Review Article

Diagnostic Mesothelioma Biomarkers in Effusion Cytology

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Malignant mesothelioma is a rare malignancy with a poor prognosis whose development is related to asbestos fiber exposure. An increasing role of genetic predisposition has been recognized recently. Pleural biopsy is the gold standard for diagnosis, in which the identification of pleural invasion by atypical mesothelial cell is a major criterion. Pleural effusion is usually the first sign of disease; therefore, a cytological specimen is often the initial or the only specimen available for diagnosis. Given that reactive mesothelial cells may show marked atypia, the diagnosis of mesothelioma on cytomorphology alone is challenging. Accordingly, cell block preparation is encouraged, as it permits immunohistochemical staining. Traditional markers of mesothelioma such as glucose transporter 1 (GLUT1) and insulin-like growth factor 2 mRNA-binding protein 3 (IMP3) are informative, but difficult to interpret when reactive proliferations aberrantly stain positive. BRCA1-associated protein 1 (BAP1) nuclear staining loss is highly specific for mesothelioma, but sensitivity is low in sarcomatoid tumors. Cyclin-dependent kinase inhibitor 2A (CDKN2A)/p16 homozygous deletion, assessed by fluorescence in situ hybridization, is more specific for mesothelioma with better sensitivity, even in the sarcomatoid variant. The surrogate marker methylthioadenosine phosphorylase (MTAP) has been found to demonstrate excellent diagnostic correlation with p16. The purpose of this review is to provide an essential appraisal of the literature regarding the diagnostic value of many of these emerging biomarkers for malignant mesothelioma in effusion cytology. ***Cancer Cytopathol*** **2021;129:506-516**. *© 2021 American Cancer Society*.

KEY WORDS: biomarker; cytology; immunohistochemistry; mesothelioma; mesothelium; pleural effusion.

INTRODUCTION

Malignant mesothelioma arises from the serosal surfaces of the pleural, peritoneal, and pericardial cavities.[1] Oncogenesis is related to exposure to asbestos fibers; however, it has been shown recently that genetic predisposition to mesothelioma also plays a role.[2,3] Malignant pleural mesothelioma (MPM) is the most frequent mesothelioma encountered, and it has a poor prognosis, with an overall survival of less than 18 months.[1,4] Although considered a rare cancer, the incidence of MPM is increasing; in some European countries, a peak is expected during the next decade, taking into account the long latency between asbestos exposure and malignancy development. A definitive diagnosis of MPM is usually reached with a tissue biopsy or on surgical resection specimens. However, pleural effusion is often the first sign of malignancy. Afflicted patients are usually elderly or unfit to tolerate invasive procedures such as thoracoscopic surgery to obtain a diagnostic biopsy. A cytological examination of pleural fluid, on the other hand, is far less invasive and can be readily performed.[4,5] In fact, cytology samples are often the only available material to establish a diagnosis. However, the cytological features of MPM are not always straightforward, as reactive mesothelial cells can have an atypical appearance that overlaps with MPM. Moreover, the cytologic appearance of MPM in effusions can sometimes be deceptively bland (representative examples of reactive mesothelium and mesothelioma are shown in Figure 1). Not surprisingly, the cytological diagnosis of mesothelioma in

