Research on the Distribution and Morphologic Feature of Lymph Vessels in Gastric Carcinoma by PAS Staining*

Huidong Liu, Yulan Li, Yunfang Zhang, Yimin Li, Xiaodong Li, Linghui Zhao

Department of Anatomy, Harbin Medical University, Harbin 150086, China

Abstract **Objective** To research the lymphatic metastasis mechanism of gastric carcinoma. **Methods** Specimens were obtained in 20 patients with gastric cancer. PAS staining allowed that lymphatic and blood capillaries could be distinguished objectively. The morphologic features of lymphatics were observed in the periphery region of carcinoma and normal region under microscopy. By using computer image analysis system, area number density and medium diameter of lymphatics were measured both in periphery region and normal region of carcinoma. **Results** The number density and medium diameter of lymphatics in periphery area of gastric cancer was higher than that in normal region. The lymphatics were dilated and their walls were disintegrated in periphcry area of carcinoma. **Conclusion** proliferation and deformity of lymphatics existed in periphery region of carcinoma, which increase the probability of cancer dissemination via lymphatic system route. PAS staining provide a method for research on distribution of lymph vessels and the lymphtic metastasis mechanism of carcinoma.

Key Words gastric carcinoma; PAS staining; lymph vessel

Gastric carcinoma, as one of the most common human malignant tumor worldwide, ranks the first in frequency among human cancer in china [1]. Metastasis is the primary mechanism leading to death of the patient with gastric carcinoma. The lymph–borne metastasis is a major pathway for gastric cancer. However, the purely descriptive accounts of some of the most critical phases of the metastatic process are still lacking. Mechanisms of lymph–borne metastasis are also the least understood. Accordingly, to research the lymphatic metastasis mechanism of gastric carcinoma, we used PAS method that allowed lymphatic and blood capillaries be distinguished objectively and studied the distribution, density, and morphologic and structural features of the lymphatic vessels in specimens removed from patients with gastric cancer.

*This item was supported by grant from the National Natural Science
Foundation of China (No.30270697) and the National Natural Science
Of HeilongJiang(D01–07)
Correspondence to: Huidong Liu
Department of Anatomy,
Harbin Medical University, 150086,Harbin China;
Tel:0451–86674508
E–mail: huidongliu2003@yahoo.com.cn

MATERIAL AND METHODS

Tumor samples

Experimental specimens were obtained from 20 patients with gastric cancer. Specimens were respectively cut in the center, periphery and normal regions of the postoperative tissue.

PAS Staining

Samples were fixed in 10% formalin, embedded in paraffin wax, cut (6μm)using a sliding microtome and stained by PAS method [2]. Using this method paraffin–sections were oxygenated by periodic acid, then were reacted by colored fuchsion. Under light microscope, the morphology and structure of initial lymphatics were observed in the center, periphery region of carcinoma and normal region of gastric mucosa.

Statistical Analysis

With aid of computer–based image analyzer, we compared area number density and medium diameter of lymphtics in normal regions with that in periphery regions of carcinoma. Difference was considered significant when the P value was less than 0.05.

RESULTS

By PAS method, the lymphtics and blood ves-

sels displayed distinctly difference. The basement membranes of blood vessel appear purplish red staining and lymphatic vessels show lightish staining. Furthermore, lymphatics represented morphologic characteristics. They had wider, more irregular lumen than blood vessels and absence of a well-developed basement membrane[3-5] and the endothelium obviously protrudes into the cavity. On the basis of the above distributions, lymphatic could be distinguished from blood capillaries objectively. In periphery region of carcinoma, Lumina of lymphatics were dilated(Fig.1); carcinoma cells were readily evident invading initial lymphatics (Fig.2); walls were often dissolved and destroyed, whereas cancer cells located near the lymphatics. Lymphatic vessels were eroded by invading cancer cells and they contained many lymphocytes as part of immuno-responsiveness to the adjacent cancer (Fig.3). Lymphtics were not obvious change in normal region.

By mean of computer image analysis system, area number density and medium diameter of lymphtics in normal and periphery regions of carcino-

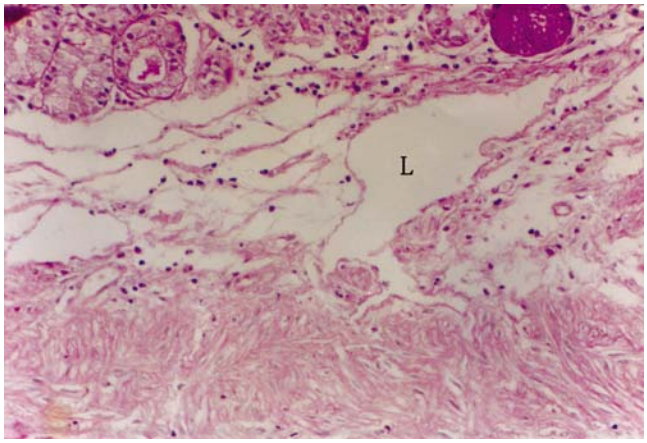

Fig.1 Lymph capillary in mucosa was dilated in periphery region of gastric carcinoma.
PAS staining ×66 L: Lymph capillary

