Co-Overexpression of Cyclooxygenase-2 and Microsomal Prostaglandin E Synthase-1 Adversely Affects the Postoperative Survival in Non-small Cell Lung Cancer

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Introduction: Cyclooxygenase (COX)-2 and microsomal prostaglandin E synthase (mPGES)-1 have been found to be overexpressed in non-small cell lung cancer (NSCLC). The aim of this study was to investigate the expression profiles of COX-2 and mPGES-1 and their correlation with the clinical characteristics and survival outcomes in patients with resected NSCLC.

Methods/Results: Seventy-nine paired adjacent normal-tumor matched samples were prospectively procured from patients undergoing surgery for NSCLC. The protein levels of COX-2 and mPGES-1 were assessed by Western blot analysis. Overexpression in the tumor sample was defined as more than twofold increase in protein expression compared with the corresponding adjacent normal tissue. Co-overexpression of COX-2 and mPGES-1 were further confirmed by immunohistochemistry. COX-2 was overexpressed in 58% and mPGES-1 in 70% of the tumor samples ($p < 0.0001$). Co-overexpression of mPGES-1 and COX-2 was noted in 43%, and they were unrelated to each other ($p = 0.232$). Co-overexpression of both proteins was significantly associated with less tumor differentiation ($p = 0.046$), tumor size larger than 5 cm ($p = 0.038$), and worse survival status during the follow-up ($p = 0.036$). Multivariate analysis showed that in addition to overall stage, co-overexpression of both proteins adversely affected the overall (hazard ratio, 2.40; $p = 0.045$) and disease-free survivals (hazard ratio, 2.27; $p = 0.029$).

Conclusions: Overexpression of either COX-2 or mPGES-1 is common but unrelated in NSCLC. Co-overexpression of both COX-2 and mPGES-1 adversely affects postoperative overall and disease-free survivals.

Key Words: Non-small cell lung cancer, Cyclooxygenase-2, Microsomal prostaglandin E synthase-1, Survival.

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Lung cancer is the leading cause of cancer death among both men and women worldwide, and non-small cell lung cancer (NSCLC) accounts for more than 80% of all lung cancer. Surgery is the mainstay of treatment for early-stage NSCLC; however, result of surgical treatment alone remains unsatisfactory.[1] It is imperative to identify biomarkers that can predict patients' outcome after surgery.

Epidemiologic studies have shown that long-term use of nonsteroidal anti-inflammatory drugs decreases the risks of several cancers, including NSCLC.[2,3] Cyclooxygenase (COX), the key enzyme in the biosynthesis of prostaglandins (PGs), is the main target of nonsteroidal anti-inflammatory drugs.[4] Two isoforms of COX, COX-1 and COX-2, each encoded by separate genes, have been identified. In the biosynthesis of PGs, arachidonic acid is first mobilized from membrane glycerophospholipids by the action of phospholipase A_2. The COX enzymes then catalyze the formation of an intermediate PGG_2, followed by reduction to PGH_2. PGH_2 is subsequently converted to several structurally related PGs, including PGE_2, PGD_2, $PGF_{2\alpha}$, PGI_2 and thromboxane A_2 by the activity of specific PG synthases. Among the several kinds of PGs, increased production of PGE_2 has been found in various malignancies.[5,6] Many studies have indicated that PGE_2 plays a key role in carcinogenesis and cancer progression.[7,8] Increased expression of COX-2 has been found in a variety of human malignancies, including lung cancer.[9,10]

The PGE synthase (PGES), which catalyzes the formation of PGE_2, was first identified by Jakobsson et al. in 1999.[11] There are at least three PGES isoenzymes, namely the cytosolic PGES and microsomal PGES (mPGES)-1 and -2.[12,13] Similar to COX-1, cytosolic PGES has been demonstrated to be constitutively expressed in many tissues and

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functionally coupled with COX-1 in the maintenance of tissue homeostasis,[12] whereas mPGES-1 was found to be inducible and act in concert with COX-2 and contribute to a variety of physiologic and pathologic conditions, such as fever, inflammation, and reproduction.[14] Overexpression of mPGES-1 has been shown in about 65 to 80% of patients with NSCLC.[15–17]

We have previously reported that mPGES-1 overexpression was not significantly associated with either overall or disease-free survival in 93 patients undergoing surgical resection for NSCLC.[15] Additionally, there was no significant association between the expression of mPGES-1 and COX-2 in 30 cases. In this study, we have investigated the co-overexpression of COX-2 and mPGES-1 in surgical specimens prospectively procured from 79 NSCLC patients, and elucidated its role with the clinical characteristics and survival outcome.

