Low expression of S100P is associated with poor prognosis in patients with clear cell adenocarcinoma of the ovary

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Abstract

Objective: The S100P protein stimulates cell proliferation and survival, thereby contributing to cancer progression. The purpose of this study was to evaluate S100P expression in ovarian clear cell adenocarcinoma and to determine whether S100P

expression was correlated with the clinicopathological features or prognoses of patients with clear cell adenocarcinoma.

Methods/materials: We examined S100P expression in 30 ovarian clear cell adenocarcinoma specimens using immunohistochemistry analysis. The Kaplan-Meier method was used for analysis of overall survival (OS), and comparisons were made based on the log-rank test.

Results: Negative staining for nuclear S100P was associated with a poor prognosis as compared with that of positive staining for nuclear S100P in specimens from patients with clear cell adenocarcinoma.

Conclusions: These data suggested that S100P may serve as an independent prognostic factor and marker for acquired resistance to chemotherapeutic drugs in clear cell adenocarcinoma.

Keywords: Clear cell adenocarcinoma; ovary; S100P; prognosis; immunohistochemistry

Introduction

Clear cell adenocarcinoma of the ovary (CCA) has been recognized as a distinct histologic entity in the World Health Organization classification of epithelial ovarian carcinoma (EOC) since 1973 (1). Although CCA represents less than 10% of all EOC diagnosed in the United States (2), the incidence of CCA has been shown to account for more than 15% of all EOC in Japan (3). In addition, at the time of diagnosis, approximately half of CCA cases are early-stage tumors without intraperitoneal lesions. Nevertheless, owing to their potential resistance to chemotherapeutic agents, the clinical outcomes of patients with CCA are generally poorer than those of patients with other types of EOC, such as serous adenocarcinoma (4). Therefore, it is critical to identify clinical indicators of CCA in order to develop strategies for monitoring tumor status and improving prognoses (5).

The human S100P protein belongs to the S100 subfamily of calcium-binding proteins, which share a common Ca^{2+}-binding structural motif, termed the EF-hand (6). Twenty members of the S100 subfamily have been identified to date, and S100P, a 95-amino acid protein first purified from the placenta, is one of the least characterized

members of this family. Despite this, the molecular structure of S100P has been studied extensively. Interestingly, S100P has been shown to have a restricted cellular distribution; S100P is initially produced in the cytoplasm and then remains localized within the cytoplasm and/or nucleus (7). Altered expression of S100P proteins has been documented in many human diseases and cancers. For example, overexpression of S100P has been detected in pancreatic (8), colon (9), breast (10), prostate (11), lung (12), and ovarian cancers (13). Moreover, we previously described the expression of S100P in ovarian mucinous tumors (14); our data showed that S100P expression decreases with increasing degrees of atypia and polarity turbulence (benign < borderline < malignant), and the pattern of S100P expression may be useful as an auxiliary means of diagnosis for ovarian mucinous cystic tumors. Additionally, recent reports have shown that S100P contributes to chemosensitivity to carboplatin and paclitaxel in ovarian cancers (15).

In the present study, we analyzed the expression of S100P in CCA. In particular, we examined the expression of S100P in CCA tissues by immunohistochemistry to determine whether S100P expression was correlated with clinicopathological factors or prognoses of patients with CCA.

Materials and methods

Patients and tissue samples

Thirty human CCA tissues were obtained from patients who underwent surgery for CCA at National Hospital Organization Nagasaki Medical Center between 2001 and 2012. All patients provided informed consent for participation in the study and use of their tissues before surgery was performed. Our study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

The definition of CCA was based on the classification proposed by the World Health Organization, and the specimens were evaluated by two pathologists (YU and MI). We enrolled patients with CCA based on following inclusion criteria: (1) availability of complete clinical information, (2) availability of long-term follow-up data, and (3) availability of tissue slides for immunohistochemical staining. We reviewed data from 30 cases of CCA (median patient age: 53 years, range: 35–85 years). Surgical treatment consisted of total hysterectomy, bilateral salpingo-oophorectomy, and omentectomy. All patients received postoperative platinum-based chemotherapy. Tumor

recurrence/progression was defined based on clinical, radiologic, or histologic diagnosis.

