Lights on HBME-1: the elusive biomarker in thyroid cancer pathology

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ABSTRACT

Among the different ancillary immunohistochemical tools that pathologists may employ in thyroid nodules, the so-called Hector Battifora's 'MEsothelioma' 1 (HBME-1) staining is one of the most fascinating, since its real identity is currently unknown. In the present review, the different clinical applications of HBME-1 are analysed, with main emphasis on its role in thyroid pathology with overview on less impactful fields, such as haematopathology or mesothelial lesions. Different acceptable or good diagnostic performances were recorded for HBME-1 in thyroid pathology, being used in routine practice as one of the best tools to screen thyroid malignancy both in terms of sensitivity and specificity. From a speculative point of view, after many attempts to hunt the cryptic target antigen of this antibody, its identity still remains elusive. In this setting, the application of high-throughput technologies (mainly in situ proteomics) may be the exact route to improve the knowledge about the pathophysiology of HBME-1 and to finally unveil its true identity.

INTRODUCTION AND HISTORICAL PERSPECTIVE

Hector Battifora's 'MEsothelioma' 1 (HBME-1) made its first appearance in 1992 when the Department of Pathology at City of Hope National Center, California, USA, making use of a mesothelioma cell line, produced a monoclonal antibody capable of staining the microvilli of malignant mesothelial cells. This provided a helpful diagnostic tool for the interpretation of challenging pathological cases of the thoracic region. The birth of this marker caused quite a sensation due to the possible employment on formalin-fixed paraffin-embedded (FFPE) tissue sections, unlike many other monoclonal antimesothelioma antibodies under study at that time. Although formerly used to define the human bone marrow endothelial cell line, HBME-1 refers to 'membrane epitope' for some authors due to the distinct membranous pattern of this staining, even if the cytoplasmic positivity is not unusual in specific settings (figure 1).[1] This antibody does not immunoprecipitate/react on western blot analysis and its target epitope is still under investigation. However, being a mouse monoclonal antibody of IgM class, authors postulated that it could be directed against a polysaccharide antigen or a carbohydrate determinant on a glycoprotein of the cellular membrane.[2] Tissue microarray analyses on both benign and malignant thyroid tissues presented interesting information regarding its subcellular localisation, alongside the membranous antibody CK19: in 'lymph node metastatic' and 'extrathyroidal tissue invasive' papillary cancer, these two antibodies showed a stronger membranous staining and a weaker cytoplasmic positivity than those found in non-metastatic thyroid carcinomas.[3] These findings suggest a possible pathophysiological link between these two molecules, stressing a potential role of HBME-1 in cytoskeletal and myofibrils organisation. Following these theories, Crescenzi *et al* demonstrated that, compared with their normal counterparts, neoplastic cells upregulate specific saccharide residues on their membranes, underlining the role of surface glycoconjugates as constituents of membrane receptors and their involvement in cell-cell and cell-matrix interaction (figure 2).[4] A recently introduced in situ proteomic technique, matrix-assisted laser/desorption ionisation mass spectrometry imaging, could significantly help in deciphering the elusive epitope targeted by HBME-1 antibody, providing a fresh point of view in the application of such diagnostic tool in thyroid pathology.[5] Moreover, a combined application of lipidomics in this investigation could potentially cover the subgroup of glycosphingolipids and cholesterol which enrich the microvilli and brush border of cell membranes, opening the door for a lipidomics-based approach to this issue.[6]

HBME-1 antibody in action

General performances

In the years following the discovery of HBME-1, many studies tested its diagnostic performance, highlighting an 85% average sensitivity (Sn) and a 42% average specificity (Sp) in the differential diagnosis between epithelioid mesothelioma and carcinoma at histology on FFPE samples.[7] Moreover, 93%–98% Sn and 71%–83% Sp, respectively, were found in the ability to differentiate reactive mesothelial cells from neoplastic malignant epithelial cells in serous effusion specimens.[8 9] These results pointed out that HBME-1 had a moderate diagnostic performance for mesothelial cells and hence could be included in the immunohistochemical panel performed for the differentiation between mesothelial and epithelial cells, either on cytological and histological samples. Nevertheless, its current popularity has largely declined in this setting owing to the rising of more reliable, specific and handy markers of mesothelial origin, such as calretinin, Wilms Tumour 1 (WT-1), D2-40/podoplanin, Heart Development Protein

