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ORIGINAL PAPER

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Changes in the expression of E-cadherin repressors, Snail, Slug, SIP1, and Twist, in the development and progression of ovarian carcinoma: the important role of Snail in ovarian tumorigenesis and progression

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Abstract Changes in the expression of E-cadherin have been reported to be important in the tumorigenesis and progression of epithelial ovarian carcinoma. To further examine the mechanisms regulating E-cadherin expression in ovarian tumorigenesis, we investigated the immunohistochemical expression of transcriptional repressors for E-cadherin, such as Snail, Slug, SIP1, and Twist, in the ovarian surface epithelium (OSE) and 95 cases of epithelial ovarian tumors. OSE cells were negative for SIP1 and Slug, whereas weak expression of Snail and Twist was observed in 8 (73%) and 3 (27%) cases, respectively. Of 95 ovarian tumors, the expression of Snail, Slug, SIP1, and Twist increased stepwise in benign, borderline, and malignant tumors. Among them, the expression of Snail showed significantly inverse correlation with that of E-cadherin. Regarding the FIGO stage classification, the expressions of Snail and Twist were significantly increased in advanced cases. The prognosis of ovarian carcinoma patients positive for Snail expression was poorer than that of negative patients. Our results indicate that the expression of E-cadherin transcriptional repressors increased with malignancy in ovarian epithelial neoplasms and that the expression of E-cadherin and its negative regulators is altered during ovarian cancer development and peritoneal dissemination.

Key words Snail · Ovarian carcinoma · E-cadherin repressors · Slug · Twist

Introduction

Ovarian carcinoma is the leading cause of death from female genital malignancies, and more than half of all patients are diagnosed at the advanced stage with widespread dissemination in the peritoneal cavity.[1] The prognosis of advanced carcinoma patients is most likely related to the degree of peritoneal dissemination. Although the process of cancer metastasis appears to be regulated by a variety of gene products, little is known about the molecular aspects for the progression of ovarian carcinoma cells. Peritoneal dissemination is a unique metastatic process in which cancer cells detach from the primary tumor, attach to the peritoneum, and regrow at this site.[2] One of the important events for metastasis is the reduction of adhesion between tumor cells, and E-cadherin is known to play a key role in epithelial cell adhesion.[3] In malignant neoplasms, inactivation of E-cadherin has been reported to occur via various mechanisms such as gene mutation, promoter hypermethylation, post-translational modification, and transcriptional repression.[4–7]

In epithelial ovarian tumors, however, upregulation of E-cadherin has been reported to be essential in the development of epithelial neoplasms from their histogenetic origin, ovarian surface epithelium (OSE).[8] On the other hand, reduced expression of E-cadherin has been observed in ovarian carcinomas, being associated with tumor progression.[9–11] We reported that the expression of E-cadherin along with that of α-, β-, and γ-catenins was significantly decreased in ovarian carcinomas with an advancing stage of disease. Interestingly, we also found that E-cadherin expression in metastatic lesions was re-elevated.[12] Thus, the expression of E-cadherin seems to change drastically during ovarian carcinoma development and progression; however, the molecular mechanism for the change in E-cadherin expression is largely unknown. Recent studies identified transcriptional repressors such as Snail, Slug, SIP1, and Twist, and the expression of these molecules has been reported to show an inverse correlation with that of E-cadherin in various malignancies.[6,13–15] All these molecules have been reported to be important in epithelial-mesenchymal transition (EMT) in cancer cells.[7,16] In ovarian carcinoma cells, our study first demonstrated that a hypoxic microenvironment plays an important role in downregulation of E-cadherin via upregulation of the transcriptional repressor Snail, suggesting the transcriptional regulation of E-cadherin in ovarian tumorigenesis.[17]

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In this study, to address the possible importance of E-cadherin repressors in the development and progression of ovarian carcinoma, we systematically examined the expressions of Snail, Slug, SIP1 and Twist, along with E-cadherin expression, in normal OSE and benign, borderline, and malignant ovarian tumors using immunohistochemistry, reverse transcription-polymerase chain reaction (RT-PCR), and Western blot analyses. In addition, we compared the expressions of these molecules between primary and metastatic lesions of advanced ovarian carcinoma. Finally, we also analyzed the prognostic significance of the expression of these molecules in ovarian carcinoma patients.

Materials and methods

Tissue samples

A total of 95 primary epithelial ovarian tumors were examined for immunohistochemistry. Sixty-eight consecutive patients with ovarian carcinoma visited Shinshu University Hospital and underwent surgery. None had received preoperative chemotherapy or radiotherapy. Specimens from these tumors were fixed in 10% phosphate-buffered formalin and embedded in paraffin. Serial sections of 3-μm thickness were made for hematoxylin and eosin staining and for immunohistochemistry. Histologically, 14 of the 95 tumors were benign (5 serous and 9 mucinous cystadenomas), 13 were borderline (4 serous and 9 mucinous tumors), and 68 were carcinomas (23 serous, 7 mucinous, 17 endometrioid, and 21 clear cell adenocarcinomas). Of the 68 carcinomas, 37 were classified as stage I (2 serous, 6 mucinous, 11 endometrioid, and 18 clear cell adenocarcinomas), 10 as stage II (6 serous and 4 endometrioid adenocarcinomas), 18 as stage III (13 serous, 1 mucinous, 1 endometrioid, and 3 clear cell adenocarcinomas), and 3 as stage IV (2 serous and 1 endometrioid adenocarcinomas) according to the International Federation of Gynecology and Obstetrics (FIGO) classification. For RT-PCR and Western blotting, fresh surgical specimens were obtained from 13 women with epithelial ovarian tumors who underwent operation and stored at –80°C. Each tissue was used with the approval of the Ethics Committee of Shinshu University, after obtaining written informed consent from the patient.

