Thoracic Cancer

Open Access

Thoracic Cancer ISSN 1759-7706

ORIGINAL ARTICLE

Clinical significance of calcium-binding protein S100A8 and S100A9 expression in non-small cell lung cancer

He Huang, Qingdong Huang, Tingyu Tang, Liang Gu, Jianzong Du, Zhijun Li, Xiaoling Lu & Xiaoxi Zhou

Respiratory Department, Zhejiang Hospital, Hangzhou, China

Keywords
Clinical characteristics; non-small cell lung cancer; S100.

Correspondence
Xiaoxi Zhou, Department of Respiratory, Zhejiang Hospital, No. 12 Lingyin Road, Hangzhou City, Zhejiang Province 310013, China.
Tel: +86 571 8798 7373
Fax: +86 571 8798 7372
Email: riverjinbo@126.com

Received: 3 March 2018;
Accepted: 1 April 2018.

doi: 10.1111/1759-7714.12649

Thoracic Cancer **9** (2018) 800–804

Abstract

Background: The purpose of this study was to evaluate the correlation between calcium-binding protein S100A8 and S100A9 expression in non-small cell lung cancer (NSCLC) and patients' clinical features.

Methods: Fifty-two NSCLC patients who underwent surgery at Zhejiang Hospital from February 2014 to January 2016 were included in this study. Calcium-binding protein S100A8 and S100A9 expression patterns in cancer and para-cancer tissues were examined by immunohistochemistry assay. The correlation between calcium-binding protein S100A8 and S100A9 expression patterns and NSCLC patients' clinical characteristics, including age, gender, tumor node metastasis stage, and pathology type, were evaluated.

Results: S100A8 and S100A9 were generally expressed on the cytoplasm and nucleus of NSCLC cells, mainly located in the cytoplasm, stained with brown particles, and distributed evenly. The positive expression rates of S100A8 and S100A9 in cancer tissues were 71.2% and 76.9%, respectively, which were significantly higher than in para-cancer tissues at 11.5% and 19.2%, respectively, with statistical significance ($P < 0.05$). S100A8 and S100A9 positive expression was associated with tumor differentiation degree ($P < 0.05$) but were not correlated with age, gender, smoking history, tumor diameter, pathology type, tumor node metastasis stage, or pleural effusion ($P_{all} > 0.05$).

Conclusion: S100A8 and S100A9 positive expression in cancer tissues was significantly higher than in para-cancer tissues and was correlated with tumor differentiation, which may be a potential marker for poor prognosis.

Introduction

Lung cancer, one of the leading causes of cancer-related death globally, is a significant malignancy that threatens human health.[1] Prognosis is generally poor, with low five-year survival rates, partly because effective lung cancer screening and early diagnostic methods are lacking.[2] Many published studies have evaluated the dependent factors related to lung cancer prognosis.[3,4] Calcium-binding proteins S100A8 and S100A9, members of the S100 family of proteins, contain 2 EF hand calcium-binding motifs. S100 genes include at least 13 members, which are located as a cluster on chromosome 1q21.[5] This protein may inhibit the casein kinase. In this region, chromosome deletion, translocation, and overlap often occur and stability is poor, factors that are closely related to the occurrence and development of several cancers, including gastric,[6] pancreatic,[7] liver, and ovarian cancers. However, the correlation between calcium-binding protein S100A8 and S100A9 expression in non-small cell lung cancer (NSCLC) and patients' clinical features is seldom reported. In our study, we examine S100A8 and S100A9 protein expression in NSCLC and para-cancer tissues and evaluate their correlation with patients' clinical characteristics.

Methods

Patients

Fifty-two NSCLC patients who underwent surgery at Zhejiang Hospital from February 2014 to January 2016 were

included in this study. S100A8 and S100A9 expression was analyzed via immunohistochemistry assay. The inclusion criteria were: NSCLC diagnosis confirmed via postoperative pathology, no preoperative chemoradiation or biotherapy performed, availability of complete follow-up data, and informed patient consent for the use of tissue specimens. The exclusion criteria were: small cell lung cancer cases, patients who received neoadjuvant chemoradiotherapy, malignant tumors in other systems, and incomplete data.