PATIENTS AND METHODS

Patients and Tissue Procurement

Seventy-nine patients with NSCLC who underwent surgical resection in a tertiary medical center between March 1999 and June 2003 were enrolled in this study. The tissue procurement protocol was approved by the institutional review board, and written informed consent was obtained from all patients. The patients included 56 (70.9%) men and 23 (29.1%) women, with a mean age of 66.9 ± 11.2 years (mean ± standard deviation; range, 30–83 years). Tumor histology and stages were classified according to the World Health Organization classification and the international staging system for lung cancer.[18,19] Forty-seven (59.5%) tumors were classified as adenocarcinoma, 22 (27.8%) as squamous-cell carcinoma, 7 (8.9%) as large-cell carcinoma, and 3 (3.8%) as pleomorphic carcinoma. Lobectomy was performed in 60 (75.9%) patients, bilobectomy in 8 (10.1%), pneumonectomy in 5 (6.3%), segmentectomy in 3 (3.8%), and wedge resection in 3 (3.8%). Mediastinal lymph node sampling or dissection was done for all patients, with the mean number of removed lymph nodes being 17.8 ± 10.4 (mean ± standard deviation) per patient. After the surgery, 22 (27.8%) patients received adjuvant therapy; 12 (15.2%) patients received chemotherapy, 8 (10.1%) patients received radiotherapy, and 2 (2.5%) patients received chemoradiation therapy. Samples from non-necrotic area of the tumor and from adjacent nontumor tissue were obtained from at the time of surgery. These tissues were immediately frozen in liquid nitrogen, and stored at −80°C until analysis. After surgery, the patients were followed up regularly with physical examination and imaging study. The study follow-up ended in March 2006, and the mean follow-up duration was 27.2 ± 12.4 months (surgical mortality included; range, 0.3–47.8 months). The length of follow-up was defined as the interval between the date of surgical resection and the date of either death or the last follow-up. There was no difference in the overall follow-up time between patients with co-expression of COX-2 and mPGES-1 and patients without co-expression.

Protein Extraction and Western Blot Analysis

Protein extraction was carried out as previously described[20] with some modifications. In brief, frozen tissue was homogenized and thawed in ice-cold radioimmunoprecipitation buffer added with 100 μg/ml phenylmethylsulfonyl fluoride, 25 μg/ml aprotinin, 25 μg/ml lupeptin, 10 μg/ml soybean trypsin inhibitor, and 1 mM sodium orthovandate. The lysate was left on ice for 20 minutes and then centrifuged at 12,000 rpm for 10 minutes. The clarified supernatant was collected, and the protein concentration was measured using Bio-Rad protein assay kit (Bio-Rad, Hercules, CA). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed as previously described.[21] Fifty micrograms of protein from each sample was run in sodium dodecyl sulfate-polyacrylamide gel electrophoresis using a Bio-Rad Mini-Protean system (Bio-Rad), with an 8% resolving gel and 4% stacking gel. The resolved proteins were transferred onto Immobilon polyvinyl difluoride membranes (Millipore Corp., Bedford, MA). Ponceau S (Sigma Chemical, St. Louis, MO) staining of the membranes was performed to assess the equivalence of sample loading and gel transfer. Computer densitometry was used to determine the relative loading. The membranes were then destained by several washes with tap water. After blocking with 5% skimmed milk in Tris-buffered saline containing 0.1% Tween 20, the membranes were incubated with rabbit anti-human mPGES-1 polyclonal antibody (1:500; Cayman Chemical, Ann Arbor, MI) and goat anti-human COX-2 polyclonal antibody (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA), respectively. The blots were then incubated with anti-rabbit horseradish peroxidase-conjugated secondary antibody for mPGES-1 (1:2000; Amersham Pharmacia Biotech, Buckinghamshire, UK) or anti-goat horseradish peroxidase-conjugated secondary antibody for COX-2 (1:5000; Santa Cruz Biotechnology). Subsequently, membranes were developed using the Pierce SuperSignal chemiluminescent detection reagents (Pierce Biotechnology, Rockford, IL) according to the manufacturer's instructions and exposed to NEN Renaissance x-ray film (New England Nuclear, Boston, MA) with intensifying screens. The linear-range signal intensity of each specific band on the fluorogram is quantitated by a densitometric scanning system and comparison of proteins of interest is performed after normalization to the densitometric scanning of the Ponceau S staining. The control value of Ponceau S was assigned an arbitrary unit of 1, and the expression of each protein was denoted as arbitrary densitometry units (ADU) relative to the corresponding value of Ponceau S stain. Overexpression of a specific protein was defined as more than 100% increase of the ADU in tumor sample compared with the nontumor sample.

