Nuclear Factor-κB (NF-κB) Is Frequently Expressed in Lung Cancer and Preneoplastic Lesions

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BACKGROUND. Nuclear factor-κB (NF-κB), a key transcription factor thought to play a major role in carcinogenesis, regulates many important signaling pathways involved in tumor promotion. Although NF-κB can be activated in lung cancer cell lines by tobacco exposure, there have been no studies of the expression of NF-κB in lung cancer pathogenesis.

METHODS. The immunohistochemical expression of NF-κB p65 was investigated in 394 lung cancers (370 nonsmall cell lung carcinomas [NSCLC]; and 24 small cell lung carcinomas [SCLC]) and 269 lung normal epithelium and preneoplastic lesions, including hyperplasias, squamous metaplasias, dysplasias, and atypical adenomatous hyperplasias.

RESULTS. High levels of nuclear immunohistochemical expression of NF-κB p65 were detected in the lung cancers, with significantly higher levels in SCLCs compared with NSCLCs ($P < .0001$). In adenocarcinomas the NF-κB p65 expression level was significantly higher in advanced TNM stages (III-IV) than in earlier stages (I-II) ($P < .0001$), and when NF-κB p65 is dichotomized using 50% as the cutoff point (high vs low), a higher NF-κB p65 expression level was detected in tumors having either *K-RAS* ($P = .02$) or *EGFR* ($P = .009$) mutations compared with wild-type tumors. A relatively high level of nuclear NF-κB p65 expression was detected in normal and mildly abnormal epithelium, and a progression with increasing histology severity was detected in preneoplastic lesions.

CONCLUSIONS. NF-κB p65 nuclear expression is an early and frequent phenomenon in the pathogenesis of lung cancer. The findings indicate that NF-κB activation plays an important role in lung cancer pathogenesis and is a suitable target for the development of new lung cancer therapies and chemoprevention strategies. ***Cancer* 2006;107:2637–46.** © *2006 American Cancer Society.*

KEYWORDS: NF-κB, lung cancer, inflammation, lung preneoplasia, squamous dysplasia, *EGFR* mutation, *K-RAS* mutation.

Despite intense research and improvements in preventive, diagnostic, and therapeutic approaches, lung cancer remains the leading cause of cancer-related deaths in the US.[1] Lung cancer is a highly complex neoplasm,[2] consisting of several histologic types, the most frequent being small cell lung carcinoma (SCLC) and 2 types of nonsmall cell lung carcinoma (NSCLC): squamous cell carcinoma and adenocarcinoma.[3] NSCLCs are believed to arise after the progression of sequential preneoplastic lesions, including bronchial squamous dysplasias for squamous cell carcinoma and atypical adenomatous hyperplasias (AAH) for a subset of adenocarcinomas.[4] An increased understanding of the molecular mechanisms involved in the pathogenesis and progression of lung cancer may lead to new and more effective strategies for risk assessment, prevention, early detection, and targeted treatment.

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Nuclear factor-κB (NF-κB), initially discovered as a transcription factor in the nucleus of B cells that binds to the enhancer of the kappa light chain of immunoglobulin, has since been shown to be expressed ubiquitously in the cytoplasm of all types of cells.[5] NF-κB translocates to the nucleus only when activated, where it regulates the expression of more than 200 genes that control inflammation and cell growth.[6] Most commonly, NF-κB exists as a p65/p50 heterodimer that is retained in its inactive state by its association with IκBα, an inhibitory protein. NF-κB is activated by a wide variety of stimuli that phosphorylate IκBα.[7] This phosphorylation results in dissociation of IκBα from p65/p50 heterodimers. Then, the NF-κB protein, freed from its inhibitory regulator, is transported to the nucleus, where it regulates transcription. Thus, the nuclear localization of p65 protein is a marker of NF-κB activation in cells.[8]

NF-κB activation has been associated with the initiation and progression of several human cancers[6,9–13] and is considered a molecular link between chronic inflammation and cancer development.[13–16] Thus, there is an increasing level of interest in developing inhibitors of NF-κB for novel chemoprevention and treatment strategies of human cancers. A number of lines of evidence suggest that chronic inflammation contributes to the process of lung carcinogenesis through activation of a number of molecular pathways, including NF-κB.[17,18] In NSCLC cell lines, it has been demonstrated that tobacco components stimulate NF-κB-dependent survival,[19] and the cyclooxygenase (COX)-2 inhibitor celecoxib suppresses NF-κB p65 nuclear immunolocalization induced by various carcinogens.[20] To date, however, the expression of nuclear NF-κB has not been studied comprehensively in lung cancer tumors and lung preneoplastic lesion tissues.

To better understand the importance of NF-κB activation in lung cancer pathogenesis and progression, we investigated nuclear NF-κB p65 immunohistochemical expression in a large series of NSCLC and SCLC tumor tissue specimens and in normal and abnormal lung bronchial and alveolar epithelial foci, using tissue microarray (TMA) specimens, and we correlated those findings with clinical-pathologic features of lung cancer patients.

