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Original Article

Expression of Extracellular Matrix Proteins in Ovarian Serous Tumors

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Summary: The aims of this study were to perform a comprehensive expression analysis of the genes encoding extracellular matrix proteins and to investigate the expression pattern in one of these proteins, syndecan 1, in normal ovarian epithelium as well as benign and malignant ovarian serous tumors. Gene expression of 16 different extracellular matrix proteins was analyzed in ovarian serous tumors based on serial analysis of gene expression database. Semiquantitative reverse transcription–polymerase chain reaction was used to validate the serial analysis of gene expression result from each gene. As compared with normal ovarian surface epithelium, we found overexpression of syndecan 1, collagen type IV alpha 2, elastin microfibril interfase located protein 1, and laminin 5 in ovarian serous carcinomas. Syndecan 1 was selected for further study as it has not been well characterized in ovarian cancer and the syndecan 1 antibody was available for immunohistochemistry. Using a syndecan 1–specific monoclonal antibody, we demonstrated that syndecan 1 was expressed in 30.4% of high-grade serous carcinomas, 29.7% of low-grade carcinomas and serous borderline tumors, but none of benign serous cystadenomas and ovarian surface epithelium. Although both high-grade and low-grade serous carcinomas had a similar percentage of syndecan 1–positive cases, the immunointensity in high-grade carcinoma was significantly higher than that in low-grade carcinomas and serous borderline tumors (P = 0.007). In summary, ovarian carcinomas exhibit up-regulated expression of several extracellular matrix proteins, and syndecan 1 represents a novel tumor-associated marker in ovarian serous carcinomas.
Key Words: Syndecan 1—Ovarian serous tumors—Extracellular matrix proteins.

The extracellular matrix (ECM) is a group of complex structural proteins that surround cells and support their microenvironment in tissue. It is composed of structural and specialized fibrillary proteins and proteoglycans. The ECM is essential for tissue remodeling, and many of its components provide a physical structure support to the surrounding cells. More importantly, ECM proteins play a key role in regulating survival, motility, proliferation, and morphology of normal cells and contribute to a variety of cellular functions, including organ development, wound healing, and metabolism (1). Dysregulation of the ECM, on the other hand, has been implicated in many diseases including the development of breast, ovarian, colorectal, and pancreatic carcinomas (2–5). In ovarian cancer, it has been shown that ECM proteins including tenascin C, collagen type VI, and procollagen types I and III are present in the tumor microenvironment and are thought to facilitate tumor development (6–8). In addition, it is expected that more ECM genes are expressed in ovarian

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cancer, and some of them may contribute to its pathogenesis. Serial analysis of gene expression (SAGE) has become one of the established methods for genome-wide analysis of gene expression. SAGE provides several unique features as compared with hybridization-based techniques. For example, SAGE can analyze virtually all transcripts, known or unknown, in a tissue sample. This method provides a digital readout that precisely measures the copy number of a transcript species. With the centralized deposition of SAGE libraries, the SAGE data can be compared among different SAGE libraries from time to time and can be used to analyze gene expression pattern in silica. In this study, we analyzed the SAGE database from the public domain together with newly made ovarian cancer SAGE libraries comparing the ECM gene products between ovarian surface epithelium and ovarian carcinomas. We used an algorithm to select those ECM proteins that are highly expressed in ovarian carcinomas but not in their benign counterparts. Quantitative real-time polymerase chain reaction (PCR)