Immunohistochemistry

Tissue sections (4 μm) were prepared from paraffin-embedded tumor blocks using a microtome and mounted on Superfrost Plus slides (Dako, Kyoto, Japan). Sections were deparaffinized in xylene, rehydrated in graded alcohol, and washed in distilled water. Antigen retrieval was performed by steaming the sections in 10 mM citric acid (pH = 6.0) for 30 minutes. Subsequently, endogenous peroxidase activity was

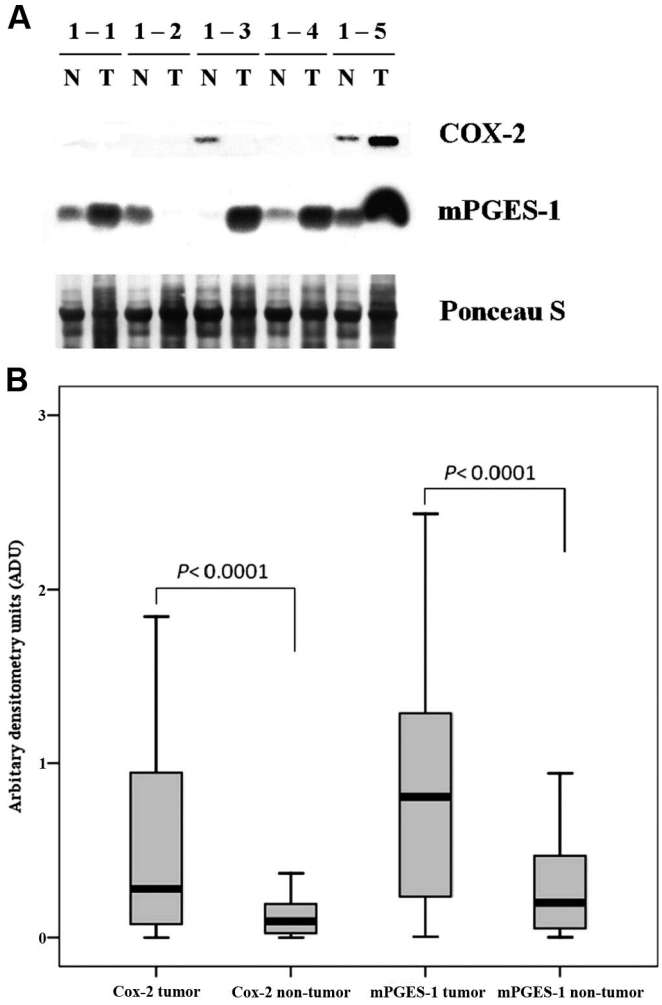

FIGURE 1. Western blot analysis of cyclooxygenase (COX)-2 and microsomal prostaglandin E synthase (mPGES)-1 in 79 patients with tumor and nontumor matched tissues. *A,* Representative Western blotting of adjacent normal (N)-tumor (T) matched tissues from five subjects. Ponceau S staining was used as a loading control. *B,* Boxplot showing the semiquantitative results of the COX-2 and mPGES-1 protein levels (in arbitrary densitometry units) in 79 patients with normal-tumor matched tissues. The bottom of each box is the 25th percentile; the top is the 75th percentile; and the band in the middle is the 50th percentile. The whiskers represent the minimum and maximum of all the data. Paired Student *t* test was used to compare between tumor and nontumor samples.

blocked with 3.0% hydrogen peroxide. The slides were washed three times in phosphate-buffered saline (PBS) and blocked for 20 minutes with 3% bovine serum albumin in PBS. Tissue sections were then incubated with rabbit anti-human mPGES-1 polyclonal antibody (Cayman Chemical) or goat anti-human COX-2 polyclonal antibody (Santa Cruz Biotechnology) at a 1:50 dilution for 1 hour at room temperature. Control sections were incubated with preimmune serum. After being washed three times with PBS, the secondary antibody Dako Link (Dako LSAB2 kit; Dako) was applied for 20 minutes and then rinsed with Tris-buffered saline. Additional washing was followed by incubation with streptavidin horseradish peroxidase (Dako LSAB2 kit; Dako) for 20 minutes. Immunoreactivity was visualized by incubation of sections with 3-amino-9-ethylcarbazole (Sigma Chemical). Subsequently, the slides were rinsed in tap water and counterstained with hematoxylin. The slides were then dehydrated with ethanol, rinsed with xylene, and mounted.

