

Morphological analysis of lymph vessels and capillaries in gastric carcinoma

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Abstract

AIM: To investigate the morphology of lymph vessel capillaries in both human gastric carcinomas and their peritumoral tissues, as well as the relation of this morphology to lymphatic metastasis.

METHODS: The morphology and fine distribution of both lymph vessels and capillaries in and around the primary foci of gastric carcinoma were studied using the 5'Nase Alpace double staining method. The total amount of opened vessels and the opening rate of lymph vessels and capillaries were counted using a light microscope (100 × magnification), and the maximal luminal area, perimeter and diameter were measured using an image analysis technique.

RESULTS: Lymph vessels and capillaries displayed strong 5'Nase-positive staining (brown and dark brown), while blood vessels and capillaries revealed significant Alpace activity (blue). There were many lymph vessels, capillaries and solid strip-like tissues found in the gastric carcinoma samples analyzed. The total amount of lymphatics in the metastatic group (gastric carcinoma *vs* peritumoral tissue) and non-metastatic group was 26.9 ± 14.2 *vs* 10.4 ± 4.0 , 11.4 ± 3.4 and 9.7 ± 3.2 , $P < 0.01$, respectively. Their opening rates were

21.2 *vs* 47.5 and 40.4 *vs* 46.0 , $P < 0.01$, respectively. Their maximal luminal areas were 1502.98 ± 1236.91 *vs* 5526.80 ± 4853.42 ; 1918.14 ± 2299.24 *vs* 3836.16 ± 3549.16 ; 5526.80 ± 4853.42 *vs* 3836.16 ± 3549.16 , $P < 0.05$, their perimeters were 220.33 ± 130.25 *vs* 441.43 ± 276.51 ; 241.79 ± 171.13 *vs* 333.80 ± 199.66 ; 441.43 ± 276.51 *vs* 333.80 ± 199.60 , $P < 0.05$, and their diameters were 28.80 ± 14.98 *vs* 59.39 ± 28.53 ; 25.37 ± 15.79 *vs* 46.22 ± 20.85 ; 59.39 ± 28.53 *vs* 46.22 ± 20.85 , $P < 0.05$, respectively. In summary, the lymphatics found in gastric carcinoma samples from the metastatic group were significantly lower than those of the other groups.

CONCLUSION: There are newly formed lymph capillaries found in gastric carcinoma. Dilation of lymph capillaries may be related to edema found in peritumoral connective tissues. The observed lymph node metastases from gastric carcinoma occur through mature lymph capillaries that invade in and around primary gastric carcinoma foci.

Key words: Stomach neoplasms; Lymphatic metastasis; Lymphatic system/pathology

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INTRODUCTION

Lymph node metastases from human gastric carcinoma typically occur through lymphatic channels, however its mechanism remains unclear. Some researchers^[1] believe that the probability of lymph node metastasis increases with an accompanying increase in the number of lymph vessels and capillaries in the invaded peritumoral tissues. Nevertheless, others^[2] think that the opening of lymph vessels and capillaries due to edema in peritumoral connective tissues facilitates cancer cell metastasis. Previous reports have suggested that the stomachs of mice exhibit a fine distribution of lymphatics. This study was performed in order to both profile the distribution, quantity and morphologic changes of lymph vessels and capillaries in gastric carcinoma, as well as to further illuminate the metastatic mechanism underlying gastric cancer.

MATERIALS AND METHODS

Materials

Fresh, surgically resected gastric carcinoma specimens were collected from 32 cases (22 metastatic and ten non-metastatic) and carefully examined. All light microscopy specimens were fixed in 10% forma-

Table 1 Lymph vessel and capillary counts ($\bar{x} \pm s$)

Group	Total	Opened No.	Opening rate (%)
Metastatic			
Gastric carcinoma	26.9 ± 14.2	5.7 ± 5.0	21.2
Peritumoral tissue	10.4 ± 4.0	4.9 ± 2.5	47.5
Non metastatic			
Gastric carcinoma	11.4 ± 3.4	4.6 ± 3.2	40.5
Peritumoral tissue	9.7 ± 3.2	4.5 ± 2.3	46

Table 2 Morphological quantitative analysis of lymphatics ($\bar{x} \pm s$)

Group	n	Lumen area (μm^2)	Perimeter (μm)	Diameter (μm)
Metastatic				
Gastric carcinoma	43	1502.98 ± 1236.91	220.33 ± 130.25	28.80 ± 14.98
Peritumoral tissue	61	5526.80 ± 4853.42	441.43 ± 276.51	59.39 ± 28.53
Non metastatic				
Gastric carcinoma	47	1918.14 ± 2299.24	241.79 ± 171.13	25.37 ± 15.79
Peritumoral tissue	58	3836.16 ± 3549.16	333.80 ± 199.66	46.22 ± 20.85

lin and paraffin-embedded. Sections were stained with hematoxylin and eosin., Gastric carcinoma and their peritumoral tissues were simultaneously frozen at -20°C and sectioned using a cryostat. All frozen sections with a $6\text{ }\mu\text{m}$ thickness were fixed in preparation for 5' NaseAlpase double staining. 5'adenosine monophosphate (AMP, No. A-1752, Sigma), naphthol AS-MX phosphate (No. N-5000, Sigma), fast blue BB (No. F-3578, Sigma), Tetramisol (No. T-1512, Sigma).

