VEGF –C Expression in Gastric Cancer and It´s Role in Lymphatic Metastasis

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Abstract **Objective** To study the expression of VEGF-C in gastric cancer and its role in lymphatic metastasis. **Methods** One gastric cell line SGC-7901 and 56 specimens with primary gastric cancer were examined by immunohistochemical staining. **Results** Positive immunohistochemical staining for VEGF-C was observed in the cytoplasm of cancer cells of 18 specimens(32%) and the SGC-7901 gastric cell line. The expression of VEGF-C was conspicuously higher in lymph node-positive group than in node-negative group. **Conclusions** VEGF-C may play an important role in lymphatic metastasis of gastric cancer.

Key words VEGF-C; Stomach neoplasms; Lymphatic metastasis; Immunohistochemistry

Vescular endothelial growth factors (VEGFs) and their receptors (VEGFRs) are important regulators of blood and lymphatic vessel growth and vascular permeability. VEGF-C is a new member in this family, and functions specifically to induce the development and maintenance of lymphatic vessels. Its receptor VEGFR-3 is predominantly expressed in the endothelium of lymphatic vessels [1,2], so VEGF-C is considered to be both a specific marker for lymphatic endothelial cells and a specific factor for lymphangiogenesis. About the mechanism of lymphogenous metastasis of maliganant tumors is still unclear so we examined the relationship between expression of VEGF-C and clinicopathological features with immunohistochemistry techniques, to probe the mechanism of lymph geneus metastasis in cases of gastric cancer.

MATERIALS AND METHODS

Tumor samples

Primary gastric cancer tissues were obtained from 56 patients (40 men and 16 women) in the Second Hospital of Shandong University. The mean age of patients was 61 years (31~85); 32 were lymph node-positive, and 24 were lymph node negative. The histological types of the tumors were: well-differentiated 6, middle-differentiated 18, poorly-differentiated 32.

SGC–7901 gastric cancer line

bought from the medical academy of Shandong province.

Immunohistochemial staining

The primary(first) antibody used in this study was a goat monoclonal antibody at a 1:100 dilution for VEGF-C (Santa Cruz Biotechnology company, USA). Paraffin sections were deparaffinized and immunohistochemical staining was performed using SABC method. That is, endogenous peroxidase was blocked by treatment with 0.3% hydrogen peroxide in methanol for 20 min, and the specimens were washed with PBS(ph7.4). The sections were incubated with normal rabbit serum for 10~20 min at room temperature to achieve blocking. Not washed, the sections reacted with antibodies for 12 h at 4℃, Then, they were washed with PBS(2min×3) and reacted with biotin-labelled rabbit anti-goat immunoglobulin for 20 min at room temperature. After they were washed with PBS(2min×3), then reacted with SABC for 20 min at room temperature and washed with PBS(5min×4). Color was developed by DAB(about 5 min) and washed with distilled water. Counterstaining was done with haematoxylin. The negative control used all of the reagents except for the primary antibody.

Statistical methods

T test and x^2 test were used to analyze the result.

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Table 1 correlation between VEGF-C and sex, histological types, lymph node metastasis

	Sex		differentiation			metastasis	
VEGF-C	male	female	well	middle	poorly	positive	negative
positive	14	4	1	4	13	16	2
negative	26	12	5	14	19	16	22

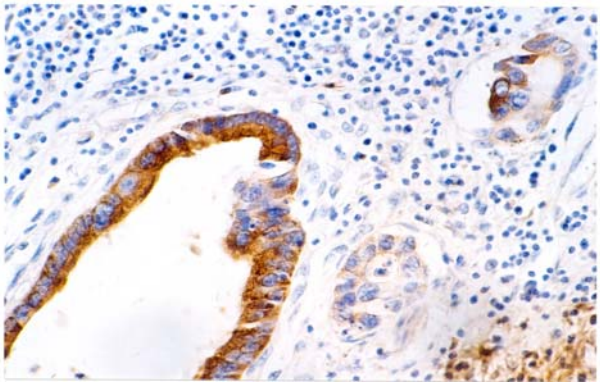
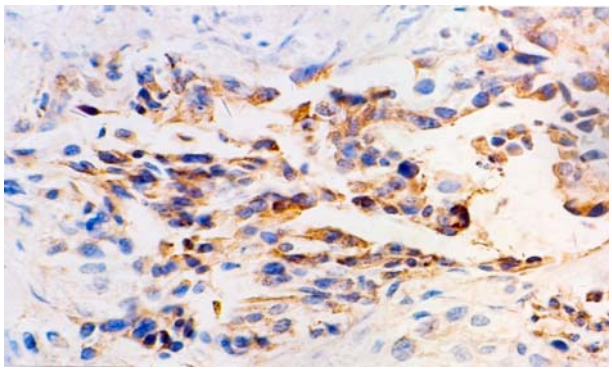


Fig.1,2 Expression of VEGF-C in gastric cancer, the positive brown staining granules are seen in cytoplasm of cancer cells by immunohistochemistry, peritumor tissue are negative.(SABC×400)



