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Original Research Article

Overexpression of S100A8 is Associated with Aggressive Biological Behaviour of Lung Adenocarcinoma

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Abstract

The inflammatory proteins S100A8 and S100A9 form a heterocomplex associated with the prognosis of various cancers. This study aims to examine the association between S100A8 and S100A9 expressions and various pathological variables in primary lung adenocarcinoma (AC). Serial paraffin-embedded tissue sections from 150 patients with lung AC resected at the Shizuoka General Hospital were immunostained and assessed. In patients with invasive lung AC (n = 132), an overlap between S100A8 and S100A9 immunopositivities was observed. S100A8 alone was not reported, but both immunopositivities were associated with high histological grade ($P < 0.05$), and S100A8 immunopositivity was associated with vessel permeation, poor pT categories, node metastasis, and poor pStage ($P < 0.05$). In patients with AC *in situ* (n = 18), only limited S100A9 immunopositivity was observed. The overexpression of S100A8 (S100A8/S100A9 up-regulation) might be a poor prognostic factor in lung AC.

Keywords: S100A8, S100A9, Lung adenocarcinoma, Immunohistochemistry, Immunopositivity, pTNM, pStage, Lung squamous cell carcinoma.

INTRODUCTION

Lung cancer is a leading cause of cancer-related deaths worldwide [1]. In Japan, it is the first leading cause of cancer-related death regardless of gender [2]. Non-small-cell lung cancer is associated with increased expressions of several members of the S100 protein family [3]. S100 proteins belong to the superfamily of calcium-binding proteins with a helix-loop-helix domain [3, 4]. To date, 21 different S100 proteins have been identified, and they are expressed in a controlled tissue- or cell-type-specific manner [3, 4]. This protein family is considered to be involved in various biological processes [3, 4]. Further, changes in the expressions and/or functions of S100 proteins represent key steps during cancer development or progression [3, 4].

S100A8 and S100A9 were originally identified in myeloid cells, and they are up-regulated during inflammatory conditions [5-10]. S100A8 and S100A9 naturally form a stable functional heterodimer, and the functions of both proteins are mainly dependent on the heterocomplex [4-10]. The overexpression of S100A8 and S100A9 correlates with several pathological parameters that suggest poor prognosis in breast carcinoma [11]. This overexpression has also been reported to be associated with adenocarcinoma (AC) progression in various organs [3, 4, 12-15]. It was indicated that high mRNA expression levels of S100A8 and S100A9 are associated with poor overall survival in lung AC. However, the relationships between each protein and pathological parameters have not been studied in detail [16]. The overexpression of S100A8 and S100A9 in AC can lead to aggressive biological behavior. We aimed to examine the relationships between S100A8 and S100A9 expressions and various pathological parameters in primary lung AC.

MATERIALS AND METHODS

Study design

The mRNA expressions of S100A8 and S100A9 were assessed in lung carcinoma cell lines. The expression of each protein was examined by immunohistochemistry in tissue sections of lung cancer, and the relationships of the expression with various pathological parameters influencing prognosis were assessed.

Cells and Culture

The human lung AC cell line PC-3 (JCRB0077) and human lung squamous cell carcinoma (SCC) cell line LC-1 sq (JCRB0258) were obtained from Japan Health Sciences Foundation (Tokyo, Japan). Cells were grown in F12 medium supplemented with

10% heat-inactivated foetal bovine serum (Sigma, St. Louis, MO, USA) and 100 ug/ml each of sodium ampicillin and kanamycin sulphate at 37°C under humidified 5% CO_2/95% air [17, 18].

Reverse transcription-polymerase chain reaction (RT-PCR) of cultured lung carcinoma cells

Total RNA was isolated using Trizol solution (Invitrogen, Tokyo, Japan), and first-strand cDNA was synthesized using the SuperScript III kit (Invitrogen) and oligo $(dT)_{20}$ primer, as previously described [17]. PCR amplification was performed for S100A8 and S100A9 using appropriate primers. The PCR protocol and primers have been described elsewhere [17, 19].

Antibodies against S100A8 or S100A9

Monoclonal antibodies (mAbs) against S100A8 and S100A9 (clone #83 and mAb60B8, respectively) were prepared [19]. These antibodies showed no cross-reaction with other members of the S100 protein family [18].

