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Versican expression in human cervical cancer

J. Kodama[a,*], *Hasengaowa*[b], *T. Kusumoto*[a], *N. Seki*[a], *T. Matsuo*[a], *K. Nakamura*[a], *A. Hongo*[a], *Y. Hiramatsu*[a]

[a]*Department of Obstetrics and Gynecology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama 700-8558, Japan*
[b]*Department of Obstetrics and Gynecology, Inner Mongolia Agriculture University Hospital, Zhaowudalu 306, Huhehaote 010018, China*

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ABSTRACT

Versican expression may enhance tumour invasion and metastasis. However, the expressions of versican in cervical cancer have seldom been characterised. The aim of this study was to investigate versican expression in human cervical cancers. We immunohistochemically investigated the expression of versican protein in 174 cervical cancers and analysed the correlation with various clinicopathological features, including patient outcome. Stromal versican expression was significantly higher in patients with lymph node metastasis ($p < 0.0001$). Epithelial versican expression was significantly higher in patients with non-squamous cell cercinoma ($p = 0.0003$), lymph-vascular space invasion ($p = 0.046$), lymph node metastasis ($p = 0.009$) and ovarian metastasis ($p = 0.0001$). Multivariate analysis showed that high epithelial versican expression was an independent prognostic factor for disease-free survival. Versican enrichment of the tumour tissue may be associated with progression in cervical cancer. Versican expression can serve as an indicator of poor prognosis in patients with cervical cancer.

1. Introduction

Tumour cell invasion and subsequent metastasis via the bloodstream and lymph vessels are critical steps in the progression of malignant tumours, including cervical cancer. It is well known that the tumour environment is one of the major factors that determine the behaviour of malignant cells. Remodelling of the extracellular matrix (ECM) through altered expression of molecules integrated in the functional network of cell-to-cell and cell-to-matrix interactions is essential for local tumour cell invasion and metastasis.[1]

Proteoglycan is one of the components of the ECM that has the ability to alter cell function. Versican is a member of the large aggregating chondroitin sulphate proteoglycan (CSPG) family.[2] Structurally, versican is composed of an N-terminal G1 domain, a glycosaminoglycan (GAG) attachment region and a C-terminal G3 domain. Alternative splicing generates at least four isoforms of versican, named V0, V1, V2 and V3.[3–5] V0, the largest versican isoform, contains two GAG binding regions called the CSδ and CSβ domains. The V1 isoform contains a CSβ domain, and the V2 isoform contains a CSδ domain. Versican V3 is solely composed of the G1 and G3 domains, lacking all potential GAG attachment sites. Versican is highly expressed in the early stages of tissue development, and its expression decreases after tissue maturation. Its expression is also elevated during wound repair and tumour growth.[6–8] An increase in versican expression in the ECM facilitates local tumour invasion and metastasis by decreasing the cell–ECM adhesion.[9] In fact, it has been demonstrated that versican expression is related to tumour progression in some types of malignant tumours.[10–15]

* *Corresponding author:* Tel.: +81 862357320; fax: +81 862259570.
E-mail address: kodama@cc.okayama-u.ac.jp (J. Kodama).
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Thus, versican expression may enhance tumour cell invasion and metastasis, although its expression in human cervical cancer has seldom been characterised. Therefore, we investigated the expression of the versican protein in 174 cervical cancers. We then analysed its correlation with various observed clinicopathological features, including patient outcome.

2. Patients and methods

2.1. Patients and tissue samples

In this study, we examined 174 patients presenting with the international Federation of Gynecology and Obstetrics (FIGO) stages IB–IIB cervical cancers. Each of these patients underwent radical hysterectomy and pelvic lymphadenectomy at the Department of Obstetrics and Gynecology of the Okayama University Hospital. Tumour specimens were obtained at the time of surgery and were immediately fixed in 10% neutral-buffered formalin and embedded in paraffin. Informed consent was obtained from each patient prior to sample collection. Histological cell typing was conducted according to the WHO classifications as follows: 102 were classified as squamous cell carcinomas, 49 as adenocarcinomas and 23 as adenosquamous carcinomas. Clinical staging was assessed based on the FIGO staging system as follows: 92 were allocated to stage IB, 4 to stage IIA and 78 to stage IIB. The median age at the time of surgery was 46 years (range 25–67 years). Patients with lymph node metastasis, parametrial involvement, deep stromal invasion or marked lymph-vascular space involvement were treated with an adjuvant external whole-pelvic irradiation, combination chemotherapy or chemoradiation. Disease-free and overall survival rates were defined as the interval from the initial surgery to clinically or radiologically proven recurrence and death, respectively. The end date of the follow-up study for conducting the analysis was 31st July 2006, and the median duration of the follow-up was 60.5 months (range, 1–143 months).

