Tumor-Infiltrating Foxp3+ Regulatory T Cells are Correlated with Cyclooxygenase-2 Expression and are Associated with Recurrence in Resected Non-small Cell Lung Cancer

Katsuhiko Shimizu, MD, PhD, Masao Nakata, MD, PhD, Yuji Hirami, MD, PhD, Takuro Yukawa, MD, Ai Maeda, MD, and Kazuo Tanemoto, MD, PhD

Background: Cyclooxygenase-2 (COX-2) is constitutively overexpressed in a variety of epithelial malignancies and is usually associated with a poor prognosis. COX-2-derived prostaglandin E_2 transforms CD4+CD25+ T regulatory (Treg) cells (Tregs), and Tregs are thought to moderate the antitumor immune response. Herein, we investigated the prognostic value of tumor-infiltrating Treg cells and their correlation with COX-2 expression in resected non-small cell lung cancer (NSCLC).

Material and Methods: Intratumoral COX-2 and Treg expression were retrospectively assessed using immunohistochemistry in paraffin-embedded samples from 100 patients who had undergone complete resections for NSCLC. The expressions of COX-2 and Foxp3, which was most specific Treg cell marker, were compared with the clinicopathological variables, and the correlation between Foxp3+ Tregs and COX-2 expression was analyzed.

Results: The recurrence-free survival (RFS) of patients with elevated COX-2 expression was significantly worse than that of patients without COX-2 expression. Tumor-infiltrating Foxp3-positive lymphocytes were positively correlated with COX-2 expression. The median count for Foxp3-positive lymphocytes was 3 (minimum-maximum, 0–24) in 10 high-power fields. The RFS of patients with tumors containing ≥3 Foxp3-positive cells (Foxp3 expression group) was significantly worse than that of patients with tumors containing <3 Foxp3-positive cells. In a multivariate analysis, only nodal status was an independent predictor of a significantly shorter RFS. However, in node-negative NSCLC, Foxp3 expression was an independent predictor of a significantly shorter RFS.

Conclusions: Tumor-infiltrating Foxp3+ Tregs were positively correlated with intratumoral COX-2 expression and were associated with a worse RFS, especially among patients with node-negative NSCLC.

Division of Thoracic and Cardiovascular Surgery, Department of Surgery, Kawasaki Medical School, Kurashiki, Japan.
Disclosure: The authors declare no conflict of interest.
Address for correspondence: Katsuhiko Shimizu, MD, PhD, 577 Matsushima, Kurashiki Okayama 701-0192, Japan. E-mail: kshimizu@med.kawasaki-m.ac.jp

ISSN: 1556-0864/10/0505-0585

Key Words: Foxp3, Regulatory T cell, Cyclooxygenese-2, Non-small cell lung cancer.

(*J Thorac Oncol.* 2010;5: 585–590)

Lung cancer is a major cause of death in many developed countries. Surgical resection continues to play an important role in the treatment of this disease, especially during the early stages of lung cancer. Even when patients are diagnosed at an early stage, however, the relapse rate is high as 15 to 35% after surgical resection in Japan.[1] Despite having identical radiologic and histologic features, many patients with presumed localized disease also have undetectable metastases at the time of diagnosis. Thus, the current clinical-pathologic staging system is inadequate.

Cyclooxygenase (COX) is the key enzyme required for the conversion of arachidonic acid to prostaglandins (PGs). Two COX isoforms have been identified and are referred to as constitutive COX (COX-1) and inducible COX (COX-2),[2] COX-1 is constitutively expressed in many tissues and plays an important role in the control of homeostasis. Conversely, COX-2 is an inducible enzyme that is activated in response to extracellular stimuli such as growth factors and proinflammatory cytokines.[3] Some investigators have demonstrated that COX-2 is constitutively overexpressed in a variety of epithelial malignancies such as lung, breast, pancreas, colon, esophagus, and head and neck cancers, and COX-2 overexpression is usually associated with a poor prognosis.[4–9]

T regulatory (Treg) cells (Tregs) were initially characterized as having a CD4+CD25+ phenotype, and these cells are thought to modulate the antitumor immune response.[10] Tregs can suppress the activity of cytotoxic T cells through direct cell-to-cell contact or through the release of cytokines. The most specific Treg cell marker identified to date is a nuclear transcription factor known as Foxp3.[11,12] A high density of tumor-infiltrating Foxp3+ Tregs is reportedly associated with a higher risk of recurrence and a poor overall survival among patients with certain types of malignant neoplasms.[13–18] Thus, Treg cells within the tumor microenvironment might play a significant role in the suppression of local antitumor immune responses.

