Received: 1 April 2021 | Accepted: 15 April 2021

DOI: 10.1111/1759-7714.13985

ORIGINAL ARTICLE

WILEY

BAI1 nuclear expression reflects the survival of nonsmoking non-small cell lung cancer patients

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Abstract

Background: Smoking- and nonsmoking-associated lung cancers have different mechanisms of carcinogenesis. We divided non-small cell lung cancer (NSCLC) patients into nonsmoking and smoking groups with the aim of trying to understand the utility of brain-specific angiogenesis inhibitor 1 (BAI1) expression in the separate groups.

Methods: Clinicopathological data were obtained from 148 patients who had undergone surgery for NSCLC of the lung. Tissue microarray blocks were made of samples from NSCLC patients. Two pathologists graded the intensity of BAI1 expression as high or low expression in the cancer cells of patients in the smoking and nonsmoking groups.

Results: NSCLC nonsmokers with higher BAI1 nuclear expression had poor disease-specific survival (DSS) (hazard ratio: 2.679; 95% confidence interval [CI]:1.022–7.022, $p = 0.045$). The Kaplan–Meier survival curve confirmed that higher BAI1 expression was significantly associated with poor DSS ($p = 0.034$) in the nonsmoking group.

Conclusions: We divided NSCLC patients into nonsmoking and smoking groups and found that nuclear BAI1 expression was related to patient survival in nonsmoking NSCLC patients. We suggest BAI1 expression as a predictive marker of

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nonsmoking-associated NSCLC and recommend that it be evaluated as an AJCC staging criterion in the future.

KEYWORDS
brain-specific angiogenesis inhibitor 1, non-small cell lung cancer, nonsmoking

INTRODUCTION

Lung cancer is one of the leading causes of cancer death worldwide, and its incidence is strongly related to smoke-related carcinogens. When determining lung cancer treatment, the National Comprehensive Cancer Network (NCCN) guidelines are the most reliable and are technically based on the American Joint Committee on Cancer (AJCC) and World Health Organization (WHO) classifications. According to the NCCN guidelines for NSCLC patients, smoking history is the most important patient factor in the initial assessment of NSCLC.[1] In addition, smoking-associated and nonsmoking-associated lung cancers have different mechanisms of carcinogenesis, including different signaling pathways and distinct tumor immune microenvironments. However, whether the patient smokes has not yet been specifically investigated in many studies of NSCLC tumor metabolic activity or genomic differences or correlated with TNM staging according to the AJCC.[1–3]

According to the International Agency for Research on Cancer, more than 60 out of 4000 identified chemicals in cigarette smoke are carcinogens.[4] Among the strongest and most prevalent carcinogens, nicotine-derived nitrosaminoketone (NNK) is the most important carcinogen associated with lung cancers.[5] Although nicotine itself is not a carcinogen, it is a surrogate agent and promotes uptake of other carcinogens and toxicants in cigarette smoke.[6] Nicotine addiction and persistent smoking cause unreactive carcinogens covalently bind to DNA to generate "DNA adducts". When these DNA adducts are not repaired and accumulate, they cause DNA miscoding and finally stimulate driver mutations of lung cancers and other genetic changes in the K-RAS, p53, and RB genes. In addition, cocarcinogens, inflammation, oxidative damage, and gene promotor methylation eventually inhibit apoptosis, enhance angiogenesis and promote the loss of normal growth control mechanisms, contributing to lung carcinogenesis, from mild dysplastic change to invasive carcinoma.[5,6]

In this study, we investigated brain-specific angiogenesis inhibitor 1 (BAI1) expression in NSCLC patient samples. BAI1 is a member of the adhesion type G-protein coupled receptor (GPCR) family, and has been suggested to be an engulfment receptor on macrophages and fibroblasts that takes up apoptotic cells.[7] As in cancer cells, downregulation of BAI1 mRNA has been found in many cancers, including lung adenocarcinoma,[8] primary glioma, and advanced brain tumors.[9] BAI1 appears to have an antitumor effect on malignant tumors; however, the detailed mechanism by which BAI1 regulates cancer cells has not yet been reported. In a recent study in 2020, the authors demonstrated the effectiveness of BAI1 expression in NSCLC.[3] They conducted experiments with NSCLC TMA blocks, a mouse model, and an in vitro model to reveal that negative BAI1 expression was related to poor prognosis in NSCLC patients.[3] However, they did not separate NSCLC patients into smoking and nonsmoking groups. Herein, we expanded these findings and explored the utility of BAI1 expression among nonsmoking NSCLC patients. We divided NSCLC patients into nonsmoking and smoking groups and found that nuclear BAI1 expression was related to patient survival among nonsmoking NSCLC patients.

