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Gwi Eon Kim,[1] Yong Bae Kim,[1] Nam Hoon Cho,[3] Hyun-Cheol Chung,[2] Hong Ryull Pyo,[1] Jong Doo Lee,[3] Tchan Kyu Park,[5] Woong Sub Koom,[1] Mison Chun,[6] and Chang Ok Suh[1]

Departments of [1]Radiation Oncology, [2]Medical Oncology, Yonsei Cancer Center, [3]Pathology, [4]Nuclear Medicine, [5]Obstetrics and Gynecology, Brain Korea 21 Project for Medical Science, Yonsei University, College of Medicine, Seoul, Korea, and [6]Department of Radiation Oncology, Ajou University, College of Medicine, Suwon, Korea

ABSTRACT

Purpose: **To evaluate the potential of the new prognostic information gained by analyzing the coexpression of epidermal growth factor receptor (EGFR) and cyclooxygenase-2 (COX-2) in cervical cancer patients.**

Experimental Design: **Sixty-eight patients with International Federation of Gynecology and Obstetrics stage IIB squamous cell carcinoma of the uterine cervix, who underwent concurrent chemoradiotherapy between 1993 and 1996, were divided into the following four groups according to their immunoreactivities for EGFR and COX-2 in paraffin-embedded sections: (*a*) the EGFR-negative/COX-2-negative group ($n = 11$); (*b*) the EGFR-negative/COX-2-positive group ($n = 8$); (*c*) the EGFR-positive/COX-2-negative group ($n = 27$); and (*d*) the EGFR-positive/COX-2-positive group ($n = 22$). The clinical features, patterns of treatment failure, and survival data in the four groups were compared.**

Results: **Positive immunoreactivity for EGFR and COX-2 was observed in 49 of 68 (72%) and 19 of 68 (28%), respectively. However, no strong correlation was found between the levels of EGFR and COX-2 immunopositivity ($R^2 = 0.05$, $P = 0.07$). Patients in the EGFR-positive/COX-2-positive group had a higher likelihood of locoregional recurrence than those in the other three groups ($P = 0.02$). Of the patients in the four groups, patients positive for both oncoproteins were found to have the worst prognosis with an overall 5-year disease-free survival rate of 55% compared with 91% for the EGFR-negative/COX-2-negative patients, 88% for the EGFR-negative/COX-2-positive patients, and 69% for the EGFR-positive/COX-2-negative patients ($P = 0.05$, log-rank test). In addition, the synchronous coexpression of the EGFR and COX-2 oncoproteins was found to be an independent prognostic factor by univariate and multivariate analyses (relative risk = 4.0, $P = 0.03$).**

Conclusions: **Given these observations, we conclude that the coexpression of EGFR and COX-2 immunoreactivity may be used as a potent molecular risk factor for predicting the poor survival of patients with the International Federation of Gynecology and Obstetrics stage IIB squamous cell carcinoma of the uterine cervix.**

INTRODUCTION

Because the molecular mechanisms of tumor aggressiveness are usually dependent on the proliferative stimuli induced by various tumor promoters, numerous proto-oncogenes and oncogenes regulating tumor cell growth, differentiation, and motility have been investigated to identify molecular targets that might be used as potential predictors of survival in the management of cancer (1, 2). Of a number of biological markers, epidermal growth factor receptor (EGFR) has received much attention during the last decades. It is a *trans*-membrane glycoprotein, which consists of the following three distinct domains: (*a*) an external ligand-binding domain; (*b*) a short intramembranous segment; and (*c*) a cytosolic domain with tyrosine kinase activity (2–7). Ligand-induced dimerization and autophosphorylation of EGFR activates a signal transduction pathway by activating intrinsic protein tyrosine kinase activity and the phosphorylation of several target proteins, which lead to increased DNA replication and the stimulation of cellular differentiation and proliferation (2). Increased EGFR expression has been observed in many experimental cell lines and human tumors, such as head and neck cancer (3), esophageal cancer (4), gastric cancer (5), and cervical cancer (6–14). In the case of uterine cervical cancer, the incidence of EGFR overexpression has been variably reported to occur in 6–85% of cases according to the detection techniques and methods used previously (7). Although there have been a few contradictory results (14), the majority of reports show that elevated levels of EGFR correlate with a more aggressive biological behavior and are clinically relevant to poor prognosis in cervical cancer patients (7–13).

On the other hand, cyclooxygenase (COX)-2, a key enzyme that catalyzes the conversion of arachidonic acid to prostaglandins and other eicosanoids, alters several cellular responses involved in cell cycle regulation, the inhibition of apoptosis, extracellular matrix deposition, or pathological angiogenesis (15–21). It promotes carcinogenesis, tumor proliferation, and the growth and spread of cancer by mediating the pathological processes that affect mitogenesis, cellular adhe-

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Requests for reprints: Gwi Eon Kim, Department of Radiation Oncology, Yonsei Cancer Center, Yonsei University, College of Medicine Seodaemoon-Gu, Shinchon-Dong 134 Seoul 120–752, Korea. Phone: (82)-2-361-7631; Fax: (82)-2-312-9033; E-mail: gekim@yumc.yonsei.ac.kr.

sion, and immune surveillance (15–18). Therefore, COX-2 overexpression has also been considered an indicator of tumor invasiveness and aggressiveness as well as a predictor of metastatic potential in various types of cancers (16, 17, 22), including uterine cervical cancer (23, 24). A previous report showed that elevated levels of COX-2 expression are closely linked to a higher incidence of parametrial invasion and lymph node metastases in early-stage uterine cervical cancer (23). Moreover, increased COX-2 expression has been proposed recently as a putative prognostic determinant of uterine cervical cancer (23–25), consistent with EGFR overexpression.