5'-Nase Alpase double staining^[3-8]

The Alpase reaction to label blood vessels was followed by 5' Nase staining to label the lymphatics. Following incubation for 60 min at 37°C , the slides with treated with standard medium containing Wachstein, incubated for two minutes. at room temperature in yellow ammomium sulfide, and incubated for 15 min. at room temperature in standard medium with Burston. Control slides were incubated without substrate (AMP or naphthol AS-MX phosphated).

Quantification of lymph vessels and capillaries

The total number of opened vessels and the opening rate (percentage = the opened/the total) of both lymph vessels and capillaries in the submucosa of peritumoral and carcinoma tissues were studied under a light microscope ($\times 100$ magnification). Lymph vessels and capillaries in tissue cross sections were counted, while those in longitudinal and oblique sections were excluded.

Morphological quantitative analysis of lymph vessels and capillaries

The maximal luminal area, perimeter and diameter of both lymph vessels and capillaries containing opened lumens were measured using an image analysis device. This device included both light and fluorescent microscopes (Nikon, Japan), a digital brand, a computer, and a morphologic program (stereology, United States).

RESULTS

Swelling and metastatic lymph nodes

There were 15 lymph nodes within the lesser curvature of the stomach, 23 within the greater curvature of the stomach and 10 at other sites, and all these lacked signs of lymph node metastases. Additionally, there were 39 lymph nodes within the lesser curvature, 41 within the greater curvature, and 10 at other sites that accounted for 52 metastases (59.4%).

Enzyme histochemistry

There were significant differences detected between the lymphatic and blood vessel staining. Using 5'Nase Alpase double staining, Mg^{2+} as an activator and lead as a capture agent, the reaction product of 5'Nase activity was detected as a brown or dark brown precipitate of lead sulfide on lymph vessel and capillary walls following incubation in 5'Nase standard medium. When tissue sections were then incubated in azo dye reaction medium for Alpase activity, the coloration of the blood vessels and capillaries changed to blue. The lymph vessels were morphologically irregular, with large lumens and thin-walled vessels, while the blood vessels were regular and

thick-walled. The slides demonstrated 5'Nase activity of both lymph vessels and capillaries. The slides only showed Alpase-positive staining of blood vessels and capillaries, with no sign of Alpase-positive lymph vessels and capillaries. The control slides that were incubated without 5' AMP or naphthol AS MX monophatate demonstrated no lymph, blood vessel, or capillary staining.

Lymph vessel and capillary distribution and morphology

Many lymph capillaries were identified in the mucosal layer of peritumoral tissues, mostly between the bottom of the gastric gland and mucosal muscularis. Some lymph capillaries extended into the connective tissues within the gastric gland, while others extended into the mucosal muscularis or submucosa. There were many lymph vessels and capillaries in the submucosa, with the majority of lymph capillaries found near the mucosal muscularis and the majority of lymph vessels found near the muscularis. Many lymph vessels and capillaries were found in the muscularis, and many more between the oblique, circular and longitudinal muscles. The lymph vessels and capillaries found in the serosa were also seen in connective tissues near the longitudinal muscles, which were also surrounded by large lymph vessels.

All of the lymph vessels and capillaries in the gastric carcinoma tissues exhibited irregular morphologic shapes and branches. Lymph capillaries were partially opened with small lumens and had either lobular or triangle shapes. Some appeared strip-like with enlarged light brown endothelial cells that were irregularly arranged. It is possible that these strip-like tissues are in fact newborn lymph capillaries. A few strip-like tissues that stained positive for 5'Nase were seen in peritumoral tissues, which could be collapsed lymph capillaries. In and around the primary foci of gastric carcinoma, the carcinoma cells appeared to destroy the lymph capillary walls. These capillaries contained small and regularly shaped 5'Nase-positive endothelial cells that invaded the lumen. The lumens were large with irregular morphologies. These lymph capillaries may therefore be mature lymph capillaries.

