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Original Articles

Syndecan-1 serves as a marker for the progression of epithelial ovarian carcinoma

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Summary

Purpose: Syndecan-1 (SDC-1) promotes the proliferation of cancer cells and plays a role in angiogenesis by binding to a variety of extracellular effectors. The present study was designed to compare the expression of SDC-1 in the normal ovary and in ovarian tumors, to better understand its roles in the progression of epithelial ovarian carcinoma (EOC). *Materials and Methods:* The expression of SDC-1, fibroblast growth factor 2 (FGF-2), and FGF receptor 1 (FGFR1) and their transcripts in 65 samples including the normal ovary, benign tumors, borderline ovarian tumors, and EOC was assessed using immunohistochemistry and the reverse transcription-polymerase chain reaction. The influence of FGF-2 on the expression of *SDC-1* mRNA syndecan-1 in a human ovarian carcinoma cell line was determined using an FGF-2-neutralizing antibody. *Results:* SDC-1 was not detected in normal ovarian tissue but was present in the epithelial cells of benign or borderline tumors and in ovarian adenocarcinomas. The levels of expression were significantly different in ovarian tissues derived from benign or malignant cases. Coordinate stromal expression of SDC-1 and its mRNA was detected at the original site of the tumor, as well as in metastatic foci in the greater omentum of ovarian adenocarcinomas. FGF-2 reduced the level of expression of *SDC-1* mRNA when added exogenously to SKOV3 cells. This effect was abolished in the presence of an FGF-2-neutralizing antibody. *Conclusion*: SDC-1 contributes to the role of FGF-2 in proliferation and angiogenesis but may also play a role in the invasive properties of EOC. To the present authors' knowledge, this study is the first to report the presence of distinct patterns of expression of SDC-1 in local and metastatic foci in the greater omentum in patients with EOC. These data reinforce the role of the tumor stroma in the invasive properties of ovarian adenocarcinoma and suggest that stromal changes in the expression of SDC-1 may originate from the stroma and contribute to the pathogenesis and metastatic potential of EOC.

Key words: Syndecan-1; FGF-2; FGFR1; Ovarian carcinoma.

Introduction

Syndecans are members of a family of cell surface proteoglycans that play regulatory roles in wound healing, inflammation, angiogenesis, and neuronal patterning. There are four members of the syndecan family (syndecan-1, syndecan-2, syndecan-3, and syndecan-4), each comprising an ectodomain carrying heparan sulfate- or chondroitin sulfate-rich glucosaminoglycan chains, a transmembrane domain, and a short cytoplasmic tail. The syndecans can bind structural proteins of the extracellular matrix and growth factors, such as basic fibroblast growth factor (FGF-2) [1, 2]. Syndecan-1 (SDC-1) expression is associated with poorly differentiated tumors, and loss of expression of SDC-1 by epithelial cells correlates with poor clinical outcomes in patients with squamous cell carcinoma of the head and neck [3–5], mesothelioma [6], poorly differentiated non-small cell lung cancer [7, 8] and in patients with hepatocellular carcinoma with high metastatic potential [9]. In gastric cancer [10, 11] and in breast carcinoma [12], the stromal expression of SDC-1 correlates with a poor prognosis. In contrast, pancreatic adenocarcinoma cells overexpress SDC-1 compared with normal pancreatic cells [13], indicating that SDC-1 plays different roles in the growth of tumors.

Epithelial ovarian cancer (EOC) is the second most common female genital tract malignancy after endometrial cancer. They account for approximately 15,000 annual deaths in the United States. Patients often present with disseminated disease because they may be asymptomatic. This can be explained by containment of the tumor cells within the ovary, as well as by the early dissemination of tumor cells to the peritoneal cavity. SDC-1 is expressed by ovarian cells and can be detected within the extracellular matrix [14]. The goal of the present study was to clarify the relationship between the expression of SDC-1 and the progression of EOC.

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Materials and Methods

Subjects and tissues

Patients' clinical data included those for age, menarche, menstrual cycle, pregnancy and childbirth history, serum CA125, mass size, clinical stage, and clinical characteristics. The tumors were staged according to the guidelines of the International Federation of Gynecologists and Obstetricians (FIGO) stage, based on surgical and histological assessments. Samples of frozen ovarian tissue were taken from biopsies of patients admitted to the China Medical University's Shengjing Hospital for Cancer Research during 2003 to 2007. The tissues were cut into five-mm-diameter cubes, frozen in liquid nitrogen within one hour of resection, and stored at –80°C. Samples of normal ovarian tissues were obtained from the archive of the Shengjing Hospital of China Medical University. The Ethics Committee of the China Medical University approved this study. Histological samples were reviewed by a histopathologist specializing in gynecological oncology. Samples that were included in the study were classified as those obtained from the normal ovary, benign ovarian tumors, epithelial tumors of borderline malignancy, or primary ovarian adenocarcinomas. Histological grade was assessed as moderately or poorly differentiated.

Immunohistochemistry

The antibodies used for immunochemistry were as follows: mouse anti-human syndecan-1 antibody, CD138 Ab-1(5F7), rabbit anti-fibroblast growth factor 2 (FGF-2), and rabbit anti-FGF receptor 1 (FGFR1) PV9000 kit.

Embedded tissue samples were cut into seven-µm sections. The slides were placed in a staining rack at room temperature and deparaffinized with dimethyl benzene and rehydrated in a graded alcohol series. After washing with PBS, 3% hydrogen peroxide was used to block non-specific endogenous peroxidase activity. High temperature and pressure were used for antigen retrieval. After blocking with 10% normal serum, slides were incubated with primary antibody overnight at 4°C. Slides were washed in phosphate-buffered saline (PBS), incubated with secondary antibody, and then treated with PV9000 reagents, followed by staining with 3,3-diaminobenzidine and counterstaining with hematoxylin. Slides were dehydrated in a graded alcohol series and mounted for analysis.