A recent report has shown that tumor-derived COX-2/PGE_2 induces the expression of the Foxp3 gene and increases Treg cell activity in lung cancer.[19] In this study, we investigated the prognostic value of intratumoral COX-2 expression and tumor-infiltrating Foxp3+ Tregs and determined whether a correlation exists between the expression of COX-2 and Foxp3+ Tregs in non-small cell lung cancer (NSCLC).

PATIENTS AND METHODS

Study Population

One hundred patients with NSCLC who underwent resection at our institution were retrospectively studied. All the patients had undergone a lobectomy and lymph node dissection and had followed up for at least 2 years. Patients who had received induction therapy or who had another malignancy were excluded. The baseline demographics, histopathologic data, RFS period, and pathologic specimens preserved in paraffin were available for all the patients. A written informed consent was obtained from each patient before surgery, and this study was approved by the institutional Ethics Committee of Kawasaki Medical School.

Immunohistochemistry

Immunohistochemical analyses were performed using resected, paraffin-embedded lung cancer tissues. After microtome sectioning (4 μm), the slides were processed for COX-2 and Foxp3 staining using an automated immunostainer (Nexes; Ventana, Tucson, AZ). The streptavidin-biotin-peroxidase detection technique using diaminobenzidine as a chromogen was applied. The primary antibodies were used according to the manufacturer's instructions (COX-2: DakoCytomation clone CX-294, 1/50 dilution; Foxp3: Abcam, clone 22510, 1/100 dilution).

The slides were examined by an investigator who had no knowledge of the corresponding clinicopathologic data. To evaluate the COX-2 immunostaining, the reactions in smooth muscles and vascular endothelial cells, which were present in all the specimens, were used as internal built-in controls. Cases with tumor cells that exhibited a significantly more intense staining pattern than an internal control cells were recorded as positive[20] (Figure 1*A*). To evaluate Foxp3 immunostaining, 10 high-power fields (HPFs) digital images of the tumor areas were selected, and the absolute number of Foxp3-positive lymphocytes in these 10 HPF digital images was determined. The number of immunostained Foxp3 cells was then determined by averaging the 10 HPF digital image cell counts[14] (Figure 1*B*).

Statistical Analysis

Given that there are no widely accepted standard cutoff points for defining clinical outcome according to the number of Tregs in the present setting and consistent with the method used in previous studies, we selected the median intratumor Foxp3+ T cell count of the entire group as the cutoff value. We then assessed the associations among COX-2 expression, Treg number, clinicopathologic features, and RFS. The following patient characteristics were investigated: age, sex, histology, tumor size, and nodal involvement of lung cancers. A statistical analysis examining significant differences among the categorized groups and possible correlations between COX-2 expression and the clinicopathologic features were performed using the Fisher's exact test or the χ^2 test, as appropriate. An unpaired *t* test was used for the comparison of continuous data. Multivariate analyses were performed using a logistic regression analysis. To explore the association between RFS and COX-2 expression or the Foxp3+ Treg count, a Kaplan-Meier survival analysis was performed by stratifying significant predictor variables identified in the Cox proportional hazards model. All data were analyzed using the Stat View software program, version 5.0 (SAS Institute Inc., Cary, NC). All statistical tests were two -sided, and probability values <0.05 were regarded as statistically significant.

RESULTS

The patient characteristics and immunohistochemical variables are shown in Table 1. The tumors were staged according to the 1997 tumor, node, metastasis staging system.