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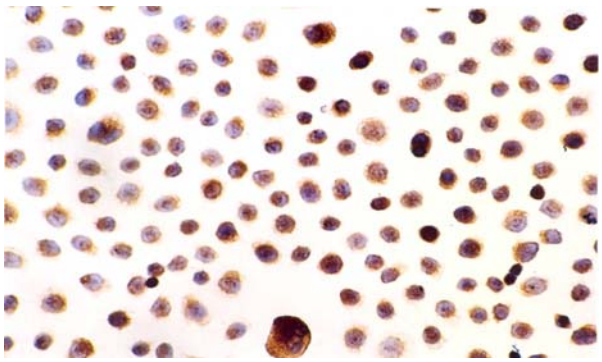

Fig.3 Expression of VEGF-C in gastric line SGC-7901, the positive brown staining
granules are seen in cytoplasm of cancer cells by immunohistochemistry(SABC×400)

RESULTS

Immunohistochemistry

The positive expression for VEGF-C was found in the gastric cancer cells of 18 specimens from 56 specimens, the positive ratio is 32%. Positive staining structures were brown granules in cytoplasm of cancer cells (fig.1,2), peritumor tissues are negative. The SGC-7901 gastric cancer line was also observed the strong positive staining (fig.3).

Correlation with ages and tumor diameter

In the 18 positive patients for VEGF-C, the mean age is 60.2±13 y, the mean tumor diameter is 59±19.8mm; in the 38 negative patients, the mean age is 62±11.2, the mean tumor diameter is 56±24mm. There were not statistic significance in this two groups for the ages and tumor diameter by t test(p>0.05).

Correlation with the sex, differentiation, lymph node metastasis

The table 1 showed: no correlation between the positive ratio for VEGF-C and the sex of the patients (x^2=0.524 p>0.05). But the expression of VEGF-C was conspicuously higher in lymph node-positive group than in node-negative group(x^2=13.2 p<0.01), and in the poorly differentiated gastric cancer cases than in the middle and well differentiated (x^2=10.64 p<0.01).

DISCUSSION

The VEGF family is mediated by three known tyrosine kinase receptors, VEGFR-1, 2, and 3. VEGFR-1 and 2 are expressed on vascular endothelial cells and VEGFR-3 differs from VEGFR-1 and 2 in that it is mainly expressed in the endothelium of lymphatic vessels, which means that it may be specific marker for lymphatic endothelial cells [3]. VEGF-C is a ligand for VEGFR-3, and is thought to be a specific factor inducing lymphangiogenesis[1].

VEGF-C was detected in several types of human malignant tumors, the expression of VEGF-C was

strongly associated with lymph nodes metastasis and lymphangiogenesis. Fellmer PT[4] found that the ratios of VEGF-C gene expression by Northern blot analysis were significantly higher in papillary thyroid carcinoma than in follicular cancer; expression of the VEGF-C gene was observed by situ hybridization in cells of papillary thyroid carcinoma but not in those of follicular carcinoma. As we know, papillary thyroid carcinoma characteristically metastasizes to regional lymph nodes, whereas follicular thyroid carcinoma commonly spreads hematogenously. In cases of human prostatic carcinoma, the expression of VEGF-C, examined using the situ hybridization, showed that VEGF-C mRNA was significantly stronger in the lymph node-positive group than in the lymph node-negative group[5]. In the cases of human colorectal cancers had the same results[6]. All these studies showed that the expression of VEGF-C was related with the lymph nodes metastasis.

In this study, we observed that VEGF-C was expressed in the cytoplasm of gastric cancer cells of 18 specimens (32%), especially in poor-differentiated SGC-7901 gastric cancer line, the VEGF-C expression was stronger staining by immunohistochemistry. The results suggested that expression of VEGF-C was associated with lymph nodes metastasis and the differentiation of gastric cancer, and no correlation with the age, sex, and the tumor size. The lymph nodes metastasis and the differentiated grades both are the important prognostic parameters in the gastric cancer. So VEGF-C may also be a valuble parameter value in gastric cancer, the patients with a high expression of VEGF-C may have a significantly poorer prognosis than those with low VEGF-C expression.

Relatively little is known about the mechanisms of malignant tumors metastasis via the lymphatic system. The lymphatic vessels do not contain tight junctions or continuous basal laminae, their penetration may only require tumor cell adhesion to the endothelium and transmigration through intracellular gaps[7].It is not yet known how such interendothelial gaps are regulated. VEGF has been shown to increase microvascular permeability by enhancing the transmigration activity of the vesicular-vacuolar organelles[8]. Both VEGF and VEGF-C are strong vascular permeability factors, and VEGF-C might also have a similar function in the lymphatic system. In general, VEGF and VEGFR-3 could be component of a paracrine signaling network between cancer cells and the endothelium, and they may be involved in modifying the permeabilities of the lymphatic vessels and metastasis formation. So interrupting the secretion of VEGF-C from cancer cells or inhibiting the lymphangiogenesis, decrease the permeabilities of the lymphatic vessels, the tumor metastasis through the lymphatic system may be controlled.

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