Immunohistochemistry

Samples

Samples were obtained from 150 patients with primary lung AC (98 males; mean age, 69 years) who underwent surgery at Shizuoka General Hospital between January 2001 and December 2015. All study participants provided informed consent, and the study design was approved by the Ethics Committee of Shizuoka General Hospital (approval number: SGHIRB#2018091). All tumor samples were fixed in 20% neutral buffered formalin. The histological type was classified according to the criteria of WHO [20]. Special types of lung AC were excluded. There were 132 cases of invasive, but not minimally invasive, lung AC (mean invasion size, 3.1 cm; range, 0.6–11.5) and 18 cases of non-mucinous AC *in situ* (AIS; mean diameter, 1.8 cm; range, 1–3). The 132 patients with invasive lung AC were subcategorized into lepidic AC (n = 36), papillary AC (n = 45), acinar AC (n = 28), and solid AC (n = 23). Vascular invasion and pleural involvement in these tumors were judged using the Elastica van Gieson stain. The mitotic count was determined as the number of mitoses per 10 high-power fields (HPFs) at ×400 magnification (field area, 0.196 mm^2) in areas with the highest density of mitotic figures. The mitotic index was categorized into the following grades: grade 1, ≤4 mitotic cells/10 HPFs; grade 2, 5–9/10 HPFs; and grade 3, ≥10/10 HPFs [21]. The 132 cases were staged according to the TNM classification proposal of the International Association for the Study of Lung Cancer [22]. S100A9 protein expression is related to the differentiation of lung AC [23]. In the present study, histological grade was classified as follows: low grade, lepidic AC (n = 36); intermediate grade, papillary and acinar ACs (n = 73); and high grade, solid AC (n = 23) [20].

In total, 75 patients with primary lung SCC (including 65 males; mean age, 72 years; mean diameter, 4.0 cm; range, 1.0–10.3) were included as controls. Histological grading of these lung SCCs was performed in accordance with the 2004 WHO classification [24], and the cases were classified into well, moderately, and poorly differentiated SCC (n = 4, 33 and 38, respectively).

Staining of Tissue Sections

Immunohistochemical analysis was performed on serial sections prepared from formalin-fixed, paraffin-embedded tissues. The protocols have been described elsewhere [11]. The sections were subjected to heat antigen retrieval in 10 mM citrate buffer (pH 6.0) for 30 min at 98°C (S100A8 staining) and pretreated with 0.4 mg/mL proteinase K (DAKO, Glostrup, Denmark) for 5 min (S100A9 staining). After the inhibition of endogenous peroxidase activity, the sections were incubated with mAbS100A8 (#83) or mAb60B8 for 30 min. The labeled antigens were detected using ChemMate EnVision (DAKO), and the reaction products were visualized with 3,3′-diaminobenzidine.

Ki-67 antigen expression was examined in all samples. Immunostaining for the Ki-67 antigen and the estimation of the Ki-67 labeling index were performed as previously described [11].

Evaluation of S100A8 and S100A9 immunostaining in tumor cells

The percentage of positively stained tumor cells was scored as follows: score 0, non-reactive (no immunoreactive tumor cells); score 1, <10% positive cells; score 2, ≥10% but <50% positive cells, and score 3, ≥50% positive cells [11, 18, 23].

Statistical Analysis

All statistical analyses were performed using SPSS software version 21 (IBM Corp., Armonk, NY, USA). Categorical data were studied using chi-squared test, and measurement data were analyzed using Student's *t*-test or Welch's *t*-test, as appropriate. A P-value of <0.05 was considered significant.

RESULTS AND DISCUSSION

Gene expressions of S100A8 and S100A9 in cultured lung carcinoma cells

Gene expressions of S100A8 and S100A9 were detected in lung AC and SCC cells (Fig-1).

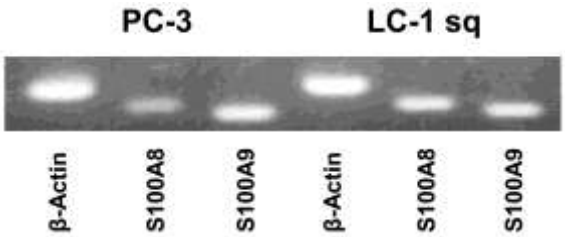

Fig-1: Expression of S100A8 and S100A9 in human lung carcinoma cell lines

The transcripts of S100A8 and S100A9 genes are amplified using RT-PCR in both adenocarcinoma cell line PC-3 and squamous cell carcinoma cell line LC-1 sq.

