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Imaging, Diagnosis, Prognosis

Clinical Cancer Research

Cyclooxygenase-2 Genetic Variants Are Associated with Survival in Unresectable Locally Advanced Non–Small Cell Lung Cancer

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Abstract

Purpose: Cyclooxygenase-2 (COX-2) plays important roles in the modulation of apoptosis, angiogenesis, immune response, and tumor invasion. Elevated COX-2 expression has been reported to be correlated with reduced survival after radiotherapy. This study examined whether genetic variations in the *COX-2* gene are associated with different survival in inoperable locally advanced non–small cell lung cancer (NSCLC) treated with chemoradiotherapy or radiotherapy alone.

Experimental Design: One hundred and thirty-six patients with inoperable stage IIIA-B NSCLC receiving thoracic irradiation between 2004 and 2007 were recruited in this study. Five functional *COX-2* polymorphisms were genotyped using DNA from blood lymphocytes. Kaplan-Meier methods were used to compare survival by different genotypes. Cox proportional hazards models were used to identify independently significant variables.

Results: During the median 22.4 months of follow-up, the favorable *COX-2* −1195GA and GG genotypes were significantly correlated with better overall survival (20.2 months versus 15.7 months; $P = 0.006$; hazard ratio (HR), 0.58; 95% confidence interval (CI), 0.39-0.86) and with longer progress-free survival (11.9 months versus 9.5 months; $P = 0.034$) compared with the −1195AA genotype. No significant associations were found among other *COX-2* polymorphisms and clinical outcomes. In the multivariate Cox proportional hazards model, *COX-2* −1195G/A polymorphism was independently associated with overall survival after adjusting the clinicopathologic factors ($P = 0.008$; HR, 0.58; 95% CI, 0.39-0.87).

Conclusion: *COX-2* −1195G/A polymorphism is a potential predictive marker of survival in locally advanced NSCLC patients treated with chemoradiotherapy or radiotherapy alone. *Clin Cancer Res; 16(8); 2383–90. ©2010 AACR.*

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Non–small cell lung cancer (NSCLC) accounts for >80% of primary lung cancers and about one third of NSCLC patients are diagnosed at a locally advanced stage (1). Treatment of these patients is usually based on a multidisciplinary strategy, including a combination of radiotherapy and chemotherapy. However, results of these treatments were unsatisfactory with a 3-year overall survival (OS) being 10% to 20% (2). The classic prognostic determinants for lung cancer include the tumor-node-metastasis staging system, performance status, sex, and weight loss. Unfortunately, all these factors are far less than sufficient to explain the patient-to-patient variability. Therefore, identification of new biomarkers for more accurate prognostic and predictive assessment is warranted and could be helpful to highlight the possibility of patient-tailored decisions (3).

Cyclooxygenase (COX, also known as prostaglandin endoperoxide synthase) is a key enzyme that catalyzes the conversion of arachidonic acid into prostaglandins (4).

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Translational Relevance

Cyclooxygenase-2 (COX-2) is a key enzyme involved in cancer development and progression, and elevated COX-2 expression was related to unfavorable survival after radiotherapy. Several functional single nucleotide polymorphisms have been identified in the *COX-2* regulation region. In this study, we found for the first time that the *COX-2* −1195G/A polymorphism, resulting in significantly increased COX-2 expression, was independently associated with poor progress-free survival and overall survival among inoperable locally advanced non–small cell lung cancer patients treated with chemoradiotherapy or radiotherapy alone. Therefore, the *COX-2* −1195G/A single nucleotide polymorphism may be a prognostic biomarker for non–small cell lung cancer after radiotherapy. Additionally, this study might also help to optimize cancer treatment by identifying different responders to COX-2 target therapy in patients treated with conventional therapy combined with COX-2 inhibitors.