Immunohistochemistry

Immunohistochemical staining for SIP1 was performed by the streptavidin–biotin–peroxidase complex method using a Histofine SAB-PO kit (Nichirei, Tokyo, Japan). After deparaffinization and rehydration, the sections were boiled in antigen retrieval solution (Nichirei) at 120°C for 20 min and then treated with 0.3% hydrogen peroxide and incubated with 10% normal goat serum to block nonspecific binding. The primary antibody, anti-SIP1:L-20 goat polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), was used at a dilution of 1:25.

For Snail, Slug, and Twist immunostaining, a Simple Stain MAX-PO kit was used. After deparaffinization and rehydration, the sections were boiled in 0.01 M citrate buffer (pH 6.0) for 25 min in a microwave oven. The primary antibodies used were polyclonal anti-Snail:H-130, polyclonal anti-Slug:H-140, and polyclonal anti-Twist:H-81(Santa Cruz Biotechnology), which were used at a dilution of 1:50. After incubation with the primary antibody at 4°C overnight, the sections were washed in phosphate-buffered saline (PBS) and incubated with biotinylated antigoat or antirabbit immunoglobulin G (IgG), followed by treatment with peroxidase-conjugated streptavidin, and stained with diaminobenzidine and 0.15% hydrogen peroxidase. Counterstaining was performed with hematoxylin.

Staining scores were established by semiquantitative optical analysis, using the product of the percentage of positive cells and staining intensity from 1 to 3 (1 weak, 2 moderate, and 3 strong) and therefore ranging from 0 to 300. Immunostaining was evaluated by two independent observers (J.Y. and A.H.) who were unaware of the patients or the tissue sites. The results of immunostaining were classified as negative (–) when the staining score was 0 to 30, weakly positive (+) when the staining score was 31 to 150, and strongly positive (++) when the staining score was 151 to 300. Cytoplasmic immunostaining was observed sporadically in the tumor cells.

Western blotting

Extracts equivalent to 50 μg total protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (8% acrylamide) and transferred onto polyvinylidene difluoride (PVDF) membranes (Invitrogen, Carlsbad, CA, USA). The primary antibodies against Snail (H-130), Slug (H-140), SIP1 (L-20), Twist (H-81) (Santa Cruz), and β-actin (Sigma-Aldrich) were used in Tris-buffered saline Tween-20 (TBST) containing 5% nonfat dry milk. The membranes were then incubated with donkey antigoat or goat antirabbit IgG (Amersham Biosciences, NJ, USA) in TBST containing 2% nonfat dry milk. Bound antibodies were detected with an enhanced chemiluminescence system (Amersham Biosciences).

RT-PCR

RNA was extracted using Trizol (Invitrogen) according to the manufacturer's protocol. One microgram of total RNA was treated with 1 U/10 μl DNase (Life Technologies, Gaithersburg, MD, USA). Reverse transcription (RT) was carried out using an RNA polymerase chain reaction (PCR) kit (Tanaka Shuzo, Otsu, Japan). Then, 1 μl RT products, containing 50 ng reverse-transcribed total RNA, was amplified by PuReTaq Ready-To-Go PCR Beads (Amersham Biosciences) with 0.2 μM of oligonucleotide primer sets. Primers were synthesized to encompass a specific segment of the cDNA sequence of the Snail (sense 5′-TTCCAG CAGCCCTACGACCAG-3′ and antisense 5′-CGGACT

Table 1. Immunohistochemical expression of Snail, Slug, SIP1, Twist, and E-cadherin in normal ovarian surface epithelium (OSE) and epithelial ovarian neoplasms

	Total	SNAIL			SLUG			SIP			TWIST			E-cadherin		
		–	+	++	–	+	++	–	+	++	–	+	++	–	+	++
Normal OSE	11	3 (27)	5 (45)	3 (27)	11 (100)	0	0	11 (100)	0	0	8 (72)	0	3 (27)	11 (100)	0	0
Benign cystadenoma	14	5 (35)	4 (28)	5 (35)	11 (78)	3 (27)	0	13 (93)	1 (7)	0	12 (86)	2 (14)	0	5 (36)	3 (21)	7 (50)
Borderline tumor	13	5 (38)	6 (46)	2 (16)	8 (61)	5 (38)	1 (8)	7 (53)	6 (46)	0	7 (53)	6 (46)	0	4 (30)	4 (30)	5 (38)
Malignant tumor	68	12 (18)	30 (44)	26 (38)	23 (34)	26 (38)	19 (28)*	43 (63)	22 (32)	3 (4)	27 (40)	24 (35)	17 (25)*	16 (24)	32 (47)	20 (29)
Histological type																
Serous	23	3 (13)	8 (35)	12 (52)	6 (26)	13 (57)	4 (17)	19 (83)	4 (17)	0	6 (26)	5 (22)	12 (52)*	8 (34)	9 (39)	6 (26)
Mucinous	7	2 (29)	5 (71)	0	1 (14)	2 (29)	4 (57)	4 (57)	3 (43)	0	2 (29)	3 (43)	2 (29)	1 (14)	3 (43)	3 (43)*
Endometrioid	17	4 (24)	8 (47)	5 (29)	5 (29)	6 (35)	6 (35)	9 (53)	8 (47)	0	8 (47)	7 (41)	2 (12)	2 (12)	8 (47)	7 (41)
Clear cell	21	3 (14)	9 (43)	9 (43)	11 (52)	5 (24)	5 (24)	11 (52)	7 (33)	3 (14)	11 (52)	9 (43)	1 (5)	5 (24)	12 (57)	4 (19)
FIGO stage																
I	37	6 (16)	21 (56)	10 (27)	14 (38)	11 (30)	12 (32)	23 (62)	12 (32)	2 (5)	17 (46)	15 (41)	5 (14)	8 (22)	19 (51)	10 (27)
II	10	2 (20)	3 (30)	5 (50)	1 (10)	6 (60)	3 (30)	6 (60)	4 (40)	0	4 (40)	2 (20)	4 (40)	2 (20)	5 (50)	3(30)
III	18	3 (17)	8 (44)	7 (39)**	7 (39)	8 (44)	3 (17)	11 (61)	6 (33)	1 (6)	5 (28)	7 (39)	6 (33)*	3 (17)	6 (33)	9 (50)*
IV	3	1 (33)	0	2 (67)	1 (33)	1 (33)	1 (33)	3 (100)	0	0	1 (33)	0	2 (67)	2 (67)	1 (33)	0