METHODS

Case selection

Representative hematoxylin and eosin (H&E) slides from 148 consecutive patients with NSCLC were reviewed by two experienced pathologists. The patients underwent surgery for NSCLC at the Gyeongsang National University Hospital, Jinju, Korea, between January 2002 and December 2009. Electronic medical records were reviewed, and clinicopathological data, including age, sex, smoking history, histological type, TNM T stage, N stage, M stage, mean survival, and five-year survival rate, were collected (Table 1). The pathological stages of cancer were determined according to the eighth edition of the AJCC guidelines.

Tissue microarray and immunohistochemistry

Representative H&E-stained glass slides containing intratumoral lesions from the 148 NSCLC specimens were selected for analysis. To obtain the tissue sample for staining, a core was obtained from the front of the invasive tumor on each representative paraffin block and transplanted into the recipient tissue microarray (TMA) block. Immunohistochemical staining was performed on 4-μm sections of the TMA block samples. When attached to glass slides, the sections were deparaffinized, rehydrated, and incubated in 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity, which can result in non-specific background staining. Sections were then heated for 20 min in 10 mM citrate buffer (pH 6.0) in a microwave oven (700 W). After incubation with Ultra V block (Lab Vision Corporation) for 7 min at room temperature to block background staining, the slides were incubated with a primary antibody specific to BAI1 (1:200 dilution, ab135907; Abcam), and an ultraview Universal DAB detection kit was

TABLE 1 Clinicopathological information of 148 patients with pulmonary non-small cell lung carcinoma (NSCLC)

Variable	Value (%)
Age, mean (range)	64.85 (31–77)
Gender (M/F)	125/23 (84.5/15.5)
Follow-up period, mean (month)	41.56
Smoking history[a]	
Nonsmoker	50(33.8)
Smoker	97(65.5)
Surgery	
Lobectomy	130 (87.8)
Pneumonectomy	15 (10.1)
Bilobectomy or sleeve operation	3 (2.0)
TNM stage	
IA	37 (25.0)
IB	26 (17.6)
IIA	9 (6.1)
IIB	51 (34.5)
IIIA	20 (13.5)
IIIB	2 (1.4)
IVA	3 (2.0)
Adenocarcinoma	37
Acinar	15 (40.5)
Solid	6 (16.2)
Papillary	8 (21.6)
Micropapillary	3 (8.1)
Lepidic	3 (8.1)
Mucinous	2 (5.4)
Squamous cell carcinoma	96
Well	15 (15.6)
Moderately	58 (60.4)
Poorly	23 (24)
Others[b]	15
BAI1 expression[c]	
Nuclear high expression	44 (30.8)
Cytoplasmic high expression	22 (15.4)
Total number of patients	148

[a]Smoking history was unavailable in one adenocarcinoma patient.
[b]In others, eight patients had large cell neuroendocrine carcinoma and seven patients pleomorphic carcinoma and mucoepidermoid carcinoma.
[c]Specimens of five patients were unavailable because of loss of cores in tissue microarray.

used (760–500, Ventana) according to the manufacturer's recommendations for visualization. 3, 3′-diaminobenzidine was used to detect the protein. The sections were then counterstained with hematoxylin.

Evaluation of BAI1 expression

The immunohistochemical staining pattern of BAI1 was evaluated for each of 148 cores from the TMA blocks (except five cores lost during tissue processing). Distinct nuclear or cytoplasmic staining for BAI1 was considered to represent positivity. BAI1 nuclear expression was evaluated by comparison with lymphoid cells in the stroma as a positive internal control. In addition, BAI1 cytoplasmic expression was evaluated by comparison with collagenous stroma as a negative internal control. The nuclear staining intensity of BAI1 was defined as positive when nuclear expression was as stronger than that of the stromal immune cells used as the positive control and negative when nuclear expression was weaker than that of the stromal immune cells used as the negative control. The cytoplasmic staining intensity of the stained tumor cells was scored as follows: when the cytoplasm stained more weakly than the collagenous stroma, the cells were scored as negative and when the cytoplasm stained more strongly than the stroma, the cells were scored as positive.