Quantification of lymph vessels and capillaries

In the metastatic group, the total number of lymph vessels and capillaries in the primary foci of gastric carcinoma were significantly higher ($P < 0.01$) and their opening rates were significantly lower ($P < 0.01$) than that of the peritumoral tissues of this same group. Additionally, the total numbers in the gastric carcinoma and peritumoral tissues of the metastatic group were significantly higher than those of the non-metastatic group ($P < 0.01$), whereas the differences in the number of openings were not significant between the two groups ($P > 0.05$) (Table 1).

Morphological quantitative analysis of lymph vessels and capillaries

There were significant differences ($P < 0.05$) in the maximal luminal area, perimeter and diameter of lymph vessels and capillaries between gastric carcinoma and their peritumoral tissues in metastatic group, gastric carcinoma and their peritumoral tissues in non metastatic group, and between peritumoral tissues in metastatic and non metastatic group, whereas the difference of the maximal luminal area, perimeter and diameter ($P < 0.05$) between gastric carcinoma in metastatic group and in non metastatic group was not significant. Statistically the maximal lumen area, perimeter and diameter of lymph vessels and capillaries in carcinoma tissues in metastatic group were significantly smaller than those in other groups (Table 2).

DISCUSSION

While lymph capillaries were observed to be thin-walled vessels with only a single layer of endothelium, the newborn capillaries were lined only by a single endothelial layer and thin basement membrane that lacked smooth muscle. This therefore made it considerably challenging to distinguish between the lymph capillaries and newborn capillaries. Using a light microscope, the composition of the lumen and the morphological characteristics of vessels were usually used for analysis. The lymph vessels were thin-walled with large

lumens that lacked red blood cells and contained irregularly projected endothelial nuclei. The lumens of these vessels also contained a few proteins and white blood cells. Using the 5'Nase Albase double staining method, lymph vessels and capillaries appeared brown in color, while blood vessels and capillaries appeared blue. Therefore, compared with traditional lymphatic injection, glass mounting and H and E staining of slides, the 5'Nase Albase double staining method accurately demonstrates the fine distribution of lymph vessels and capillaries. This provides an experimental method and morphological basis for performing research on the morphological shapes of lymph vessels and capillaries in human gastric carcinoma, as well as its relation to lymph node metastasis of gastric carcinoma.

Previous research postulated that the absence of lymphatic metastasis in early gastric carcinoma is due to the lack of lymphatics in superficial layers of the mucosa. With the discovery of tumor infiltration, we now believe that the increase in lymph vessels and capillaries around the primary foci of the tumor may be one of the critical factors that elevate the rate of lymph node metastasis^[9]. Lubach *et al.*^[11] determined that melanoma invasiveness significantly increased the number of lymph vessels and capillaries in surrounding tissues as well as metastatic activities in lymph vessels. These results suggest that lymph node metastasis in carcinomas are related to the increase in the number of lymph vessels and capillaries found in peritumoral tissues, which is due to the distribution of lymph vessels and capillaries that are not newborn. This study shows that the total number of lymph vessels and capillaries in gastric carcinoma is larger than those of their peritumoral tissues. Some capillaries, either partially opened or solid strip-like with enlarged, brown and irregularly arranged endothelial cells, may be newborn lymph capillaries. Upon comparing the morphologic characteristics of lymph capillaries in carcinoma and peritumoral tissues, it appears that lymph capillaries are not induced by the opening of collapsed lymph capillaries, an event caused by edema in connective tissues. Ryan^[10] believes that angiogenesis should encompass not only blood vessels but also lymphatics. It remains unclear whether lymph capillary proliferation is induced by active secretions from tumor angiogenesis or by the host's reaction to tissue damage caused by the tumor.

Under normal conditions, most lymphatics exist in a collapsed state because only a portion of the entire lymphatic system is actively involved in the transportation process. The majority of lymphatics are therefore inactive, functioning as a potential reservoir for increased lymph flow^[11]. Rapid tumor and edema growth among peritumoral tissues increased the pressure within the tissue, which dilates most lymph capillaries. During each stage of human and animal tumor invasion, severe edema was observed in peritumoral tissues and favored tumor cell infiltration. As a result, many tumor

cells accumulated in the afferent lymphatic vessels of the lymph node, particularly at drainage areas.

Among the several factors that induce edema in peritumoral tissues, the most important one may be the increase of vascular permeability and ineffective circulation of the lymphatic fluid within the tumor area^[2]. High fluid pressure was induced due to a large amount of fluid accumulation within the tumor stroma and this fluid was transported from the center to the edge of the tumor. The edema in the peritumoral tissue widened and opened the lumen between the connective tissue bundles. Furthermore, the tissue fluid pushed the tumor cells to lymph capillaries, and both the tumor cells and tissue fluid entered into the lumen of the lymph capillaries. Lymph capillaries in gastric carcinoma showed light staining, low opening rates and small lumens, which indicated that lymph node metastases occur by invading through mature lymph capillaries that are located in and around the primary foci of human gastric carcinomas.

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