New Markers for Separating Benign From Malignant Mesothelial Proliferations Are We There Yet?

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Context.—The separation of benign from malignant mesothelial proliferations is crucial to patient care but is frequently morphologically difficult.

Objective.—To briefly review adjunctive tests claimed to be useful in this setting and to examine in detail 2 new tests: p16 fluorescence in situ hybridization (FISH) and BRCA1-associated protein 1 (BAP1) immunohistochemistry.

Design.—Literature review with emphasis on p16 FISH and BAP1 immunohistochemistry.

Results.—Glucose transporter-1, p53, insulin-like growth factor 2 messenger RNA–binding protein 3 (IMP-3), desmin, and epithelial membrane antigen have all been claimed to mark either benign or malignant mesothelial processes, but in practice they at best provide statistical differences in large series of cases, without being useful in an individual case. Homozygous deletion of p16 by FISH or loss of BAP1 has only been reported in malignant mesotheliomas and not in benign mesothelial proliferations. BAP1 appears to be lost more frequently in epithelial than mixed or sarcomatous mesotheliomas. Homozygous deletion of p16 by FISH is seen in pleural epithelial, mixed, and sarcomatous mesotheliomas, but it is much less frequent in peritoneal mesothelioma. The major drawback to both these tests is limited sensitivity; moreover, failure to find p16 deletion or BAP1 loss does not make a mesothelial process benign.

Conclusions.—In the context of a mesothelial proliferation, the finding of homozygous deletion of p16 by FISH or loss of BAP1 by immunohistochemistry is, thus far, 100% specific for malignant mesothelioma. The limited sensitivity of each test may be improved to some extent by running both tests.


Whether a patient has a malignant mesothelioma or a benign mesothelial reaction is a distinction that is crucial to patient care and prognosis, but one which, on biopsy or effusion cytology specimens, is sometimes exceedingly difficult. The basic morphologic features that enable this separation have been reviewed in detail.1–4 While morphology is diagnostic in many instances, a significant proportion of cases have equivocal morphology, making it necessary to resort to various ancillary (largely immunohistochemical) tests. This article considers the use of ancillary tests for separating benign from malignant mesothelial proliferation.

A variety of immunohistochemical stains have been claimed to be useful in this context and 5 of them have been reported in multiple studies. Of these, glucose transporter 1 (GLUT-1), a putative marker of malignancy, probably makes the most mechanistic sense, since it is increased in a very wide variety of malignant tumors. Unfortunately, GLUT-1 staining of mesothelial cells is confounded by staining of red cells, making interpretation difficult; and, more important, although some authors5–7 claim that GLUT-1 staining is never seen in benign mesothelial reactions, our experience and that of others8–11 has been positive for GLUT-1.

p53 is a critical tumor suppressor; it has a role in genomic integrity, cell cycle arrest, and apoptosis. Many types of malignancies have mutated p53, suggesting, at first glance, that p53 immunostaining might be helpful in the diagnosis of mesothelioma. However, most of the reports describing p53 as a marker of mesothelioma predate the realization that some degree of p53 staining, typically fairly weak and patchy, can be seen in any process with proliferating cells, the so-called wild-type p53 staining pattern. Judging by other types of malignancies, the decision that abnormal p53 staining is present requires either strong staining of a very large proportion of cells (>50% or >75%) in a tumor, or staining of no cells at all.12,13 Using these guidelines for interpretation, p53 immunohistochemistry is a strong predictor of p53 mutational status.14

In fact, p53 is infrequently mutated in mesotheliomas,15 and what has been described as positivity for p53 in...
mesotheliomas is, in retrospect, probably a mixture of a (mostly) normal and an occasional abnormal immunophilotype. For example, in one older study it was reported that 20 of 27 mesotheliomas (74%) showed staining of fewer than 25% of cells and the staining was of low intensity, which is the typical picture of normal p53 expression, while only 2 mesotheliomas demonstrated staining of more than 75% of cells. Attanoos et al summarized the p53 immunostaining literature from 1992 to 2001. Seven of 9 reports (78%) failed to find p53 staining in benign reactions, while 2 found staining in 62% and 85% of cases.

In equivocal cases, overall survival can serve as a surrogate marker for the diagnosis of mesothelioma versus a benign reactive mesothelial proliferation. Our own experience is that, in a series of atypical mesothelial reactions, 30% of patients with greater than 10% of cells staining for p53 were alive 5 years after diagnosis, implying that, at least as commonly interpreted, p53 staining is not helpful.