Immunohistochemical staining and evaluation

Tumor samples were fixed with 10% formaldehyde, embedded in paraffin, and sectioned into 3-μm-thick slices. Immunohistochemical staining was performed using the EnVision system (Dako, Tokyo, Japan). Endogenous peroxidase activity was inhibited, and antigen retrieval was performed in a pressurized chamber in citrate buffer (pH 6.0). Primary antibody staining was then carried out using rabbit polyclonal anti-S100P antibodies (sc-98913, 1:100 dilution; Santa Cruz Biotechnology, CA, USA). The sections were counterstained with hematoxylin and mounted. Evaluation of the immunohistochemical results was scored by two pathologists (YU and MI), who were blinded to the clinical data. To evaluate the expression of CCA, 10 representative fields, including cancerous and noncancerous tissues, were selected for each slide. The proportion of S100P-positive cells was semiquantitatively measured using the following scale according to the percentage of positive tumor cells: 0 (< 1% positive cells), 1+ (1–

10% positive cells), 2+ (11–50% positive cells), and 3+ (> 51% positive cells). The expression of S100P in cancer cells was defined as positive when the immunohistochemical score was 2+ or 3+ for the nucleus or cytoplasm; nuclear and cytoplasmic staining were separately evaluated. For the survival analysis, total expression of S100P was classified as negative (0/1+ nuclear expression and 0/1+ cytoplasmic expression) or positive (2+/3+ nuclear expression and/or 2+/3+ cytoplasmic expression).

Statistical analysis

Statistical analysis of group differences was carried out using $\chi 2$ tests and Fisher's exact tests. The Kaplan-Meier method was used for analysis of overall survival (OS), and comparisons were made based on the log-rank test. Differences or correlations with *P* values of less than 0.05 were considered statistically significant. OS was defined as the time between the date of surgery and the last date of follow-up or the date of death due to any cause. Statistical analysis was performed using GraphPad Prism 5.0d.

Results

Immunohistochemical expression of S100P in CCA tissues

As shown in FIGURE 1, S100P immunoreactivity varied among the tissue sections. There was little S100P detected in the tumor stroma. Of the 30 CCA specimens examined in this study, S100P was expressed in the nucleus and cytoplasm in all cases, although the extent of expression varied. Of the 30 carcinomas, nine (30%) were negative for nuclear S100P (0/1+ nuclear expression), while 21 (70%) were positive for nuclear S100P (2+/3+ nuclear expression; TABLE 1). In the cytoplasm, five cases (17%) were negative for S100P (0/1+ cytoplasmic expression), while 25 cases (83%) were positive for S100P (2+/3+ cytoplasmic expression).

Correlations with clinicopathological features, including age, International Federation of Gynecology and Obstetrics (FIGO) stage, and recurrence, are shown in TABLE 2.

Analysis of the association between S100P expression and survival in patients with CCA

Patient survival was evaluated for a mean follow-up period of 50 months (range, 6–101 months). None of the patients were lost to follow-up. At the end of the follow-up period, eight patients (27%) had recurrence and died of the disease. The survival rates of patients with CCA having negative nuclear S100P expression were significantly lower than those of patient with CCA having positive nuclear S100P expression ($p = 0.001$, FIGURE 2A). There were no significant differences between the survival rates of patients with CCA having positive cytoplasmic S100P expression and negative cytoplasmic S100P expression ($p = 0.135$, FIGURE 2B). The hazard ratios were 0.0636 (95% confidence interval [CI], 0.0124–0.3260) in the nuclear S100P group and 0.1617 (95% CI, 0.0148–1.765) in the cytoplasmic S100P group.

Clinical features and site of recurrence in deceased patients

TABLE 3 shows the clinical features in the eight deceased patients. Of these eight patients, six (75%) exhibited negative nuclear S100P expression.

Discussion

In general, patients with CCA, even early-stage CCA, have a greater risk of recurrence and poorer survival than those with other histologic types of EOC, despite administration of platinum-based adjuvant chemotherapy. S100P has been shown to be a potential target molecule for therapeutic applications. In this study, we found that S100P was expressed at low levels in specimens from patients with aggressive CCA. Patients with CCA lacking nuclear S100P expression showed poorer prognosis after surgery than those with positive nuclear S100P expression. Thus, our results suggested that the immunohistochemical detection of S100P expression in CCA may be a useful indicator of prognosis and clinical outcomes.

