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COX-2/EGFR expression and survival among women with adenocarcinoma of the lung

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Previous studies suggest that cyclooxygenase-2 (COX-2) expression may predict survival among patients with non-small cell lung cancer. COX-2 may interact with epidermal growth factor receptor (EGFR), suggesting that combined COX-2/EGFR expression may provide predictive value. The extent to which their independent or combined expression is associated with prognosis in women with adenocarcinoma of the lung is unknown. In the present study, we examined relationships between COX-2 expression (n = 238), EGFR expression (n = 158) and dual COX-2/EGFR expression (n = 157) and survival among women with adenocarcinoma of the lung. Overall survival was estimated by constructing Cox proportional hazards models adjusting for other significant variables and stratifying by stage at diagnosis and race. Clinical or demographic parameters were not associated with either COX-2 or EGFR expression. Patients with COX-2-positive tumors tended to have poorer prognosis than did patients with COX-2-negative tumors [hazard ratio (HR) 1.67, 95% confidence interval (CI) 1.01–2.78]. African-Americans with COX-2-positive tumors had a statistically non-significant higher risk of death than African-Americans with COX-2-negative tumors (HR 5.58, 95% CI 0.64–48.37). No association between COX-2 expression and survival was observed among Caucasians (HR 1.29, 95% CI 0.72–2.30). EGFR expression was associated with a 44% reduction in the risk of death (HR 0.56, 95% CI 0.32–0.98). COX-2−/EGFR+ tumor expression, but not COX-2+/EGFR+ tumor expression, was associated with survival when compared with other combined expression results. In conclusion, COX-2 and EGFR expression, but not combined COX-2+/EGFR+ expression, independently predict survival of women with adenocarcinoma of the lung.

Introduction

Lung cancer is the second leading type of cancer in both men and women in USA and the leading cause of cancer-related mortality. The low overall 5 year survival rate of 15% underscores the need to develop new treatment options (1). Identification of molecular pathways involved in lung carcinogenesis may lead to novel-targeted therapies. Two such pathways involve cyclooxygenase-2 (COX-2) and epidermal growth factor receptor (EGFR); both proteins are expressed in non-small cell lung cancer (NSCLC) and may predict prognosis. These proteins may affect lung carcinogenesis both individually and, as recently suggested, synergistically (2). Additionally, they have been targeted simultaneously for treatment of lung cancer (3–8). However, the prognostic significance of the individual and combined expression of these proteins with the patient's clinical, epidemiological and demographic characteristics is poorly characterized. NSCLC in women has distinct features including a higher prevalence of adenocarcinoma, a lower frequency of current smokers and distinct mutation profile, making women with NSCLC an important subgroup to study (9,10).

Abbreviations: CI, confidence interval; COX-2, cyclooxygenase-2; EGFR, epidermal growth factor receptor; HR, hazard ratio; IHC, immunohistochemistry; mRNA, messenger RNA; NSAID, non-steroidal anti-inflammatory drug; NSCLC, non-small cell lung cancer; TKI, tyrosine kinase inhibitor.

COX-2 is one of the two COX enzymes that catalyze the conversion of arachidonic acid into prostanoids including prostaglandin E_2, which has been associated with angiogenesis, cell motility and invasion, immunosuppression and inhibition of programmed cell death, all processes that are vital to tumor progression (11–13). Between 54 and 100% of NSCLC tumors are COX-2 positive as measured by immunohistochemistry (IHC), reverse transcription–polymerase chain reaction and *in situ* hybridization (14–16). Yuan *et al.* (14) found that high COX-2 messenger RNA (mRNA) expression is correlated with high COX-2 protein expression. Studies examining the relationship between COX-2 tumor expression and survival among lung cancer patients have been inconsistent, with reports of an inverse relationship with survival (14,17–19), no association (20), or a direct association with survival (21). COX-2 expression has been most consistently associated with poorer survival among stage I and II NSCLC patients (17–19,22). This result was replicated in a meta-analysis of 14 studies (23). Tsubochi *et al.* (18) found that COX-2 tumor expression predicted survival in men, patients <65 years of age and patients with adenocarcinomas. High COX-2 mRNA expression is associated with a shorter relapse time and poorer 5 year survival even after taking histology into account (17). These studies suggest a role of COX-2 in tumor progression.