Relation between the Expression Status of COX-2 and Clinicopathologic Characteristics

Of the 100 patients, 65 exhibited markedly more intense COX-2 immunoreactivities in their tumor cells, whereas the remaining 35 cases did not show an increase in COX-2 expression. A significant association between elevated COX-2 expression and nodal involvement was observed ($p = 0.033$), but no significant associations with age ($p = 0.212$), sex ($p = 0.285$), histology ($p = 0.129$), tumor size ($p = 0.739$), or disease stage ($p = 0.130$) were noted (Table 1). The RFS of patients with elevated COX-2 expres-

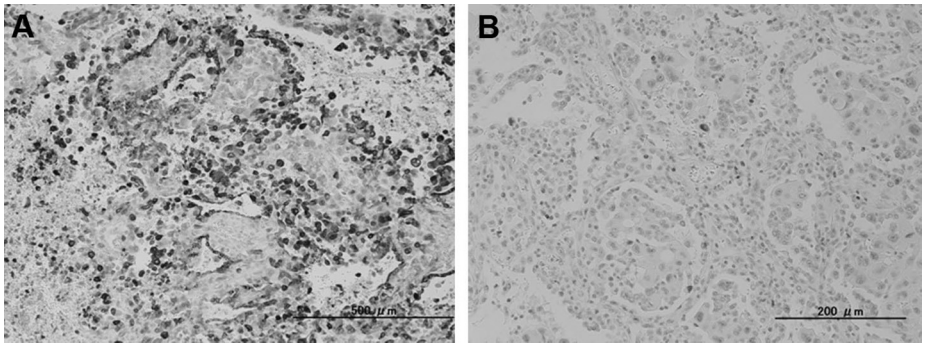

FIGURE 1. Immunohistochemical staining for COX-2 (A) and Foxp3 (B).

TABLE 1. The Patient Characteristics by COX-2 and Foxp3

	COX-2 Expression			Foxp3-Positive Cells		
Characteristics	**Positive**	**Negative**	***p***	**≥3**	**<3**	***p***
Patients, number	65	35		51	49	
Age (yr)	68 ± 11	66 ± 9	0.212	69 ± 10	67 ± 10	0.192
Gender						
Male	42	18	0.285	31	29	0.999
Female	23	17		20	20	
Histology						
Adenocarcinoma	41	28	0.129	33	36	0.465
Squamous cell carcinoma	24	7		18	13	
Primary tumor (pT)						
T1	31	18	0.739	25	24	0.844
T2	25	14		19	20	
T3–4	9	3		7	5	
Nodal involvement (pN)						
N0	43	31	0.033	34	40	0.081
N1	9	3		6	6	
N2	13	1		11	3	
Disease stage						
IA	23	16	0.130	18	21	0.255
IB	16	13		13	16	
II(A+B)	11	3		7	7	
III(A+B)	15	3		13	5	

COX, cyclooxygenese-2.

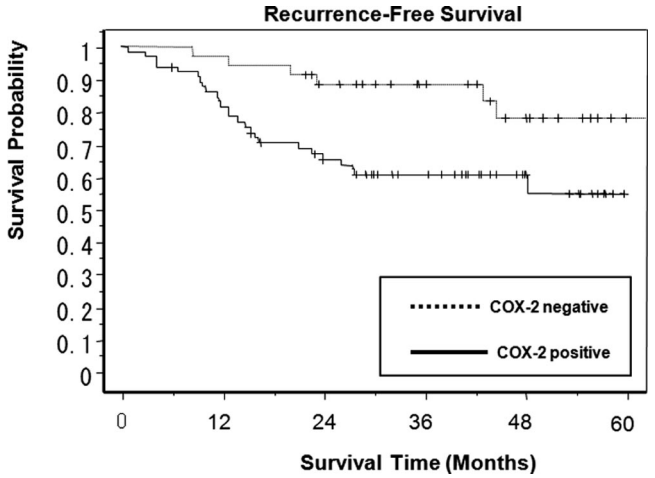

FIGURE 2. Kaplan-Meier recurrence-free survival curve according to COX-2 expression, log-rank $p = 0.017$.

