Manchana et al., 2006; Dursun et al., 2007; Khunamornpong et al., 2009). Few studies that were limited to surgically-treated cervical squamous cell carcinoma indicated an association of COX-2 expression with LVSI or lymph node metastasis (Kim et al., 2003; Dursun et al., 2007; Khunamornpong et al., 2009). COX-2 expression was significantly associated with the risk of tumor recurrence and survival in surgically treated patients who did not receive postoperative adjuvant therapy (Khunamornpong et al., 2009).

High LVSI count has been reported to be a risk factor for lymph node metastasis in surgically treated patients, but there was no association between tumor COX-2 expression and the quantity of LVSI (Khunamornpong et al., 2009). In this study, LV-COX-2 expression strongly correlated with high LVSI count of tumors ($p<0.001$), whereas the association between LV-COX-2 expression and tumor COX-2 expression was only of marginal significance ($p=0.050$). As there was a strong correlation between LV-COX-2 expression and high LVSI count, the larger number of neoplastic cells within the lymphovascular spaces may, at least partially, contribute to an increased chance of finding COX-positive cells. With regard to the prognosis, LV-COX-2 expression was not significantly associated with lymph node metastasis or tumor recurrence.

When the subgroups of tumors were analyzed, LV-COX-2 expression was independently associated with an increased rate of lymph node metastasis in tumors showing high LVSI count ($p=0.038$). However, the promoting effect of LV-COX-2 expression for lymph node metastasis was not observed in tumors with low LVSI count. There was also a tendency for the COX-2-positive tumors to have a higher rate of lymph node metastasis when LV-COX-2 expression was identified in comparison with the absence of LV-COX-2 expression, but the difference was not significant. The presence of LV-COX-2 expression in cases with negative parametrium was also marginally associated with lymph node metastasis ($p=0.051$).

These findings suggest that LV-COX-2 expression might be a reflection of increased metastatic potential of neoplastic cells. The evaluation of LV-COX-2 expression may provide additional prognostic value to the conventional evaluation of tumor COX-2 expression in certain selected subgroups, particularly those showing high LVSI count. Further investigations, particularly in vitro, may clarify the possible mechanisms for increased metastatic potential for COX-2-positive cells of cervical squamous cell carcinoma.

In conclusion, expression of COX-2 in neoplastic cells within lymphovascular spaces may provide additional prognostic value for lymph node metastasis in cervical squamous cell carcinoma with high LVSI count.

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## References

Dursun P, Yuce K, Usubutun A, et al (2007). Cyclooxygenase-2 expression in cervical intraepithelial neoplasia III and squamous cell cervical carcinoma, and its correlation with clinicopathologic variables. *Int J Gynecol Cancer*, **17**, 164-73.

Ferrandina G, Lauriola L, Distefano MG, et al (2002). Increased cyclooxygenase-2 expression is associated with chemotherapy resistance and poor survival in cervical cancer patients. *J Clin Oncol*, **20**, 973-81.

Gaffney DK, Holden J, Zempolich K, et al (2001). Elevated COX-2 expression in cervical carcinoma: reduced cause-specific survival and pelvic control. *Am J Clin Oncol*, **24**, 443-6.

Haffty BG, Yang Q, Moran MS, et al (2008). Estrogen-dependent prognostic significance of cyclooxygenase-2 expression in early-stage invasive breast cancers treated with breast-conserving Surgery and Radiation. *Int J Radiat Oncol Biol*

*Phys*, 74, 1006-13.
Kakiuchi Y, Tsuji S, Tsujii M, et al (2002). Cyclooxygenase-2 activity altered the cell-surface carbohydrate antigens on colon cancer cells and enhanced liver metastasis. *Cancer Res*, **62**, 1567-72.
Khunamornpong S, Settakorn J, Sukpan K, et al (2009). Cyclooxygenase-2 expression in squamous cell carcinoma of the uterine cervix is associated with lymph node metastasis. *Gynecol Oncol*, **112**, 241-7.
Kim MH, Seo SS, Song YS, et al (2003). Expression of cyclooxygenase-1 and -2 associated with expression of VEGF in primary cervical cancer and at metastatic lymph nodes. *Gynecol Oncol*, **90**, 83-90.
Kim YB, Kim GE, Cho NH, et al (2002). Overexpression of cyclooxygenase-2 is associated with a poor prognosis in patients with squamous cell carcinoma of the uterine cervix treated with radiation and concurrent chemotherapy. *Cancer*, **95**, 531-9.
Liu JF, Jamieson G, Wu TC, et al (2006). Cyclooxygenase-2 expression in squamous cell carcinoma of the esophagus. *Dis Esophagus*, **19**, 350-4.
Manchana T, Triratanachat S, Sirisabya N, et al (2006). Prevalence and prognostic significance of COX-2 expression in stage IB cervical cancer. *Gynecol Oncol*, **100**, 556-60.
Munkarah A, Ali-Fehmi R (2005). COX-2: a protein with an active role in gynecological cancers. *Curr Opin Obstet Gynecol*, **17**, 49-53.
Roman LD, Felix JC, Muderspach LI, et al (1998). Influence of quantity of lymph-vascular space invasion on the risk of nodal metastases in women with early-stage squamous cancer of the cervix. *Gynecol Oncol*, **68**, 220-5.
Ryu HS, Chang KH, Yang HW, et al (2000). High cyclooxygenase-2 expression in stage IB cervical cancer with lymph node metastasis or parametrial invasion. *Gynecol Oncol*, **76**, 320-5.
Young JL, Jazaeri AA, Darus CJ, et al (2008). Cyclooxygenase-2 in cervical neoplasia: a review. *Gynecol Oncol*, **109**, 140-5.