TABLE 1. The Association Between COX-2 and mPGES-1 Protein Expression in 79 Paired Samples[a]

Variable	mPGES-1 Overexpression (+)	mPGES-1 Overexpression (−)	Total
COX-2 overexpression (+)	34	12	46 (58%)
COX-2 overexpression (−)	21	12	33 (42%)
Total	55 (70%)	24 (30%)	

[a] $P = 0.232$ by Fisher's exact test (one-sided).
COX-2, cyclooxygenase-2; mPGES-1, microsomal prostaglandin E synthase-1.

Statistical Analyses

Between paired tumor and nontumor samples, the ADU of COX-2 and mPGES-1 protein expression were compared using the paired Student *t* test. The association between the expression of COX-2 and PGES in tumor tissue was analyzed with Fisher's exact test. The associations between various clinicopathologic parameters, and any one of COX-2 overexpression or mPGES-1 overexpression or co-expression of both proteins were analyzed with Fisher's exact test. All 79 subjects were included in the survival analysis. However, not every subject had complete measurements for all variables that included possible prognostic factors and important outcomes. The overall survival is defined from the date of operation to the date of death, including surgical mortality. Disease-free survival was measured from the date of operation to the date of first documented recurrence, or to date of last follow-up if no recurrence had occurred. Survival probability was calculated by Kaplan-Meier method. Univariate Cox proportional hazard regression model was applied to quantify the hazard ratio of each prognostic factor. The prognostic factors with survival probability at a significance level of 0.20 or less were considered in a multivariable Cox proportional hazard regression analysis. Variable selection with backward stepwise method was used to choose the optimal independent prognostic factors. A *p*-value less than 0.05 was considered statistically significant. All the analyses were performed with SPSS software version 13.0 (SPSS Inc., Chicago, IL).

RESULTS

Expression of COX-2 and mPGES-1 in NSCLC Tumor Samples

Western blot analysis was performed in all paired tumor and nontumor samples to assess the expression of COX-2 and mPGES-1. A representative blot of five pairs of