Two isoforms, COX-1 and COX-2, have been identified. COX-1 is constitutively expressed in normal tissues, which is thought to be responsible for multiple physiologic functions (5). COX-2 is usually undetectable but is rapidly induced by various stress-related stimulations, including cytokines, growth factors, and irradiation (4, 5). It has been shown that COX-2 plays a significant role in carcinogenesis, which is thought to modulate cellular adhesion and apoptosis, stimulate angiogenesis, and suppress immune response (6–8). Overexpression of COX-2 has been reported in many malignancies including NSCLC, suggesting its involvement in pulmonary tumorigenesis (9–11). Increased COX-2 expression is also associated with more aggressive tumor behavior and poor prognosis in NSCLC patients (12). Interestingly, recent reports found that elevated COX-2 expression is correlated with reduced survival in both breast cancer and cervix carcinoma after radiotherapy (13, 14). These observations suggest that COX-2 may also play a part in patient survival after ionizing radiation. Moreover, COX-2 inhibitors have been reported to have radiosensitizing and chemosensitizing activities both *in vitro* (15, 16) and *in vivo* (17, 18). Therefore, reduced COX-2 expression may predict response to radiotherapy and act as a prognostic factor for locally advanced NSCLC survival.

Several functional single nucleotide polymorphisms (SNP) in the *COX-2* gene have been identified, which may contribute to different gene expression or different enzyme activities (19–21) and, thus, could at least partially contribute to individual variability in arachidonic acid metabolism. Our previous studies have identified several functional *COX-2* SNPs in Chinese population. We showed that some of them are associated with increased risk of malignancies, including esophageal cancer and pancreatic cancer (20–23). These SNPs include −1290A/G (rs689465), −1195G/A (rs689466), and −765G/C (rs20417) in the promoter region; 1759G/A (rs3218625) in exon 10; and 8473T/C (rs5275) in the 3′ untranslated region of the gene. Although some *COX-2* SNPs, such as the 8473T/C polymorphisms, are significantly associated with NSCLC risk (24, 25). However, little is known about their prognostic effects on NSCLC.

As a potential prognostic factor, the concept of germline variation is appealing, especially for inoperable patients. Because only a little blood sample is needed for the test, genotyping of germline variation is more practical and useful in the clinical practice. Recently, several studies have shown associations between host genetic polymorphisms and survival of NSCLC (26–28). In this setting, genetic variations in the *COX-2* gene that alter gene expression and/or protein activity may be potential candidates to predict prognosis and treatment response in NSCLC. Here, we performed a cohort study to elucidate whether functional SNPs in the *COX-2* gene, alone or in combination, are associated with a biologically different survival advantage in inoperable locally advanced NSCLC treated with chemoradiation or radiation alone.

Materials and Methods

Patients. Between January 2004 and December 2007, patients with unresectable and locally advanced stage IIIA-B NSCLC were recruited at the Cancer Hospital, Chinese Academy of Medical Sciences (Beijing). The eligible patients were those with histologically or cytologically confirmed NSCLC; with measurable lesions; with Karnofski performance status (KPS) of ≥70; and with normal results of complete blood counts, liver function, and urinalysis. Patients with malignant pleural effusion were excluded. Pretreatment staging involved a thorough history and physical examination, chest X-ray, and computed tomography scans of the chest and upper abdomen, with the adjunctive use of magnetic resonance imaging or positron emission tomography scanning when available.

All the patients underwent thoracic radiation with or without platinum-based chemotherapy. None of them received epidermal growth factor receptor– or vascular endothelial growth factor receptor–targeted therapy as their systemic treatment. In the conventional radiotherapy group, the field area contained the primary tumor plus the ipsilateral hilum, the mediastinum superior, or entire mediastinum. In the three-dimensional conformal radiotherapy group, only the primary tumor and positive lymph node–draining area, with a short axis diameter of at least 1 cm on computed tomography or fluorodeoxyglucose uptake on positron emission tomography, were included in the clinical target area. A total dose of 40 to 70 Gy was given with 2 Gy per fraction, 5 d weekly. In fact, the original dose prescription was no less than 60 Gy. However, there were four patients whose actual dose prescriptions were below 60 Gy. The detailed information was as follows: two patients received <60 Gy (40 and

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50 Gy, respectively) because of severe radiation-induced pneumonitis and two patients received 50 Gy because of progressive disease during radiation. The toxicity was graded according to the Common Terminology Criteria for Adverse Events v3.0. Survival information was collected every 3 mo from hospital medical records and/or by phone.