The intensity of staining was scored as negative (–) to indicate immunostaining score less than 30, positive (+) to indicate immunostaining score 30–150 and strong positive (++) to indicate immunostaining score 150–300

* $P < 0.01$; ** $P < 0.05$

CTTGGTGCTTGTGGA-3′) (BC012910), Slug (sense 5′-AAGCATTTCAACGCCTCCAA-3′ and antisense 5′-AAGGTAATGTGTGGGTCCGA-3′) (NM003068), SIP1 (sense, 5′-GCGGCATATGGTGACACA-3′ and antisense, 5′-TGCCACTAAACCCGTGTGTA-3′) (AB056507), Twist (sense 5′-GAGGCGCCCCGCTCTTCTCC-3′ and antisense 5′-AGCTCCTCGTAAGACTGCGGAC-3′) (NM000474), and E-cadherin (sense 5′-TTCCTCCCAA TACATCTCCCTTCACAGCAG-3′ and antisense, 5′-CGAAGAAACAGCAAGAGCAGCAGAATCAGA-3′) (AB025106). The corresponding cDNA fragments were denatured at 94°C for 1 min, annealed at 50°–56°C for 1 min, and extended at 72°C for 1 min for 30–40 cycles.

Statistical analysis

Values represent the means ± SD. Fischer's exact test, the Kruskal–Wallis test, and Mann–Whitney's *U* test were used to assess differences. Spearman's rank correlation was used to determine whether there was a positive or negative correlation. The log-rank test was used to evaluate significant predictors of survival. Cumulative survival was also analyzed by the Kaplan–Meier method. Differences were considered significant at $P < 0.05$. These analyses were performed using the StatView system (Abacus, Berkeley, CA, USA).