2.2. Immunohistochemistry and staining evaluation

Four micrometer thick sections from several representative areas of the tumour specimens were placed onto glass slides and immunostained according to the labelled streptavidin biotin procedure of the Dako LSAB kit (Dako North America Inc., CA, USA). Briefly, after the slides were dewaxed in xylene and rehydrated in an alcohol series, antigen retrieval was performed in a microwave oven in 10 mM citric acid buffer (pH 6.0) for 3×10 min. The sections were incubated with 0.3% hydrogen peroxide to block endogenous peroxidase activity, followed by incubation with normal horse serum for 5 min at room temperature. Immunostaining was then performed by incubation with a 1:500 dilution of mouse monoclonal anti-human versican (clone: 2B1; Seikagaku Corporation Ltd., Tokyo, Japan) that recognises all forms of versican for 2 h at room temperature. The sections were next incubated for 20 min with biotinylated goat anti-mouse immunoglobulin followed by incubation with peroxidase-conjugated streptavidin for 20 min, and with 0.05% 3,3′-diaminobenzidine tetrahydrochloride (Wako Pure Chemical Industries Ltd., Osaka, Japan) containing hydrogen peroxide for 10 min. Finally, the slides were counterstained with Mayer's hematoxylin and mounted in aqueous mounting medium. At each step, the slides were washed carefully in phosphate-buffered saline (pH 7.4). As negative controls, the sections were incubated with normal mouse serum at a concentration of 10 μg/ml.

2.3. Staining evaluation

The level of versican immunoreactivity in the stroma was expressed by classifying the area of peri- and intra-tumoural versican-positive stroma into four groups: strong, more than 50% of the stroma stained; moderate, 10–50% of the stroma stained; weak, less than 10% of the stroma stained; and negative, no staining. We also evaluated versican-positive cancer cells. Any case with cancer cell-associated versican staining was considered as positive. Microscopic analyses were evaluated independently by two of the authors who had no prior knowledge of the clinical data. Final decisions in questionable cases were made using a conference microscope.

2.4. Statistical analyses

The χ^2 test was used to examine the association between clinicopathological factors and versican expression. The survival rates were calculated by the Kaplan–Meier method, and the differences between the survival curves were examined by using the log-rank test. Factors found to be significant were then analysed by a stepwise Cox's multivariate proportional hazard model to decide their prognostic values. These analyses were performed by utilising the Stat-View 5.0 software (Abacus Concepts Inc., CA, USA). Probability values less than 0.05 were considered to be statistically significant.

3. Results

3.1. Stromal versican expression

Fig. 1B–F illustrate the representative immunostaining of stromal versican in the cervical cancers. Strong stromal staining was observed in 19 tumours (11%); moderate staining, in 35 tumours (20%); weak staining, in 36 (21%) tumours; and no staining, in 84 tumours (48%). The association between stromal versican staining and clinicopathological factors is shown in Table 1. Stromal versican expression was significantly higher in cases with lymph node metastasis ($p < 0.0001$). Stromal versican expression was higher in cases with elderly age, deep stromal invasion and ovarian metastasis, although there was no statistical significance. In some cases, we noticed that versican staining was also observed in the normal cervical stroma surrounding the tumours (Fig. 1A). The frequency of versican staining in normal cervical stroma was significantly associated in cases with stromal versican staining in tumour stroma ($p = 0.003$).