A total of 144 patients agreed to participate in this study and had adequate blood DNA for genotyping and 136 had complete follow-up and clinical information. There was no significant difference in the distributions of demographic information between patients enrolled and patients who did not. Written informed consent was obtained from each patient for the use of their DNA and clinical information. The study was approved by the Institutional Review Board of the Cancer Institute and Hospital.

SNP genotyping. Genomic DNA was extracted from 5-mL blood sample that was collected from each patient upon recruitment. The *COX-2* −1290A/G, −1195G/A, −765G/C, 1759G/A, and 8473T/C polymorphisms were genotyped by PCR-based RFLP methods as previously described (21, 29). Genotypes were confirmed by direct DNA sequencing of the PCR products. A 15% blind, random sample of study subjects was genotyped twice by different persons (N. Bi and M. Yang) and the reproducibility was 100%.

Statistical analysis. For each polymorphism, the Hardy-Weinberg equilibrium was tested by the Pearson χ^2 test. Student's *t* test or χ^2 test was used to calculate the difference of patient clinical characteristics. OS was calculated as the time to death from the date of diagnosis. Progression-free survival (PFS) was calculated as the time to progression or death without progression from the date of diagnosis. Survival distributions were estimated with the Kaplan-Meier method and were compared with the log-rank test. Multivariate Cox proportional hazards models were applied to estimate the effect of prognostic factors on OS and PFS, using proverbial clinical factors, including age, sex, smoking status, KPS, weight loss, histology, clinical stages, radiation technique and dosage, and chemotherapy. Statistical significance was set at a level of 0.05 and all the analyses were done using the SPSS software package (version 11.5, SPSS, Inc.).

Results

Patient characteristics and clinical outcomes. The distribution of demographic and clinical characteristics of patients is presented in Table 1. By the time of the final analysis (February 2009), the median follow-up time of the patients was 22.4 months. One hundred and seventeen patients (86.0%) died and the median survival time was 18.1 months (range, 2.7-60.3 months). The 1-, 2-, 4-, and 5-year OS rates were 74%, 34%, 13%, and 7%, respectively.

Comparison of survival according to baseline characteristics of patients. To test whether various clinical characteristics contribute to survival, patients were grouped according to age, sex, smoking status, KPS, weight loss, histology, clinical stages, or treatment parameters. Both OS and PFS were compared among different groups. For OS, only radiation technique significantly influenced patient prognosis ($P = 0.001$). The median OS for patients who had undergone three-dimensional radiotherapy (3D-RT)

Table 1. Patient clinical and treatment characteristics

Characteristics	*n* (%)
Age, y	
Median	60.0
Range	26.0-83.0
Sex	
Male	115 (84.6)
Female	21 (15.4)
Smoking status	
Nonsmoker	35 (25.7)
Smoker	101 (74.3)
KPS	
≥80	112 (82.4)
<80	24 (17.6)
Weight loss	
<5%	111 (81.6)
≥5%	25 (18.4)
Histology	
Squamous carcinoma	83 (61.0)
Adenocarcinoma	42 (30.9)
Others*	11 (8.1)
Tumor stage at diagnosis	
IIIA	34 (25.0)
IIIB	102 (75.0)
Radiation technique	
2D-RT	21 (15.4)
3D-RT	115 (84.6)
Radiation dose	
Median	60
Range	40-70
Treatment†	
Sequential chemoradiation	40 (29.4)
Concurrent chemoradiation	62 (45.6)
Radiation alone	34 (25.0)
GTV (cm^3)‡	
Median	112.0
Range	4.0-481.3

Abbreviations: 2D-RT, two-dimensional radiotherapy; GTV, gross tumor volume.

*Others include large cell carcinoma and adenosquamous carcinoma.

†Chemotherapy consisting of two or more cycles of platinum-based regimen.

‡Only patients receiving three-dimensional conformal radiotherapy had gross tumor volume measured.

