ORIGINAL RESEARCH

Clinical implications of transforming growth factor-beta–induced gene-h3 protein expression in lung cancer

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Aim: The clinical implications of transforming growth factor-beta–induced gene-h3 (beta-IGH3) protein expression in lung cancer remain unclear. This study investigated beta-IGH3 protein expression levels and biological function, as well as lung cancer prognosis.

Methods: Beta-IGH3 protein expression levels were measured in 236 lung cancers and were matched with adjacent noncancerous tissues by immunohistochemical staining. Subsequently, the relationship between beta-IGH3 protein expression, clinical–pathological parameters, and lung cancer prognosis was evaluated.

Results: Beta-IGH3 protein expression was significantly higher in lung cancer tissues compared with adjacent noncancerous tissues (61.86% vs 22.88%; P=0.01). Of the 236 enrolled cases, 146 (61.86%) showed high beta-IGH3 levels. Tumor size, clinical stage, and lymph node metastasis were significantly related to beta-IGH3 protein expression in univariate analysis (P=0.001, 0.044, and 0.029, respectively), whereas age, sex, and histological type were not (P=0.038, 0.756, and 0.889, respectively). Finally, a Cox regression model also identified beta-IGH3 as an independent prognostic factor (P=0.01).

Conclusion: Beta-IGH3 is highly expressed in lung cancers and may be a potential target for lung cancer treatments.

Keywords: lung cancer, beta-IGH3 protein, lymph node, metastasis, prognosis

Introduction

The primary types of lung cancer include small-cell lung carcinoma and non-small-cell lung carcinoma.[1,2] Despite development of various treatments including surgery, radiotherapy, chemotherapy, molecular targeted therapy, and other biological agent therapies, recurrence and metastasis still account for most cancer-related deaths.[3] In 1992, Skonier et al[4] first cloned and characterized transforming growth factor-beta (TGF-beta)–induced gene-h3 (beta-IGH3). They built a complementary DNA (cDNA) library from messenger RNA (mRNA) isolated from a human lung adenocarcinoma cell line (A549) that had been treated for 3 days with TGF-beta. Beta-IGH3 was isolated after the library was screened by differential hybridization of a cDNA clone.[4] Beta-IGH3 RNA has been detected in several cell lines and tissues, and it may be involved in mediating the signals of this multifunctional growth modulator.[4,5]

Sasaki et al[6] evaluated the clinical significance and biological function of beta-IGH3 in lung cancer. These reverse transcription polymerase chain reaction experiments measured beta-IGH3 expression in 71 lung cancer specimens, demonstrating that beta-IGH3 was highly expressed in lung cancers.[6] However, until now, no studies have assessed the clinical implications of beta-IGH3 expression in lung cancer.

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Therefore, this study investigated beta-IGH3 expression, its clinical implications, and prognosis in order to facilitate lung cancer management.

Methods
Lung tissue specimens
Matched benign and malignant lung tissues for immunohistochemical staining were obtained from patients undergoing surgical treatment at Harbin University from January 2001 to January 2005. The stages of lung cancer were determined according to the International Association for the Study of Lung Cancer criteria.[7] All the patients were female, with a mean age of 60.68 years (range 35–79 years). Written informed consent was obtained from all of the individual patients, and the experimental protocol was approved by the Ethics Committee of Harbin Medical University.

Immunohistochemical staining
Lung cancer tissue samples were fixed in 10% neutralized formalin (pH 7.0) and paraffin embedded. The paraffin-embedded tissue sections (4 µm) were dewaxed, rehydrated, and treated with 3% H_2O_2 in methanol, followed by overnight incubation with primary antibodies against beta-IGH3 (Catalog number: 10188-1-AP; Proteintech Group, Inc., Rosemont, IL, USA). Subsequently, tissue sections were incubated with multi-link biotinylated swine anti-goat/mouse/rabbit immunoglobulin G (Dako, Carpinteria, CA, USA). After washing, bound antibodies were detected with horseradish peroxidase–conjugated avidin-biotin conjugates (1:1000 dilution; Vector Laboratories, Burlingame, CA, USA), and visualized using 3,3-diaminobenzidine. The sections were then counterstained with Gill's hematoxylin.[8]

