

Original Article

Different expression of S100A11 in normal ovarian epithelium, serous benign/borderline tumor, and low-grade serous ovarian carcinoma

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Received March 26, 2016; Accepted May 28, 2016; Epub July 1, 2016; Published July 15, 2016

Abstract: S100A11 is an EF hand-type Ca^{2+} -binding protein involved in various biological processes such as cell proliferation and differentiation. It acts as either a tumor suppressor or promoter in many different types of cancers. Low-grade serous ovarian carcinoma (LGSC) is a less common subtype of ovarian cancer which is considered to develop in a stepwise fashion. To further understand the role of S100A11 in LGSC, we investigated immunohistochemical expression of S100A11 on a series of benign, borderline and malignant serous tumors as well as normal ovarian epithelia. Moreover, the association between S100A11 expression and various clinical pathological parameters of LGSC was also evaluated. The results showed that S100A11 expression was significantly increased from normal ovarian epithelium to benign and borderline tumors, and then to LGSC. S100A11 overexpression was associated with more advanced FIGO stage ($P = 0.018$) and increased residual disease ($P = 0.043$) in LGSC. Higher S100A11 expression was associated with poorer overall survival ($P = 0.043$), while multivariate analysis revealed S100A11 to be an independent prognostic factor for overall survival ($P = 0.011$) in patients with LGSC. Results suggest that S100A11 expression may contribute to the initiation, promotion and progression of LGSC; it might be a useful biomarker for the outcome prediction of LGSC.

Keywords: Serous ovarian cancer, S100A11, immunohistochemistry, serous borderline tumor

Introduction

Based on distinctive morphologic and molecular genetic features, a dualistic model of ovarian carcinogenesis has been proposed which classifies ovarian carcinomas into 2 groups: Type I and Type II. Type I tumors include low-grade serous carcinomas (LGSC), low-grade endometrioid carcinomas, clear cell and mucinous carcinomas, and Brenner tumors. Type II group is composed of high-grade serous carcinomas (HGSC), high-grade endometrioid carcinomas, malignant mixed mesodermal tumors (carcinosarcomas), and undifferentiated carcinomas [1]. LGSC accounts for a small proportion (9%) of all serous ovarian carcinomas [2]. It is less common and aggressive than HGSC, yet exceptionally difficult to treat when chemotherapy and hormonal therapy fail after surgery. The molecular pathogenesis of LGSC is poorly understood. It is thought to arise from cortical

inclusion cysts within the ovarian parenchyma underlying and develop in a stepwise fashion, sequentially from ovarian epithelial inclusions or serous cystadenoma, then to serous borderline tumor (SBT), and eventually to invasive carcinomas [3-5]. Atypical proliferative serous tumors (APSTs) and non-invasive MPSCs (SBT, micropapillary variant) are precursors of LGSCs [1, 6].

S100A11, which is also called S100C or calgizarin, is an EF hand-type Ca^{2+} -binding protein belonging to the family of S100 multitasking proteins. As one of the less well-known members of S100 family, it was first discovered in 1989 [7], and then purified from porcine cardiac muscle [8] and chicken gizzard smooth muscle [9]. S100A11 has been indicated to play a role in endo- and exocytosis, regulation of enzyme activity, cell growth, apoptosis, and inflammation [10]. In cancer research, S100A11

Table 1. Expression of S100A11 in different epithelial lesions

	Total N = 237	S100A11 expression		P
		Low	High	
Normal ovarian epithelium	54	46	8	0.000 ^a
Benign serous cystadenoma	59	39	20	0.019 ^b 0.027 ^c
SBT	55	25	30	0.000 ^d 0.025 ^e
LGSC	69	18	51	0.000 ^f 0.000 ^g

^aP value of four groups, $\chi^2 = 47.679$; ^bBenign serous cystadenoma vs normal ovarian epithelium, Z = -2.337; ^cBenign serous cystadenoma vs SBT, Z = -2.210; ^dNormal ovarian epithelium vs SBT, Z = -4.332; ^eSBT vs LGSC, Z = -2.242; ^fNormal ovarian epithelium vs LGSC, Z = -6.484; ^gBenign serous cystadenoma vs LGSC, Z = -4.523.

seems to act as a dual mediator. It appears to have distinct roles depending on the tumor involved. S100A11 is observed to be overexpressed in a variety of carcinomas [11-15] and involved in cancer cell proliferation and migration [16, 17], suggesting it may play a role in carcinogenesis and could be a tumor promoter. Meanwhile, other findings suggest S100A11 possesses tumor suppressing abilities [18, 19]. Very little is known regarding the expression of S100A11 in benign and malignant ovarian tissues. Furthermore, genetic changes affecting the 1q21 chromosomal segment (to which S100A11 maps) have been found in a high proportion of ovarian cancers [20, 21]. Therefore, further study was warranted to measure S100A11 expression pattern in ovarian tissues.