A member of the ErbB family of cell surface tyrosine kinase receptors, EGFR binds to growth factor ligands, triggering intracellular signal transduction cascades associated with the activation of genes involved in antiapoptotic mechanisms, cell cycle progression, angiogenesis, gene transcription and increased cellular proliferation (24). EGFR has been found to be overexpressed at the protein level in NSCLC tissue at all tumor stages in comparison with uninvolved lung tissue, and closely associated with EGFR protein expression in NSCLC tumors are *EGFR* gene copy number and EGFR mRNA transcript level (25,26). EGFR expression intensity has been shown to increase with stage at diagnosis, and EGFR overexpression has been found more frequently in stages II and III disease than in stage I disease (27,28). Moreover, lymph node metastasis has been associated with higher EGFR protein expression as measured by competitive ligand binding assay and IHC (28). EGFR protein expression has also been associated with well-differentiated tumors (26,29). Conflicting studies, however, report that EGFR expression is not related to stage, grade, tumor size, sex, age, smoking status or other clinicopathologic parameters (26,27,30–32). Estimates of the prevalence of EGFR-positive lung tumors range from 13 to 80% (33). While EGFR overexpression appears to be more intense and more prevalent among squamous cell carcinomas (27), a meta-analysis reported that an average of 46.2% of adenocarcinomas were EGFR positive (33). While this meta-analysis of the relationship between EGFR expression and survival among lung cancer patients found a 13% increased risk of death [hazard ratio (HR) 1.13, 95% confidence interval (CI) 1.00–1.28], it did not examine this relationship by stage (33). The question remains whether the relationship between EGFR tumor expression and survival varies by stage at diagnosis.

EGFR inhibitors have been approved to treat lung cancer since 2004. In recent years, researchers have begun to study the efficacy of combined treatment with a COX-2 inhibitor and an EGFR inhibitor. A phase I trial found combined therapy to be safe with a partial response in 33% and stabilization of disease in another 24% of the patients studied (n = 22) (3). However, recently published clinical trials investigating the efficacy of combined therapy with a COX-2 inhibitor and the EGFR inhibitors gefitinib or erlotinib found no additional benefit of combined treatment in platinum therapy unresponsive or chemotherapy-naive patients in comparison with results from

previous studies involving treatment with gefitinib or erlotinib alone (4–7). These studies did not test the efficacy of gefitinib or erlotinib alone as a comparator arm. These results contrast with a reported benefit of combined therapy *in vitro* in cell lines with *EGFR* mutations, suggesting that the benefit of such treatment may only be seen when tumors with *EGFR* mutations are selected (8). Only one previously published study has examined the relationship between combined COX-2/EGFR tumor expression and survival among a small number of patients with lung cancer (34). The authors report finding no association between combined COX-2/EGFR tumor expression and survival.

The current study was undertaken to determine the prognostic significance of individual or combined expression of COX-2 and EGFR in women with adenocarcinoma of the lung and to study whether epidemiological or demographic features affect such expression.

Materials and methods

Study population

The study population and data collection methods have been described elsewhere (35). Briefly, subjects were identified through the population-based Metropolitan Detroit Cancer Surveillance System (MDCSS), a participant in the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program. Women aged 18–74 years and diagnosed with primary NSCLC in the tri-county (Wayne, Macomb and Oakland) area between 1 November 2001 and 31 October 2005 were eligible to participate. Ascertainment was originally focused on adenocarcinoma histology but was broadened after 1 November 2004 to include all NSCLC histologies. No proxy interviews were conducted; therefore, women deceased at ascertainment or first contact were ineligible. Five hundred and seventy-seven cases completed an interview (54%), and paraffin-embedded tumor samples were obtained for 402 cases (69.7%).

Data collection

All local institutional and review boards approved this study. Informed consent was obtained from each subject prior to study participation. Trained interviewers conducted in-person interviews to collect demographic information, medication history (aspirin, acetaminophen and non-steroidal anti-inflammatory drug (NSAID)), smoking history, health history and lifetime estimates of environmental tobacco smoke exposure. Ex-smokers were individuals quitting >2 years before diagnosis. Never smokers included those who smoked <100 cigarettes in their lifetime. Medical history included self-report of physician diagnoses of asthma, emphysema, allergies, pneumonia, bronchitis, chronic obstructive pulmonary disease, tuberculosis and cancer. Family history of lung cancer was coded as yes or no based on the detailed first-degree family history information collected. Regular pill use (aspirin, acetaminophen and NSAID) was defined as taking at least one pill, three times per week or more for at least 1 month during the participant's lifetime. For aspirin, participants were asked whether they took baby/senior citizen aspirin (81 mg) or adult-strength aspirin (325 mg). Lung cancer diagnosis dates, clinical data including histological type, grade, American Joint Committee on Cancer stage, localized/regional/distant staging, nodal involvement and date of death or date last known alive were obtained through the MDCSS. Staging was defined according to SEER guidelines as follows: localized disease is confined to the carina, hilus or main stem bronchus or a single tumor confined to one lung; regional disease has invaded surrounding structures, including spread to regional ipsilateral lymph nodes, involved multiple masses in the same lobes or main stem bronchus; distant disease has spread to distant lymph nodes or involved separate tumor nodules in different lobe, metastasized to the contralateral lung, abdominal organs, heart, vertebrae, skeletal muscle or was associated with malignant pericardial or pleural effusion. The MDCSS maintains yearly follow-up of all patients with a follow-up rate of 98%.