MATERIALS AND METHODS

Tumor and Respiratory Epithelium Case Selection and Tissue Microarray Construction

We obtained archival, formalin-fixed and paraffin-embedded (FFPE) material from surgically resected lung cancer specimens (lobectomies and pneumonectomies) containing tumor and adjacent normal and abnormal epithelium tissues from the Lung Cancer SPORE Tissue Bank at M. D. Anderson Cancer Center from 1997 to 2001 (Houston, TX). Tumor tissue specimens obtained from 394 lung cancers were histologically examined, classified using the 2004 World Health Organization (WHO) classification,[3] and selected for TMA construction. They consisted of 24 (6%) SCLCs, 254 (64%) adenocarcinomas, and 116 (29%) squamous cell carcinomas (Table 1). All adenocarcinomas were mixed histology subtype, except for 20 bronchioloalveolar carcinomas (BAC). This study was approved by the University of Texas M. D. Anderson Cancer Center Institutional Review Board.

Detailed clinical and pathologic information, including demographic, smoking history (never- and ever-smokers) and status (never, former, and current smokers), clinical and pathologic TNM staging, overall survival, and time of recurrence was available in most cases (Table 1). Patients who had smoked at least 100 cigarettes in their lifetime were defined as smokers, and smokers who quit smoking at least 12 months before lung cancer diagnosis were defined as former smokers. Tumors were pathologic TNM stages I-IV according to the revised International System for Stating Lung Cancer.[21] In a subset of 75 adenocarcinomas, *K-RAS* (codons 12 and 13) and *EGFR* (exons 18–21) gene mutation data were available. After histologic examination, tumor TMAs were prepared using triplicate 1-mm-diameter cores per tumor, obtaining tissue from central, intermediate, and peripheral tumor areas.

From surgically resected NSCLC FFPE specimens, 269 bronchial and bronchiolar epithelium specimens from 88 patients having normal histology ($n = 56$), hyperplasia ($n = 61$), squamous metaplasia ($n = 19$), squamous dysplasia and carcinoma in situ ($n = 62$), and peripheral lung tissue with AAH ($n = 71$) were identified and selected for analysis (Table 1). Histological classification of epithelial lesions was performed using the 2004 WHO classification[3] of lung cancer preneoplastic lesions. For NF-κB p65 expression analysis, squamous dysplasias were arranged in 2 groups: 1) low-grade, mild, and moderate dysplasias; and, 2) high-grade, severe dysplasia, and carcinoma in situ. For the TMA construction of epithelial foci, in an attempt to capture most of the small preneoplastic lesions in the TMA cores single 2-mm cores were used.

NF-κB p65 Immunohistochemical Analysis

Five-micron-thick FFPE tissue histology sections were deparaffinized, hydrated, heated in a steamer for 10 minutes with 10 mM sodium citrate (pH 6.0) for antigen retrieval, and washed in Tris buffer. Peroxide

TABLE 1
Demographic and Clinical-Pathologic Data Regarding the Lung Cancer and Respiratory Epithelial Samples Studied for NF-κB Immunohistochemical Expression

		Sex		Stage*				Smoking history†		Smoking status‡		
Type and histology of samples	No.	Men	Women	I	II	III	IV	Yes	No	Never	Former	Current
Total cancers	394	208	186	—	—	—	—	—	—	—	—	—
SCLC*	24	7	17	—	—	—	—	24	0	0	3	7
NSCLC	370	201	169	242	69	49	10	243	111	111	137	94
Adenocarcinoma	234	143	91	158	33	36	7	145	83	83	77	59
BAC	20	13	7	17	0	2	1	10	6	6	5	3
Squamous cell carcinoma	116	45	71	67	36	11	2	88	22	22	55	32
Total epithelial foci	269	123	146	—	—	—	—	215	49	49	97	108
Normal epithelium	56	29	27	—	—	—	—	43	13	13	21	22
Hyperplasia	61	16	45	—	—	—	—	53	6	6	28	25
Squamous metaplasia	19	8	11	—	—	—	—	14	2	2	7	7
Mild dysplasia	2	2	0	—	—	—	—	2	0	0	1	1
Moderate dysplasia	9	2	7	—	—	—	—	9	0	0	5	4
Severe dysplasia	10	1	9	—	—	—	—	10	0	0	0	1
Carcinoma in situ	41	12	29	—	—	—	—	36	5	5	17	19
AAH	71	53	18	—	—	—	—	48	23	23	18	29

SCLC indicates small cell lung carcinoma; NSCLC, nonsmall cell lung carcinoma; BAC, bronchioloalveolar carcinoma; AAH, atypical adenomatous hyperplasia.

* Staging is shown only for NSCLC cases. All SCLCs were limited stage.

† Smoking history was not available in 16 NSCLCs and 5 epithelial lesions.