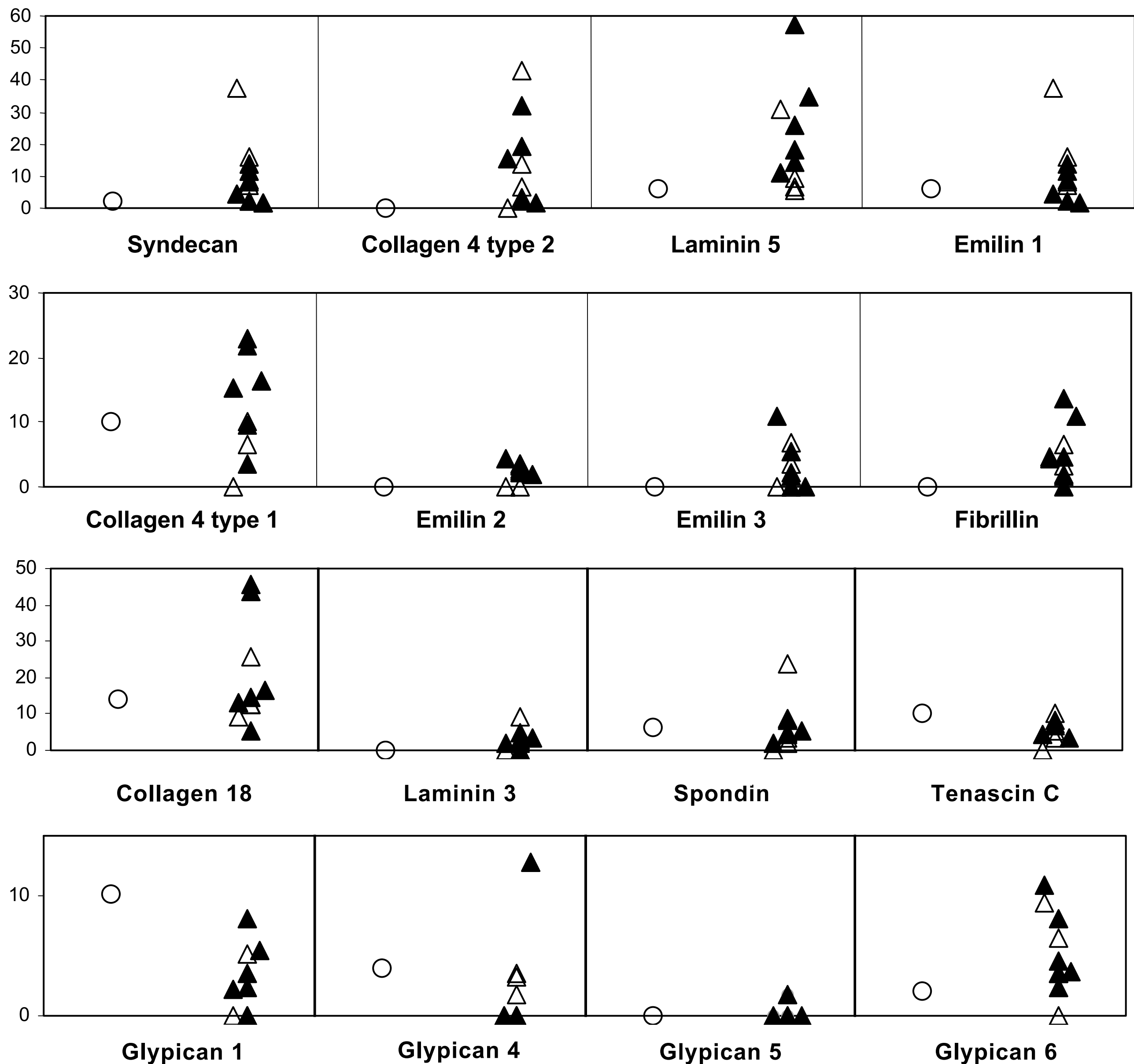

FIG. 1. Serial analysis of gene expression for 16 ECM proteins. The *y* axis represents the number of tags per million, which represents the level of gene expression for individual genes. Syndecan 1 is overexpressed in the ovarian cancer cell lines and tumors as compared with the ovarian surface epithelial cells (HOSE4). The HOSE4 cell line is represented by ○, cancer cell lines are represented by △, and tumor tissues are represented by ▲.

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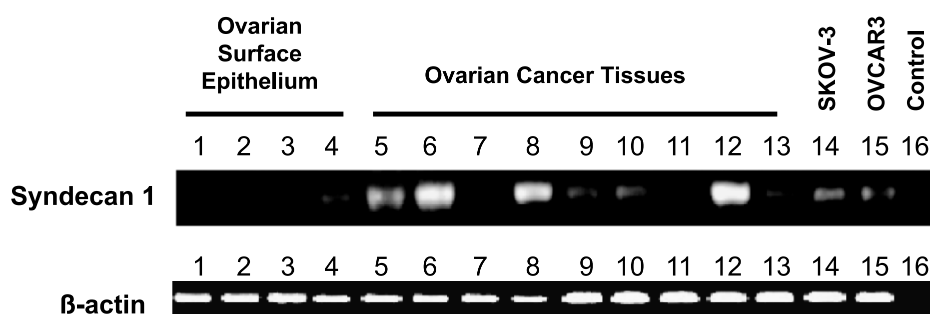

FIG. 2. Results of real-time PCR for syndecan 1 and β-actin (control). Normal ovarian epithelial samples (lanes 1–4), ovarian cancer samples (lanes 5–13), ovarian cancer cell lines, SKOV3 and OVCAR3 (lanes 14 and 15), and a negative control (lane 16) are shown.

was then performed to validate the SAGE results. We then focused on one of the most preferentially expressed ECM proteins, syndecan 1, by performing immunohistochemistry on a relatively large number of ovarian serous carcinoma tissues to assess its biological significance. Our results suggest that syndecan 1 is a highly specific tumor-associated ECM that has potential as a diagnostic marker and a molecular target for therapy of ovarian serous carcinomas.