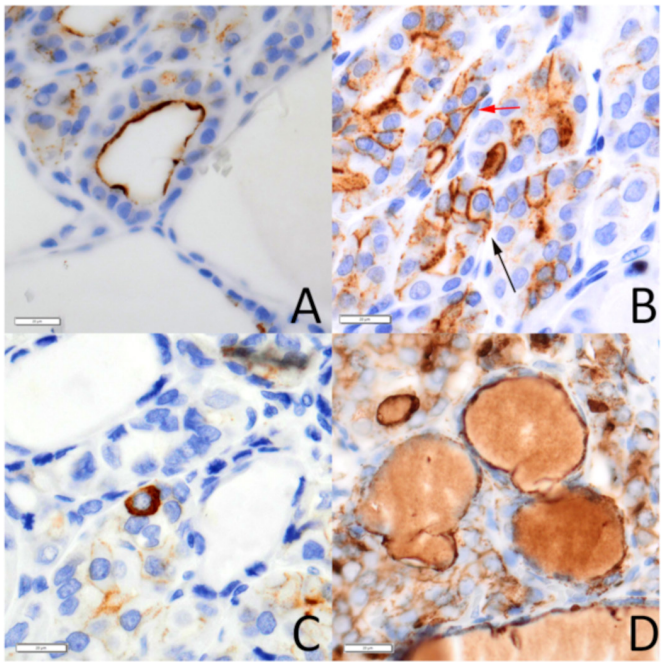

Figure 1 Different staining patterns of Hector Battifora's' MEsothelioma' 1 (HBME-1). (A) An eminently apical pattern of staining, supporting the possible polysaccharide nature of the target antigen against which HBME-1 antibody is directed, possibly located in the microvillar region (×40). (B) The alternative membrane pattern with basolateral (so-called 'cup-like', black arrow) or circumferential (red arrow) staining of neoplastic cells (×40). Although being rare, a granular cytoplasmic staining can be seen, alone or in combination with more 'classic' patterns (C), ×40. Finally, in thyroid tissue faint staining can be seen in the lumen of follicles, suggesting a possible release of the antigen from the apical side of thyroid cells (D), ×40.

With EGF Like Domains 1 (HEG-1), CK5/6 and GATA Binding Protein 3 (GATA-3), often used in combination with glycoprotein markers, to create immunohistochemical panels in which HBME-1 is rarely employed. Furthermore, the need of a more detailed biological characterisation of mesothelial malignancies is shifting the attention towards the assessment of precise genetic

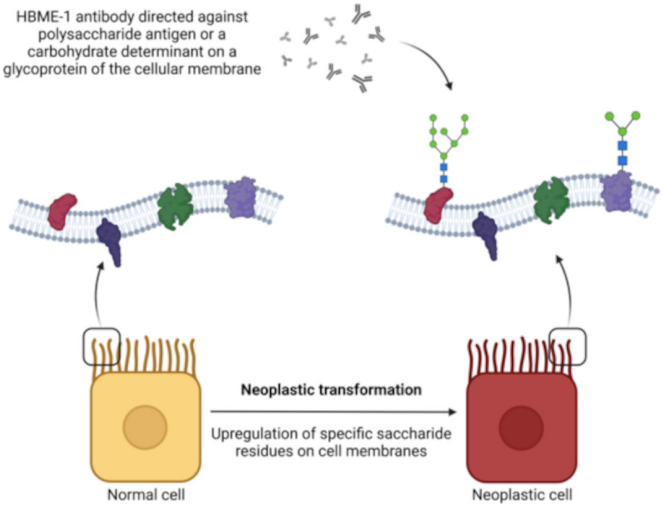

Figure 2 Schematic representation of the putative antigen against which Hector Battifora's 'MEsothelioma' 1 (HBME-1) antibody could be directed. Created with BioRender.

