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IMMUNOHISTOCHEMICAL EVALUATION OF LYMPH NODE MICROMETASTASES FROM BREAST CANCER

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The lymph node micrometastasis was retrospectively immunohistochemically investigated in 185 patients with node-negative breast cancer and compared with the routine conventional findings. The monoclonal antibodies to epithelial membrane antigen (EMA) and mucinous-like carcinoma antigen (MCA) were used. Of 19 EMA-positive cases, 16 were false positive. Therefore only 3 cases were found to show staining with both EMA and MCA. The patients are all living and well and free of recurrence. No micrometastasis was immunhistochemically demonstrated in 11 node-negative breast cancer patients with subsequent metastases. The results of the present study indicate that the detection rate containing occult cells afforded by conventional routine evaluation is sufficient, at least in Japanese.

The presence of lymph node metastasis is considered to be the most important prognostic factor in patients with breast cancer (1–3). However, even though there is no lymph node metastasis, 20–30% of the node-negative patients show recurrence within 10 years (4–6).

When compared with node-negative breast cancer, the presence of even one single micrometastasis in a lymph node is associated with a significant difference in terms of recurrence and survival (7). Therefore, the detection of micrometastases may be important in the prediction of recurrence of breast cancer. Currently, the capacity to detect micrometastases is not adequate and it is sometimes not possible to identify any malignant cells by hematoxylin and eosin staining methods.

As means of improving the detection rate, serial sectioning of the lymph node has been used to decrease sampling error and immunohistochemical procedures have been adopted to detect small foci of metastases which might otherwise escape detection (7–11). The present study was undertaken to determine whether the use of immunohistochemical staining would increase the detection rate of occult breast cancer metastases in axillary nodes, to evaluate and compare the efficacy of monoclonal antibodies, and to assess whether the occult micrometastases are related to the survival rate.

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Material and Methods

One hundred and eighty-five consecutive patients were surgically treated for primary cancer of the breast with histological diagnosis of negative axillary nodes between 1985 and 1992 at the Fukushima Medical College Hospital. The frequency of node-negative breast cancer during the same period was 60.3%. The mean age of the patients at operation was 51.3 years (range 21–80). The diagnosis was confirmed by reviewing the pathological reports and records.

Eighteen women had intraductal carcinoma, one lobular carcinoma in situ and 166 invasive ductal carcinoma. There were 7 T0, 80 T1, 88 T2 and 10 T3 tumors. A total of 3 227 lymph nodes were examined, and the mean number of lymph nodes examined in each patient was 17.4 (range 5–45). The assessment of presence or absence of tumor cells in the lymph nodes was based on the official pathology report.

All paraffin blocks containing lymph node tissues from the node-negative patients were recut into three serial (consecutive) 4 μm sections for immunohistochemical study. These tissue sections were stained, using the avidin-

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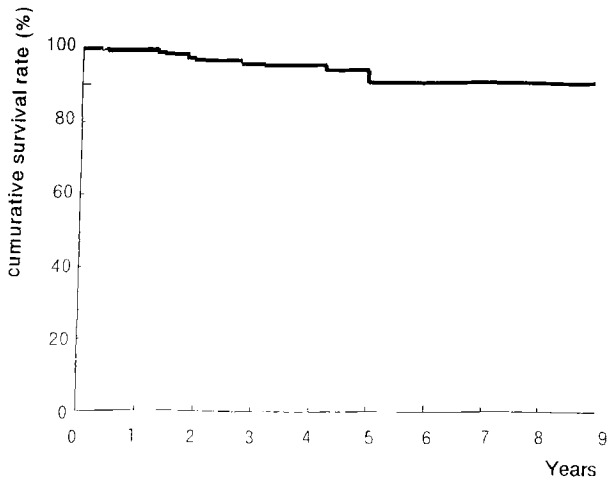

Fig. 1. Disease-free survival of node-negative breast cancer.

biotin-peroxidase complex technique (12), two monoclonal antibodies; one directed against epithelial membrane antigen (EMA) (Dako Japan, Tokyo, Japan) and the other against mucinous-like carcinoma associated antigen (MCA) which was kindly provided by Roche Diagnostics (Bern, Switzerland). EMA is a breast cell-surface component, and immunohistochemical studies have indicated that MCA is expressed on mucinous epithelial surfaces and in mucinous secretions of normal and breast cancer epithelia (13). The primary monoclonal antibodies were added at a dilution of 1:100 for EMA and 1:200 for MCA in 0.05 mol/l phosphate-buffered saline for 1 h at room temperature, and the sections were incubated for 40 min with biotin-labelled goat antibody IgG (Vector Lab., Burlingame, CA, USA). The negative controls consisted of sections incubated with normal mouse serum instead of the primary specific antibody. The nodes used for immunostaining had in the original diagnostic slides been stained with hematoxylin and eosin. Follow-up status was evaluated at the end of July, 1994. The survival curve was culculated by the Kaplan-Meier method.

Results

In sections stained with monoclonal antibody to EMA, stained cells were found in the regional nodes in 19 of the 185 patients. However, the review of the original hematoxylin and eosin-stained sections revealed that the stain products seen mainly in the cytoplasm were identified as plasma cells in 16 of the 19 patients with positive staining. Thus only three patients were found to have microscopic foci of metastatic carcinoma in the axillary lymph nodes. In all patients the tumor foci were small and tumor cells were usually present in the subcapsular portion in the medullary sinuses of the nodes.

