Versican Expression in Tumor Epithelial Cells Is Correlated with a Good Prognosis in Gastric Cancer

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Abstract. *Background/Aim: Versican expression has been reported to have prognostic value in several cancers. The aim of the present study was to investigate the prognostic significance of versican expression in gastric cancer. Materials and Methods: In total, 105 gastric cancer patients who received gastrectomy were included in the study. Versican expression in the epithelial and stromal components of the tumors was determined by immunohistochemistry. Results: Versican was expressed in 21.0% of tumor epithelial cells and 44.8% of stromal cells. Patients with versican expression in tumor epithelial cells had significantly better 5-year disease-free (p=0.021) and overall (p=0.034) survival rates, whereas versican expression in stromal cells was not associated with disease-free (p=0.532) and overall (p=0.876) survival. Multivariate analysis showed that versican expression in tumor epithelial cells was an independent prognostic indicator for better clinical outcome in disease-free and overall survival. Conclusion: Versican expression in tumor epithelial cells predicts a good prognosis for gastric cancer patients.*

Gastric cancer is one of the most common malignancies worldwide and the second leading cause of cancer-related death (1, 2). Cancer progression and metastasis, which remain significant obstacles to the treatment of gastric cancer, are complicated systemic multi-step processes affected by tumor cell characteristics and the environment surrounding the tumor. The extracellular matrix (ECM) is a highly organized, three-dimensional structure with many physiological and pathological roles (3). The ECM also regulates cell migration, cellular differentiation and proliferation, and furthermore provides a reservoir of cytokines and growth factors. There is increasing evidence to suggest that ECM components play an active role in tumor progression and metastasis (3, 4).

The epithelial-mesenchymal transition (EMT) is considered a key step toward cancer metastasis. The EMT characterized by a gain of mesenchymal cell markers and a loss of epithelial markers is a process whereby cells acquire molecular alterations that facilitate cell motility and invasion (5, 6). In addition, EMT leads to generation of cancer stem cells with self-renewal and tumor-initiating capabilities, as well as resistance to apoptosis and chemotherapy (7). Versican, known as chondroitin sulfate (CS) proteoglycan, is the largest member of the lectican family providing cells with an anti-adhesive environment (8-10). The versican-rich extracellular matrix, which versican is combined with other ECM components such as hyaluronan and type I collagen, possesses anti-adhesive properties. Thus, versican can modulate proliferation and migration. Through this mechanism, versican participates in the EMT (4).

Previous studies of ovarian, breast and prostate cancer have revealed that versican plays a structural and biomechanical role predicting a dismal outcome (11-14). However, few studies have reported the role of versican in gastric cancer. The aim of this study was to investigate the potential prognostic value of versican expression in gastric cancer.

Materials and Methods

Patients and samples. A total of 105 gastric cancer patients who underwent gastrectomy combined with lymph node dissection at the Uijeongbu St. Mary's Hospital between 2001 and 2005 were enrolled in the present study. Operative details and pathological data of all enrolled patients were collected retrospectively from the gastric cancer registry of Uijeongbu St. Mary's Hospital. The pathological stage was classified according to the Seventh American Joint Cancer Committee (AJCC) TNM classification. The histological type was categorized as differentiated or undifferentiated. Poorly-differentiated tubular adenocarcinoma, signet ring cell adenocarcinoma and mucinous adenocarcinoma were assigned to the undifferentiated group.

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The patients comprised of 75 males and 30 females aged from 29 to 89 years (median, 62.0 years). Sixty-eight and thirty-seven patients received subtotal and total gastrectomies, respectively. Stage III was most common in the final pathological diagnosis (Table I). Tissue samples were obtained from paraffin-embedded, resected specimens of the stomach after histopathological diagnosis.

Regular follow-up programs were conducted; these involved evaluation of tumor markers, abdominal computed tomography (CT) and endoscopic examination according to our standard protocol (every 3 and 6 months for advanced and early gastric cancer, respectively, for the first 3 years; every 12 months thereafter). The mean follow-up period was 66.6±48.4 months (range, 0-151 months). The survival results were repeatedly identified using registration data from the Korea National Statistical Office and the patients' medical records.