The relative levels of beta-IGH3 expression were evaluated by semiquantification. Briefly, two blinded investigators evaluated the intensities of positive anti-beta-IGH3 staining in ten randomly selected high-power fields (magnification ×400). The percentage of stained tumor cells in a given field was scored as 0 (no stained cells), 1 (up to 10% of cells stained), 2 (10%–50% of cells stained), or 3 (over 50% of cells stained). The staining intensity of a given field was scored as 0 (no staining), 1 (weak staining, appearing as light yellow), 2 (moderate staining, appearing as yellowish-brown), or 3 (strong staining, appearing as brown). The staining index of individual sections was calculated as the average staining intensity score multiplied by the score of the proportion of stained cells. The cut-off value for anti-beta-IGH3 staining was determined by measuring heterogeneity. Accordingly, a staining index of 4 (the cut-off value) was used to distinguish between negative (<4) and positive (≥4) beta-IGH3 expressions.

Statistical analysis
All the analyses were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). The data are presented as mean ± standard error of the mean. The difference between beta-IGH3 expressions in tumor and normal tissues was determined using unpaired Student's t-tests. The correlation between beta-IGH3 expression and clinical–pathological characteristics was analyzed using chi-square tests and Spearman's correlation analyses. Disease-free and overall survivals were estimated using the Kaplan–Meier method and Cox regression analyses. $P<0.05$ was considered significant.

Results
Beta-IGH3 expression in lung cancer
The clinical–pathological factors of the 236 lung cancer patients are shown in Table 1. Immunohistochemical staining was used to quantify the expression of beta-IGH3 in each lung cancer clinical specimen. Two independent pathologists validated the beta-IGH3 expression scoring, and beta-IGH3 protein was identified in the cytoplasm and membranes of lung cancer cells. Beta-IGH3 expression was significantly higher in lung cancer tissues compared with that of adjacent noncancerous tissues (61.86% vs 22.88%; $P=0.01$; Figure 1).

Relationship between beta-IGH3 protein expression and clinical–pathological characteristics
Of the 236 enrolled cases, 146 (61.86%) showed high beta-IGH3 expression. Tumor size, clinical stage, and lymph node metastasis were significantly related to beta-IGH3 protein expression in universal analysis ($P=0.001$, 0.044, and 0.029, respectively), whereas age, sex, and histological type were not ($P=0.038$, 0.756, and 0.889, respectively). Multiple logistic analyses were performed to exclude the influence of confounding factors. Finally, tumor size, clinical stage, and lymph node metastasis were identified as factors significantly related to beta-IGH3 expression ($P=0.01$, 0.001, and 0.001, respectively).

Prognosis analysis
In this study, cases that expressed beta-IGH3 protein tended to have poor prognosis. Compared with 36 (40.00%) patients with no detectable beta-IGH3 expression, 86 (58.90%) of

Table 1 The relationship between beta-IGH3 expression and the clinical–pathological factors of lung cancer (n=236)

Variables	Patients, n	Beta-IGH3 positive, n (%)	X^2	P-value
Age			0.771	0.380
<60 years	81	47 (58.02)		
≥60 years	155	99 (63.87)		
Sex			0.097	0.756
Male	173	106 (61.27)		
Female	63	40 (63.49)		
Tumor size			60.665	0.001
T1	57	12 (21.05)		
T2	87	58 (66.67)		
T3	41	30 (73.17)		
T4	51	46 (90.20)		
Histological type			0.236	0.889
Adenocarcinoma	153	95 (62.09)		
Squamous cell carcinoma	57	36 (63.16)		
Others	26	15 (57.69)		
Clinical stage			8.1113	0.044
I	96	50 (53.13)		
II	48	29 (60.42)		
III	39	27 (69.23)		
IV	53	40 (73.58)		
Lymph node metastasis			4.478	0.029
Negative	161	92 (57.14)		
Positive	75	54 (72.00)		

Abbreviation: Beta-IGH3, transforming growth factor-beta–induced gene-h3.

