Original Article

# Expression of VEGF-C and VEGFR-3 in human colorectal adenocarcinoma and its significance

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**ABSTRACT** **Objetive:** To study the expression of vascular endothelial growth factor C (VEGF-C) protein and vascular endothelial growth factor receptor 3 (VEGFR-3) in colorectal adenocarcinoma tissue and lymph nodes, and to determine the effects of VEGF-C in the onset, development and lymphatic metastasis of colorectal adenocarcinoma. **Methods:** The expression of VEGF-C and VEGFR-3 protein was detected in 31 paraffin-embedded cases of colorectal adenocarcinoma tissue and 14 paraffin-embedded cases of lymph nodes with immunohistochemistry method. **Result:** Among 31 cases of colorectal adenocarcinoma, VEGF-C protein expression was detected in cytoplasm of cancer cells in 17 cases (54.8%), and VEGFR-3 protein expression was detected in 20 cases (64.5%). Among 14 cases of metastasized lymph nodes, VEGF-C protein expression was detected in 10 cases (71.4%.), and VEGFR-3 protein expression in 9 cases (64.3%).

**Conclusion:** The positive rates of VEGF-C and VEGFR-3 protein expression are high both in colorectal adenocarcinoma and in metastasized lymph nodes.Probably, the conjugation of VEGF-C and VEGFR-3 will promote the proliferation and migration of lymphatic endothelial cells and the formation of new lymphatic vessels, and induce the metastasis of cancer cells.

Key Words: VEGF-C; VEGFR-3; Colorectal adenocarcinoma; Immunohistochemistry

The colorectal adenocarcinoma, which endanger human health seriously, is a common cancer of digestive tract and its incidence is on the rise in recent years. The effect of surgery in early stages of the disease is better, but it is worse in the advanced cases because of lymph node metastasis. As a result, the prevention of cancer metastasis is the key to improve the cure rate. We detect the expression of VEGF-C protein and its receptor VEGFR-3 protein in human colorectal adenocarcinoma cells and lymph nodes using immunohistochemical methods, in order to determine the effects of VEGF-C and VEGFR-3 in the onset, the development and lymphatic metastasis of colorectal adenocarcinoma. It is valuable to clarify the mechanism for the lymphatic metastasis of colorectal cancer, and to provide new ideas for the therapy of colorectal adenocarcinoma.

## MATERIALS AND METHODS

### *Specimens*

Thirty-one cases of paraffin-embedded colorectal carcinoma specimens were obtained from department of Pathology of Affiliated Hospital of Luzhou Medical College, the specimens included 15 male and 16 female, their ages ranged from 26 to 72 years (average 54.4 years). Of them, 10 cases with Dukes A , 12 cases with Dukes B, 9 cases with Dukes C; 9 cases with lymph node metastasis and without metastasis 22 cases. Fourteen cases lymph nodes were obtained from the 31 patients, include 9 cases with Dukes C, 5 cases with Dukes B. Check 5 cases normal colorectal tissue of cancer patients (from the cancer tissue area for more than 5 cm) served as controls.

The authors have no commercial,proprietary,or financial interest in the products or companies described in this article.

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ISSN:1538-5124/$-see front matter 

*Reagent*

Rabbit anti-human VEGF-C antibody, rabbit anti-human VEGFR-3 antibody, biotinylated goat anti-rabbit secondary antibody, concentrated SABC immunohistochemistry kit, DAB reagent (brown), were purchased from Wuhan Boster Biological Engineering Co., Ltd.

*Methods*

Immunohistochemical staining for VEGF-C and VEGFR-3 were performed with SABC method. Sections (5 μm) of formalin-fixed and paraffin embedded tissue were affixed to glass slides coated with APES. After deparaffinization in xylene and rehydration through graded alcohol baths, endogenous peroxidase activity was blocked after incubated with 3% hydrogen peroxide for 20 min at room temperature. Slides were autoclaved at 121°C in 0.1 M citrate buffer for 10 min, and incubated in 10% normal goat serum for 20 min. Subsequently, slides were incubated with 1:100 rabbit polyclonal anti-human VEGF-C (VEGFR-3) antibody for 30 min in wet box at 37 °C, then overnight at 4°C. After washing with 0.01 mol/l PBS, slides were incubated with biotinylated goat anti-rabbit antibody (1: 100) for 30 min. At last, slides were incubated with SABC complex for 30 min in wet box at 37 °C, then were covered by DAB till cells were stained under microscope. The data were analysed by x2 test, and $P<0.05$ was considered statistically significant.

