

HBME-1 Immunostaining in Thyroid Tumors Especially in Follicular Neoplasm

TAKAHIRO MASE, HIROOMI FUNAHASHI*, TAKASHI KOSHIKAWA**, TSUNEO IMAI*,
YOSHIHARU NARA***, YUJI TANAKA# AND AKIMASA NAKAO*

Department of Surgery, Atsumi Hospital, Tahara, Aichi 441-3415, Japan

**Department of Surgery II, Nagoya University School of Medicine, Nagoya 466-8550, Japan*

***Aichi Prefecture College of Nursing and Health, Nagoya 463-8502, Japan*

****Department of Pathology, Yokkaichi Municipal Hospital, Yokkaichi 510-8567, Japan*

#Department of Surgery, Tajimi Daiichi Hospital, Tajimi 507-0039, Japan

Abstract. It is generally known that even with permanent sections, the differential diagnosis between follicular adenoma and follicular carcinoma is often difficult to determine. It is not unusual to encounter patients diagnosed with benign follicular adenoma whose diagnoses have to be changed to malignancies because of recurrence or metastasis. As the monoclonal antibody HBME-1 produced by mesothelioma cells has been shown to have reactivity in thyroid carcinomas, we investigated the diagnostic usefulness of HBME-1 in follicular neoplasms. Immunohistochemical staining for HBME-1 was performed on 205 various thyroid tumors using the labeled streptavidin biotin peroxidase method. When hematoxylin-eosin (HE) staining was performed again for this study and all cases were examined in accordance with the WHO Histological Classifications 2nd Edition, 87.2% (54/62) of adenomatous goiter and 72.6% (45/62) of follicular adenoma were negative. On the other hand, 84.6% (33/39) of follicular carcinoma and 97.2% (35/36) of papillary carcinoma were positive. All anaplastic (2/2) and medullary (4/4) carcinoma were negative. Examination in follicular neoplasms had a sensitivity of 84.6%, specificity of 72.6%, positive predictive value of 66.0% and overall accuracy of 77.2%. Among the cases treated as follicular adenoma clinically, the diagnosis of 13 cases was changed to follicular carcinoma, and 6 cases to papillary carcinoma for this study. These cases showed strong HBME-1 positivity. Two of the follicular carcinoma cases experienced recurrence. We conclude that immunohistochemical staining with HBME-1 may be useful clinically to pick out cases with a high risk of recurrence in follicular carcinoma, and that benign adenoma cases need close follow-up.

Key words: Thyroid follicular neoplasm, HBME-1, Immunohistochemical staining

(*Endocrine Journal* 50: 173–177, 2003)

IT is generally known that in follicular neoplasm, pre-operative examination including fine-needle aspiration cytology (FNAC) and intraoperative pathologic examination with frozen sections (FS) show low accuracy. Even with postoperative examination with permanent sections stained with hematoxylin-eosin (HE), the differential diagnosis between follicular adenoma and follicular carcinoma is often difficult to determine. It

is not unusual to encounter patients diagnosed with benign follicular adenoma based on pathologic examinations of extirpated tissue samples who later experience recurrence or metastasis and whose clinical diagnoses have to be changed to malignancies. Thus, how a thyroid follicular neoplasm is treated becomes a clinical or pathological issue, and the development of new methods that can distinguish benign tumors from malignant ones more simply and accurately is greatly desired.

The antihuman mesothelial cell mouse monoclonal antibody HBME-1 (Hector Battifora Mesothelial (cell)) was shown by Battifora *et al.* to be produced by mesothelioma cells [1, 2]. Although the target antigen

Received: October 7, 2002

Accepted: December 24, 2002

Correspondence to: Dr. Takahiro MASE, Department of Surgery, Atsumi Hospital, 1-1 Kanbe Akaishi, Tahara, Atsumi-gun, Aichi 441-3415, Japan

is an as yet unelucidated membrane antigen that exists in the microvilli of mesothelioma cells, Miettinen *et al.* performed HBME-1 immunohistochemical staining on thyroid tumors and reported HBME-1 positivity in thyroid carcinomas [2–4]. Therefore, we conducted the present study of immunohistochemical staining using HBME-1 antibody on various thyroid tumors and investigated their usefulness in differential diagnoses. Particularly in follicular neoplasms, the staining results were compared with long-term clinical progress.