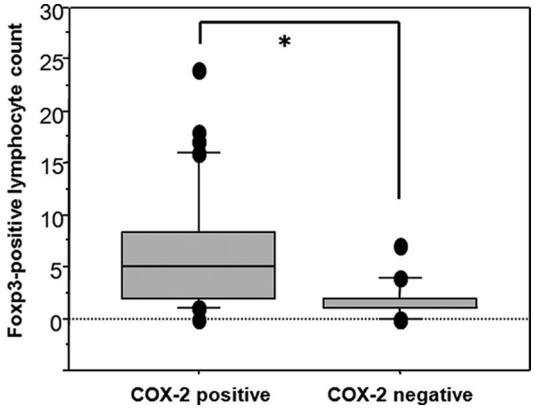

FIGURE 3. Relationship between the expression status of COX-2 and tumor-infiltrating Foxp3+ regulatory T-cells, $*p < 0.001$.

sion was significantly worse than that of patients without COX-2 expression ($p = 0.017$ according to a log-rank test; Figure 2).

Relation between the Expression Status of COX-2 and the Foxp3-Positive Lymphocyte Count

In the COX-2 positive group, the mean Foxp3-positive lymphocyte count of 10 HPF was 6.5 ± 5.5. Conversely, in the COX-2 negative group, the mean Foxp3-positive lymphocyte count of 10 HPF was 1.7 ± 1.5. Tumor-infiltrating Foxp3-positive lymphocytes were positively correlated with COX-2 expression ($p < 0.001$) (Figure 3).

Relation between the Expression Status of Foxp3 and Clinicopathologic Characteristics

Among the 100 cases, the median count of Foxp3-positive lymphocytes was 3 (minimum-maximum, 0–24). The number of cases with Foxp3 cells <3 and the number with ≥3 (Foxp3 expression group) were 49 and 51, respectively. No significant associations between an elevation in Foxp3 expression and age ($p = 0.192$), sex ($p = 0.999$),

histology ($p = 0.465$), tumor size ($p = 0.844$), nodal involvement ($p = 0.081$), or disease stage ($p = 0.255$) were noted (Table 1). The RFS of patients with tumors containing ≥3 Foxp3 cells (Foxp3 expression group) was significantly worse than that of patients with tumors containing <3 Foxp3 cells ($p = 0.004$ according to a log-rank test; Figure 4).

Prognostic Value of COX-2 and Tregs

A univariate analysis revealed that nodal status ($p = 0.001$), COX-2 expression ($p = 0.017$), and Foxp3 expression ($p = 0.004$) were independent risk factors associated with RFS. Nevertheless, a multivariate analysis revealed that only nodal status was an independent risk factor ($p = 0.004$) and that COX-2 expression ($p = 0.320$), and Foxp3 expression ($p = 0.107$) were not independent risk factors (Table 2). In the node-negative group (N = 76), the RFS of patients with elevated COX-2 and Foxp3 expression levels was significantly worse than that of patients without COX-2 expression ($p = 0.024$) or without Foxp3 expression ($p < 0.001$) (Figure 5). Conversely, in the node-positive NSCLC group, the RFS of patients elevated COX-2 and Foxp3 expression levels was not significantly worse than that of patients without COX-2 expression ($p = 0.933$) or without Foxp3 expression ($p = 0.668$). A multivariate analysis revealed that only Foxp3 expression was an independent predictor of RFS ($p = 0.016$) (Table 2).

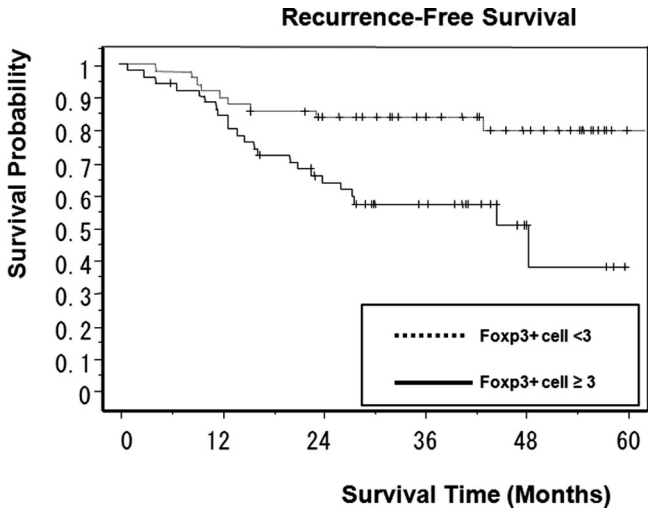

FIGURE 4. Kaplan-Meier recurrence-free survival curve according to Foxp3 expression, log-rank $p = 0.004$.