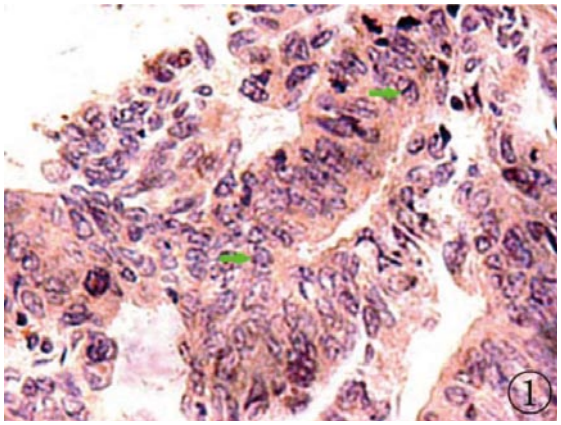
**RESULTS**

*Expression of VEGF-C protein in colorectal adenocarcinoma tissues*

Among the 31 cases of colorectal adenocarcinoma, brown particles of VEGF-C protein expression were showed in the cytoplasm of adenocarcinoma cells in 17 cases(54.8%, 2 cases in Dukes A, 7 cases in Dukes B, 8 cases in Dukes C) (Figure 1). In specimens of normal colorectal tissues, no VEGF-C protein was stained.

*Expression of VEGFR-3 protein in colorectal adenocarcinoma tissues*

Among the 31 cases of colorectal adenocarcinoma, brown particles of VEGFR-3 protein expression were showed in 20 cases (64.5%, 4 cases in Dukes A, 7 cases in Dukes B, 9 cases Dukes C) in the cytoplasm of adenocarcinoma cells (Figure 2). In specimens of normal colorectal tissues, no VEGFR-3 protein was stained.



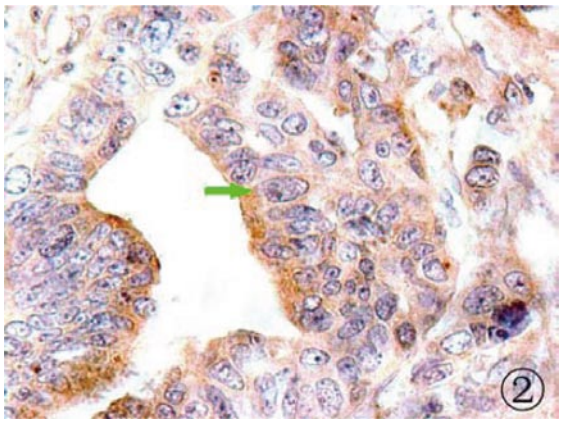
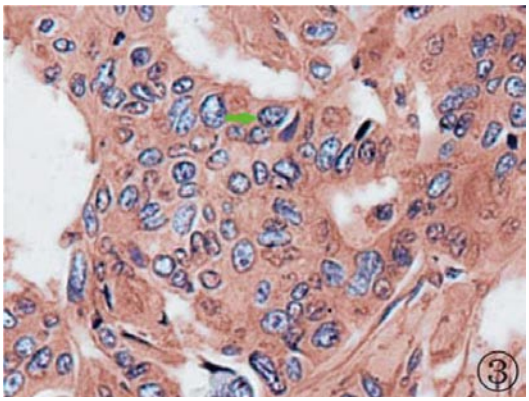




Figure 1. The expression of VEGF-C protein in rectal adenocarcinoma tissue, arrow indicating cancer cells,x 400.

Figure 2. The expression of VEGFR-3 protein in rectal adenocarcinoma tissue, arrow indicating cancer cells,x 400.