3.2. Epithelial versican expression

Fig. 1D illustrates the characteristic immunostaining of epithelial versican in a cervical cancer. Epithelial versican

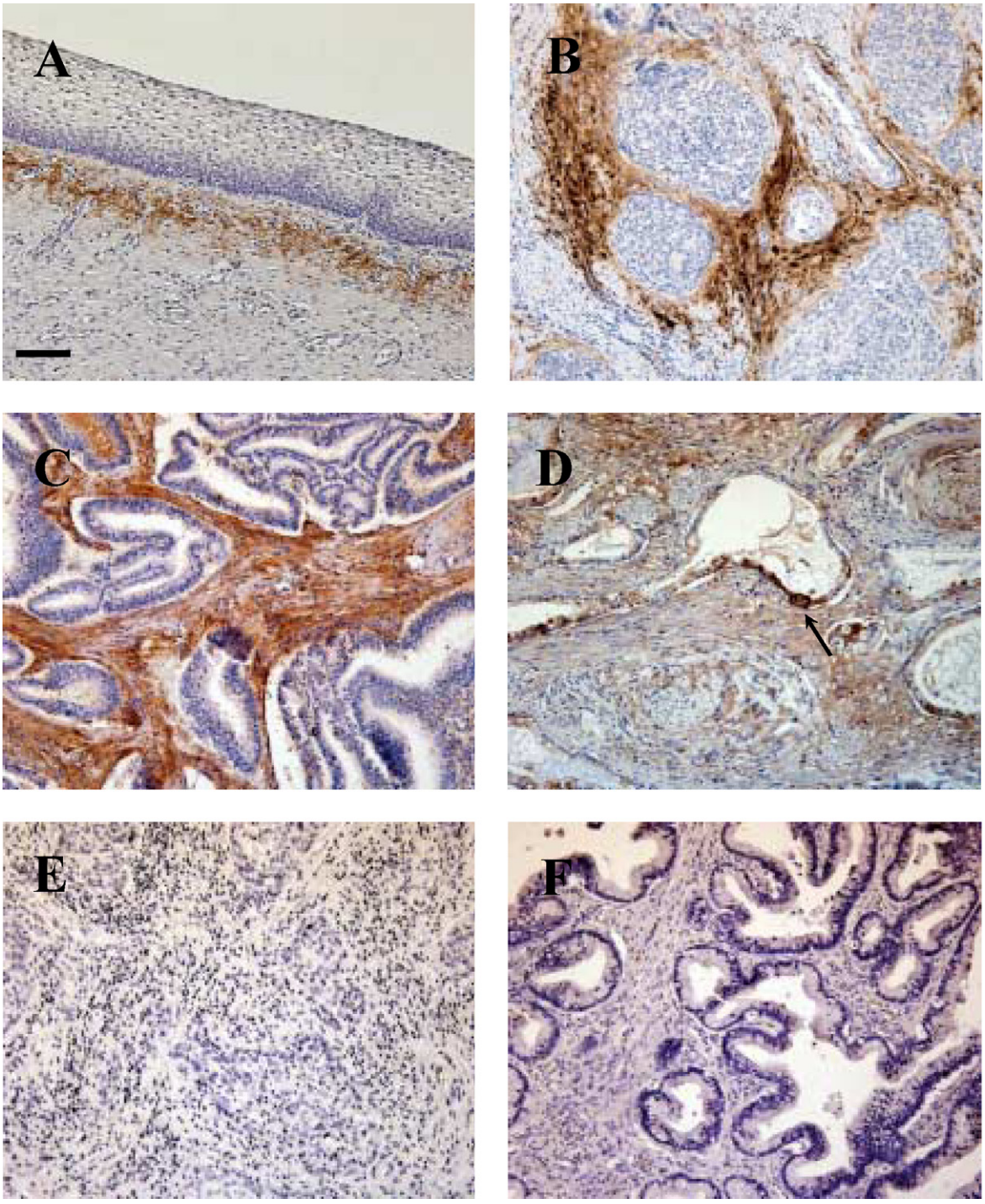

Fig. 1 – Immunohistochemical staining of versican in cervical cancer specimens using the anti-human versican 2B1. Scale bar = 50 μm. (A) Versican staining in normal cervical stroma. (B) Strong stromal versican staining in squamous cell carcinoma. (C) Strong stromal versican staining in adenocarcinoma. (D) Moderate stromal and intracellular staining of versican in adenocarcinoma. Epithelial accumulation of versican is indicated by an arrow. (E) Negative versican staining in squamous cell carcinoma. (F) Negative versican staining in adenocarcinoma.

staining was observed in 22 tumours (13%); and no staining, in 152 tumours (87%). The frequency of epithelial versican staining was significantly associated in cases with stromal versican staining ($p = 0.0006$). The association between epithelial versican staining and clinicopathological factors is shown in Table 2. Epithelial versican expression was significantly higher in patients with non-SCC ($p = 0.0003$), lymph-vascular space involvement ($p = 0.046$), lymph node metastasis ($p = 0.009$) and ovarian metastasis ($p = 0.0001$).