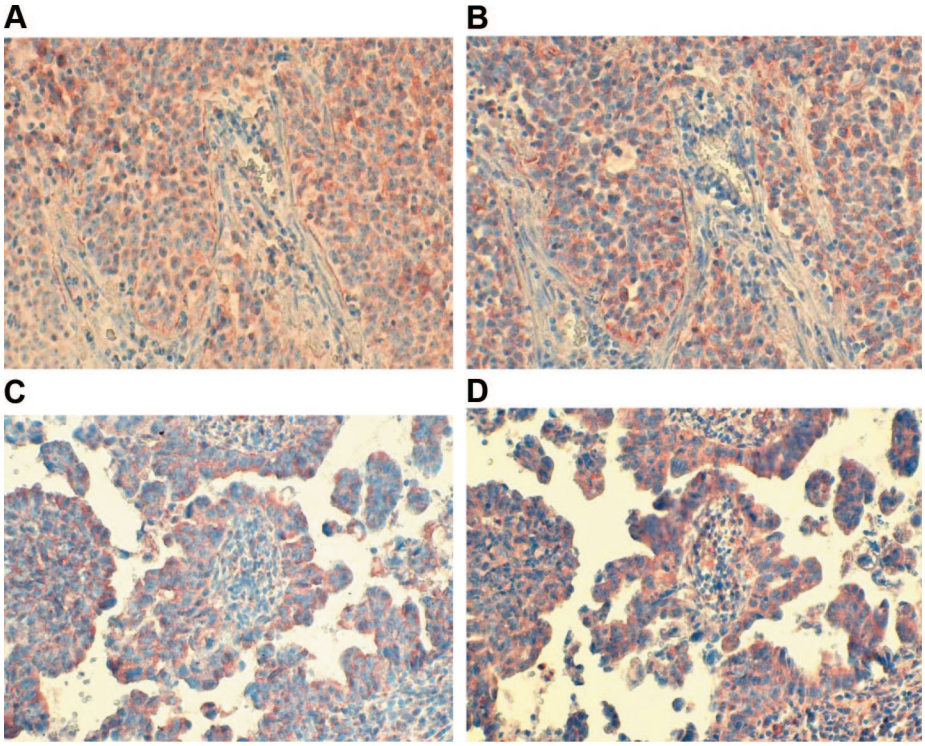

FIGURE 2. Representative results of cyclooxygenase (COX)-2 (*A* and *C*) and microsomal prostaglandin E synthase (mPGES)-1 (*B* and *D*) expressed on tissue sections of lung squamous-cell carcinoma (*A* and *B*) and adenocarcinoma (*C* and *D*) by immunohistochemistry. If Western blotting results showed co-expression of COX-2 and mPGES-1, immunohistochemistry was performed to confirm their localizations. Diffuse cytoplasmic immunoreactivity for COX-2 and mPGES-1 were observed in the cancer cells. Brown indicates positive staining of COX-2 and mPGES-1; blue indicates counterstaining with hematoxylin (original magnification, ×200).

NSCLC tumor and nontumor samples is shown in Figure 1*A*. The ADU of each protein in 79 paired tumor and nontumor tissues were depicted with box plots in Figure 1*B*. Both COX-2 and mPGES-1 exhibited significant overexpression in the tumor tissues ($p < 0.0001$, respectively). As shown in Table 1, COX-2 overexpression was noted in 46 samples, and mPGES-1 overexpression was noted in 55 samples. There were 34 (43%) samples showing co-overexpression of both proteins. Co-overexpression of both mPGES-1 and COX-2 in those samples were confirmed by immunohistochemistry (Figure 2). However, there was no significant association between the overexpression of both proteins by Fisher's exact test ($p = 0.232$).

Survival Outcomes

Among the 79 patients enrolled for study, the overall median survival was 27.7 ±12.4 months, with the 1-, 3- and 5-year cumulative survival rates of 84.8%, 76.2%, and 66.8%, respectively. At the last follow-up session, 54 (68.4%) patients were alive (16 patients with recurrent cancer); 17 (21.5%) patients died of cancer; 6 (7.6%) patients died of surgery or other causes without evidence of tumor recurrence; and 2 patients (2.5%) were lost to follow-up.

The relationship between the clinicopathologic parameters and protein expression in tumor sample is shown in Table 2. COX-2 overexpression was significantly more often observed in patients with tumors larger than 5 cm ($p = 0.002$). No significant correlations were observed between the clinicopathologic parameters and the expression of mPGES-1. Co-overexpression of both proteins was significantly associated with less tumor differentiation ($p = 0.046$), tumor size larger than 5 cm ($p = 0.038$), and worse survival status during the follow-up ($p = 0.036$).

As in Table 3, the univariate survival analysis demonstrated that either COX-2 or mPGES-1, when analyzed separately, did not affect overall or disease-free survival. However, co-overexpression of COX-2 and mPGES-1, pathologic assessment of primary tumor (pT), pathologic assessment of lymph node (pN), and overall stage did have an impact on overall and disease-free survivals.

Multivariate analysis showed co-overexpression of both proteins and overall stage significantly affected the overall and disease-free survivals (Table 4). A significant difference in overall survival and disease-free survival was observed between patients with co-overexpression of both proteins or not (Figure 3*A*, $p = 0.045$; and Figure 3*B*, $p = 0.029$, respectively).

DISCUSSION

Both COX-2 and mPGES-1 are often up-regulated in clinical cancer samples, but the relative degrees of overexpression vary, suggesting that the regulation of the two enzymes is not identical. We have previously reported that no significant association existed between the expression of COX-2 and mPGES-1 in 30 paired NSCLC tumor-nontumor samples.[15] In this study, we have further confirmed that there is no significant correlation between the expressions of mPGES-1 and COX-2 in 79 paired NSCLC samples.

Our previous study has shown that overexpression of mPGES-1 was not a prognostic indicator in patients with resected NSCLC. In this study, because of the increased sample

TABLE 2. Relationship Between the Clinicopathologic Parameters and COX-2 and mPGES-1 Protein Expression

