HBME-1 Expression in Follicular Tumor of the Thyroid: an Investigation of Whether it Can be Used as a Marker to Diagnose Follicular Carcinoma

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Abstract. Background: HBME-1 has been recognized as a useful marker for diagnosing thyroid carcinoma. In this study, we investigated whether it has a diagnostic value for discriminating follicular carcinoma from adenoma. Materials and Methods: We investigated HBME-1 expression in 138 follicular carcinomas, 155 follicular adenomas, 98 adenomatous nodules and 37 papillary carcinomas, using anti-HBME-1 monoclonal antibody. Results: HBME-1 was positive in 60.9% of follicular carcinoma and the incidence was significantly higher (p<0.0001) than that of follicular adenoma, 30.3%. In adenomatous nodules, only 17.3% were classified as positive, which was lower even than that of follicular adenoma (p=0.0257). All papillary carcinomas examined were positive for HBME-1. We calculated the positive predictive value of HBME-1 in discriminating follicular carcinoma from adenoma as 64.2%. Conclusion: These results suggest that, although HBME-1 contributes to the diagnosis of papillary carcinoma, it could not be applied in the preoperative diagnosis of follicular carcinoma, for example, using fine-needle aspiration biopsy samples.

Thyroid carcinoma is one of the most common malignancies originating from the endocrine organs. There are two prominent histological types of thyroid neoplasms originating from follicular cells: papillary carcinoma and follicular carcinoma, the latter being comparably rare (1). Follicular carcinoma is associated with iodine deficiency, suggesting that this type of tumor can develop from glands under chronic proliferative circumstances(2). Follicular carcinoma may arise from preexisting follicular adenoma, although this hypothesis has not yet been confirmed. It is extremely difficult to discriminate between follicular carcinoma and follicular adenoma before surgery, because the morphology of carcinoma cells and adenoma cells obtained by fine-needle aspiration biopsy (FNAB) are similar. The final diagnosis should be determined by postoperative pathological examination by the evaluation of the specific characteristics of follicular carcinoma such as vascular invasion, capsular invasion and lymph node metastasis.

If we could preoperatively diagnose follicular carcinoma, it would facilitate determination of surgical indication for follicular tumor. To date, studies have been intensively performed to find markers able to discriminate follicular carcinoma from adenoma which can be applied to preoperatively obtained specimens, such as immunostaining of FNAB samples. Recently, galectin-3 has raised interest as a potential tool for such discrimination (3), but more recent results were contradictory (4, 5).

HBME-1 is an antigen on the surface of mesothelial cells with unclear function (6, 7). In thyroid neoplasms, HBME-1 was positive in tissue sections as well as FNAB specimens of papillary and follicular carcinomas (8-12). However, no studies have been performed to determine whether HBME-1 is a useful diagnostic tool for distinguishing follicular carcinoma. In this study, therefore, we investigated HBME-1 expression in 138 cases of follicular carcinoma and 155 cases of follicular adenoma in order to elucidate this point.

Materials and Methods

Tissue specimens. Tissue specimens were obtained from patients undergoing surgery in the Department of Surgery, Kuma Hospital, Japan. The specimens comprised 138 follicular carcinomas, 155 follicular adenomas, 98 adenomatous nodules and 37 papillary carcinomas. The follicular carcinoma specimens were selected from patients undergoing surgery between 2000 and 2003, while the remainder were obtained from patients treated...
between 2002 and 2003. Ten follicular carcinomas were diagnosed as widely invasive carcinoma and the remaining 128 were diagnosed as minimally invasive carcinoma. Of the 37 papillary carcinomas, 18 were 1.0 cm or less in maximal diameter and classified as papillary microcarcinoma by WHO classification. This project was approved by the ethical committee of the hospital and informed consent was obtained from the participating patients. For immunohistochemical study, tissues were fixed with 10% formalin and paraffin-embedded.

**Antibody.** The mouse monoclonal antibody, HBME-1, was purchased from Dako (Copenhagen, Denmark). It was applied at a dilution of 1:50.

**Immunohistochemistry.** Tissue sections 4 μm thick were dewaxed and endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 15 min. After rinsing in phosphate-buffered saline pH 7.2 (PBS), 10% bovine serum (Wako, Osaka, Japan) was applied for 20 min to block nonspecific reactions. Sections were then incubated with a primary antibody at 4°C overnight. After rinsing in PBS, sections were treated with peroxidase-labelled anti-mouse and anti-rabbit immunoglobulins (Nichirei, Tokyo, Japan) for 30 min. The peroxidase reaction was visualized by incubating the sections with 0.02% 3,3'-diaminobenzidine tetrahydrochloride in 0.05 M Tris buffer with 0.01% hydrogen peroxide (Nichirei, Tokyo, Japan). The sections were counterstained with hematoxylin. Sections for negative control were prepared using mouse immunoglobulins instead of the primary antibody.

**Immunohistochemical evaluation.** We regarded cells as immunoreactive for HBME-1 when the signal was clearly observed in the cytoplasms. We classified all cases as negative or positive for HBME-1 on the basis of whether any HBME-1 immunoreactive tumor cells could be found. Furthermore, quantification of HBME-1-positive cases was performed by classifying them into three groups; +/−, less than 10% of cells were immunoreactive; +, 11 to 50% of cells were immunoreactive; ++, more than 50% of cells were immunoreactive.

**Statistical analyses.** Fisher's exact probability test was adopted to examine the relationship between the variables. A p value less than 0.05 was considered significant.