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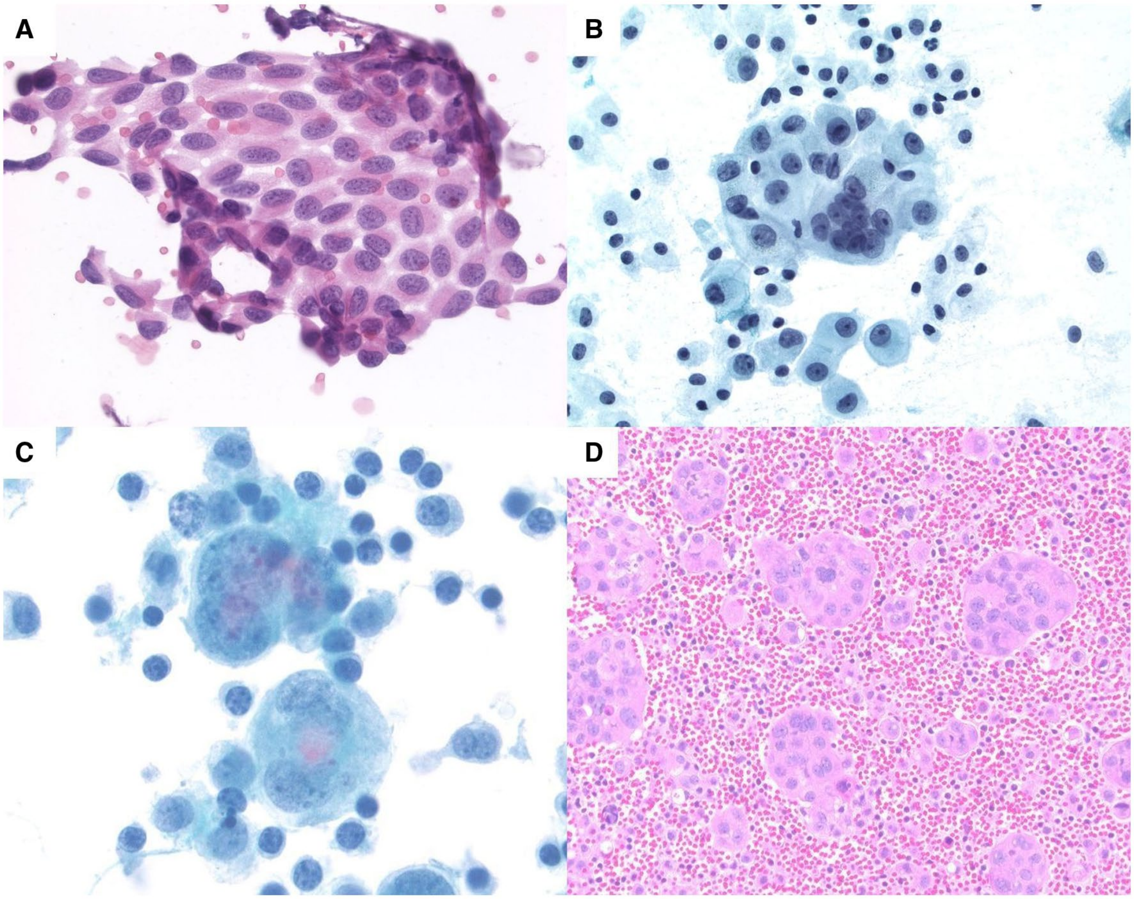

Figure 1. (A) Direct smear showing a sheet of benign mesothelial cells (Papanicolaou stain, original magnification ×400). (B) Pleural effusion showing reactive mesothelial cells, including a multinucleated cell (Papanicolaou stain, original magnification ×400). (C) ThinPrep of pleural effusion showing malignant mesothelial cells with a chronic inflammatory background (Papanicolaou stain, original magnification ×400). (D) Cell block of a pleural effusion showing several large clusters of atypical mesothelial cells (hematoxylin and eosin stain, original magnification ×100).

pleural effusion material was previously believed to be unreliable, where the published diagnostic sensitivity ranged from 30% to 75%.[6] Today, the availability of immunocytochemistry (ICC) with newer biomarkers has greatly enhanced the diagnostic yield of cytology. Some of these emerging markers are currently recommended in the International Mesothelioma Interest Group guidelines to establish mesothelial lineage and diagnose malignancy.[7,8]

The purpose of this review is to summarize the published evidence concerning diagnostic biomarkers to discriminate pleural mesothelioma from reactive benign mesothelium in pleural effusion cytology specimens. Given that systematic and formal quantitative analysis of the diagnostic performance of a single biomarker would require additional and targeted work, this is considered a state-of-the-art review in a recognized framework of review methodology.[9] An essential summary of literature evidence on the diagnostic biomarkers is provided in Table 1.

GENERAL CONSIDERATIONS REGARDING EFFUSION CYTOLOGY SPECIMENS

Cytological material derived from a pleural effusion is often the only available specimen to establish a diagnosis in patients with suspected mesothelioma. Hence, adequate sampling and specimen processing is of paramount importance.[10] The conventional belief that the greater the volume of effusion fluid sent to the laboratory, the higher the likelihood of having a positive cytological diagnosis, has not been firmly supported in the literature.[11] Nevertheless, the availability of large amounts of material can allow for the production of cell blocks with higher cellularity, thereby enabling

TABLE 1. Systematic Evidence on Diagnostic Performance of Malignant Pleural Mesothelioma Markers

