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Clinicopathological significance of overexpression of TSPAN1, KI67 and CD34 in gastric carcinoma

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ABSTRACT

To investigate the expression of TSPAN1 (Gene ID: 10103), Ki67 and CD34 in gastric carcinomas and the clinicopathological significance, the expression of TSPAN1, Ki67 and CD34 was detected in 86 cases of gastric carcinoma, paraffin-embedded sections using an immunohistochemical method. The rates of overexpression of TSPAN1, Ki67 and CD34 in gastric carcinomas were 56.98%, 74.42%, and 62.79%, respectively. The overexpression of these markers was positively correlated with clinical stage and negatively correlated with survival rates (at 3 and 5 years). The overexpression of TSPAN1 and Ki67 was negatively correlated with carcinoma differentiation, and the overexpression of TSPAN1 and CD34 was positively correlated with infiltration and lymph node status of the tumor. Thus, overexpression of TSPAN1, Ki67 and CD34 in gastric cancer tissues is associated with development of the cancer. The detection of expression of TSPAN1, Ki67 and CD34 in gastric cancer may provide useful prognostic information for patients with the disease.

Introduction

Gastric carcinoma is now the second commonest cancer in the world [1]. Every year the number of new cases is increased by 800,000, and 650,000 die of the disease[1]. Among them, 60% occurs in developing countries like China[2]. The incidence in Japan is twenty times than that in western countries and is thought to result from a combination of environmental factors and accumulation of specific genetic alteration[3]. So far, the mechanism underlying the pathogenesis and development of the cancer is unclear. In addition, there are less efficient markers for the prognosis in the clinic.

TSPAN1 (or NET-1, Gene ID: 10103) is a new member of the tetraspanin super family[4], which plays an important role in cell signal transmission, regulation, adherence, mobility, proliferation and differentiation. It can be expressed in many kinds of human cells and tumors. Serru *et al.*[5] detected the expression of seven new tetraspans from TSPAN1 to NET-7 in various kinds of human cells using reverse-transcriptase polymerase chain reaction and showed that TSPAN1 was expressed in various kinds of tumor cells including cervical carcinoma, lung carcinoma, squamous cell carcinoma, colon carcinoma and carcinoma of the breast. Xu *et al.*[6] identified a differentially expressed TSPAN1 gene in human prostate tissues and prostate cancer using cDNA database subtraction and microarray. Evidence from our previous study suggested that TSPAN1 overexpression might contribute to liver carcinoma cellular proliferation and alterable differentiation[7]. Recently, Volker *et al.*[8] reported the significance of detecting the expression of TSPAN1 protein in cervical carcinoma using TSPAN1 mouse polyclonal antibodies. They found TSPAN1 expressions had an obvious correlation with pathological grading and clinical staging of cervical carcinoma. Combined with analysis of the follow-up, they noted that the expression of TSPAN1 may be an index for the prognosis of cervical carcinoma.

Key words: *CD34, gastric carcinoma, Ki67, prognosis, TSPAN1.*

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Ki67 is an important cell proliferation marker that is overexpressed in many cancers. It is closely correlated to malignant biological behavior and prognosis of many cancers[9,10].

As a membrane glycoprotein, CD34 is mainly expressed in hematopoietic cells and vascular endothelial cells. It is also called human hepatopoietic progenitor cell antigen. It is closely correlated to angiogenesis and is a marker of endothelial cells. CD34 has a stable and sensitive expression in capillaries and small vascular endothelial cells in tumors. Angiogenesis evaluation depending on CD34 is an important clue to estimate metastasis and prognosis of malignant tumors[11].

In the present study, we examined the expression of TSPAN1 combined with Ki67 and CD34 in tissues of 86 cases with gastric cancer. The relationship between the overexpression of TSPAN1, Ki67 and CD34 with the clinicopathological factors and the prognosis of gastric carcinoma was explored.