The transcripts of S100A8 and S100A9 genes were amplified using RT-PCR in both adenocarcinoma cell line PC-3 and squamous cell carcinoma cell line LC-1 sq.

Immunohistochemical analysis of S100A8 and S100A9 expressions in invasive lung AC

Among 132 patients with invasive lung AC, immunostaining revealed S100A8 and S100A9 expressions in 84 (63.6%) and 129 (97.7%) patients, respectively (Table-1).

S100A8 alone was not detected in any case (Table-1). S100A8-immunopositive cases expressed both proteins. Immunoreactivity for S100A8 showed heterogeneity among tumor cells similar to that for S100A9 [11, 13, 18, 23]. In invasive lung ACs that showed immunopositivity for both proteins, an overlap between S100A8 and S100A9 staining patterns was observed. However, S100A9 immunopositivity was also observed in S100A8-negative tumor cells (Fig-2).

Table-1: Relationship of the immunoreactivity between S100A8 and S100A9 in 132 cases of pulmonary invasive adenocarcinoma

S100A8 immunoreactivity				
S100A9 immunoreactivity	Score 0	Score 1	Score 2	Score 3
Score 0	3 (6)	-	-	-
Score 1	20 (42)	8 (17)	-	-
Score 2	23 (48)	28 (58)	12 (46)	-
Score 3	2 (4)	12 (25)	14 (54)	10 (100)

Immunoreactivity score: score 0, non-reactive; score 1, positively stained tumor cells <10%; score 2, ≥10% but <50%; and score 3, ≥50%. The numbers in parentheses represent the percentage of positively stained tumor cells.

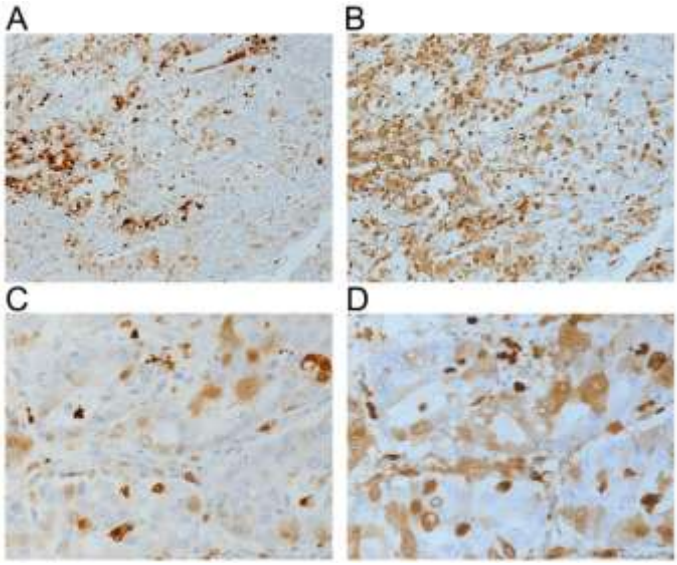

Fig-2: Immunohistochemistry for S100A8 (A, C) and S100A9 (B, D) on the serial sections of lung adenocarcinoma (C and D are high-power views of A and B, respectively)

An overlap between S100A8 and S100A9 staining patterns was observed. However, S100A9 positivity is also found in S100A8-negative tumor cells (Original magnifications, A and C, ×100; B and D, ×400).

Relationships between the immunoreactivity of each S100 protein and pathological parameters in invasive lung AC.

The relationships between the immunoreactivity of each S100 protein and pathological parameters are presented in Table-2.

Table-2: Relationships between the immunoreactivity of each S100 protein and pathological parameters in 132 cases of pulmonary invasive adenocarcinoma