Table 1 – Association between stromal versican expression and clinicopathological factor in cervical cancer

Variable	Stromal versican expression				p-Value[a]	Variable	Stromal versican expression				p-Value[a]
	No	Weak	Moderate	Strong			No	Weak	Moderate	Strong	
Age (year)					0.087	*Vaginal invasion*					0.970
<50	60	22	18	9		Negative	72	31	31	16	
≧50	24	14	17	10		Positive	12	5	4	3	
FIGO stage					0.300	*Parametrial invasion*					0.393
I	49	20	14	9		Negative	67	25	29	13	
II	35	16	21	10		Positive	17	11	6	6	
Histological type					0.795	*LVS involvement*					0.313
Non-SCC	35	15	16	6		Negative	44	18	12	8	
SCC	49	21	19	13		Positive	40	18	23	11	
Tumour size (cm)					0.198	*Lymph node metastasis*					<0.0001
≦4	72	25	27	16		Negative	78	29	23	9	
>4	12	11	8	3		Positive	6	7	12	10	
Stromal invasion					0.069	*Ovarian metastasis*					0.051
≦2/3	49	18	12	7		Negative	84	35	34	17	
>2/3	35	18	23	12		Positive	0	1	1	2	

LVS, lymph-vascular space.
a χ^2 test.

Table 2 – Association between epithelial versican expression and clinicopathological factors in cervical cancer

Variable	Epithelial versican expression		p-Value[a]	Variable	Epithelial versican expression		p-Value[a]
	(–)	(+)			(–)	(+)	
Age (year)			0.918	*Vaginal invasion*			0.194
<50	95	14		Negative	133	17	
≧50	57	8		Positive	19	5	
FIGO stage			0.456	*Parametrial invasion*			0.609
I	82	10		Negative	118	16	
II	70	12		Positive	34	6	
Histological type			0.0003	*LVS involvement*			0.046
Non-SCC	55	17		Negative	76	6	
SCC	97	5		Positive	76	16	
Tumour size (cm)			0.864	*Lymph node metastasis*			0.009
≦4	122	18		Negative	126	13	
>4	30	4		Positive	26	9	
Stromal invasion			0.393	*Ovarian metastasis*			0.0001
≦2/3	77	9		Negative	151	19	
>2/3	75	13		Positive	1	3	

LVS, lymph-vascular space.
a χ^2 test.

3.3. *Univariate survival analysis*

Fig. 2 presents both the disease-free and overall survival curves for the 174 patients displaying cervical cancer, according to the stromal versican expression status. The disease-free and overall survival rates of patients exhibiting positive stromal versican expression were significantly lower than those of patients exhibiting negative stromal versican expression ($p = 0.0009$ and $p = 0.009$, respectively). Fig. 3 presents both disease-free and overall survival curves, according to the epithelial versican expression status. The disease-free and overall survival rates of patients exhibiting epithelial versican expression were significantly lower than those of patients exhibiting negative epithelial versican expression ($p < 0.0001$ and $p = 0.031$, respectively) Table 3.

3.4. *Multivariate survival analysis*

Multivariate analysis showed that lymph node metastasis was the strongest independent prognostic factor for disease-free survival; this was followed by positive epithelial versican expression (Table 4). In addition, non-SCC histology was the only independent prognostic factor for overall survival (Table 4).