Material and methods

Clinical data of the patients

Formalin-fixed, paraffin-embedded tissues from 10 non-neoplastic mucosa at the gastric ulcer margin and 86 primary gastric carcinomas that had been surgically resected from December 1994 to December 1996 were retrieved from the files of the Department of Pathology, the People's Hospital of Haimen City, Jiangsu Province, PRC. None of the patients had received chemotherapy or radiotherapy before surgery. The patients ranged in age from 32 to 79 years (mean, 57.6). There were 57 males (66.28%) and 29 females (33.72%). The tumors were located in the gastric antrum, cardiac, lesser curvature, and other locations, and they ranged from 4 to 16 cm in diameter (median, 7.5). A total five slides was examined for each case. Histologic types were classified into papillary adenocarcinoma, tubular adenocarcinoma, mucoid adenocarcinoma, signet-ring cell carcinoma, and the other types including small cell and undifferentiated carcinoma[12,13]. Metastasis based on lymph node status and pathological grading[14] (grade 1, well-differentiated, *versus* grades 2 and 3, intermediate or poorly differentiated) and depth of myoinvasion (in or out of the serosa) were assessed on hematoxylin-eosin-stained sections. Clinical stages of the tumors were determined according to the TNM Classification System of the AJCC from I-IV[15]. The follow-up period of the patients ranged from 60 to 90 months.

Preparation of TSPAN1 polyclonal antibody

TSPAN1 gene was screened in amino an acid/protein database through the computer, then the antigenicity, hydrophilicity, flexibility, amphiphilic, helicity, and amphiphilic lamellar surface probability of the TSPAN1 gene were analyzed comprehensively. Antigen signal was found in the 140~200 amino acid sequence range in TSPAN1 protein, and other markers were in the ideal range. We identified 15 amino acids from the 153 to 168 amino acid sequence, which was "NYTDFED-SPYFKENS", as ideal antigenic determinants when they all had antigenicity. To increase antigenicity, C amino acid was added to the N-terminal of the peptide. While verifying the homology in the amino acid/protein database (protein Blast), no other homology with other proteins was found. Polyclonal antibody was prepared by immunizing rabbits. Negative antibody against TSPAN1 protein was used as negative control.

Immunohistochemical examination of expression of TSPAN1, Ki67 and CD34

Immunohistochemical Envision+/DAB method was performed on formalin-fixed, paraffin-embedded 5-μm sections from all patients for the detection of TSPAN1 and Ki67 in cancer cells and CD34 in stromal microvessels. Five consecutive slides were prepared from each tissue block and stained. The paraffin slides were dewaxed in xylene and microwaved. For antigen retrieval, they were heated at 95 °C for 10 min in sodium citrate buffer (10 mM sodium-citrate monohydrate, pH 6.0). Slides were allowed to cool for 20 min at room temperature and then incubated in Envision+peroxidase blocking solution (Dakocytomation, Glostrup, Denmark) for 5 min and rinsed with 0.05% Tris-buffered saline (TBS)/Tween 20 buffer, pH 7.4. The slides were then incubated with primary antibodies for 30 min at room temperature (primary antibodies including TSPAN1 antibody and negative antibody were devised by the author and prepared under the cooperation of the American San Francisco Gene Biological Company, San Francisco, Ca, USA). Mouse anti-human monoclonal antibody ki67 was purchased from Zymed Laboratories (USA). Mouse anti-human hematopoietic stem cell monoclonal antibody was obtained from CD34 (Zymed). Slides were washed with 0.05% Tween 20 in TBS (pH 7.4). Detection was achieved with the DAKO Envision+/HRP system (Dakocytomation). The color was developed by a 15-min incubation with a diaminobenzidine (DAB) solution (DAB kit IL1-9032), and sections were weakly counterstained with hematoxylin.

The immunohistochemical trimarker double-staining method was performed using the Envision double-labeling system (Dakecytomation) according to the manufacturer's instructions. Briefly, slides were subjected to high-temperature antigen retrieval (20 min of microwaving in citrate buffer (10 mM sodium-citrate monolydrate, pH 6.0), then incubated in Envision+peroxidase blocking solution (Dakecytomation) for 5 min, rinsed in water and transferred to TBS. Sections were incubated at 37°C for 60 min with the first primary antibody Ki67(1/100) and rinsed in TBS. A second-

ary linking polymer antibody bound to goat antimouse IgG/alkaline phosphatase (PV-6001) was then applied for 30 min. Sections were rinsed with TBS and stained by blue-black BCIP/NBT color reagent (Zymed Histostain®-Dskit) at 37°C for 30 min. After completion of the primary staining sequence, slides were rinsed in TBS and incubated in a double-stain block solution for 3 min (Dakecytomation), followed by rinsing in TBS. TSPAN1 was applied (1/100) to the tissue sections at 37 °C for 60 min and incubated at 4 °C overnight. A secondary linking antibody bound to goat antirabbit IgG/horseradish peroxidase (PV-6002) was added for 30 min at 37 °C followed by rinsing in TBS. Sections were developed with a liquid DAB plus for 10 min (for a brown color). After completion of the double staining, slides were rinsed in TBS and incubated in the third stain block solution for 3 min (Dakecytomation), followed by rinsing in TBS. The slides were incubated with CD34 antibody at 37 °C for 60 min and at 4 °C overnight. A secondary linking polymer antibody bound to goat antimouse IgG/horseradish peroxidase (PV-6002) was added at 37 °C and incubated for 30 min. Slides were stained by AEC color reagent (Histostain-Dskit) for 10 min (for a red color), rinsed in TBS, counterstained in Mayer's hematoxylin for 10 s, and mounted in aqueous mounting medium.