	COX-2 Overexpression			mPGES-1 Overexpression			Overexpression of Both COX-2 and mPGES-1		
	Yes	No	p^a	Yes	No	p^a	Yes	No	p^a
Age			0.356			0.565			0.522
≥65 yr	29	23		36	16		22	30	
<65 yr	17	10		19	8		12	15	
Sex[a]			0.481			0.214			0.380
Male	32	24		37	19		23	33	
Female	14	9		18	5		11	12	
Smoking index			0.506			0.430			0.522
≥20 pack-year	19	14		22	11		20	25	
<20 pack-year	27	18		32	13		14	19	
Histologic type			0.151			0.463			0.424
Squamous cell	15	7		14	8		11	11	
Adenocarcinoma	26	21		35	12		18	29	
Large cell	4	3		5	2		4	3	
Other cell types	1	2		1	2		1	2	
Differentiation			0.276			0.096			0.046
Well	0	4		3	1		0	4	
Moderate	32	18		38	12		26	24	
Poor	13	10		13	10		7	16	
Angiolymphatic invasion			0.071			0.281			0.310
Yes	19	7		17	9		21	30	
No	27	24		38	13		13	13	
Tumor size			0.002			0.467			0.038
≥5 cm	18	3		14	7		13	37	
<5 cm	28	30		41	17		21	8	
pN status			0.135			0.415			0.055
Positive	19	9		19	9		16	12	
Negative	25	23		35	13		17	31	
pM status			0.300			0.482			0.368
Positive	4	1		3	2		31	43	
Negative	42	32		52	22		3	2	
Pathologic stage			0.363			0.344			0.420
Stage I	20	20		29	11		14	26	
Stage II	9	3		6	6		5	7	
Stage III	13	9		17	5		12	10	
Stage IV	4	1		3	2		3	2	
Adjuvant therapy			0.565			0.324			0.509
Yes	13	9		14	8		9	13	
No	33	24		41	16		25	32	
Tumor recurrence			0.076			0.535			0.077
Yes	21	11		22	10		17	29	
No	22	22		31	13		15	15	
Follow-up status			0.145			0.598			0.036
Dead	16	7		16	7		14	9	
Alive	30	26		39	17		20	36	

[a] The p values were calculated by Fisher's exact test.

COX-2, cyclooxygenase-2; mPGES-1, microsomal prostaglandin E synthase-1; pN, pathologic assessment of lymph node; pM, pathologic assessment of distant metastasis.

size, we were able to explore and compare clinicopathologic parameters in patients with and without co-overexpression of COX-2 and mPGES-1. Moreover, we have used Western blot analysis to quantitate the protein expression and immunohistochemical study to confirm the co-overexpression of both proteins. Co-overexpression of both COX-2 and mPGES-1 proteins was associated with worse clinical outcome in terms of overall and disease-free survivals.

Many studies, including our previous report, have shown that mPGES-1 is overexpressed in NSCLC.[15,17,22]

TABLE 3. Univariate Cox Proportional Hazards Model for Survival Analysis

	Overall Survival			Disease-Free Survival		
Variable	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
COX-2 overexpression (+ vs. −)	1.752	0.720–4.260	0.216	1.703	0.824–3.517	0.150
mPGES-1 overexpression (+ vs. −)	1.184	0.484–2.894	0.711	1.251	0.591–2.651	0.558
Co-expression COX-2 and mPGES-1	2.612	1.125–6.064	0.025	2.385	1.190–4.782	0.014
Age (≥65 vs. <65)	2.778	0.944–8.179	0.064	0.933	0.455–1.912	0.849
Smoking index (≥20 vs. <20)	1.921	0.841–4.391	0.121	0.812	0.403–1.636	0.560
Sex (female vs. male)	0.783	0.308–1.989	0.607	1.415	0.685–2.924	0.349
Differentiation grade[a] (+1[b])	1.930	0.931–4.083	0.085	1.156	0.626–2.134	0.644
Angiolymphatic invasion (+ vs. −)	2.095	0.922–4.460	0.077	1.694	0.835–3.435	0.144
Adjuvant therapy (yes vs. no)	1.052	0.433–2.560	0.910	1.463	0.718–2.298	0.294
pT stage[a] (+1[b])	1.619	1.063–2.464	0.025	1.812	1.257–2.612	0.001
pN stage[a] (+1[b])	2.226	1.452–3.414	<0.001	2.214	1.488–3.293	<0.001
Overall stage[a] (+1[b])	2.005	1.346–2.985	0.001	2.128	1.517–2.965	<0.001

[a] Differentiation grade, pT, pN, and overall stage were assessed as continuous variables.
[b] Scoring was assessed for each unit increase.
COX-2, cyclooxygenase-2; mPGES-1, microsomal prostaglandin E synthase-1; HR, hazard ratio; CI, confidence interval.