Immunohistochemistry

In total, 292 and 183 tumor blocks were assessed for COX-2 expression and EGFR expression, respectively. Five micron sections were cut and stained using standard avidin–biotin techniques with antibody COX-2 clone 229 (Zymed, South San Francisco, CA) and EGFR antibody clone 31G7 (Ventana Medical Systems, Tuscon, AZ), respectively, manually (COX-2) or with the Ventana automated slide stainer (EGFR). For COX-2, heat-induced epitope retrieval was used to unveil the reactivity of chemical groups that have been masked by formalin fixation. For EGFR, proteolytic enzyme digestion was performed using sodium citrate. The COX-2 antibody, a mouse monoclonal antibody, was diluted 1:50 and incubated for 2 h at room temperature. The monoclonal EGFR antibody was pre-optimized; primary EGFR antibody incubation was for 32 min. Positive and negative controls were included with each run. Positive controls included colon cancer (COX-2) and cytoplasmic and invasive breast ductal carcinoma tissue (EGFR). Negative controls were obtained by omitting the primary antibody. The pathologist was blinded to all subject characteristics. Sections were scored for percent positivity of tumor cells (0, <10, 11–50 and 51–100%) and for staining intensity (none, faint, moderate and intense). Sections were considered positive if percent positivity was >10% and staining intensity was faint or higher.

Statistical analysis

To test for differences between positive and negative tumor expression, Student's *t*-tests were performed for continuous variables; χ^2 analysis was conducted for categorical variables. Stepwise logistic regression was performed to test for associations between clinical and risk factor variables and COX-2, EGFR and combined COX-2/EGFR expression.

Cox proportional hazards models were used to estimate the relationship between COX-2 and EGFR tumor expression and prognosis among women with NSCLC. Age, race, stage and other variables significant in univariate analyses were included in these models. Stepwise regression was performed to select variables for inclusion; criteria to stay in the model was $P = 0.10$. Cox regression plots were constructed for COX-2+ versus COX-2− and EGFR+ versus EGFR−. Analysis was stratified by stage (localized and regional/distant) and race (African-American and Caucasian). The following additional comparisons were made: COX-2+/EGFR+ versus COX-2−/EGFR−, COX-2+/EGFR+ versus other (COX-2−/EGFR−, COX-2+/EGFR− and COX-2−/EGFR+) and COX-2+/EGFR+ versus COX-2−/EGFR+. Additionally, COX-2−/EGFR+ was compared with other (COX-2−/EGFR−, COX-2+/EGFR− and COX-2+/EGFR+) to analyze the association between combined tumor expression relative to the lowest risk group (COX-2−/EGFR+). For each of these comparisons, the proportional hazards assumption was tested by plotting the ln(−ln(S(t))) versus ln(t). Analysis of the relationships between COX-2 and EGFR staining intensity and percent positive staining and survival were also conducted.

Results

IHC results

Of the 402 tumor blocks obtained, the distribution of histological type is as follows: 33 (8.2%) squamous cell carcinoma, 304 (75.4%) adenocarcinoma, 10 (2.5%) large cell carcinoma and 55 (13.7%) NSCLC unspecified. As the majority of cases were adenocarcinoma and to control for variability in underlying disease processes, subsequent analyses were restricted to cases with this histological type. As nine of the adenocarcinoma tumor blocks were not lung tissue, these cases were excluded from analyses. In 53 and 60 blocks, there was insufficient material for COX-2 or EGFR analysis, respectively. The remainder of the blocks were not available at the time of staining for COX-2 or EGFR. One hundred and fifty-nine (66.8%) of the adenocarcinomas were COX-2+ and 108 (68.4%) were EGFR+. Dual COX-2/EGFR expression was available for 157 adenocarcinoma cases with the following results: 18 (11.5%) COX-2−/EGFR−, 32 (20.4%) COX-2+/EGFR−, 30 (19.1%) COX-2−/EGFR+ and 77 (49.0%) COX-2+/EGFR+.