Positive controls (normal lymph node proliferating genetic center with strong nuclear Ki67, hepatocellular carcinoma cytoplasmic TSPAN1, and breast cancer stromal microvessel endothelia CD34) and negative controls (TBS was substituted for primary antibody at the same concentration) were performed for each immunohistochemical run.

Staining patterns and evaluation standard

The percentage of tumor cells expressing the various antigens under investigation was assessed semiquantitatively at x200 magnification. We used a grading system based on the intensity and extent of cytoplasmic/membranous and nuclear immunostaining of TSPAN1 and Ki67 proposed and applied in a series of previous studies by other authors[9,16] (Table 1). The counts were performed on the active tumor area in all available fields of view. The mean value was used as a cutoff point to define cases of over and low expression. A cutoff point of 50% for TSPAN1 cytoplasmic/membranous reactivity and a cutoff point of 10% for Ki67 nuclear reactivity were used to define an overexpression group *versus* a low expression group.

Tumor angiogenesis was assessed by microvessel density (MVD) counting using CD34 immunostaining. Three areas of high vascular density were selected at the invading tumor front with an image-analyzed system. The final microvessel score was the mean of the vessel counting number obtained from these fields. Only blood vessels with a clearly defined lumen or a linear vessel shape, but not single endothelial cells, were taken into account. For MVD, a cutoff point of 15 under high power view (×200) was used to define the overexpression group *versus* the low expression group.

In immunohistochemical trimarker double staining, TSPAN1 expression was shown by a brown color (DAB), Ki-67 with blue-black color (BCIP/NBT), and CD34 with a red color (AEC).

In all cases, the assessment of TSPAN1, Ki67and CD34 was performed independently by two observers on a multihead microscope.

Statistical analysis

Differences of the positive expression rates of TSPAN1, Ki67 and CD34 and clinicopathological parameters were analyzed by the chi-squared test for categorical variables. The correlations of TSPAN1, Ki67 and CD34 expression were statistically analyzed by Spearman grade correlation analysis with Stata 9.0. Fisher's exact test was used to assess the relationship of the positive expressions in different groups and the clinicopathological variables. Survival curves were estimated using the method of Kaplan-Meier, and the logrank test was used to determine statistical differences between life tables. *P* values <0.5 were considered significant.

Table 1 - A grading system based on the intensity and extent of cytoplasmic and nuclear immunostaining of TSPAN1 and Ki67

Grading	Score
Complete absence of reactivity	Negative (low)
Weak cytoplasmic reactivity in less than 50% of tumor cells	Low
Nuclear expression in sporadic tumor cells (<10% of cells)	Low
Strong cytoplasmic expression in more than 50% of tumor cells	Over
Nuclear expression in more than 10% of tumor cells	Over

Results

Expression patterns of TSPAN1, Ki67 and CD34 in gastric carcinoma tissues

The expression of TSPAN1 in cancer cells varies from cytoplasm to membrane, or mixed. Figure 1 shows the expression patterns of TSPAN1 in gastric carcinomas. The expression of Ki67 was mainly in the nucleus (Figure 2). CD34 was mainly expressed in endothelial cell microvessels with a scattered pattern. The most dense staining zones were around carcinoma nests (Figure 3) and were reduced significantly in the center of carcinoma nests. The mean value of MVD expressed by CD34 in gastric carcinoma was 27.8 ± 10.3 (4-52). All positive control groups showed the best expression staining of