Preclinical *in vitro* and *in vivo* studies have demonstrated that the gene amplification and/or the protein expression of COX-2 is rapidly induced in response to various tumor promoters, growth factors, oncogenes, and carcinogens (15, 26–32). In particular, activation of the EGFR signaling pathway enhances the transcription of the rate-limiting COX-2 gene in several cell types (15, 27–30). Although both EGFR and COX-2 enzyme are known to play a vital role in oncogenic transformation, carcinogenesis, and tumor invasiveness (3–12, 15–17), little information is available regarding the relationship between EGFR and COX-2 in cultured cervical carcinoma cell lines or in human cervical cancer. Moreover, the prognostic significance of the coexpressions of EGFR and COX-2 has not been investigated despite the fact that the separate overexpression of EGFR (7–13) or COX-2 (23–25) is known as an independent prognostic indicator in uterine cervical cancer patients. The aim of the present study was to evaluate the potential of the new prognostic information gained by analyzing the coexpression of EGFR and COX-2 immunoreactivities in patients with squamous cell carcinoma of the uterine cervix.

MATERIALS AND METHODS

Patients and Treatment. Of the patient population identified through a search of the Tumor Registry Database maintained by the Department of Radiation Oncology at the Yonsei Cancer Center, Yonsei University, College of Medicine (Seoul. Korea), we chose 68 consecutive patients with stage IIB invasive squamous cell carcinoma of the uterine cervix, who were treated with concurrent chemoradiotherapy between 1992 and 1996. The clinical staging and histological classification used for uterine cervical cancer were based on the International Federation of Gynecology and Obstetrics classification and the WHO (Geneva, Switzerland) classification. To make the study population more homogeneous, those with small cell carcinomas or adenocarcinomas were excluded from the study. All the International Federation of Gynecology and Obstetrics stage IIB patients who were treated with neoadjuvant chemotherapy and radiation or with radiotherapy alone were also excluded. Eligible criteria for the concurrent chemoradiotherapy protocol included patients with high risk factors, such as tumor size >4 cm, lymphovascular permeation, or lymph node metastases on abdominopelvic computed tomography or magnetic resonance imaging. All patients received a combination of external irradiation and high-dose-rate intracavitary irradiation by a remote afterloading system using Ir-192 sources (γ-Med II). External irradiation was delivered to the whole pelvis at a dose of 1.8 Gy/fraction, five times/week to a midline dose of 27–45 Gy, before high-dose-rate intracavitary radiation involving six insertions (twice per week) with a fractional dose of 5 Gy to a total dose of 30 Gy at point A. After the high-dose-rate intracavitary radiation, the patients received a second course of external irradiation with central shielding up to a total external dose of 45–50.4 Gy. Three cycles of chemotherapy were given concomitantly in weeks 1, 4, and 7 of radiotherapy. The chemotherapy regimens used were cisplatin and 5-fluorouracil. Cisplatin was injected as a daily bolus dose of 100 mg/m^2, and 5-fluorouracil was administered as a 24-h continuous infusion at a dose of 1,000 mg/m^2/day for 5 days. After completing the treatment, patients were followed-up at 3-month intervals for at least 5 years or until death. The follow-up period for all patients ranged from 8 to 108 months, with a median of 60 months in the entire patient group and a median of 66 months in the surviving group.

Immunohistochemical Staining for EGFR and COX-2. For immunohistochemical staining, the tissue array blocks were made from 4 μm-thick paraffin-embedded tissue blocks, which were obtained from punch biopsy at the pretreatment period. After an evaluation of the H&E-stained slides, donor blocks were prepared. Using a 2-mm core needle, representative cancer regions were chosen from the matching donor blocks and transplanted to the recipient blocks made with purified agar in 3.8 $\times$ 2.2 $\times$ 0.5 cm frames. The recipient blocks were framed in the mold, which was used to frame the conventional paraffin block. Holes measuring 2 mm were made in the recipient blocks using a core needle, and the agar core was discarded. Subsequently, paraffin was added to the frame. Consecutive 4 μm-thick sections were cut from the recipient blocks using an adhesive-coated slide system (Instrumedics Inc., Hackensack, NJ), which supported the cohesion of the 2-mm array elements on the glass. Monoclonal mouse antihuman EGFR antibody and rabbit polyclonal antibody specific for human COX-2 were purchased from DAKO (Carpinteria, CA) and Cayman (Ann Arbor, MI), respectively. Immunostaining for EGFR and COX-2 oncoproteins was performed using a modified avidin-biotin immunoperoxidase technique, as described previously (29). Briefly, after deparaffinization with graded ethanol solutions, endogenous peroxidase activity was blocked by immersion of slides in methanol with 0.3% hydrogen peroxide for 30 min. Antigen retrieval was then performed using a 0.01 M sodium citrate-buffered saline (pH 6.0) in a microwave processor for 30 min at 95°C. The slides were then rinsed in PBS for 5 min and blocked with a solution of 10% normal rabbit serum in PBS for 10 min at room temperature. Both antibodies to the EGFR and COX-2 oncoproteins used for immunostaining the tumor specimens were incubated at 4°C overnight. Each antibody was preheated in a microwave (650 W) for 10 min in a citrate buffer (pH 6.0). Nonspecific mouse immunoglobulin G_1 monoclonal antibodies (03001D; PharMingen; 1 mg/ml) were used as a negative control. Tissues were incubated with biotinylated horse antimouse secondary antibodies at a 1:500 dilution (Vector Laboratories, Burlingame, CA), followed by extensive washing and then treatment with avidin-biotin peroxidase complex at 1:25 dilution. Diaminobenzene was used as the chromogen, and hematoxylin was used as the nuclear counterstain.