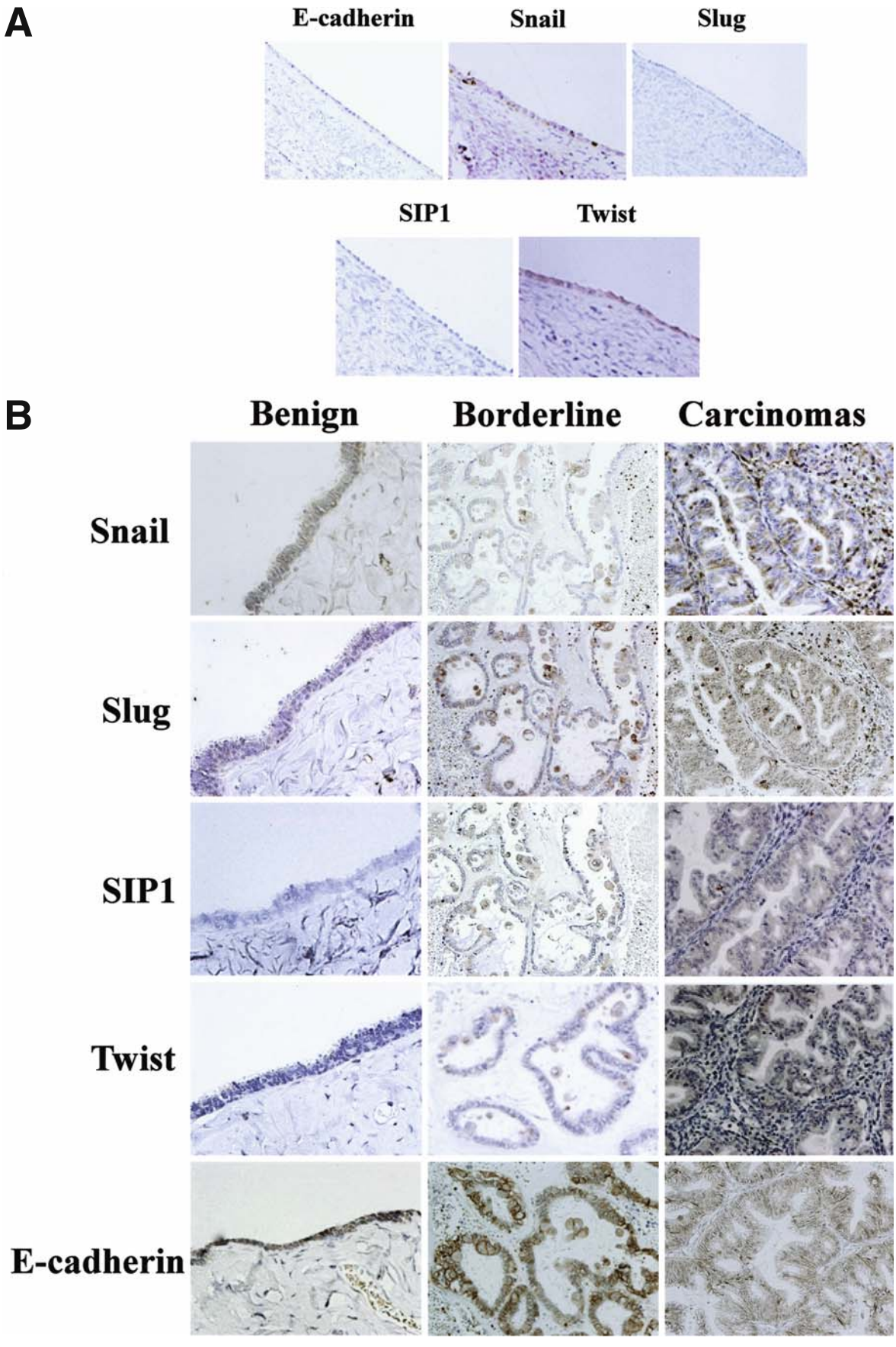
Results

Immunohistochemical expression of Snail, Slug, SIP1, and Twist was increased stepwise in benign, borderline, and malignant tumors

Representative profiles of immunostaining for Snail, Slug, SIP1, Twist, and E-cadherin are shown in Fig. 1A,B. Expression of Snail, Slug, SIP1, and Twist was observed mainly in cytoplasm.[18] Immunostaining for Slug and Snail was observed in cytoplasmic or perinuclear staining, consistent with a previous report.[19] Stromal cells were stained negative for Slug but stained weakly for Snail.

Normal OSE cells of 11 patients were investigated by immunohistochemical analysis. For Slug, SIP1, and E-cadherin, expression was negative in all OSE. In some cases, staining for Snail and Twist was observed at the cell surface of OSE (Fig. 1A). The immunohistochemical expression of Snail in OSE was observed strongly positive in 3 (27%), weakly positive in 5 (45%), and negative in 3 (27%). For Twist, the expression was strongly positive in 3

Fig. 1. A Immunohistochemical staining of E-cadherin, Snail, Slug, SIP1, and Twist in ovarian surface epithelium (OSE). OSE cells showed positive staining for Snail and Twist. ×400. **B** Immunohistochemical staining of Snail, Slug, SIP1, Twist, and E-cadherin in epithelial ovarian tumors. In ovarian tumor cells, immunostaining of Snail, Slug, SIP1, Twist, and E-cadherin is mainly detected in the cytoplasm. Serous benign tumor, ×400; serous borderline tumor, ×100; mucinous adenocarcinoma, ×100


A
E-cadherin
Snail
Slug
SIP1
Twist
B
Benign
Borderline
Carcinomas
Snail
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E-cadherin

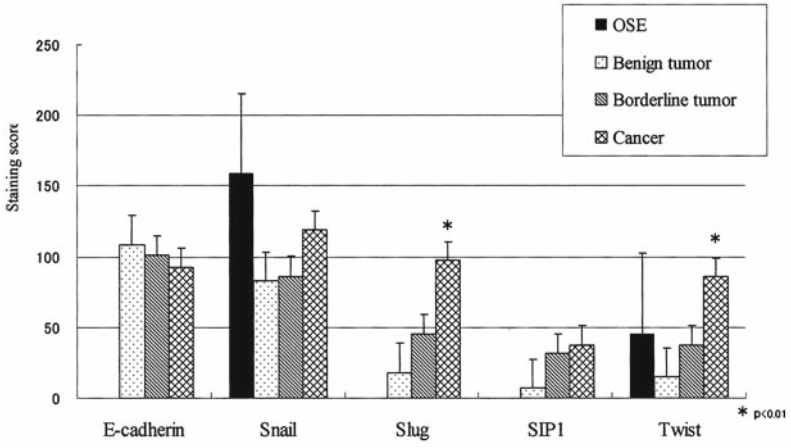

Fig. 2. Immunohistochemical expression of E-cadherin, Snail, Slug, SIP1, and Twist in ovarian surface epithelium (OSE) and epithelial ovarian tumors. The expression of Slug and Twist in carcinomas is significantly increased compared to in benign and borderline tumors ($P = 0.0021$; $P = 0.0015$, respectively). Values represent the mean ± SD