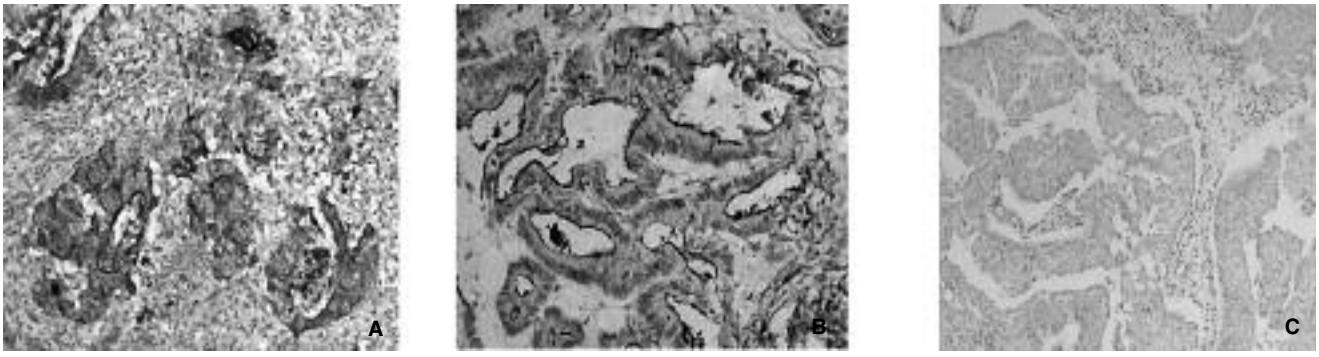

Figure 1 - Immunohistochemical images of TSPAN1 reactivity. A) Poorly differentiated gastric carcinoma tissue with deeper myoinvasion showes cytoplasmic staining. Magnification ×200; B) Well-differentiated gastric carcinoma tissue shows membranous staining. C) Negative control. Magnification ×200.

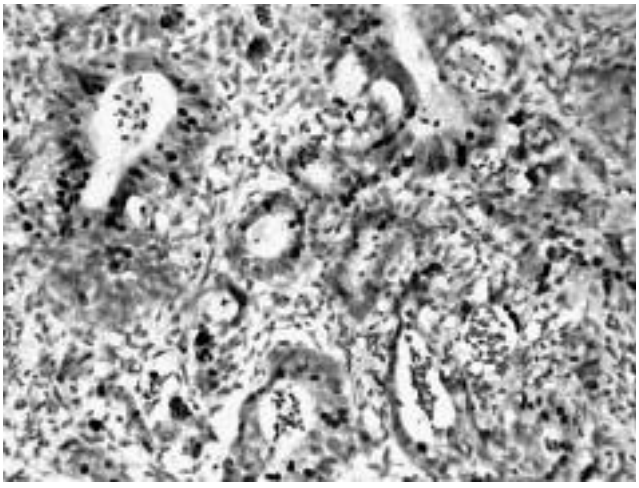

Figure 2 - Immunohistochemical images of Ki67 reactivity. Poorly differentiated gastric carcinoma tissue with deeper myoinvasion shows nuclear staining with an overexpression. Magnification ×200.

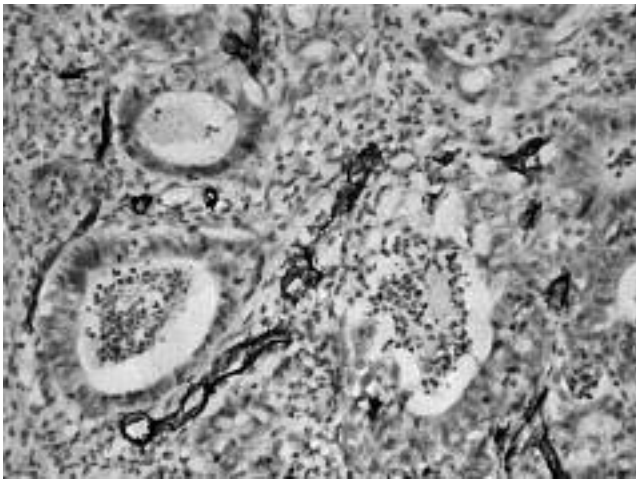

Figure 3 - Immunohistochemical images of CD34 reactivity. Poorly differentiated gastric carcinoma tissue with deeper myoinvasion shows CD34 overexpression in stromal microvessels. Magnification ×200.

TSPAN1, Ki67 and CD34, and no positive staining was found in negative control groups.

Observation of localization of TSPAN1, Ki67 and CD34 by immunohistochemical trimarker double staining

For more precise localization, three-marker immunohistochemical staining was performed in 46 positive cases. Three markers were well expressed on the same slide by different color reagents. TSPAN1, Ki67 and CD34 displayed strong localization in tumor cell cytoplasm or membrane, in tumor cell nucleus, and in stroma vessels, respectively (Figure 4). Three markers were positively correlated *in situ*.