Two independent observers who were not aware of the clinicopathological information inspected all slides. The staining patterns were scored using a semiquantitative scoring system. Scores of 0–3 were given according to the intensity and the percentage of cells stained as follows: 0 = no staining, 1 = weak staining, 2 = moderate staining, and 3 = strong staining.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from tissues using the RNAout kit by following the manufacturer's instructions and was stored at -80ºC. Samples (100 mg) were mixed with one ml RNAout dissolved in RNase-free water. Primer sequences were as follows: *SDC-1*, sense: agctgaccttcacactcc and anti-sense: tcggctcctccaaggagt; *FGF2*, sense: agcggctgtactgcaaaaac and anti-sense: cccaggtcctgttttggat; *FGFR1*, sense: ctgggtagcaacgtggagtt and anti-sense: accatgcaggagatgaggaa; *β-actin*, anti-sense: gtggggtgcaccaggcacca and anti-sense: ctccttaatgtcacgcacgatttc.

RT-PCR reactions were performed as follows: rcDNA synthesis was performed in a total volume of ten µl ($MgCl_2$, two µl; 10×RNA buffer, one µl; RNase free dH_2O, 3.75 µl; dNTP, one µl; RNase inhibitor, 0.25 µl; avian myeloblastosis virus reverse transcriptase, 0.5 µl; oligo-dT 0.5 µl; RNA, one µl). The conditions used for the procedure were as follows: 42ºC for 30 minutes, 99ºC for five minutes, and 5ºC for five minutes. For PCR amplification of *β-actin*, *FGF2*, and *FGFR1*, the following conditions were used: total volume, 25 µl (5×RNA Buffer, five µl; dH_2O, 13.75 µl; dNTP, two µl; each primer, 0.5 µl; Taq polymerase, 0.25 µl; cDNA, three µl). The PCR protocol for *β-actin*, *FGF2*, and *FGFR1* was as follows: 94ºC for two minutes; 30 cycles of 94ºC for 30 seconds, 56ºC for 30 seconds, 72ºC for 45 seconds; and 72ºC at seven minutes. The PCR protocol for *SDC-1* was as follows: 94ºC for two minutes; 35 cycles of 94ºC for 45 seconds, 52ºC for 60 seconds, 72ºC for 60 seconds; and 72ºC for seven minutes. PCR products were electrophoresed using 1.5% agarose gels. Data were analyzed using a GIS-2020 gel image analytical system with *β-actin* as the standard.

Total RNA was extracted from cultured cells using the RNAout kit by following the manufacturer's instructions. RT-PCR reactions were performed as follows: Reverse transcription was performed using the reagents described above; the *SDC-1* primers 5'-CCCTGA AGA TCA AGA TGG CTC T-3' (sense) and 5'-CCC GAG GTT TCA AAG GTG AAG T-3' (antisense) (563 bp) and the β-actin primers gtggggtgcaccaggcacca (sense) and ctccttaatgtcacgcacgatttc (anti-sense) were used. PCR reaction conditions for *SDC-1* and *β-actin* were as follows: 30 cycles of 30 seconds at 94˚C and 30 seconds at 55˚C; and extension for one minute at 72˚C. PCR products were analyzed as described above.

Cell culture

SKOV3 cells were cultured in Ham's F-12 medium supplemented with 10% fetal bovine serum (FCS), 50 IU/ml penicillin, 50 µg/ml streptomycin, and 50 µg/ml L-ascorbic acid (F12/FCS); incubated at 37ºC in an atmosphere containing 5% CO_2; and used at passages 2–6. SKOV3 cells grown in 12-well plates (2.5×10^4 cells/well) were serum-starved for 48 hours. FGF-2 (100 pg/ml, one ng/ml, 10 ng/ml) or 10% FCS were then added to each well in each group for 24 hours. Media and growth factors were changed daily.

Statistical analysis

Chi-square tests were performed to compare the frequency of SDC-1 staining in benign and malignant cases by using SPSS version 13.0. A $p < 0.05$ was considered statistically significant. Two independent samples were analyzed as appropriate to compare relative optical densities of benign and malignant cases, and differences between positive staining and negative staining cases were evaluated using SPSS version 13.0. A $p < 0.05$ was considered statistically significant.

Results

Clinical characteristics of patients

The mean age of subjects was 34.4 years (range, 21–63). The ages of members of the EOC group were higher than those of patients with benign or borderline lesions. The values of serum CA 125 of the EOC group were significantly higher than those of the other groups, and there were significant differences in the sizes of foci of patients with EOC compared with those of the other three cases. The most common symptom of EOC was abdominal distension. The majority of the lesions were benign, followed by 60% and 36% frequencies of Stage III and Stages I-II, respectively (Table 1).

