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• GASTRIC CANCER •

Association of VCAM-1 overexpression with oncogenesis, tumor angiogenesis and metastasis of gastric carcinoma

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Abstract

AIM: To investigate the relationship between the expression of vascular cell adhesion molecule-1 (VCAM-1) and oncogenesis, tumor angiogenesis and metastasis in gastric carcinoma, and to evaluate the clinical significance of serum VCAM-1 levels in gastric cancer.

METHODS: Specimens from 41 patients with gastric cancer, 8 patients with benign gastric ulcer, and 10 healthy subjects were detected for the expression of VCAM-1 by immunohistochemistry. Microvessel density (MVD) was measured by counting the endothelial cells immunostained with the monoclonal antibody CD34 at x200 magnification. Serum VCAM-1 concentrations were measured by an enzyme linked immunosorbent assay in the 41 gastric cancer patients before surgery, and at 7 days after surgery as well as in 25 healthy controls. The association between preoperative serum VCAM-1 levels and clinicopathological features, and their changes following surgery was evaluated. In addition, serum carcinoembryonic antigen (CEA) was also examined.

RESULTS: Of the 41 gastric cancer tissues, 31 (75.6 %) were VCAM-1 positive. The VCAM-1 positive gastric cancers were more invasive and classified in the more advanced stage than the VCAM-1 negative ones. The VCAM-1 positive cancers were associated with more lymph node metastases than VCAM-1-negative ones ($P<0.05$). The expression of VCAM-1 was detected in tissues of two of the eight patients with gastric ulcer and two of the 10 healthy controls. The expression of VCAM-1 in gastric cancer patients was significantly more frequent than that in the healthy controls and ulcer group (both $P<0.05$). MVD in VCAM-1 expressing tissues was higher than that in VCAM-1 negative tissues ($t=2.13$,$P<0.05$). Serum VCAM-1 levels in gastric cancer patients were significantly higher than those in controls ($t=3.4$, $P<0.05$). There was a significant association between serum VCAM-1 levels and disease stage, as well as invasion depth of the tumor and the presence of distant metastases. The concentrations of serum CEA in gastric cancer were higher than normal controls. Both serum VCAM-1 and CEA levels decreased significantly after radical resection of the primary tumor ($P<0.05$). Furthermore, the serum levels of VCAM-1 were positively correlated with the expression of VCAM-1 in the tumor tissue ($r=0.85$, $P<0.05$).

CONCLUSION: The expression of VCAM-1 is closely related to oncogenesis, tumor angiogenesis and metastasis in gastric carcinoma. Serum VCAM-1 level in gastric cancer patients is significantly increased compared with normal controls, which decreases significantly after radical resection of the primary tumor. The serum concentration of VCAM-1 may be considered as an effective marker of tumor burden of gastric cancer. Moreover, overexpression of VCAM-1 in gastric cancer tissue is likely a major source of serum VCAM-1.

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INTRODUCTION

Solid tumors are composed of two distinct but interdependent compartments, malignant cells themselves and the vascular and connective tissue stroma induced by malignant cells where malignant cells are dispersed. Stroma provides the vascular supply that tumors require for obtaining nutrients, gas exchange, and waste disposal. Thus, any increase in tumor mass, either primary or metastatic, must be accompanied by angiogenesis formation[1,2]. The mechanism by which tumors induce stroma has caused considerable attention in recent years. Emphasis is increasingly placed on tumor angiogenesis. Tumor angiogenesis has been linked to tumor progression and metastasis. Many reports have suggested that cell adhesion molecules (CAM) not only play key roles in various stages of tumor angiogenesis, but also are involved in tumor progression and metastasis[3-5].

CAMs are cell-surface glycoproteins which are critical for cell- to-cell interactions. Intercellular adhesion mediated by CAMs directly influences differentiation, and disruption of normal cell-cell contacts has been noted in neoplastic transformation and in metastasis. CAMs expressed on lymphocytes and vascular endothelial cells are thought to play an important role in lymphocyte trafficking for immune anti-infection response. There is evidence that vascular cell adhesion molecule-1 (VCAM-1) may be involved in tumor progression and metastasis[5,6].

VCAM-1 belongs to the immunoglobulin super family group of adhesion molecules, and is one of the most important adhesion molecules which plays a crucial role in this process. VCAM-1 is an 110 KDa glycoprotein that is constitutively expressed on tissue macrophage, dentric cells and epithelial cells, as well as on the surface of stimulated endothelial cells. Thus, VCAM-1 is a widely distributed protein. It is possible that VCAM-1 is a candidate for mediating tumor cell adhesion to vascular endothelial cells and promoting the metastatic process. Recent reports have shown that angiogenesis favors tumor growth and facilitates entry of cells into the circulation[7,8].

It has recently been reported that the microvessel density (MVD) in a tumor correlates with tumor progression, hematogenous metastasis and recurrence of gastric carcinoma.