Demographic characteristics

None of the following demographic characteristics were significantly different between COX-2+ and COX-2− or between EGFR+ and EGFR− lung cancer patients: race, age at diagnosis, education level, body mass index, passive cigarette smoke exposure as a child or as an adult at work or home, smoking pack-year history, smoking status, history of chronic obstructive lung disease or family history of lung cancer (Table I).

Clinical characteristics

Neither COX-2 tumor expression nor EGFR tumor expression was associated with the following clinical parameters: stage, nodal involvement, grade, radiation treatment or surgery (Table II). The percentage of cells staining positive for COX-2 was greater in African-Americans than in Caucasians ($P = 0.0089$): 0% (19.6 versus 34.8%), <10% (2.2 versus 13.0%), 11–50% (28.3 versus 19.0%) and 51–100%

Table I. Participant characteristics by COX-2 and EGFR tumor expression in women with adenocarcinoma of the lung

	COX-2			EGFR		
	Positive, *n* (%)	Negative, *n* (%)	*P* value	Positive, *n* (%)	Negative, *n* (%)	*P* value
n (%)	159 (66.8)	79 (33.2)		108 (68.4)	50 (31.6)	
Race						
Caucasian	117 (73.6)	67 (84.8)		79 (73.2)	38 (76.0)	
African-American	36 (22.6)	10 (12.7)		25 (23.2)	9 (18.0)	
Other	6 (3.8)	2 (2.5)	0.15	4 (3.7)	3 (6.0)	0.62
Age						
Mean (SD)	59.7 (9.5)	60.7 (8.9)	0.46	59.6 (9.4)	57.7 (9.7)	0.24
Smoking status						
Never	17 (10.7)	12 (15.2)		13 (12.0)	5 (10.0)	
Former	50 (31.4)	24 (30.4)		28 (25.9)	13 (26.0)	
Current	92 (57.9)	43 (54.4)	0.41	67 (62.0)	32 (64.0)	0.74
Pack-years among smokers						
Mean (SD)	44.4 (29.1)	41.6 (23.0)	0.45	45.7 (29.3)	42.9 (22.4)	0.54
History of COPD[a]						
No	109 (68.6)	57 (72.2)		73 (67.6)	32 (64.0)	
Yes	50 (31.4)	22 (27.8)	0.57	35 (32.4)	18 (36.0)	0.66
Family history of lung cancer						
No	113 (71.1)	60 (76.0)		76 (70.4)	37 (74.0)	
Yes	46 (28.9)	19 (24.0)	0.43	32 (29.6)	13 (26.0)	0.64

[a]COPD, chronic obstructive pulmonary disease, including chronic bronchitis and emphysema.

Table II. Clinical characteristics by COX-2 and EGFR tumor expression in women with adenocarcinoma of the lung

	COX-2			EGFR		
	Positive, *n* (%)	Negative, *n* (%)	*P* value	Positive, *n* (%)	Negative, *n* (%)	*P* value
Stage						
Localized	72 (45.3)	40 (51.3)		49 (45.4)	21 (42.0)	
Regional	64 (40.2)	25 (32.0)		39 (36.1)	23 (46.0)	
Distant	23 (14.5)	13 (16.7)	0.70	20 (18.5)	6 (12.0)	0.80
Nodal involvement						
No	112 (71.3)	56 (72.7)		76 (71.0)	31 (64.6)	
Yes	45 (28.7)	21 (27.3)	0.82	31 (29.0)	17 (35.4)	0.42
Grade						
Well differentiated	26 (16.4)	23 (29.1)		18 (16.7)	13 (26.0)	
Moderately differentiated	57 (35.8)	29 (36.7)		38 (35.2)	22 (44.0)	
Poorly differentiated	57 (35.8)	17 (21.5)		39 (36.1)	11 (22.0)	
Unknown	19 (12.0)	10 (12.7)	0.50	13 (12.0)	4 (8.0)	0.18
Radiation						
No	117 (73.6)	64 (81.0)		76 (70.4)	38 (76.0)	
Yes	42 (26.4)	15 (19.0)	0.21	32 (29.6)	12 (24.0)	0.46
Surgery						
No	22 (13.8)	8 (10.1)		15 (13.9)	2 (4.0)	
Yes	137 (86.2)	71 (89.9)	0.42	93 (86.1)	48 (96.0)	0.10

(50.0 versus 33.2%). There was a trend toward higher COX-2 staining intensity among African-Americans in comparison with Caucasians ($P_{trend} = 0.0270$). Percentage of cells staining positive for EGFR and EGFR staining intensity did not differ between African-Americans and Caucasians ($P = 0.46$ and $P = 0.74$, respectively).