**Results**

HBME-1 immunoreactivity was not observed in normal follicular epithelium, but was observed in scattered histiocytes (not shown). In tumor cells, HBME-1 signal was detected predominantly in the cytoplasm. We investigated HBME-1 expression in various thyroid neoplasms originating from follicular cells. The results are summarized in Table I. Eighty-four out of 138 follicular carcinomas (60.9%), including 5 of the 10 widely invasive type (50.0%), were classified as positive for HBME-1 (Figure 1a), showing a significantly higher incidence (p<0.0001) than that in follicular adenomas, which showed antigen positivity in only 30.3% of the cases. Forty-eight cases of follicular carcinoma (34.8%) diffusely expressed HBME-1, since more than 50% of tumor cells were HBME-1 immunoreactive, whereas this finding was observed only in 16 cases of adenoma (10.3%) (Figure 1b). Of the 98 adenomatous nodules, positive HBME-1 staining was observed in only 17 cases (17.3%) and the incidence was significantly lower (p=0.0257) than that in follicular adenoma. We also investigated HBME-1 expression in 37 papillary carcinomas and HBME-1 immunoreactivity was found in all these cases, including 18 papillary microcarcinomas (Figure 1c).

Statistical analyses of HBME-1 immunostaining for discriminating follicular carcinoma and follicular adenoma are shown in Table II. All variables, including positive predictive value (PPV), were between 60 and 70%.

**Discussion**

To date, the most notable finding concerning HBME-1 expression in thyroid neoplasms is that papillary carcinoma is very frequently reactive for this antigen. Miettinen et al. demonstrated that all of the 145 cases of this carcinoma they examined were positive for this antigen (12). Recently, Hirokawa et al. showed that HBME-1 immunostaining was useful in distinguishing between intranodal benign thyroid tissue and metastatic papillary carcinoma in lymph nodes (10). Furthermore, Casey et al. investigated HBME-1 expression in 30 cases of papillary carcinoma and papillary thyroid hyperplasia and showed that all papillary carcinomas, but only one hyperplasia, were positive for HBME-1, suggesting that HBME-1 may be helpful in distinguishing papillary carcinoma from hyperplasia in diagnostically difficult cases (11). In this study, we investigated HBME-1 immunoreactivity in 37 cases of papillary carcinoma and found that all cases, including 18 papillary microcarcinomas, were positive for this antigen, showing findings very similar to those of previous studies.

**Table I. Expression of HBME-1 in thyroid tumors.**

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<tr>
<th>HBME-1 expression</th>
<th>Number of case (%)</th>
<th>Positive staining quantification</th>
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<tbody>
<tr>
<td><em>Follicular carcinoma</em></td>
<td>54 (39.1)</td>
<td>84 (60.9)</td>
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<tr>
<td><strong>Follicular adenoma</strong></td>
<td>108 (69.7)</td>
<td>47 (30.3)</td>
</tr>
<tr>
<td>Adenomatous nodule</td>
<td>81 (82.7)</td>
<td>17 (17.3)</td>
</tr>
<tr>
<td>Papillary carcinoma</td>
<td>Microcarcinoma</td>
<td>0</td>
</tr>
<tr>
<td>Larger size</td>
<td>0</td>
<td>19 (100)</td>
</tr>
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*p<0.0001 (Negative vs. Positive)

*p=0.0257 (Negative vs. Positive)
Figure 1a. Diffuse HBME-1 expression in thyroid follicular carcinoma. b. Absence of HBME-1 signal in follicular adenoma. c. Diffuse HBME-1 expression in microcarcinoma. Original magnification: 750.
However, HBME-1 expression and its diagnostic value in follicular tumor, especially in follicular carcinoma, have not been studied in depth. To date, the study by Miettinen et al. is the most extensive in this respect. They showed that all 27 follicular carcinomas were positive for HBME-1, although this phenomenon could be observed in only 28% of follicular adenoma (12). In this study, we obtained a similar incidence of HBME-1 expression in follicular adenoma, that is, 30.3% of follicular adenoma in our series were positive for HBME-1. However, 39.1% of follicular carcinoma in our series were negative for HBME-1, which may be because the number of follicular carcinoma we examined was larger than that in the previous study. In our series, there was a significant difference ($p<0.0001$) in the incidence of HBME-1 positivity between follicular carcinoma and adenoma. When we calculated PPV for HBME-1 discriminating follicular carcinoma from adenoma, using surgical specimens including a similar number of follicular carcinoma and adenoma, the PPV was 64.2%. However, this value is likely to further decrease when HBME-1 staining is applied to preoperative diagnosis of FNAB specimens, because the incidence of follicular adenoma as well as adenomatous nodule is much larger than that of follicular carcinoma at that stage of thyroid nodule screening.

In summary, we demonstrated that HBME-1 was a useful tool for diagnosing papillary carcinoma, but its use should not be suggested as a diagnostic marker for follicular carcinoma in preoperative screening using FNAB specimens. By combining other markers with HBME-1, PPV may increase and this possibility should be investigated in the future.

Table II. Discrimination between follicular adenoma and follicular carcinoma by immunodetection of HBME-1.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Positive HBME-1 expression</th>
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<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>60.9</td>
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<tr>
<td>Specificity (%)</td>
<td>69.7</td>
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<tr>
<td>Positive predictive value (%)</td>
<td>64.2</td>
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<tr>
<td>Negative predictive value (%)</td>
<td>66.7</td>
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<tr>
<td>Diagnostic accuracy (%)</td>
<td>65.5</td>
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References


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