Assessment of Immunostaining. To eliminate the possibility of individual bias, sections were examined microscopically by an experienced investigator unaware of the clinical

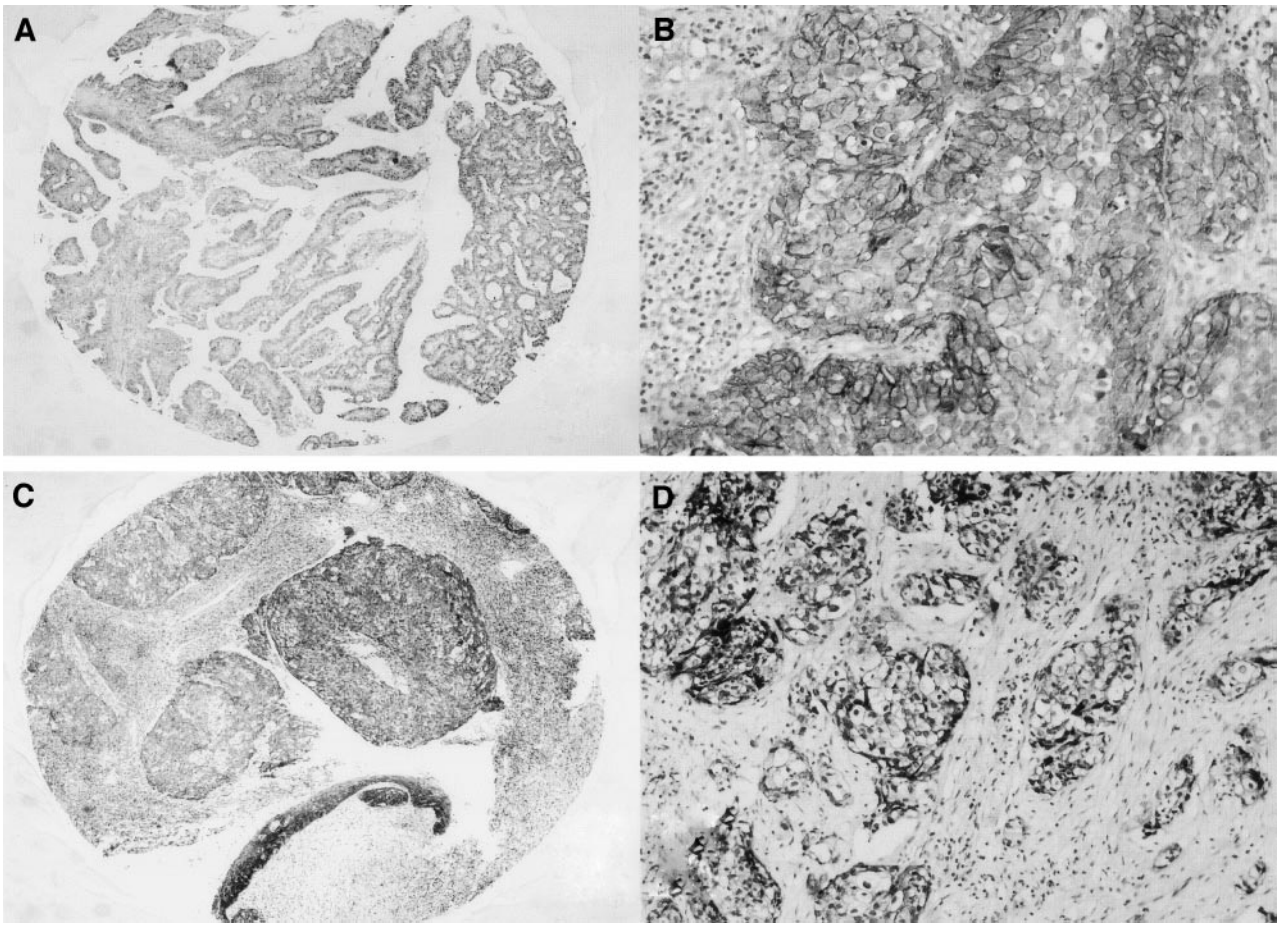

Fig. 1 Epidermal growth factor receptor (EGFR) immunoreactivity was most abundant along the membranes of tumor cells although the staining pattern was variable within the tumor nest. Some cases showed intense staining patterns along an invading margin of the tumor nest (*A–B*), whereas others revealed diffuse immunoreactivity within the entire tumor nest (*C–D*).

information. The same pathologist reexamined all slides after a 1-month interval. Discrepancies in staining analysis were <5%. Immunoreactivities were classified as a continuum from the undetected level (0%) to diffuse and homogeneous staining (100%).