Table 1 Immunohistochemical HBME-1-positive and HBME-1-negative tissues[12–16]

Tissues showing positivity to HBME-1	Tissues eminently negative to HBME-1
Lung	*GI tract epithelium*
Normal bronchial cells	*Squamous epithelium*
Lung alveolar lining cells	*Liver*
Adenocarcinoma (cytoplasmic staining)	*Kidney*
Uterus	*Testis*
Endocervical epithelium	*Thyroid (normal thyroid cells)*
Endometrial glands	*Placenta*
Adenomatoid tumour	*Benign connective tissue*
Cartilage	*Muscle*
Normal cartilage	*Skin*
Chordoma	*Lymphoid tissue*
Chondrosarcoma	*(only scattered histiocytes)*
Breast	
Myoepithelial cells	
Ductal carcinoma	
Pleura	
Normal mesothelial cells	
Mesothelioma	

GI, gastrointestinal; HBME-1, Hector Battifora's 'MEsothelioma' 1.

alterations, for which immunohistochemical and in situ hybridisation (FISH) surrogate markers are progressively being applied (eg, BAP1, MTAP and IMP3, or FISH for CDKN2A homozygous deletion).[10 11]

Applications in pathology

Further studies found that HBME-1 also stains bronchial and endocervical epithelium, cartilage, lung alveolar lining cells, breast ducts myoepithelium, endometrial glands (apical staining pattern) and scattered histiocytes in lymphoid tissue (table 1).[12–16] Moreover, variable stain positivity has been described in lung, breast and pancreatic adenocarcinomas, as well as in ovarian serous carcinomas.[12 13] Finally, even some mesenchymal tumours, such as chordoma, chondrosarcoma and synovial sarcoma may show HBME-1 positivity. It is interesting to note that each of these tumours displays some kind of microvillous membrane projections, stressing once again the possible nature of the target antigen.[14 15 17] HBME-1 negativity is observed in the epithelium of the gastrointestinal tract, squamous epithelium, liver, kidney, testis, normal thyroid, placenta, connective tissue, muscle, skin epidermis/dermis and lymphoid tissue.[16]

The great lack of specificity and the development of more reliable antibodies, such as brachyury in chordoma, or the shift to genomic tests, such as the assessment of rearrangements in synovial sarcoma, clipped the wings of HMBE-1 with regard to its use in these settings.

Haematopathology

It is well known that HBME-1 marks scattered pronormoblast in normal bone marrow, but never marks mature normoblast and erythrocytes: nevertheless, in dyserythropoietic bone marrow, HBME-1 also labels nucleated erythroid precursors, with higher intensity in immature forms. The comparison of the HBME-1/CD235a-positive cells ratio between dyserythropoietic bone marrow and normal samples suggests a left-shifted erythroid maturation when ≥10%, giving to HBME-1 a potential useful dyserythropoiesis recognition role.[18] Another potential use for HBME-1 may be the detection of indolent lymphoproliferation processes in villous B lymphocytes, such as hairy cell leukaemia