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Table 2. Associations between *COX-2* genotypes and PFS and OS

Genotype	No.	PFS				OS			
		Median (mo)	*P*	HR	95% CI	Median (mo)	*P*	HR	95% CI
−1290A/G									
AA	72	10.6		1.00		19.2		1.00	
AG	32	12.3	0.870	1.04	0.67-1.61	15.0	0.140	1.40	0.89-2.18
GG	1	5.3	NC	NC	NC	21.2	NC	NC	NC
AG+GG	33	13.0	0.900	0.90	0.60-1.34	15.0	0.140	1.41	0.91-2.19
−1195G/A									
AA	56	9.5		1.00		15.7		1.00	
AG	49	11.9	0.130	0.72	0.47-1.10	17.5	0.075	0.68	0.44-1.04
GG	24	12.1	0.020	0.52	0.30-0.91	27.7	0.002	0.42	0.24-0.73
GG+GA	73	11.9	0.034	0.66	0.45-0.97	20.2	0.006	0.58	0.39-0.86
−765G/C									
GG	119	10.5		1.00		19.1		1.00	
GC	17	11.2	0.810	0.93	0.52-1.66	15.1	0.360	1.31	0.73-2.34
CC	—	—	NC	NC	NC	—	NC	NC	NC
GC+CC	17	11.2	0.810	0.93	0.52-1.66	15.1	0.360	1.31	0.73-2.34
1759G/A									
GG	95	10.9		1.00		18.5		1.00	
GA	19	10.0	0.930	1.02	0.60-1.76	21.7	0.900	0.97	0.56-1.66
AA	—	—	NC	NC	NC	—	NC	NC	NC
GA+AA	19	10.0	0.930	1.02	0.60-1.76	21.7	0.900	0.97	0.56-1.66
8473T/C									
TT	92	10.9		1.00		20.4		1.00	
TC	38	10.5	0.850	0.96	0.64-1.45	14.7	0.150	1.35	0.89-2.05
CC	5	27.8	0.300	0.54	0.17-1.73	11.8	0.660	1.23	0.49-3.05
TC+CC	43	11.2	0.610	1.11	0.74-1.66	14.7	0.150	1.33	0.90-1.98

Abbreviation: NC, not calculated.

was 6 months longer than that of patients undergone two-dimensional radiotherapy (18.9 months versus 13.9 months). However, other baseline characteristics did not affect OS (all $P > 0.05$). In addition, PFS was not influenced by any patient characteristics and treatment (Table 3).

Effects of SNPs on OS and PFS. The allelic frequencies for the *COX-2* variants are summarized in Table 2. All observed genotype frequencies in patients conformed to the Hardy-Weinberg equilibrium. No statistical associations were observed between any of these polymorphisms and age, sex, smoking status, KPS, weight loss, histology, clinical stages, or treatment parameters (data not shown).

Interestingly, the *COX-2* -1195G/A polymorphism was significantly associated with patient survival. As shown in Table 2, patients with the −1195GG genotype had 12 months longer survival [median OS, 27.7 months; 95% confidence interval (CI), 21.3-34.1 months) than those with the −1195AA genotypes (median OS, 15.7 months; 95% CI, 12.1-19.3 months; $P = 0.0016$ for log-rank test). Moreover, the median OS for patients with the −1195GA + GG genotypes (median OS, 20.2 months, 95% CI, 14.4-26.0 months) was also significantly longer than that for patients with the AA genotype (median OS, 15.7 months; 95% CI, 12.1-19.3 months; $P = 0.006$ for log-rank test; Fig. 1A). Similarly, significant difference in PFS was observed in lung cancer patients related to −1195G/A genotypes ($P = 0.034$ for log-rank test; Table 2; Fig. 1B). The median PFS for the −1195 AA genotype and GA + GG genotypes were 9.5 months and 11.9 months, respectively.

Because 75.0% of NSCLC patients in this cohort also underwent chemotherapy, we further examined whether the association between the *COX-2* −1195G/A SNP and survival could be influenced by different treatments. As shown in Fig. 2, the median OS of the −1195AA genotype was shorter than that of the −1195GA + GG genotypes both in patients treated with chemoradiation (median OS, 15.9 months versus 18.9 months; $P = 0.04$ for log-rank test) and in patients treated with radiation alone (median OS, 14.7 months versus 24.8 months; $P = 0.09$ for log-rank test). We further tested whether this association between the polymorphism and OS still existed in patients treated with concurrent chemoradiation and the results were also positive (median OS, 15.0 months versus 20.2 months; $P = 0.03$ for log-rank test).