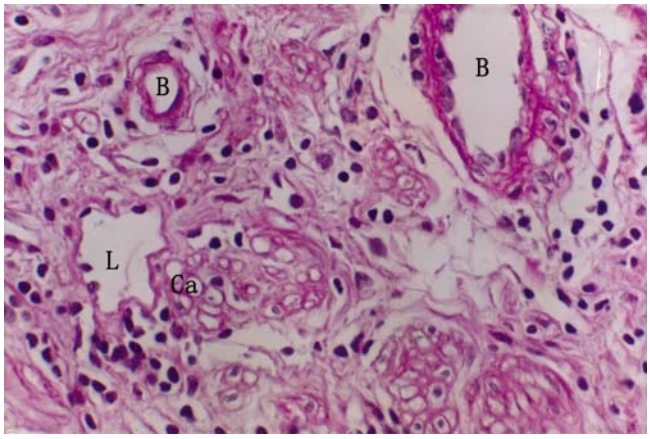

Fig.2 Gastric cancer cells located near lymph capillary in periphery region of gastric carcinoma.
PAS staining ×132 Ca: cancer; L: Lymph capillary; B: Blood

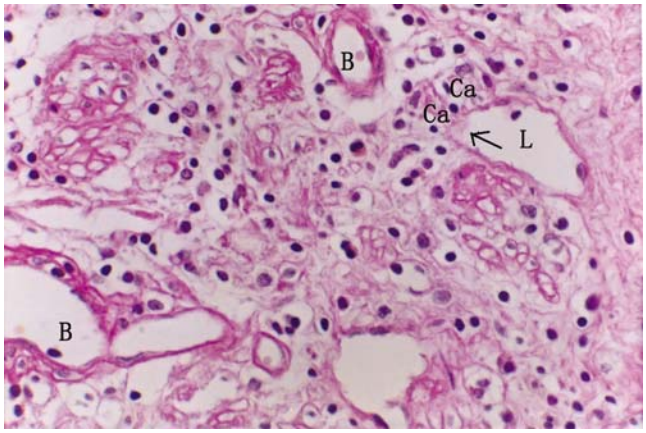

Fig.3 Wall of lymph capillary was partly dissolved(↑) in periphery of gastric carcinoma.
PAS staining ×132, Ca: cancer cells

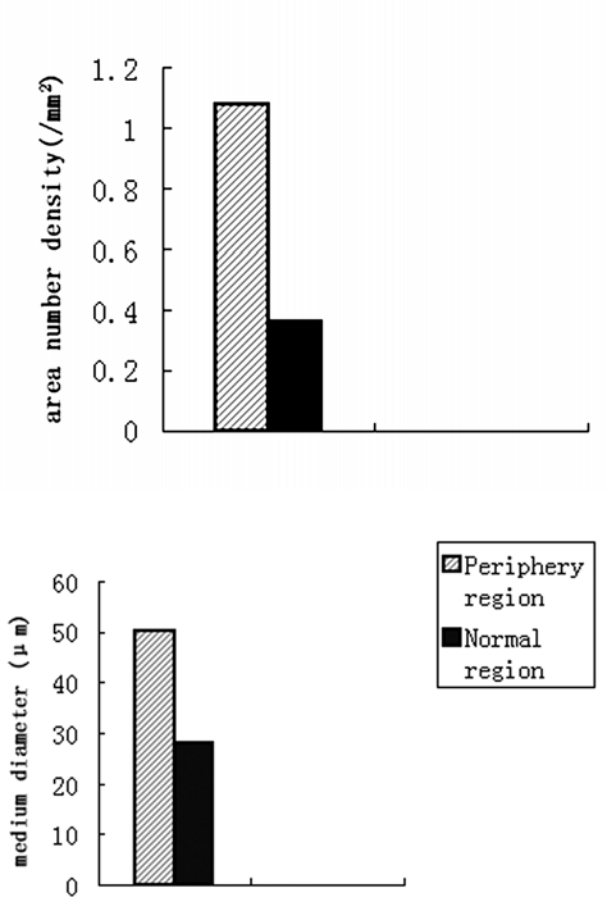

Fig.4 Comparisons of data about the lymph vessels

ma were summarized in (Fig. 4). There was significant difference between the two groups (P<0.05).

DISCUSSION

In studies on lymph vessels, researchers have applied means such as 5´–Nase and ALPase stain[6] and immunohistochemical method [7] to distinguish lymph vessels and blood vessels. We used firstly PAS method to obtain direct and objective view of lymphatic capillaries. By this method, the staining of tissues and cells around lymphtics vessels were not influenced as similar as the routing HE. Compared with transmission electron microscopy, under light section with PAS staining there were bigger visual field. So there were more informations to be observed. PAS method was also easier and more economical than other methods.