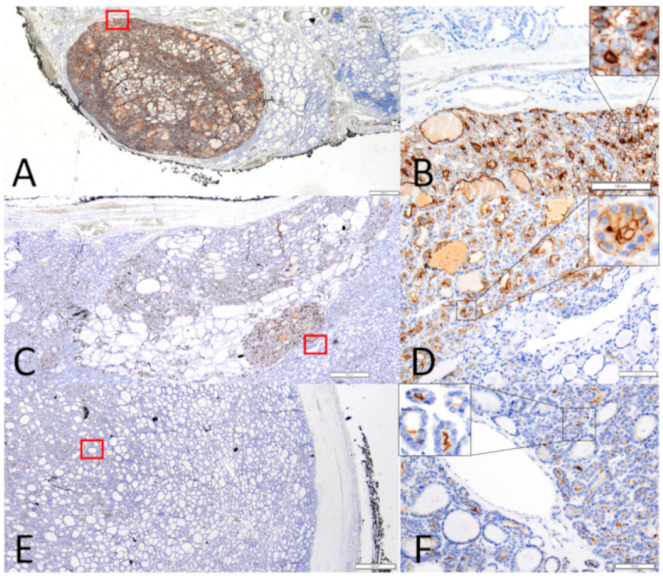

Figure 3 Hector Battifora's 'MEsothelioma' 1 (HBME-1) in different thyroid neoplasms. An encapsulated follicular variant of papillary thyroid carcinoma demonstrating strong and diffuse apical/membranous positivity to HBME-1 (A), ×2, (B), ×10 and inset on nuclear inclusions. More heterogeneous the expression of HBME-1 in another case of 'sprinkling' Non-Invasive Follicular Thyroid neoplasm with Papillary-like nuclear features (C), ×2, with alternance of areas with even strong expression (inset, upper right) associated with completed negative follicles (D), ×10. Finally, rare cases of follicular tumour with uncertain malignant potential can show faint apical positivity, especially in regions with dense, microfollicular growth patterns ((E), ×2 and (F), x10 with magnification in the upper left inset).

(HCL). A normal proportion of hyperplastic B lymphocytes with villous membrane projections, which show HBME-1 staining, is commonly found in normal lymphoid tissue. The sharing of this feature with B lymphocytes of hairy cell leukaemia allowed this marker to be useful for the diagnosis of this disease (96% of cases show positivity), while it had a less relevant impact on other diseases of the same family (39% of cases of HCL variants and 50% of cases of splenic diffuse red pulp small B cell lymphoma).[17]

Thyroid cancer landscape: the last HBME-1 action field

HBME-1 finds its most widespread use in thyroid neoplasms (figure 3). The first data about its ability to distinguish benign thyroid conditions from malignancies, especially papillary thyroid carcinoma (PTC), were published in 1996, when the combined application of HBME-1 and CD15 has been proposed to highlight cellular glycoconjugates changes related to malignant transformation in differentiated thyroid carcinomas.[19]

The overall Sn and Sp for HBME-1 immunohistochemistry are 77% and 83%, respectively, with a high variation in Sn among the reports according to the different entities tested (table 2).[20] Generally, it stains follicular-derived malignant tumours: most PTC show intense staining in 88% of cases, with a reduction of percentages (from 45% to 82%) in the follicular variant of PTC. In the thyroid pathology setting, this marker experienced a further renaissance due to the recent introduction of Non-Invasive Follicular Thyroid neoplasm with Papillary-like nuclear features, which often shows positivity to HBME-1, in about 78% of cases.[21] In follicular neoplasms, this marker demonstrated a range of Sn and Sp of 61%–85% and 70%–73%,[22 23] respectively, with lower Sn (53%) and higher Sp (88%) in oncocytic lesions.[24]

Table 2 Diagnostic performances of HBME-1 in different entities encountered in thyroid pathology

Diagnostic entity	Sensitivity	Specificity
Classic PTC[32]	95%	77%
fvPTC[37]	95%	94%
NIFTP[21]	78%	53%
Follicular neoplasm[22 23]	61%–85%	70%–73%
Oncocytic carcinoma[24]	53%	88%
Overall	77%	83%

fvPTC, follicular variant of papillary thyroid carcinoma; HBME-1, Hector Battifora's 'MEsothelioma' 1; NIFTP, Non-Invasive Follicular Thyroid neoplasm with Papillary-like nuclear features; PTC, papillary thyroid carcinoma.

Anaplastic and poorly differentiated carcinomas express HBME-1 in 67%–91% and 0%–50% of cases, respectively.[16] Nevertheless, HBME-1 may stain benign conditions too, such as adenomatous goitre (3%–12%) and follicular adenomas (0%–27%).[22] Considering the high Sp of this marker, some studies stressed the need of active surveillance for benign cases showing focal HBME-1 staining, for their possible degeneration towards an incipient malignant neoplasm.[16 22 25]

A further useful application for HBME-1 could be in the setting of indeterminate fine-needle aspiration biopsies (follicular proliferations), where it manifested an 80% Sn and a 96% Sp. Furthermore, the association of additional markers (eg, galectin-3) can improve its Sn to 94%, with a high negative predictive value (92%) retaining moderate Sp and positive predictive value (73% and 50%, respectively). This can be of help to rule out follicular carcinoma in cytology when both are negative.[26 27]