DISCUSSION

Previous reports have shown that the total number of tumor-infiltrating lymphocytes (TILs) is positively associated with patient prognosis in lung cancer and other cancers.[21–23] However, whether all TILs have an antitumor effect is unclear. In 2001, Woo et al.[24] showed that large populations of CD4+CD25+ T cells were present among the TILs of patients with NSCLC. They demonstrated that CD4+CD25+ T cells selectively inhibit the host immune response and therefore could contribute to the progression of lung cancer.[25] Since then, malignancies such as lung cancer have also been noted to have increased numbers of Treg cells and Treg activity levels within the peripheral blood and within TIL populations.[26–29] In addition, a high density of tumor-infiltrating Foxp3+ Tregs is reportedly associated with a higher risk of recurrence and a poor overall survival in patients with stage I NSCLC.[13] Our studies also showed that the number of tumor-infiltrating Foxp3+ Tregs was associated with a worse RFS, especially among patients with node-negative NSCLC.

Meanwhile, a report published nearly a decade ago showed that COX-2 overexpression was associated with a poor prognosis and a short survival period in NSCLC.[4] To date, several investigators have intensively studied the contribution of COX-2 to tumorigenesis. Several mechanisms are thought to mediate the tumorigenic activity of COX-2 as follows: (1) PGs directly stimulate the proliferation of cancer cells[30,31]; (2) COX-2 acts as an angiogenic stimulator and has been shown to increase the production of angiogenic factors and the migration of endothelial cells[32,33]; (3) COX-2-derived PGs function to prevent apoptosis induced by anticancer drugs[34,35]; (4) COX-2 expression might increase the invasive ability of tumor cells, promoting cancer metastasis[36]; and (5) PGs are immunoregulatory molecules that can suppress antitumor activity.[37,38] Our studies also showed that COX-2 expression was associated with a worse RFS.

Of note, the number of tumor-infiltrating Foxp3+ Tregs was positively correlated with intratumoral COX-2 expression in this study. To our knowledge, only one other report has shown that the number of Tregs is positively correlated with intratumoral COX-2 expression. Li et al.[39]

TABLE 2. Prognostic Value of Recurrence-Free Survival

	Univariate Analysis		Multivariate Analysis	
Variables	**Unfavorable/Favorable**	***p***	**HR (95% CI)**	***p***
All cases				
Nodal involvement	N1–2/N0	0.001	2.89 (1.40–5.98)	0.004
COX-2 expression	Positive/negative	0.017	1.64 (0.62–4.37)	0.320
Foxp3 expression	≥3/<3	0.004	2.02 (0.86–4.74)	0.107
Node-negative cases				
COX-2 expression	Positive/negative	0.024	1.92 (0.50–7.39)	0.344
Foxp3 expression	≥3/<3	<0.001	5.38 (1.38–21.07)	0.016

HR, hazard ratio; CI, confidence interval.