	S100A8 immunoreactivity					S100A9 immunoreactivity				
	Non-reactive	Reactive (n = 84)				Non-reactive	Reactive (n = 129)			
	Score 0 (n = 48)	Score 1 (n = 48)	Score 2 (n = 26)	Score 3 (n = 10)		Score 0 (n = 3)	Score 1 (n = 28)	Score 2 (n = 63)	Score 3 (n = 38)	
Histological grade										
Low (n = 36)	24 (50)	11 (23)	1 (4)	-	P < 0.01	2 (67)	14 (50)	20 (32)	-	P < 0.01
Intermediate (n = 73)	21 (44)	30 (63)	17 (65)	5 (50)		1 (33)	14 (50)	35 (56)	23 (61)	
High (n = 23)	3 (6)	7 (15)	8 (31)	5 (50)		-	-	8 (13)	15 (39)	
Mitotic counts										
Grade 1 (n = 90)	41 (85)	30 (63)	13 (50)	6 (60)	P = 0.019	3 (100)	26 (93)	40 (63)	21 (55)	P < 0.01
Grade 2 (n = 11)	1 (2)	5 (10)	3 (12)	2 (20)		-	-	4 (6)	7 (18)	
Grade 3 (n = 31)	6 (13)	13 (27)	10 (38)	2 (20)		-	2 (7)	19 (30)	10 (26)	
Vascular invasion										
Absent (n = 61)	31 (65)	19 (40)	10 (38)	1 (10)	P < 0.01	1 (33)	18 (64)	35 (56)	7 (18)	P < 0.01
Present (n = 71)	17 (35)	29 (60)	16 (62)	9 (90)		2 (67)	10 (36)	28 (44)	31 (82)	
Lymphatic permeation										
Absent (n = 66)	33 (69)	25 (52)	7 (27)	1 (10)	P < 0.01	3 (100)	17 (61)	34 (54)	12 (32)	P = 0.017
Present (n = 66)	15 (31)	23 (48)	19 (73)	9 (90)		-	11 (39)	29 (46)	26 (68)	
Pleural involvement										
Absent (n = 61)	25 (52)	24 (50)	10 (38)	2 (20)	P = 0.241	1 (33)	14 (50)	33 (52)	13 (34)	P = 0.318
Present (n = 71)	23 (48)	24 (50)	16 (62)	8 (80)		2 (67)	14 (50)	30 (48)	25 (66)	
pT categories										
pT1 (n = 44)	19 (40)	19 (40)	6 (23)	-	P = 0.035	1 (33)	9 (32)	25 (40)	9 (24)	P = 0.374
≥pT2 (n = 88)	29 (60)	29 (60)	20 (77)	10 (100)		2 (67)	19 (68)	38 (60)	29 (76)	
Node metastasis										
Absent (n = 90)	39 (81)	30 (63)	18 (69)	3 (30)	P = 0.011	3 (100)	23 (82)	42 (67)	22 (58)	P = 0.136
Present (n = 42)	9 (19)	18 (38)	8 (31)	7 (70)		-	5 (18)	21 (33)	16 (42)	
pStage										
pStage IA (n = 42)	19 (40)	18 (38)	5 (19)	-	P = 0.026	1 (33)	9 (32)	23 (37)	9 (24)	P = 0.594
pStage ≥IB (n = 90)	29 (60)	30 (63)	21 (81)	10 (100)		2 (67)	19 (68)	40 (63)	29 (76)	
Ki-67 labelling index Mean ± SD (%)	Score 0 and 1 21.5 ± 24.1		Score 2 and 3 31.9 ± 28.3		P = 0.058	Score 0 and 1 13.4 ± 13.1		Score 2 and 3 27.9 ± 27.6		P = 0.0001

Immunoreactivity score: score 1, positively stained tumor cells <10%; score 2, ≥10 but <50%; score 3, ≥50%. Mitotic counts: grade 1, 0–4/10 high-power fields (HPFs); grade 2, 5–9/10 HPFs; and grade 3, ≥10/10 HPFs. The numbers in parentheses represent the percentage of positively stained tumor cells.

S100A8 immunopositivity was significantly correlated with high histological grade, mitotic activity, vascular invasion, lymphatic permeation, poor pT categories, node metastasis, and poor pStage ($P < 0.01$, $P = 0.019$, $P < 0.01$, $P < 0.01$, $P = 0.035$, $P = 0.011$, and $P = 0.026$, respectively, using chi-squared test) but not with pleural involvement ($P = 0.241$). Conversely, S100A9 immunopositivity was significantly correlated with high histological grade, mitotic activity, vascular invasion, and lymphatic permeation ($P < 0.01$, $P < 0.01$, $P < 0.01$, and $P = 0.017$, respectively, using chi-squared test) but not with pleural involvement, poor pT categories, node metastasis, and poor pStage ($P = 0.318$, $P = 0.374$, $P = 0.136$, and $P = 0.594$, respectively). In S100A8 and S100A9, no significant difference was found between papillary AC and acinar AC (data not shown). The Ki-67 labeling index was higher in cases with S100A9-positive tumor cell scores of 2 or 3 than in cases with scores of 0 or 1. However, such a significant difference was not identified for S100A8 immunoreactivity.