MVD also reflects tumor angiogenesis[2-4,9]. In the present study, the expression of VCAM-1 and the density of microvessels were examined by immunohistochemistry in patients with gastric cancer. The association between the expression of VCAM-1 and oncogenesis, tumor angiogenesis and metastasis of gastric carcinoma was evaluated. Meanwhile we also detected the concentration of soluble forms of VCAM-1 and serum levels of carcinoembryonic antigen (CEA) in patients with gastric cancer and investigated its relation to clinical and pathological features.

MATERIALS AND METHODS

Patients

A total of 41 patients with histologically confirmed gastric cancer who had undergone curative gastrectomy at our department from March 1999 to March 2000 were included in this study. They consisted of 26 male and 15 female patients ranging in age from 35 to 74 years (mean, 58.4 years). The clinicopathologic findings were determined according to the principles set by the Japaneese Society Committee on Histologial Classifiation of Gastric Cancer. Of the 41 patients, 28 had lymph node metastasis. The patients had not received either chemotherapy or radiation therapy before operation. A tumor sample and a normal part of the stomach were obtained during surgical resection. The samples were each divided into two pieces, which were subjected to fixation in 10 % formalin for histological examination and immunohistological test. All patients had been performed distal partial gastrectomy, proximal partial gastrectomy, or total gastrectomy with regional lymph node dissection to group 1(D1), group 2(D2), group 3 (D3) with a curative intention. Gastric specimens from eight patients with gastric ulcer and 10 patients with normal gastric tissues were obtained by fiber gastroscope.

Both the expression of VCAM-1 and the density of microvessels were examined by immunohistochemical staining. Meanwhile, blood samples from the 41 gastric cancer patients were obtained before any treatment and at 7 days after surgery. There were no infections in all these patients. Additionally, blood samples were also obtained from 25 healthy subjects as controls. The mean age of these subjects was 39.1 years (age range 20-55 years), with a ratio of male to female of 12:8.

Immunohistochemistry

Specimens were fixed in a 40 g· L^{-1} formaldehyde solution and embedded in paraffin. Four-micrometer thick sections were cut and mounted on glass slides. Immunohistochemistry was performed using the avidin-biotin complex (ABC) method. Sections were dewaxed in xylene, dehydrated in ethanol, washed by phosphate-buffered saline solution (PBS, pH=7.4) and then heated in a microwave oven for 10 minutes to retrieve the antigens. Endogenous peroxidase activity was blocked by incubation of samples with 3 % hydrogen peroxide in methanol for 30 minutes. After being washed with PBS, 50 μl 10 % normal goat serum was added to glass slides for 10 minutes to reduce nonspecific antibody binding. Specimens were then incubated with a 1:250 dilution of anti-VCAM-1 antibody overnight at 4 ℃, followed by three washes with PBS. Sections were then incubated with biotinylated goat antimouse immunoglobulin G (Nanjing Sangon Biotechnology Co.) at a dilution of 1:50 for 2 hours followed by three washes. Slides were then treated with the complex of reagent A and reagent B (ABC kit, Nanjing Sangon Biotechnology Co.) for 2 hours at a dilution of 1:50 and were washed with PBS three times. Finally, slides were incubated in PBS containing diaminobenzidine and 300 mL· L^{-1} hydrogen peroxide for 10 minutes. Normal mouse immunoglobulin-G was substituted for primary antibody as the negative control. Immunoreactivity was graded as follows: +, more than 10 % of carcinoma cells were stained; -, no detectable expression or fewer than 10 % of carcinoma cells were stained.

Microvessel detection and counting

A detection procedure for microvessels was performed using anti-CD34 monoclonal antibody (Nanjing Sangon Biotechnology Co.). Envision-labeled polymer reagent was applied for immunoreaction. A single microvessel was defined as any brown immunostained endothelial cells, and other connective tissue elements. The stained sections were screened at ×100 magnification under a light microscope to identify the five regions of the section with the highest number of microvessels. The image was visualized on a computer display through a color video camera module and color image freezer. Microvessels were counted in this area at ×200 magnification, and the average number of microvessels in these five regions was recorded, and defined as MVD. The visualized area on the display was determined to be 0.075 mm^2.

Assay of soluble VCAM-1

For assay of soluble adhesion molecules venous blood samples. were collected into plain tubes, allowed to clot for up to 1 hour and centrifuged at 5 000 g for 5 min. The serum was removed, aliquoted and stored at -70 ℃ until assayed. Concenrations of soluble VCAM-1were measured with commercially available sandwich ELISA kits based on dual monoclonal antibodies. CEA test, a routine test in our department, was also performed.

Statistical analysis

The data were presented as $\bar{x}\pm s$. Differences in categorical variables between groups were determined by chi-square test, and differences in enumeration data between groups were determined by *t* test or by analysis of variance. Correlations between the levels of soluble adhesion molecules and the clinical and pathological variables were determined by Spearman rank correlation method.

