IMMUNOHISTOCHEMICAL STUDY ON ANGIOGENESIS OF AXILLARY LYMPH NODE METASTASIS IN HUMAN INVASIVE BREAST CARCINOMA

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ABSTRACT

Objective: To study angiogenesis of the axillary lymph node metastases including micrometastases in breast carcinoma and the relationship between microvessel density (MVD) and metastasis. Methods: Thirty-seven breast cancer tissues and 121 metastatic axillary lymph nodes were collected from the patients and studied immunohistochemically. MVD was counted by means of microvideo system under 100 magnification. The diameter of each micrometastasis was measured with a micrometer. Results: The mean diameter of 13 micrometastatic foci was 210±37 μm. No blood vessel formation was found. MVD of the primary tumor and that of metastatic tumor in the axillary lymph node were 93.8±21.8 and 89.3±18.4, respectively ($P<0.05$). The distribution of microvessels of the metastatic tumor in the lymph node and that of the primary tumor was similar, being higher at the periphery than at the center. Conclusion: Micrometastatic foci of breast carcinoma in the axillary lymph node do not have new blood vessel formation. Their further growth, however, depends on neoangiogenesis. Treatment based on inhibition of angiogenesis may be efficacious in the prevention of micrometastatic foci from developing into metastatic tumor in lymph node.

Key words: Breast cancer, Lymph node metastasis, Angiogenesis, Immunocytochemistry

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The axially lymph node is one of the earliest and most common metastatic positions of breast carcinoma. Hence, axillary lymph node status (metastasis or no) and the number of metastatic lymph nodes are considered to be important indicators which affect prognosis of breast cancer patients. It has been widely confirmed that malignant solid tumor growth must be dependent upon angiogenesis/neovascularization. Considerable evidence has shown that microvessel density (MVD) of breast cancer tissue correlates with metastasis including axillary lymph node metastasis.[1,2] However, there is up-to-date no systemic research on angiogenesis of secondary tumors, especially of the axillary lymph node metastasis. During the clinical trials of an angiogenesis inhibitor (AI-6), co-authors found that it can not only suppress the primary tumor growth significantly but also have marked antimetastatic effect. More interestingly, some lymph node metastases, such as supraclavicular lymph node metastases of esophageal and pulmonary cancers, diminished obviously and even disappeared (unpublished data). Therefore we take breast cancer as an example, to investigate angiogenesis of the axillary lymph node metastasis. Moreover, we attempt to determine whether the micro-metastasis possesses its blood supply. In this study, quantification of angiogenesis in breast carcinoma and axillary lymph node metastasis was undertaken using antibody against von Willebrand factor (ⅧF) which is present in vascular endothelial cells.

MATERIALS AND METHODS

Clinical Data

Thirty-seven invasive breast cancer tissues and 121 axillary metastatic lymph nodes were collected from the patients who were undergone either radical or modified

radical mastectomy in Jinan Central Hospital, Shandong Province, China. 326 axillary lymph nodes just removed from breast cancer patients were first stained with routine hemotoxylin and eosin. Among them, 121 lymph nodes were metastatic (including micrometastasis), the other 205 lymph nodes were nonmetastatic. The metastatic lymph nodes and breast cancer tissues were embedded in paraffin and cut into 5 μ sections, and then were immunocytochemically stained using monoclonal antibody for Factor eight related antigen (ⅧF-RAg).

Immunostaining Method

The breast cancer tissues and metastatic lymph nodes were immunostained using ABC assay with some modifications.[1] Rabbit anti-factor Ⅷ related antigen, ABC kit, and DAB were purchased from Zhongshan Biotech. Inc., Beijing.

Microvessel Count Criteria

According to Weidner's method[3], hot spots, densest microvascular area under 100 magnification were selected. We counted the microvessels on the monitor through a microvideo system (Panasonic Co., Japan). Three investigators were counted simultaneously in order to reduce the error to the minimum. EC was stained brown. Whether there was a tube or not, an isolated EC or a clump of ECs was considered to be a microvessel. If there was a tube, only the vessel less than 8 red blood cells was counted as a microvessel.

Diameter Caliber of Axillary Lymph Node Micrometastases

The diameter of each micrometastasis was measured by means of an Olympus objective micrometer and then averaged.

Statistical Method

Microvessel density was expressed as $\bar{x}\pm s$, t test.

RESULTS

The mean diameter of 13 micrometastases in the 121 lymph node metastases was 210±37 μm, the maximum diameter of micrometastasis was less than 300 μm. Angiogenesis of such micrometastases was not found (Figure 1). Whereas the diameter of metastases was largely greater than 300 μm, and a number of microvessels whose ECs were full of brown granules could be seen (Figure 2). In preparing tissue sections, only can part of the metastatic tumor be cut because of large volume. So it was difficult to see the complete structure of a whole lymph node.

The microvessels in the lymph node metastases and breast cancer tissues distributed in a similar manner. The MVD in the periphery of either the primary or the metastatic tumor was higher than that in the center. Comparison of MVD in the lymph node metastasis and in breast cancer, their MVDs had no significant difference (Table 1).