Interesting results came from the correlation between HBME-1 immunohistochemical expression and genetic mutations, with a trend noted in follicular carcinomas with peroxisome proliferator-activated receptor (PPAR) rearrangements that fail to show HBME-1 immunoreactivity in 59% of cases, in contrast to RAS mutated cases that are often positive (62% of cases).[28] This strict relation with the underlying genetic background of thyroid neoplasms culminates in PTC, in which a direct positive correlation between BRAFV600E mutation and HBME-1 expression has been noted.[29]

In the next generation sequencing (NGS) era it is lawful to ask what role HBME-1 may play in thyroid cancer, if there is one. An interpretation to this question came recently from a paper that, starting from small samples of thyroid nodules, matched the expressions of several immunohistochemical markers with a large NGS panel.[30] This report suggests that a satisfactory level of reliability in non-follicular lesions may be reached, even in complex cases, either with only an immunohistochemical panel including CK-19, galectin-3, HBME-1 and CD56, or with NGS alone. The combination of these two approaches proved to be non-cost effective. On the other hand, considering only follicular lesions, neither immunohistochemical panel nor NGS alone seemed to have adequate diagnostic performances, while their combination seemed to be useful just in some difficult cases.

IS THE APPLICATION OF HIGH-THROUGHPUT TECHNOLOGIES THE LOGICAL SOLUTION FOR THE HBME-1 IDENTITY DISCLOSURE?

The generally limited scope for the application of HBME-1 in routine pathology is nowadays known, but possible usefulness in

thyroid pathology is still under discussion. Differentiating benign from malignant thyroid neoplasms and establishing their biological aggressiveness could be a hard task for pathologists in some challenging cases, either for the presence of complex morphological criteria to assess and the lack of reliable immunohistochemical markers.[31 32] In this context, HBME-1 staining has a good Sn, but has a low Sp, in particular when used as a standalone marker.

The mystery about its precise origin and its unknown biological role recently aroused interest surrounding this gradually underrated marker, indeed in-depth studies concerning its precise subcellular location and related immunohistochemical expression may reveal its true role, both in normal biological processes and in the mechanisms which underpin cancer development. We already know that important functions, such as cell-to-cell interaction, intracellular homeostasis and numerous signalling pathways are usually regulated by membrane proteins and that, unsurprisingly, cancer progression is widely correlated to these protein dysfunctions.[33] Recent analytical approaches and subcellular fractionations made it possible to study the plasma membrane proteome in more detail; moreover, receptor-protein interactions are independent of protein synthesis, making proteomics the principal approach, as opposed to genomics and transcriptomics, for analysing membrane-expressed molecules.[34 35] Mass-spectrometry is a high-throughput system employed for the identification of new biomarkers, especially in neoplastic settings: the identification of proteomic patterns associated with disease development has become a promising approach in untargeted tumours. However, in the past, proteomic analysis in the setting of membrane protein expression has been a particularly challenging task due to their hydrophobicity.[36] In addition, membrane proteins often undergo various post-transcriptional modifications such as glycosylation, which is the most common, and cancer cells frequently display glycoproteins with increased branching of the glycan structures, making their studies even more inquisitive.[34] Nonetheless, recent advantages and novel proteomic technologies have rendered this challenge of thoroughly investigating the real essence of HMBE-1 affordable, and the stage appears to be finally set for the riddle of its diagnostic role in thyroid neoplasms to be solved.

CONCLUSIONS

The antibody HBME-1, directed against an elusive membrane marker, has a limited role in different settings of diagnostic pathology, although can still play a role in thyroid cancer thanks to its good performances alone or in combination with other immunohistochemical stains. The application of high-throughput technologies (mainly in situ proteomics) may be the exact route to improve the knowledge about the pathophysiology of HBME-1 and identify it definitively.

Take home messages

- ⇒ Hector Battifora's 'MEsothelioma' 1 (HBME-1) staining is one of the most fascinating in pathology, with a wide range of applications from mesothelioma to thyroid cancer, although its real identity is currently unknown.
- ⇒ Different acceptable or good diagnostic performances were recorded for HBME-1 in thyroid pathology, being used in routine practice as one of the best tools to screen thyroid malignancy both in terms of sensitivity and specificity.
- ⇒ The application of high-throughput technologies (mainly Tin situ proteomics) may be the exact route to improve the knowledge about the pathophysiology of HBME-1 and to finally unveil its true identity.

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