Aspirin and NSAID use

In multivariate analysis, no relationship was observed between a history of regular (ever/never) aspirin (adult or baby strength), acetaminophen or other NSAID use or duration of use and COX-2 tumor expression (data not shown). For every 1 year increase in time between first regular use of adult-strength aspirin and diagnosis, there was a 6.2% decrease in the probability of having a COX-2+ tumor after adjusting for race, age at diagnosis, smoking pack-year history and grade (odds ratio 0.94, 95% CI 0.89–0.98). Time between first regular pill use and diagnosis was not significantly associated with probability of having a COX-2-positive tumor for baby aspirin, acetaminophen or NSAIDs (data not shown).

Tumor expression and survival

Of the 239 adenocarcinoma cases followed for whom COX-2 and/or EGFR results were available, only one participant was lost to follow-up. Median survival times were 37.5 months for cases with COX-2− tumors and 33.0 months for cases with COX-2+ tumors. Median survival times for cases with EGFR− tumors were 35 months and 40 months for cases with EGFR+ tumors. Patients with COX-2+ tumors had a statistically significant poorer prognosis in comparison with patients with COX-2− tumors (HR 1.67, 95% CI 1.01–2.78) (Table III; Figure 1). When analysis was stratified by stage at diagnosis, COX-2 expression was no longer significantly associated with survival time for patients diagnosed at a localized stage or for those diagnosed at a regional/distant stage (data not shown). A statistically significant reduction in the risk of death was observed for cases with EGFR+ tumor status (HR 0.56, 95% CI 0.32–0.98) (Table III; Figure 2). When analysis was stratified by stage (localized versus regional/distant), EGFR was associated with a statistically non-significant reduction in risk of death among patients diagnosed at a regional or distant stage

Table III. Estimated risk of death associated with EGFR and COX-2 positivity in women with adenocarcinoma of the lung

Strata	All stages	African-American	Caucasian
	HR[a] (95% CI)	HR[b] (95% CI)	HR[b] (95% CI)
COX-2	**1.67 (1.01–2.78)**[c]	5.58 (0.64–48.37)	1.29 (0.72–2.30)
EGFR	**0.56 (0.32–0.98)**[d]	0.93 (0.20–4.23)	0.53 (0.27–1.04)
COX-2+/EGFR+ versus other	1.10 (0.65–1.85)	1.68 (0.39–7.26)	0.80 (0.41–1.58)
COX-2+/EGFR+ versus COX-2−/EGFR−	0.72 (0.29–1.77)	—	0.58 (0.21–1.60)
COX-2+/EGFR+ versus COX-2+/EGFR−	0.64 (0.32–1.31)	0.80 (0.16–3.94)	0.68 (0.27–1.70)
COX-2−/EGFR+ versus other	**0.43 (0.20–0.92)**[e]	0.33 (0.03–3.30)	0.63 (0.25–1.58)

[a]Adjusted for age at diagnosis, race, surgery, stage, family history of lung cancer, smoking pack-year history, history of chronic obstructive pulmonary disease and radiation.
[b]Adjusted for age at diagnosis, surgery, stage, family history of lung cancer, smoking pack-year history, history of chronic obstructive pulmonary disease and radiation.
[c]$P = 0.0470$.
[d]$P = 0.0431$.
[e]$P = 0.0298$.

(HR 0.54, 95% CI 0.28–1.03, $P = 0.06$) but not among patients diagnosed at a localized stage (HR 0.95, 95% CI 0.26–3.47). The data were reanalyzed excluding four participants who died of causes unrelated to lung cancer; results did not change.