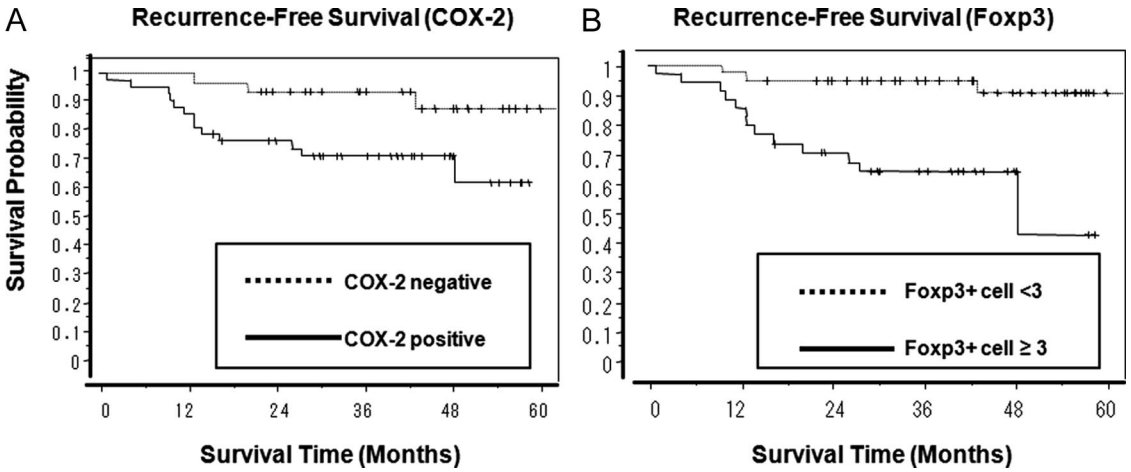

FIGURE 5. Kaplan-Meier recurrence-free survival curve in node-negative non-small cell lung cancer (NSCLC) according to (*A*) COX-2 expression and (*B*) Foxp3 expression in node negative patients, log-rank $p = 0.024$ and $p < 0.001$.

demonstrated that an increase in the number of peritumoral Tregs was associated with a worse prognosis and was positively correlated with intratumoral COX-2 expression in patients with renal cell carcinoma. Our results using tissues from patients with NSCLC are similar to this previous report examining renal cell carcinoma and represent the first report of these phenomena in NSCLC.

A recent study reported that tumor-induced Treg cell activity can be downregulated by COX-2 inhibition, leading to the restoration of antitumor responses. Shama et al.[19] documented a COX-2-dependent immunosuppressive network in the NSCLC microenvironment in mice, and tumor-derived COX-2/PGE_2 induced the expression of the Treg cell-specific transcription factor Foxp3, resulting in an increase in Treg cell activity. In addition, they documented that COX-2/PGE2 suppressed antigen presentation, decreases maturation of dendritic cells and potently induced IL-10 transcription while reducing IL-12.[40] Baratelli et al.[41] reported that PGE_2 is involved in the modulation of T cell function and differentiation in vitro, and an increase in T cell function and differentiation could contribute to tumor-induced immunosuppression. Mahic et al.[42] also described Treg cells expressed COX-2 and Foxp3 in vitro, and Treg cells produced PGE_2 and suppressed effector T cell responses in a manner that is reversed by COX-2 inhibitors and PGE_2 receptor-specific antagonist. Recently, they described that Treg cells expressed cell surface markers consistent with activated phenotype and secreted high levels of TGF-ß and IL-10 excluding PGE_2.[43] Our present study using resected tissue supported these basic experiments, confirming a correlation between Tregs and COX-2 expression in NSCLC. Future cancer treatments targeting both the control of COX-2 and Treg might be feasible. In fact, recent clinical trial by Cancer and Leukemia Group B demonstrated that patients with increased COX-2 expression receiving COX-2 inhibitor had better survival than did COX-2-expressing patients not receiving drug.[44]

In summary, the present results indicate that the number of tumor-infiltrating Foxp3+ Tregs is positively correlated with intratumoral COX-2 expression. Among patients with node-negative NSCLC, in particular, Foxp3 expression (tumors containing ≥3 Foxp3 positive cells in 10 HPFs) was an independent prognostic factor in a multivariate analysis. Thus, COX-2 expression might suppress antitumor activity by tumor-infiltrating Tregs. A COX-2 inhibitor might be beneficial for the treatment of patients with COX-2 overexpression. Further studies examining other types of cancer are necessary.

ACKNOWLEDGMENTS

The authors thank Keiko Isoda for technical assistance.