‡ Smoking status was not available in 14 SCLCs, 28 NSCLCs, and 15 epithelial lesions.

blocking was performed with 3% H_2O_2 in methanol at room temperature for 15 minutes, followed by 10% bovine serum albumin in TBS-t for 30 minutes at room temperature. The mouse monoclonal antihuman antibody against C-terminus of human NF-κB p65 protein (1:250 dilution; BD Pharmingen, San Diego, CA) was used and incubated for 1.5 hours at room temperature. Incubation with the secondary antibody (Envision+ dual-link labeled polymer; DAKO, Carpinteria, CA) was performed for 30 minutes, followed by application of diaminobenzidine chromogen for 5 minutes. Although cell cytoplasmic expression was detected in most normal, hyperplastic, preneoplastic, and tumor cells examined, only distinct nuclear immunostaining for NF-κB p65, which is considered activated NF-κB, was quantified. In each case, 200 tumor and epithelial cells were quantified by light microscopy using a ×20 magnification objective, and a score (range, 0–100) expressing the percentage of positive cells was obtained. For the tumor TMAs, scores from the three cores examined were averaged. For the epithelial samples, only 1 score was obtained. As a positive control, FFPE pellets from lung cancer cell lines having NF-κB p65 overexpression (as indicated by Western blot analysis) were used. As a negative control, paraffin-embedded positive lung cancer and cell line pellets were subjected to NF-κB p65 immunostaining by using specific NF-κB p65 blocking peptide and omitting the primary antibody, which was replaced with phosphate-buffered saline (PBS) buffer.

Statistical Analysis

Summary statistics, including frequency tabulation, means, standard deviations, median, and range, were provided to describe subject characteristics. Correlations between the NF-κB p65 immunostaining expression scores for tumor and epithelial samples and clinical-pathologic and genetic (*K-RAS* and *EGFR* mutation) features were analyzed using the Kruskal-Wallis test and Wilcoxon rank-sum test as appropriate. The Fisher exact and chi-square tests were applied to test the association between 2 categorical variables, such as *EGFR* mutation and NF-κB p65 (high vs low). Repeated measures analysis of variance (ANOVA), taking multiple measures from each patient into consideration, was used to test the difference in NF-κB p65 expression among the preneoplastic lesions. Logistic regression model was employed to model the effect of histology type and pathologic T and N stage on the high expression of NF-κB p65 ($\geq$50). A Cox model was fitted to estimate the effect of prognostic factors on survival. All statistical tests were 2-sided, and 2-sided *P*-values of .05 or less were considered statistically significant. Statistical analysis was performed with standard statistical software, including SAS Release 8.1 (SAS Institute, Cary, NC) and S-Plus 2000 (MathSoft, Seattle, WA).