MATERIALS AND METHODS

Tissue Samples

A total of 138 ovarian serous carcinomas (112 high grade and 26 low grade), 17 ovarian serous borderline tumors (SBT), also referred to as atypical proliferative serous tumors, 22 ovarian serous cystadenomas, and 12 normal ovaries were retrieved from the surgical pathology files at the Department of Pathology. The local Institutional Review Board approved the acquisition of anonymous paraffin tissues. Tissue microarrays were made with 1.5-mm diameter for each core, and 3 representative cores were selected from each tissue specimen. In addition, a variety of normal tissues including breast, colon, gall bladder, lung, lymph node, and brain were included as controls.

Serial Analysis of Gene Expression

The SAGE database was used to obtain the tags in which the number in each library was normalized per million tags. The SAGE libraries studied include OVT6, OVT7, OVT8, ES2, A2780, OVCA3, and HOSE4 deposited at the web site http://www.hlm.nih.gov/SAGE/, MPSC-1 previously reported by us (9), and new SAGE libraries, OV4663 and OV92, established in this study. Long SAGE was performed with 2-μg messenger RNA using the standard SAGE protocol that has been detailed at the web site http://www.sagenet.org/sage_protocol.htm with the modifications previously described (10). We searched tags and counted their number corresponding to 16 ECM molecules: syndecan 1 (Hs.224607), collagen type XVIII alpha 1 (Hs.517356), spondin-1 (Hs.445818), laminin 5 (Hs.473256), collagen type IV alpha 1 (Hs.17441) and alpha 2 (Hs.508716), fibrillin 1 (Marfan syndrome) (Hs.146447), laminin 3 (Hs.436367), glypican 1 (Hs.328232), glypican 4 (Hs.58367), glypican 5 (Hs.546254), glypican 6 (Hs.444329), elastin microfibril interfase located protein (EMILIN) 1 (Hs.63348), EMILIN 2 (Hs.532815), EMILIN 3 (Hs.25897), and tenascin C (hexabrachion) (Hs.143250).

Immunohistochemistry

Paraffin sections in tissue microarrays were used for immunohistochemistry with a syndecan 1–specific monoclonal antibody, DL-101 (Santa Cruz, California, CA), at a dilution of 1:100 followed by the avidin-biotin peroxidase method. The immunoreactivity was defined by discrete brownish chromogen deposit in the cytoplasm and cell surface A specimen was scored according to the percentage of cells staining positive for syndecan 1. Syndecan 1 immunointensity was scored as 0 (undetectable), 1+ (weak), 2+ (moderate), and 3+ (intense).

Reverse Transcription–polymerase Chain Reaction

Reverse transcription-PCR was used to assess the transcript levels of syndecan 1 in OSE2A, OSE2B, OSE7,

TABLE 1. *Syndecan immunoreactivity in the ovarian serous tumor and normal ovaries*

	Total No. Cases	Immunointensity			
		0	1+	2+	3+
Normal ovary	12	12 (100)	0	0	0
Serous cystadenoma	22	18 (82)	4 (18)	0	0
Low-grade carcinoma/ SBT	43	26 (60)	6 (14)	9 (21)	3 (7)
High-grade carcinoma	112	63 (56)	15 (13)	11 (10)	23 (21)

Values in parenthesis are in percentages.