Instruments and reagents

An Ultra-thin Semiautomatic Microtome (Leica Inc. Co. Ltd., Wetzlar, Germany), an electron microscope (Olympus Co. Ltd., Tokyo, Japan), an electrothermal thermostat (Rong Ke Inspection Instrument Co. Ltd., Shanghai, China), a centrifuge (Beckman Coulter, Fullerton, CA, USA), S100A8 and S100A9 rabbit monoclonal antibodies (Abcam, Cambridge, UK), and an immunohistochemical kit (Bioengineering Co. Ltd., Shanghai, China) were purchased for the study.

Immunohistochemistry assay

Paraffin specimens were sliced continuously to obtain a thickness of 3 μm. The paraffin tissue sections were dewaxed and dehydrated. Endogenous peroxidase was eliminated and heat antigen repair was performed. Normal goat serum was used to close the tissue non-specific antigen for 30 minutes. Specific anti-S100A8 or S100A9 rabbit monoclonal antibodies were added at 4°C overnight. Phosphate buffered saline and drops of horseradish peroxidase-labeled goat anti-rabbit immunoglobulin G at 37°C were added the following day. After the specimens were incubated for 30 minutes, DAB color development, hematoxylin complex dyeing, dehydration, transparency, and sealing film were applied or performed.[8] For the negative control, normal goat serum was used instead of the antibody.

Positive criteria

Two individuals blinded to patient information and the experimental purpose analyzed the film and the same pieces of tissues under a microscope. After the hematoxylin and eosin-stained tissue section was identified as tumor tissue, immunohistochemically-stained sections were observed and counted. Brown-yellow granules in the cytoplasm, nucleus, or cell membrane of the tumor cells were considered positive cells. Each slice was randomly selected from 10 high-magnification fields (400×), with at least 1000 cells per magnification. Scores were based on the degree of staining strength of the slices: 0 for no staining, 1 for light yellow, 2 for tawny, and 3 for brown. The rate of positive cells in the slices was scored as follows: 0 for < 5% positive cells, 1 for 5–25% positive cells, 2 for 25–50% positive cells, 3 for 50–75% positive cells, and 4 for > 75% positive cells. The scores of the degrees of staining strength and the rates of positive cells were added to obtain the total score, with a minimum value of 0 and a maximum value of 7. The total scores were interpreted as follows: < 2, "–";