Table 1. Comparison of MVD in the lymph node metastasis and in breast cancer

Position	Microvessel density (MVD) ($\bar{x}\pm s$)	P
Axillary lymph node	89.34±18.42	
Breast cancer	93.82±21.76	>0.05

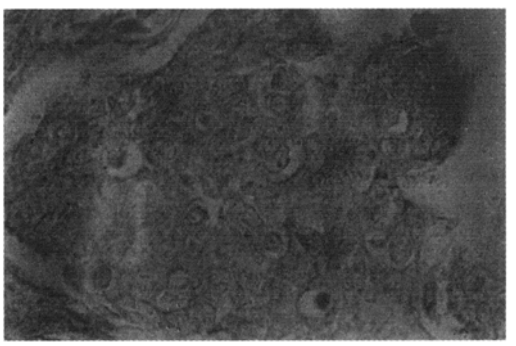

Fig. 1. No angiogenesis was observed in the lymph node micrometastasis of beast carcinoma after immunostaining. 400×

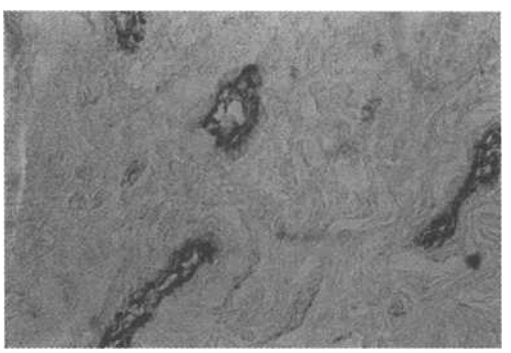

Fig. 2. Microvessels could be seen clearly in the lymph node metastasis after immnunostaining using antibody against ⅧF, endothelial cells were stained to be brown. 400×

DISCUSSION

Breast cancer cells which has entered the axillary

lymph nodes may have at least four fates: (1) "Guest", namely, they don't stay at the local lymph nodes, instead of travelling along the lymphatic stream; (2) Cancer cells may be destroyed by host immunosystem or die automatically during travel; (3) Cancer cells may live as a dormant state, and lack of angiogenesis, such dormant tumor nodule is the so-called micrometastasis; (4) Another possibility of these cancer cells is that they may grow to become large tumors – "metastasis" after they have established their own blood supply. In a sense, angiogenesis of the lymph node metastasis increases the opportunity of re-metastases. Although the lymph node metastasis does not threaten patient's life directly, it may spread cancer cells into the blood stream and then form distant metastases such as lung, bone, brain, liver and so on to cause death. Therefore, antilymphatic metastasis is thought to be one challenge as well in the antimetastatic research area.

The process of "micrometastasis—angiogenesis—macrometastasis" indicates that lymph node metastatic tumor growth must also be dependent upon angiogenesis which supplies oxygen and nutrients. This phenomenon is consistent with that of primary tumor. So it is suggested that antiangiogenesis therapy can not only control primary tumor growth and antimetastasis,[4-6] but also suppress the metastatic tumor growth in lymph nodes. It is beneficial to explain why angiogenesis inhibitor AI-6 can make some lymph node metastases shrunk or disappeared.

The microvessels of lymph node metastasis and primary tumor distributed in a similar manner. MVD in the periphery of either metastatic or primary tumor was significantly higher than that in the center. Folkman had such an explanation that cancer cells might be classified into two groups: one is angiogenic cancer cell, the other is nonangiogenic cancer cell. But these two kinds of cancer cells proliferate in dramatically different speed.[7]

Although ⅧF is thought to be a specific marker of vascular endothelium, it is hard to determine with certainty whether an individual endothelium belongs to a lymphatic or blood microvessel. Some authors proposed that lymphatics originate as buds from the venous system (centripetal theory) whereas others favored an independent origin from tissue mesenchyme (centrifugal theory), an issue still unresolved. Anyway, both lymphatic and blood microvessels possess Weibel-Palade body which lies in the endothelial cytoplasm. ⅧF is synthesized and secreted in such bodies.[8] Hence, there is a possibility that not all endothelia counted in this study belong to blood microvessels, they may include some lymphatic endothelia. But it did not affect the result because there is good evidence to suggest that there are not (at least not functional) lymphatics within tumors. As a matter of fact, it is difficult to make the morphological distinction between lymphatic and vascular endothelium within the tumor substance with current technology.

Micrometastatic tumor does not have angiogenesis and exists in a dormant state. Holmgren thought that the apoptosis rate of avascular tumor cells was balanced with the proliferation rate. Whereas in the vascularized tumor, acquired blood supply, the cancer cells proliferated at a higher speed than they apoptosized.[9] So it indicates that antiangiogenesis therapy might suppress metastatic tumor growth through inhibiting its angiogenesis.[10] And the micrometastasis because of lacking angiogenesis might keep in dormant stage for an unlimited period. Furthermore, we proposed that antiangiogenesis may be a promising approach to treat lymph node metastasis.

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