The expression of SDC-1, FGF-2, and FGFR1 in local foci of ovarian lesions

SDC-1 was detected in three cases (10%) of benign lesions (two endometrial cysts, one teratoma) and was present around

Table 1. — *Clinical data*

	Normal (2)	Benign (27)	Borderline (5)	Epithelial ovarian cancer (26)
Age	43 ± 12.7	34.4 ± 12.8	42.8 ± 16.7	54.4 ± 9.4
Menarche	13.5 ± 0.7	14.1 ± 1.8	14 ± 1.2	15.1 ± 2.3
Cycle	30 ± 0	28.3 ± 2.3	31.6 ± 4.8	29.2 ± 1.6
Focus	4.2 ± 1.4	7.5 ± 4.6	9.7 ± 6.1	10.5 ± 4.4
Gravidity	1 ± 0	1.7 ± 1.4	1.8 ± 1.1	2.7 ± 1.3
Parity	0.5 ± 0.7	0.6 ± 0.6	1 ± 1.2	1.4 ± 0.9
Serum CA 125 (u/ml)	10.4 ± 4.7	57.8 ± 86.8	132.8 ± 105.8	846.5 ± 623.1
Stage I				7
II				2
III				15
IV				1
Chief complaint				
Asymptomatic		15	3	
Abdominal pain		6		5
Menstruation disorder		3	2	1
Dysuria		1		
Abdominal distension		4		14
Mass		1		3
Anorexia				1
Low-grade fever				1
Types of disease				
Teratoma		9		
Endometrial cyst		10		
Cystadenoma		2		
Serous cyst		2		
Simple cyst		2		
Mucous cyst		3		
Adenofibroma		2		
Boundary cystadenoma			5	
Cystadenocarcinoma				25

the membrane and in the cytoplasm of glandular epithelial cells, and staining was scored as 1–2. In one case (25%) of a borderline lesion, staining was present in the cytoplasm of glandular epithelial cells and scored as 1. In eight cases (32%) of EOC (Stage III), staining was localized to membranes and, in seven cases, to the cytoplasm of the glandular epithelial cells. Staining in two cases was localized to the stroma and was assigned scores of 1–2. The expression of SDC-1 was undetectable in normal ovarian tissue (Figure 1).

The expression of FGF-2 was detected in three (10%) patients with benign lesions (one endometrial cyst, two teratomas) and staining (scored 1–2) was localized to the cytoplasm of glandular epithelial cells. In eight (32%) patients with EOC (three, Stage I; five, stage III), staining was localized to the cytoplasm of glandular epithelial cells (scored 2–3). FGF-2 expression was undetectable in either normal ovarian tissue or in borderline lesions.

The expression of FGFR1 was be detected in two (6.7%) patients with benign lesions (two endometrial cysts), and staining was localized to the cytoplasm of glandular epithelial cells (scored 1–2). In six (32%) patients with EOC (two, Stage I; four, Stage III), staining was localized to the cytoplasm of glandular epithelial cells (scored 1–2). FGFR1 expression was undetectable in normal ovarian tissue or in borderline lesions (Figure 2).

Levels of expression SDC-1, FGF2, and FGFR1 in the primary foci of ovarian lesions

The differences in the levels *SDC-1* and *FGF2* mRNAs between benign lesions and EOC agreed with the results of the immunohistochemical analyses. In contrast, the level of expression of *FGFR1* mRNA differed between those of patients with benign lesions and with EOC ($P < 0.05$). There was also a significant difference between staining patterns and the levels of mRNA expression of the positive and negative groups (Figure 3).

The expression of syndecan-1, FGF-2, and FGFR1 in metastatic foci present in the greater omentum

SDC-1 was detected in eight patients with EOC (one, Stage IV; seven, Stage III), among which staining was localized to the glandular epithelium in four cases, the stroma in seven cases (+), and to both the glandular epithelium and stroma in three cases (score for glandular epithelium = 2, score for the stroma = 2–3). The expression of FGF-2 was detected in four patients with EOC (Stage III) and in one

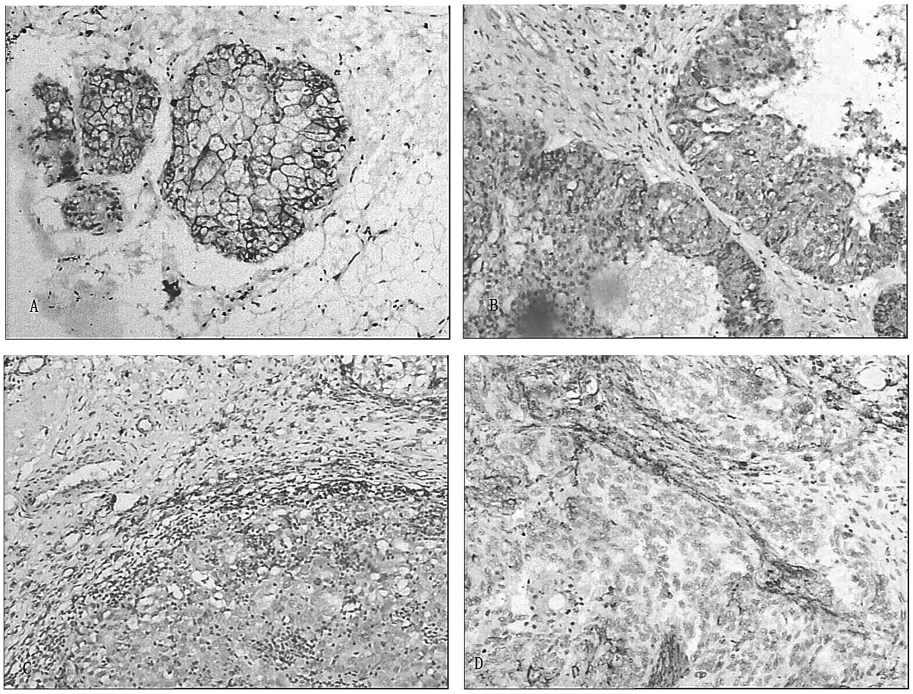

Figure 1. — SDC-1 expression. A) Epithelial ovarian carcinoma (FIGO Stage III) showing strong membrane and cytoplasmic staining of the adenoepithelium; the procedure involved an anti-SDC-1 antibody. B) Moderate (grade 2) staining of the membranes of focal epithelial cells and moderate (grade 2) staining of stromal cells in a frozen section of a local focus of a poorly differentiated Stage III serous ovarian adenocarcinoma. C) Moderate (grade 2) stromal cell-specific staining in a frozen section of a local lesion of a poorly differentiated Stage III serous ovarian adenocarcinoma. D) Moderate (grade 2) stromal cell staining of a frozen section of a metastatic focus present in the greater omentum of a poorly differentiated Stage III serous ovarian adenocarcinoma. The epithelial cells are negative.