TABLE 4. Multivariate Cox Proportional Hazards Model for Survival Analysis

	Overall Survival			Disease-Free Survival		
Variable	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***P***
Co-expression of COX-2 and mPGES-1	2.399	1.019–5.651	0.045	2.268	1.089–4.722	0.029
Overall stage[a] (+1[b])	1.971	1.310–2.966	0.001	2.094	1.451–3.022	<0.001

[a] Overall stage was assessed as continuous variable.
[b] Scoring was assessed for each unit increase.
COX-2, cyclooxygenase-2; mPGES-1, microsomal prostaglandin E synthase-1; HR, hazard ratio; CI, confidence interval.

However, expression of mPGES-1 has been demonstrated in bronchial epithelial cells in clinical specimen by immunohistochemical study.[17] In transgenic mice experiment, overexpression of mPGES-1 in bronchial epithelial cells did not lead to lung tumorigenesis.[23] Interestingly, tumor necrosis factor-α induced mPGES-1 in NSCLC cell lines, but not in bronchial epithelial cell line.[22] Martey et al.[24] have shown that cigarette smoke induced the expression of COX-2 and mPGES-1 in human lung fibroblasts. Therefore, the expression of mPGES-1 in lung tissues could be related to cellular transformation, cytokine stimulation, and inflammatory process.

Yoshimatsu et al.[16] observed marked differences in the extent of mPGES-1 and COX-2 expression in individual paired samples from NSCLC patients. In NSCLC cell lines, treatment with cytokines, such as IL-4 and tumor necrosis factor-α, has shown to cause differential expression between COX-2 and mPGES-1.[16,25] Lack of correlation or marked difference between mPGES-1 and COX-2 expression in clinical tumor samples has also been demonstrated in breast and colon cancers.[22,26] Similar finding on lack of coupling of mPGES-1 and COX-2 expression has been identified in brain and chondrocytes.[27,28]

Murakami et al.[29] showed that cells co-expressing COX-2 and mPGES grew faster, produced more PGE2, and exhibited aberrant morphology compared with cells in which either COX-2 or mPGES overexpressed. In a study investigating markers involved in PG biosynthetic pathway, it has been shown that COX-2, mPGES, and other markers may act synergistically and promote tumor metastasis and angiogenesis in patients with NSCLC.[30] It is plausible that co-overexpression of both proteins leads to higher level of PGE2 and cellular addiction, and ultimately makes cancer cell more aggressive in metastasis and invasion.

In an exploratory analysis with complementary DNA microarray, we have preliminarily identified no significantly shared expression patterns between overexpression of COX-2 and mPGES-1 (data not shown). Additionally, we have identified different genetic profiles responsible for the overexpression of COX-2 and mPGES-1 in NSCLC. Further study is in progress to characterize novel markers identified from microarray analysis and study the roles of these markers in the mPGES-1- and COX-2-related carcinogenesis and drug-resistant mechanism.

The surgical treatment of early-stage NSCLC remains unsatisfactory, with 5-year survival rates for pathologic stages II and I disease ranging from 39 to 67%.[1] In patients with resected stage II or III NSCLC, adjuvant chemotherapy with vinorelbine and cisplatin can provide approximately 8 to 15% survival benefit at 5 years.[31,32] The use of adjuvant chemotherapy in stage I NSCLC remains controversial. Identification and validation of prognostic and predictive markers