146 patients with samples positive for beta-IGH3 protein expression had postoperative distant metastasis (P=0.001). Survival analysis revealed that high beta-IGH3 protein expression was significantly associated with poorer postoperative disease-specific survival than those patients without beta-IGH3 protein expression (P=0.01; Figure 2). Finally, a Cox regression model also identified beta-IGH3 protein as an independent prognostic factor (P=0.01; Table 2).

Discussion

Lung cancer is one of the most common malignant tumors worldwide.[9,10] Although current antitumor therapies have greatly improved the postoperative prognosis of patients with lung cancer, local recurrence and distant metastasis of lung cancer after surgical resection of the primary tumor are often incurable, which leads to patient mortality.[11] The mechanisms underlying the metastasis of lung cancer are
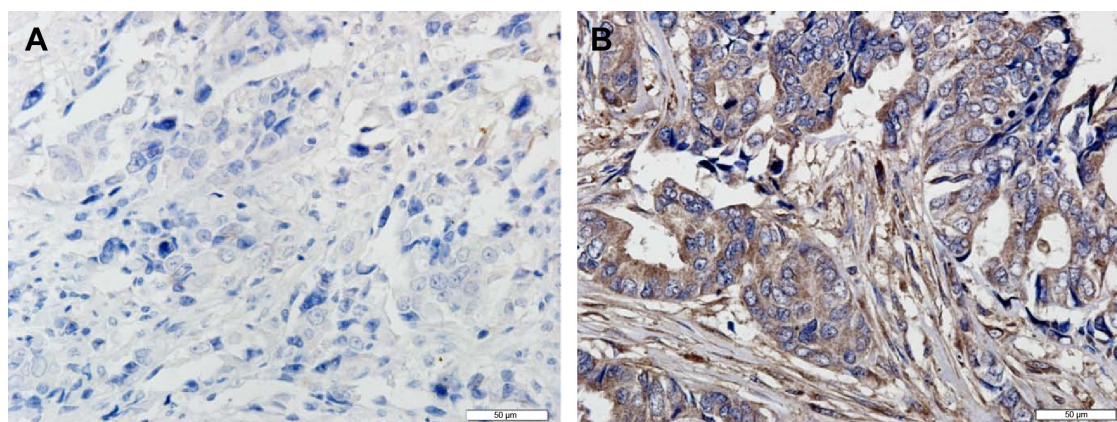
Figure 1 Immunohistochemical assessment of beta-IGH3 expression in lung cancer specimens.
Notes: (**A**) Negative anti-beta-IGH3 staining in lymph node-negative cases (magnification ×400). (**B**) Strong positive anti-beta-IGH3 staining in lymph node-positive tumors (magnification ×400).
Abbreviation: Beta-IGH3, transforming growth factor-beta–induced gene-h3.
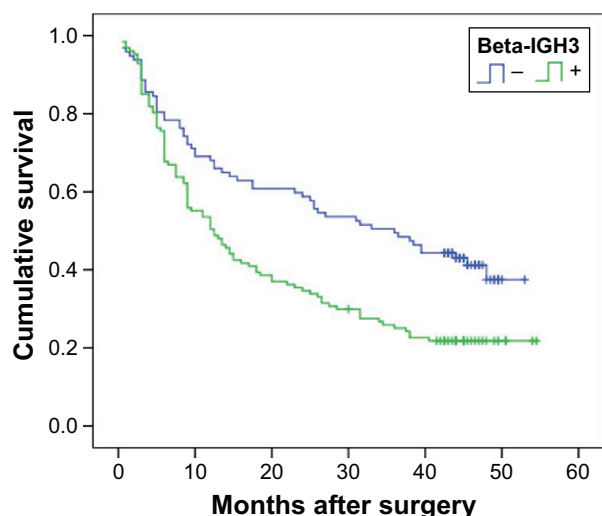
Figure 2 Kaplan–Meier curves of overall survival revealed beta-IGH3 expression to be an independent prognostic factor in Cox regression analysis (P=0.01).
Abbreviations: −, negative; +, positive; Beta-IGH3, transforming growth factor-beta–induced gene-h3.

not fully understood.[12] Therefore, development of new screening markers for early diagnosis, prognostic factors, and potential therapeutic targets for lung cancer would be of great significance.[13]