Materials and Methods

Two hundred five specimens excised between January 1973 and April 1998 at the Department of Surgery II, Nagoya University School of Medicine, and affiliated institutions were studied. Because considerable time had passed since some of these cases had undergone the operation, HE staining was performed again using paraffin-embedded sections for this study, and all cases were examined by three pathologists independently in accordance with the WHO Histological Classifications, 2nd Edition, issued in 1988 [5] with a consensus diagnosis being reached after discussion among the pathologists when a disagreement occurred. This diagnosis was performed without telling the pathologists about the result of immunohistochemical staining. Consequently the histologic diagnoses in these patients were 62 cases of follicular adenoma (FA), 39 cases of follicular carcinoma (FC), 36 cases of papillary carcinoma (PC), 62 cases of adenomatous goiter (AG), 4 cases of medullary carcinoma (MC) and 2 cases of anaplastic carcinoma (AC).

The immunohistochemical staining was performed using the labeled streptavidin biotin peroxidase method from a commercially available kit (Histofine SAB-PO (M) kit, Nichirei, Tokyo, Japan) with the anti-human mesothelial cell mouse monoclonal antibody HBME-1 (Dako, Carpinteria, CA.) at a dilution of 1:50. A tissue that was clearly positive in a preliminary experiment was prepared as a positive control. Antigen activation, such as by microwave irradiation, was not performed.

Immunoreactivity was measured semiquantitatively using a scale from (–) to (4+) by a pathologist collaborating in the study; (–) indicates absolutely no immunostaining, (1+) represents 0–25% of cells reactive, (2+) represents 25–50% of cells reactive, (3+) repre-

sents 50–75% of cells reactive, and (4+) represents 75–100% of cells reactive. Finally, scales of (–) and (1+) were judged as negative staining, and (2+), (3+) and (4+) were judged as positive.

FC patients, as diagnosed based on the method mentioned above, were followed over 12 to 300 months (mean 127.1 months). Twenty-four of the FA patients diagnosed based on the same method were followed up for 5–174 months (mean 47.6 months).

Results

Immunoreactive cells were distributed mainly in the cell membranes and cytoplasm (Fig. 1). Thirty-three (84.6%) of the 39 FC patients were positive; the high positive rate is similar to the staining result of the PC patients (97.2%). However, seventeen (27.4%) of the 62 FA patients were positive, and eight (12.9%) of the 62 AG patients stained positive (Table 1). All of the MC and AC cases were negative (data not shown).

Among the 17 positively-stained FA patients, 6 cases showed a strong positivity (4+), and 11 cases showed mild positivity (3+ or 2+). In the 33 positively-stained FC cases, 27 (81.8%) of them showed strong positivity (4+) (Table 1).

Among the cases diagnosed as FA at the time of operation, two cases experienced recurrence during follow-up observation. Both of them were the cases classified into FC in this study and showed strong HBME-1 positivity (4+). In the three recurrent cases of FC, two of them showed strong positivity (4+). But no relationship was found between the positive staining part and the part with vascular invasion or capsular invasion found as a results of staining.

Discussion

Recent reports in the literature have indicated the sensitivities of preoperative fine needle aspiration cytology (FNAC) to be between 33.3% and 58.3% [6–10]. The reason for this is considered to be that benign follicular adenoma contains hyalinizing trabecular adenoma and atypical adenoma, among others, whose differential diagnoses are extremely difficult to establish by cytology alone. Furthermore, in many reports the sensitivities of frozen sections (FS) indicated 50% or less [7, 11, 12].