Marker	Sensitivity and Specificity in Systematic Reviews		Notes
	Sensitivity (CI)	Specificity (CI)	
Soluble			
Mesothelin/SMRP	0.79 (0.75-0.83)[27] 0.69 (0.64-0.72)[28]	0.85 (0.83-0.87)[27] 0.90 (0.85-0.94)[28]	• Different cutoffs of the studies included • No subgroup analysis for different MPM subtypes • Diagnostic performance is usually studied in differential against both lung cancer and reactive atypical mesothelium
Fibulin-3	0.73 (0.54-0.86)[31]	0.80 (0.60-0.91)[31]	
IHC and FISH			
GLUT1	0.83 (0.71-0.90)[36]	0.90 (0.79-0.96)[36]	• Marker of malignancy, not of MPM • Informative only when positive • Stains also red blood cells
IMP3	No systematic review; reported values ranging 37-94%		• Oncofetal protein used as marker of malignancy, not of MPM • Few studies dealing with cytology[37,38]
BAP1	0.58 (0.50-0.65)[44] 0.547 (0.512-0.716)[45]	0.96 (0.89-0.99)[44] 0.957 (0.939-0.971)[45]	• The sensitivity is reported to be higher in epithelioid mesothelioma and very low (0-0.22) in sarcomatoid mesothelioma • Some carcinomas and melanoma could also show BAP1 loss • Reliable to assess in cytology specimens, particularly cell blocks
p16 HD	0.72 (0.67-0.76)[65]	1.00 (0.94-1.00)[65]	• Available review not up-to-date; most recent studies point toward a very high specificity but still overall unsatisfactory sensitivity, even though p16 HD has shown the highest sensitivity for sarcomatoid mesothelioma • High specificity should be considered to apply to MPM diagnosis only when mesothelial lineage of atypical cells has been established • Reliable to assess in cytology specimen, particularly cell block • Still some heterogeneity in the cutoff for establishing HD (10%-20% of nuclei) • Less available and more expensive as an IHC marker; to be used in a diagnostic panel if BAP1 nuclear staining is retained
MTAP	No systematic reviews		• IHC surrogate of p16 HD • Similar diagnostic performance profile • Recent studies show high correlation with p16 HD and reliability of assessment in cytological specimens
BAP1+p16 or MTAP	No systematic reviews		• The specificity is virtually 100% • The sensitivity is increased, but still suboptimal
miRNAs	No systematic reviews; highly heterogeneous studies		• A panel of circulating and tissue miRNAs constitutes a diagnostic signature of MPM[80] • Most studies deal with circulating or tissue miRNAs, only 3 with pleural effusion cytology[77-79] • Large amount of data, but highly heterogenous studies in terms of study design, type of control patients, specimens, quantification methods, and pool of miRNAs explored; hence, the value of these biomarkers remains unclear[74,81]

Abbreviations: BAP1, BRCA1-associated protein 1; FISH, florescence in situ hybridization; GLUT1, glucose transporter 1; HD, homozygous deletion; IHC, immunohistochemistry; IMP3, insulin-like growth factor 2 mRNA-binding protein 3; miRNAs, microRNAs; MPM, malignant pleural mesothelioma; MTAP, methylthioadenosine phosphorylase; SMRP, soluble mesothelin-related peptides.

ICC to be performed, as well as preservation of material for future investigation or biobanking. Mesothelioma guidelines recommend that cell block preparation should be performed whenever possible.[4] Cytology material prepared from effusions is also suitable for molecular studies and, accordingly, is being used increasingly for the determination of prognostic and predictive markers including those used (eg, CDKN2A/p16) in MPM.[12,13]

IMMUNOHISTOCHEMICAL DISTINCTION OF MESOTHELIOMA FROM METASTATIC CARCINOMA

Given the rarity of mesothelioma, lung carcinoma involving the pleura—which is much more frequent—needs to always be excluded in a patient manifesting with a malignant pleural effusion. The epithelioid subtype of mesothelioma is most likely to mimic carcinoma, which is also the variant most frequently encountered in cytology specimens, as it sheds mesothelial malignant cells in the pleural fluid—unlike sarcomatoid mesothelioma, which is less prone to this phenomenon. Apart from infrequent subtypes such as mesothelioma with abundant signet ring cells that could aberrantly express markers more typical for an adenocarcinoma,[14,15] several immunohistochemical (IHC) markers are now widely and reliably used to separate mesothelioma from carcinoma or other discohesive malignancies.[14] Current guidelines recommend the use of an IHC panel comprising 2 mesothelial lineage markers and 2 epithelial (nonmesothelial) markers.[16] Typical mesothelial lineage markers include calretinin, CK5/6, WT-1, and podoplanin (D2-40), while historical markers of epithelial differentiation include BerEP4, TAG72 (B72.3), and MOC-31.[17] These markers should always be evaluated as part of a panel in combination with other markers. Calretinin and CK5/6 are not entirely specific for mesothelium and perform best when lung adenocarcinoma is the only entity in the differential diagnosis. Careful consideration should apply to employing D2-40 in situations wherein there may be a metastasis from a tumor that is also known to express this marker, such as salivary gland neoplasms or squamous cell carcinoma. Given the potential for overlapping staining of WT-1 with serous ovarian carcinoma, the use of this marker may be problematic when routinely working up peritoneal, but not pleural, effusions. A newer marker of mesothelial origin is heart development protein with EGF-like domains 1 (HEG1), which has been shown in recent studies to exhibit higher sensitivity and specificity than calretinin, D2-40, and WT-1 for mesothelial lineage, with 100% specificity for epithelioid mesothelioma and 64% to 78% sensitivity for sarcomatoid mesothelioma.[18] However, there is currently limited commercial availability of HEG1.[17] While BerEP4 and MOC-31 are highly specific (90%-100%) for epithelial lineage and sensitive (85%-100%) for adenocarcinoma, they lack sensitivity in carcinomas with sarcomatoid differentiation. Claudin-4 is a relatively newer marker,[17] with increasing consensus that it may be the best epithelial marker for diagnostic use,[19-23] demonstrating up to 92% to 100% sensitivity and 94% to 100% specificity for epithelial cells.[22,23]