Statistical analysis

The relationship between BAI1 expression and clinicopathological characteristics, including age, sex, smoking history, histological type, T stage, N stage, and M stage, was evaluated using Pearson's chi-square test. Disease-free survival (DFS) and disease-specific survival (DSS) were evaluated with a multivariate Cox proportional hazard regression model. In addition, DFS and DSS were analyzed by the Kaplan–Meier method with the log-rank test. *p*-values of less than 0.05 were considered statistically significant. SPSS version 24.0 (IBM) was used for all statistical analyses.

Ethics statement

Informed consent was submitted by all participants when they were enrolled. This study was approved by the Institutional Review Board of the Gyeongsang National University Hospital (GNUH-2020-04-005). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

RESULTS

Patient characteristics

A total of 148 NSCLC patients were enrolled in the study. The clinicopathological data of the NSCLC patients is summarized in Table 1. The mean age of the patients was 64.85 years. Among them, 125 (84.5%) were male patients, and 97 (65.5%) had a history of smoking. Of all the patients, 130 patients (87.8%) underwent lobectomy, while the remaining 18 patients (11.1%) underwent more invasive

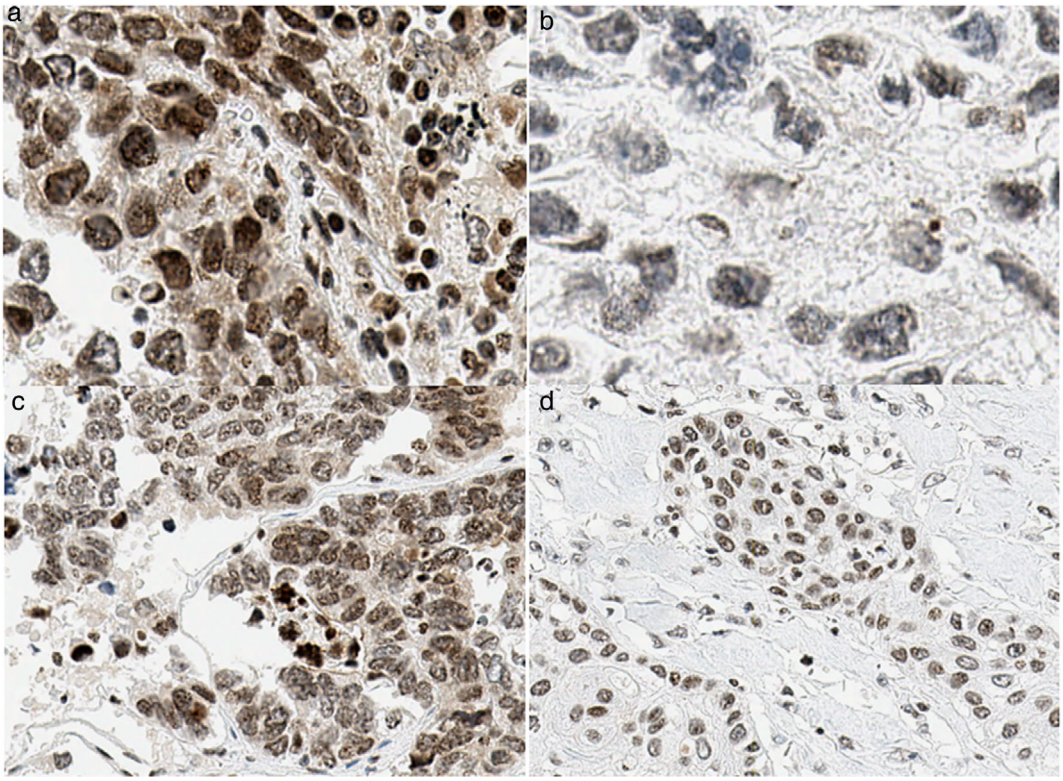

FIGURE 1 In squamous cell carcinoma, tumor cells with high BAI1 nuclear expression showed distinct nuclear intensity as high as that of stromal immune cells (a), whereas tumor cells with low BAI1 nuclear expression showed lower nuclear intensity than stromal immune cells (b). Tumor cells with high BAI1 cytoplasmic expression showed stronger cytoplasmic intensity than did the collagenous stromal matrix (c), whereas tumor cells with low BAI1 cytoplasmic expression showed weaker cytoplasmic intensity than the collagenous stroma (d)

