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 neo-angiogenesis. 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

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 (WF) 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 hematoxylin 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 (VIII-F-RAg).

**Immunostaining Method**

The breast cancer tissues and metastatic lymph nodes were immunostained using ABC assay with some modifications. 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, 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>
<thead>
<tr>
<th>Position</th>
<th>Microvessel density (MVD) $\bar{x} \pm s$</th>
<th>$P$</th>
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<tbody>
<tr>
<td>Axillary lymph node</td>
<td>89.3±18.42</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>93.8±21.76</td>
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DISCUSSION

Breast cancer cells which has entered the axillary