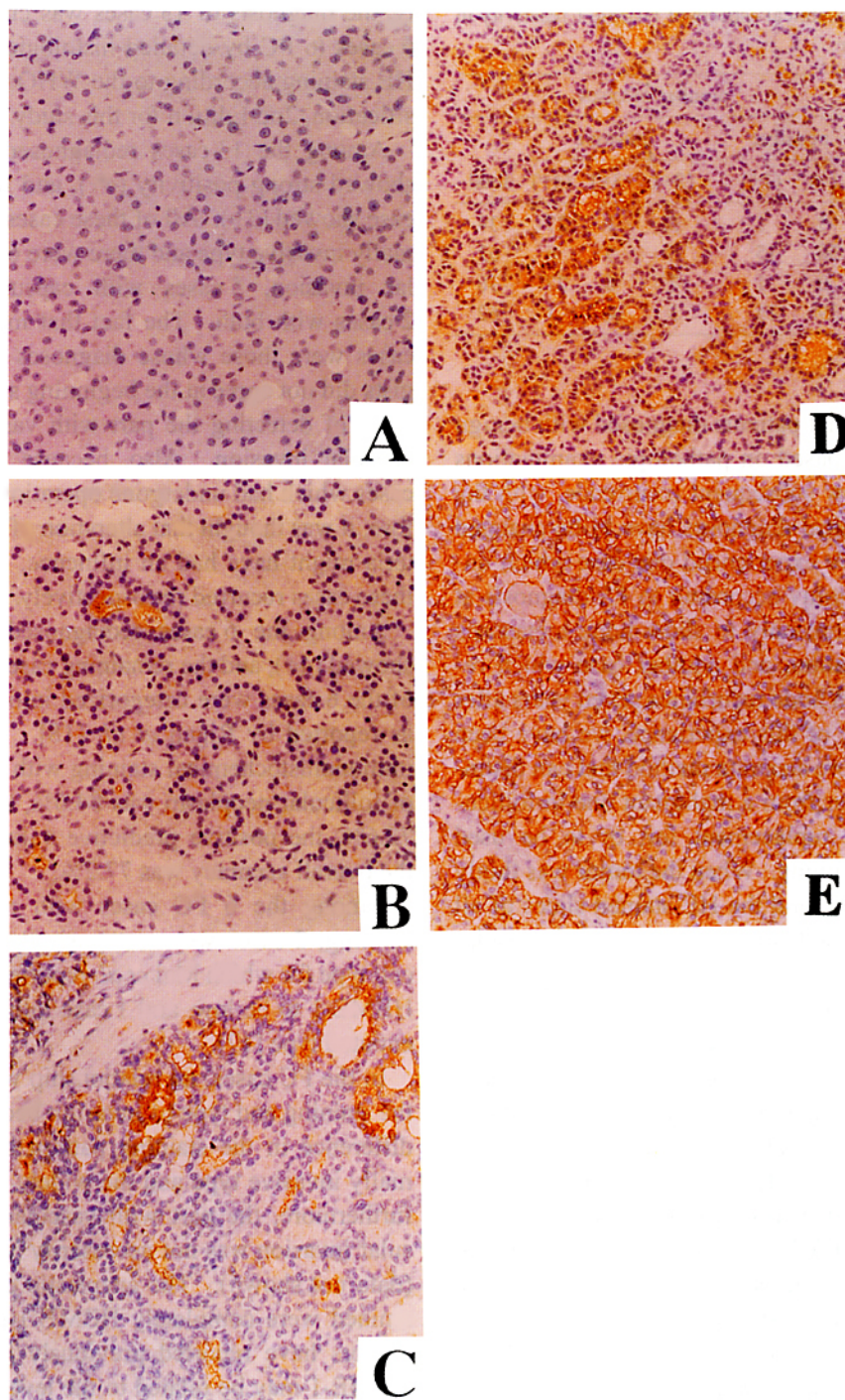


Fig. 1. Immunohistochemical distribution of HBME-1 positive cells in thyroid follicular neoplasms. The labeled streptavidin biotin peroxidase with haematoxylin counter stain (original magnification 20X). (A) Follicular thyroid adenoma showing no reactive cell: (-). (B) Follicular thyroid adenoma showing about 5% lesional cells reactive: (1+). (C) Follicular thyroid carcinoma showing about 30% cells reactive: (2+). (D) Follicular thyroid carcinoma showing about 60% cells reactive: (3+). (E) Follicular thyroid carcinoma showing almost all cells reactive: (4+). (A) and (B) were judged negative. (C), (D) and (E) were judged positive staining.