procedures, including bilobectomy, sleeve lobectomy (3), and pneumonectomy (15). In terms of TNM stage, 63 (42.6%) samples were stage I, 60 (40.6%) were stage II, 22 (14.9%) were stage III, and three (2.0%) were stage IV. The pathological differentiation of the tumors was as follows among adenocarcinomas: acinar pattern, 15 (40.5%); solid pattern, six (16.2%); papillary pattern, eight (21.6%); micropapillary pattern, three (8.1%); lepidic pattern, three (8.1%); and mucinous pattern, two (5.4%). Among squamous cell carcinomas, 15 (15.6%) specimens were well differentiated, 58 (60.4%) were moderately differentiated, and 23 (24.0%) were poorly differentiated.

Identification of BAI1 expression

BAI1 was expressed in the nucleus and cytoplasm of NSCLC cells. A total of 44 out of 148 cores showed high nuclear BAI1 expression (30%), and 22 out of 148 cores showed high cytoplasmic BAI1 expression (15%). For both adenocarcinoma (ADC) and squamous cell carcinoma (SQCC), there were a wide variety of manifestations, from positive to aberrant or negative expression. In SQCC, tumor cells with high BAI1 nuclear expression showed distinct nuclear intensity as high as that of stromal immune cells (Figure 1(a)), whereas tumor cells with low BAI1 nuclear expression showed nuclear intensity lower than that of stromal immune cells (Figure 1(b)). Tumors cells with high BAI1 cytoplasmic expression showed stronger cytoplasmic intensity than did the collagenous stromal matrix (Figure 1(c)), whereas tumor cells with low BAI1 cytoplasmic expression showed weaker cytoplasmic intensity than did the collagenous stroma (Figure 1(d)). In ADC, tumor cells with high and low BAI1 expression showed the same nuclear and cytoplasmic expression patterns observed for SCC (Figure 2(a): nuclear high, 2(b): nuclear low, 2(c): cytoplasmic high 2(d): cytoplasmic low).

Correlations between BAI1 expression and clinicopathological characteristics and survival data

Among the investigated clinical and pathological factors (age, sex, smoking, histological type, and pathological TNM stage), there were no factors that showed a statistically significant relationship with the nuclear or cytoplasmic expression of BAI1 by Pearson's chi-square test. (Table 2). To confirm BAI1 expression as an independent prognostic marker, multivariate Cox proportional analyses were performed. Male NSCLC patients had poor DSS (hazard ratio = 0.324, 95% confidence interval = 0.116–0.911,