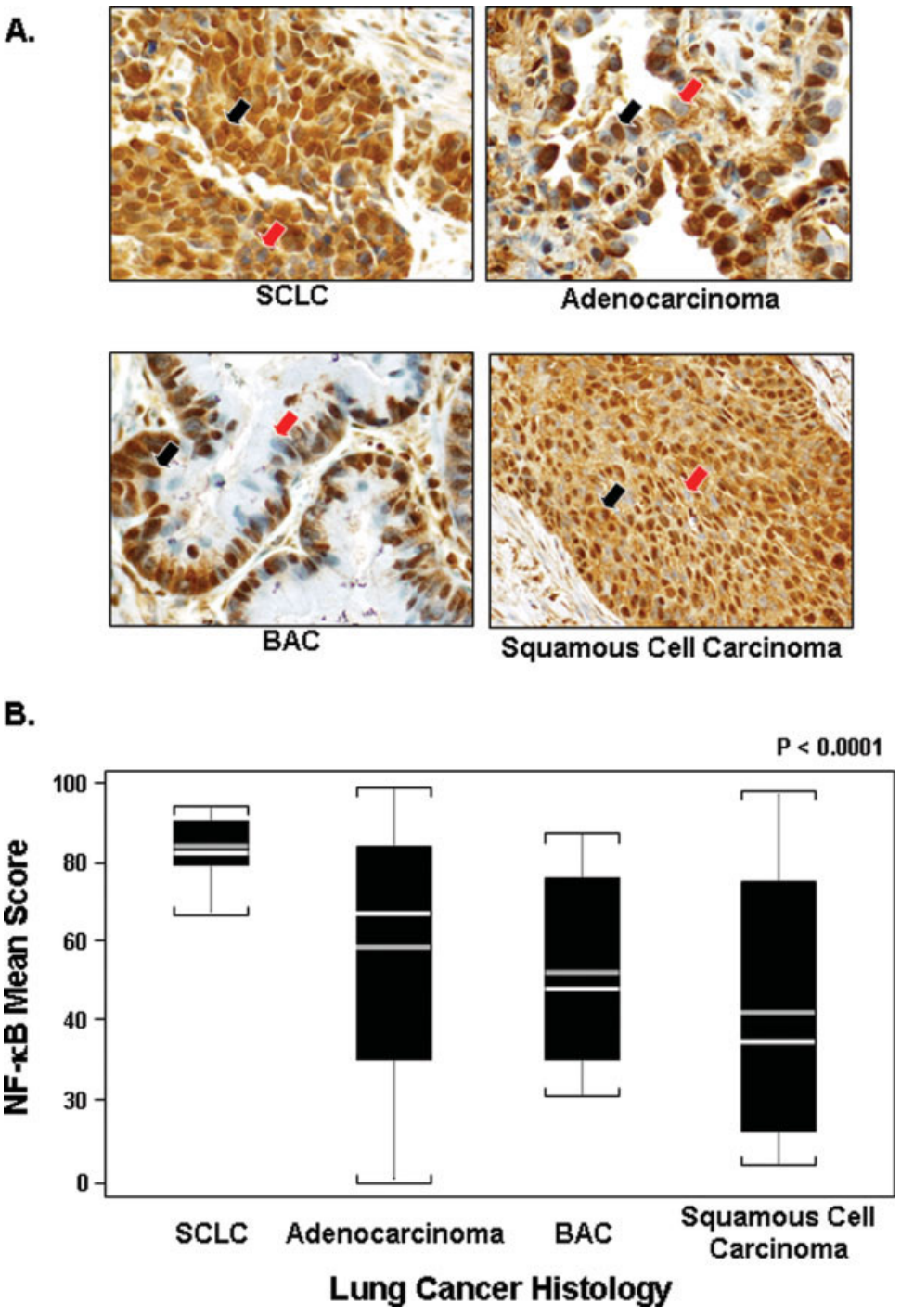

FIGURE 1. Nuclear factor (NF)-κB p65 immunohistochemical expression in lung cancers. (A) Microphotographs (×200) showing NF-κB p65 immunostaining in the 4 lung cancer histologies examined. Notice the dark brown nuclear staining in a subset of tumor cells (black arrow), whereas negative nuclei demonstrate blue counterstaining only (red arrow). (B) Scores of nuclear NF-κB p65 expression by lung cancer histology. SCLC, small cell lung carcinoma; BAC, bronchioloalveolar carcinoma (gray line, mean score; white line, median score).

RESULTS

NF-κB p65 Immunohistochemical Expression in Lung Cancer Specimens

Overall, lung cancers demonstrated high levels of activated nuclear NF-κB p65 expression (Fig. 1). The mean score of nuclear NF-κB p65 expression in all lung tumors examined (n = 394) was 55.2 (range, 0–99.0). Graphical distribution analysis of the NF-κB p65 nuclear scores obtained from all tumors examined indicated the existence of 2 distinct groups of tumor cases: 1) low level of expression (tumors with $< 50\%$ positive cells), and 2) high level of expression (tumors with $\geq 50\%$ positive cells). Using this cutoff level, 56.6% of the lung tumors (223 of 394) demonstrated a high level of nuclear NF-κB p65 expression. A statistically significant difference ($P < .0001$, Kruskal-Waiils test) in NF-

TABLE 2
Correlation Between Nuclear NF-κB Immunohistochemical Expression and Clinical-Pathologic Features in Lung Cancer

			NF-κB Immunohistochemical expression							
						Low level (< 50% cells)		High level (≥50% cells)		
Category	Feature	N	Mean score	SD	*P*	N	%	N	%	*P*
Histology	SCLC	24	83.7	8.4	< .0001*	0	0	24	100.0	< .0001*
	NSCLC	370	53.3	30.6		171	46.2	199	53.8	
	Adenocarcinoma	234	59.2	29.5	< .0001†	86	36.8	148	63.2	< .0001†
	BAC	20	51.8	22.6		11	55.0	9	45.0	
	Squamous cell carcinoma	116	41.8	30.9		74	63.8	42	36.2	
TNM stage in NSCLC‡										
Adenocarcinoma	T1-2	216	57.6	29.7	.007	85	39.4	131	60.6	.004
	T3-4	18	77.7	18.5		1	5.6	17	94.4	
Squamous cell carcinoma	T1-2	103	41.1	30.8	NS§	67	65.0	36	35.0	NS
	T3-4	13	46.7	32.5		7	53.9	6	46.2	
Adenocarcinoma	N0-1	205	55.8	29.6	< .0001	85	41.5	120	58.5	< .0001
	N2	29	83.0	13.7		1	3.4	28	96.6	
Squamous cell carcinoma	N0-1	109	41.1	30.7	NS	71	65.1	38	34.9	NS
	N2	7	51.4	34.6		3	42.9	4	57.1	
Adenocarcinoma	I-II	191	54.1	29.8	< .0001	85	44.5	106	55.5	< .0001
	III-IV	43	81.5	13.5		1	2.3	42	97.7	
Squamous cell carcinoma	I-II	103	41.3	31.0	NS	67	65.0	36	35.0	NS
	III-IV	13	45.8	30.6		7	53.9	6	46.2	
Mutation status in adenocarcinomas‖										
EGFR (exon 18–21)	No	56	66.8	29.1	.39	15	26.8	41	73.2	.009
	Yes	19	78.1	14.2		0	0	19	100.0	
K-RAS (codon 12–13)	No	57	65.5	28.8	.06	15	26.3	42	73.7	.02
	Yes	18	82.8	10.0		0	0	18	100.0	