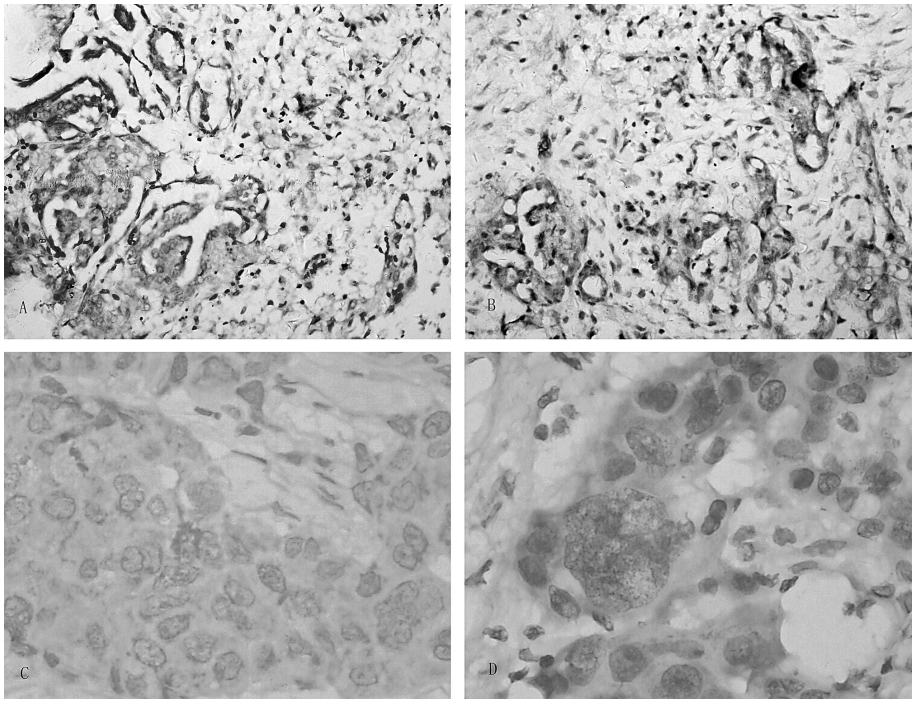

Figure 2. — FGF-2 and FGFR1 expression. A) Ovarian adenocarcinoma (FIGO Stage III) showing strong staining by an antibody against FRFR1 of the cytoplasm of glandular epithelial cells. B) Ovarian adenocarcinoma (FIGO Stage III) showing moderate staining of the cytoplasm of glandular epithelial cells by an antibody against FGR-2. C) Moderate staining of the cytoplasm of glandular epithelial cells by an antibody against FGF2 in a frozen section of a metastatic focus located in the greater omentum of a poorly differentiated (FIGO Stage III) serous ovarian adenocarcinoma. D) Moderate staining of the cytoplasm of glandular epithelial cells by an antibody against FGFR1 in a frozen section of a metastatic lesion located in the greater omentum of a poorly differentiated (FIGO Stage III) serous ovarian adenocarcinoma.

patient with a boundary lesion (score = 1–2, with staining localized to the cytoplasm of glandular epithelial cells). The expression of FGFR1 was detected in nine patients with EOC (Stage III) and one patient with a boundary lesion (score = 1–2, with staining localized to the cytoplasm of glandular epithelial cells (Table 2).

The influence of FGF-2 on the expression of SDC-1 in SKOV3 cells

When cultured in the presence of 10% FCS, SKOV3 cells expressed moderate levels of *SDC-1* mRNA, which increased with time. When FGF-2 was added to the culture, the expression of *SDC-1* mRNA was inhibited and its level

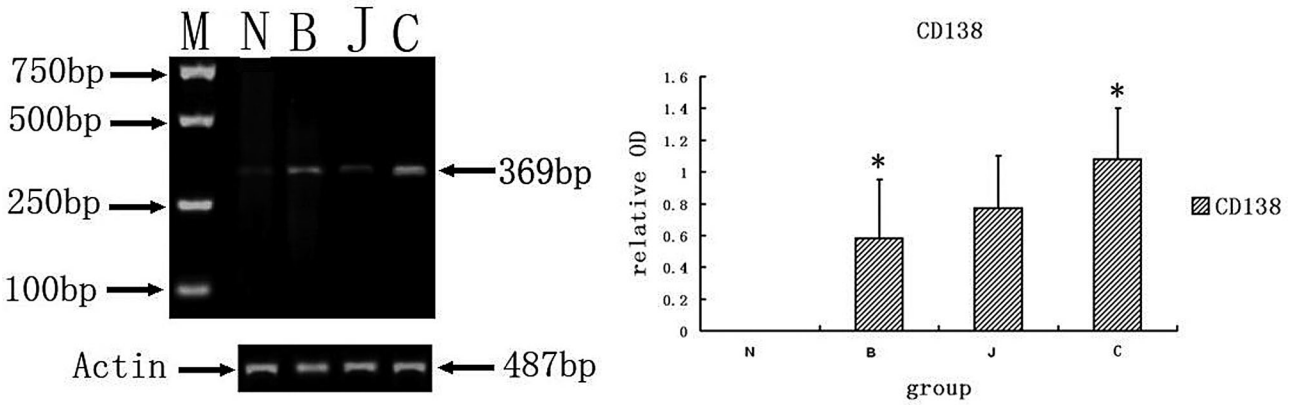

Figure 3. — Detection of *SDC-1* mRNA. M, Marker; N, normal ovarian tissue. mRNA was not detected. B. benign ovarian tumor; J. borderline ovarian cystoadenoma; C. epithelial ovarian carcinoma; *significance difference between B and C.