This study was approved by the Institutional Review Board of the Ethics Committee of the College of Medicine, Catholic University of Korea (UC14SISI0070)

Immunohistochemistry. Immunohistochemistry for human gastric tumors was performed on formalin-fixed, paraffin-embedded, 4-μm-thick tissue sections using the avidin-biotin-peroxidase complex method. Briefly, the sections were deparaffinized three times for 10 min each in xylene and then dehydrated using a graded ethanol series. Endogenous peroxidase activity was halted by the administration of 3% hydrogen peroxidase and methanol for 10 min. For antigen retrieval, the sections were treated with 10 mM citrate buffer (pH 6.0) at 98˚C for 15 min in a microwave oven and allowed to cool for 1 h at room temperature. After incubation for 10 min in a blocking solution (Histo-Plus kit; Zymed, San Francisco, CA, USA) containing 10% normal serum in phosphate-buffered saline (PBS), the sections were incubated at 4˚C overnight in a humidified chamber with versican mouse anti-human monoclonal antibody (diluted 1:500; LifeSpan Biosciences, Seattle, WA, USA) as the primary antibody. A biotinylated secondary antibody (Histo-Plus kit; Zymed) was used for the detection of primary antibodies and the sections were incubated for 10 min at 45˚C. The sections were rinsed three times in PBS and incubated with streptavidin-horseradish peroxidase complex (Histo-Plus kit; Zymed) for 10 min. Antigen localization was revealed using 3,3'-diaminobenzidine tetrahydrochloride as chromogen and the slides were counterstained with hematoxylin.

Evaluation of immunoscores. Versican expression was separately measured in the epithelial and stromal components of the tumor. Both the extent and intensity of immunopositivity were considered in each component when scoring versican protein expression. The intensity of positive staining was scored as follows: 0, negative; 1, weak; 2, moderate; 3, strong. The extent of positive staining was scored according to the percentage of positive cells in the respective lesions: 0, 0%; 1, 1-10%; 2, 11-25%; 3, 26-50%; 4, 51-75%; 5, 76-90%; 6, >90%. The final score was obtained by multiplying the extent of positivity and intensity scores, yielding a range from 0 to 18. Versican expression was considered positive when the scores totaled ≥9.

Statistical analysis. Differences between groups were analyzed using the Student's *t*-test for continuous variables and χ^2 test or Fisher's exact test for proportions. Survival analysis was performed using the Kaplan-Meier method with the log-rank test for univariate analyses. Multivariate analysis for survival was performed using a Cox proportional hazards model with the 'Backward LR' method.

Table I. *Patients' characteristics*.

Variable	n=105
Gender	
Male	75
Female	30
Age in years	
Median (range)	62.0 (29-89)
Extent of resection	
Subtotal	68
Total	37
Lymph node dissection	
D1+	6
D2	53
More than D2	46
Retrieved lymph nodes	
Mean±SD (range)	27.7±13.4 (15-76)
Reconstruction	
Billroth-I	9
Billroth-II	59
Roux-en-Y	37
Pathological stage (7th AJCC)	
I	5
II	32
III	66
IV	2

SD, Standard deviation; AJCC, American Joint Cancer Committee.

Statistical analyses were performed using the SPSS (Statistical Package for the Social Sciences, Chicago, IL, USA) version 13.0, and a *p*-value less than 0.05 indicated statistical significance.

Results

Versican expression in gastric cancer. Versican expression was observed in the cytoplasm of tumor cells and/or tumor stromal cells (Figure 1). The cytoplasmic staining pattern was diffusely granular (Figure 1b). Tumor stromal fibroblasts were the most prominent cell type among versican-positive stromal cells (Figure 1c). The positive versican expression in tumor cells and stroma was found in 22 (21.0%) and 47 (44.8%) of 105 cases studied, respectively.

Correlation between versican expression and clinico-pathological features. Versican expression in the stromal compartment was significantly correlated with the occurrence of serosal exposure (p=0.035) and perineural invasion (p=0.041). However, stromal versican expression was not correlated with tumor stage. No clinicopathological factor, including pathological stage, was associated with versican expression in tumor epithelium (Table II).