Statistical Analysis. Patient's characteristics and patterns of treatment failure were compared by ANOVA and Fisher's exact test. The correlation between EGFR and COX-2 expression was analyzed using the Pearson's correlation. Although different cutoff values of EGFR and COX-2 were tested for the survival analyses, we decided to choose the best prognostic cutoff point value as significant discriminators. If the distributions of immunoreactivity exceeded 10%, the sample was classified as positively stained. The probability of overall actuarial survival and disease-free survival for each positive or negative group was calculated by the Kaplan-Meier method, and the difference between survivals was compared using the log-rank test. Cox's proportional hazards model was used to identify prognostic factors influencing survival. Prognostic variables that showed statistical significance by univariate analysis were then included in the multivariate analysis to define variables of independent significance. $P \leq 0.05$ was considered statistically significant.

RESULTS

Immunohistochemical Findings. Fig. 1 illustrates characteristic membrane staining for EGFR. Immunoreactivity for EGFR was most abundant along the cytoplasmic membranes of tumor cells. However, the staining pattern was variable within the tumor nest. Some cases showed intense staining-patterns along an invading margin of the tumor nest (Fig. 1*A–B*), whereas others revealed diffuse immunoreactivity within the entire tumor nest (Fig. 1*C–D*). Although essentially no immunoreactivity for EGFR was found within normal cells, immunostaining for anti-EGFR monoclonal antibody was occasionally observed in the normal basal and parabasal squamous epithelium. On the other hand, COX-2 immunopositivity was entirely restricted to the cytoplasms of tumor cells (Fig. 2), although perinuclear immunoreactivity was also observed in some tumor cells. Focal or diffuse COX-2 staining was detected in the front margin of the tumor nest (Fig. 2*A–B*) or within entire infiltrating tumor nests (Fig. 2*C–D*). However, the cells of