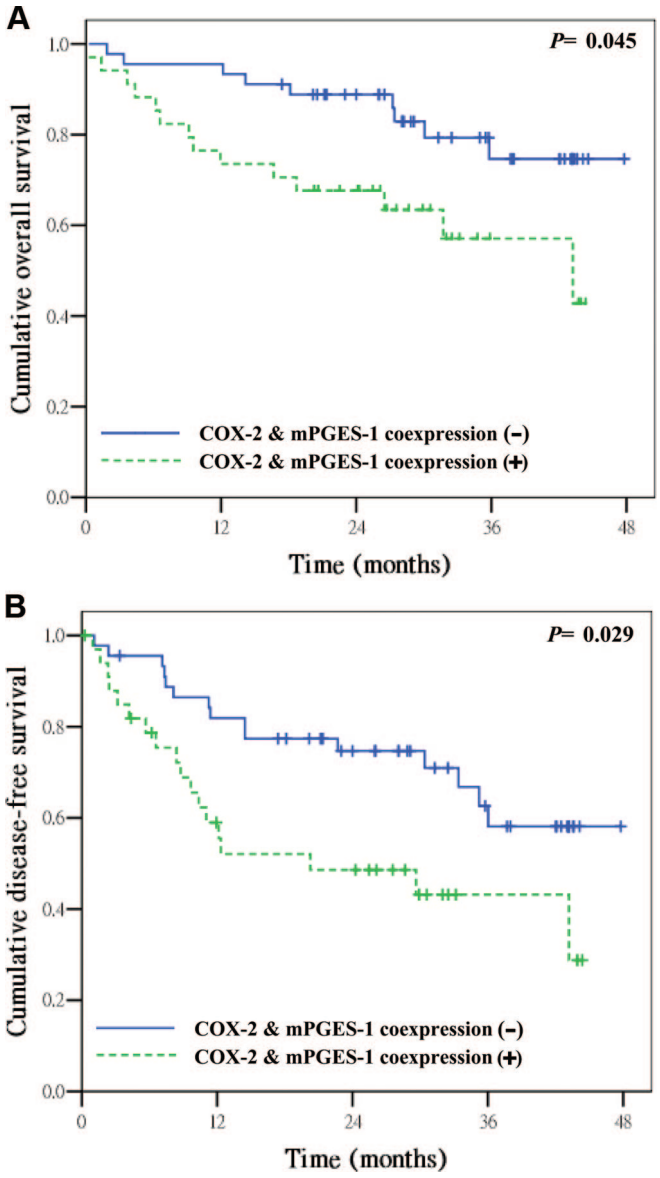

FIGURE 3. Kaplan-Meier analysis showing cumulative overall survival (*A*) and disease-free survival (*B*) with regard to the co-expressed effects of cyclooxygenase (COX)-2 and microsomal prostaglandin E synthase (mPGES)-1. A significant difference in overall survival and disease-free survival was observed between patients with co-expression and patients without co-expression (p = 0.045 and 0.029, respectively, by multivariable Cox proportional hazard regression analysis).

for patients with resected NSCLC are currently under active investigation.[33] Prognostic markers can be used to select patients with increased risk of recurrence who may derive more benefit from adjuvant treatment. Predictive markers can be used to select agents to which patients' tumors are most likely to respond. Many prognostic markers have been investigated in NSCLC, including k-ras mutation, p53 mutation, excision repair cross-complementation group 1 (ERCC1), ribonucleotide reductase subunit M1 (RRM1) expression, gene-expression array, and single nucleotide polymorphisms.[34–39]

Although both COX-2 and mPGES-1 are frequently overexpressed in NSCLC, the prognostic value of either COX-2 or mPGES-1 remains to be determined. A meta-analysis of 10 studies involving more than 1200 patients has shown that only in patients with stage I NSCLC, COX-2 overexpression is associated with reduced survival (combined hazard ratio, 1.64; 95% confidence interval, 1.21–2.24).[40] Increased COX-2 expression leading to elevated PGE2 level has been demonstrated in patients receiving chemotherapy for NSCLC.[41] Inhibition of COX-2 with celecoxib, when given concurrently with chemotherapy, attenuated the surge of PGE2 level in lung tumors and enhanced the response of preoperative paclitaxel and carboplatin chemotherapy in early-stage NSCLC.[42] In Cancer and Leukemia Group B Trial 30203, Edelman et al.[43] have demonstrated in patients with advanced NSCLC that chemotherapy plus COX-2 inhibitor therapy can improve the survival in patients with tumors overexpressing COX-2, compared with chemotherapy alone. In this study of 79 patients receiving surgery for NSCLC, multivariate analysis has shown that, in addition to overall stage, co-overexpression of COX-2 and mPGES-1 adversely affected the overall and disease-free survivals. The prognostic and predictive roles of COX-2 and mPGES-1 co-expression should be further explored in patients receiving postoperative chemotherapy in conjunction with inhibition of COX-2 or PGE2.

In conclusion, we have shown that there in no association between the overexpression of COX-2 and mPGES-1 in NSCLC. Co-overexpression of both COX-2 and mPGES-1 adversely affects the overall and disease-free survivals in patients receiving surgery for NSCLC. The limitations of our findings are mainly attributed to the heterogeneous patient population in terms of stages (stage I, 40; stage II, 12; stage III, 21; and stage IV, 5 patients), histologic types (adenocarcinoma, 47; squamous cell, 22; large cell, 7; and other cell type, 3 patients), and adjuvant therapy (observation, 57; chemotherapy, 12; radiotherapy, 8; and chemoradiotherapy, 2 patients). Our finding can be used to identify patients at risk after surgery for NSCLC, and to develop novel therapeutic strategies to improve outcome. We are planning to incorporate this finding with other biomarkers, such as expression of ERCC1 and RRM1 to design novel adjuvant treatment study for patients with resected early-stage NSCLC.

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