RESULTS

Expression of VCAM-1 in gastric cancer, gastric ulcer and normal gastric tissue

VCAM-1 was positively stained in the cytoplasm or the membrane of vascular endothelial cells in brown or yellow in gastric cancer tissue. VCAM-1 was expressed not only in vascular endothelial cells, but also in the majority of gastric cancer cells. Generally, VCAM-1 expression was intense throughout the tumor, especially in keratin pearl of gastric cancer. VCAM-1positive vessels were preferentially found in vascular-rich tumor areas. VCAM-1 expression was present in 31 (75.6 %) out of 41gastric cancer tissues and in 5 (12.2 %) adjacent normal gastric tissue. The rate of expression of VCAM-1 in gastric caner tissue was significantly higher than that in adjacent normal gastric tissue (χ^2=52.1, P<0.05).

VCAM-1 expression was present in 25 % (2/8) of gastric ulcer tissue, and in 20 % (2/10) of healthy subjects (χ^2=0.1,P> 0.05). However, The rate of VCAM-1 expression in gastric cancer was remarkably higher than that in gastric ulcer and healthy subjects (both P<0.05).

CD34 was positively stained mainly in the cytoplasm of vascular endothelial cells as brown or yellow granules. MVD ranged from 21.5 to 71.2, with a mean value of 30.2 in gastric cancer tissues, and ranged from 2 to 14, with a mean value of

11.5 in normal gastric tissues. MVD in tumor tissue was significantly higher than that in normal tissues (t=3.1, P<0.05).

Association of the expressions of VCAM-1 and MVD with pathological features of gastric carcinoma

As shown in Table 1, VCAM-1 expression was present in 26 (92.9 %) of the 28 gastric cancer patients with lymph node metastasis, but in only 5 (34.5 %) of the 13 gastric cancer patients without lymph node metastasis. The rate of VCAM-1 in patients with lymph node metastasis was significantly higher than that in patients without lymph node metastasis (χ^2=11.4, P<0.05). Meanwhile VCAM-1 expression in the gastric cancer patients was also associated with clinicopathological stage and depth of infiltration (both P<0.05).

Furthermore, microvessel count in patients with gastric cancer was related to clinicopathological stage, depth of infiltration and lymph node metastasis (all P<0.05,). The rate of expression of VCAM-1 and MVD in gastric carcinoma tissue had no significant differences among the size, location, age and gender (data not shown).

Table 1 Association of expressions of VCAM-1 and MVD with pathological features of gastric carcinoma

Pathological chacteristics	*n*	Positive VCAM-1(%)	MVD($\bar{x}$+*s*)
Size of tumor			
<3 cm	10	6	37.1±12.1
3 cm	31	25	39.5±12.7
Location			
Lower third	10	7	39.4±7.3
Middle third	20	17	36.2±11.3
Upper third	11	7	41.4±13.3
Depth of invasion			
Mucosa and submucosa	11	5	28.4±8.1
Muscle and subserosa	12	9	36.9±10.7
Serosa	18	17[a]	49.6±15.1[b]
Clinicopathologic stage			
I	7	3	29.3±3.54
II	12	7	33.9±9.3
III	16	15	46.3±10.3
IV	6	6[c]	55.4±8.1[d]
Lymph node metastasis			
Present	28	26	45±9.8
Absent	13	5[e]	28.5±5.5[f]

MVD and expression of VCAM-1 had no significant difference in the size, location. The rate of positive VCAM-1 expression and MVD were positively correlated with depth of invasion ([a]P<0.05, [b]P<0.05 respectively). There was a significant difference between the rate of positive VCAM-1 expression, MVD and clinic pathological stage ([c]P<0.05, [d]P<0.05). The rate of positive VCAM-1 expression and MVD were significantly higher than that in those without lymph node metastasis ([e]P<0.05, [f]P<0.05 respectively).

Correlation between expression of VCAM-1 and MVD

MVD was 46.5±11.3 in VCAM-1 positive tissue in gastric cancer, while it was 31.2±8.4 in negative VCAM-1 tissue. MVD in VCAM-1-positive tumors was significantly higher than that in VCAM-1-negative tumors (t=2.13, P<0.05).

Association of soluble VCAM-1 with gastric cancer

In gastric cancer patients, soluble VCAM-1 was significantly elevated in comparison with that in healthy subjects (878±46 μg/lm *vs* 297±35 μg/ml, P<0.05, Table 2). The difference in serum concentration of soluble VCAM-1 was also significant between patients with stage I-II and those with stage III-IV gastric cancer, indicating that soluble VCAM-1 concentration correlated well with the staging of gastric cancer. No significant difference was found between ulcer group and controls (P>0.05). Concentrations of serum CEA in gastric cancer patients were higher than those in control group and ulcer group (both P<0.05). But no significant difference was found between stage I-II and stage III-IV gastric cancer.

Table 2 Soluble levels of VCAM-1, CEA in gastric cancer patients, ulcer patients and control group

	n	Soluble VCAM-1(μg / ml) ($\bar{x}$±*s*)	CEA
Gastric cancer	41	878±46[a]	4.7±0.43
I-II	17	764±24[b]	4.1±0.23
III-IV	24	1006±78[c]	4.9±0.31
Ulcer	8	301±21	4.5±0.41
Control	25	297±35	2.3±0.28

[a]P<0.05, [b]P<0.05, [c]P<0.05, (*vs* control group).