Immunohistochemical analysis in non-mucinous AIS.

S100A9 immunopositivity in tumor cells was observed in 12 of the 18 patients with non-mucinous AIS. However, in all cases, positivity was barely visible and was scored 1. There was no correlation between S100A9 immunoreactivity and the Ki-67 labeling index (S100A9-negative, 1.9% ± 1.1%; S100A9-positive, 3.6% ± 4.6%; P = 0.211). No S100A8 immunopositivity in tumor cells was found.

Immunohistochemical analysis of S100A8 and S100A9 expressions in lung SCC.

Among the 75 patients with lung SCC, S100A8 and S100A9 were detected in 68 (90.7%) and 75 (100%) patients, respectively (Table-3).

Table-3: Relationship of the immunoreactivity between S100A8 and S100A9 in 75 patients with pulmonary squamous cell carcinoma

S100A8 immunoreactivity				
S100A9 immunoreactivity	Score 0	Score 1	Score 2	Score 3
Score 0	-	-	-	-
Score 1	3 (43)	5 (28)	-	-
Score 2	4 (57)	12 (67)	14 (54)	-
Score 3	-	1 (6)	12 (46)	24 (100)

Immunoreactivity score: score 0, non-reactive; score 1, positively stained tumor cells <10%; score 2, ≥10 but <50%; score 3, ≥50%. The numbers in parentheses represent the percentage of positively stained tumor cells.

Immunopositivity for each protein was mainly detected in the spinous layer of tumor cell nests, and it decreased or was undetectable in the basal layer (Fig-3).

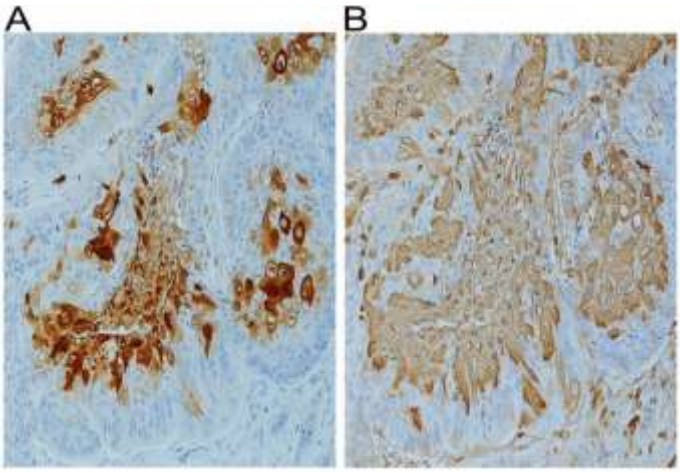

Fig-3: Immunohistochemistry for S100A8 (A) and S100A9 (B) on the serial sections of lung squamous cell carcinoma

An overlap between S100A8 and S100A9 staining patterns was observed in squamous cell carcinoma. Both S100A8 and S100A9 are chiefly immunolocalized in the spinous layers of the tumor cell nests (Original magnification: ×200).

The staining pattern of each protein in lung SCC was similar to that in invasive lung AC in the following respects: 1) staining heterogeneity among tumor cells; 2) no case showing S100A8 immunopositivity alone; and 3) an overlapping expression pattern in corresponding tissue areas (Fig-3).

Relationships between the immunoreactivity of each S100 protein and pathological parameters in lung SCC

The results are summarized in Supplementary Table-1.

Immunopositivities of S100A8 and S100A9 (especially S100A8) were inversely correlated with poor tumor differentiation (P < 0.01 and P = 0.012, respectively) in contrast to the results for invasive lung AC. Vascular invasion was associated with S100A9 immunopositivity (P = 0.027) but not with S100A8 immunopositivity (P = 0.151). Neither immunopositivities showed correlations with the following pathological parameters: mitotic activity, lymphatic permeation, pleural involvement, pT categories, node metastasis, pStage, and the Ki-67

labeling index (each P-value is shown in Supplementary Table-1).