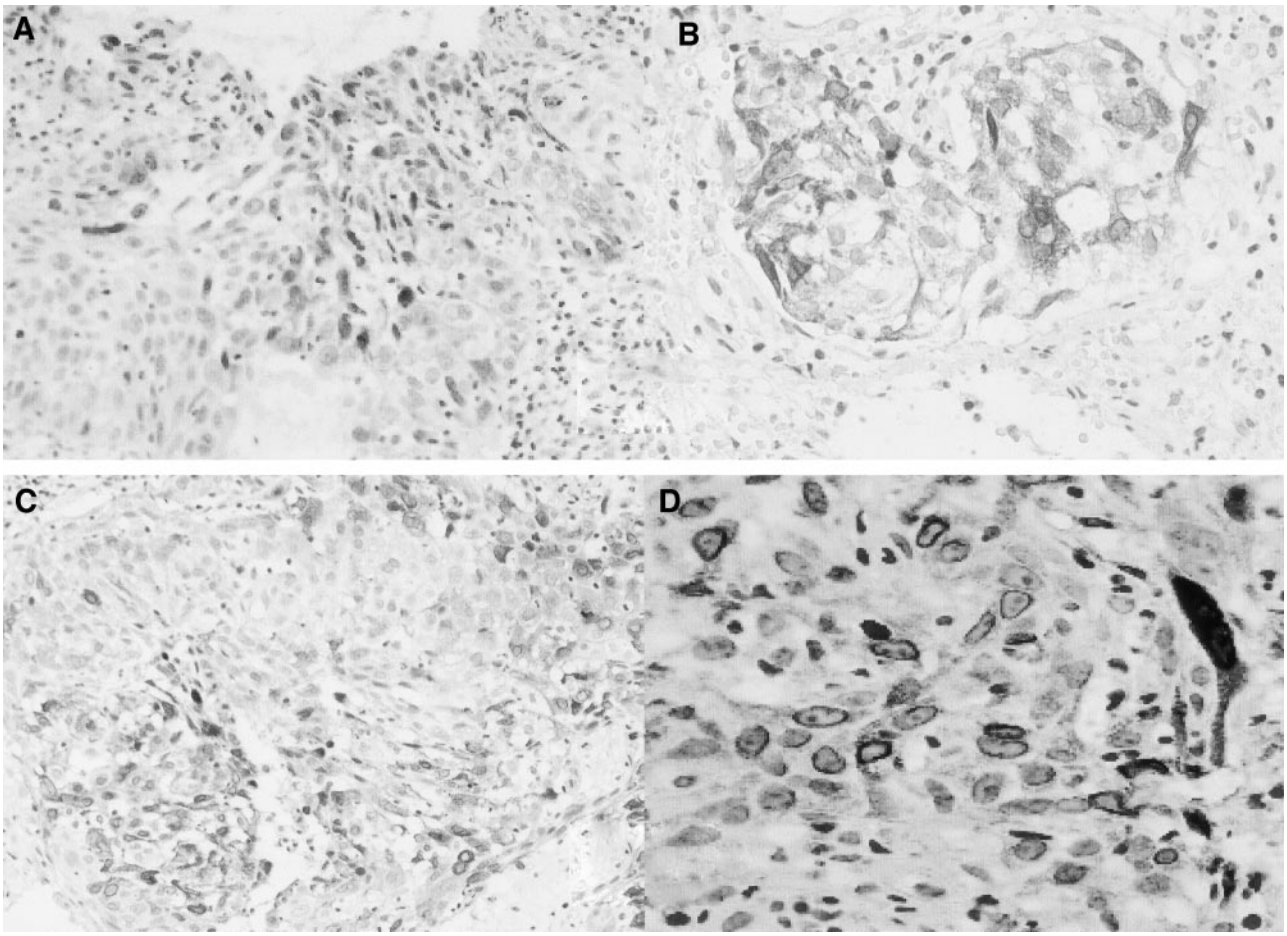

Fig. 2 The immunoreactivities for cyclooxygenase (COX)-2 restricted to tumor cells within the cytoplasms. Focal or diffuse COX-2 staining was detected in the front margin of the tumor nest (*A–B*) or within entire infiltrating tumor nests without preference (*C–D*). However, the cells of histologically normal areas adjacent to tumor did not show any COX-2 immunoreactivity.

histologically normal areas adjacent to tumor did not show any COX-2 immunoreactivity. The levels of EGFR and COX-2 expression represent continuous variables from 0% to 100%. When positive immunoreactivity was defined as the extent of the distribution of immunostaining that exceeded 10%, overall positivity for EGFR and COX-2 was found in 49 of 68 (72%) and 19 of 68 (28%), respectively. Of the 68 patients, 22 (32%) showed the coexpression of EGFR and COX-2, whereas 11 (16%) expressed neither EGFR nor COX-2.

Clinical Features. All patients were divided into the following four groups according to their EGFR and COX-2 expression status: (*a*) the EGFR-negative/COX-2-negative group ($n = 11$); (*b*) the EGFR-negative/COX-2-positive group ($n = 8$); (*c*) the EGFR-positive/COX-2-negative group ($n = 27$); and (*d*) the EGFR-positive/COX-2-positive group ($n = 22$). No significant differences in the ages or in the performance status were found between the patients of the four groups. We attempted to identify relationships among several clinical parameters, but no significant association was found in terms of pathological subtype, tumor shape, tumor size, extent of parametrial involvement, or pelvic lymph node status in the four groups. The patient characteristics of groups are listed in Table 1.

Relationship between Levels of EGFR and COX-2 Expressions. On comparing the frequency of COX-2 expression in the EGFR-positive and EGFR-negative group, no relationship was found between EGFR status and COX-2 expression. Of the 49 EGFR-positive tumors, 22 of 49 (45%) were positive for COX-2, compared with 8 of 19 (42%) of the EGFR-negative tumors (Fisher's exact test, $P = 0.527$). To investigate the distribution of COX-2 level according to EGFR level, linear regression analysis was performed; however, only a weak correlation was observed (Pearson correlation, $R^2 = 0.05$, $P = 0.07$; Fig. 3).

Patterns of Treatment Failure. At the time of this analysis, a total of 23 patients suffered tumor recurrence. The majority of recurrences and/or metastatic diseases evolved within 3 years of completing treatment. We classified the patterns of treatment failure into the following three categories: locoregional recurrence, extrapelvic lymph node metastasis, and hematogenous metastasis. Patients in the EGFR-positive/COX-2-positive group had a higher likelihood of locoregional recur-

Table 1 Patient characteristics

Characteristics	EGFR[a] (−)		EGFR (+)		Statistical significance
	COX (−)	COX (+)	COX (−)	COX (+)	
No. of patients	11	8	27	22	
Age (yrs)					NS
Median	59	50	55	51	
Range	31–75	34–67	29–71	32–67	
Karnofsky performance status					NS
Median	80	80	80	80	
Range	60–90	60–90	60–90	60–90	
Pathology					NS
LCK	3	2	10	6	
LCNK	8	6	17	16	
Tumor shape					NS
Exophytic	1	2	7	9	
Infiltrative	10	6	20	13	
Tumor size (cm)					NS
≤4	7	4	14	12	
>4	4	4	13	10	
Extent of parametrial involvement					NS
Unilateral/medial[b]	4	1	14	8	
Bilateral/lateral[c]	7	7	13	14	
Pelvic LN involvement	1	3	1	7	NS
CT cycle (median)	3	3	3	3	NS
RT dose (Gy)					NS
Median	84	84	84	84	
Range	75–86.8	75–84	75–93	75–102	

[a] EGFR, epidermal growth factor receptor; COX, cyclooxygenase; LCK, large cell keratinizing; LCNK, large cell nonkeratinizing; LN, lymph node; CT, chemotherapy; RT, radiotherapy; NS, not significant.
[b] Unilateral and medial-half extension.
[c] Bilateral or lateral-half extension.