The current study was designed to investigate the expression of S100A11 in various serous ovarian epithelial lesions, including normal epithelia, benign cystadenomas, SBT as well as LGSC, and simultaneously to determine the association between S100A11 expression and established clinicopathological parameters of patients with LGSC.

Materials and methods

Tissue specimens

A total of 237 paraffin-embedded specimens were obtained for immunohistochemical analysis, including 54 normal ovarian epithelia, 59 benign serous cystadenomas, 55 SBTs (30

APSTs and 25 non-invasive MPSCs), and 69 LGSCs. The specimens were obtained from patients with complete clinical data in the Shanghai General Hospital, Shanghai Jiao Tong University, School of Medicine, China. The LGSC cases were collected from September 21, 2005 to January 05, 2013. The median age at diagnosis was 55 years (range, 37-64). All of them received cytoreductive surgery followed by taxol and platinum combination chemotherapy. Patient clinical parameters, including age, clinical stage, and performance status, clinical response to chemotherapy, ascitic fluid volume, residual disease, and CA125 level before resection were obtained. After surgery, all patients were reviewed every 3 to 6 months for 2 year, and annually thereafter. The follow-up periods for survivors ranged from 11 to 108 months (median, 51 months) after surgery. A total of 35 patients (50.7%) died within the study period and 46.4% were alive at the last follow-up. Two patients were lost to follow-up. Tumors were classified according to the 2014 World Health Organization (WHO) classification. Clinical stage of carcinomas was determined according to the established International Federation of Gynecology and Obstetrics (FIGO) standards. All the pathological diagnoses were verified by two gynecological oncology pathologists. None of the cases were treated with chemotherapy, immunotherapy or radiotherapy prior to tumor resection. For the use of these specimens for research purpose, prior patients' consents and approval from the Institutional Research Ethics Committee were obtained.

Immunohistochemistry

Serial sections (4 μ m) from paraffin-embedded blocks were cut for each case, and one section stained with H&E was used to confirm the histopathological diagnosis. Only sections containing representative and sufficient sample of tumor or epithelium were considered for the study. Immunohistochemical staining was performed with a rabbit polyclonal antibody against S100A11 (sigma, USA) at a dilution of 1:200, according to the previously described procedures [22]. In detail, the paraffin sections were deparaffinized, blocked with 3% hydrogen peroxide for 10 min and subjected to antigen retrieval with microwave treatment in 10 mM citrate buffer (PH 6.0) for 15 min. Endogen peroxidase activity was blocked with 3% hydrogen