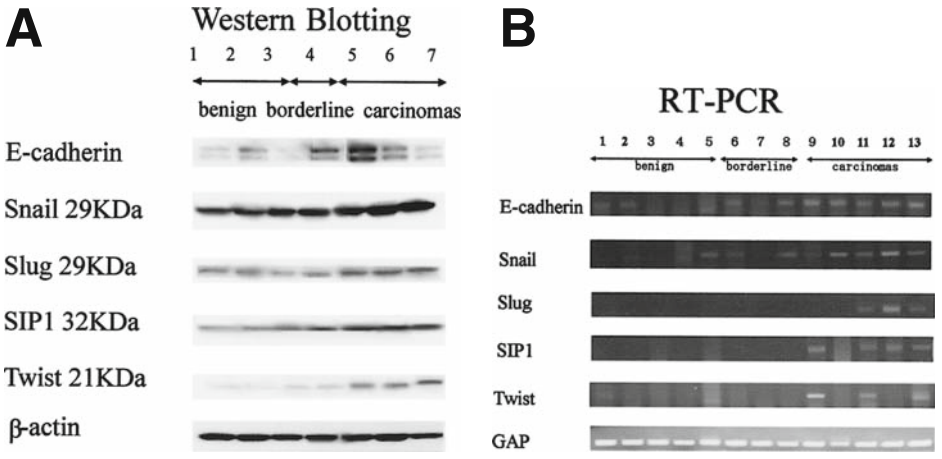

Fig. 3. **A** Western blotting for E-cadherin, Snail, Slug, SIP1, and Twist in epithelial ovarian tumors. Protein expression of E-cadherin, Snail, Slug, SIP1, and Twist is detected by Western blotting. In ovarian carcinomas, the expression of Snail, Slug, SIP1, and Twist is most increased compared to that in benign and borderline tumors. β-Actin expression served as an internal control. **B** Reverse transcription polymerase chain reaction for E-cadherin, Snail, Slug, SIP1, and Twist in epithelial ovarian tumors. The mRNA expression of E-cadherin, Snail, Slug, SIP1, and Twist is detected by reverse transcription polymerase chain reaction. In ovarian carcinomas, the expression of Snail, Slug, SIP1, and Twist is increased more than those in benign and borderline tumors. Glyceraldehyde phosphate dehydrogenase (GAPDH) expression served as an internal control

(27%), weakly positive in 0 (0%), and negative in 8 (72%) (Table 1).

In ovarian neoplasms, the expression of Snail, Slug, SIP1, and Twist was increased stepwise in benign, borderline, and malignant tumors (Fig. 1B). In particular, the expression of Slug and Twist in carcinomas was significantly increased compared to that in benign and borderline tumors ($P = 0.0032$ and $P = 0.0026$; Fig. 2). The expression of Snail, Slug, SIP1, and Twist in borderline tumors was between that of benign and malignant tumors, but the differences were not significant (Fig. 2).

To confirm the results of immunohistochemical staining, we examined the expression of Snail, Slug, SIP1, Twist, and E-cadherin using Western blot and RT-PCR in tissue samples (Fig. 3A,B). By Western blotting, specific bands for SIP1, Slug, Snail, and Twist were detected. In ovarian carcinomas, the expression of SIP1, Slug, Snail, and Twist was increased compared to benign and borderline tumors. In particular, Slug and Twist were most increased in cancers compared to benign and borderline tumors. β-Actin was expressed in all samples. In addition, we examined mRNA expressions of Snail, Slug, SIP1, Twist, and E-cadherin using RT-PCR. Although glyceraldehyde phosphate dehydrogenase (GAPDH) was expressed equally in all samples, the band density of Snail, Slug, SIP1, and Twist in ovarian carcinomas was higher than in benign and borderline tumors. These finding are compatible with the results of immunostaining in tissues of ovarian neoplasms. However, E-cadherin expression of mRNA and protein level did not show the same tendency as immunopositivity of E-cadherin; this might be caused by the tissue sample, which included not only epithelial cells but also stromal cells.

According to the histological type, the expressions of SIP1, Slug, Snail, and Twist were compared (Fig. 4A). All transcriptional repressors showed different expression patterns in each histological type. The strong expression of Twist was observed in 12 of the 23 serous carcinomas (52%),