SOLUBLE BIOMARKERS IN EFFUSIONS

Soluble biomarkers have been investigated extensively for the diagnosis of mesothelioma in high-risk groups, such as asbestos-exposed populations. The most important soluble markers researched include mesothelin/soluble mesothelin-related peptides (SMRP), fibulin-3, and osteopontin. These markers are usually assessed using an enzyme-linked immunosorbent assay or chemiluminescent immunoassay that can be performed on serum, plasma, or serous effusion samples. Most research was focused on blood samples to develop a rapid diagnostic tool. However, cytology samples collected at the time of thoracentesis for cytological examination could potentially be used as well.

Mesothelin is a 40-kDa glycoprotein attached to the cell surface of neoplastic cells in mesothelioma and other cancers. The gene mesothelin (*MSLN*) produces a precursor protein that generates both soluble mesothelin (SMRP and C-ERC/mesothelin) and the cytokine megakaryocyte potentiating factor (also known as N-ERC/mesothelin).[24] Soluble mesothelin is considered one of the most promising markers, and thus far is the only biomarker for malignant mesothelioma that has been approved by the US Food and Drug Administration.[25,26] In 2014, Cui et al[27] reported mesothelin measurements in pleural effusions from 11 studies showing a pooled sensitivity of 0.79 (95% CI, 0.75-0.83) and specificity of 0.85 (95% CI, 0.83-0.87) and, when compared with serum measurement, concluded that this soluble marker is equally effective for the diagnosis of MPM. In another review, however, Gao et al[28] reported that mesothelin had unsatisfactory diagnostic performance with poor sensitivity (0.69 [95% CI, 0.64- 0.72]) albeit high specificity (0.90 [95% CI, 0.85-0.94]) for MPM.

Additional soluble biomarkers that have been investigated are fibulin-3 and osteopontin, both of which are extracellular glycoproteins involved in mesothelioma cell migration and proliferation.[29,30] Fibulin-3 has been reported to be unsatisfactory for diagnostic use,[24] but this finding is based largely on studies using plasma or serum specimens. Ren et al[31] reported a pooled sensitivity of 0.73 (95% CI, 0.54-0.86) and specificity of 0.80 (95% CI, 0.60-0.91) for fibulin-3 in pleural effusions, but these data are derived from only a small number of studies. Studies evaluating the diagnostic role of osteopontin has thus far dealt only with blood levels.[32]

Finally, methods such as detection of cell-free DNA in body fluids and circulating tumor cells (CTCs) are being studied increasingly.[33] Antigens detected in CTCs appear to be able to reveal the presence of malignant cells, but with most applications targeted to early discovery of metastasis.[34] Next-generation sequencing appears to be the most promising technique to detect molecular alterations diagnostic of malignancy, with potential future application also to MPM.[33]

GENERAL IMMUNOHISTOCHEMICAL MARKERS FOR MESOTHELIOMA

Several IHC markers have been investigated to discriminate between mesothelioma and atypical reactive mesothelium. They include epithelial membrane antigen (EMA), p53, desmin, glucose transporter-1 (GLUT1) and insulin-like grow factor II mRNA-binding protein 3 (IMP3). As reported by Churg et al,[35] these markers work well "in a statistical sense," because while a significantly greater proportion of mesotheliomas stain positively with these markers (the reverse applies to desmin), a larger quota of benign mesothelial lesions also yield a stain that is "wrong" with these markers, thus reducing their diagnostic utility.[35]