We found that the area number density of lymphatic capillaries in periphery regions were higher than that in normal region (P<0.05). Our data support that amount of lymph vessels increased in carcinomatous peripheral tissues. How was the result caused? Recently an important advance about VEGF–C is finding. Experiments in transgenic mice have shown that VEGF–C is a growth factor for developing lymphatic vessels. In the skin of transgenic mice, overexpression of the VEGF–C cDNA has been shown to selectively induce lymphatic endothelial cell proliferation and hyperplasia of the lymphatic vasculature. In differentiated chick chorioallantoic membrane, purified marure VEGF–C also induced growth of lymphatic vessels, having very little effect on blood capillaries[8–9]. Furthemore, expression of VEGF–C mRNA has been detected also in malignant human tumors, including nearly half of the breast cancers analyzed[10]. Lubach D[11] revealed that the increase of malignant aggressiveness is directly related to the arrangement of the initial lymphatic vessels in the different layers of the skin. Sudden increase in the density of lymphatic vessels permits a corresponding increment of malignant seeding via the lymphatic system. According to above descriptions, we proposed that there were proliferation of lymphtics in periphery of carcinoma, a great number of lymphatics nearby the tumor tissue facilitated tumor cells to enter the lymphatic lumina.

In addition, medium diameter of lymphtics in periphery regions of carcinoma was also higher than that in normal regions (P<0.05). The data implied lumina of lymphatics were dilated in periphery region of carcinoma. Result of this study also proved lymphatics were dilated, dissolved and distingrated in periphery regions. Previously studies provided explanations for our results. It is likely that with ongoing angiogenesis and cancer growth, lymphatic anchoring filaments are activated and lymphatic capillaries become dilated, which facilitating the migration of cancer cells from the interstitium into initial lymphatics[12–14]. Serial examination described following process of the tumor cells invading lymphtics: tumor cells first aligned themselves along the lymphatic vessels, then they penetrate the abluminal fibrous network to occupy the perilymphatic space, then fuse partially with the endothelial cell wall to destroy it. The process whereby neroplastic cells breach the endothelial layer are unknown, but possible factors may include mechanical pressure exerted by motile tumor cells and the local release of substances which cause endothelial retraction or inflict minute lesions on walls of lymphtics[15–16].

In conclusion, we thought PAS method provided a reliable new approach for researching on the lymphtics and the lymphtic metastatic mechanism. Our investigation suggested that the important factors involved in lymph–borne metastasis were increased peritumoral lymphatic density, distinct dilatation and disrupted lymphatic structure.

REFERENCES

1. DENG DJ. Progress lf gastric cancer etiology: N–ni–tros–amides 1999s. World J Gastroenterol, 2000, 6: 613–618.
2. LING Qi–bo. Practical pathology special stain and photo–chemistry technique. Guangdong Advanced Education Press, 1988, 201–211.
3. Casley–Smith JR. The structure and functioning of the blood vessel, interstitial tissues and lymphatics. Lymphan–giology, 1983, 2:27–143.
4. Ryan TJ.Structure and function of lymphatics, J Invest Dermatol, 1989, 92:185–245.
5. Braverman JM. the role of blood vessels and lymphatics in cutaneous inflammatory processes, Br J Dermatol, 1983, 109:88–98.
6. Werner JA. Histochemical visualization of lymphatic capillaries in the rat. Arch Histol Jap, 1987, 50(3): 505.
7. Ezaki T, Matsuno k, Fujii H, et al. A new approach for identification of rat lymphatic capillaries using a mono–clonal antibody. Arch Histol Cytol, 1990, 53:77.
8. Jeltsch M, Kaipainen A, Jorkov V, et al: Hyperplasia of lymphatic vessels in VEFG–c transgenic mice. Science, 1997, 276: 1423–1425.

9. Oh S, Jeltsch M, Birkenhager R, et al: VEGF and VEGF–C: specific induction of angiogenesis and lym–phan giogenesis in the differentiated avian chorioallantoic membrane. Devbiol, 1997, 188: 96–109.
10. Salven P, Lymboussaki A, Heikkila P, et al. Vascular endothelial growth factors VEGF–B and VEGF–C are expressed in human tumors. Am J Pathol, 1998, 153: 103–108.
11. Lubach D, Berens von Rautenfeld D, Kaiser HE. The possible role of the initial lymph vessels of the skin during metastasis of malignant tumors. In Vivo, 1992, 6 (4): 443–450.
12. Leak LV, JF Burke. Ultrastructure studies on the lym–phatic anchoring filament. Cell Biol, 1968, 36: 129–149.
13. Leak LV, Burke JF. Fine structure of the lymphatic capillary and adjoining connective tissue area. Am J Anat, 1996, 118(3): 785–809.
14. Gerli, R. Ilbba, CFrwchellin. Ultrastructural cytochemistry of anchoring filaments of human lymphatic capillaries and their relation to elastic fibers. Lymphology, 1991, 24: 105–112.
15. Deutsch A, Lubach D, Nissen S, et al. Ultrastructural studies on the invasion of melanomas in initial lymphat–ics of human skin. Invest Dermatol, 1992, 98(1): 64–67.
16. RL Cater. Some aspects of the metastatic process. Jclin Pathol, 1982, 35:1041–1049.