The concentration of soluble VCAM-1 was higher in the patients with lymph node metastasis than that in those without lymph node metastasis (P<0.05). Positive correlation was found between concentration of soluble VCAM-1 and depth of invasion of gastric cancer. In contrast, no significant association was found between concentration of soluble VCAM-1 and location, size, age and gender (data not shown).

Levels of postoperative soluble VCAM-1 in gastric cancer patients were reduced significantly compared to preoperative levels (578±39 μg/lm *vs* 878±46 μg/ml, P<0.05, Table 3). It was noteworthy that concentration of soluble VCAM-1 was positively correlated with expression of VCAM-1 in the tumor tissue (r=0.85, P<0.05). The level of serum CEA was also decreased significantly after radical resection of the primary tumor (P<0.05).

Table 3 Concentrations of soluble VCAM-1 and CEA at pre-operative and postoperative stages

	$\bar{x}$±*s* (ng / L)	*P*
sVCAM-1		
preoperative	878±46	
postoperative	578±39	0.0001
CEA		
preoperative	10.3±18.4	
postoperative	6.3±11.5	0.0031

Serum VCAM-1 and CEA levels decreased significantly after radical resection of the primary tumor (P<0.05).

DISCUSSION

Gastric carcinoma, as one of the most common human malignant tumors, ranks worldwide the first leading cause of gastrointestinal cancer-related mortality. In China, it now ranks the second among all malignant tumors. Recent important advance in oncology is the finding that tumor angiogenesis plays an important role in tumor genesis, growth and metastasis, and an increasing number of studies have proven that vascular targeting therapy is very effective[9-11]. Many factors are involved in tumor angiogenesis, one of the most important factors is VCAM-1, Which is capable of promoting and maintaining the establishment of tumor vascular system[12]. Thus it can directly stimulate tumor growth and metastasis. Generation of new blood vessels, or angiogenesis, plays a key role in the growth

of malignant disease and has drawn great interest in developing agents that inhibit angiogenesis. Angiogenesis is characterized by invasion, migration, and proliferation of endothelial cells, processes that depend on cell interactions with extracellular matrix components[12-14]. Our current study has shown VCAM-1 is a key player by providing a vasculature-specific target for antiangiogenic treatment strategies.

Expression of VCAM-1 may facilitate oncogenesis

Recent advances in molecular biology and genetic technology studies on adhesion molecules have demonstrated that cell-cell and cell-extracellular matrix interactions play an important role in cancer metastasis. In addition to activation of oncogenes and inactivation of tumor suppressor genes, alteration of adhesion molecules seems to be critical for the development of gastric cancer[15]. Tumour development is a multi-step process during which genetic and epigenetic events determine the transition from a normal to a malignant cellular state. In the past decade, extensive effort has been made not only to define the molecular mechanisms underlying progression to malignancy but also to predict the development of the disease and to identify possible molecular targets for therapy. Common to most tumours, several regulatory circuits are altered during multistage tumour progression of gastric cancer, which includes control of proliferation, balance between cell survival and programmed cell death (apoptosis), communication with neighbouring cells and extracellular matrix, induction of tumour neovascularization (angiogenesis) and tumour cell migration, invasion and metastatic dissemination. De-regulation of each of these processes represents a rate-limiting step for tumour development and, hence, has to be achieved by tumour cells in a highly selective manner during tumour progression. In this complex process, more attention has been placed on adhesion molecules, because adhesion molecules are necessary to mediate cell-matrix and cell-cell interactions, metabolism, and differentiation. Moreever, adhesive interactions between tumor cell surface receptors and endothelial cell adhesion molecules are thought to contribute to tumor cell arrest and extravasation during hematogenous metastasis[14-17]. Changes in expression and function of adhesion molecules are important characteristics in the development of gastrointestinal malignancies and might be used in future as prognostic factors or as new targets in diagnosis and therapy. VCAM-1, one of the adhesion molecules involved in malignant tumor, is found to be expressed mainly in activated endothelial cells, and dentric cells.

Maurer *et al*. confirmed that VCAM-1 protein was over-expressed in colorectal cancers at the mRNA level by Northern blotting, In comparison to normal controls, the expression of VCAM-1 mRNA was increased by 3.4 fold in colorectal cancers. Our study found that the expression of VCAM-1 in gastric tumor tissue was higher than that in adjacent-cancer tissue, and the ratio of positive VCAM-1 in gastric cancer tissue was higher than that in gastric ulcer and normal mucosa, suggesting that VCAM-1 may play a key role in the growth of gastric cancer.

However, we noteed that VCAM-1 expression was positive in one healthy subject, and in two patients with gastric ulcer, indicating that there are other factors involved in the expression of VCAM-1. Some studies believed that *Helicobacter pylori* infection of gastric mucosa was one of the causes responsible for VCAM-1 expression[18,19].