Table 1. Immunohistochemical aspects of HBME-1 reactivity in thyroid tumor

	number of cases					total
	negative		positive			
	(-)	(1+)	(2+)	(3+)	(4+)	
follicular carcinoma	5	1	5	1	27	39
follicular adenoma	30	15	6	5	6	62
papillary carcinoma	0	1	2	6	27	36
adenomatous goiter	39	15	3	4	1	62

It is well known that patients diagnosed with benign follicular adenoma based on pathologic examinations by HE staining show subsequent recurrence, with the result that their clinical diagnoses have to be changed to malignancies. We experienced one case which underwent only lobectomy according to FS diagnosis at the primary operation, only to show bone metastasis 18 years after operation, and another case which resulted in neck lymph node recurrence seven years after operation. Because of this experience, we have been seeking a newer, more accurate method that can distinguish a benign tumor from a malignant one in follicular neoplasm as a replacement for, or to be used together with HE staining. When the tumor was thinly cut to about 2–3 mm, and the HE-stained slices of all sections were searched for the existence of capsular invasion or vascular invasion in accordance with the WHO Histological Classifications, 2nd Edition, we were able to obtain an accurate diagnosis. However, practically speaking, it is almost impossible to examine all cases by this method, and another simpler differentiating technique is to be desired.

HBME-1 is an antibody originally produced by a mesothelioma cell suspension by Battifora [1, 2]. The target antigen of HBME-1 exists in the microvilli of mesothelioma cells. Although it is a useful marker to detect mesothelial neoplasms, it shows positive for the normal tracheal epithelium and adenocarcinoma of the lung, pancreas and mammary gland [1–4, 13–16].

In almost all reports of HBME-1 immunohistochemical staining of thyroid tissue, the staining showed positive for nearly all cases of papillary carcinoma and follicular carcinoma, but there was nothing described in the literature focusing on the relationship between the differential diagnosis of follicular neoplasms and the results of staining [2–4, 13, 17–19].

In our study, among the cases treated as FA clinical-

ly, the diagnosis of 13 cases was changed to FC, and 6 cases to PC by detailed histological examination of HE staining according to the WHO Histological Classification, 2nd Edition for this study.

Among the 13 cases rediagnosed as FC, 12 cases showed strong positivity (4+), and the one remaining case showed (3+). All of these cases were of the minimally invasive type and there were 6 cases with vascular invasion, 5 cases with capsular invasion and 2 cases with both. The fact that many of the 13 FC cases were those whose diagnoses by three pathologists did not agree at the beginning suggested the difficulty of detecting the part with vascular invasion or capsular invasion. Also, because they included the cases which had undergone operation considerably before, it was thought that alteration of the diagnostic criteria might have had an influence. Moreover two of these cases recurred retrospectively.

Among the 6 cases rediagnosed as PC, 5 cases showed (4+), and the remaining case showed (3+). All of the 6 cases were categorized into follicular variant, as reported by Rigau *et al.* [18]. The sensitivity of immunohistochemical staining with HBME-1 in cases of follicular neoplasm was 84.6%, the specificity was 72.6%, the positive predictive value was 66.0% and the overall accuracy was 77.2%.

Among the 6 FC cases with negative HBME-1 staining, 2 cases were categorized into the oxyphilic cell type for which Mai *et al.* reported the reactivity for HBME-1 to be low, and 1 case into the poorly differentiated type [17].

In the FC cases, no relationship was found between the staining and type of invasion because there was no difference in the staining pattern between the minimally invasive type and the widely invasive type, and furthermore, the part with vascular invasion or capsular invasion was not necessarily indicated positively (data not shown). The anaplastic carcinomas we examined were completely HBME-1-negative, as some authors have reported previously [3, 4, 19]. Thus, HBME-1 may be related to the level of differentiation of thyroid carcinomas.

Seventeen (27.4%) FA cases and 8 (12.9%) AG cases showed HBME-1 positivity and 6 (15.4%) FC cases were negative. The present criteria of FC is determined by the existence of invasion and the *in situ* lesion with nuclear atypia is not taken into consideration. These facts may be a reason why these benign tumor cases showed positive staining.

However these results point to the necessity for a strict long-term follow up for HBME-1 staining positive cases that have been diagnosed as benign by HE staining.

In conclusion, immunohistochemical staining with HBME-1 has a few exceptions in that a negative result

is obtained in malignancy and a positive in benign cases thus, it may not be suitable for differential diagnosis, but may be useful clinically to pick out cases with a high risk of recurrence in follicular carcinoma and cases that need a close follow-up in benign adenoma.