SD indicates standard deviation; SCLC, small cell lung carcinoma; NSCLC, nonsmall cell lung carcinoma; BAC, bronchioloalveolar carcinoma.
* SCLC vs NSCLC.
† Comparison of NSCLC histologies.
‡ Only adenocarcinoma and squamous cell carcinoma (N = 350 cases).
§ NS = not significant.
‖ *EGFR* and *K-RAS* mutations were detected only in adenocarcinoma histology.

κB p65 nuclear expression was detected among the three major types of lung cancer histologies examined: SCLC, adenocarcinoma, and squamous cell carcinoma (Fig. 1B). SCLC histology demonstrated the highest level (mean score, 83.7; range, 66.5–94.0) of expression, squamous cell carcinomas (mean score, 41.8; range, 4.5–97.5) had the lowest, and adenocarcinomas (mean score, 59.2; range, 0–99.0) were intermediate (Fig. 1B). Whereas all 24 SCLC specimens showed a high level of nuclear expression (≥50% tumor cells positive), this phenomenon was detected in 63.2% (148 of 234) of adenocarcinomas and in only 36.2% (42 of 116) of squamous cell carcinomas ($P < .0001$, Fisher exact test; Table 2). The difference between adenocarcinomas and squamous cell carcinomas was also statistically significant ($P < .0001$, chi-square test). BAC, a noninvasive subtype of lung adenocarcinoma histology, expressed a slightly lower level of NF-κB p65 expression than did invasive adenocarcinomas, with a mean score of 51.8 and 45% (9 of 20) of the tumors with a high level of expression.

Correlation Between NF-κB p65 Immunohistochemical Expression in Lung Cancer and Clinical-Pathologic Features

Using mean score and level (low vs high) of expression, no statistically significant correlation was detected between NF-κB p65 expression and demographic data, including sex, age, ethnicity, and smoking history. In lung adenocarcinomas (n = 234), a significant higher mean score of NF-κB p65 and level of nuclear expression was detected in cancers with more advanced local growth (TNM pathologic T3-T4 compared with T1-T2), more advanced regional lymph node metastasis status (TNM pathologic N0-1 compared with N2), and more advanced TNM stage (stages I-II compared with III-IV; Table 2). However, such correlations were not detected for squamous cell carci-

noma histology (Table 2). Logistic regression model of NF-κB p65 levels (high vs low) considering tumor histology (adenocarcinoma vs squamous cell carcinoma) and pathologic T and pathologic N indicated that the significantly higher frequencies of high-level NF-κB p65 nuclear expression observed for adenocarcinoma histology ($P < .0001$), T3-T4 ($P = .008$), and N2 ($P = .0003$) were independent of each other.

Overall survival analysis for NF-κB p65 nuclear expression was performed using 298 patients with NSCLCs with a median follow-up of 3.05 years. Univariate and multivariate survival analyses demonstrated that NF-κB p65 nuclear expression did not significantly influence the overall survival or disease-free survival of NSCLC patients (data not shown).

Correlation Between NF-κB p65 Immunohistochemical Expression and *EGFR* and *K-RAS* Mutation Status in Lung Cancer

To investigate the correlation between nuclear NF-κB p65 expression and *K-RAS* and *EGFR* mutations in lung cancer, we selected 75 lung adenocarcinomas for which information was available for *K-RAS* mutation at codons 12 and 13 and for *EGFR* mutation at exons 18 to 21. Eighteen cases had mutation in *K-RAS* and 19 had mutation in *EGFR*, and both mutations were mutually exclusive. *K-RAS* and *EGFR* mutated adenocarcinomas demonstrated a significantly higher level of nuclear NF-κB p65 expression compared with wildtype tumors (Table 2). Whereas statistically nonsignificant higher mean scores for nuclear NF-κB p65 expression were detected in mutated compared with wildtype tumors, 100% of the 18 *K-RAS* and the 19 *EGFR* mutated adenocarcinomas expressed high levels ($\geq$50% tumor cells positive) of NF-κB p65 immunostaining compared with 73.7% and 73.2% of the wildtype tumors, respectively (Table 2). These differences were statistically significant.