In a meta-analysis of 24 studies including 969 mesothelioma patients and 1080 patients with reactive mesothelial cells, Zhong et al[36] showed that GLUT1 has a pooled sensitivity and specificity of 0.73 (95% CI, 0.62-0.81) and 0.95 (95% CI, 0.91-0.98), respectively. These authors concluded that the diagnostic performance of GLUT1 is high both in histology and cytology specimens, with high diagnostic odds ratio and high positive likelihood ratio, but low negative likelihood ratio not low enough to exclude a diagnosis. This implies that GLUT1 is useful and informative only when positive, as the absence of staining does not exclude malignancy.[24] IMP3 is reported to have a higher specificity and sensitivity, ranging from 37% to 94%.[24] However, the number of studies evaluating this marker is lower, especially those regarding stain performance in cytology material.[37,38] Nonetheless, IMP3 is not affected by high background staining, as occurs with GLUT1. Using the aforementioned markers in combination can improve their diagnostic yield.[39] Employing such panels of IHC stains is usually easier to perform and assess when one marker has nuclear staining and others show membranous/cytoplasmatic positivity, or they are visualized with different detection systems. Moreover, GLUT1 is available both as monoclonal and polyclonal antibody, while the most commonly used IMP3 clone in cytological specimen is clone 69.1; furthermore, in comparison with GLUT1, IMP3 has the advantage of not staining red blood cells, thus being perceived as "cleaner."

BRCA-ASSOCIATED PROTEIN 1

BRCA1-associated protein 1 (BAP1) is a tumor suppressor gene located at chromosome 3p21.1 that encodes a nuclear deubiquitinating enzyme that regulates several cellular functions such as chromatin remodeling, cellular differentiation, DNA damage response, growth suppression, and apoptosis. Germline mutations of BAP1 cause a reduced level of the active protein, leading to the accumulation of genetic mutations and ultimately malignancy.[40,41] Carriers of these germline mutations are more prone to several types of cancer, most commonly uveal melanoma, and BAP1 loss detected with IHC has been shown to be concordant with a genetic mutation.[42] BAP1 loss has emerged in recent years as a virtually 100% specific marker of malignancy in mesothelial proliferations.[17,43] However, BAP1 is also somewhat insensitive, with studies showing 50% to 65% sensitivity in pleural mesotheliomas, with higher percentages in the epithelioid subtype, intermediate results in biphasic tumors, and a very low diagnostic yield in sarcomatoid mesotheliomas. Recent systematic reviews confirm the high specificity of this marker. In 2017, Wang et al[44] reported a pooled specificity of 0.96 (95% CI, 0.89-0.99) and sensitivity of 0.58 (95% CI, 0.50-0.65) compared with Mlika et al,[45] who reported a specificity of 0.957 (95% CI, 0.939-0.971) and sensitivity of 0.547 (95% CI, 0.512-0.716).

BAP1 loss (ie, negative nuclear immunoreactivity) is highly specific not only in the differential diagnosis between mesothelioma and reactive atypical mesothelium, but also with metastatic malignancy involving serous cavities. Of note, other malignancies such as renal cell carcinoma and melanoma can also have BAP1 loss. Chapel et al[17] recently reported a preponderance of evidence indicating that BAP1 loss is highly specific for mesothelioma and that it can be assessed accurately in cell block preparations. However, some studies have shown that the specificity of this marker in cytology specimens is not that high,[46] and further data are needed regarding sensitivity in various subtypes of mesothelioma. Indeed, most of evidence points toward a high specificity of BAP1 for the diagnosis of malignant mesothelioma, both against reactive atypical mesothelial cells and against metastatic malignancy; however, some

