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S100P is a useful marker for differentiation of ovarian mucinous tumors

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Summary

The S100P protein stimulates cell proliferation and survival, thereby contributing to tumor progression. The purpose of this study was to evaluate S100P expression in the three subtypes of mucinous cystic tumors, cystadenomas, borderline tumors, and adenocarcinomas. The authors examined nuclear S100P expression in 60 mucinous ovarian tumor specimens, including 24 specimens of mucinous cystadenoma, 15 of borderline tumors, and 21 of adenocarcinomas. Immunohistochemistry revealed S100P expression followed one of three patterns: (1) Expressed in most nuclei of mucinous epithelial cells, (2) sporadic (spotted or patchy) expression, or (3) absent or rarely expressed in the nuclei of mucinous epithelial cells. Most adenomas showed the first expression pattern, and borderline tumors often showed a patchy expression pattern. Adenocarcinomas generally demonstrated absence of S100P expression. These data suggest that S100P is a useful histological marker to differentiate between benign, borderline, and malignant mucinous tumors of the ovary.

Key words: Mucinous tumor; Ovary; S100P; Immunohistochemistry.

Introduction

Morphological differentiation of ovarian mucinous cystic tumors is sometimes difficult, and distinction between benign, borderline, and malignant lesions is important for determining prognosis and treatment. For instance, pathologists waver in diagnosis between mucinous benign tumors and slightly atypical borderline tumors or between highly atypical borderline tumors and adenocarcinomas. The present authors examined the usefulness of S100P immuno- histochemistry in improving the diagnostic accuracy of ovarian cancer.

S100P protein is a member of the S100 subfamily of calcium-bound proteins; its expression is predominantly observed in the placenta, as well as various human tissues and tumors. S100P promotes cancer progression via its specific roles in cell proliferation, survival, angiogenesis, and metastasis. Signal transduction pathways and the regulatory molecules that mediate these effects include Ca^{2+} ions [1], the receptor for advanced glycation end products (RAGE)-dependent pathway proteins [2], ezrin [3], calcyclin-binding protein/Siah-1-interacting protein (CacyBP/SIP) [4], and cathepsin D [5].

Although the molecular function of S100P is hitherto unclear, an association between S100P overexpression and poor prognosis of pancreatic [6], colorectal [7], lung [8], breast [9], and prostate [10] cancers has been reported. However, only one report has demonstrated S100P expression in the ovaries; Surowiak *et al.* briefly described expression of this protein in malignant ovarian tumors [11].

The present authors investigated S100P expression in the three subgroups of ovarian mucinous cystic tumors, namely, cystadenoma, borderline tumors, and adenocarcinomas, in order to characterize S100P expression during tumor progression toward malignancy and to assess whether this protein can serve as a clinical biomarker for differentiation of benign, borderline, and malignant tumors.

Materials and Methods

Tissue samples

Tumor tissue specimens were collected from patients who underwent standard surgical treatment and histopathological examination at the National Hospital Organization Nagasaki Medical Center between 2001 and 2010. The present study protocol conformed to the ethics guidelines of the 1975 Declaration of Helsinki, and informed consent was obtained from all patients before these specimens were used for the study.

The examined specimens of ovarian mucinous tumors included 24 cystadenomas, 15 borderline tumors, and 21 adenocarcinomas. Tumor definition was based on the World Health Organization classification and the specimens were evaluated by two pathologists (Y.U. and M.I.).

When a given specimen contained a mixture of two or more tumor subtypes, the histological diagnosis with the higher or highest malignancy level was adopted, respectively. Patients with ovarian and multiple other coexistent cancers and those who had undergone preoperative chemotherapy were excluded.