In this study, the overexpression of S100A8 in lung AC was correlated with several poor pathological parameters. In lung cancer of category pT2, the frequency of node metastasis has been shown to increase 2.4 times compared with that in category pT1 [22]. In lung cancer with node metastasis, pStage is classified as >IIB, and the 5-year survival rate has been predicted to be <60% [22]. Accordingly, the overexpression of S100A8 is considered a poor prognostic factor of lung AC [11, 13, 16].

In myeloid cells, S100A8 is ordinarily bound to S100A9 [5–7, 9], and heterocomplex formation with S100A9 is thought to be essential for the stability of S100A8 [5, 6]. In this study, S100A8-positive tumor cells were localized with S100A9-positive tumor cells irrespective of the histological carcinoma type, and no cells expressing only S100A8 were found. The co-immunolocalization of both proteins has also been observed in breast carcinoma, prostatic AC, and thyroid carcinoma, suggesting the formation of a S100A8/S100A9 complex in carcinoma cells [11-13]. S100A8 in carcinoma cells acquire stability through S100A8/S100A9 complex formation, as in myeloid cells. Thus, the overexpression of S100A8 is considered to indicate S100A8/S100A9 complex up-regulation.

In tissue sections, S100A9 immunopositivity was found in S100A8-negative tumor cells, suggesting that S100A9-positive tumor cells include cells with the S100A8/S100A9 complex and those without the complex. This has also been found in breast and thyroid carcinoma [11, 13]. The S100A8/S100A9 complex is a functionally relevant form of S100A8 and S100A9, and each protein has individual functions [6–8]. S100A8 was considered as an active component of the S100A8/S100A9 complex in inflammatory cells, whereas S100A9 was regarded as a regulator of S100A8 function [7]. S100A8 and S100A9 change the biological behavior of inflammatory and carcinoma cells co-operatively or individually. The present findings suggest that S100A8 and S100A9 are overexpressed in lung AC because they are related to high histological grade. S100A8/S100A9 complex up-regulation leads to aggressive biological behaviors, such as severe stromal invasion and lymphatic spread, and the overexpression of S100A9 is associated with cell proliferation. In breast cancer, the overexpression of S100A8 was related to poor histological grade, high mitotic counts, poor pT categories, node metastasis, vessel invasion, overexpression of HER2, and poor pStage [11]. In thyroid cancer, S100A8 expression was associated with cancer dedifferentiation [13]. The S100A8/S100A9 complex promotes tumor cell migration/invasion, vessel permeation, and metastasis in carcinomas of glandular cell origin, and S100A8 is the active component of the heterocomplex in inflammatory and carcinoma cells.

Mitogen-activated protein kinase, nuclear factor-κB, the receptor of advanced glycation end products, Toll-like receptor 4, and CD147 are known mediators of S100A8/S100A9 function [4, 6, 7, 9, 10, 25]. These signaling proteins also play important roles in inflammatory and immune responses [6, 7, 9, 10]. Elucidation of S100A8/S100A9-triggered signaling in myeloid cells would be useful for clarifying that in AC cells.

In lung SCC, expressions of S100A8 and S100A9 (especially S100A8) were inversely correlated with poor tumor differentiation. Similar results have been reported in oesophageal SCC and uterine cervical intraepithelial neoplasia [26, 27]. The S100A8/S100A9 complex in SCC is inseparably linked to the maintenance of squamous differentiation [26, 27]. The differences in both protein expressions between SCC and AC suggest different expression mechanisms and biological roles of these S100 proteins according to the histological carcinoma type.

S100A8 and S100A9 secreted by myeloid and endothelial cells in tumoral environments induce tumor cell migration/invasion and metastasis [4, 9, 10, 28, 29]. AC cells can also enhance their aggressiveness through the S100A8/S100A9 complex.

The present study had some limitations, including a retrospective design, relatively small sample size, and lack of matching or regression to adjust for potential confounders and mediators in between-group characteristics. However, the findings are consistent with those of previous studies conducted on S100 proteins [3, 4, 6, 10-16, 25]. Large cohort studies are needed to confirm the present results.