VCAM-1 expression stimulates tumor angiogenesis

Angiogenesis is of key importance in the process of tumour progression in a number of tumour types. Angiogenesis is a biological process by which new capillaries are formed from pre-existing vessels. It occurs in physiological conditions such as embryo development, and cyclically in wound repair of female genital system, and during pathological conditions, such as arthritis, diabetic retinopathy and tumors. In the both physiological conditions, angiogenesis is mediated accurately by feedback system. However, in solid tumor growth, a specific ally critical turning point is the transition from the vascular to the vascular phase. Having developed an intrinsic vascular network, the neoplastic mass is able to grow indefinitely (unlike other forms of cell growth, tumor angiogenesis is not limited in time) both *in situ* and at distant sites (metastasis). Thus, tumor angiogenesis cannot be controlled by feedback. Angiogenesis is capable of providing continuous nutrients, gas exchange, and waste disposal for tumors growth. Any increase in tumor mass, either primary or metastatic, must be accompanied by vascular formation. Therefore, tumor angiogenesis is a very complex process. At present, an increasing number of studies have shown that abnormal adhesion molecules contribute to tumor angiogenesis by stimulating neoplastic cells to produce growth factors specific for endothelial cells and able to stimulate growth of the host' s blood vessels[6,8,13,15]. Recent studies have demonstrated that VCAM-1 may contribute to tumor angiogenesis. MVD, a reliable index of tumor angiogenesis, has been confirmed to be linked to tumor progression, hematogenous metastasis, and tumor recurrence.

Our study indicated that MVD of gastric cancer tissues with VCAM-1 expression was significantly higher than that in tissues negative for VCAM-1, suggesting that VCAM-1 expression may be one of the factors mediating the activation of angiogenesis. Several reports have observed that gastric cancer cells are capable of activating vessel endothelial cells, which leads to expression of VCAM-1, and thus the induction of tumour neovascularization. Maeds *et al*. proposed that VCAM-1 binding to its ligand VLA4 not only results in activation of vessel endothelial cells, but also leads to shedding of tumor cells and invasion of adjacent tissue[20,21]. Byrne *et al*. suggested that VCAM-1 was expressed on endothelial cells as a result of stimulation by vascular endothelial growth factor (VEGF). In general, these findings suggest that VCAM-1 may be used for sustained angiogenesis and tissue invasion and metastasis via autocrine/paracrine manners[7].

Howerever, in some tumors, high MVD VCAM-1 expression was not detected, which suggests that other factors such as extracellular matrix, and metabolic and mechanical factors also contribute to tumor angiogenesis. Hence anti-angiogenesis therapy should utilize multiantiangiogenesis tactics[23,24].

MVD may be different according to the observer, and cut-off values are also different. In addition, it has been reported that different MVD could be obtained with different antibodies used in different studies. Therefore, MVD that was observed in this study may be different from those previously reported.

VCAM-1 favors lymph node metastasis of gastric cancer

It is generally accepted that the presence or absence of regional lymph node involvement is one of the important factors influencing survival in respectable gastric cancer. Lymph node metastasis occurs even in some patients with early gastric cancer. Recently, studies have confirmed that prognosis of gastric cancer patients with lymph node metastasis is very poor. Gastric caner patients with lymph node metastasis have been commonly reported to have poorly differentiated adenocarcinoma, and deeper invasion, in comparison with gastric cancer patients without lymph node metastasis. In our present study, we observed that VCAM-1 expression in 26 of 28 cases with lymph node metastasis, whereas in only 5 of 13 gastric cancer patients without lymph node metastasis. There

was a significant difference between the two groups ($P<0.05$), indicating that expression of VCAM-1 may be associated with lymph node metastasis of gastric cancer. VCAM-1 is predominantly expressed on gastric carcinoma cells, giving these tumor cells the ability to perform lymph-node metastasis. Meanwhile, expression of VCAM-1 significantly alters malignant transformation[11,20,21].

Tumor metastasis involves the release of cells from primary tumor due to a firmly formed cluster of tumor cells probably by detaching from the tumor nests with unstable adhesiveness, followed by their migration in extracellular matrix, adhesion to vessel walls, arrest in microcirculation of distant organs, and subsequent extravasation. Extravasation of metastatic tumor cells from bloodstream of the tissue space in a secondary organ is related specific binding to determinants on endothelial cell surface. Each step requires cell adhesive interactions involving specific adhesion molecules and receptors. Several families of adhesion molecules have now been identified, some of which are promising candidates for a role in neoplasia. So tumor metastasis is a very complex process, Colette *et al*. thought that VCAM-1 overexpression stimulated tumour neovascularization, and angiogenesis was necessary for tumor growth and metastasis, VCAM-1 expression was linked to lymph node metastasis[2,3,21-24].

We observed that VCAM-1 expression was present in 5 of the 13 cases without lymph node metastasis, which may be related to lymph node micrometastasis. It has been demonstrated that micrometastases consisting of one to a few cells in lymph nodes resected during gastrectomy are difficult to identify using conventional hematoxylin and eosin (H&E) stains[25,26].