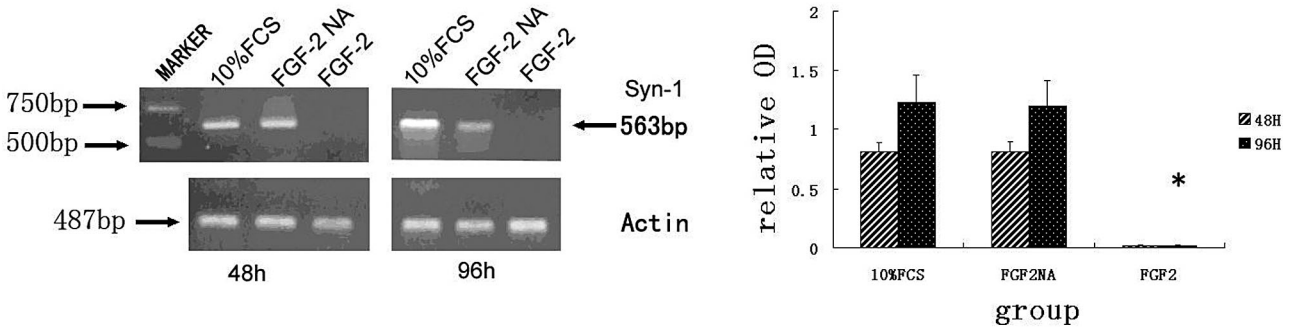

Figure 4. — Detection of *SDC-1* mRNA in SKOV3 cells. 1) *SDC-1* was expressed at moderate levels in the presence of 10% FCS at 48 hours and at a higher level at 96 hours. 2) *SDC-1* mRNA was not detectably expressed by SKOV3 cells treated with FGF-1 for 48 hours and 96 hours. 3) *SDC-1* mRNA was expressed by SKOV3 cells treated with FGF-2 and an FGF-2-neutralizing antibody at 48 hours and was moderately expressed after 96 hours.

did not change with time. When an FGF-2-neutralizing antibody was added, the level of expression of *SDC-1* mRNA was restored to that without FGF-2 and became weaker with time (Figure 4).

Discussion

Epithelial ovarian cancer can occur in female individuals as young as 15 years; however, the mean age at presentation age is 56 years. The median age for presentation of ovarian adenocarcinoma is between 60 and 65 years. In the present study, the age range for patients with EOC was 21–63 years. Childbearing and contraception reduces the risk of developing ovarian cancer. In the present study, the gravidity and parity of patients with EOC were 2.7 ± 1.3 and 1.4 ± 0.9, respectively, and were not significantly different from their values in the benign group.

The levels of CA125 in serum serve as a prognostic marker for EOC. In the present study, the levels of CA 125 were 57.8 ± 86.8 μl/ml, 132.8 ± 105.8 μl/ml, and 846.5 ± 623.1 μl/ml for benign lesions, borderline adenomas, and EOC, respectively. Because the location and characteristics of the ovary, many patients remain asymptomatic. In the present study, the first common symptom of patients with EOC was abdominal distension (56%), and the five-year survival rate of patients presenting with advanced disease (64%, 16/25) was lower (14%–38%).

Table 2. — *Immunohistochemical analysis of tumor foci in the greater omentum of patients with EOC or borderline ovarian lesions (30 cases).*

Protein	Site	HSCORE			
		0	1	2	3
SDC-1	Glandular epithelium	26	0	4	0
	Stroma	23	1	3	3
FGF-2	Glandular epithelium	25	4	1	0
FGFR-1	Glandular epithelium	20	7	3	0

Syndecans are members of the cell-surface heparan sulfate peptidoglycan family and are ubiquitously expressed in a wide range of cells. They play important roles in a variety of cellular functions, including cell adhesion, differentiation, and migration. SDC-1 was first detected by RT-PCR analysis of mRNAs isolated from patients with myeloma, and its expression by all myelomas was subsequently confirmed by immunohistochemical analysis with a monoclonal antibody [15, 16]. As a cell surface receptor, SDC-1 is intimately involved in normal and pathological events by regulating cell–cell and cell–matrix interactions, cell migration, development, neovascularization, microbial pathogenesis, and tumorigenesis [17-20]. Alterations in SCD-1 expression are associated with strongly aggressive phenotypes of some cancers and indicates poor prognosis of patients with breast, ovarian, and pancreatic cancers as well as in those with gliomas [21-27].

In the present study, using immunohistochemical analysis, the authors detected the expression of SDC-1 in the glandular epithelium in 8/25 cases of EOC with a mean staining score of 1.04. In contrast, SDC-1 was detected in only 3/30 patients with benign lesions of the ovary, with a mean staining score of 0.43. These results are consistent with those described for tissues taken from patients with pancreatic [28] or prostate cancer [29]; however, they do not correlate with the expression patterns of gastric, lung, cervical, and head-and-neck cancers [30-33]. Therefore, SDC-1 might facilitate the development of EOC through its regulation at the transcriptional level because the levels of SDC-1 and its mRNA change coordinately [34].