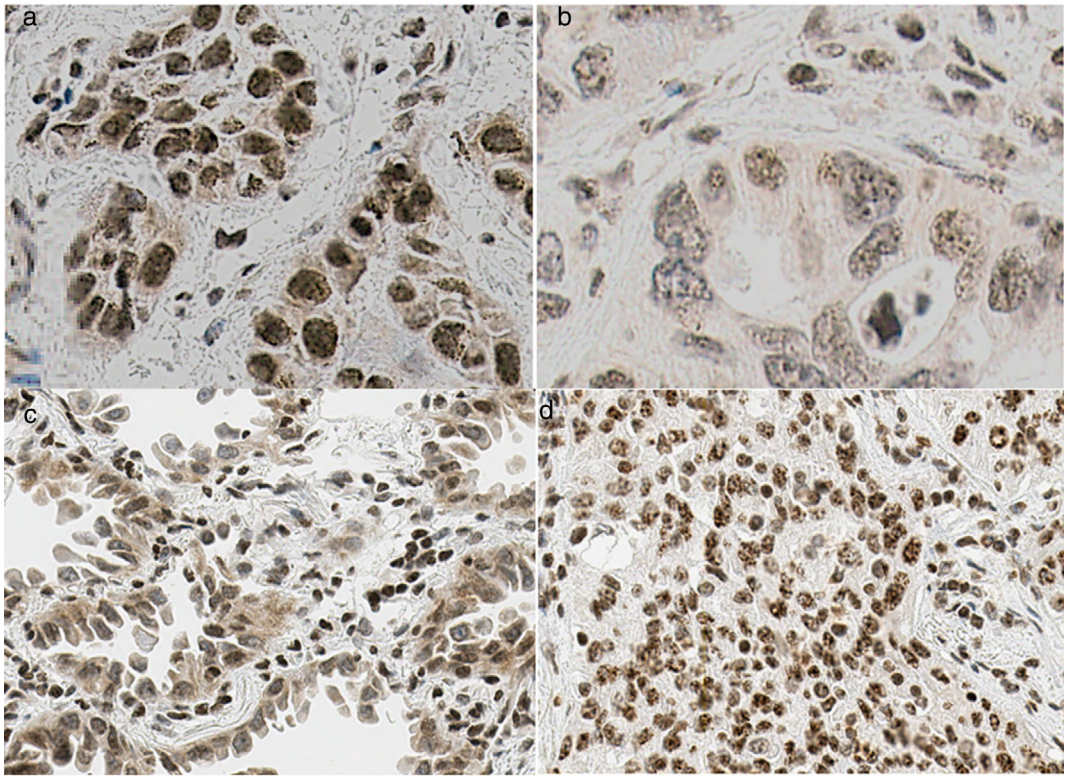

FIGURE 2 (a) In adenocarcinoma, tumor cells with high BAI1 nuclear expression showed strong nuclear expression as high as that of stromal immune cells. (b) Tumor cells with low BAI1 nuclear expression showed lower nuclear intensity than stromal immune cells. (c) Tumor cells with high BAI1 cytoplasmic expression showed stronger cytoplasmic expression than did the collagenous stromal matrix. (d) Tumor cells with low BAI1 cytoplasmic expression showed weaker cytoplasmic intensity than did of the collagenous stroma

TABLE 2 Correlations among clinical factors and BAI1 expression

	Cytoplasmic expression of BAI1			Nuclear expression of BAI1		
Variables	**Low[a]**	**High**	***p***	**Low**	**High[b]**	***p***
Age (years)			0.295			0.366
<65	53	7		44	16	
≥65	68	15		55	28	
Gender			0.110			0.699
Male	105	16		83	38	
Female	16	6		16	6	
Smoking[c]			0.106			0.405
Nonsmoker	45	4		36	13	
Smoker	76	17		62	31	
TNM stage			1.000			0.765
≤II	100	19		83	36	
≥III	21	3		16	8	
Pattern of ADC			0.396			1.000
Solid and micropapillary	8	1		7	2	
Others[d]	19	8		22	5	
Differentiation of Sqcc			0.261			0.293
Well, moderately	65	7		46	26	
Poorly differentiated	17	4		16	5	

Abbreviations: ADC, adenocarcinoma; *p*, *p*-value; Sqcc, squamous cell carcinoma.
[a]This group contains cases with BAI1 cytoplasmic expression of tumor cells, not more than collagenous stroma.
[b]This group contains cases with BAI1 nuclear expression of tumor cells, not less than stromal immune cells.
[c]Smoking history was unavailable in one adenocarcinoma patient. Specimens of five patients were unavailable because of loss of cores in tissue microarray.
[d]Others includes patients with acinar, papillary, lepidic, or mucinous patterns.