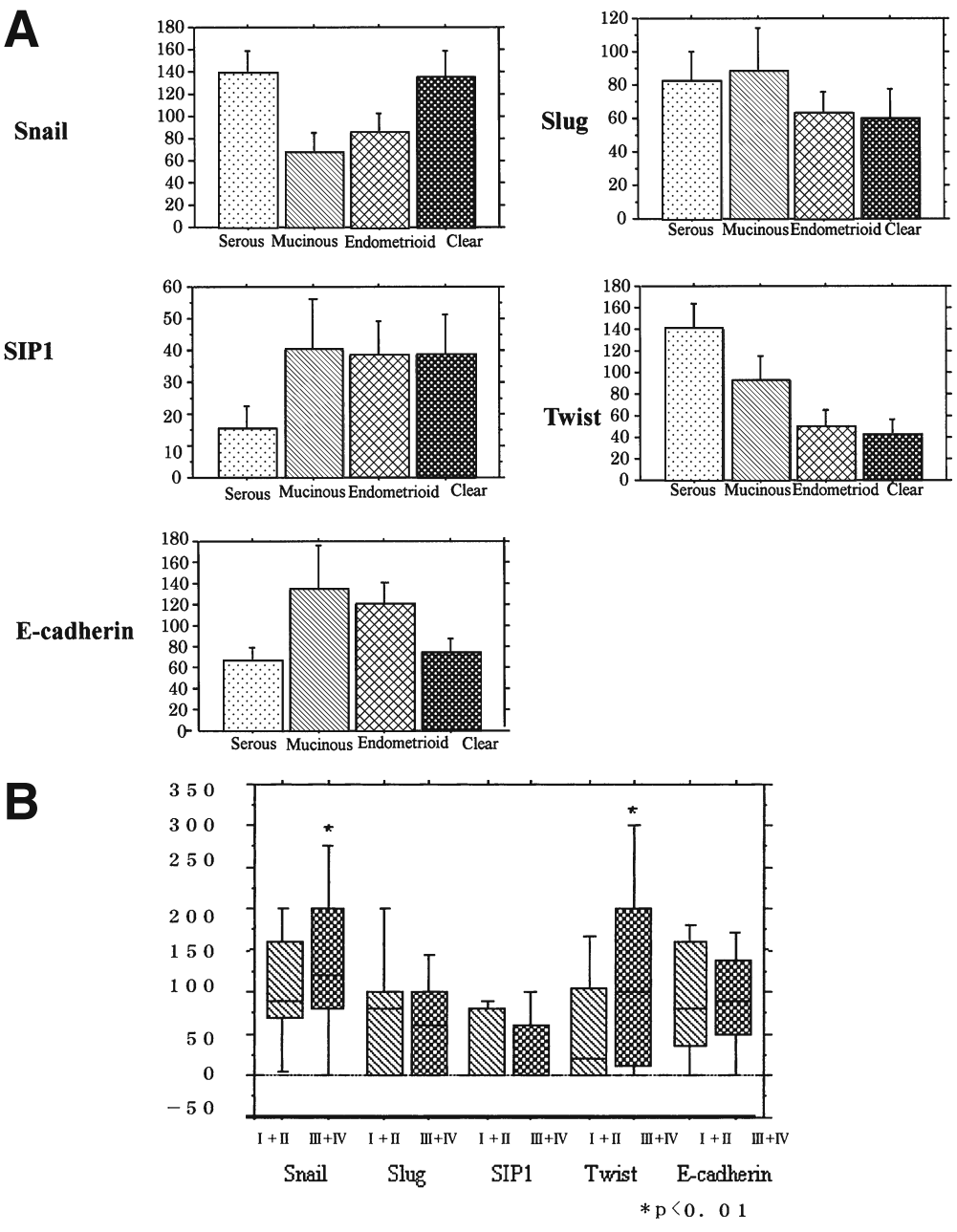

Fig. 4. **A** Immunohistochemical expression of Snail, Slug, SIP1, Twist, and E-cadherin in carcinomas according to histological type. The expression of Twist is significantly highest in serous carcinoma. The expression pattern of Snail showed an inverse relationship with E-cadherin expression pattern. **B** Immunohistochemical expression of Snail, Slug, SIP1, Twist, and E-cadherin in carcinomas according to FIGO stage classification. Positivity for Twist and Snail is significantly increased in stage III + IV tumors compared to stage I + II tumors ($P = 0.041$ and $P = 0.047$, respectively)

1 of the 7 mucinous carcinomas (14%), 2 of the 17 endometrioid carcinomas (12%), and 1 of the 21 clear cell carcinomas (5%). Twist expression in serous adenocarcinomas was significantly higher than in other histological types ($P = 0.0019$). Snail, Sip1, and Slug did not show significant differences among histological types (see Table 1, Fig. 4A). The expression pattern of Snail showed an inverse relationship with E-cadherin expression pattern.

According to the FIGO stage classification, we compared SIP1, Slug, Snail, and Twist expression (see Table 1, Fig. 4B). A strong expression of Twist was observed in 9 of the 47 stage I + II tumors (19%) and 8 of 21 stage III + IV tumors (38%) (Fig. 4B). Positivity for Twist and Snail was significantly increased in stages III + IV tumors compared to stage I + II tumors ($P = 0.041$ and $P = 0.047$, respectively) (Fig. 4B).

The expression of Snail showed a negative correlation with E-cadherin expression in primary lesions

Next, we studied the association of positivity between E-cadherin and transcriptional repressors. By Spearman's rank correlation test, the expression of Snail showed significantly weak negative correlation with E-cadherin ($\delta = -0.209$, $P = 0.042$). Positivity for Slug, SIP1, and Twist did not show a significant correlation with E-cadherin expression (Table 2).