Versican expression and clinical outcomes. No significant difference was found in disease-free (p=0.532) and overall survival (p=0.876) between patients who were positive for

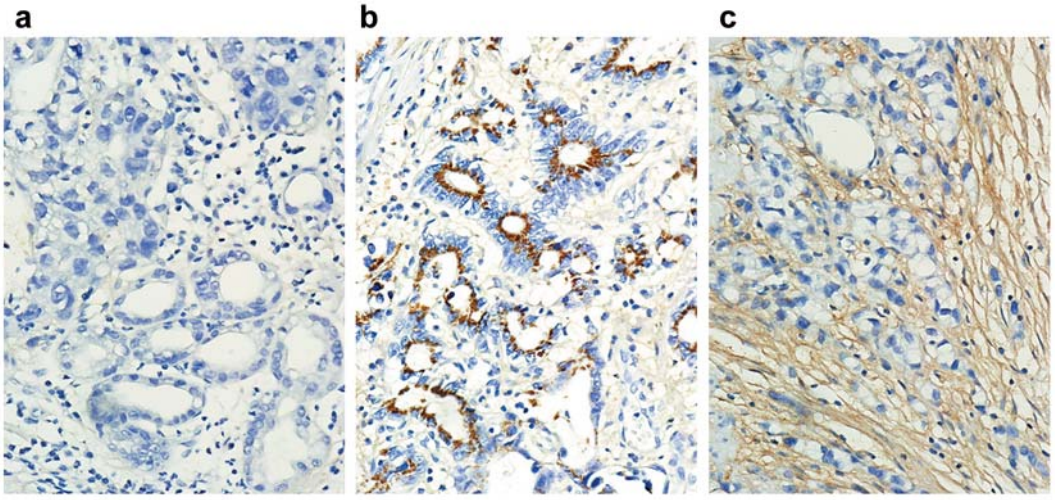

Figure 1. *Immunohistochemical staining of versican in gastric cancer: (a) negative (×400); (b) granular, cytoplasmic staining of tumor cells (×400); (c) diffuse staining of stromal cells (×400).*

versican in the stromal cells and those who were negative (Figure 2). Conversely, the 5-year disease-free survival rate of epithelial versican-positive patients was significantly better than that of epithelial versican-negative patients (67.4% *versus* 32.8%, respectively; p=0.021) (Figure 3a). Additionally, the epithelial versican-positive patients had a significantly better 5-year overall survival rate than the epithelial versican-negative patients (68.2% *versus* 43.4%, respectively; p=0.034; Figure 3b). In multivariate analysis of disease-free and overall survival using a Cox proportional hazards regression model, versican expression in tumor epithelial cells was an independent factor predictive of a good clinical outcome, in contrast to AJCC TNM stage (seventh edition classification system), which was predictive of a poor clinical outcome for gastric cancer (Tables III and IV).

Discussion

The present study revealed that epithelial versican expression in the tumor area predicts a good prognosis in gastric cancer and is an independent factor predictive of a good clinical outcome. Stromal expression was correlated with serosal exposure and perineural invasion; however, stromal versican expression was not associated with cancer prognosis. Most previous studies have demonstrated that versican expression is predictive of poor prognosis in many cancers, including breast, ovarian, cervical, prostate, endometrial, non-small-cell lung cancer and astrocytoma (11, 15-22). Thus, the stromal expression of versican has been described as a biomarker for poor prognosis in ovarian cancer, breast cancer and oral squamous cell carcinoma (15, 16, 23). Although epithelial expression has also been reported in endometrial, cervical, ovarian, prostate and colon cancers (4, 11, 15, 18, 24), a few studies of ovarian and colonic cancers have reported opposite effects; *i.e.*, epithelial expression of versican was correlated with a good prognosis (11, 24). As the present and other studies have shown, the role of versican in cancer progression is unclear.

The characteristics of epithelial and mesenchymal cells have been identified based on their visual appearance and the morphology of multi-cellular structures that abut each other. Cell-to-cell junctions and adhesions hold epithelial cells tightly and inhibit their movement. Internal adhesiveness allows an epithelial sheet to enclose and provide mechanical rigidity. Conversely, mesenchymal movement differs in that mesenchymal cell structures are irregular in shape and non-uniform in composition. Thus, mesenchymal cells can move individually and leave the region of the adhesion (25). EMT is a series of processes involving alterations in morphology, cellular architecture, adhesion and migration capacity (25,26). This process is evoked during tumor invasion and metastasis. EMT allows epithelial tumor cells to acquire the capacity of mesenchymal cells to infiltrate the surrounding tissue and metastasize to distant sites (7, 25). Molecular signaling pathways in EMT have been identified (7, 26, 27). Versican is known to participate in the EMT (4).