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There were no associations between the other four *COX-2* polymorphisms and OS or PFS. However, it is notable that the variant CC and TC genotypes of the 3′ untranslated region 8473T/C SNP showed a tendency to be associated with poor OS compared with the TT genotype, although the association was not statistically significant (median OS, 14.7 months versus 20.4 months; $P = 0.15$ for log-rank test).

Multivariate analysis. In the multivariate Cox proportional hazards model, after adjustment for the clinicopathologic factors including age, sex, smoking status, KPS, weight loss, histology, clinical stages, chemotherapy, or radiation dosage, the prognostic significance of both *COX-2* −1195G/A polymorphism and radiation technique still existed (both $P = 0.008$; Table 3). The hazard ratios (HR) of patients with the −1195GA + GG genotypes or treated with 3D-RT on OS were 0.58 (95% CI, 0.39-0.87) and 0.47 (95% CI, 0.27-0.82).

Discussion

In the present study, we examined whether genetic polymorphisms in the *COX-2* gene are associated with survival in a cohort of 136 locally advanced NSCLC patients treated with chemoradiotherapy or radiotherapy alone. We found that the AA genotype of −1195G/A SNP in the *COX-2* promoter region significantly contributed to poor OS and PFS compared with the −1195GG genotype. After adjusting for clinicopathologic factors, the *COX-2*−1195G/A variant was still significantly associated with OS, showing that the −1195G/A polymorphism was an independent survival predictor. Additionally, 3D-RT was also independently associated with OS of NSCLC patients in the cohort.

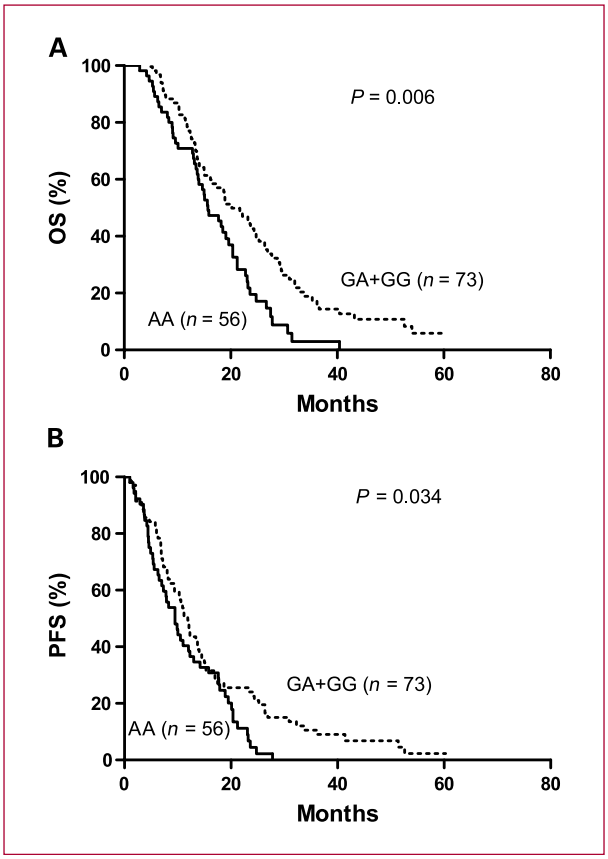

Fig. 1. Kaplan-Meier curve of estimated (A) OS ($P = 0.006$ for log-rank test) and (B) PFS ($P = 0.034$ for log-rank test) for the entire cohort with different *COX-2* −1195G/A genotypes.