Median survival times in months among cases with adenocarcinoma of the lung by combined COX-2/EGFR expression were as follows: COX-2−/EGFR−, 39.0 months; COX-2+/EGFR−, 33.0 months; COX-2−/EGFR+, 40.0 months and COX-2+/EGFR+, 40.0 months. Probability of survival was no different in patients with COX-2+/EGFR+ tumors than it was for patients with all other tumor results combined (COX-2+/EGFR−, COX-2−/EGFR+ or COX-2−/EGFR−) (HR 1.10, 95% CI 0.65–1.85) or for patients with COX-2−/EGFR− tumors (HR 0.72, 95% CI 0.29–1.77) (Table III). When patients with COX-2+/EGFR+ tumors were compared with those who had COX-2+/EGFR− tumors, addition of EGFR expression was associated with a statistically non-significant reduction in risk of death (HR 0.64, 95% CI 0.32–1.31). Risk of death was significantly reduced in women with COX-2−/EGFR+ compared with women with other tumor results (COX-2−/EGFR−, COX-2+/EGFR− and COX-2+/EGFR+) (HR 0.43, 95% CI 0.20–0.92, $P = 0.03$).

When analyses were stratified by race, COX-2+ tumor expression was associated with a greater reduction in median survival time among African-American women (41.5 versus 28 months) than among Caucasian women (37 versus 33 months). However, the risk of death associated with COX-2 tumor expression was not statistically significant among African-American women (HR 5.58, 95% CI 0.64–48.37) or Caucasian women (HR 1.29, 95% CI 0.72–2.30) after adjustment for age at diagnosis, surgery, stage, family history of lung cancer, smoking pack-year history, history of chronic obstructive pulmonary disease and radiation (Table III). Conversely, EGFR expression was associated with an improvement in median survival time among Caucasian women (33 versus 40 months) but not among African-American women (36 versus 33 months). EGFR tumor expression was associated with a statistically non-significant decrease in survival for Caucasian women (HR 0.53, 95% CI 0.27–1.04). When cases with COX-2+/EGFR+ tumors were compared with cases with other expression results (COX-2+/EGFR−, COX-2−/EGFR+ and COX-2−/EGFR−), no association was found with risk of death in African-Americans or Caucasians.

There was no trend in the association between COX-2 staining intensity and survival ($P_{trend} = 0.40$) (data not shown). Moderate EGFR staining intensity was associated with a 55% reduction in the risk of death (HR 0.45, 95% CI 0.22–0.92). There was no trend in the relationship between EGFR percent positivity and survival ($P_{trend} = 0.23$) (data not shown). Percent COX-2 positivity was associated with a statistically significant increase in the risk of death among patients with tumors staining positive for 11–50% of the cells (HR 1.98, 95% CI 1.04–3.76).

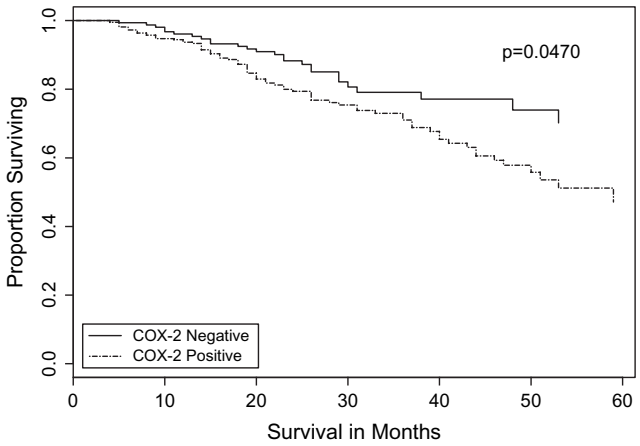

Fig. 1. Cox proportional hazards estimates of overall survival among patients with COX-2+ versus COX-2− tumors adjusted for age at diagnosis, race, surgery, stage at diagnosis, family history of lung cancer, smoking pack-year history, history of chronic obstructive pulmonary disease and radiation.

Discussion

While the relationships between COX-2 and EGFR and survival have been studied independently, only one study examining the relationship between dual COX-2/EGFR tumor status and survival among lung cancer patients has been published previously (34). It reported no association between combined COX-2/EGFR tumor status and survival. As it involved lung cancer samples from 23 COX-2−/EGFR−, 9 COX-2−/EGFR+, 14 COX-2+/EGFR− and 7 COX-2+/EGFR+ patients ($n = 53$), their analysis may not have had sufficient power to detect statistically significant differences. Our considerably larger sample size ($n = 157$), however, yielded similar results.