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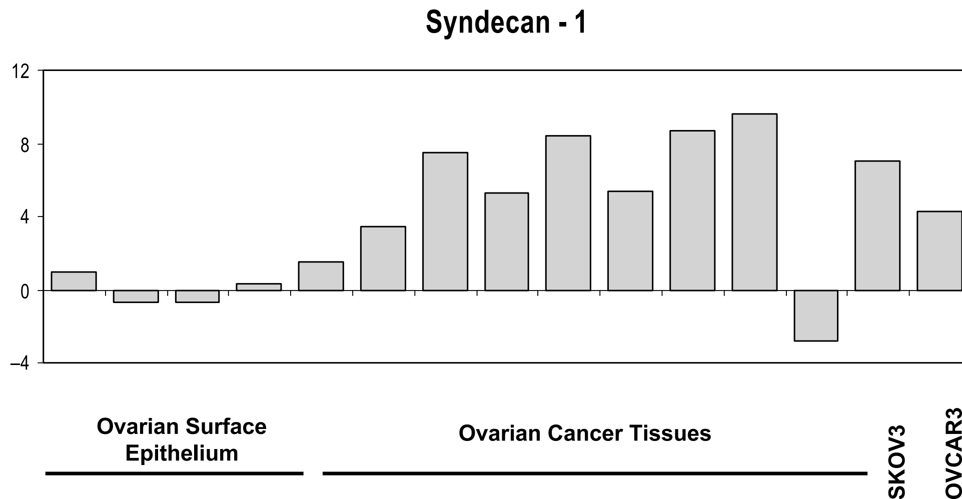

FIG. 3. Overexpression of syndecan 1 is demonstrated in ovarian carcinoma samples as compared with normal ovarian epithelium when evaluating the differences between the Ct (threshold cycle) for each sample from the Ct for the control gene.

OSE10, 9 ovarian carcinoma tissues, and 2 ovarian carcinoma cell lines, OVCAR3 and SKOV3. Cell pellets from the cell culture or tissue fragments were placed in the TRIzol reagent (Invitrogen, Carlsbad, CA). Total RNA was isolated and contaminating genomic DNA was removed using the DNA-free kit (Ambion, Austin, TX) following the protocol supplemented by the manufacturer. Complementary DNA was prepared using oligo dT primers and diluted for PCR. For PCR, 2 μL of complementary DNA was added to the PCR cocktails (23 μL) containing all the essential reagents. The sequences for the syndecan 1 PCR primers were forward 5′-ATGAGG-CGCGCGGCGCTCT-3′ and reverse 5′-CGTGGGAA TAGCCGTCAGGAG-3′. The PCR product was separated by 1% agarose gel and was visualized by ethidium bromide staining.

Statistical Methods

Fisher exact test was used for comparing benign and malignant samples and syndecan 1 expression. Statistical significance was accepted as values less than or equal to 0.05 ($P \leq 0.05$).

RESULTS

To analyze gene expression profiles of ECM proteins in ovarian serous carcinomas, we used the candidate gene approach by counting the sequence tags that represented individual ECM transcripts in ovarian SAGE libraries. In this study, we focused on 16 ECM genes and demonstrated that syndecan 1, laminin 5, collagen type IV alpha 2, and Emilin 1 were consistently up-regulated in ovarian carcinoma libraries as compared with normal ovarian surface epithelial cells. The results of SAGE are shown in Figure 1. Expression of collagen type IV alpha 2 and laminin 5 in ovarian cancer have been described in the literature, and no antibody was available for EMILIN 1 (11,12). Thus, syndecan 1 was selected for further study.

The expression of syndecan 1 in ovarian carcinoma was validated by semiquantitative real-time PCR. As shown in Figure 2, the syndecan 1 PCR products were much more abundant in 10 of 11 ovarian carcinoma samples including 9 ovarian serous carcinoma tissues

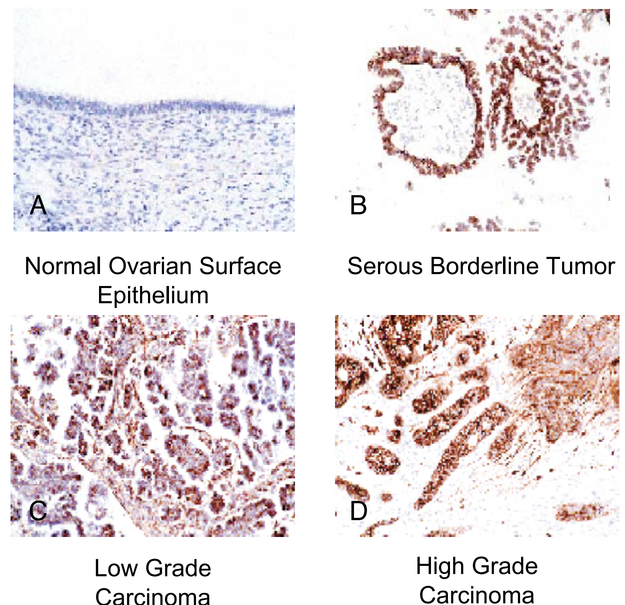

FIG. 4. Syndecan 1 immunoreactivity in ovarian tumors and normal ovarian surface epithelium. (A), Absent expression in normal serous epithelium (magnification, ×10). (B), Immunoreactivity in SBT (magnification, ×10). (C), Immunoreactivity in low-grade carcinoma (magnification, ×40). (D), Immunoreactivity in high-grade carcinoma (magnification, ×40).