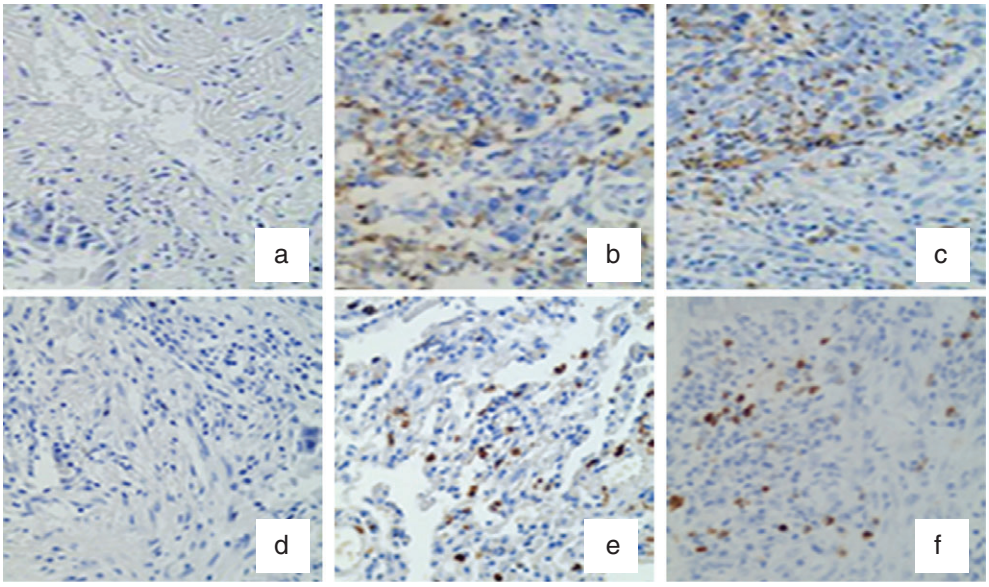

Figure 1 S100A8 and S100A9 expression detected by immunohistochemical assay (400×): (**a**) negative control of S100A8; positive S100A8 expression in (**b**) adenocarcinoma and (**c**) squamous cell carcinoma; (**d**) negative control of S100A9; positive S100A9 expression in (**e**) adenocarcinoma and (**f**) squamous cell carcinoma.

Table 1 S100A8 and S100A9 positive expression rates in cancer and normal tissues (*n*, [%])

	Cancer (*n* = 52)		Normal (*n* = 52)	
Factors	Positive	Negative	Positive	Negative
S100A8	37 (71.2)	15 (28.8)	6 (11.5)	46 (88.5)
S100A9	40 (76.9)	12 (23.1)	10 (19.2)	42 (80.8)

2–3 points (excluding 3 points), "+"; 3–6 points (excluding 6 points), "++"; and 6–7 points, "+++". Cases with "–" and "+" were classified as protein-negative expression groups, whereas cases with "++" and "+++" were categorized as protein-positive expression groups.

Statistical method

SPSS version 17.0 (http://www-01.ibm.com/software/analytics/spss/) was used to analyze all data. Measurement data were expressed by $\overline{x} \pm s$ and comparisons between groups were made based on a *t*-test of the sample mean. Enumeration data were expressed with a relative number (*n*, %), and comparison between groups was made based on χ^2 or Fisher's exact tests. $P < 0.05$ indicated statistical difference.

Results

S100A8 and S100A9 expression

S100A8 and S100A9 were expressed in the cytoplasm and nucleus of the NSCLC cells, mainly located in the cytoplasm, stained with brown particles, and distributed evenly (Fig 1).

S100A8 and S100A9 expression patterns between cancer and para-cancer tissue

The positive expression rates of S100A8 and S100A9 in cancer tissues were 71.2% and 76.9%, respectively, which were significantly higher than in para-cancer tissues at 11.5% and 19.2%, respectively, with statistical significance ($P < 0.05$) (Table 1).

Association of S100A8, S100A9 expression and patients clinical features

S100A8 and S100A9 positive expression was associated with tumor differentiation degree ($P < 0.05$). The S100A8 and S100A9 positive expression rates in poorly differentiated patients were significantly higher than in moderate/well-differentiated patients (Table 2). However, S100A8

Table 2 Correlation between S100A8 expression and clinical features in NSCLC patients (*n*, [%])

		S100A8 expression			
Features	*n* = 52	Negative (*n* = 15)	Positive (*n* = 37)	Chi-square	*P*
Age				0.16	0.69
≥ 60	30	8 (53.3)	22 (59.5)		
< 60	22	7 (46.7)	15 (40.5)		
Gender				0.44	0.51
Male	31	10 (66.7)	21 (56.8)		
Female	21	5 (33.3)	16 (43.2)		
Smoking				0.43	0.51
Positive	28	7 (46.7)	21 (56.8)		
Negative	24	8 (53.3)	16 (43.2)		
Tumor diameter (cm) (*n*, [%])				1.83	0.18
≤ 5	38	9 (60.0)	29 (78.4)		
> 5	14	6 (40.0)	8 (21.6)		
Pathology type				0.35	0.84
Squamous cell carcinoma	21	7 (46.7)	14 (37.8)		
Adenocarcinoma	27	7 (46.7)	20 (54.1)		
Other	4	1 (6.6)	3 (8.1)		
Differentiation				4.30	**0.04**
Well/moderately differentiated	30	12 (80.0)	18 (48.6)		
Poor	22	3 (20.0)	19 (51.4)		
TNM stage				2.48	0.11
I/II	33	12 (80.0)	21 (56.8)		
IIIA	19	3 (20.0)	16 (43.2)		
Pleural effusion				0.11	0.74
Yes	43	12 (80.0)	31 (83.8)		
No	9	3 (20.0)	6 (16.2)		

*$P < 0.05$ between S100A8 negative and positive groups. TNM, tumor node metastasis.