REFERENCES

1. Asamura H, Goya T, Koshiishi Y, et al. A Japanese Lung Cancer Registry study: prognosis of 13,010 resected lung cancers. *J Thorac Oncol* 2008;3:46–52.
2. Griswold DE, Adams JL. Constitutive cyclooxygenase (COX-1) and inducible cyclooxygenase (COX-2): rationale for selective inhibition and progress to date. *Med Res Rev* 1996;16:181–206.
3. Dubois RN, Abramson SB, Crofford L, et al. Cyclooxygenase in biology and disease. *FASEB J* 1998;12:1063–1073.
4. Hida T, Yatabe Y, Achiwa H, et al. Increased expression of cyclooxygenase 2 occurs frequently in human lung cancers, specifically in adenocarcinomas. *Cancer Res* 1998;58:3761–3764.
5. Hwang D, Scollard D, Byrne J, et al. Expression cyclooxygenase-1 and cyclooxygenase-2 in human breast cancer. *J Natl Cancer Inst* 1998;90: 455–460.
6. Okami J, Yamamoto H, Fujiwara Y, et al. Overexpression of cyclooxygenase-2 in carcinoma of the pancreas. *Clin Cancer Res* 1999;5:2018–2024.
7. Ogino S, Kirkner GJ, Nosho K, et al. Cyclooxygenase-2 expression is an independent predictor of poor prognosis in colon cancer. *Clin Cancer Res* 2008;14:8221–8227.
8. Takatori H, Natsugoe S, Okumura H, et al. Cyclooxygenase-2 expression is related to prognosis in patients with esophageal squamous cell carcinoma. *Eur J Surg Oncol* 2008;34:397–402.
9. Peng JP, Chang HC, Hwang CF, et al. Overexpression of cyclooxygenase-2 in nasopharyngeal carcinoma and association with lymph node metastasis. *Oral Oncol* 2005;41:903–908.
10. Curiel TJ. Tregs and rethinking cancer immunotherapy. *J Clin Invest* 2007;117:1167–1174.
11. Kim JM, Rudensky A. The role of the transcription factor Foxp3 in the development of regulatory T cells. *Immunol Rev* 2006;212:86–98.

12. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 2003;299:1057–1061.
13. Petersen RP, Campa MJ, Sperlazza J, et al. Tumor infiltrating Foxp3+ regulatory T-cells are associated with reccurence in pathologic stage 1 NSCLC patients. *Cancer* 2006;107:2866–2872.
14. Perrone G, Ruffini PA, Catalano V, et al. Intratimoural FOXP3-positive regulatory T cells are associated with adverse prognosis in radically resected gastric cancer. *Eur J Cancer* 2008;44:1875–1882.
15. Gao Q, Qiu SJ, Fan J, et al. Intratumoral balance of regulatory and cytotoxic T cells is associated with prognosis of hepatocellular carcinoma after resection. *J Clin Oncol* 2007;25:2586–2593.
16. Bates GJ, FOX SB, Han C, et al. Quantification of regulatory T cells enables the identification of high-risk breast cancer patients and those at risk of late relapse. *J Clin Oncol* 2006;23:5373–5370.
17. Hiraoka N, Onozato K, Kosuge T, et al. Prevalence of FOXP3+ regulatory T cells increases during the progression of pancreatic ductal adenocarcinoma and its premalignant lesions. *Clin Cancer Res* 2006;12: 5423–5434.
18. Salama P, Phillips M, Grieu F, et al. Tumor-Infltrating FOXP3+ T regulatory cells show strong prognostic significance in colorectal cancer. *J Clin Oncol* 2009;27:186–192.
19. Shama S, Yang SC, Zhu L, et al. Tumor cyclooxygenase-2/prostaglandin E_2-dependent promotion of FOXP3 expression and $CD4^+CD25^+$ T regulatory cell activities in lung cancer. *Cancer Res* 2005;65:5211–5220.
20. Achiwa H, Yatabe Y, Hida T, et al. Prognostic significance of elevated cyclooxygenase 2 expression in primary, resected lung adenocarcinomas. *Clin Cancer Res* 1999;5:1001–1005.
21. Kataki A, Scheid P, Piet M, et al. Tumor infiltrating lymphocytes and macrophages have a potential dual role in lung cancer by supporting both host-defense and tumor progression. *J Lab Clin Med* 2002;140: 320–328.
22. Schumacher K, Haensch W, Roefzaad C, et al. Prognostic significance of activated CD8(+) T cell infiltrations within esophageal carcinomas. *Cancer Res* 2001;61:3932–3936.
23. Naito Y, Saito K, Shiiba K, et al. CD8+ T-cells infiltrated within cancer cell nests as a prognostic factor in humon colorectal cancer. *Cancer Res* 1998;58:3491–3494.
24. Woo EY, Chu CS, Goletz TJ, et al. Regulatory T CD4(+)CD25(+) T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. *Cancer Res* 2001;61:4766–4672.
25. Woo EY, Yeh H, Chu CS, et al. Cutting edge: regulatory T cells from lung cancer patients directory inhibit autologous T cell proliferation. *J Immunol* 2002;168:4272–4276.
26. Liyanage UK, Moore TT, Joo HG, et al. Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinomas. *J Immunol* 2002;169:2756–2761.
27. Sasada T, Kimura M, Yoshida M, et al. CD4+CD25+ regulatory T cells in patients with gastrointestinal malignancies. *Cancer* 2003;98:1089–1099.
28. Okita R, Saeki T, Takashima S, et al. CD4+CD25+ regulatory T cells in the peripheral blood of patients with breast cancer and non-small cell lung cancer. *Oncol Rep* 2005;14:1269–1273.
29. Ormandy LA, Hillemann T, Wedemeyer H, et al. Increased populations of regulatory T cells in peripheral blood of patients with hepatocellular carcinoma. *Cancer Res* 2005;65:2457–2464.
30. Mutoh M, Watanabe K, Kitamura T, et al. Involvement of prostaglandin E receptor subtype EP(4) in colon carcinogenesis. *Cancer Res* 2002;62: 28–32.
31. Krysan K, Reckamp KL, Daiwadi H, et al. Prostaglandin E2 activates mitogen-activated protein kinase/Erk pathway signaling and cell proliferation in non-small cell lung cancer cells in an epidermal growth factor receptor-independent manner. *Cancer Res* 2005;65:6275–6281.
32. Tsujii M, Kawano S, Tsuji S, et al. Cyclooxygenases regulates angiogenesis induced by colon cancer cells. *Cell* 1998;93:705–716.
33. Gately S, Li WW. Multiple role of COX-2 in tumor angiogenesis: a target for antiangiogenic therapy. *Semin Oncol* 2004;31:2–11.
34. Lin MT, Lee RC, Yang PC, et al. Cyclooxygenase-2 inducing Mcl-1-dependent survival mechanism in human lung adenocarcinoma CL1.0 cells. *J Biol Chem* 2001;276:48997–49002.
35. Sorokin A. Cyclooxygenase-2: potential role in regulation of drug efflux and multidrug resistance phenotype. *Curr Pharm Des* 2004;10:647–657.
36. Costa C, Soares R, Reis-Filho JS, et al. Cyclooxygenase2 expression is associated with lymph node metastasis in human breast cancer. *J Clin Pathol* 2002;55:429–434.
37. Huang M, Sharma S, Mao JT, et al. Non-small cell lung cancer-derived soluble mediators and prostaglandin E2 enhance peripheral blood lymphocyte IL-10 transcription and protein production. *J Immunol* 1996; 157:5512–5520.
38. Huang M, Stolina M, Sharma M, et al. Non-small cell lung cancer cyclooxygenase2-dependent regulation of cytokine balance in lymphocytes and macrophages: up-regulation of interleukin 10 and down-regulation of interleukin 12 production. *Cancer Res* 1998;58:1208–1216.
39. Li JF, Chu YW, Wang GM, et al. The prognostic value of peritumonal regulatory T cells and its correlation with intratumoral cyclooxygenese-2 exprssion in clear cell renal cell carcinoma. *BLU Int* 2008;103:399–405.
40. Shama S, Stolina M, Yang SC, et al. Tumor cyclooxygenase 2-dependent suppression of dendritic cell function. *Clin Cancer Res* 2003;9: 961–968.
41. Baratelli F, Lin Y, Zhu L, et al. Prostaglandin E_2 Induces *FOXP3* gene expression and T regulatory cell function in human $CD4^+$ T cells. *J Immunol* 2005;175:1483–1490.
42. Mahic M, Yaqub S, Johansson CC, et al. FOXP3+CD4+CD25+ adaptive regulatory T cells express cyclooxygenase-2 and suppress effector T cells by a prostaglandin E2-dependent mechanism. *J Immunol* 2006;177:246–254.
43. Mahic M, Yaqub S, Bryn T, et al. Differentiation of naive CD4+ T cells into CD4+CD25+FOXP3+ regulatory T cells by continuous antigen stimulation. *J Leukoc Biol* 2008;83:1111–1117.
44. Edelman MJ, Watson D, Wang X, et al. Eicosanoid modulation in advanced lung cancer: cyclooxygenase-2 expression is a positive predictive factor for celecoxib + chemotherapy—Cancer and Leukemia Group B Trial 30203. *J Clin Oncol* 2008;26:848–855.