TABLE 3 Cox proportional hazards multivariate analysis of albumin expression in 148 pulmonary non-small cell lung cancer (NSCLC) patients

	NSCLC (148)[a]				Nonsmoker (50)				Smoker (97)[b]			
	DFS		DSS		DFS		DSS		DFS		DSS	
Variables	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age (years) (<65 vs. ≥65)	1.355 (0.831–2.209)	0.224	1.175 (0.695–1.986)	0.548	1.384 (0.591–3.238)	0.454	1.218 (0.462–3.214)	0.690	1.311 (0.689–2.496)	0.409	1.090 (0.554–2.144)	0.802
Gender (Male vs. Female)	0.555 (0.250–1.230)	0.147	**0.324** (0.116–0.911)	**0.033**	0.376 (0.113–1.250)	0.110	0.338 (0.087–1.315)	0.118	0.598 (0.138–2.597)	0.492	0.223 (0.029–1.694)	0.147
Operation (lob vs. more[a])	1.271 (0.664–2.436)	0.469	1.243 (0.618–2.503)	0.542	1.384 (0.435–4.400)	0.582	2.327 (0.698–7.759)	0.169	1.342 (0.603–2.987)	0.471	1.004 (0.413–2.442)	0.994
TNM stage (≤II vs. ≥III)	**2.216** (1.275–3.851)	**0.005**	**1.905** (1.036–3.504)	**0.038**	1.828 (0.686–4.875)	0.228	1.378 (0.438–4.329)	0.584	**2.840** (1.376–5.851)	**0.005**	**3.013** (1.429–6.352)	**0.004**
BAI1 nuclei (low vs. high)	0.898 (0.537–1.504)	0.684	1.018 (0.587–1.764)	0.950	1.976 (0.846–4.618)	0.116	**2.679** (1.022–7.022)	**0.045**	0.592 (0.300–1.169)	0.131	0.636 (0.313–1.289)	0.209
BAI1 cyto (low vs. high)	1.319 (0.700–2.484)	0.392	1.601 (0.818–3.134)	0.169	2.329 (0.399–13.488)	0.349	1.803 (0.329–9.869)	0.497	1.562 (0.724–3.374)	0.256	2.175 (0.983–4.811)	0.055

Note: Bold values indicate that the *P*-values of less than 0.05 were considered statistically significant.

Abbreviations: CI, confidence interval; cyto, cytoplasmic expression; DFS, disease-free survival; DSS, disease-specific survival; HR, hazard ratio; lob, lobectomy; more, bilobectomy or sleeve lobectomy or pneumonectomy; nuclei, nuclear expression.

[a]Smoking history was unavailable in one adenocarcinoma patient.

[b]Smoker means patients with a smoking history.

FIGURE 3 The Kaplan–Meier survival curve confirmed that high BAI1 nuclear expression was significantly associated with poor DSS ($p = 0.034$). low; high; low-censored; high-censored

$p = 0.033$), and NSCLC patients with a higher TNM stage (≥III) had poor DFS (hazard ratio = 2.216, 95% confidence interval = 1.275–3.851, $p = 0.005$) and poor DSS (hazard ratio = 1.905, 95% confidence interval = 1.036–3.504, $p = 0.038$). NSCLC smokers with a higher TNM stage (≥III) had poor DFS (hazard ratio = 2.840, 95% confidence interval = 1.376–5.851, $p = 0.005$) and poor DSS (hazard ratio = 3.013, 95% confidence interval = 1.429–6.352, $p = 0.004$). NSCLC nonsmokers with high BAI1 nuclear expression had poor DSS (hazard ratio = 2.679, 95% confidence interval = 1.022–7.022, $p = 0.045$) (Table 3). The Kaplan–Meier survival curve confirmed that high BAI1 nuclear expression was significantly associated with poor DSS ($p = 0.034$) (Figure 3).

DISCUSSION

To date, the most credible source for determining the Lung Cancer Treatment Guidelines is the National Comprehensive Cancer Network (NCCN). According to the NCCN guidelines from 2018, when incidentally suspicious nodules for lung cancer are found, the first thing to do with a multidisciplinary evaluation is smoking cessation counseling.[1] After initial evaluation, including pathological reviews, the most important factors for clinical staging for lung cancer treatment are dependent on the AJCC pathological TNM stage. However, smoking and nonsmoking groups have not been considered for separate classification in the TNM staging system from the AJCC so far.[1,2] Lung cancer can be divided into various categories, and smoking is the most representative. Since nicotine addiction can supply various kinds of carcinogens either directly or indirectly, many