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and 2 ovarian cancer cell lines as compared with ovarian surface epithelial cell cultures ($P < 0.001$) (Fig. 3). To further examine the role of the syndecan 1 in ovarian carcinomas, we performed immunohistochemistry for syndecan 1 on normal, benign, and malignant ovarian tissues, and the results were summarized in Table 1. Because syndecan 1 immunoreactivity was always diffuse, we used the intensity scores to assess syndecan 1 expression in this study. Syndecan 1 staining was detected in 30.4% of high-grade serous carcinomas and 27.9% of low-grade carcinomas and SBT. Syndecan 1 expression in ovarian carcinomas was found to be significantly higher than serous cystadenomas and normal ovarian surface epithelium, in which no syndecan 1 expression was detected ($P = 0.009$). Syndecan 1 immunoreactivity in representative ovarian tissues was shown in Figure 4. The percentage of cases with an immunointensity score of syndecan 1 more than or equal to 2 was significantly higher in high-grade than low-grade carcinomas or SBT ($P = 0.007$) (Table 1).

DISCUSSION

This study is the first to analyze 16 different ECM proteins in ovarian serous carcinoma using SAGE and specifically provides a focus on syndecan 1 immunodistribution in ovarian serous tumors. Many proteins in the ECM, including syndecan 1, have been shown to have a potential to serve as both prognostic and diagnostic markers in different human cancers. Here we show that syndecan 1 is absent from benign ovarian surface epithelium and stroma and is expressed in ovarian serous carcinoma. Furthermore, the expression level of syndecan 1 is significantly higher in high-grade serous carcinomas than low-grade serous carcinomas and SBT.

Syndecan 1 is a heparan sulfate proteoglycan present in the ECM and on cell surfaces. Syndecan 1 has been shown to participate in proliferation, migration, and cell-matrix interactions (13) and is normally found on the surface of many types of cells, including epithelial cells and fibroblasts (1). This proteoglycan has the ability to interact with fibrous proteins of the ECM and the ability to bind and sequester growth factors including fibroblast growth factors (1). The binding of growth factors may lead to the enhancement or inhibition of functions of growth factors and results in altered expression of syndecan 1 (1).

The expression of syndecan 1 has been evaluated in many tumor types as a potential maker associated with tumorigenesis. Several studies have shown syndecan 1 immunoreactivity in tumor tissue and reported that syndecan 1 is a prognostic factor associated with a favorable clinical outcome in endometrial, gastric, pancreatic, and colorectal cancers (14–17). In contrast, other studies have demonstrated that syndecan 1 expression was associated with a poor prognosis. For example, Barbareschi et al. (18) reported that increased expression of syndecan 1 was found in 42% of patients with breast carcinoma and that there was a significant correlation of syndecan 1 expression with a poor clinical outcome. Another study demonstrated that overexpression of syndecan 1 expression was associated with a good prognosis (19).

The role of syndecan 1 has not been well established in ovarian cancer. A study by Davies et al. (20), reported the absence of syndecan 1 in normal ovarian tissue and positive expression in ovarian carcinomas, which is consistent with our findings. They also demonstrated that increased syndecan 1 expression was a poor prognostic factor for survival in ovarian cancer. In contrast, we did not observe a correlation between syndecan 1 expression and clinical behavior, including overall survival and disease-free interval (data not shown). The demonstration of syndecan 1 as a tumor-associated marker in ovarian serous carcinomas may have clinical implications. Because syndecan 1 is present on the tumor cell surface and its microenvironment, it can be used as a marker in conjunction with other cell surface markers for tumor detection and therapeutic targeting.

In conclusion, we have reported up-regulation of several ECM proteins in ovarian serous carcinomas based on analysis of gene expression in silica. In addition, we demonstrated that syndecan 1 immunoreactivity is much higher in high-grade serous carcinoma compared with low-grade carcinoma and SBT (atypical proliferative serous tumors). Our findings lend further support to the view that ECM expression is associated with tumor development and suggest that syndecan 1 is a tumor-associated marker in ovarian serous carcinomas.

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