MCA-positive cells were demonstrated to be among the epithelial cells, although the staining intensity was rather weak. Positive MCA staining was found in the three patients in whom staining with EMA was also seen. Occult metastases consisted of invasive ductal carcinoma in three patients, in all of whom involvement of one lymph node was seen.

During the follow-up period the disease recurred in 13 patients, of whom 2 died. The immunohistochemical examination of the lymph nodes of these 13 patients with node-negative breast cancer who showed subsequent metastases failed to demonstrate any micrometastasis. The figure shows the disease-free survival, calculated by the Kaplan-Meier method for patients without nodal metastases by the conventional method. The three patients with occult micrometastases are at present alive and well and free of recurrence 6.2, 5.2 and 2.9 years after diagnosis.

Discussion

The importance of axillary node involvement in the prognosis of breast cancer patients has been repeatedly emphasized. However, it is also recognized that routine evaluation of axillary lymph nodes fails to detect a sizable number of metastases (8, 14) due to both inadequate sampling and pathological limitation in detecting small foci of carcinoma using conventional hematoxylin and eosin staining.

Several studies have shown that the examination of serial sections of lymph nodes could increase the accuracy of metastases detection (4, 7, 15), and micrometastases have been detected by incidence in 9 to 24% (4, 16, 17). The occult metastases in the lymph nodes may thereby have an important role in prognosis. These micrometastases are visible by conventional staining, but routinely cutting multiple serial sections is not practical and requires extra labor and expense. Therefore immunohistochemical analysis has been adopted to improve the detectability of micrometastases in lymph nodes.

More recently, several studies have shown that immunohistochemical analysis can be used to detect tumor cells in tissues that are regarded as free of tumor based on examination of conventionally stained sections (7–11). The results obtained using immunohistochemical staining to detect minute tumor foci have also been impressive. Among 2 400 patients with breast cancer, approximately 13% conversion from node-negative to node-positive status was observed by using immunohistochemistry (17). Thus a substantial proportion of women with node-negative breast cancer is considered to be understaged by the conventional method.

A specific stain for EMA is always associated with cells of epithelial origin and the EMA-positive cells seen in lymph nodes are considered to be derived from the breast cancer. Although Sloane et al. (18) found no occult metastases using antisera to EMA in a group of 23 patients, the detection of occult metastases using antisera to EMA has

been reported in 10 to 15% of lymph nodes which were found to be negative on initial conventional examination (8, 11). The cells immunostained with anti-EMA staining in the present study showed false positive, and were regarded as plasma cells (19). Bussolati et al. (9) observed that the EMA-reactive material was also present in macrophages, thereby suggesting no sufficient evidence of metastasis by single immunohistochemical approach.

The MCA antigen is a member of the mucin-like glycoproteins expressed by breast cancer which include the CA antigen, CA15-3 and the human milk fat globule, and has been regarded as a sensitive marker for breast cancer (20, 21). These characteristics of the anti-MCA antibody immunoreactivities make it an available tool for the detection of breast cancer micrometastases, even though it is not absolutely specific for breast cancer cells (13). The MCA-immunostained lesions in the lymph nodes of the present cases were determined by very small foci of metastatic cells which were hardly detected in the routine histopathological examination.

The present study, however, did not focus on the diagnostic value of immunohistochemistry in detecting lymph node micrometastases. The choice of antibodies for such immunohistochemical evaluation is wide. Various monoclonal antibodies reacting with human mammary cancer have been described (8–11, 20). Using the cocktail of five monolonal antibodies, Trojani et al. (10) found 14% of micrometastases in 142 node-negative breast cancer, and stressed the utility to ensure an optimal intensity of staining. A difference in the incidence of occult micrometastases between this study and others may therefore depend on the number of reagents investigated, and a further investigation is required. Recently, polymerase chain reaction has come into use to detect micrometastases (22, 23), but this method is not suitable for routine assessment.

One reason for the lower incidence of micrometastases in this study compared to that in other reports, may also be some differences in biological behavior between the breast cancer in Western countries and that in Japan. While approximately 30% of the node-negative patients in Western countries recurred within 10 years (6), the recurrence rate among Japanese patients is reported to be around 10% (24). The incidence of lobular carcinoma in Japan is also very low and significantly different from that in the Western countries (24). The 10-year survival rate in the patients with no lymph node metastasis was 82.6% in the Cancer Institute Hospital, Tokyo and 64.0% in the Vanderbilt University Hospital, USA (25). However, there are few reports concerning the micrometastases in Japanese patients with breast cancer, and the incidence and clinical importance of micrometastases pertinent to those in Western countries is obscure.

A significant difference for both survival and tumor recurrence rate was noted between patients with occult micrometastases and those without metastases (7, 9, 10, 14). On the other hand, there are some reports that patients without micrometastases show no significant difference in survival from those with micrometastases, as assessed either by routine procedures (26) or by serial section (4, 15). The survival data acquired in the present study, though based on a comparatively short follow-up period, show that the detection of occult tumor cells in the regional nodes is not associated with any increased risk of developing visceral metastases.

We concluded, based on the results of the present study, that at least in Japanese patients with breast cancer, the nodes containing occult cells afforded by routine evaluation is sufficient.

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