Correlation of the overexpression of TSPAN1, Ki67 and CD34 in gastric carcinoma

In all tissues studied, the overexpression rates of TSPAN1, Ki67 and CD34 were 56.98% (49/86), 74.42% (64/86), and 62.79% (54/86), respectively. Table 2 shows the association among the expression of TSPAN1, Ki67 and CD34 in 86 cases of gastric carcinomas. There was a significant relationship between the overexpression of TSPAN1 and Ki67 (Cramer's V = 0.4192, P = 0.000) and between the overexpression of TSPAN1 and CD34 (Cramer's V = 0.5134, P = 0.000), whereas no significant relationship was found between the overexpression of Ki67 and CD34 (Cramer's V = 0.1703, P = 0.173).

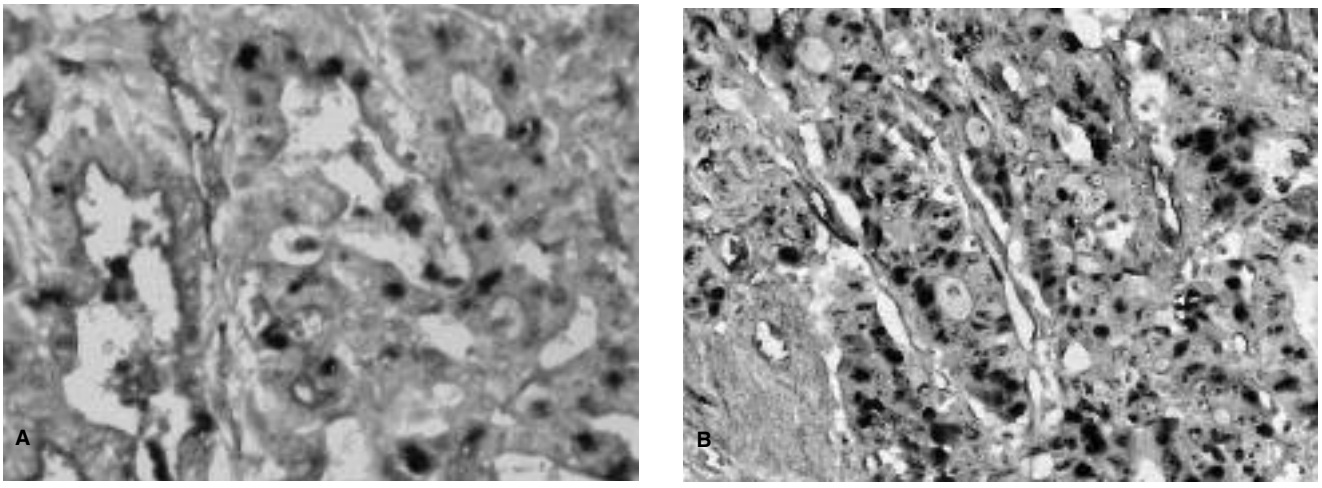

Figure 4 - Immunohistochemical trimarkers double staining demonstrated a co-distribution of TSPAN1, Ki67 and CD34 in gastric carcinomas tissue. A) Well-differentiated gastric carcinoma tissue overexpresses TSPAN1 in cytoplasm or on membrane at the edge of a glandular cavity, overexpresses Ki67 in the nucleus, and shows stromal microvessels stained by CD34. Magnification ×400. B) Poorly differentiated gastric carcinoma tissue shows TSPAN1 staining in tumor cytoplasm, Ki67 in tumor cell nucleus, and CD34 in stromal microvessels with equal expression intensity. Magnification ×400.

Table 2 - Correlation of the overexpression of TSPAN1, Ki67 and CD34 in gastric carcinoma

Index	Expression							Cramer's V	*P**
	Negative		Low		High				
	No.	%	No.	%	No.	%			
TSPAN1[a]	0	0.00	37	22.09	49	56.98	a *vs* b	0.4192	0.000
Ki67[b]	8	9.30	14	16.28	64	74.42	a *vs* c	0.5134	0.000
CD34[c]	0	0.00	32	37.21	54	62.79	b *vs* c	0.1703	0.173

*Fisher exact test.