Concentration of soluble VCAM-1 may be one of the important markers for gastric carcinoma

Our study demonstrated that serum concentration of VCAM-1 was elevated in patients with gastric cancer before treatment in comparison with the healthy group; hence, the concentration of soluble VCAM-1 may be of significance in diagnosis of gastric carcinoma. In addition, a positive correlation was observed between levels of soluble VCAM-1 and tumor stage, and invasion depth. More important was that the concentration of soluble VCAM-1 in patients with lymph node metastasis was significantly higher than that in patients without lymph node matastasis. These findings suggest that the levels of soluble VCAM-1 are linked to tumor growth and metastasis. Our results are consistent with the findings by Velikova *et al*, who also reported elevated serum levels of VCAM-1 in gastric cancer, and proposed that patients with elevated serum VCAM-1 have a poorer survival rate. Measurement of circulating VCAM-1 may bring additional prognostic information for patients with gastric cancer in relation to different stages and tumor pathology, and it should be included in future large multivariate analyses of prognostic factors should be performed whenever possible[27,28]. Animal experiments have confirmed that soluble VCAM-1 promotes angiogenesis in rat cornea, which is supported by our findings[29,30].

Additionally, It was first observed that postoperative concentrations of soluble VCAM-1 decreased significantly in patients with gastric cancer in comparison with preoperative concentrations ($P<0.05$). This finding suggests that measurement of circulating VCAM-1 may play a critical role in prognosis of gastric cancer patient. It is likely that tumor burden in patients with gastric cancer is decreased after operation, so the concentration of soluble VCAM-1 is reduced. If the levels of soluble VCAM-1 increase again after operation, we should pay great attention to tumor recurrence. Therefore, soluble VCAM-1 may be considered as a marker for tumor burden. Some investigators have reported a positive correlation between serum concentration of VCAM-1 and age of patients, but no such a correlation was found in healthy subjects. Our study did not confirm this although we noted that the median age in healthy group was lower than that of gastric cancer patients. Therefore, the effect of age on serum levels of VCAM-1 can not be excluded, and future studies should take it into account[31].

In our department, CEA is one of the routine tests in gastric cancer. It is well accepted that CEA is one of the most important marks of gastrointestinal carcinoma, and preoperative positivity for CEA is an independent risk factor for hematogenous recurrence of gastric carcinoma. Our finding on CEA is consistent with those previously reported[32,33].

Expression of VCAM-1 in tumor tissue is one of the sources of soluble VCAM-1 in gastric cancer

The source of soluble VCAM-1 is not yet known. VCAM-1 is known to be expressed predominantly on activated endothelial cells, dendritic cells and renal proximal tuble cells. We found that strong VCAM-1 expression occurred in gastric cancer cells and endothelial cells in tumor tissues, especially in vessel abundant regions. Some studies have reported that VCAM-1 has been found in malignant epithelial tissue, including metastasis gastric cancer cells, melanoma cell lines and hepatocellular cells. In our study, a strong correlation was found between expression of VCAM-1 in gastric cancer tissue and serum concentration of soluble VCAM-1 ($r=0.85$, $P<0.05$). Increased expression of VCAM-1 in gastric cancer cells and their shedding into circulation may be the factor accounting for the significantly elevated serum levels of VCAM-1. Other studies have reported that soluble VCAM-1 may be related to the white cell count. To eliminate this possibility, blood tests were performed, and gastric cancer patients with normal blood tests were included. We observed that expression of VCAM-1 in gastric carcinoma tissue was positively correlated with concentration of soluble VCAM-1, indicating that expression of VCAM-1 in gastric cancer tissue is a major source of soluble VCAM-1. VCAM-1 is expressed on endothelial cells as a result of vascular endothelial growth factor (VEGF) stimulation[4,6,19-21,34,35].

In conclusion, VCAM-1 may be involved in the progression of human gastric carcinoma, particularly via lymphangiogenesis. VCAM-1 expression at invading edge of gastric carcinoma may be a sensitive marker for metastasis to lymph nodes. Differentially expressed vascular molecules may influence the functional characteristics of extravasating leukocytes and represent new targets in anti-gastric cancer therapy. In general, an increasing number of studies on a variety of malignant diseases have suggested that VCAM-1 may play a role in the process of adhesion of tumor cells to endothelial cells and neovascularization. Expression of VCAM-1 is associated with oncogenesis, tumor angiogenesis and metastasis in gastric carcinoma. MVD in gastric carcinoma tissue is closely associated with lymph node metastasis, clinical stage and depth of invasion. Expression of VCAM-1 is closely related to MVD in gastric cancer, and thus VCAM-1 can act as an important index reflecting the biological behaviors of gastric carcinoma. VCAM-1 may be used as a metastasis marker and/or a target for antiangiogenic therapy. VCAM-1 has been shown to be a key player by providing a vasculature-specific target for antiangiogenic treatment strategies. Concentration of soluble VCAM-1 correlates well with tumor growth and metastasis. Expression of VCAM-1 in gastric cancer tissue is positively correlated with concentration of soluble VCAM-1. Elevated soluble VCAM-1 is decreased significantly in gastric caner patients after operation, and thus soluble VCAM-1 may be one of the markers for gastric carcinoma tumor burden, and

serum VCAM-1 may be considered as an effective diagnostic and prognostic factor.