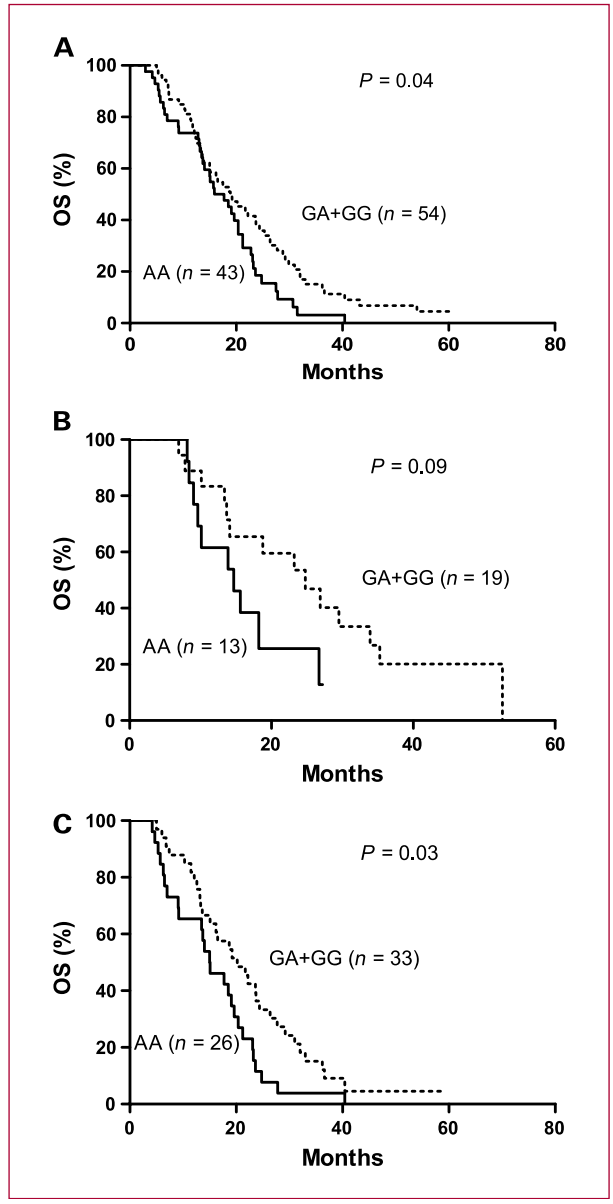

Fig. 2. Kaplan-Meier curve of estimated OS for the patients with different *COX-2* −1195G/A genotypes receiving (A) chemoradiation ($P = 0.04$ for log-rank test), (B) radiation alone ($P = 0.09$ for log-rank test), or (C) concurrent chemoradiation ($P = 0.03$ for log-rank test).

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Table 3. Univariate and multivariate Cox-regression analyses for OS

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age	1.09 (0.76-1.57)	0.650	1.02 (0.64-1.61)	0.950
Sex	1.05 (0.64-1.75)	0.840	0.86 (0.44-1.70)	0.660
Smoking status	1.04 (0.69-1.58)	0.850	1.42 (0.79-2.55)	0.240
KPS	0.74 (0.45-1.22)	0.240	0.76 (0.42-1.37)	0.370
Weight loss	0.93 (0.57-1.49)	0.750	0.84 (0.51-1.38)	0.490
Histology	1.04 (0.93-1.16)	0.530	1.04 (0.93-1.67)	0.490
Tumor stage	0.90 (0.58-1.39)	0.620	0.88 (0.55-1.40)	0.600
Radiation technique	0.44 (0.26-0.72)	0.001	0.47 (0.27-0.82)	0.008
Radiation dose	0.76 (0.51-1.15)	0.190	0.82 (0.53-1.27)	0.380
Chemotherapy	1.14 (0.74-1.77)	0.560	1.45 (0.82-1.56)	0.200
COX-2 −1195G/A	0.58 (0.39-0.86)	0.006	0.58 (0.39-0.87)	0.008

It was shown that inhibition of COX-2 enzyme with selective inhibitors enhances tumor response to radiation and chemotherapeutic agents (30). Pyo et al. (31) showed that, compared with drug nontreated tumor-bearing control mice, selective COX-2 inhibitors enhanced the effect of radiation on tumors that express COX-2, such as NCI-H460 cells, but not on COX-2–lacking tumors. An association between COX-2 expression and responses to radiation has also been observed in patients with cervical or breast cancer (32, 33).