Correlation of the overexpression of TSPAN1, Ki67 and CD34 with clinicopathological factors in gastric carcinomas

The overexpression of TSPAN1 was negatively related to cancer cell differentiation (χ^2 = 10.6143, P = 0.005) and tumor metastasis (χ^2 = 10.6407, P = 0.001) (Table 3). The overexpression rate in clinical stages III-IV was 35%, significantly higher than the rate, 14%, for stages I-II (χ^2 = 5.7267, P = 0.017). There was a significant difference between the intermediate and low differentiation groups (c^2 = 6.7308, P = 0.009). The overexpression of Ki67 was negatively related to tumor differentiation (χ^2 = 12.1325, P = 0.002). Its expression in clinical stages III-IV was significantly higher than that in stages I-II (χ^2 = 4.7295, P = 0.030). There was a significant difference between the intermediate group (positive rate = 65%) and low differentiation group (positive rate = 90%) (χ^2 = 5.7509, P = 0.016). The overexpression of CD34 was significantly and positively related to cancer metastasis (χ^2 = 8.8367, P = 0.003) and deep tumor invasion (χ^2 = 6.013, P = 0.049). There was a significant difference between the myoinvasion (<1/2 and >1/2) groups (χ^2 = 44.2392, P = 0.000). The overexpression of CD34 in clinical stages III-IV was significantly higher than that in stages I-II (χ^2 = 5.957, P = 0.015).

Survival analysis

The relationship between the overexpression of TSPAN1, Ki67 and CD34 and the 3- and 5-year survival rates is shown in Table 4. The survival rates of the TSPAN1 overexpression within 3 years group and within 5 years group were 44.9% and 26.53%, respectively, significantly lower than those of the low expression groups, which were 67.57% and 56.76%, respectively (P = 0.049 and P = 0.007). The survival rates of Ki67 overexpression within 3 years group and within 5 years group were 45.31% and 35.94%, considerably lower than those of

Table 3 - Correlation of the overexpression of TSPAN1, Ki67 and CD34 with clinicopathological factors in gastric carcinoma

Clinicopathological factor	No. of cases	TSPAN1[a]		Ki67[b]		CD34[c]		*P*-value*: low *vs* over
		low	over	neg/low	over	low	over	
Location								
Gastric antrum	43	20	23	14	29	16	27	a 0.924
Cardiac part	15	6	9	3	12	7	8	b 0.524
Lesser curvature	12	5	7	2	10	5	7	c 0.638
Other	16	6	10	3	13	4	12	
Myoinvasion								
<1/2	10	5	5	4	6	7	3	a 0.425
>1/2	25	13	12	8	17	10	15	b 0.274
Serosa	51	19	32	10	41	15	36	c 0.049
Histological types								
Papillary	25	9	16	6	19	8	17	a 0.694
Tubular	20	11	9	8	12	10	10	b 0.331
Mucoid	18	8	10	5	13	8	10	c 0.507
Signet-ring cell	19	8	11	3	16	5	14	
Other types	4	1	3		4	1	3	
Differentiations								
Well	15	10	5	8	7	7	8	a 0.005
Intermediate	26	15	11	9	17	13	13	b 0.002
Low	45	12	33	5	40	12	33	c 0.104
Metastasis								a 0.001
N0	22	16	6	6	16	14	8	b 0.833
N1-3	64	21	43	16	48	18	46	c 0.003
Clinical stage								a 0.017
I-II	34	20	14	13	21	18	16	b 0.030
III-IV	52	17	35	9	43	14	38	c 0.015

* Chi-squared test.

Table 4 - Correlation between the overexpression of TSPAN1, Ki67 and CD34 and 3- and 5-year survival rates

Index	No. of cases	Survival rate (%)		Low/Neg *vs* over: *P**	
		3 years[a]	5 years[b]		
TSPAN1 low	37	67.57	56.76	a	0.049
TSPAN1 over	49	44.90	26.53	b	0.007
Ki67 neg/low	22	77.27	72.73	a	0.013
Ki67 over	64	45.31	35.94	b	0.003
CD34 low	32	62.50	56.25	a	0.040
CD34 over	54	51.85	31.48	b	0.376

*Fisher exact test.

the negative or low expression groups, which were 77.27% and 72.73%, respectively (P = 0.013 and P = 0.003). The 5-year survival rate of the CD34 overexpression group was significantly lower than that of the low expression group (P = 0.04). There was no significant difference in 3-year survival rates between the CD34 overexpression and low expression groups (P = 0.376).

The comparison of Kaplan-Meier survival curves is presented in Figure 5. The survival rate of the TSPAN1 overexpression group was significantly lower than that of the low expression group (logrank test, χ^2 = 4.55, P = 0.0328 <0.05). The survival rate of the Ki67 overexpression group was significantly lower than that of negative or low expression groups (logrank test, χ^2 = 5.79, P = 0.0161). The survival rate of the CD34 overexpression group was significantly lower than that of the low expression group (logrank test, χ^2 = 4.13, P = 0.042).