REFERENCES

1 **Ghossein RA**, Bhattacharya S, Rosai J. Molecular detection of micrometastases and circulating tumor cells in solid tumors. *Clin Cancer Res* 1999; **5**: 1950-60

2 **Liu DH**, Zhang XY, Fan DM, Huang YX, Zhang JS, Huang WQ, Zhang YQ, Huang QS, Ma WY, Chai YB, Jin M. Expression of vascular endothelial growth factor and its role in oncogenesis of human gastric carcinoma. *World J Gastroenterol* 2001; **7**: 500-505

3 **Saito H,** Tsujitani S, Kondo A, Ikeguchi M, Maeta M, Kaibara N. Expression of vascular endothelial growth factor correlates with hematogenous recurrence in gastric carcinoma. *Surgery* 1999; **125**: 195-201

4 **Ren J,** Dong L, Xu CB, Pan BR. The role of KDR in the interactions between human gastric carcinoma cell and vascular endothelial cell. *World J Gastroenterol* 2002; **8**: 596-601

5 **Alexiou D,** Karayiannakis AJ, Syrigos KN, Zbar A, Kremmyda A, Bramis I, Tsigris C. Serum levels of E-selectin, ICAM-1 and VCAM-1 in colorectal cancer patients: correlations with clinicopathological features, patient survival and tumour surgery. *Eur J Cancer* 2001; **37**: 2392-2397

6 **Maurer CA,** Friess H, Kretschmann B, Wildi S, Muller C, Graber H, Schilling M, Buchler MW. Over-expression of ICAM-1, VCAM-1 and ELAM-1 might influence tumor progression in colorectal cancer. *Int J Cancer* 1998; **79**: 76-81

7 **Byrne GJ**, Bundred NJ. Surrogate markers of tumoral angiogenesis. *Int J Biol Markers* 2000; **15**: 334-339

8 **Li DY,** Sorensen LK, Brooke BS, Urness LD, Davis EC, Taylor DG, Boak BB, Wendel DP. Defective angiogenesis in mice lacking endoglin. *Science* 1999; **284**: 1534-1537

9 **Kakeji Y,** Koga T, Sumiyoshi Y, Shibahara K, Oda S, Maehara Y, Sugimachi K. Clinical significance of vascular endothelial growth factor expression in gastric cancer. *J Exp Clin Cancer Res* 2002; **21**: 125-129

10 **Tao HQ,** Lin YZ, Wang RN. Significance of vascular endothelial growth factor messenger RNA expression in gastric cancer. *World J Gastroenterol* 1998; **4**: 10-13

11 **Takahashi N,** Haba A, Matsuno F, Seon BK. Antiangiogenic therapy of established tumors in human skin / severe combined immunodeficiency mouse chimeras by anti-endoglin (CD105) monoclonal antibodies, and synergy between anti-endoglin antibody and cyclophosphamide. *Cancer Res* 2001; **61**: 7846-7854

12 **Muller AM,** Weichert A, Muller KM. E-cadherin, E-selectin and vascular cell adhesion molecule: immunohistochemical markers for differentiation between mesothelioma and metastatic pulmonary adenocarcinoma? *Virchows Arch* 2002; **441**: 41-46

13 **Brandvold KA,** Neiman P, Ruddell A. Angiogenesis is an early event in the generation of myc-induced lymphomas. *Oncogene* 2000; **19**: 2780-2785

14 **Langley RR,** Carlisle R, Ma L, Specian RD, Gerriten ME, Granger DN. Endothelial expression of vascular cell adhesion molecule-1 correlates with metastatic pattern in spontaneous melanoma. *Microcirculation* 2001; **8**: 335-345

15 **Maehara Y,** Kabashima A, Koga T, Tokunaga E, Takeuchi H, Kakeji Y, Sugimachi K. Vascular invasion and potential for tumor angiogenesis and metastasis in gastric carcinoma. *Surgery* 2000; **128**: 408-416

16 **Park HJ,** Lee YW, Hennig B, Toborek M. Linoleic acid-induced VCAM-1 expression in human microvascular endothelial cells is mediated by the NF-kappa B-dependent pathway. *Nutr Cancer* 2001; **41**: 126-134

17 **Seon BK,** Takahashi N, Haba A, Matsuno F, Haruta Y, She XW, Harada N, Tsai H. Angiogenesis and metastasis marker of human tumors. *Rinsho Byori* 2001; **49:** 1005-1013

18 **Ohnita K,** Isomoto H, Mizuta Y, Maeda T, Haraguchi M, Miyazaki M, Murase K, Murata I, Tomonaga M, Kohno S. *Helicobacter pylori* infection in patients with gastric involvement by adult T-cell leukemia / lymphoma. *Cancer* 2002; **94**: 1507-1516