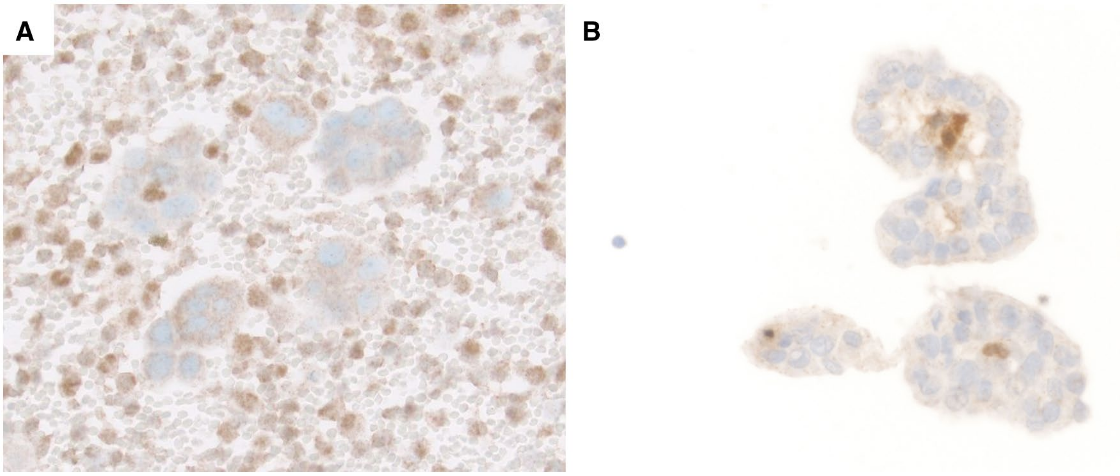

Figure 2. (A) Cell block showing BAP1 loss in mesothelioma cell clusters (BAP1 immunohistochemical stain, original magnification ×200). (B) Cell block showing MTAP loss in mesothelioma cell cluster (MTAP immunohistochemical stain, original magnification ×600).

contradictory results have been reported, and sensitivity appears to depend greatly on mesothelioma subtype.[43] By far, the most used clone is clone C4 by Cell Marque, and it has been noted that alcohol fixative tends to leach antigen, while formalin fixation is more reliable.[43] BAP1 is ideally considered lost when tumor nuclear staining is absent in the presence of a positive internal control, which may not always be present in scant cytology samples (representative example of BAP loss is shown in Figure 2). Cytoplasmic-only staining may be seen in a subset of cases but is still considered BAP1 loss for diagnostic purposes.[17,43] To improve diagnostic yield, it is recommended that BAP1 be used in combination with other markers.[43,47-49] To that end, a 117-gene expression panel, based on Nanostring technology, able to differentiate epithelioid MPM from mesothelial reactive hyperplasia in pleural tissues better than BAP and p16 has been proposed.[47]

ENHANCER OF ZESTE HOMOLOG 2

Enhancer of zeste homolog 2 (EZH2) is a component of a nuclear repressive complex that plays a central role in the epigenetic suppression of gene expression through polymethylation of specific histone residues. Overexpression of EZH2 is reported in several malignancies, and a link with a loss of the BAP1 gene suggests a role in mesothelioma development and progression.[50] EZH2 staining is nuclear, which allows it to be easily used in combination with other membranous/cytoplasmic markers. The first 2 reports of EZH2 involved tissue specimens and showed a sensitivity of 45% with 100% specificity if used alone to discriminate malignant mesothelioma from reactive atypical mesothelium,[51] and a sensitivity of 90% with 100% specificity when used in combination with BAP1 loss.[52] More recent data evaluating its application on cell blocks revealed a sensitivity of 45% with 100% specificity for EZH2 alone, while a combination of EZH2 with other markers (BAP1, methylthioadenosine phosphorylase [MTAP], or p16 fluorescence in situ hybridization [FISH]) increased sensitivity to 77.5%, reaching 87.5% when 3 markers were used.[53] However, the degree of EZH2 overexpression appears not to be uniform among mesothelioma cases and showed expression in up to 30% of reactive cases.[53] EZH2 should perhaps be considered a marker of malignancy, and not specifically a marker of mesothelioma, given its overexpression in other metastatic effusions.[54-56] Hence, EZH2 is to be used only to support the diagnosis of mesothelioma when a mesothelial lineage has already been established.[17]

5-HYDROXYMETHYLCYTOSINE

5-Hydroxymethylcytosine (5-hmC) is a modified nucleotide produced from 5-methylcytosine by a DNA hydroxylase as the first step of DNA demethylation. The role of 5-hmC in the tumorigenesis of mesothelioma is a relatively recent discovery. Its diagnostic use for pleural mesothelioma has only been reported in histological specimens.[57] Staining interpretation is similar