These findings in lung cancer are in direct contrast with the studies of cervical and esophageal cancer that suggest that COX-2 and EGFR expression may be associated with tumor progression and survival among cancer patients. A comparison of patients with normal tissue, Barrett's esophagitis and esophageal adenocarcinoma revealed increasing expression of COX-2 and EGFR from normal tissue to Barrett's esophagitis to adenocarcinoma (36). Patients with COX-2+/EGFR+ squamous carcinoma of the uterine cervix had a poorer 5 year disease-free survival rate and higher probability of locoregional recurrence in comparison with patients with COX-2+/EGFR−, COX-2−/EGFR+ or COX-2−/EGFR− tumors (37). These results support

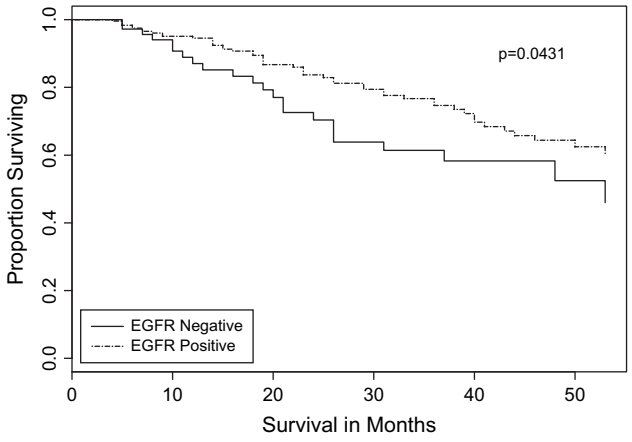

Fig. 2. Cox proportional hazards estimates of overall survival among patients with EGFR+ versus EGFR− tumors adjusted for age at diagnosis, race, surgery, stage at diagnosis, family history of lung cancer, smoking pack-year history, history of chronic obstructive pulmonary disease and radiation.

the interactions between COX-2 and EGFR in progression of esophageal and cervical cancer; however, this conclusion may not apply to adenocarcinoma of the lung as both cancers differ from NSCLC in etiology and pathogenesis.

The relationship between COX-2 expression and survival for NSCLC has not been evaluated previously in African-Americans and only sparingly in women. Previous studies included between 20 and 50% women in their analyses, and the majority of these studies were conducted in populations outside of USA. Only one study conducted in Japan examined this association stratified by sex (18); they reported a significant decrease in survival among men, but not women, with COX-2+ tumors. We observed that COX-2 expression was associated with a poorer prognosis overall, with higher risk seen among African-Americans than Caucasians; however, these results were not statistically significant. While the number of African-Americans included in this sample was small, these results suggest that further race-specific research needs to be conducted. Among lung cancer patients, African-Americans have a poorer prognosis in comparison with Caucasians (38). This difference in survival rates has been associated with decreased probability of having surgical treatment and poorer performance status (39,40); however, when universal care is available, racial disparities in survival among NSCLC patients are eliminated (41). At the same time, potential racial differences in mechanisms underlying lung cancer development and progression have not been explored. Our data suggest that COX-2-positive tumor status is associated with decreased survival, and potentially more aggressive disease, in African-American women. The increased percent COX-2-positive staining and intensity of staining among African-Americans may be associated with smoking behavior. Cigarette smoking is associated with induction of *COX2* gene expression. While African-Americans smoke fewer cigarettes per day than their Caucasian counterparts and start smoking later in life, they are less likely to quit smoking and more likely to smoke non-filtered cigarettes with a high tar yield (42). In our sample, African-Americans reported smoking fewer cigarettes per day in comparison with Caucasians (16.08 ± 10.28 versus 25.44 ± 12.12, $P < 0.0001$). In contrast with the literature, in our sample there was no difference between African-American women and Caucasian women in the number of years smoked (34.68 ± 11.97 versus 36.31 ± 11.70, $P = 0.43$) or in the age smoking began (16.80 ± 4.33 versus 17.02 ± 4.28, $P = 0.77$). The increased COX-2 tumor staining among African-American women may be related to their continued smoking. At the time of interview, African-American women reported a lower smoking pack-year history (29.12 ± 24.71 versus 46.82 ± 27.17, $P = 0.0002$) and more frequently reported being current smokers than Caucasian women (69.6 versus 53.8%, $P = 0.0651$). Few studies have examined the association between cigarette smoking and COX-2 expression in people with and without cancer. Cigarette smoking, but not number of cigarettes smoked per day, is related to an increase in COX-2 mRNA levels in patients with bladder cancer and controls (43). In contrast, Laga *et al.* (19) report no association between COX-2 protein expression as measured by IHC and smoking pack-year history among NSCLC patients. Thus, this relationship between current smoking status and COX-2 expression and racial differences in outcome needs to be explored further.