Table 3 Correlation between S100A9 expression and clinical features in NSCLC patients (*n*, [%])

Features	*n* = 52	S100A9 expression		Chi-square	*P*
		Negative (*n* = 12)	Positive (*n* = 40)		
Age				1.64	0.20
≥ 60	30	5 (41.7)	25 (62.5)		
< 60	22	7 (58.3)	15 (37.5)		
Gender				0.01	0.92
Male	31	7 (58.3)	24 (60.0)		
Female	21	5 (41.7)	16 (40.0)		
Smoking				0.93	0.33
Positive	28	5 (41.7)	23 (57.5)		
Negative	24	7 (58.3)	17 (42.5)		
Tumor diameter (cm) (*n*, [%])					
≤ 5	38	8 (66.7)	30 (75.0)	0.32	0.57
> 5	14	4 (33.3)	10 (25.0)		
Pathology type				0.68	0.71
Squamous cell carcinoma	21	6 (50.0)	15 (37.5)		
Adenocarcinoma	27	5 (41.7)	22 (55.0)		
Other	4	1 (8.3)	3 (7.5)		
Differentiation				4.20	**0.04**
Well/Moderately differentiated	30	10 (83.3)	20 (50.0)		
Poor	22	2 (16.7)	20 (50.0)		
TNM stage				0.07	0.79
I/II	33	8 (66.7)	25 (62.5)		
IIIA	19	4 (33.3)	15 (37.5)		
Pleural effusion				0.65	0.42
Yes	43	9 (75.0)	34 (85.0)		
No	9	3 (25.0)	6 (15.0)		

*$P < 0.05$ between S100A9 negative and positive groups. TNM, tumor node metastasis.

and S100A9 expression patterns were not correlated with age, gender, smoking history, tumor diameter, pathology type, tumor node metastasis stage, or pleural effusion ($P_{all} > 0.05$) (Table 3).

Discussion

Lung cancer is one of the most commonly diagnosed malignant carcinomas, especially in China.[9–11] A recent publication of cancer statistics reported approximately 14.1 million new cancer cases globally, with lung cancer accounting for approximately 1.82 million cases.[12] Approximately 8.2 million new cancer-related deaths occur each year, with lung cancer-related death accounting for 1.58 million.[13] Clinical studies have shown that approximately 80% of lung cancer patients are diagnosed at advanced stage, and are thus ineligible for surgery.[14] The prognosis of advanced stage NSCLC is poor, with a five-year survival rate of < 15%.[15] Therefore, determining the pathogenesis of lung cancer and the development of reasonable treatment plans have become hot spots in research.

S100A8 and S100A9 are members of the S100 protein family. Most members of protein families with a molecular weight of 10–20 kDa are specifically expressed in vertebrates.[16] Studies have shown that 25 members constitute this family, and most are associated with inflammation, tumor cell proliferation, apoptosis, and invasion.[17] Some S100 family members, such as S100A2, S100A4, and S100A6, have significantly higher expression levels in lung cancer cells than in normal cells.[18,19] Thus, the S100 protein family is closely related to the occurrence and prognosis of lung cancer.

In the present study, we used an immunohistochemical method to detect S100A8 and S100A9 protein expression in 52 NSCLC cancer and adjacent para-cancer tissues to explore the correlation between S100A8 and S100A9 expression and NSCLC patients' clinical characteristics, including age, gender, pathological type, and clinical stage. Positive expression of S100A8 and S100A9 in cancer tissues was significantly higher than in para-cancer tissues. S100A8 and S100A9 positive expression was associated with tumor differentiation degree ($P < 0.05$). The S100A8 and S100A9 positive expression rates in poorly differentiated patients were significantly higher than in moderate/well-differentiated patients. This result indicates that S100A8 and S100A9 may play important roles in the development of NSCLC and may represent potential markers to distinguish poorly and well-differentiated tumors. However, no correlation

between S100A8 and S100A9 expression and patient age, gender, clinical stage, pathology type, or smoking history were found, consistent with the results of a study published by Su *et al*.[8]