**Expression of VEGF-C protein in lymph nodes**

In 14 cases of lymph node tissues, VEGF-C protein expression (brown particles) were examined in 10 cases (71.4%, 8 cases of 9 in metastasis, 2 cases of 5 in non-metastasis). Brown particles were observed in the cytoplasm of cancer cells (Figure 3) and lymphocytes (Figure 4).



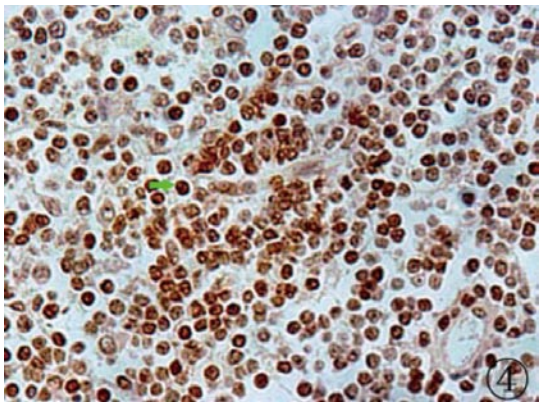
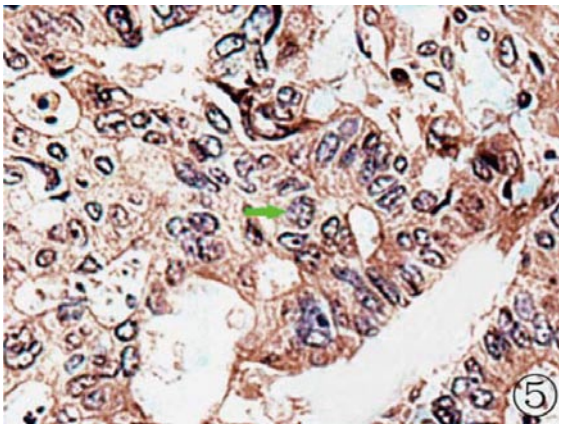


Figure 3 the expression of VEGF–C protein in lymph node, arrow indicating cancer cells,x400.

Figure 4 the expression of VEGF–C protein in lymph node, arrow indicating lymphocytes,x400.

*Expression of VEGFR–3 protein in lymph nodes*

In 14 cases of lymph node tissues, VEGFR-3 protein expressions (brown particles) were examined in 9 cases (64.3%, 5 cases of 9 in metastasis, 4 cases of 5 in non-metastasis). Brown particles were observed in the cytoplasm of cancer cells (Figure 5) and lymphocytes (Figure 6).



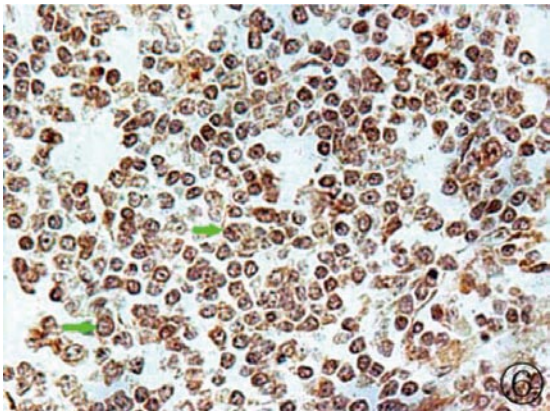


Figure 5 the expression of VEGFR–3 protein in lymph node, arrow indicating cancer cells,x400.

Figure 6 the expression of VEGFR–3 protein in lymph node, arrow indicating lymphocytes,x400.

*The relationship between the VEGF–C, VEGFR–3 and sex, age, stages, lymph node metastasis (Table)*

Table. The relationship between VEGF-C, VEGFR-3 and sex, age, stages, lymph node metastasis

| Item | n | VEGF-C postive | P | VEGFR-3 postive | P |
|---|---|---|---|---|---|
| Sex | | | | | |
| male | 15 | 7 | | 10 | |
| female | 16 | 10 | 0.376 | 10 | 0.809 |
| Age | | | | | |
| ≥50 | 19 | 9 | | 11 | |
| <50 | 12 | 8 | 0.293 | 9 | 0.332 |
| Stage | | | | | |
| Dukes A | 10 | 2 | | 4 | |
| Dukes B | 12 | 7 | 0.01 | 7 | 0.02 |
| Dukes C | 9 | 8 | | 9 | |
| Lymph node | | | | | |
| Metastasis | 9 | 8 | | 9 | |
| No metastasis | 22 | 9 | 0.015 | 11 | 0.008 |

From the table, we can see, VEGF-C and VEGFR-3 protein expression was related with stage and lymph node metastasis ($P < 0.05$), but was not related to sex and age ($P > 0.05$).