SDC-1 participates in proliferation, migration, and cell-matrix interactions [35], and localizes to the cell surface as well as intracellular compartments [36, 37]. SDC-1 binds and sequesters growth factors, including members of the fibroblast growth factor family [38] and acts as a coreceptor to facilitate signaling through FGFRs. FGFs stimulate not only mitogenesis but also angiogenesis, which is required for tumors to grow larger than two mm. FGFs mediate their biological effects by binding to FGFRs, which are high-affinity cell-surface receptors with protein tyrosine kinase activity. The most widespread expression was observed for FGFR1 and FGFR2. For example, high levels of immunoreactive FGFR1 were detected in the skin, cornea, lung, heart, placenta, kidney, and urethra, and moderate levels were detected in the testis and ovary. FGFR2, FGFR3, and FGFR4 are expressed at relatively low levels in ovaries [39].

FGF-2 plays an important role in oncogenesis [40-43]. Fujimoto *et al.* reported that increased levels of the expression of FGF-2 in advanced primary ovarian cancers indicate that FGF-2 may accelerate the growth of ovarian cancer cells [44]. SDC-1 regulates cell growth and differentiation in part by modulating the interactions of growth factors with their cellular receptors [45]. In the present study, analysis of ECOs revealed higher levels of FGF-2 and FGFR1 and their transcripts in the cytoplasm of epithelial cells. It was thought that SDC-1 together with FGF-2/FGFR1 as a coreceptor played a role in the development of EOC. Further, coexpression of SDC-1 and FGF-2/FGFR1 was detected in epithelial cells in only one case of Stage III EOC. In another study, no significant relationship was noted between expression of SDC-1 and FGF-2 in malignant mesotheliomas in vivo [46].

Here, when FGF-2 was added to cultures of SKOV3 cells, the expression of *SDC-1* mRNA was inhibited, indicating that upregulation of *SDC-1* expression occurred before the level of expression of FGF-2 changed. Metastatic foci in the greater omentum are known to proliferate at higher rates than primary tumor cells, leading to more rapid progression of tumor dissemination. The current analyses of the levels of SDC-1 expression in the glandular epithelium and stroma indicate that SDC-1 likely contributes to the invasiveness of EOC.

Reciprocal interactions between epithelial tumor cells and stroma play a very important role in facilitating tumor cell growth and migration in patients with breast cancer [47]. The induction of SDC-1 expression in reactive stromal fibroblasts creates a favorable microenvironment for accelerated tumor cell growth and angiogenesis. Thus, SDC-1 joins a group of molecules that are aberrantly expressed in the stromal compartment and contribute to carcinoma progression [48]. Cancer-associated stroma may contribute to tumor cell invasion and the development of metastasis. Here, the authors found moderate levels of expression of SDC-1 in stroma in two cases of local foci derived from patients with Stage III EOC. Staining was weakly positive in the cytoplasm of glandular epithelial cells but was not detectable in those of the other groups. The intensity of SDC-1-staining in metastatic foci of the greater omentum in patients with EOC was more frequent and intense compared with the glandular epithelium. The present authors believe it is therefore reasonable to conclude that the changes in the levels of expression of SDC-1 in local versus metastatic foci indicate that SDC-1 plays a role in the invasiveness of EOCs.

The present study also shows that the elevated levels of expression of FGF-2 (5/30) and FGFR1 (10/30) in metastatic foci in the greater omentum indicate more active mitogenesis and angiogenesis than those seen in localized tumors. The current results wherein exogenously added FGF-2 was found to downregulate the expression of *SDC-1* mRNA in SKOV3 cells lead the authors to speculate that stromal SDC-1 may arise from ectodomain shedding [49-51] from the cell membranes of EOC cells or from stromal cells. However, only four cases of ECO were positive for SDC-1-staining in local and metastatic foci. Therefore, they conclude that SDC-1 is not shed but is expressed by the stromal cells. Growth factors, such as FGF-2, and the accumulation of SDC-1 within the tumor stroma, may contribute to extensive angiogenesis and stromal proliferation.

Conclusion

In summary, the authors present novel data on the expression of SDC-1 in local and metastatic foci in patients with EOC. These findings implicate and reinforce the contribution of stromal expression and changes in the expression of SDC-1 in the pathogenesis and metastasis of ovarian cancer.