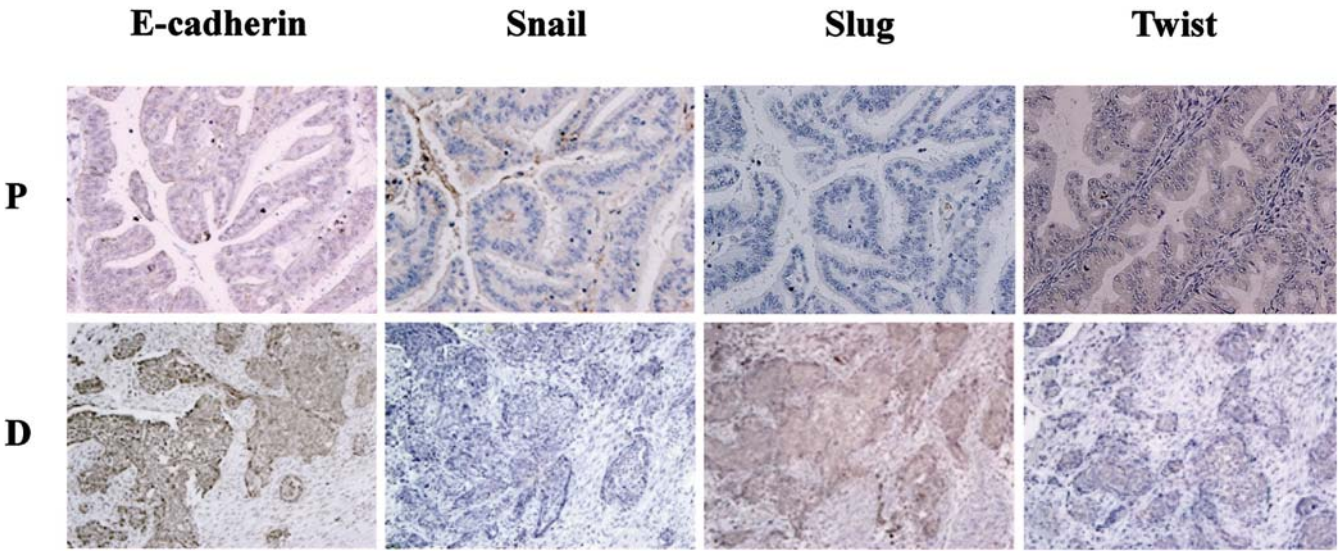

Fig. 5. Immunohistochemical expression of E-cadherin, Snail, Slug, and Twist in primary and disseminated lesions. *P*, primary lesion; *D*, disseminated lesion. The expression of Snail and Twist in peritoneal dissemination was decreased compared to in primary lesions. E-cadherin and Slug expression was increased at metastatic sites

Table 2. Spearman's correlations between the immunostaining of Snail, Slug, SIP1, Twist, and E-cadherin

Marker	Correlation	Snail	Slug	SIP1	Twist	E-cadherin
Snail	Correlation coefficient	1.000				
	P value	0.000				
Slug	Correlation coefficient	0.142	1.000			
	P value	0.168	0.000			
SIP1	Correlation coefficient	0.242	0.286	1.000		
	P value	0.019	0.006	0.000		
Twist	Correlation coefficient	0.322	0.533	0.241	1.000	
	P-value	0.002	0.000	0.020	0.000	
E-cadherin	Correlation coefficient	–0.209	0.053	0.074	–0.007	1.000
	P value	0.042*	0.61	0.471	0.947	0.000

Spearman's rank correlation was used to determine whether there was a positive or negative correlation. The expression of Snail showed significantly negative correlation with E-cadherin
* < 0.05

The expression of E-cadherin transcriptional repressors in peritoneal dissemination was decreased compared to that in primary lesions

We compared the expression of Snail, Slug, SIP1, Twist, and E-cadherin between primary and metastatic lesions of ovarian carcinoma (Fig. 5). Immunostaining results for matched primary and metastatic lesions in 20 cases of advanced ovarian carcinoma are given in Table 2. The expression of Snail, SIP1, and Twist in peritoneal dissemination was decreased compared to that in the primary lesions. Cases with weaker expression in metastatic lesions showed significantly frequent Snail ($P = 0.007$) and Twist ($P < 0.0001$) expression, respectively (Table 3). In contrast, Slug expression was increased in metastatic sites (Table 3).

Table 3. Summary of the expression of Snail, Slug, SIP1, Twist, and E-cadherin in the primary and metastatic lesions of ovarian carcinoma

	P > D	P = D	P < D	*P* value
Snail	16	3	1	0.007
Slug	6	3	11	0.16
SIP1	8	10	2	0.08
Twist	18	1	1	<0.0001
E-cadherin	2	11	7	0.06

P, primary lesion; D, disseminated lesion

Expression of Snail is a poor prognostic factor in ovarian carcinoma patients

All 13 borderline patients were alive with no evidence of recurrence at the last follow-up. Of the 68 patients with

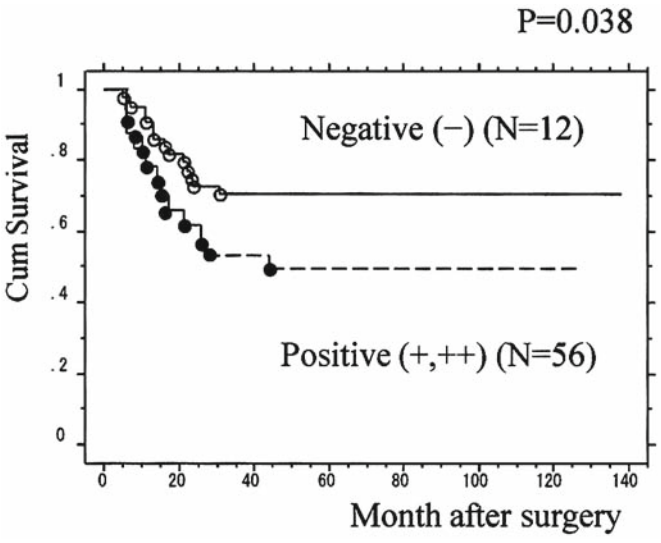

Fig. 6. Overall survival of ovarian carcinoma patients according to the expressions of Snail. Kaplan–Meier analysis showed that prognosis was significantly poorer in patients with strong positive immunostaining for Snail

carcinoma, 26 had died of the disease and 42 were alive. Prognosis was significantly poorer in patients with advanced FIGO stages (overall survival, stage I + II, 62.7 ± 5.3 months versus stage III + IV, 27.9 ± 5.4 months, $P = 0.0001$). In the 68 patients with ovarian carcinoma, the prognostic significance of Snail, Slug, SIP1, Twist, and E-cadherin expression was analyzed using the Kaplan–Meier method. The results obtained by log-rank test showed that prognosis was poorer in patients with positive immunostaining for Snail (strongly positive, 44.9 ± 7.5 months versus negative/weakly, 57.6 ± 5.6 months, $P = 0.038$) (Fig. 6). The prognosis in patients with positive immunostaining for Twist was poorer than that with negative/weakly immunostaining for Twist (strongly positive, 44.9 ± 7.5 months versus negative/weakly, 57.6 ± 5.6 months, $P = 0.082$), but it was not significant. There were no prognostic differences according to immunostaining for Slug, SIP1, or E-cadherin.