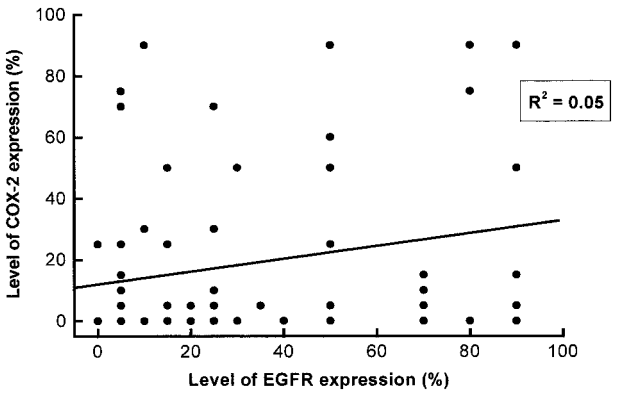

Fig. 3 Linear regression analysis between levels of epidermal growth factor receptor (EGFR) and cyclooxygenase (COX)-2 expressions. No strong correlation was observed between EGFR level and COX-2 immunopositivity (Pearson correlation, $R^2 = 0.05$, $P = 0.07$).

rence (32%) than those in the other three groups (9%; $P = 0.02$). However, there were no significant differences in the rates of extrapelvic lymph node failure including paraaortic and/or supraclavicular node metastases among the four groups. In particular, EGFR-positive/COX-2-positive patients did not show a tendency to more frequently develop hematogenous metastases. Table 2 shows the patterns of treatment failure for patients in the four groups.

Survival and Prognostic Factors. On the basis of a median follow-up of 60 months, the 5-year overall actuarial and disease-free survival rates for the 68 patients who were treated with radiotherapy and concurrent chemotherapy were 82% and 79%, respectively. When these patients were stratified into four groups according to their EGFR and COX-2 expression statuses, patients positive for both oncoproteins were found to have the worst prognosis with an overall 5-year actuarial survival rate of 58% compared with 100% for the EGFR-negative/COX-2-negative patients, 100% for the EGFR-negative/COX-2-positive patients, and 82% for the EGFR-positive/COX-2-negative patients ($P = 0.009$, log-rank test). Most strikingly, approximately two-thirds of the EGFR-positive/COX-2-positive patients died of pelvic recurrence and/or extrapelvic metastases within 3 years. A similar trend was also observed in the disease-free survivals of the patients in the four groups. Five-year disease-

Table 2 Patterns of treatment failure

Patterns of failure	No. of patients (%)		Statistical significance
	Negative or either positive[a] ($n = 46$)	Both positive[b] ($n = 22$)	
Locoregional recurrence	4 (9%)	7 (32%)	0.02
Extrapelvic LN[c] metastasis	3 (7%)	4 (18%)	NS
Hematogenous metastasis	4 (9%)	1 (5%)	NS

[a] Negative staining or positive immunoreactivities of either EGFR or COX-2.
[b] Positive immunoreactivities of both EGFR and COX-2.
[c] LN, lymph node; NS, not significant; EGFR, epidermal growth factor receptor; COX, cyclooxygenase.

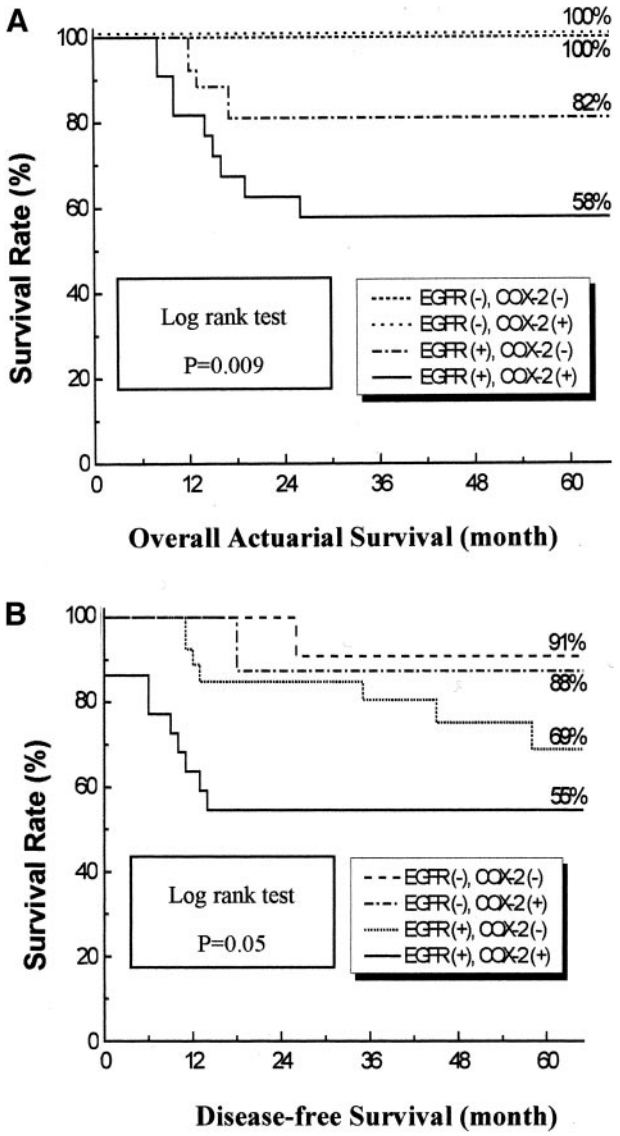

Fig. 4 Comparison of overall actuarial survival rates (*A*) and disease-free survival rates (*B*) according to epidermal growth factor receptor (EGFR) and cyclooxygenase (COX)-2 overexpression (Kaplan-Meier method). Patients showing positive immunoreactivity for both EGFR and COX-2 (*solid line*) showed the worst prognosis.