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References

[1] Blackhall F.H., Merry C.L., Davies E.J., Jayson G.C.: "Heparan sulfate proteoglycans and cancer". *Br. J. Cancer,* 2001, *85*, 1094.
[2] Sanderson R.D.: "Heparan sulfate proteoglycans in invasion and metastasis". *Semin. Cell. Dev. Biol.*, 2001, *12*, 89.
[3] Gonzalez A.D., Kaya M., Shi W., Song H., Testa J.R., Penn L.Z., Filmus J.: "OCI-5/GPC3, a glypican encoded by a gene that is mutated in the Simpson-Golabi-Behmel overgrowth syndrome, induces apoptosis in a cell line-specific manner". *J. Cell. Biol.*, 1998, *141*, 1407.
[4] Filmus J.: "Glypicans in growth control and cancer". *Glycobiology*, 2001, *11*, 19R.
[5] Lin H., Huber R., Schlessinger D., Morin P.J.: "Frequent silencing of the GPC3 gene in ovarian cancer cell lines". *Cancer Res.*, 1999, *59*, 807.
[6] Midorikawa Y., Ishikawa S., Iwanari H., Imamura T., Sakamoto H., Miyazono K., *et al.*: "Glypican-3, overexpressed in hepatocellular carcinoma, modulates FGF2 and BMP-7 signalling". *Int. J. Cancer*, 2003, *103*, 455.
[7] Powell C.A., Xu G., Filmus J., Busch S., Brody J.S., Rothman P.B.: "Oligonucleotide microarray analysis of lung adenocarcinoma in smokers and nonsmokers identifies GPC3 as a potential lung tumor suppressor". *Chest*, 2002, *12*, 6S.
[8] Xiang Y.Y., Ladeda V., Filmus J.: "Glypican-3 expression is silenced in human breast cancer". *Oncogene*, 2001, *20*, 7408.
[9] Kokenyesi R.: "Ovarian carcinoma cells synthesize both chondroitin sulfate and heparan sulfate cell surface proteoglycans that mediate cell adhesion to interstitial matrix". *J. Cell. Biochem.*, 2001, *83*, 259.
[10] International Collaborative Ovarian Neoplasm Group: "Paclitaxel plus carboplatin versus standard chemotherapy with either single-agent carboplatin or cyclophosphamide, doxorubicin, and cisplatin in women with ovarian cancer, the ICON3 randomised trial". Lancet, 2002, *360*, 505.
[11] Kato M., Wang H., Kainulainen V., Fitzgerald M.L., Ledbetter S., Ornitz D.M., Bernfield M.: "Physiological degradation converts the soluble syndecan-1 ectodomain from an inhibitor to a potent activator of FGF-2". *Nat. Med.*, 1998, *4*, 691.
[12] Fitzgerald M.L., Wang Z., Park P.W., Murphy G., Bernfield M.: "Shedding of syndecan-1 and -4 ectodomains is regulated by multiple signaling pathways and mediated by a TIMP-3-sensitive metalloproteinase". *J. Cell. Biol.*, 2000, *148*, 811.
[13] Presta M., Leali D., Stabile H., Ronca R., Camozzi M., Coco L., *et al.*: "Heparin derivatives as angiogenesis inhibitors". *Curr. Pharm. Des.*, 2003, *9*, 553.
[14] Davies E.J., Blackhall F.H., Shanks J.H., David G., McGown A.T., Swindell R., *et al.*: "Distribution and clinical significance of heparan sulfate proteoglycans in ovarian cancer". *Clin. Cancer Res.*, 2004, *10*, 5178.
[15] Ridley R.C., Xiao H.Q., Hata H., Woodliff J., Epstein J., Sanderson R.D.: "Expression of syndecan regulates human myeloma plasma cell adhesion to type I collagen". *Blood*, 1993, *81*, 767.
[16] Wijdenes J., Vooijs W.C., Clément C., Post J., Morard F., Vita N.,et al.: "A plasmocyte selective monoclonal antibody (B-B4) recognizes syndecan-1". *Br. J. Haematol.*, 1996, *94*, 318.
[17] Perrimon N., Bernfield M.: "Specificities of heparan sulphate proteoglycans in developmental processes". *Nature*, 2000, *404*, 725.
[18] Rapraeger A.C.: "Molecular interactions of syndecans during development". *Semin. Cell. Dev. Biol.*, 2001, *12*, 107.
[19] Carey D.J.: "Syndecans: multifunctional cell-surface co-receptors". *Biochem. J.*, 1997, *327*, 1.
[20] Bernfield M., Gotte M., Park P.W., Reizes O., Fitzgerald M.L., Lincecum J., Zako M.: "Functions of cell surface heparan sulfate proteoglycans". *Ann. Rev. Biochem.*, 1999, *68*, 729.
[21] Liebersbach B.F., Sanderson R.D.: "Expression of syndecan-1 inhibits cell invasion into type I collagen". *J. Biol. Chem.*, 1994, *269*, 20013.
[22] Ohashi M., Kusumi T., Sato F.: "Expression of syndecan-1 and E-cadherin is inversely correlated with poor patient's prognosis and recurrent status of extrahepatic bile duct carcinoma". Biomed. Res., 2009, *30*, 79.
[23] Kato M., Saunders S., Nguyen H., Bernfield M.: "Loss of cell surface syndecan-1 causes epithelia to transform into anchorage-independent mesenchyme-like cells". *Mol. Biol. Cell.*, 1995, *6*, 559.
[24] Barbareschi M., Maisonneuve P., Aldovini D., Cangi M.G., Pecciarini L., Angelo Mauri F., *et al.*: "High syndecan-1 expression in breast carcinoma is related to an aggressive phenotype and to poorer prognosis". *Cancer* 2003, *98*, 474.
[25] Davies E.J., Blackhall F.H., Shanks J.H., David G., McGown A.T., Swindell R., *et al.*: "Distribution and clinical significance of heparan sulfate proteoglycans in ovarian cancer". *Clin. Cancer Res.*, 2004, *10*, 5178.
[26] Conejo J.R., Kleeff J., Koliopanos A., Matsuda K., Zhu Z.W., Goecke H., *et al.*: "Syndecan-1 expression is up-regulated in pancreatic but not in other gastrointestinal cancers". *Int. J. Cancer*, 2000, *88*, 12.
[27] Watanabe A., Mabuchi T., Satoh E., Furuya K., Zhang L., Maeda S., Naganuma H.: "Expression of syndecans, a heparan sulfate proteoglycan in malignant gliomas: participation of nuclear factor-kappaB in upregulation of syndecan-1 expression". *J. Neurooncol.*, 2006, *77*, 25.
[28] Conejo J.R., Kleeff J., Koliopanos A., Matsuda K., Zhu Z.W., Goecke H., *et al.*: "Syndecan-1 expression is up-regulated in pancreatic but not in other gastrointestinal cancers". *Int. J. Cancer*, 2000, *88*, 12.
[29] Chen D., Adenekan B., Chen L.: "Syndecan-1 expression in locally invasive and metastatic prostate cancer". *Urology*, 2004, *63*, 402.
[30] Anttonen A., Heikkila P., Kajanti M., Jalkanen M., Joensuu H.: "High syndecan-1 expression is associated with favourable outcome in squamous cell lung carcinoma treated with radical surgery". *Lung Cancer*, 2001, *32*, 297.
[31] Numa F., Hirabayashi K., Kawasaki K., Sakaguchi Y., Sugino N., Suehiro Y., *et al.*: "Syndecan-1 expression in cancer of the uterine cervix: association with lymph node metastasis". *Int. J. Oncol.*, 2002, *20*, 39.
[32] Rintala M., Inki P., Klemi P., Jalkanen M., Grénman S.: "Association of syndecan-1 with tumor grade and histology in primary invasive cervical carcinoma". *Gynecol. Oncol.*, 1999, *75*, 372.
[33] Inki P., Stenback F., Grenman S., Jalkanen M.: "Immunohistochemical localization of syndecan-1 in normal and pathological human uterine cervix". *J. Pathol.*, 1994, *172*, 349.
[34] Leppä S., Vleminckx K., Van Roy F.: "Syndecan-1 expression in mammary epithelial tumor cells is E-cadherin-independent". *J. Cell Sci.*, 1996, *109*, 1393.
[35] Mennerich D., Vogel A., Klaman I., Dahl E., Lichtner R.B., Rosenthal A., *et al.*: "Shift of syndecan-1 expression from epithelial to stromal cells during progression of solid tumours". *Eur. J. Cancer,* 2004, *40*, 1373.