Versican is encoded by the *VCAN* gene, located on chromosome 5q (12-14). Versican mRNA generates four isoforms: V0, V1, V2 and V3 (28, 29). Each isoform contains globular domains at the amino terminus (G1) and carboxyl terminus (G3) (9). The G1 domain is composed of an immunoglobulin-like motif, followed by two proteoglycan tandem repeats that bind hyaluronan (HA) (30). The G3 domain contains two epidermal growth-factor-like repeats, a

Table II. *Correlations between versican expression and clinicopathological characteristics.*

Variable	Stromal expression		*p*-Value	Epithelial expression		*p*-Value
	Positive n=47	Negative n=58		Positive n=22	Negative n=83	
Age in years (mean±SD)	58.5±12.5	60.3±11.2	0.431	58.6±13.6	59.7±11.3	0.678
Gender, n (%)						
Male	35 (46.7)	40 (53.3)	0.535	16 (21.3)	59 (78.7)	0.879
Female	12 (40.0)	18 (60.0)		6 (20.0)	24 (80.0)	
Histological type, n (%)						
Differentiated	13 (40.6)	19 (59.4)	0.572	9 (28.1)	23 (71.9)	0.232
Undifferentiated	34 (46.6)	39 (53.4)		13 (17.8)	60 (82.2)	
Lauren classification, n (%)						
Intestinal type	14 (40.0)	21 (60.0)		9 (25.7)	26 (74.3)	
Diffuse type	24 (44.4)	30 (55.6)	0.555	10 (18.5)	44 (81.5)	0.683
Mixed type	9 (56.3)	7 (43.8)		3 (18.8)	13 (81.3)	
Lymphatic invasion, n (%)						
Present	43 (47.8)	47 (52.2)	0.128	17 (18.9)	73 (81.1)	0.300
Absent	4 (26.7)	11 (73.3)		5 (33.3)	10 (66.7)	
Vascular invasion, n (%)						
Present	3 (37.5)	5 (62.5)	0.729	1 (12.5)	7 (87.5)	1.000
Absent	44 (45.4)	53 (54.6)		21 (21.6)	76 (78.4)	
Perineural invasion, n (%)						
Present	32 (53.3)	28 (46.7)	0.041	12 (20.0)	48 (80.0)	0.782
Absent	15 (33.3)	30 (66.7)		10 (22.2)	35 (77.8)	
Serosal exposure, n (%)						
Absent	4 (22.2)	14 (77.8)	0.035	4 (22.2)	14 (77.8)	1.000
Present	43 (49.4)	44 (50.6)		18 (20.7)	69 (79.3)	
Lymph node metastasis, n (%)						
N0	13 (43.3)	17 (56.7)		6 (20.0)	24 (80.0)	
N1	8 (38.1)	13 (61.9)	0.612	4 (19.0)	17 (81.0)	0.963
N2	14 (56.0)	11 (44.0)		5 (20.0)	20 (80.0)	
N3	12 (41.4)	17 (58.6)		7 (24.1)	22 (75.9)	
Pathological stage, n (%)						
I	0 (0.0)	5 (100.0)		1 (20.0)	4 (80.0)	
II	14 (43.8)	18 (56.3)	0.085	7 (21.9)	25 (78.1)	1.000
III	33 (50.0)	33 (50.0)		14 (21.2)	52 (78.8)	
IV	0 (0.0)	2 (100.0)		0 (0.0)	2 (100.0)	

SD, Standard deviation.

carbohydrate recognition domain and complement-binding protein-like sub-domains with structural similarity to the selectin family (9). Versican can regulate many cellular processes—such as adhesion, proliferation and apoptosis—and participates in the EMT, including migration and invasion, through interactions of the G1 and G3 domains with other proteins (4, 9). In addition, versican binds to ECM components and cell surface molecules such as P- and L-selectin, CD-44 and integrin beta1 and can regulate chemokine function (31-34).