Discussion

Gastric cancer is the second most common cause of cancer-related death in the world[1,2]. Many studies have examined the molecular genetics of gastric cancer in general, but no study on the clinicopathological significance between gastric carcinoma and the expression of TSPAN1 has yet been reported.

We examined the overexpression of TSPAN1, a new member of the tetraspanin super family[4-8], in 86 cases of gastric carcinoma; 56.98% of them expressed the TSPAN1 protein. The expression of TSPAN1 in cancer cells displayed cytoplasmic or membranous patterns, or mixed membranous/cytoplasmic patterns, which

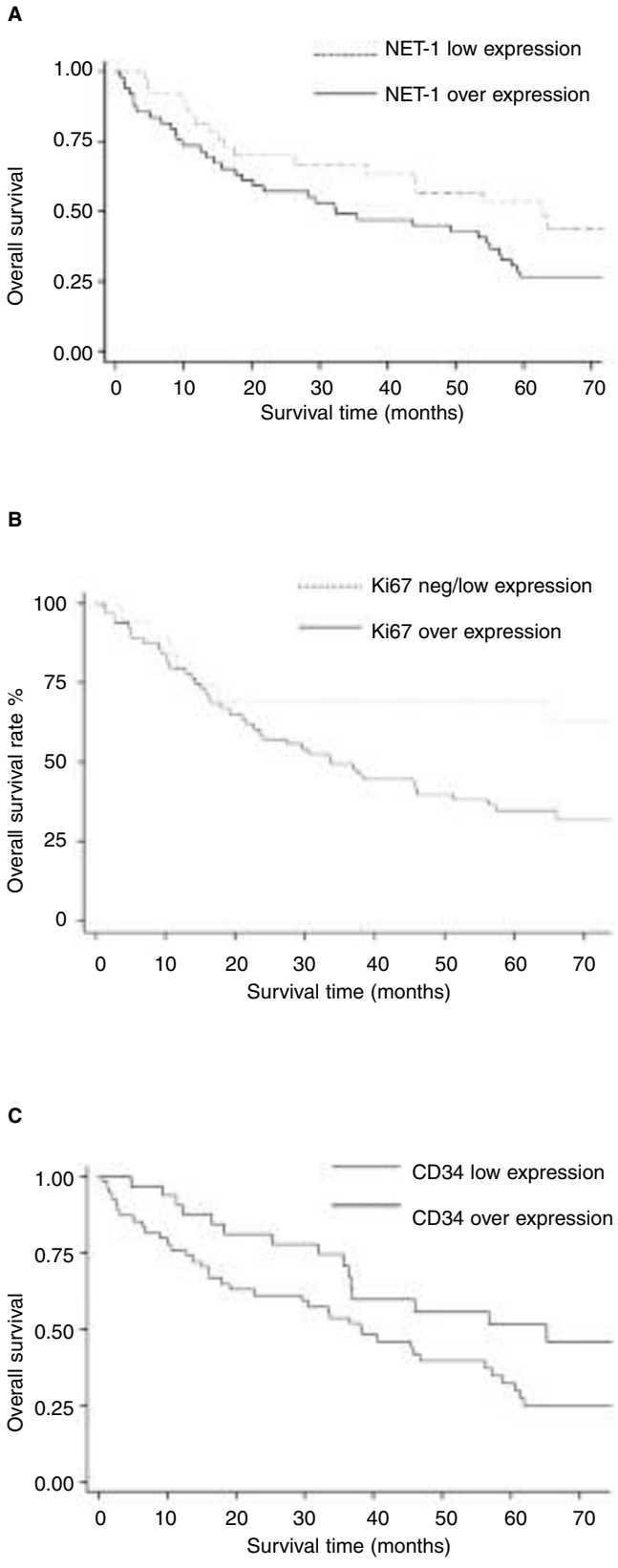

Figure 5 - Kaplan-Meier survival curves in gastric carcinomas stratified for TSPAN1 (A), Ki67 (B) and CD34 over *vs* low expressions (C).

showed the distribution and functional sites of the TSPAN1 molecule in cells. The molecule may accept extracellular signals when located on the membrane and carry out functions in the cytoplasm. The overexpression of TSPAN1 was linked to tumor clinical stage, survival rate, pathologic grade and cancer metastasis. A higher expression of TSPAN1 implied a lower cell differentiation and higher clinical staging. It is noteworthy that the molecule was related to the differentiation of cancer cells. Cancer cells with low differentiation displayed an increase or accumulation of the TSPAN1 protein in cells. It raises the possibility that the transcription activity of the gene encoding the TSPAN1 protein was elevated in cancer cells with low differentiation, or that the production or function of the protein in cells was changed. This could be valuable for further study. However, the survival rates of the TSPAN1 overexpression group within 3 and 5 years were significantly lower than those of the low expression group.