NF-κB p65 Immunohistochemical Expression in the Sequential Pathogenesis of Lung Cancer

We investigated the expression of nuclear NF-κB p65 expression in histologically normal (n = 56), mildly abnormal (hyperplasia and squamous metaplasia; n = 80), and dysplastic (n = 62) bronchial epithelium obtained from 88 lung cancer patients. A relatively high level of nuclear NF-κB p65 expression was detected in histologically normal epithelium (mean, 21.8% of positive cells; range, 2.0–57.5) in bronchial and bronchiole structures. An increasing level of NF-κB p65 nuclear expression with increasing severity of histological abnormalities was detected (Fig. 2). Repeated measures ANOVA indicated that significantly higher scores of nuclear NF-κB p65 expression were detected in mildly abnormal (hyperplasia and squamous metaplasia), low-grade, and high-grade dysplasias compared with normal epithelium (Fig. 2B). Severe bronchial dysplasia and carcinoma in situ combined demonstrated significantly higher levels than did lower grades of bronchial dysplasia (moderate and mild dysplasia) ($P = .002$, repeated measures ANOVA). AAH lesions, a putative precursor lesion for adenocarcinomas with BAC features,[4] demonstrated significantly higher levels of nuclear NF-κB p65 expression than did normal epithelium from bronchial and bronchiolar origin ($P < .0001$, repeated measures ANOVA). Of interest, AAH lesions demonstrated a lower NF-κB p65 expression score than did the BAC type of lung adenocarcinoma.

DISCUSSION

Our finding of frequent and high levels of NF-κB p65 nuclear expression in lung tumor tissues largely confirmed the previously reported NF-κB activation observed in a limited panel of NSCLC cell lines.[19,20,22] We demonstrate for the first time differences in the level of NF-κB p65 nuclear expression across different lung cancer histologies, with SCLCs having significantly higher levels of expression than NSCLCs, and with adenocarcinomas in the latter group demonstrating significantly greater levels of high expression than squamous cell carcinomas. High levels of nuclear NF-κB p65 expression have been reported in other epithelial tumors, including breast, prostate, gastric, and esophageal carcinomas.[6,9–12,23] In prostate cancer, NF-κB p65 expression has been associated with poor prognosis[24] and the presence of lymph node metastasis.[25] In our study, NF-κB p65 expression in lung adenocarcinomas significantly correlated with more advanced tumor stage (stages III and IV) and, in particular, locally more advanced tumors and lymph node metastasis; however, no correlation between NF-κB p65 expression and overall survival or disease-free survival was detected. Although the utilization of TMA methodology allowed us to perform immunohistochemical expression analysis in a large series of lung tumor specimens using a very controlled setting for immunohistochemistry,[26] the levels of NF-κB p65 protein in lung tumors could be underestimated compared with standard tumor histology sections.

An interesting implication for the presence of high levels of NF-κB p65 expression in epithelial tumors such as lung cancer is the finding reported from several in vivo and in vitro studies that NF-κB inhibits chemotherapy-induced apoptosis of tumor cells[9] by regulating either directly or indirectly the transcription of several genes whose protein products have an antia-