An association between EGFR protein expression and survival among NSCLC patients has been shown previously (29,44); however, other studies have found either no significant association (25,31,45,46) or an inverse relationship (47,48) between EGFR protein expression and survival. These findings remain controversial with only a handful of studies using quantitative methods to measure protein expression. The majority of studies used IHC to measure protein expression by scoring percent positivity and/or staining intensity, and at present there is no standard scoring method or definition of positivity. Furthermore, antibody detection method varied across studies, and many of these studies did not adjust for factors such as stage, age at diagnosis, comorbidities and smoking status. In contrast, our study did address the relationship between IHC score and clinical–epidemiological variables.

The improvement in survival among patients with EGFR+ tumors when tumor expression (positive/negative) was analyzed may have been associated with their enhanced responsiveness to EGFR tyrosine kinase inhibitors (TKIs). EGFR TKI therapy has been shown to be most effective among women, Asians, never smokers and adenocarcinoma cases. As our study included only women with adenocarcinoma, this argument may hold some validity. However, as chemotherapy information was not collected through this study, this question could not be addressed directly. Among NSCLC patients, increased *EGFR* gene copy number has been associated with enhanced protein expression (49,50), and gene copy number, mRNA expression and protein expression have been associated with improved response to treatment with a TKI and overall survival (51–53). Hirsch *et al.* (52) report that NSCLC cases with high *EGFR* copy number who were not treated with gefitinib had a far worse prognosis than cases with high *EGFR* copy number who were treated with gefitinib. However, some studies report an association between *EGFR* copy number and survival among NSCLC patients treated with gefitinib but not between EGFR protein expression and prognosis or survival among these cases (53,54). An analysis of cases with adenocarcinoma only revealed a significant association between a response to treatment with gefitinib and moderate to intense EGFR protein expression (32). While some studies have found no association between EGFR protein expression and response to TKI therapy or survival among patients treated with TKIs, these univariate analyses did not take into account the important prognostic factors such as stage and age at diagnosis (50,55). One study by Clarke *et al.* (56) that did utilize multivariate analysis reported a greater decrease in the risk of death with erlotinib treatment among EGFR+ NSCLC cases (HR 0.65) than among EGFR− cases (HR 0.83); however, this interaction between treatment and EGFR expression was not statistically significant. The converse pattern has also been shown; patients with tumors negative for *EGFR* amplification and EGFR protein expression have not responded to gefitinib or erlotinib in clinical trials (51).

Strengths and limitations

Our study has a number of strengths. It examines the relationship between COX-2 and EGFR tumor expression and survival in women and by race. As lung tumors of different histology have significant differences in clinical and biological features, analyses were limited to adenocarcinomas instead of including cases with any NSCLC histology. Only in-person interviews regarding risk factors were performed, follow-up was standardized and all cancer diagnoses were confirmed histologically by the same pathologist, eliminating inconsistencies secondary to interobserver variability. The collection of risk

factor data and clinical data allowed for adjustment of these variables in the survival analysis. Moreover, the substantial proportion of cases who were African-American (19% for COX-2 analysis and 22% for EGFR analysis) permitted race-specific analysis. Additionally, our sample size was sufficient to stratify COX-2 and EGFR analyses by stage and race. However, while we were able to include presence or absence of surgery and radiation treatment history in the proportional hazards models, we did not collect data on specific chemotherapeutic treatments. In addition, our results may only apply to adenocarcinomas of the lung rather than to all NSCLC histological types.

In our study, women with COX-2+ tumors had a poorer prognosis than did women with COX-2− tumors. On stratified analysis, a greater reduction in survival time was observed among African-American women with COX-2-positive tumors than among Caucasian women. EGFR+ tumor status was associated with improved survival time. Combined COX-2/EGFR tumor expression did not predict survival among women with adenocarcinoma of the lung in our analysis.

While our results and those of Brattstrom *et al.* (34) suggest that combined COX-2/EGFR tumor expression does not predict survival among lung cancer patients, larger studies should be conducted to analyze whether this relationship varies by stage at diagnosis. Additional studies with larger numbers of African-Americans need to be conducted to more fully evaluate the relationship between COX-2 tumor status and survival stratified by race, stage at diagnosis and smoking status. In summary, while combined COX-2/EGFR expression does not predict survival among women with adenocarcinoma of the lung, COX-2 expression and EGFR expression may predict survival separately.

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