The association and prognosis between the expression of TSPAN1 and gastric carcinoma in this study were similar to those found in the study of Volker *et al.*[8] in cervical carcinomas. The expression of TSPAN1 was elevated in gastric carcinoma compared to non-neoplastic tissues.

The Ki67 antigen was discovered as a nuclear antigen that was expressed in proliferating cells by Gerdes *et al.*[17] in 1983. It is also a gene marker of nuclear proliferation which exists in all stages of the cell cycle except stage G_o. It is expressed in stage G_1 in the cell cycle and increased in stage S and stage G_2, reaching the peak in stage M and disappearing rapidly at the late stage of division. Since its half life calculation is short, it degrades quickly after breaking away from the cell cycle. Ki67 has become the most reliable marker to determine the proliferating activity of tumor cells[17,18].

Ki67 monoclonal antibody was once used in the study on the proliferation of non-Hodgkin lymphoma, sarcoma, inner ear cholesteatoma and various kinds of brain tumors. It was found that Ki67, similar to other proliferation markers, could indicate the proliferation rate of a tumor[17-19]. As shown in the present study, the overexpression rate of Ki67 detected in 86 cases of gastric carcinoma was 74.42%, similar to the other reports[9]. The overexpression of Ki67 in gastric carcinoma was correlated with tumor clinical stage, pathologic grade, and deep gastric wall invasion. The expression of Ki67 in clinical stages III-IV was higher than in stages I-II. The survival rate of Ki67 overexpression within 3 and 5 years was significantly lower than in the low expression group. The results confirmed the close correlation between the expression of Ki67 and proliferation and prognosis of gastric carcinoma. Ki67 was not expressed in nonneoplastic gastric mucosa, indicating a low proliferative activity in the tissues. The increased expression of Ki67 in poorly differentiated tis-

sues implied that tumor cells lost growth control in gastric carcinogenesis, leading to a DNA synthesis disorder which reflected the malignant behavior of tumor cells. This result of the study indicates that Ki67 is a good objective indicator of the proliferative ability of gastric carcinoma cells and can serve as an important index of the proliferation and differentiation of gastric carcinoma cells.

Angiogenesis plays an important role in the malignant transformation, growth, and metastasis of parenchymal tumors. Tumor angiogenesis is regulated by angiogenesis factors generated and excreted by tumor cells. Several important angiogenesis factors have been verified[11,20]. In this gastric carcinoma group, the overexpression of CD34 was 62.79%. The expression was closely correlated with tumor clinical stage, depth of infiltration and lymph node metastasis. The overexpression of CD34 was positively correlated with lymph node metastasis of cancer cells, which indicated that the CD34 antigen not only reflected objectively MVD in a carcinoma but also estimated the potential risk of metastasis. Accordingly, the 5-year survival rate of the CD34 overexpression group was significantly lower than that of the CD34 low expression group.

To further corroborate the relationship of the distributions of TSPAN1, Ki67 and CD34 in gastric carcinomas, a immunohistochemical trimarker double-staining method was performed in the cases positive for the individual markers. The method showed the relationship and accompanying conditions among three kinds of antigens, allowing an objective assessment of the more physiological co-distribution of the three markers. The results showed that, in the same tumor cell, the higher the expression of TSPAN1 in the cytoplasm, the stronger the expression of Ki-67 in the nucleus, accompanied by an increased MVD indicated by CD34 in the stroma. This phenomenon implies that the three proteins may cooperate with each other in the process of gastric carcinogenesis. In addition, the result indicated that the overexpression of TSPAN1 was related to tumor cell proliferation and up-regulated angiogenesis. Survival curves revealed that these cases with TSPAN1, Ki67 and CD34 overexpression displayed a worse clinical outcome.

In summary, for the first time, we have shown that TSPAN1 overexpression in gastric cancer is involved in cancer biological behavior including proliferation, differentiation, metastasis, clinical stage, prognosis and angiogenesis. Overexpression of TSPAN1, Ki67 and CD34 in gastric cancer tissues is associated with the development of the cancer. The detection of expression of TSPAN1, Ki67 and CD34 in gastric cancer may provide useful prognostic information for patients with the disease.

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