S100A11 protein expression in ovarian tumors

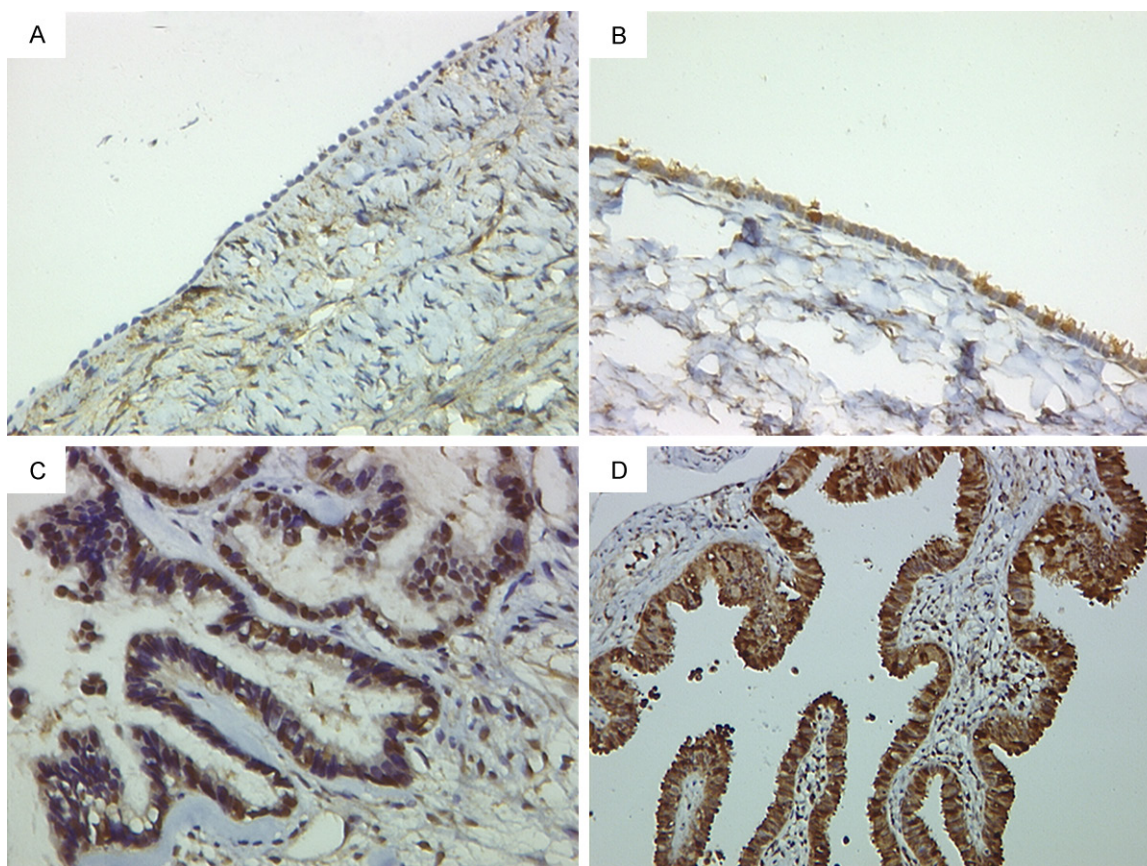


Figure 1. S100A11 expression and localization in normal ovarian epithelia (A), benign serous tumor (B), SBT (C) and LGSC (D).

peroxidase for 10 min at room temperature (RT). After immersion in 2.5% blocking serum for 30 min to reduce nonspecific binding, the slides were incubated with the anti-S100A11 antibody diluted 1:200 in Tris-buffered solution (50 mM Tris-HCl, 150 mM NaCl, pH 7.4) at RT for 1 h. Blank control sections were incubated with normal rabbit serum (Dingguo, Beijing, China) instead of primary antibody in each set of slides stained. Subsequently, the sections were incubated with Dako EnvisionTM Peroxidase (Dako Diagnostica, Hamburg, Germany) for 30 min at RT. The antibody staining was visualized with 3, 3'-diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MS). Finally, the slides were counterstained with Mayer's hematoxylin, rinsed in tap water, dehydrated, placed in xylene, and mounted at RT.

Evaluation of the staining

Stained sections were evaluated in a blind manner by two independent investigators with-

out prior knowledge of histopathologic features and patient information. The scores were determined by combining the proportion of positive cells and the intensity of staining as previously described [23, 24]. The extent of staining was scored as follows: 0 (0~4% positive cells), 1 (5~24% positive cells), 2 (25~49% positive cells), 3 (50~74% positive cells), and 4 (75~100% positive cells). The immunostaining intensity was graded as follows: 0, no yellow particles existed; 1, light yellow particles existed; 2, general yellow particles existed; and 3, deep yellow particles existed. The final score of 0~12 was obtained by multiplying the 2 scores above. We defined high-level expression of S100A11 protein as a score of 6 or more; a score from 0 to 5 denoted low or negative S100A11 expression.

Statistics

Statistical analyses were performed with SPSS 15.0 software package for Windows. The

Table 2. The association between S100A11 expression and clinicopathologic features

Variable	n	S100A11 expression		P
		Low	High	
Age (years)				0.089
0: ≤50	31	5	26	
1: >50	38	13	25	
FIGO stage				0.018*
1: I + II	13	7	6	
2: III + IV	56	11	45	
Clinical response to chemotherapy				0.108
0: PR + CR	31	11	20	
1: All others	38	7	31	
Performance status				0.698
0: No impairment	41	10	31	
1: All others	28	8	20	
Pretreatment Serum CA125 (U/ml)				0.902
0: ≤500	26	7	19	
1: >500	43	11	32	
Ascitic fluid volume (ml)				0.132
0: ≤100	28	10	18	
1: >100	41	8	33	
Residual disease (cm)				0.043*
0: ≤2	48	16	32	
1: >2	21	2	19	

Abbreviations: PR, partial remission; CR, complete remission. *Significant at the 0.05 level.