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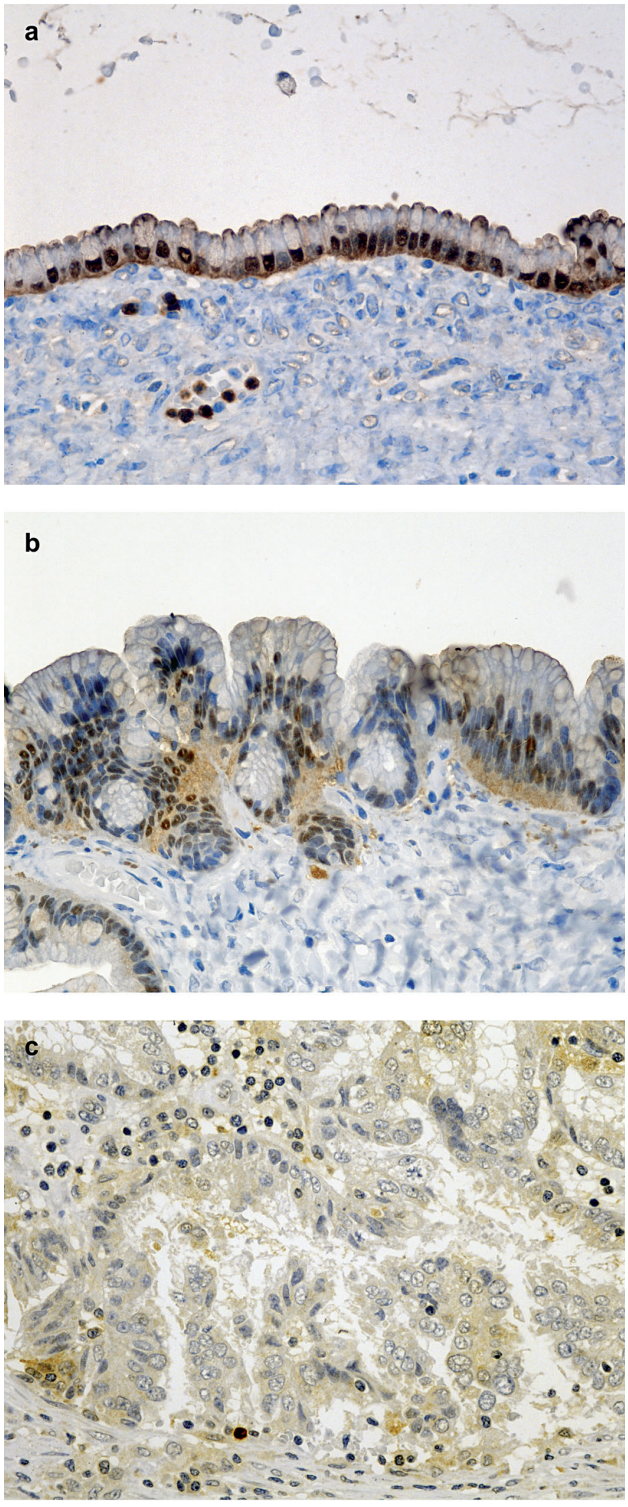

Figure 1. — Differential SP100 expression pattern in ovarian mucinous tumors. S100P expression was divided into three patterns. Benign tumors often show strong nuclear expression in the basally arranged tumor cells (a), borderline malignant tumors predominantly demonstrating a non-contiguous, patchy expression in an irregular villiform fashion (b), and malignant tumors are mainly characterized by the absence of nuclear expression in most cells (c).

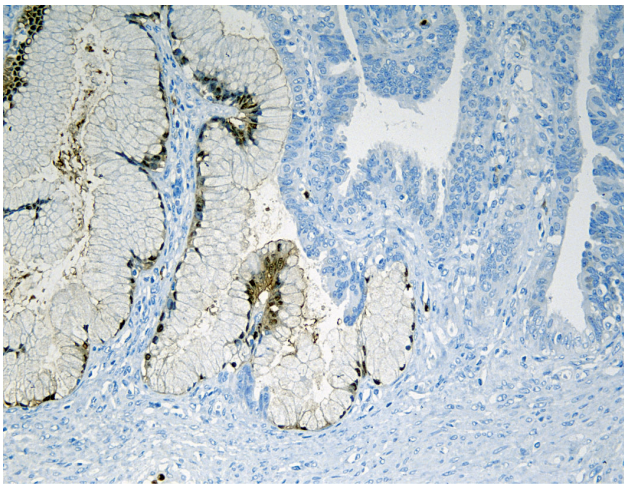

Figure 2. — A case of mucinous borderline tumor associated with carcinoma *in situ*. S100P expression is absent in the carcinoma *in situ* on the right side.

Table 1. — *Immunohistochemical staining patterns of S100P in ovarian mucinous tumors.*

	S100P expression			
	Pattern 1	Pattern 2	Pattern 3	*p*
Cystadenoma (n = 24)	19	5	0	
Borderline Tumor (n = 15)	0	13	2	<0.001
Adenocarcinoma (n = 21)	0	8	13	

Pattern 1: Positive staining in almost all neoplastic cells.
Pattern 2: Staining present in some or even most but not in all neoplastic cells.
Pattern 3: Isolated positive cells or absent in most neoplastic cells.