## DISCUSSIONS

This study showed that the expression of VEGF-C and VEGFR-3 protein in colorectal adenocarcinoma tissues and metastasized lymph nodes is high, and the expression of VEGF-C and VEGFR-3 protein was related to the stage of adenocarcinoma and lymph node metastasis ,but unrelated to patient's sex and age. The expression of VEGF-C and VEGFR-3 protein were observed in the cytoplasm of adenocarcinoma cells and lymphocytes in colorectal adenocarcinoma and metastasized lymph nodes, which corresponds with conclusions of other researchers[1-5]. On the other hand, the result of this experimental is different to other researchers in the expression rate[2] and whether related with age of the patients [5]. So it is necessary to study the factors impacting the expression of VEGF-C and VEGFR-3 protein in colorectal cancer and indicate the mechanism of lymph node metastasis by further experiments.

VEGF-C is one of the directly acting lymphangiogenic factors belonging to the VEGF family[6], it can induce proliferation of lymphatic endothelialcells in vitro[7] and lymphangiogenesis in vivo[8] by activating it's receptor VEGFR-3 which located on the lymphatic endothelial cells. Animal experimentation has shown that over-expression of VEGF-C could result in lymphatic endothelial proliferation and vessels enlargement[9], and the dilated lymphatic vessel facilitates metastasis of tumor cells to lymph nodes. In addition, the activation of lymphatics by VEGF-C is considered to induce secretion of chemokines and similar factors of the lymphatic endothelium, thus attracting and facilitating tumor cells to enter the lymphatics.

VEGF-C has been identified as regulator of lymphangiogenesis in mammals, and it's ability to drive lymphangiogenesis has been demonstrated with a range of animal-based assay systems[8,10-11]. Karpanen terhi et al[12] showed VEGF-C strongly promoted the growth of tumor-associated lymphatic vessels. Accumulating data from clinicopathologic studies suggested that the spread of tumor cells to regional lymph nodes was an early event in many solid tumors and the lymphatic vessels was the primary spreading route, and lymphangiogenesis promote lymphatic metastasis for most solid tumor cells via peritumoral and intratumoral lymphatic vessesls[13]. Although there has been considerable debate about the existing and it's functional significance of intratumoral lymphatic vessels[14-19]. Padera et al [14] showed peritumoral functional lymphatics are sufficient for promoting metastasis by offering a larger area for tumor cell escape. In our current study, we observed colorectal cancer cells in metastatic lymph nodes and detected the high expression of VEGF-C protein secreted by cancer cells, which has closely correlated with lymphatic metastasis of malignant tumor.

In most cancers, including colorectal cancer, it is the metastatic spread of tumor cells to lymph nodes and distant organs that results in patient mortality. In colorectal adenocarcinoma, tumor metastasis occurs to lymph nodes and distant organs may be via both blood vessels and lymphatic vessels by the binding of VEGF-C to VEGFR-2 and VEGFR-3. However, previous studies[8,12] suggested the main factor promoting tumor metastasis is VEGFR-3 not VEGFR-2, and inhibition of VEGFR-3 signaling can suppress tumor lymphangiogenesis and metastasis. So, we speculate lymphatic metastasis is the main approach of colorectal cancer cells metastasis. Therefore, some investigators[9-10, 20-22] have made efforts and made progress on blocking VEGF-C/VEGFR-3 signaling pathway to inhibit lymphangiogenesis and lymphatic metastasis with VEGFR-3 neutralizing antibody, soluble extracellular fragments or small molecular weight inhibitors, and so on. The success of a number of these strategies has been confirmed in animal models, which will provide a new therapeutic treatment of colorectal cancer.

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