Because the V0 isoform is prevalent during early embryonic development but is less well-represented in adult tissues (35), little information exists for the distinct functions of the V0 isoform. The V1 isoform may have a function different to that of the V2 isoform. The V1 isoform enhances cell proliferation and protects NIH-3T3 fibroblasts from apoptosis (36). Conversely, the V2 isoform exhibits opposing biological activities by inhibiting cell proliferation and lacking any association with apoptotic resistance (36). No study has directly compared the biological activity of V3 with that of the other versican isoforms. Overexpression of the V3 isoform in melanoma cancer cells was demonstrated to markedly reduce cell growth *in vitro* and *in vivo*, and promoted metastasis to the lung (15, 37). These findings suggest that the V3 isoform may play a dual role as an inhibitor of tumor growth and a stimulator of metastasis (4). The isoforms of versican are considered to be differently distributed in each organ (38, 39). Judging from our results, the V2 or V3 isoforms would be distributed mainly in the epithelial cells of gastric cancer, whereas the stromal cells of gastric cancer might display a balanced distribution of the V2 or V3 and V1 isoforms; stromal versican expression is unlikely to have prognostic significance.

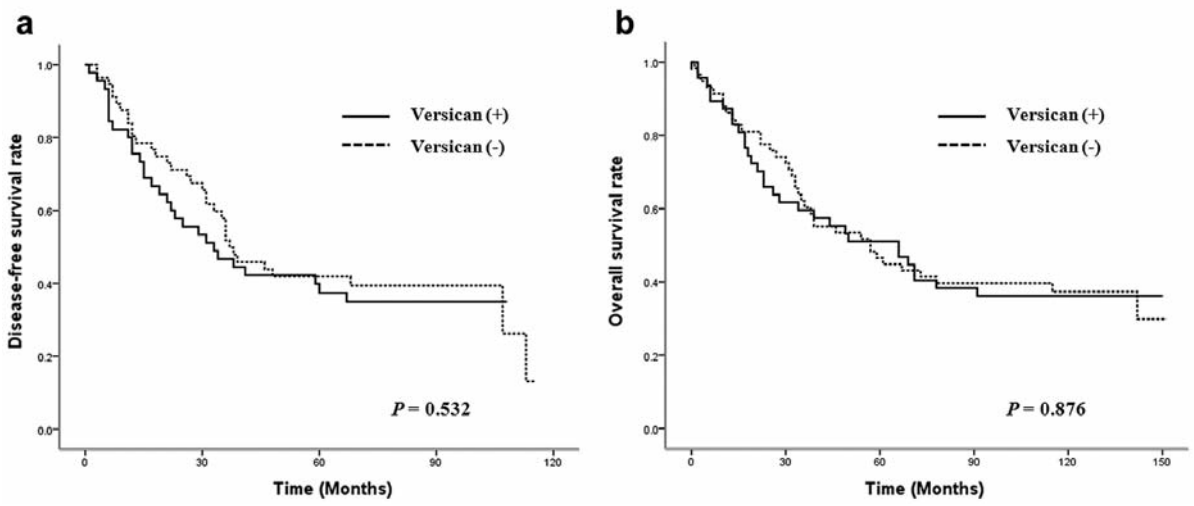

Figure 2. *Survival curves of patients according to versican expression of tumor stromal cells. No difference was found between versican-positive and -negative patients in the 5-year (a) disease-free (37.4% versus 41.9%, respectively; p=0.532) and (b) overall (51.1% versus 46.6%, respectively; p=0.876) survival rates.*