Discussion

This study showed the expression of transcriptional repressors for E-cadherin in normal ovarian surface epithelium (OSE) and various epithelial tumors of the ovary. Among repressors such as Snail, Slug, SIP1, and Twist, OSE cells expressed Snail and Twist, but not SIP1 and Slug. Our data showed that OSE cells were negative for SIP1 and Slug; however, expression of Snail and Twist was observed in 8 (73%) and 3 (27%) cases, respectively. Epithelial ovarian tumors, including ovarian carcinomas, are thought to arise in OSE covering the ovarian surface, which is a continuum of the peritoneal mesothelium. OSE cells are known to exhibit both epithelial and mesenchymal characteristics and usually do not express E-cadherin.[8,20] It has been reported that E-cadherin expression in OSE is important in mesenchymal-epithelial transition (MET), which occurs in the early step of epithelial ovarian tumorigenesis.[8] Our current study has shown that normal OSE cells do not express E-cadherin but express Snail and Twist, and that benign cystadenomas express E-cadherin along with the downregulation of Snail and Twist. These findings suggest that the changes in the expressions of Snail and Twist might play a pivotal role in the development of epithelial ovarian tumors via MET.

In our study on various epithelial ovarian neoplasms, the expressions of Snail, Slug, SIP1, and Twist showed a similar tendency of a stepwise increase from benign to borderline, and from borderline to malignant tumors. Statistically, immunopositivity for Slug and Twist was significantly higher in carcinomas than in benign and borderline tumors. Analyses using RT-PCR and Western blotting confirmed that mRNA and protein expressions of Snail, Slug, SIP1, and Twist were higher in carcinomas than in benign tumors. In addition, our study also revealed that, in ovarian carcinomas, the expression pattern of the E-cadherin repressors is different according to the histological types and the FIGO stage classification. The expression of Twist was significantly higher in serous carcinoma, which is more frequently associated with peritoneal dissemination than in other histological types.[21] The expression levels of Snail and Twist were higher in tumors of stage III + IV than stage I + II. Moreover, analysis using the log-rank test showed that the prognosis was significantly poorer in patients with positive immunostaining for Snail. Recently, Hosono et al. examined the expression of Twist in 82 ovarian carcinoma cases, reporting that Twist expression was not correlated with any clinicopathological parameter but was a predictor of poor survival.[22] In other malignancies, overexpression of Snail was shown to correlate with a higher stage or to be a marker of poor prognosis.[23,24] Those findings suggest that overexpression of Snail and Twist plays a role not only in the development of ovarian carcinoma but also in patient survival. In addition, this study implied that Snail expression might be more important than Twist expression in ovarian carcinoma prognosis. Further studies are needed to clarify the role of Snail in the aggressive behavior of ovarian carcinomas.

It is known that the expression of E-cadherin is suppressed by Snail, Slug, SIP1, and Twist; however, molecules participating in the E-cadherin suppression may differ according to the organ in each malignancy. Terauchi et al. demonstrated that the suppression of Twist expression alters cellular morphology and inhibits migration in ovarian cancer cells in vitro.[25] In this study, however, there was an inverse correlation between the expressions of E-cadherin and Snail, but not Twist, in ovarian carcinomas. Our previous study revealed that upregulation of Snail expression followed by the downregulation of E-cadherin enhances the invasiveness of ovarian carcinoma cells.[17] Kurrey et al. recently reported that the ectopic expression of Snail resulted in epithelial-mesenchymal transition (EMT) and enhanced motility and invasiveness.[7] These findings suggest that Snail is important in the suppression of E-cadherin expression and tumor progression in ovarian carcinomas.

Interestingly, our study showed that the immunoreactivity for Snail and Twist was decreased in disseminated lesions compared to that in the respective primary lesions. Such a phenomenon seems paradoxical but is consistent with the re-expression of E-cadherin in metastatic lesions. Loss of E-cadherin has been considered a key process in the initial step of metastasis, although its increased expression in metastatic sites has been reported in breast and gastric carcinomas.[26,27] In ovarian carcinomas, Davidson et al. demonstrated that the expression of E-cadherin and α-, β-, and γ-catenin was upregulated even in ascitic tumor cells of advanced cases.[28] We also previously reported that the expression of E-cadherin and α-, β-, and γ-catenin was upregulated in the tumor cells of peritoneal disseminations compared with those in primary lesion of ovarian cancers.[12] These findings strongly suggest that the expression of E-cadherin changes drastically in the process of peritoneal dissemination of ovarian carcinoma, and the specific repressor may play an important role in the change in E-cadherin expression, possibly depending on the microenvironment of metastatic sites.[29]

In summary, the dynamic changes in E-cadherin expression occur in the development of epithelial ovarian tumors from OSE, the development and progression of ovarian carcinomas, and peritoneal dissemination of ovarian carcinoma cells, and each process is associated with the respective change in the expression of transcriptional repressors. In addition, an increased expression of Snail is a poor prognostic factor in patients with ovarian carcinoma.

Conclusion

Our results indicate that the expression of E-cadherin transcriptional repressors increased with malignancy in ovarian epithelial neoplasms and that the expression of E-cadherin and its negative regulators is altered during ovarian cancer development and peritoneal dissemination.

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