free survival was 91% for the EGFR-negative/COX-2-negative patients, 88% for the EGFR-negative/COX-2-positive patients, 69% for the EGFR-positive/COX-2-negative patients, and 55% for the EGFR-positive/COX-2-positive patients ($P = 0.05$, log-rank test). The overall 5-year actuarial and disease-free survival curves for each group are shown in Fig. 4. As expected, lymph node metastasis was an important prognostic factor influencing 5-year disease-free survival rate by univariate analysis. Apart from the coexpression of EGFR and COX-2, the separate expressions of EGFR or COX-2 were also statistically significant prognostic factors. However, only the coexpression of EGFR and COX-2 was an independent predictive factor for poor overall disease-free survival by multivariate analysis (95% confidence interval; 2.7–5.3, $P = 0.03$; Table 3).

DISCUSSION

Although several studies have found that the expressions of EGFR (7–13) or COX-2 (23–25) are unfavorable prognostic markers in cervical cancer patients, synchronous coexpression of these oncoproteins in terms of their clinical and prognostic significance has not been addressed. The present study demonstrates that patients coexpressing both oncoproteins have a higher likelihood of locoregional recurrence than those expressing EGFR or COX-2 alone. Simultaneously, it was found that compared with separate expression of either oncoprotein alone, the synchronous coexpression of EGFR and COX-2 immunoreactivities was a more significant and independent prognostic factor for predicting poorer survival in the International Federation of Gynecology and Obstetrics stage IIB cervical cancer patients treated with concurrent chemoradiotherapy. These observations suggest that cervical cancers with synchronous coexpression of EGFR and COX-2 are responsible for either the more malignant phenotype with an aggressive biological behavior or the further increased resistance to standard chemotherapy and radiotherapy.

It is well recognized that the activity of the COX-2 promoter and the induction of COX-2 enzyme are stimulated by receptor-mediated signals triggered by epidermal growth factor, transforming growth factor α, and ligands of EGFR (15, 30). Although the *cis*-acting elements in the COX-2 promoter, the transcription factors modulating COX-2 expression, and the signaling pathways from activated EGFR to the COX gene are specific and independent for the pathological processes and tissue types involved (15), EGFR/Ras and phosphatidylinositol 3-kinase signaling seem likely to be principally responsible for the EGFR-mediated induction of COX-2 in squamous cell lines, which is associated with the activation of mitogen-activated protein kinase and increased activator protein 1 activity (27, 29, 31). In practice, COX-2 promoter activity is stimulated by the c-Jun, a component of the activator protein 1 transcription factor complex (29, 33–35). Conversely, the blockade of the EGFR pathway markedly reduces baseline COX-2 activities. Anti-EGFR antibodies or EGFR-selective kinase inhibitors almost totally block the induction of COX-2 enzyme in various cell lines (27, 29, 30, 36), and the pharmacological inhibition of phosphatidylinositol 3-kinase, mitogen-activated protein kinase, and p38 mitogen-activated protein kinase markedly suppressed the induction of COX-2 enzyme mediated by epidermal growth factor in cultured human uterine cervical cancer cells (29). Additional data indicate that a dominant-negative mutant for extracellular signal-regulated kinase 1, c-Jun, and p38 mitogen-activated protein kinase blocks the activation of COX-2 promoter activity, which is mediated by epidermal growth factor (29, 33, 34, 37). Given these observations, it is apparent that the activation of EGFR may be a major pathway involved in the up-regulation of COX-2 enzyme. Nonetheless, our data failed to demonstrate a strong correlation between the expressions of EGFR and COX-2; for example, patients in the EGFR-positive group showed no greater tendency toward higher COX-2 expression than those in the EGFR-negative group, nor were the

Table 3 Analysis of the prognostic factors influencing 5-year disease-free survival

Prognostic variables	No. of patients	Univariate analysis		Statistical significance	Multivariate analysis		Statistical significance
		%	95% CI[a]		RR	95% CI	
Age (yrs)							
≤55	35	63	47–79	NS	2.4	1.3–3.5	NS
>55	33	81	67–95				
Tumor size (cm)							
≤4	37	67	49–85	NS	0.7	0–1.7	NS
>4	31	74	58–90				
Pelvic LN involvement							
No	56	84	74–94	0.03	2.0	0.7–2.3	NS
Yes	12	58	30–86				
Parametrial extension							
Unilateral/Medial[b]	30	80	64–96	NS	1.8	0.8–2.8	NS
Bilateral/Lateral[c]	38	65	49–81				
EGFR expression							
Negative	19	89	75–100	0.01			
Positive	49	63	49–77				
COX-2 expression							
Negative	38	76	61–91	0.04			
Positive	30	63	45–81				
EGFR & COX-2 co-expression							
Negative or either positive	46	78	65–91	0.001	4.0	2.7–5.3	0.03
Both positive	22	55	34–76				