References

1. Sheibani K, Esteban JM, Bailey A, Battifora H, Weiss LM (1992) Immunopathologic and molecular studies as an aid to the diagnosis of malignant mesothelioma. *Hum Pathol* 23: 107–116.
2. Miettinen M, Kovatich AJ (1995) HBME-1 a monoclonal antibody useful in the differential diagnosis of mesothelioma, adenocarcinoma, and soft-tissue and bone tumors. *Appl Immunohistochem* 3: 115–122.
3. Miettinen M, Kärkkäinen P (1996) Differential reactivity of HBME-1 and CD15 antibodies in benign and malignant thyroid tumors. Preferential reactivity with malignant tumors. *Virchows Arch* 429: 213–219.
4. Sack MJ, Astengo-Osuna C, Lin BTY, Battifora H, LiVolsi VA (1997) HBME-1 Immunostaining in thyroid fine-needle aspirations: A useful marker in the diagnosis of carcinoma. *Mod Pathol* 10: 668–674.
5. Hedinger Chr, Williams ED, Sobin LH (1988) *Histological Typing of Thyroid Tumors*. 2nd ed. Geneva: WHO.
6. Lopez LH, Canto JA, Herrera MF, Gamboa-Dominguez A, Rivera R, Gonzalez O, Perez-Enriquez B, Angeles-Angeles A, Letayf V, Rull JA (1997) Efficacy of fine-needle aspiration biopsy of thyroid nodules: Experience of a Mexican institution. *World J Surgery* 21: 408–411.
7. Suzuki S, Kiman K, Furukawa H, Tsuchiya A, Abe R (1996) Clinicopathological study of follicular thyroid carcinoma. *Endocrine Surgery* 13: 217–223.
8. Kaname A, Taniguchi E, Kakudo K (1997) Reliability and pitfalls of thyroid cytology. *Endocrine Surgery* 14: 239–243.
9. Kaur A, Jayaram G (1991) Thyroid tumors: Cytomorphology of follicular neoplasm. *Diag Cytopathol* 7: 469–472.
10. Toriya K, Koshiishi H, Ito K, Ito K, Fujisawa T (1998) Aspiration cytology of thyroid follicular tumor. *Acta Cytologica* 42: 585 (Abstract).
11. Rosen Y, Rosenblatt P, Saltzman E (1990) Intraoperative pathologic diagnosis of thyroid neoplasms: Report on experience with 504 specimens. *Cancer* 66: 2001–2006.
12. Tielens ET, Sherman SI, Hruban RH, Ladenson PW (1994) Follicular variant of papillary thyroid carcinoma. A clinicopathologic study. *Cancer* 73: 424–431.
13. van Hoeven KH, Kovatich AJ, Miettinen M (1998) Immunocytochemical evaluation of HBME-1, CA 19-9, and CD-15 (Leu-M1) in fine-needle aspirates of thyroid nodules. *Diagn Cytopathol* 18: 93–97.
14. Riera JR, Astengo-Osuna C, Longmate JA, Battifora H (1997) The immunohistochemical diagnostic panel for epithelial mesothelioma. A reevaluation after heat-induced epitope retrieval. *Am J Pathol* 21: 1409–1419.
15. Wick MR (1997) Immunophenotyping of malignant mesothelioma. *Am J Surg Pathol* 21: 1395–1398.
16. Astengo-Osuna C, Lin BTY, Battifora H (1997) HBME-1 in thyroid neoplasms: An immunohistochemical study. *Mod Pathol* 10: 48A (Abstract).
17. Mai KT, Bokhary R, Yazdi HM, Thomas J, Commons AS (2002) Reduced HBME-1 immunoreactivity of papillary thyroid carcinoma and papillary thyroid carcinoma-related neoplastic lesions with Hürthle cell and/or apocrine-like changes. *Histopathology* 40: 133–142.
18. Rigau V, Martel B, Evrard C, Rousselot P, Galateau-Salle F (2001) HBME-1 immunostaining in thyroid pathology. *Annales de Pathologie* 21: 15–20.
19. Cheung CC, Ezzat S, Freeman JL, Rosen IB, Asa SL (2001) Immunohistochemical diagnosis of papillary thyroid carcinoma. *Modern Pathology* 14: 338–342.