Surowiak et al. (13) provided the first evidence that S100P expression could be an independent prognostic factor for patients with EOC. They analyzed 44 primary EOC samples and showed that S100P expression was associated with an unfavorable prognosis, with significant reductions in median progression-free survival and OS of 15 and 27 months, respectively. However, none of their patients had CCA. In the current study of 30 patients with CCA, low S100P expression was observed in 26.7% (8/30) of CCA samples, and patients with low S100P expression had markedly poorer prognoses

than those with high S100P expression. Thus, our findings indicated that immunohistochemical detection of S100P expression in CCA may be a useful indicator for selecting patients with a high risk of unfavorable clinical outcomes and for stratifying patients to facilitate better therapeutic strategies.

The role of S100P in resistance to chemotherapeutic drugs remains unclear. Cisplatin induces DNA damage, which has been reported to cause a rapid increase in S100P expression at the transcript level (16), suggesting that S100P has pro-apoptotic effects in response to cisplatin administration. In bladder cancer cells, S100P expression is associated with cisplatin sensitivity, providing a novel molecular marker for prediction of cisplatin sensitivity (17). In addition, S100P was recently found to be involved in determining the chemosensitivity of human ovarian cancer cells. In vitro analyses conducted by Wang et al. (15) and Gao et al. (18) indicated that cultured ovarian cancer cells with low S100P expression were resistant to paclitaxel while those with higher S100P expression rendered the cells more susceptible to paclitaxel and carboplatin. These reports suggested that S100P may play a role in tumor susceptibility to chemotherapy. Moreover, decreasing S100P expression has been shown to improve

cell survival in response to paclitaxel treatment, suggesting that S100P may enhance the performance of chemotherapeutic agents by reducing drug resistance. However, the expression of S100P has been shown to be an unfavorable prognostic factor in many types of cancer. In this regard, we consider our data encouraging. Paclitaxel-based adjuvant chemotherapy should be considered in patients with high S100P expression, although the effects of S100P in clinical drug resistance need to be fully elucidated in additional studies.

Our present study was very preliminary and had several limitations, such as the small number of cases, possibility of type II errors, variable follow-up periods, and different treatment protocols during the long-term study period. Thus, we propose that there may be an association between low S100P expression and poor clinical outcomes in patients with CCA. Although additional analysis is needed to clarify the molecular mechanisms of S100P in CCA cell biology, our evidence supports that S100P may be involved in CCA metastasis or chemoresistance.

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Figure Legends

FIGURE 1. Immunoreactivity of S100P observed in CCA. Immunostaining intensity was scored as follows: a (nuclear: 3+, cytoplasmic: 3+); b (nuclear: 1+, cytoplasmic: 1+). Original magnification: 200×.

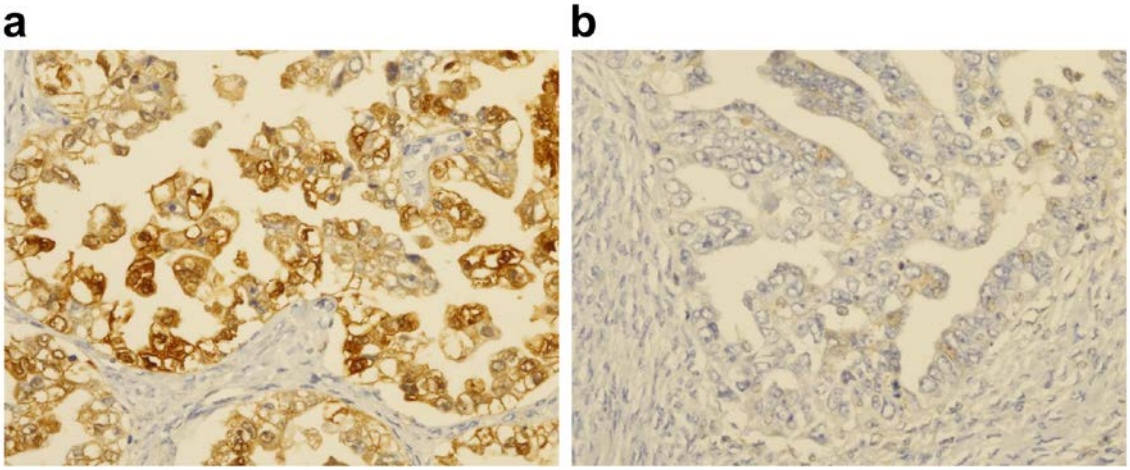

FIGURE 2. Survival curves were created based on immunohistochemical analysis in cases of nuclear (a) and cytoplasmic (b) staining.