studies have shown that lung cancer has different mechanisms depending on whether the patients are smokers. Smoking can cause lung cancer in three ways: (i) some tobacco smoke components directly bind to cellular receptors, which leads to activation of Akt, PKA and other pathways that contribute to the tumorigenic process; (ii) metabolically activated genotoxic carcinogens bind to DNA adducts and cause *KRAS* and P53 mutations; and (iii) cocarcinogens, tumor promotors, inflammatory agents, oxidative damage, and gene promotor methylation lead to tumor suppressor gene inactivation.[6] In smokers, the role of immune cells is largely influenced by the tumor microenvironment, even though many other downstream signaling pathways can be involved.[10] Among many immune cells, the activation of mast cells and CD4+ memory T cells plays an important role in smoking-induced immune dysfunction in the lung, which contributes to tumor progression. By contrast, the prognosis has been reported to be even better for nonsmokers.[10] NSCLC in nonsmokers may be a distinct tumor entity featuring a different tumor biology and tumor microenvironment than smoking-associated lung carcinomas. From an epidemiological perspective, other risk factors, such as germline mutations, may play a role, as well as metabolic syndromes that lead to lung cancer formation in certain individuals independent of exposure to carcinogens and personal lifestyle.[11] Since there have been few studies distinguishing the smoking and nonsmoking groups in the actual TMA, in our study, the aim was to further elucidate BAI1 expression to examine survival in each group of NSCLC patients and predict the effectiveness of BAI1 as a treatment. This study will be the first to explore the utility of BAI1 nuclear expression separately in smoking and nonsmoking groups of NSCLC patients.

Brain-specific angiogenesis inhibitor 1 (BAI1) is a member of the adhesion type G-protein coupled receptor (GPCR) family, and it has been suggested to be an engulfment receptor.[7] It is a 7-transmembrane protein that has both an extracellular domain and intracellular domains.[7] BAI1 has been proven to be an engulfment receptor of phagocytes (macrophages and fibroblasts) and to enhance the uptake of apoptotic cells.[7] The extracellular domain of the receptor is combined with phosphatidylserine, which sends an eat-me signal to phagocytes via a ligand on top of apoptotic cells. The intracellular domain mediates apoptotic cell uptake via direct coupling with the Rac-guanine nucleotide exchange factor (GEF) complex consisting of ELMO, Dock180, and Rac GTPase.[7,12] An important point is that uptake does not occur when any one of the cytoplasmic domains of BAI1 or extracellular domains is missing. In contrast to this study, other authors have insisted that professional macrophages do not express the phagocytic receptor BAI1/ADGRB1.[13] Similarly, colonic epithelial cells rather than professional phagocytes overexpressing BAI1 boosted apoptotic cell clearance to attenuate inflammation in vivo. BAI1 contributes as a regulator of severe inflammation by removing apoptotic debris and proinflammatory cytokines within colonic epithelial cells[14] These opposing studies motivated us to show that BAI1-mediated uptake of apoptotic cells by epithelial cells or cancer cells may open a new path for evaluating carcinogenesis mechanisms in NSCLC.

The Warberg effect is well known for its aerobic glycolysis that occurs in either malignant or benign fast-growing cells via uptake of glucose and glutamine. This not only creates a small amount of energy by producing lactate acid but also produces new fats, proteins, and nucleic acids, which in addition to cellular energy maintenance, cause cell proliferation.[15] Although BAI1 has been revealed as an angiogenesis inhibitor in several types of cancer cells,[3,8,16] particularly in lung cancers, BAI1 is involved in metabolic reprogramming and inhibits angiogenesis in the SCD1/HMGCR module.[3] Metabolic reprogramming has been previously defined as the opposite concept of the Warberg effect, and the Warberg effect was reduced when BAI1 was overexpressed.[3] In this study, we evaluated BAI1 nuclear expression and cytoplasmic expression in NSCLC cancer cells. Using NSCLC cells is thought to be suitable for evaluating the effects of phagocytosis-mediated boosting of apoptotic cell clearance or metabolic reprogramming of tumor epithelial cells. In our study, NSCLC nonsmokers with higher BAI1 nuclear expression had poor DSS (hazard ratio = 2.679, 95% confidence interval = 1.022–7.022, $p = 0.045$) (Table 3). The Kaplan–Meier survival curve confirmed that higher BAI1 expression was significantly associated with poor DSS ($p = 0.034$) in the nonsmoking group (Figure 3). This result is the opposite of that reported by Liu et al.[3] This might be due to dividing NSCLC patients into two separate groups in our study: smoking and nonsmoking groups. We suggest that BAI1 may not act as a tumor suppressor in the nonsmoking group of NSCLC patients and that there may be an opposite direction of pathway activation during the induction of carcinogenesis, that is, the Warberg effect rather than metabolic reprogramming.