[a] CI, confidence interval; LN, lymph node; NS, not significant; RR, relative risk; EGFR, epidermal growth factor receptor; COX, cyclooxygenase.
[b] Unilateral and medial-half extension.
[c] Bilateral or lateral-half extension.

levels of EGFR and COX-2 immunoreactivity closely related. This lack of positive correlation between the expressions of EGFR and COX-2 can be partly explained by the fact that the COX-2 enzyme in cancer cells is mediated by various stimuli, such as phorbol esters, cyclic AMP, hormone, or cytokines, as well as various growth factors. Matsuura *et al.* (30) observed that the activation of EGFR and the production of transforming growth factor α highly up-regulated COX-2 mRNA mediated by IFN-γ in both normal keratinocytes and squamous cell lines, although treatment with either IFN-γ or transforming growth factor α did not induce COX-2 expression in some squamous cell lines, indicating that high levels of EGFR do not necessarily result in COX-2 overexpression.

Although COX-2 transcription may be regulated by the EGFR signaling pathway as well as a number of shared or convergent EGFR-independent pathways, it is clear that autocrine/paracrine signalings of EGFR or COX-2 amplification seem likely to be closely linked to a variety of biological processes associated with cancer aggressiveness and metastatic potential (2–6). Activation of EGFR signaling in human carcinoma cell lines is responsible for an invasive phenotype achieved by increasing cell motility and by producing matrix metalloproteinase-9, which degrades the basement membrane during tumor invasion and metastasis (2). Simultaneously, COX-2 is also associated with the prevention of apoptosis (17, 18) and the induction of tumor angiogenesis (19–20), which are all crucial to the promotion of tumor progression and dissemination. However, it remains to be answered how the coordinated regulation of EGFR and COX-2 contributes to further promote their biological activities by influencing important steps of tumor invasion and metastasis. According to a few studies, COX-2 enzyme enhances mitogenesis and cell proliferation processes that are stimulated by various growth factors (26, 30, 38–40). Conversely, the inhibition of COX-2 expression or activity not only inhibits epidermal growth factor-dependent mitogenesis in mouse keratinocytes (38), but it also attenuates transforming growth factor α-dependent mitogenic effects in rat intestinal epithelial cells (26). By promoting adhesion to the extracellular matrix and transcription of the angiogenetic factors, COX-2 enzyme is likely to further enhance the mitogenesis and cancer invasiveness, mediated by EGFR activation (2, 26). These findings may, in part, explain why the synchronous coexpression of EGFR and COX-2 represents a more aggressive phenotype in uterine cervical cancer.

Perhaps the most notable result of the current study is that the synchronous coexpression of EGFR and COX-2 was identified as a new and independent prognostic marker for cervical cancer. Moreover, such patients showed poorer survival than others, irrespective of well-established clinicopathological prognostic parameters. Most strikingly, approximately two-thirds of such patients died of pelvic recurrence and/or extrapelvic metastases within 3 years. It seems likely that the aberrant expressions of EGFR and COX-2 in cancer cells, at either the quantitative or qualitative level, may be associated with the increased proliferation of tumor cells during and after radiotherapy, as well as increased tumor cell resistance to chemotherapy and radiotherapy. Therefore, controlling the EGFR and COX-2 activities of cancer cells may reduce the tumor cell repopulation and/or modulate chemosensitivity and intrinsic radiosensitivity (1, 2, 41). Numerous studies have demonstrated that either

tyrosine kinase inhibitors or nonsteroidal anti-inflammatory drugs suppress tumor angiogenesis and regress tumor growth *in vivo* by reduced VEGF production (1, 2, 41–44). When combined with cytotoxic drugs and/or radiotherapy, EGFR antibodies or small-molecule EGFR tyrosine kinase inhibitors (1, 2), as well as selective COX-2 inhibitiors (41–44) enhance the efficacy of anticancer activity in several murine carcinomas, in ways that may be of clinical relevance. Furthermore, we expect that a combination of both EGFR inhibitor and COX-2 inhibitor may be more effective than either agent alone in the management of cervical cancer patients with coexpression of both oncogenes (41). Clearly, however, this type of strategy will need to be tested in appropriate preclinical models before studies in human beings can be considered.

In summary, our data suggest that the synchronous coexpression of EGFR and COX-2 immunoreactivity is an independent prognostic factor in uterine cervical cancers. Moreover, because the immunohistochemical detection of EGFR and COX-2 coexpression usefully identifies a subset of patients with an unfavorable prognosis, the targeting of EGFR signaling pathways, in combination with the selective inhibition of COX-2, is believed to offer a novel approach for the management of such patients. However, the molecular bases of these relationships remain to be further elucidated, which is why their coexpression reflects an aggressive phenotype with increased resistance to standard chemotherapy and radiotherapy.

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