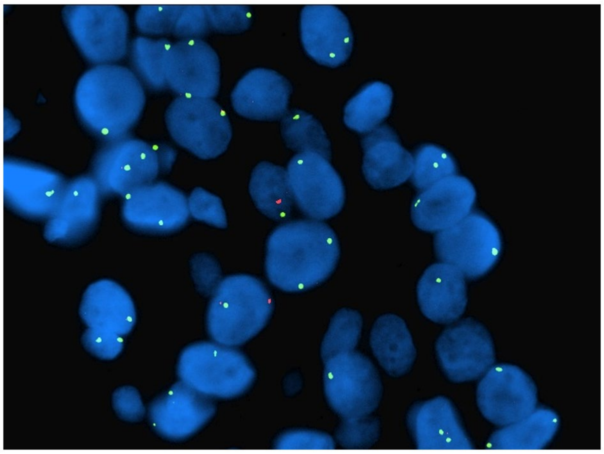

Figure 3. Fluorescence in situ hybridization (FISH) testing of a cytology sample with malignant mesothelioma with p16 deletion shows deletion of the 9p21 locus. Dual-color FISH analysis was performed using a SpectrumGreen-labeled chromosome 9 centromeric (CEP 9) probe and a *p16* (*CDKN2A*) SpectrumOrange-labeled probe (Abbott Molecular, Des Plaines, Illinois). Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI)/antifade (Vysis, Downers Grove, Illinois). Homozygous deletion can be seen in most of the tumor cell nuclei defined by loss of both *p16* gene signals and at least 1 signal for the CEP 9 probe. (Image courtesy of Juan Xing).

to that of BAP1, with loss of nuclear staining being described in more than 50% of nuclei in the presence of an internal control.[57] Studies on cytology specimens are anticipated.

FISH FOR P16 HOMOZYGOUS DELETION

CDKN2A/p16 is a cell cycle suppressor gene located at chromosome 9p21 within a cluster of genes. The down-regulation or loss of CDKN2A/p16 expression is thought to result in enhanced or aberrant proliferation. Homozygous deletion occurs commonly in all MPM subtypes: sarcomatoid (67%-100%), biphasic (69%-95%), and epithelioid (48%-70%).[58] Detection of homozygous deletion (HD) is more accurate by FISH than IHC and can be performed accurately on both cytology and histological specimens.[59-62] When identified in biphasic MPM, the HD of CDKN2A/p16 is present in both components.[63] CDKN2A/p16 HD is considered to be as highly specific as BAP1 loss for malignancy in mesothelial proliferations. Unfortunately, it has the same unsatisfactory sensitivity (0.48-0.88) for the diagnosis of mesothelioma, which is slightly higher (0.80-1.00) for sarcomatoid mesothelioma.[17,61,64]

In contrast to BAP1 loss, HD of CDKN2A/p16 is common in other cancers, thereby rendering this marker ineffective on its own to separate MPM from metastatic carcinomas.[16] The only published systematic study on the diagnostic performance of CDKN2A/p16 dates back to 2012.[65] Since then, more recent literature points toward high specificity for this marker, with highly variable sensitivity.[43] As reported by Churg et al,[43] several studies evaluating the performance of this ancillary test in cytology material all confirmed high specificity and variable sensitivity.[47,66,67] An unequivocal cutoff for HD definition has not been established.[68] Studies to date have used cutoffs of percentage of nuclei showing HD ranging from 10% to 15%.[62,66] This is in line with the intrinsic technical issue of the so-called “truncation artifact,” the rate of spurious apparent HD due to the large size of mesothelial cells; consequently, a number of at least 5 or 100 mesothelial cells is to be assessed in the sample. This is easier in histological specimens, but can be more challenging in cytological specimens with background inflammatory cells and cellular debris, requiring careful selection and pathologist expertise in reading FISH.[43] Representative example of CDKN2A/p16 HD by FISH is shown in Figure 3. CDKN2A/p16 HD by FISH is used mostly as a second-line test to confirm the diagnosis of MPM.[4,8,17] Both loss of CDKN2A/p16 protein expression by IHC and HD by FISH also appear to be associated with an adverse prognosis in MPM.[69]

MTAP

There has been much interest in identifying an IHC surrogate for CDKN2A/p16 HD detection via FISH which would be more readily available, less expensive, and easier to perform. Given that the cluster of genes involved in locus 9p21 deletion also incorporates the *MTAP* gene, the product of this gene has been extensively investigated as a surrogate of p16 HD with FISH. MTAP evaluation by IHC has shown high concordance with FISH studies and appears to carry a similar diagnostic profile, with high specificity and variable sensitivity (0.45-0.65).[17] Most MTAP studies employed cases involving cell blocks,[70-73] which differs from studies of BAP1 and p16 IHC that had a preponderance of histology samples. More than 1 clone is available, and this marker is reported to work better with formalin fixation rather than alcohol fixation, as it happens with other IHC markers/similarly to other IHC markers.[43] Some authors recommend using MTAP by IHC as an adjunctive marker to be performed in cases of retained BAP1 expression, thus reserving FISH analysis for more challenging cases.[17,43]