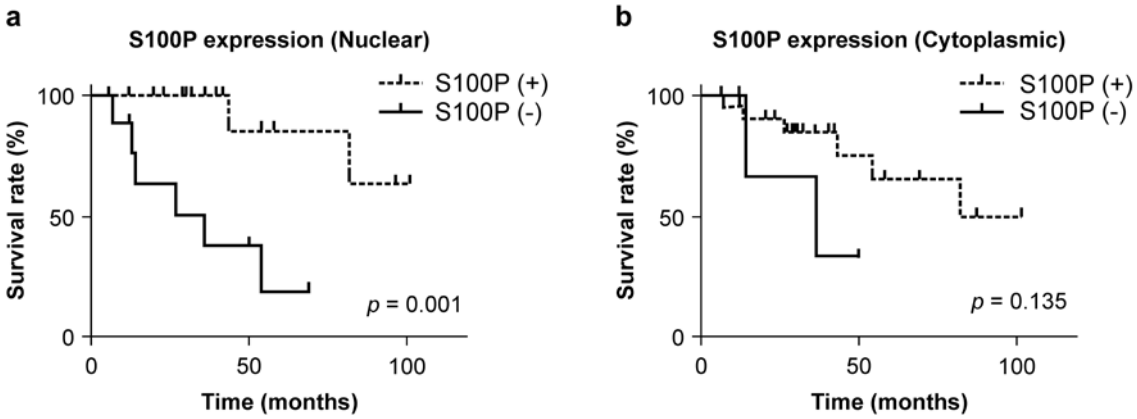

Table 1. Immunohistochemical scores and percentages of S100P-positive cells in clear cell carcinoma

	Immunohistochemical scores				
	Negative		Positive		
	0	**1+**	**2+**	**3+**	**Positive cases (%)**
S100P (nuclear)	0	9	18	3	21 (70)
S100P (cytoplasmic)	0	5	22	3	25 (83)

Table 2. Correlations between clinicopathological features and S100P expression in clear cell carcinoma

	S100P (nuclear)			S100P (cytoplasmic)		
	Positive	Negative		Positive	Negative	
	n = 21	**n = 9**	***p* value**	**n = 25**	**n = 5**	***p* value**
Age						
≤ 50	7	3	> 0.999	7	3	0.300
> 51	14	6		18	2	
FIGO stage						
I	16	4	0.115	17	3	> 0.999
II-IV	5	5		8	2	
Recurrence						
-	18	3	0.008	19	2	0.143
+	3	6		6	3	
Nuclear dysplasia						
mild	7	7	0.0457	11	3	0.642
severe	14	2		14	2	
Vascular invasion						
-	18	7	0.622	22	3	0.183
+	3	2		3	2	
Hobnail change						
-	9	6	0.427	12	3	> 0.999
+	12	3		13	2	

p53							
	-	13	5	> 0.999	15	3	> 0.999
	+	8	4		10	2	
Ki-67							
	-	18	8	> 0.999	23	3	0.119
	+	3	1		2	2	

Table 3. Clinical features and location of recurrence in deceased patients

Patient no.	**Age (years)**	**Stage**	**Initial operation**	**Chemotherapy**	**Follow-up**	**S100P expression**	
					(months)	Nuclear	Cytoplasmic
1	56	1a	TAH + BSO + OM	None	54	negative	positive
2	66	1c	TAH + BSO + OM	PTX + CBDCA	82	positive	positive
3	76	4a	TAH + BSO + OM	PTX + CBDCA	14	negative	negative
4	64	3c	TAH + BSO + OM	PTX + CBDCA	13	negative	positive
5	48	1c	TAH + BSO + OM	PTX + CBDCA	36	negative	negative
6	85	3c	TAH + BSO + OM	PTX + CBDCA	27	negative	positive
7	65	3c	TAH + BSO + OM	PTX + CBDCA	6	positive	positive
8	35	3b	TAH + BSO + OM	CPT + CDDP	7	negative	positive

TAH = Total abdominal hysterectomy; BSO = Bilateral salpingo-oophorectomy; OM = Omentectomy

PTX = Paclitaxel; CBDCA = Carboplatin; CPT = Irrinotecan; CDDP = Cisplatin