Although our and other's studies found that the *COX-2* SNPs were related to elevated risk of several cancers, including lung cancer (24, 34), few have been done to investigate if the *COX-2* SNPs are associated with cancer survival (32). Iglesias et al. (35) have recently reported that the c.3618A/G (rs4648298) polymorphism located in the 3′ flanking regions of *COX-2* was associated with improved survival of colorectal cancer patients in Caucasians. However, in our previous study, it was shown that c.3618A/G (rs4648298) variant was not a common polymorphism in Han Chinese population (major allele frequency, <0.05; ref. 21). Therefore, we did not include this polymorphism in the current study. To the best of our knowledge, this is the first study showing that the *COX-2* variants could predict survival in patients with lung cancer. Our previous functional study showed that the −1195G to A change creates a c-MYB binding site in the *COX-2* promoter region and, thus, displays a higher promoter activity (21). Compared with the −1195G-containing counterparts, the −1195AA carriers showed significantly increased COX-2 expression *in vitro* and *in vivo*. In addition, elevated expression of COX-2 has been reported in NSCLC (12, 36-38) and was associated with an invasive and more aggressive phenotype (36). Consequently, our observations are biologically plausible and consistent with these previous reports, showing that functional −1195G/A SNP, which may influence the expression of COX-2, leads to decreased survival in NSCLC independently.

Few studies have been reported thus far with regard to different *COX-2* polymorphisms and clinical outcomes of patients with NSCLC. Vogel et al. (39) showed a gene-drug interaction between the *COX-2* −1195G/A SNP and COX-2 inhibitor use in enhancing lung cancer risk. The *COX-2* −1195*A* allele was also associated with a poor response to vinorelbine-based chemotherapy in patients with NSCLC (40). All these observations suggest that the *COX-2* −1195G/A polymorphism plays an essential role in cancer development and might be a useful molecular indicator of lung cancer risk, prognosis, and response to treatment.

Our results showed that stage III NSCLC with the *COX-2* −1195AA genotype might be more aggressive and probably more radiation resistant than those with the −1195GA + GG genotypes. Therefore, a personalized more intensive treatment, i.e., a combination treatment of chemoradiotherapy and COX-2 inhibitor, might be warranted to improve the survival in patients with the unfavorable *COX-2* −1195AA genotype. Several phase II studies failed to show the value of eicosanoid inhibition in addition to other modalities, such as chemotherapy, and/or tyrosine kinase inhibitor target therapy, in unselected population of advanced NSCLC (35, 38, 41–43). However, recent evidence suggested a potential advantage for celecoxib, a selective COX-2 inhibitor, might be obtained in the certain population (44, 45). A prospective randomized clinical trail, CALGB 30203, showed that patients with moderate to high expression of COX-2 had a worse OS than those with low expression (HR, 2.51; 95% CI, 1.14-5.56; $P = 0.019$). More interestingly, combined use of COX-2 inhibitor could improve the OS of these patients significantly (HR, 0.342; 95% CI, 0.155-0.752; $P = 0.005$; ref. 44). Similarly, we found that the unfavorable *COX-2* −1195AA genotype, which results in increased COX-2 expression, was also associated with poor OS. Therefore, this genetic variant could be a novel and helpful predictive factor to identify specific NSCLC patients who may benefit from COX-2 inhibitors. Because almost all of advanced NSCLC patients are inoperatable and their tumor specimens are not

available, SNP genotyping using blood DNA samples may result in personalized combined modality and better effectiveness. Further prospective clinical trials are needed to validate this hypothesis.

Limitations of this study include its moderate sample size and retrospective design. Before being confirmed by well-designed, larger prospective studies, results from this study should not be overinterpreted. Clinical trials examining *COX-2* SNPs in patients treated with conventional therapy combined with COX-2 inhibitors may help to clarify if *COX-2* −1195G/A polymorphism could act as a predictive marker of COX-2 target therapy. Moreover, because some genetic markers are ethnic specific, our results also should be validated among different ethnic populations in the future.

In conclusion, we have reported for the first time that there were significant differences in the OS and PFS among NSCLC patients with different *COX-2* −1195G/A genotypes. Our results suggest that in addition to measures of clinicopathologic factors, the naturally occurring genetic variants in the *COX-2* gene might be useful clinical predictive factors in patients with inoperable locally advanced NSCLC receiving thoracic radiotherapy with or without chemotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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