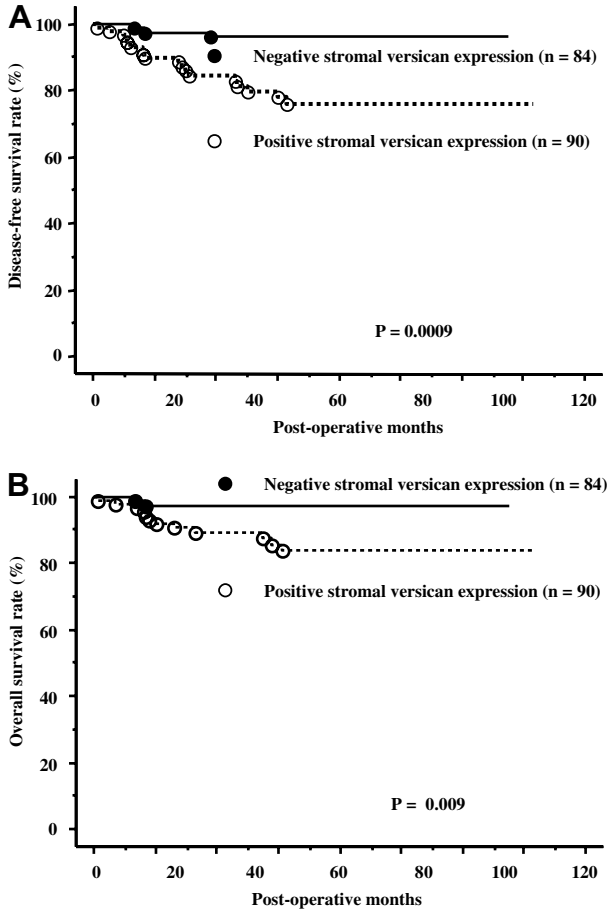

Fig. 2 – Disease-free (A) and overall (B) survival curves for the 174 patients displaying cervical cancer, according to the stromal versican expression status.

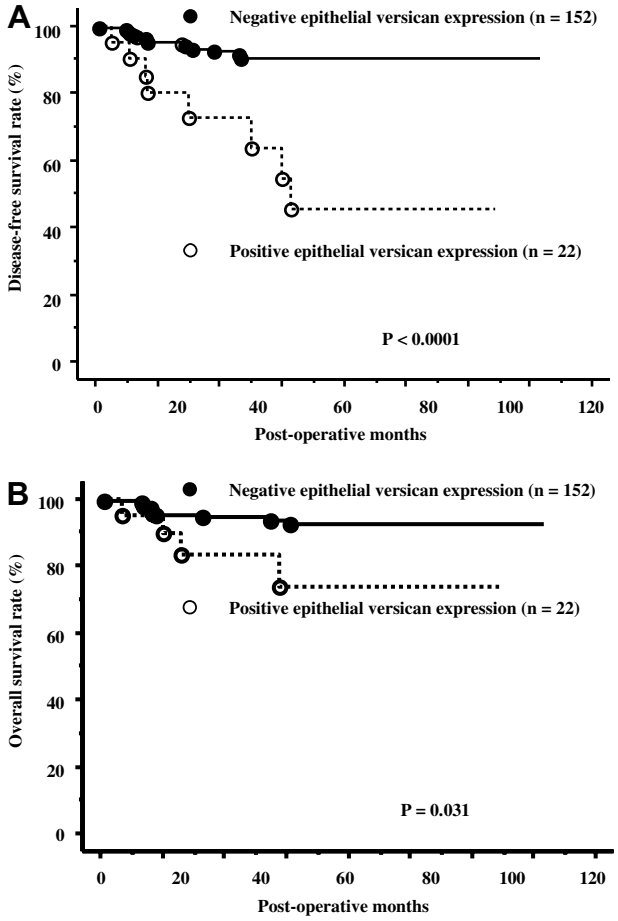

Fig. 3 – Disease-free (A) and overall (B) survival curves for the 174 patients displaying cervical cancer, according to the epithelial versican expression status.

4. Discussion

This is the first study to analyse versican protein expression in a large series of human cervical cancer specimens. We demonstrated that the overexpression of stromal versican was associated with lymph node metastasis. The overexpression of stromal versican in epithelial ovarian cancer, breast cancer, non-small cell lung cancer, prostate cancer, oral squamous cell carcinoma and endometrial cancer has been reported to be associated with tumour progression.[10–15] These results suggest that stromal versican is an important molecule in the progression of these malignant tumours. Indeed, it has been shown that the versican G1 domain can enhance cell proliferation and reduce cell adhesion in different cell types,[16,17] and the versican G3 domain enhances tumour growth and angiogenesis.[18] However, a contradictory observation has also been reported; increased stromal versican expression was found to be related to less advanced tumours in patients with pharyngeal squamous cell carcinoma.[19] The effects of stromal versican expression on tumour progression may therefore be dependent on the organ and tumour type examined.