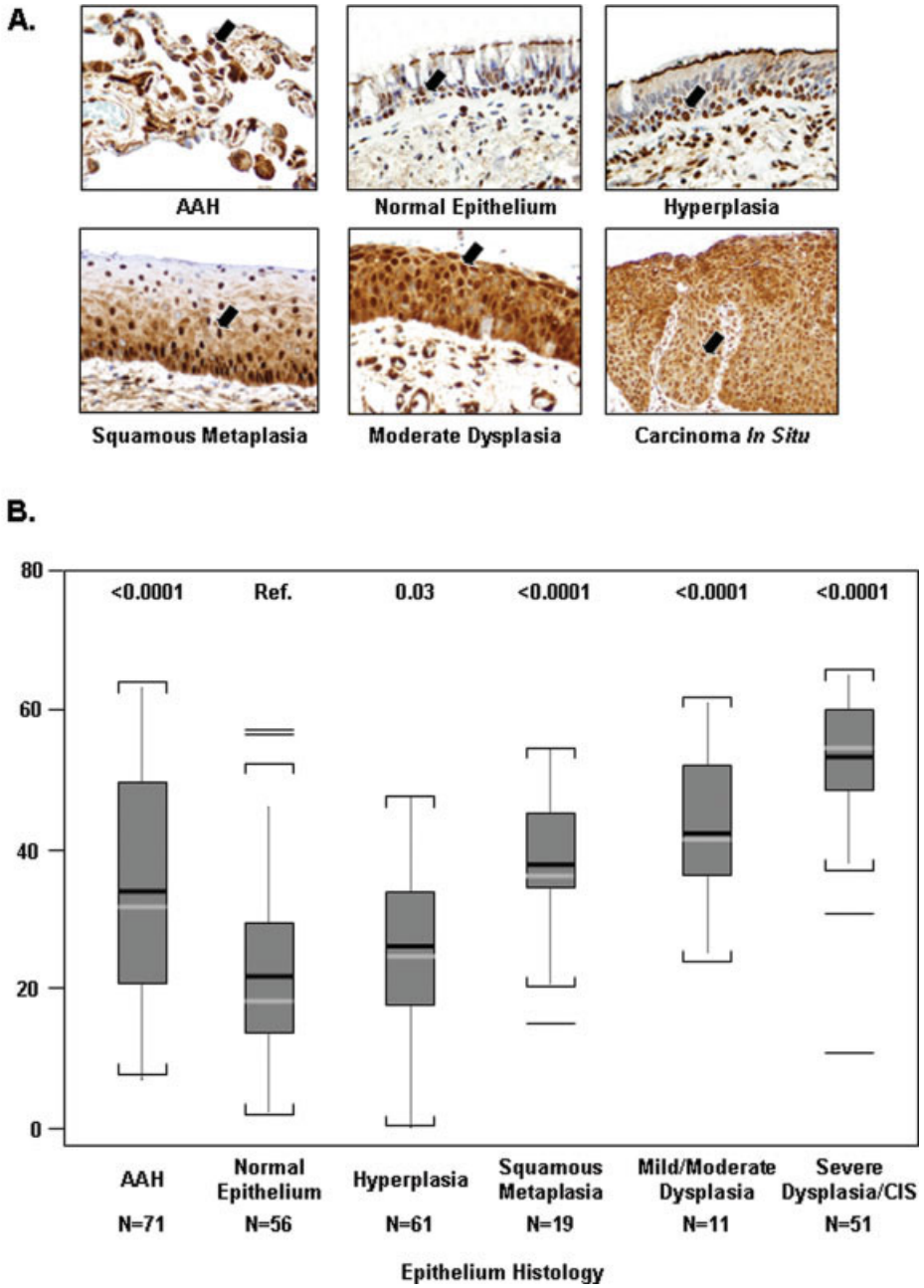

FIGURE 2. Nuclear factor (NF)-κB p65 immunohistochemical expression in normal, mildly abnormal, and preneoplastic lung epithelia. (A) Microphotographs (×200 all but carcinoma in situ, which is ×100) showing nuclear NF-κB p65 immunostaining in normal and abnormal epithelia. Notice the dark brown nuclear staining in a subset of normal and abnormal epithelial cells (black arrow), whereas negative nuclei demonstrate blue counterstaining only. (B) Scores of nuclear NF-κB p65 expression by epithelium histology. Statistically significant differences were detected, using repeated measures analysis of variance (ANOVA), on nuclear NF-κB p65 expression between normal bronchial epithelium and hyperplasia ($P = .03$), squamous metaplasia ($P < .0001$), low-grade dysplasias ($P < .0001$), high-grade dysplasia ($P < .0001$), and atypical adenomatous hyperplasia (AAH) ($P < .0001$). CIS, carcinoma in situ (black line, mean score; gray line, median score).

poptotic effect.[27–29] In addition to constitutive activation of NF-κB in tumor cells, chemotherapy and radiotherapy may themselves increase NF-κB expression,[30,31] causing or increasing treatment resistance. The finding that taxane-based chemotherapy has been shown to induce COX-2 overexpression in lung cancer by stimulating transcription[32,33] has led to the investigation of the potential additive or synergistic effects of COX-2 inhibition combined with conventional chemotherapy and radiotherapy.[34] Preliminary data have shown that celecoxib, which is known primarily as a COX-2 inhibitor but which is also a potent NF-κB inhibitor,[20] significantly decreases PGE_2 within lung tumor tissues,[34,35] suggesting that COX-2-dependent expression of genes that are deleterious to the antitumor response may also be decreased. However, NF-κB activation, which directly exerts transcriptional regulation of COX-2, has not been investigated in lung tumors treated with those therapeutic combinations. Our finding of high levels of NF-κB p65 nuclear expression in the majority (54%) of NSCLCs warrants the evaluation of this marker as a predictor of chemotherapy or radiotherapy response in lung cancer, with or without concomitant celecoxib treatment.

Although NF-κB activity seems to be required during the development of malignancy in inflammation-associated cancers,[36] whether activation of NF-κB alone is sufficient to initiate transformation remains unclear. The findings of NF-κB activation in human cancers and chemically induced rodent carcinomas have led to the hypothesis that this activity is the result of oncogenes that trigger signaling pathways leading to NF-κB activation.[36] Our findings of significantly higher levels and frequency of NF-κB p65 nuclear expression in lung adenocarcinomas demonstrating *K-RAS* and *EGFR* mutations compared with wildtype tumors support the hypothesis that NF-κB activation is triggered by genetically activated signaling pathways. Our findings are consistent with the fact that NF-κB activation has been demonstrated to be a downstream effect of oncogenic *K-RAS*[37] and *EGFR*[38] activation in tumor cells. Thus, NF-κB p65 activation seems to be a common effect of 2 distinct oncogenic pathways in lung adenocarcinoma, the *K-RAS*-smoking and the *EGFR*-nonsmoking associated pathways.[39]