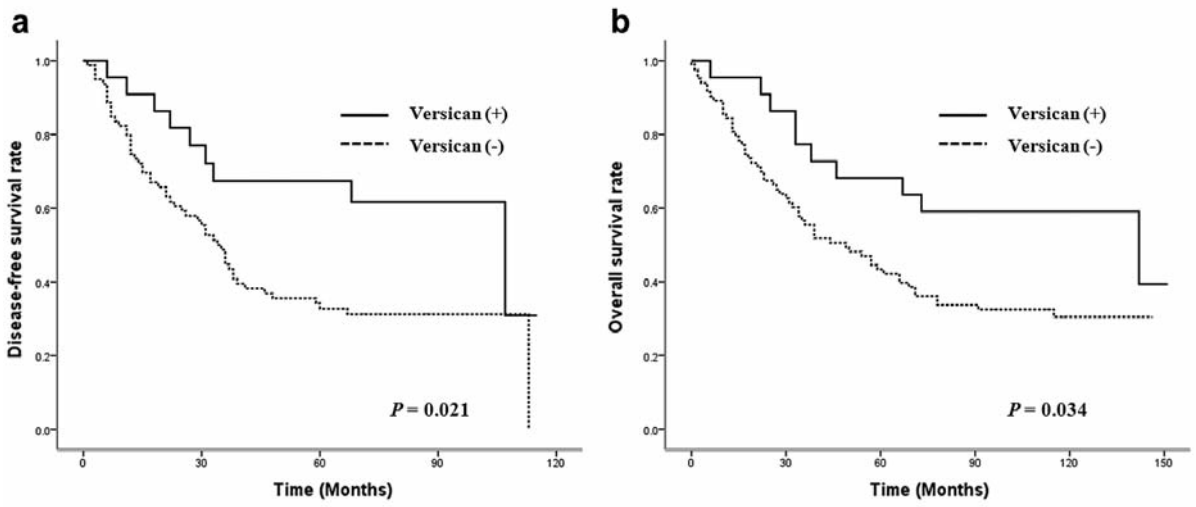

Figure 3. *Survival curves of patients according to versican expression in tumor epithelial cells. Versican-positive patients had significantly better 5-year (a) disease-free (67.4% versus 32.8%, respectively; p=0.021) and (b) overall (68.2% versus 43.4%, respectively; p=0.034) survival rates than versican-negative patients.*

Many studies have focused on the globular domains of versican. The G1 domain of versican stimulates proliferation by inhibiting cell adhesion, enhancing migration in astrocytoma cancer cells (36). However, high levels of G1 versican had opposing effects on cancer cell motility and the incidence of metastasis in lung cancer (16). Those results suggest that the G1 domain of versican can act both as a suppressor and a promoter of metastasis. Such a role of the G1 domain may explain the different prognostic effects of versican in several types of cancer.

To our knowledge, the current study is the first to clarify the prognostic value of epithelial versican expression in gastric cancer. In summary, the present study suggests that versican expression in tumor epithelial cells of gastric cancer is likely a prognostic biomarker that predicts a good outcome. By contrast, versican expression in stromal cells is not associated with the prognosis of gastric cancer. To investigate the effects of versican according to cancer type, a more accurate understanding of the role of versican isoforms and their molecular structure is required.

Table III. *Multivariate analysis of prognostic factors for disease-free survival in patients with gastric cancer.*

Variable	Coefficient	SE	Hazard ratio (95% CI)	*p*-Value
Serosal exposure				
Present/Absent	0.757	0.401	2.132 (0.971-4.681)	0.059
Lymph node metastasis				
N1/N0	0.043	0.418	1.044 (0.460-2.366)	0.919
N2/N0	0.520	0.367	1.682 (0.819-3.454)	0.157
N3/N0	0.741	0.355	2.098 (1.047-4.207)	0.037
Epithelial versican expression				
Positive/Negative	–0.817	0.361	0.442 (0.218-0.896)	0.024

SE, Standard error; CI, confidence interval.

Table IV. *Multivariate analysis of prognostic factors for overall survival in patients with gastric cancer.*

Variable	Coefficient	SE	Hazard ratio (95% CI)	*p*-Value
Serosal exposure				
Present/Absent	0.685	0.378	1.984 (0.946-4.159)	0.070
Lymph node metastasis				
N1/N0	0.176	0.389	1.192 (0.556-2.556)	0.652
N2/N0	0.527	0.354	1.694 (0.846-3.389)	0.137
N3/N0	0.632	0.338	1.881 (0.969-3.652)	0.062
Distant metastasis				
M1/M0	1.517	0.755	4.557 (1.037-20.023)	0.045
Epithelial versican expression				
Positive/Negative	–0.753	0.344	0.471 (0.240-0.925)	0.029

SE, Standard error; CI, confidence interval.

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