Immunohistochemical staining and evaluation

Each tumor tissue was fixed with 10% formalin, embedded in paraffin, and cut into three-µm slices. Immunohistochemistry was performed with a specific system and an autostainer after quenching of endogenous peroxidase with 0.3% aqueous hydrogen peroxide solution. Antigen retrieval was performed by heating samples in citrate buffer (pH 6.0) using a pressurized heating chamber. The rabbit polyclonal anti-S100P antibody (1:100 dilution) was used as the primary antibody. Immunostaining images were analyzed to determine the distribution and percentage of positively stained cells, and the intensity of chromatic responses. The results were classified into three staining patterns, as shown in Figure 1. S100P was either expressed in most nuclei of mucinous epithelial cells (Figure 1a), sporadically expressed (spotted or patchy) (Figure 1b), or absent or rarely expressed in the nuclei of mucinous epithelial cells (Figure 1c).

The stains were evaluated by two pathologists (Y.U. and M.I.) who were not informed about the clinical course (including treatment) of the concerned patients. The chromatic responses were also classified into the three aforementioned patterns.

Statistical Analysis

Intergroup differences were analyzed by Chi square analysis. A $p < 0.05$ was regarded as statistically significant. Statistical analysis was performed using the computer program Prism 5.0d.

Results

Table 1 shows the chromatic responses, which were divided into three patterns, in cystadenomas, borderline malignant tumors, and adenocarcinomas of ovarian mucinous cystic tumors. Most mucinous cystadenomas showed pattern 1 (79%, 19/24), borderline tumors predominantly demonstrated pattern 2 (86.7%, 13/15), and adenocarcinomas showed either pattern 2 (38%, 8/21) or pattern 3 (61.9%, 13/21). S100P expression patterns significantly differed between the 3 cancer subtypes ($p < 0.01$).

Figure 2 shows a case of mucinous borderline tumor associated with carcinoma *in situ*. S100P was absent in the carcinoma *in situ* region (pattern 3), and patchy expressed was observed in the borderline malignant tumor (pattern 2), clearly highlighting the immunohistochemical difference between borderline malignant tumors and adenocarcinomas.

Discussion

Morphological differentiation between ovarian mucinous cystic tumors is sometimes difficult, especially when differentiating between benign and slightly atypical borderline malignant tumors or between highly atypical borderline malignant tumors and adenocarcinomas. To achieve accurate diagnosis for mucinous cystic tumors, auxiliary diagnostic techniques, such as immunohistochemistry, if available, are extremely helpful.

This study revealed two new findings regarding S100P expression in ovarian mucinous tumors. First, S100P expression decreased with increasing degrees of atypia and polarity turbulence (benign < borderline < malignant). Second, morphological distinction between benign, borderline malignant, and malignant tumors was possible on the basis of S100P expression profile. Differences in S100P expression between the three different mucinous tumors were statistically significant (Table 1). These findings suggest that the pattern of S100P expression is useful as an auxiliary means of diagnosis for ovarian mucinous cystic tumors.

Although the exact molecular function of the S100P protein is unknown, the present results suggest a possible involvement of S100P in the differentiation and polarity of ovarian mucinous tumors.

To date, associations between increased S100P expression in tumor tissues and low survival rates have been reported for a variety of malignancies, such as pancreatic, breast, and colorectal cancers. In bile duct lesions, S100P expression increases with the degree of atypia and is reduced in cholangiocarcinomas [12]. These findings suggest that the role of S100P varies in different tumor histology and organs.

The significance of S100P expression in ovarian cancer has not been clarified. Surowiak *et al.* studied 73 cases of ovarian cancer; they reported that excessive S100P expression was associated with tumor recurrence and shortened progression-free survival, although their analysis was not based on the different histological types of ovarian cancer [11]. *In vitro* analyses conducted by Gao *et al.* [13] and Wang *et al.* [14] indicated that cultured ovarian cancer cells with low S100P expression were resistant to paclitaxel, whereas those with high S100P expression rendered the cells more susceptible to paclitaxel and carboplatin. These reports suggest that S100P may play a role in tumor susceptibility to chemotherapy. However, the molecular mechanisms of S100P and tumor susceptibility have not yet been further elucidated.

In conclusion, the present authors showed that S100P expression in ovarian mucinous tumors decreases as tumor atypia increases and that its differential expression profiles allow distinction between benign, borderline, and malignant tumors. These findings suggest that S100P is associated with tumor differentiation, and S100P immunohistochemistry is a useful marker for differentiation between the different subtypes of ovarian mucinous cystic tumors.

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