Kruskal-Wallis H and Mann-Whitney tests were applied to compare S100A11 expression among different groups. The correlation between S100A11 expression and the clinicopathologic parameters was evaluated by χ^2 X-test. Overall survival was defined as time from first day after surgery to death or last follow-up date. Survival rates were calculated by using Kaplan-Meier method and difference between survival curves was analyzed by the log-rank test. The significance of various variables for survival was assessed by the Cox proportional hazards model. $P < 0.05$ in all cases was considered statistically significant.

Results

S100A11 expression in normal ovarian epithelia, benign cystadenomas, SBTs and LGSCs

Diverse intensities of immunoreactivity to S100A11 staining were observed in the cyto-

plasm and nucleus of epithelium cells (**Table 1; Figure 1**). Low S100A11 expression was observed in 85.2% normal epithelia, 66.1% benign tumors, 45.5% SBTs and 26.1% LGSCs. Fourteen point eight percent of epithelia, 33.9% benign tumors, 54.5% SBTs and 73.9% LGSCs showed high S100A11 expression. The blank controls showed negative S100A11 staining. The staining pattern in LGSC was diffuse and widespread, and almost all of the cancer cells were stained for S100A11. Undoubtedly, clear differences in staining intensity of the four groups were observed ($P = 0.000$). Moreover, S100A11 expression was gradually increased from normal epithelium to benign and borderline serous tumors, and then to LGSC. The expression level of S100A11 was directly proportional to the degree of malignancy.

Correlation between S100A11 expression and clinicopathological parameters in LGSCs

Table 2 summarizes the relationship between different clinicopathologic characteristics and S100A11 expression in LGSCs. A significant correlation was observed between S100A11 expression and FIGO stage ($P = 0.018$). Patients with higher clinical stage exhibited stronger S100A11 staining. Besides, S100A11 expression was positively correlated with patients' residual disease in surgery ($P = 0.043$). S100A11 overexpression was associated with increased residual disease. The association between S100A11 expression and the other clinicopathological variables, such as age, clinical response to chemotherapy, performance status, pretreatment serum CA125 level and ascitic fluid volume, did not reach statistical significance.

S100A11 expression and patient survival

Kaplan-Meier analysis of this group of LGSC cases showed significant differences in overall survival ($P = 0.043$) between patients with high S100A11 expression and low S100A11 expression (**Figure 2**). The overall 5-year survival rates of patients with high and low S100A11 expression were 43.7% and 64.9%, respectively.

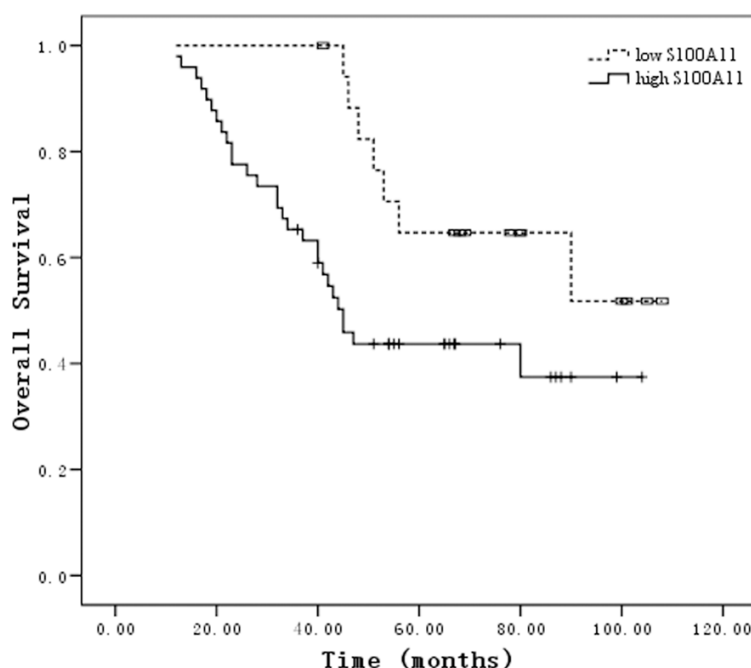


Figure 2. Survival analysis of S100A11 expression in patients with LGSC. Kaplan-Meier curve for overall survival in LGSC patients with different S100A11 expression degrees.

Univariate survival analysis showed FIGO stage, clinical response to chemotherapy, residual disease and S100A11 expression were prognostic factors for overall survival. Multivariate survival analysis revealed that FIGO stage, clinical response to chemotherapy and S100A11 expression were independent prognostic factors for overall survival (**Table 3**).