19 **Mori N,** Wada A, Hirayama T, Parks TP, Stratowa C, Yamamoto N. Activation of intercellular adhesion molecule 1 expression by Helicobacter pylori is regulated by NF-kappaB in gastric epithelial cancer cells. *Infect Immun* 2000; **68**: 1806-1814

20 **Kwon S,** Kim GS. Prognoatic significance of lymph node metastasis in advanced carcinoma of the stomach. *Br J Surg* 1996; **83**: 1600-1603

21 **Gulubova MV.** Expression of cell adhesion molecules, their ligands and tumour necrosis factor alpha in the liver of patients with metastatic gastrointestinal carcinomas. *Histochem J* 2002; **34**: 67-77

22 **Charpin C,** Garcia S, Andrac L, Horschowski N, Choux R, Lavaut MN. VCAM (IGSF) adhesion molecule expression in breast carcinoma detected by automated and quantitative immunocytochemical assays. *Hum Pathol* 1998; **29:** 896-903

23 **Hemmerlein B,** Scherbening J, Kugler A, Radzun HJ. Expression of VCAM-1, ICAM-1, E- and P-selectin and tumour-associated macrophages in renal cell carcinoma. *Histopathology* 2000; **37**: 78-83

24 **Lode HN**, Moehler T, Xiang R, Jonczyk A, Gillies SD, Cheresh DA, Reisfeld RA. Synergy between an antiangiogenic integrin alphav antagonist and an antibody-cytokine fusion protein eradicates spontaneous tumor metastases. *Proc Natl Acad Sci USA* 1999; **96**: 1591-1596

25 **Vlems FA,** Diepstra JH, Cornelissen IM, Ruers TJ, Ligtenberg MJ, Punt CJ, van Krieken JH, Wobbes T, van Muijen GN. Limitations of cytokeratin 20 RT-PCR to detect disseminated tumour cells in blood and bone marrow of patients with colorectal cancer: expression in controls and downregulation in tumour tissue. *Mol Pathol* 2002; **55**: 156-163

26 **Lee E**, Chae Y, Kim I, Choi J, Yeom B, Leong AS. Prognostic relevance of immunohistochemically detected lymph node micrometastasis in patients with gastric carcinoma. *Cancer* 2002; **94**: 2867-2873

27 **Alexiou D,** Karayiannakis AJ, Syrigos KN, Zbar A, Sekara E, Michail P, Rosenberg T, Diamantis T. Clinical significance of serum levels of E-selectin, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1 in gastric cancer patients. *Am J Gastroenterol* 2003; **98**: 478-485

28 **Karayiannakis AJ,** Syrigos KN, Polychronidis A, Zbar A, Kouraklis G, Simopoulos C, Karatzas G. Circulating VEGF levels in the serum of gastric cancer patients: correlation with pathological variables, patient survival, and tumor surgery. *Ann Surg* 2002; **236**: 37-42

29 **Velikova G,** Banks RE, Gearing A, Hemingway I, Forbes MA, Preston SR, Hall NR, Jones M, Wyatt J, Miller K, Ward U, Al-Maskatti J, Singh SM, Finan PJ, Ambrose NS, Primrose JN, Selby PJ. Serum concentrations of soluble adhesion molecules in patients with colorectal cancer. *Br J Cancer* 1998; **77**: 1857-1863

30 **Yoo NC,** Chung HC, Chung HC, Park JO, Rha SY, Kim JH, Roh JK, Min JS, Kim BS, Noh SH. Synchronous elevation of soluble intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) correlates with gastric cancer progression. *Yonsei Med J* 1998; **39**: 27-36

31 **Locker GJ,** Stoiser B, Losert H, Wenzel C, Ohler L, Kabrna E, Geissler K. Decrease of circulating hematopoietic progenitor cells During interleukin-2 treatment is associated with an increase of vascular cell adhesion molecule-1, a critical molecule for progenitor cell adhesion. *Leuk Lymphoma* 2000; **39**: 355-364

32 **Marrelli D,** Pinto E, De Stefano A, Farnetani M, Garosi L, Roviello F. Clinical utility of CEA, CA 19-9, and CA 72-4 in the follow-up of patients with resectable gastric cancer. *Am J Surg* 2001; **181**: 16-19

33 **Kinugasa T,** Kuroki M, Takeo H, Matsuo Y, Ohshima K, Yamashita Y, Shirakusa T, Matsuoka Y. Expression of four CEA family antigens (CEA, NCA, BGP and CGM2) in normal and cancerous gastric epithelial cells: up-regulation of BGP and CGM2 in carcinomas. *Int J Cancer* 1998; **76**: 148-153

34 **Kaptur S,** Riedel F, Erhardt T, Hormann K. Serum concentrations of soluble adhesion molecules sICAM-1 and sVCAM-1 in patients with malignant ENT tumors. *HNO* 2001; **49**: 910-913

35 **Ikeda M,** Furukawa H, Imamura H, Shimizu J, Ishida H, Masutani S, Tatsuta M, Kawasaki T, Satomi T. Surgery for gastric cancer increases plasma levels of vascular endothelial growth factor and von Willebrand factor. *Gastric Cancer* 2002; **5**: 137-141

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