Accumulating evidence suggests that tumor progression is governed not only by genetic changes intrinsic to cancer cells but also by epigenetic and environmental factors. Chronic inflammation has been hypothesized as 1 of the most important epigenetic factors contributing to epithelial cancer development.[40] A chronic inflammatory process enhances cell proliferation, cell survival, and cell migration in epithelial cells, as well as angiogenesis in the adjacent stroma, thereby promoting epithelial tumor development.[40] In the last decades, inflammation and related pathways have been suggested to play an important role in the pathogenesis of lung cancer, particularly in smoking-damaged respiratory epithelium.[18] However, the mechanisms involved are not well understood. NF-κB has recently been identified as a molecular link between chronic inflammation and cancer,[6,16] suggesting that NF-κB exerts its oncogenic effects in both the tumor and the microenvironment, promoting the survival of premalignant epithelial cells.[13–15] NF-κB regulates the expression of various molecules important in tumorigenesis, such as matrix metalloproteinases, COX-2, iNOS, chemokines, and inflammatory cytokines, all of which promote tumor cell invasion and angiogenesis.[7] However, it is currently unknown whether NF-κB activity itself plays a causal role in the initiation event leading to solid tumors or whether it may participate in tumor promotion and progression.

Lung cancers are believed to arise after a series of progressive pathologic changes (preneoplastic or precursor lesions) in the respiratory mucosa. Whereas the sequential preneoplastic changes have been defined for centrally arising squamous carcinomas, they have been poorly documented for adenocarcinomas and SCLCs.[4,41] Currently available information suggests that lung preneoplastic lesions and molecularly abnormal epithelial foci are frequently extensive and multifocal throughout the lung, indicating a field effect or field cancerization phenomenon by which much of the respiratory epithelium has been molecularly altered, presumably from exposure to carcinogens, including tobacco smoking components.[42] A common factor responsible for such a phenomenon in the lung airway could be the development of inflammation and the activation of inflammation-related pathways in the respiratory mucosa microenvironment, resulting in the activation of NF-κB in the respiratory epithelial cells. Our finding of frequent NF-κB p65 nuclear expression at early stages in the pathogenesis of both major types of lung cancer supports this concept. The previous reports of high levels of phosphorylated and total NF-κB p65 immunohistochemical expression in squamous cell carcinomas of head and neck[43] and esophageal adenocarcinoma,[44] respectively, and their corresponding preneoplastic lesions, suggests that NF-κB activation is a common phenomenon in the pathogenesis of aerodigestive tract tumors and may represent a relevant biological and clinical target for chemoprevention strategies for the aerodigestive cancerization field.

Mucosal changes in the large airways that may precede invasive squamous cell carcinoma include squamous dysplasia and carcinoma in situ in the central bronchial airway.[4,41] Adenocarcinomas may be preceded by morphological changes, including AAH[4,45] in peripheral airway cells. For the centrally located squamous cell carcinoma sequence, severe squamous dysplasia and carcinoma in situ of the bronchi demonstrated significantly higher levels of NF-κB p65 nuclear expression than lower grades of bronchial dysplasia (moderate and mild dysplasia) and mildly abnormal or reactive lesions (squamous metaplasia and hyperplasia). These findings greatly expand the recently reported findings of increased nuclear NF-κB p65 expression in a limited number of squamous moderate and severe dysplasias ($n = 18$) obtained from smokers without cancer compared with normal epithelium ($n = 11$).[46] Of interest, using immunohistochemistry the phosphorylated form of NF-κB p65 protein has been detected in 100% of high-grade tonsillar squamous dysplasias adjacent to invasive carcinomas.[43] However, no information on p-NF-κB p65 protein expression is available in lung specimens. Conversely, lung peripheral AAH lesions demonstrated significantly higher levels of nuclear NF-κB p65 expression than did normal epithelium from bronchial and bronchiolar origin, indicating that

this phenomenon could also be involved in the pathogenesis of peripheral lung adenocarcinomas.

The findings of NF-κB p65 nuclear expression as a frequent and early event in the pathogenesis of both major types of NSCLC make this phenomenon a very attractive candidate for inhibition in the development of novel chemoprevention strategies in lung cancer. Currently, celecoxib is being used in several ongoing lung cancer chemoprevention trials, and it has been shown capable of modulating the Ki67 proliferation index and apoptotic balance in bronchial tissue of smokers[47]; however, its effect on NF-κB activation in respiratory epithelial cells is still unknown. To circumvent the cardiovascular adverse effect potentially associated with the use of celecoxib in chemoprevention trials, attention has been focused on dietary or natural agents.[48] Among several dietary agents that have been found to be potent inhibitors of NF-κB activation,[48] curcumin,[49] a diferuloylmethane, has been shown to suppress the condensate smoking-induced NF-κB activation in human NSCLC cell lines,[7] suggesting a role for this agent in lung cancer chemoprevention strategies.

In summary, our study is the first comprehensive report showing a high level of NF-κB p65 tissue nuclear expression tissue in the major lung cancer histologies and their corresponding known preneoplastic lesions. Our findings have implications for elucidating the role of inflammation in the early pathogenesis of lung cancer and also provide a rationale for targeting NF-κB activation in therapeutic and preventive approaches to this neoplasm.

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