Discussion

In the dualistic model of ovarian carcinogenesis, the precursor of HGSC is not well characterized and therefore has been described as arising de novo from fallopian tube epithelium [25]. Conversely, the precursor of invasive LGSC has been approved. LGSC arises via transformation of benign and borderline serous tumors. However, the molecular mechanism related to the malignant transformation process is still not clear.

To date, 25 different proteins have been assigned to S100 family. Twenty-one of them are coded by genes clustered at chromosome locus 1q21, known as the epidermal differentiation complex (EDC) involving in epithelial-derived cell differentiation [26]. A few S100

proteins contribute to the malignant transformation of cells. For instance, S100A4 has been indicated to regulate cell motility and invasion in an in vitro model for breast cancer metastasis [27] and related to a more aggressive phenotype in numerous cancers [28-31]. S100A6 overexpression in colorectal cancer enhances tumor growth in vivo [32]. Overexpression of human S100A7 or its murine homologue mS100a7a15 promotes mammary tumorigenesis through upregulation of inflammatory pathways [33]. S100A11 expression differs in normal colonic epithelium, colon adenoma and colorectal carcinoma [34]. In addition, another immunohistochemistry study on a series of benign, premalignant, malignant and metastatic prostate

cancer tissues found frequent dysregulated expression of S100A11 in cancer and precursor lesions, together with an association with high histological stage, suggests that S100A11 may be involved in prostate cancer development and progression [35].

Mutations in the p53 tumor suppressor gene are common in HGSC, whereas mutations in the proto-oncogenes BRAF and K-ras are more frequently encountered in its low-grade counterpart [36, 37]. A previous study investigated the proteome modulated by oncogenic KRAS in immortalized airway epithelial cells and found S100A11 as one of the potential downstream targets, suggesting it could be an important molecule participating in RAS-mediated carcinogenesis [38].

It has also been suggested that epithelial-mesenchymal transition (EMT) is involved in the progression from non-invasive SBT to invasive LGSC [39-41]. Several members of S100A11 family have been indicated to be involved in EMT and metastasis [42-45]. A recent research found knockdown of S100A11 increased the expression of E-cadherin in ovarian cancer cell, indicating S100A11 may also be involved in EMT [46].

Table 3. Univariate and multivariate analyses for overall survival rates of individual parameters

	Univariate			Multivariate		
	HR	95% CI	P	HR	95% CI	P
Age	0.777	0.398-1.515	0.458	0.555	0.236-1.306	0.131
FIGO stage [†]	3.258	1.666-6.373	0.001*	2.357	1.159-4.791	0.018*
Clinical response to chemotherapy	3.215	1.614-6.404	0.001*	2.191	1.033-4.646	0.041*
Performance status	0.561	0.269-1.170	0.123	0.526	0.228-1.210	0.131
Pretreatment Serum CA125	0.994	0.967-1.023	0.689	0.992	0.932-1.055	0.792
Ascitic fluid volume	0.938	0.476-1.846	0.852	0.622	0.261-1.480	0.283
Residual disease	2.004	1.016-3.954	0.045*	2.351	0.729-3.479	0.243
S100A11 expression [‡]	2.374	1.029-5.477	0.043*	3.248	1.315-8.020	0.011*

Abbreviation: HR, hazard ratio; 95% CI, 95% confidence interval. [†]I + II vs III + IV. [‡]low vs high. *Significant at the 0.05 level.

In the present study, we found S100A11 expression gradually increased within four degrees of ovarian epithelial lesions (normal, benign tumor, borderline tumor and LGSC), implying S100A11 might play a role in the malignant transformation and development of ovarian epithelial cell. Though, as a limitation, the total case number of LGSC is limited because of low incidence, significant correlations were still found between S100A11 expression and FIGO stage as well as S100A11 expression and residual disease. To further evaluate the role of S100A11, the correlation between its expression and LGSC patients' survival was analyzed. We found that high level S100A11 expression was associated with decreased overall survival of patients with LGSC. Subsequent univariate and multivariate analyses showed that the S100A11 index was an independent prognosticator for LGSC patients. Our findings strongly suggested that S100A11 may be involved in the progression of LGSC and it could be a prognostic biomarker in LGSC.

Though detail mechanism related to the oncogenic of LGSC remained to be uncovered, our study strongly suggests that S100A11 may play a role in the canceration of ovarian epithelial cells and contribute to the carcinogenesis of LGSC. Our findings also may have important clinical implications in prognosis of the LGSC.

Acknowledgements

This study was supported financially by the National Natural Science Foundation of China (No. 81302252).

Disclosure of conflict of interest

None.

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