In the present study, versican was mainly present in the peri-tumoural stroma; however, tumour cell-associated versican was also observed. The occurrence of versican-positive tumour cells was significantly higher in cases with non-SCC histology, lymph-vascular space involvement, lymph node metastasis and ovarian metastasis. This is in accordance with our previous study of endometrial cancer that epithelial versican expression was significantly higher in patients with lymph node metastasis and lymph-vascular space involvement.[15] Touab et al. also reported that cell-associated versican is involved in the progression of melanomas.[20] Pirinen et al. reported that tumour cell-associated versican is not significantly associated with clinicopathological factors in non-small cell lung cancer, although the frequency of the expression was insufficient to allow statistical analysis.[12] Voutilainen et al. reported that epithelial versican expression is significantly higher in early-stage epithelial ovarian cancer.[10] Although versican is probably synthesised mostly in tumour stroma by fibroblasts, malignant cells can also synthesise versican.[20–22] It may be due to altered production, storage, degradation or cellular uptake of versican.[23] Further studies are required to clarify the mechanism and functional role of epithelial versican expression.

Our study demonstrates that both epithelial expression and stromal versican expression are associated with the reduced survival outcomes in patients with cervical cancer. Furthermore, the multivariate analysis showed that epithelial

Table 3 – Disease- free and overall survival analyses of prognostic factors using the log-rank test

Variables	No.	Estimated 5-year DFS (%)	*p*-Value[a]	Estimated 5-year OS (%)	*p*-Value[a]
Age (year)			NS		NS
<50	109	88.9		90.9	
≧50	65	82.4		92.3	
Histology			NS		0.015
SCC	102	89.0		96.4	
Non-SCC	72	83.2		84.2	
Tumour size (cm)			0.031		0.024
≦4	140	88.9		93.8	
>4	34	77.6		81.5	
Stromal invasion			0.0005		0.005
≦2/3	86	97.4		96.8	
>2/3	88	76.2		85.9	
Vaginal invasion			NS		NS
Negative	150	87.5		92.1	
Positive	24	80.8		87.5	
Parametrial invasion			0.0007		0.012
Negative	134	92.4		94.7	
Positive	40	68.7		80.7	
LVS involvement			0.0001		0.001
Negative	82	96.9		98.8	
Positive	92	77.3		84.3	
Lymph node metastasis			<0.0001		<0.0001
Negative	139	94.1		95.4	
Positive	35	56.1		75.0	
Ovarian metastasis			<0.0001		0.0003
Negative	170	88.4		92.4	
Positive	4	25.0		50.0	
Stromal versican			0.0009		0.009
Negative	84	96.0		97.5	
Positive	90	77.9		85.8	
Epithelial versican			<0.0001		0.031
Negative	152	90.5		93.4	
Positive	22	54.7		74.1	

DFS, disease-free survival; OS, overall survival; LVS, lymph-vascular space; NS, not significant.
a Kaplan–Meier test.

Table 4 – Progonostic factors for disease-free and overall survival selected by Cox's multivariate proportional hazard model analysis

	Hazard ratio	95% CI	Cox's test *p*-value
Disease-free survival			
Lymph node mestastasis	3.44	1.19–9.91	0.023
Positive epithelial versican	3.22	1.15–9.01	0.026
Overall survival			
Histology (non-scc)	4.52	1.31–15.63	0.017

versican expression is an independent prognostic factor for disease-free survival in our study population. An increase in the expression of stromal versican has been previously reported to correlate with a poor prognosis in some types of cancers.[10–15] The present study is the first to indicate that versican expression may be a promising prognostic factor for this kind of cancer.

In conclusion, versican enrichment of the tumour tissue may be associated with tumour progression in cervical cancer. Our findings also provide evidence that versican expression can serve as an indicator of poor prognosis in patients with cervical cancer.

Conflict of interest statement

None declared.

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