In our sample, distinct nuclear expression of BAI1 was found in most of the cancer cells and was highly associated with survival in the nonsmoking group in contrast to the NSCLC total and smoking groups. In the smoking group, immune cells accumulated toward bronchial epithelial cells, where they were highly activated by smoke-induced carcinogens. These immune cells run from one extreme to the other, in that they either minimize the damage to bronchial epithelial cells or weaken the bronchial epithelial cells via a harmful proinflammatory immune response. When lung cancer arises, this harmful tumor microenvironment contributes to tumor growth, tumor invasion and metastatic spread to distant organs.[11] Unlike the smoking group, in the nonsmoking group, immune cells, including mast cells and CD4+ immune T cells, were in a resting state and less influenced by chemokines and chemokine receptors, resulting in a much better prognosis in terms of immune reactions.[10] We hypothesized that in nonsmoking NSCLC patients with high BAI1 nuclear expression, apoptotic cell clearance must have been increased and inflammatory cytokine expression must have been decreased.[14] Thus, the decrease in the harmful proinflammatory immune response must have been maximized, resulting in a better prognosis for nonsmoking NSCLC patients.[11]

Another important finding was that the NSCLC total group and the NSCLC smoking group were thoroughly matched with the AJCC TNM staging system. However, the nonsmoking group tended to have no association with TNM staging. Under these circumstances, it is very encouraging that BAI1 has emerged as a meaningful marker correlated with survival in a nonsmoking group. According to the American Joint of Cancer Committee (AJCC), the smoking and nonsmoking groups have so far not been divided.[2] It is expected that in any population, the ratio of smokers to nonsmokers among NSCLC patients will be similar (smokers will have a far larger population than nonsmokers, up to 85%). Thus, the TNM stage of NSCLC might well reflect the NSCLC total group and the smoking group, but it may be obscured by the small number in the nonsmoking group, as in our study. Tumors of nonsmoking patients are distinct form of lung cancers in terms of molecular pathology, prognosis, and response to treatment. Personalized medicine, molecular assessment and targeted therapy options are needed. From a part of these observations, we suggest that BAI1 expression should become a predictive marker of the nonsmoking group of NSCLC and should be evaluated as an AJCC staging criterion in the future, just as the head and neck guidelines divided P16-positive and P16-negative cancers.[17] P16 is a tumor suppressor gene that controls cell cycle progression and has been studied since it is associated with human papillomavirus (HPV) infection in the induction of many types of cancer; one of the authors applied a new cutoff value for P16 expression in NSCLC.[18] Since BAI1 expression is associated with nonsmoking NSCLC patient survival and is more effective than currently established AJCC TNM staging guidelines, we suggest dividing smoking and nonsmoking groups differently than recommended in the AJCC criteria and applying BAI1 expression to evaluate patient survival. The limitation of this study is the small number of cases and larger case studies should be considered in the future.

In conclusion, we divided NSCLC patients into nonsmoking and smoking groups and found that nuclear BAI1 expression was related to patient survival in nonsmoking NSCLC patients. BAI1 expression is expected to be a marker to reveal the mechanism of carcinogenesis in the nonsmoking group of NSCLC and to be applied in an AJCC staging criterion in the future.

ACKNOWLEDGMENTS

The author(s) received no financial support for the research, authorship, and/or publication of this article.

CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

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How to cite this article: An HJ, Kim SH, Yang JW, et al. BAI1 nuclear expression reflects the survival of nonsmoking non-small cell lung cancer patients. *Thorac Cancer*. 2021;12:1673–1680. https://doi.org/10.1111/1759-7714.13985