In 2007, Kim et al[14] generated Alb-hss igh3 transgenic mice with liver-specific expression of human beta-IGH3 (hss igh3) under the control of the albumin (Alb) enhancer/promoter in order to investigate the influence of beta-IGH3 overexpression in the mouse eye. They concluded that beta-IGH3 may be involved in anterior segment morphogenesis and eye development in mice. In addition, this indicates that the level of normal beta-IGH3 expression must be properly maintained during ocular development. In another study, beta-IGH3 was reported to be induced by TGF-beta and to be essential for cell adhesion; it is downregulated in asbestos-induced tumorigenic human bronchial epithelial cells. Ectopic expression of the beta-IGH3 gene abrogates the tumorigenic phenotype, suggesting that the gene plays a causal role in fiber carcinogenesis.[15,16]

Table 2 Cox model regression analysis of the lung cancer prognostic factors

Variables	OR	95% CI for OR	P-value
Age	1.184	0.720–1.524	0.573
Sex	1.535	0.634–2.067	0.462
Tumor size	2.149	1.678–3.341	0.032
Histological type	1.022	0.832–1.632	0.820
Clinical stage	3.187	2.536–4.256	0.001
Lymph node metastasis	2.360	1.725–3.811	0.001
Beta-IGH3	2.173	1.594–3.507	0.010

Abbreviations: Beta-IGH3, transforming growth factor-beta–induced gene-h3; CI, confidence interval; OR, odds ratio.

One previous study hypothesized that beta-IGH3 mRNA levels could predict the development and invasion of lung cancer.[6] The authors quantified beta-IGH3 mRNA levels in 71 lung cancer samples by reverse transcription polymerase chain reaction. However, Sasaki et al[6] did not observe any significant differences in beta-IGH3 mRNA levels according to sex, age, pathological subtype, and lymph node metastasis. However, beta-IGH3 mRNA levels were elevated in stage T1–T4 lung cancer specimens (P=0.044).[6] These findings suggest that beta-IGH3 mRNA levels might be a potential marker of lung cancer aggressiveness. However, no study has investigated the relationship among beta-IGH3 protein expression, biological behavior, and prognosis in lung cancer.

The current study investigated beta-IGH3 expression in lung cancer. The protein was highly expressed in cancer tissues, but not expressed or expressed at a low level in paraneoplastic tissues. Multiple analyses revealed that tumor size, clinical stage, and lymph node metastasis were significantly associated with beta-IGH3 protein expression. Moreover, cases with high beta-IGH3 expression in the current study tended to develop distant metastasis. There were some differences between our study and the one by Sasaki et al.[6] For example, Sasaki et al[6] did not observe a significant relationship between beta-IGH3 expression and lymph node metastasis. These differences in findings might be due to differences in the detection methods and samples. First, Sasaki et al enrolled only 71 lung cancer samples, but the current study included postoperative prognosis analysis. However, the potential mechanism by which beta-IGH3 regulates the metastasis of lung cancer remains unclear.

Beta-IGH3 gene expression is reportedly increased in most highly invasive breast cancer cell lines, but not in weakly invasive cell lines.[17] Beta-IGH3 mRNA levels are positively correlated with T-status in lung cancers.[6] These data suggest that beta-IGH3 plays a role in cancer progression and invasion. Beta-IGH3 is induced by TGF-beta in H2981 (a lung adenocarcinoma cell line) and human embryonic palatal mesenchyme cells and thus may affect tumor cell proliferation and invasion as a middle player of the TGF-beta signaling pathway.[18] These findings indicate that beta-IGH3 protein may be an oncogene and a potential therapy target to inhibit the invasion and metastasis of lung cancer.

Conclusion

The results of this study showed that beta-IGH3 was overexpressed in lung cancer tissue specimens and the overexpression was significantly related to lymph node and distant

metastasis, suggesting that beta-IGH3 may be a potential biomarker to target lung tumors. However, the relationship between beta-IGH3 expression and lung cancer stem cells requires further investigation.

Acknowledgment

Disclosure

The authors report no conflicts of interest in this work.

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