POTENTIAL ROLE OF MICRORNAS

MicroRNAs (miRNAs) are short, noncoding RNAs of approximately 22 nucleotides that act as posttranscriptional regulators in physiological and pathological processes, including cancer. A differential expression of miRNAs in cancer tissues of different origins has been described, confirming their role in multiple aspects of cancer pathogenesis, ranging from tumor establishment to progression, metastasis, and resistance to therapies. Hence, they have a potential role as important diagnostic and prognostic biomarkers.[74-76] Most studies have investigated miRNAs in cell lines, histological samples, or blood specimens,[75] and only a few studies have explored the diagnostic potential of miRNAs in pleural effusions.[77-79] Micolucci et al[80] reported that a panel of circulating miRNAs (miR126-3p, miR-103a-3p, and miR-625-3p) and a panel of tissue miRNAs (miR-16-5p, miR-126-3p, miR-143-3p, miR-145-5p, miR-192-5p, miR-193a-3p, miR-200b-3p, miR-203a-3p, and miR-652-3p) were deregulated in MPM, thus constituting a potential signature with diagnostic value. Cappellesso et al[77] found that the miRNAs miR-19a, miR-19b, miR-21, and miR-126 were deregulated in cytology specimens of 29 mesotheliomas in comparison with 24 reactive atypical mesothelial cases and that single miRNAs showed an area under the curve ranging from 0.68 to 0.79, concluding that these miRNAs could be used as diagnostic markers of MPM, especially given that their sensitivity and specificity was greater than 0.80. Birnie et al[79] found another pool of miRNAs (miR-210, miR-143, miR-200c, and miR-139-5p) to be useful in the diagnosis of MPM, but their control group was comprised of both lung adenocarcinoma and benign disease.

A search for informative miRNAs requires extensive investigation of publicly available microarray data sets. An agreement on the best pool of miRNAs for the identification of MPM is still lacking.[74] Moreover, it is difficult to draw any conclusions, because many of these studies were highly heterogenous with respect to the specimen studied, the study design, and the inclusion of different control cases (eg, non-MPM patients with cancer vs healthy subjects vs patients with asbestos-related noncancer disease), thus leading to potential misinterpretation of results and preclusion of a systematic meta-analysis of the findings.[74,81] It is foreseeable, however, that in the near future even more studies with better design and focused on pleural effusion material will be performed, given that miRNAs are highly persistent and well preserved in cytology specimens.[81-83] It is also possible to retrieve miRNAs from cytology samples even 10 years after slide preparation and to isolate miRNAs from the exosome in the acellular component of a pleural effusion. For such tests to be successful, it is of great importance to assure optimal handling of the cytology material from collection to slide or cell block preparation to storage for future analysis.[81,84]

CONCLUSION

Serous effusion cytology samples are often the first and only material available for a diagnosis of mesothelioma. Rendering a diagnosis of MPM on cytomorphological grounds alone is challenging, especially when reactive atypical mesothelial cells are present. The production of cell blocks is now encouraged by several guidelines in the diagnostic workup of MPM cases, as it allows for easier performance of biomarker assessment and specimen storage. Several ICC and IHC markers are now available to help differentiate malignant versus reactive atypical mesothelium. Some of these biomarkers (eg, BAP1 and p16 homozygous deletion with FISH) are accepted by international guidelines to be incorporated within routine diagnostic panels. Importantly, all of these markers have been shown to be reliable in cell block preparations. However, while newer biomarkers such as BAP1, p16, and MTAP all have a diagnostic profile of high specificity, they still harbor unsatisfactory sensitivity, particularly for some mesothelioma subtypes. Historical biomarkers of malignancy in mesothelial proliferations such as GLUT1 and IMP3 are still used but are informative only when positive. All of these markers are available for clinical use, with slightly different proposed diagnostic workflows, while other markers such as EZH2 and 5-hmC need to be investigated extensively before they are used routinely. Systematic appraisal of the literature regarding the diagnostic performance of MPM for many of these biomarkers are still sparse and heterogeneous, comprised mostly of studies dealing with histological samples, and fail to perform subgroup analysis. Pleural effusion specimens also offer the possibility of detecting soluble biomarkers of mesothelioma, with mesothelin/SMRP being studied most extensively. Consideration should be given to storing effusion samples for future analysis with new potential biomarkers such as specific miRNAs. However, the best miRNA panel has yet to be fully established. Finally, it appears there are many biomarkers currently available to aid in the diagnosis of mesothelioma, but because none of these markers is to be relied upon alone, they are best used in combination to increase their diagnostic yield.

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