[36] Chen L., Sanderson R.D.: "Heparanase regulates levels of syndecan-1 in the nucleus". *PLoS One*, 2009, *4*, e4947.
[37] Nikolova V., Koo C.Y., Ibrahim S.A., Wang Z., Spillmann D., Dreier R., *et al.*: "Differential roles for membrane-bound and soluble syndecan-1 (CD138) in breast cancer progression". *Carcinogenesis*, 2009, *30*, 397.
[38] Krypta R.: "Cell junctions, cell adhesion, and the extracellular matrix". *In:* Alberts B., Johnson A., Lewis J., Raff M., Roberts K., Walter P., (eds). *Molecular Biology of the Cell*, 4th ed. New York: Garland Publishing, 2002, 1131.
[39] Hughes S.E.: "Differential expression of the fibroblast growth factor receptor (FGFR) multigene family in normal human adult tissues". *J. Histochem. Cytochem.*, 1997, *45*, 1005.
[40] Guddo F., Fontanini G., Reina C., Vignola A.M., Angeletti A., Bonsignore G.: "The expression of basic fibroblast growth factor (bFGF) in tumor associated stromal cells and vessels is inversely correlated with non small cell lung cancer progression". *Hum. Pathol.*, 1999, *30*, 788.
[41] Iida S., Katoh O., Tokunaga A., Terada M.: "Expression of fibroblast growth factor gene family and its receptor gene family in the human upper gastrointestinal tract". *Biochem. Biophys. Res. Commun.*, 1996, *199*, 1113.
[42] Fujimoto J., Ichigo S., Hori M., Hirose R., Sakaguchi H., Tamaya T.: "Expression of basic fibroblast growth factor and its mRNA in advanced uterine cervical cancers". *Cancer Lett.*, 1997, *111*, 21.
[43] Fujimoto J., Hori M., Ichigo S., Tamaya T.: "Expressions of the fibroblast growth factor family (FGF-1, -2 and -4) mRNA in endometrial cancers". *Tumour Biol.*, 1996, *17*, 226.
[44] Fujimoto J., Ichigo S., Hori M., Hirose R., Sakaguchi H., Tamaya T.: "Expression of basic fibroblast growth factor and its mRNA in advanced ovarian cancers". *Eur. J. Gynaecol. Oncol.*, 1997, *18*, 349.
[45] Couchman J.R.: "Syndecans: proteoglycan regulators of cell surface microdomains?" *Nat. Rev. Mol. Cell. Biol.*, 2003, *4*, 926.
[46] Kumar-Singh S., Jacobs W., Dhaene K., Weyn B., Bogers J., Weyler J., Van Marck E.: "Syndecan-1 expression in malignant mesothelioma: correlation with cell differentiation, WT1 expression, and clinical outcome". *J. Pathol.*, 1998, *186*, 300.
[47] Maeda T., Desouky J., Friedl A.: "Syndecan-1 expression by stromal fibroblasts promotes breast carcinoma growth in vivo and stimulates tumor angiogenesis". *Oncogene*, 2006, *25*, 1408.
[48] Joensuu H., Anttonen A., Eriksson M., Mäkitaro R., Alfthan H., Kinnula V., Leppä S.: "Soluble syndecan-1 and serum basic fibroblast growth factor are new prognostic factors in lung cancer". *Cancer Res.*, 2002, *62*, 5210.
[49] Mahtouk K., Hose D., Raynaud P., Hundemer M., Jourdan M., Jourdan E., et al.: "Heparanase influences expression and shedding of syndecan-1, and its expression by the bone marrow environment is a bad prognostic factor in multiple myeloma". *Blood*, 2007, *109*, 4914.
[50] Blackhall F.H., Merry C.L., Davies E.J., Jayson G.C.: "Heparan sulfate proteoglycans and cancer". *Br. J. Cancer*, 2001, *85*, 1094.
[51] Stanley M.J., Stanley M.W., Sanderson R.D., Zera R.: "Syndecan-1 expression is induced in the stroma of infiltrating breast carcinoma". *Am. J. Clin. Pathol.*, 1999, *112*, 377.

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