Supplementary Table-1: Immunopositivities of S100A8 and S100A9 (especially S100A8) were inversely correlated with poor tumor differentiation ($P < 0.01$ and $P = 0.012$, respectively), in contrast to the results for invasive lung AC. Vascular invasion was associated with S100A9 immunopositivity ($P = 0.027$), but not with S100A8 ($P = 0.151$). Neither immunopositivities showed correlations with the following pathological parameters: mitotic activity, lymphatic permeation, pleural involvement, pT categories, node metastasis, pStage and the Ki-67 labelling index (each P-value is shown in Supplementary Table-1).

	S100A8 immunoreactivity	S100A9 immunoreactivity

	Non-reactive	Reactive (n= 68)				Non-reactive	Reactive (n = 75)			
	Score 0 (n = 7)	Score 1 (n = 18)	Score 2 (n = 26)	Score 3 (n = 24)		Score 0 (n = 0)	Score 1 (n = 8)	Score 2 (n = 30)	Score 3 (n = 37)	
Tumour differentiation										
Well (n = 4)	-	-	4 (15)	-	P < 0.01	-	-	2 (7)	2 (5)	P = 0.012
Moderate (n = 33)	1 (14)	2 (11)	12 (46)	18 (75)		-	1 (13)	9 (30)	23 (62)	
Poor (n = 38)	6 (86)	16 (89)	10 (38)	6 (25)		-	7 (88)	19 (63)	12 (32)	
Mitotic counts										
Grade 1 (n = 8)	-	1 (6)	1 (4)	6 (25)	P = 0.301	-	1 (13)	-	7 (19)	P = 0.055
Grade 2 (n = 7)	-	2 (11)	3 (12)	2 (8)		-	1 (13)	2 (7)	4 (11)	
Grade 3 (n = 60)	7 (100)	15 (83)	22 (85)	16 (67)		-	6 (75)	28 (93)	26 (70)	
Vascular invasion										
Absent (n = 29)	2 (29)	4 (22)	10 (38)	13 (54)	P = 0.151	-	2 (25)	7 (23)	20 (54)	P = 0.027
Present (n = 46)	5 (71)	14 (78)	16 (62)	11 (46)		-	6 (75)	23 (77)	17 (46)	
Lymphatic permeation										
Absent (n = 30)	5 (71)	4 (22)	12 (46)	9 (38)	P = 0.148	-	5 (62)	10 (33)	15 (41)	P = 0.348
Present (n = 45)	2 (29)	14 (78)	14 (54)	15 (63)		-	3 (38)	20 (67)	22 (59)	
Pleural involvement										
Absent (n = 39)	3 (43)	8 (44)	15 (58)	13 (54)	P = 0.819	-	1 (13)	18 (60)	20 (54)	P = 0.058
Present (n = 36)	4 (57)	10 (55)	11 (42)	11 (46)		-	7 (88)	12 (40)	17 (46)	
pT categories										
pT1 (n = 12)	1 (14)	2 (11)	3 (12)	6 (25)	P = 0.836	-	1 (13)	3 (10)	8 (22)	P = 0.568
≥pT2 (n = 63)	6 (86)	16 (89)	23 (88)	18 (75)		-	7 (88)	27 (90)	29 (78)	
Node metastasis										
Absent (n = 44)	6 (86)	11 (61)	13 (50)	14 (58)	P = 0.302	-	7 (88)	17 (57)	20 (54)	P = 0.318
Present (n = 31)	1 (14)	7 (39)	13 (50)	10 (42)		-	1 (13)	13 (43)	17 (46)	
pStage										
pStage IA (n = 8)	1 (14)	1 (6)	2 (8)	4 (17)	P = 0.567	-	1 (13)	1 (3)	6 (16)	P = 0.199
– pStage ≥IB (n = 67)	6 (86)	17 (94)	24 (92)	20 (83)		-	7 (88)	29 (97)	31 (84)	
Ki-67 labelling index Mean ± SD (%)	Score 0 and 1 42.8 ± 24.3		Score 2 and 3 39.5 ± 20.5		P = 0.546	Score 0 and 1 48.0 ± 20.5		Score 2 and 3 39.6 ± 21.8		P = 0.307

CONCLUSION

In conclusion, S100A8 and S100A9 immunopositivities are associated with high histological grade, and S100A8 immunopositivity is associated with vessel permeation, poor pT categories, node metastasis, and poor pStage in invasive lung AC. The overexpression of S100A8 (S100A8/S100A9 up-regulation) might be a poor prognostic factor in lung AC.

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Declaration of Conflicting Interests

The Author declares that there is no conflict of interest.

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