In summary, S100A8 and S100A9 expression was higher in cancer tissues compared to para-cancer tissues of NSCLC patients and is related to the degree of tumor differentiation. We speculate that S100A8 and S100A9 could be used as potential molecular markers for NSCLC diagnosis and prognosis. However, the biological functions of S100A8 and S100A9 are complex, and their mechanisms in NSCLC were not evaluated in the present study. Therefore, related basic research is required to explore the molecular mechanisms of S100A8 and S100A9 in the occurrence, development, invasion, and metastasis of NSCLC to provide more efficient NSCLC treatment and prognostic methods.

Disclosure

No authors report any conflict of interest.

References

1 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin* 2018; **68**: 7–30.
2 Shi Y, Sun Y. Medical management of lung cancer: Experience in China. *Thorac Cancer* 2015; **6**: 10–6.
3 Li P, Cong Z, Qiang Y *et al.* Clinical significance of CCBE1 expression in lung cancer. *Mol Med Rep* 2018; **17**: 2107–12.
4 Murakami D, Takamori S, Kawahara A *et al.* Periostin expression in non-small cell lung cancer: Clinical significance. *Kurume Med J* 2018; **64**: 13–20.
5 Bresnick AR, Weber DJ, Zimmer DB. S100 proteins in cancer. *Nat Rev Cancer* 2015; **15**: 96–109.
6 Tian WY, Chen WC, Li R, Liu L. Markers CD40, VEGF, AKT, PI3K, and S100 correlate with tumor stage in gastric cancer. *Onkologie* 2013; **36**: 26–31.
7 Ji YF, Huang H, Jiang F, Ni RZ, Xiao MB. S100 family signaling network and related proteins in pancreatic cancer (review). *Int J Mol Med* 2014; **33**: 769–76.
8 Su YJ, Xu F, Yu JP, Yue DS, Ren XB, Wang CL. Up-regulation of the expression of S100A8 and S100A9 in lung adenocarcinoma and its correlation with inflammation and other clinical features. *Chin Med J (Engl)* 2010; **123**: 2215–20.
9 Chen W, Zheng R, Zeng H, Zhang S. Epidemiology of lung cancer in China. *Thorac Cancer* 2015; **6**: 209–15.
10 Chen W, Zheng R, Baade PD *et al.* Cancer statistics in China, 2015. *CA Cancer J Clin* 2016; **66**: 115–32.
11 Chen W. Cancer statistics: Updated cancer burden in China. *Chin J Cancer Res* 2015; **27**: 1.
12 Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; **65**: 87–108.
13 Torre LA, Siegel RL, Jemal A. Lung cancer statistics. *Adv Exp Med Biol* 2016; **893**: 1–19.
14 Nishimoto H. [Lung cancer-statistics of hospital-based cancer registry data in Japan]. *Gan To Kagaku Ryoho* 2011; **38**: 1281–4. (In Japanese.)
15 Thill PG, Goswami P, Berchem G, Domon B. Lung cancer statistics in Luxembourg from 1981 to. *Bull Soc Sci Med Grand Duche Luxemb* 2008; **2011**(2): 43–55.
16 Chen H, Xu C, Jin Q, Liu Z. S100 protein family in human cancer. *Am J Cancer Res* 2014; **4**: 89–115.
17 Heizmann CW, Fritz G, Schäfer BW. S100 proteins: Structure, functions and pathology. *Front Biosci* 2002; **7**: d1356–68.
18 De Petris L, Orre LM, Kanter L *et al.* Tumor expression of S100A6 correlates with survival of patients with stage I non-small-cell lung cancer. *Lung Cancer* 2009; **63**: 410–7.
19 Miyazaki N, Abe Y, Oida Y *et al.* Poor outcome of patients with pulmonary adenocarcinoma showing decreased E-cadherin combined with increased S100A4 expression. *Int J Oncol* 2006; **28**: 1369–74.