Insulin-like growth factor 2 messenger RNA–binding protein 3 (IMP-3) is an oncofetal protein found in fetal tissues and many types of malignant neoplasms. Some have reported that IMP-3 is only seen in mesotheliomas, but we found, as have others, that like GLUT-1, some proportion of benign reactions stain for this marker.

Two other stains that have been repeatedly examined are epithelial membrane antigen (EMA), claimed to mark mesotheliomas, and desmin, claimed to mark benign reactions. The use of these stains is purely empiric because there is no obvious underlying molecular or functional logic behind the results. Attanoos et al reviewed the literature on desmin staining up to 2003; the summarized numbers indicate that while desmin shows positivity more frequently in benign reactions, it also shows positivity in a proportion of mesotheliomas, as much as 56% in 1 study. King et al summarized the literature from 1979 to 2005, concluded that the specificity of desmin was 83%. More recently, Minato et al found that while desmin stained mostly benign reactions, a small proportion of mesotheliomas also stained positively. When we looked at the 5-year survival of patients with a biopsy diagnosis of atypical mesothelial hyperplasia, only 50% of those with positive staining for desmin were alive; suggesting that desmin staining fails to predict anything.

Epithelial membrane antigen is just as problematic. Attanoos et al reviewed 6 studies and, adding their own, found that some proportion of benign reactions were reported to stain in all but 1 report. King et al concluded that the specificity of EMA was 89%. Reports that support or refute the specificity of EMA continue to be published. We found that approximately 30% of patients with a diagnosis of atypical mesothelial hyperplasia and positive EMA staining were alive at 5 years after diagnosis.

Our point here is not to completely review the now rather extensive literature on these markers, but to point out that, for any of them, it is easy to find reports of “wrong” staining. Interpretation issues aside, the real problem with all of these stains is that, when they work at all, they work in a statistical sense; that is, overall, a greater proportion of mesotheliomas than benign reactions stain positively for GLUT-1, p53 (but that staining may be wrongly interpreted), IMP-3, and EMA, and the reverse is true for desmin. Were this a matter of dealing with one type of malignancy versus another, some of these markers might be useful. But there are too many benign or malignant processes that stain the “wrong” way to use them in an individual case when the distinction is between a tumor that in the pleura is rapidly and universally fatal and in the peritoneum requires extensive debulking and hot intraperitoneal chemotherapy, and a benign reaction that necessitates only watchful waiting and patient reassurance.

Against this background, 2 new markers have emerged that have molecular logic, and thus far, appear to have 100% specificity for separating benignity from malignancy. p16INK4a (also known as cyclin-dependent kinase inhibitor 2A [CDKN2A], referred to here as “p16”) is a member of the inhibitor of cyclin-dependent kinase 4 (INK4) family of cell cycle regulatory proteins that bind to and usually inhibit D-type cyclin-dependent kinases. p16 is a tumor suppressor gene; the normal action of its gene product is to arrest the cell cycle in G1. Conversely, the functional result of p16 deletion is enhanced cell proliferation. Loss of function via homozygous deletion, hypermethylation, or mutation of p16 has been described in a variety of human tumors.

Deletion of the 9p21 region is very common in malignant mesotheliomas, typically resulting in loss of p16 (CDKN2A) and its splice variant p14, p15 (CDKN2B), and methylthioadenosine phosphorylase (MTAP). Illei et al reported that loss of p16 gene could be detected by fluorescence in situ hybridization (FISH) in effusion cytology specimens of mesotheliomas, and Chiosea et al were the first to show that the same phenomenon could be observed in formalin-fixed, paraffin-embedded tissue.

Table 1 lists reports that have examined mesotheliomas and/or benign mesothelial reactions, using FISH for p16 in tissue sections and/or effusion cytology specimens. Thus far, with roughly 220 benign reactions reported, there are no instances of benign reactions that show homozygous loss, so the specificity of this approach is 100%, which makes it a very attractive test. An additional benefit of p16 FISH analysis is that it conveys prognostic information: loss of p16 is associated with more aggressive disease.

The 2 major drawbacks of p16 are the requirement to use FISH, because p16 immunohistochemistry does not give the same results, and, more problematic, the issue of sensitivity. As shown in Table 1, for pleural epithelial mesotheliomas, sensitivity ranges from approximately 45% to 85% (average, 64%). For peritoneal epithelial mesotheliomas sensitivity is worse, ranging from approximately 14% to 50% (mean, 38%). In some reports sarcomatous mesotheliomas fare better, with deletion reported in up to 100% of cases, but in other reports the proportion of p16-deleted sarcomatous tumors is much lower. Thus, while homozygous loss of p16 by FISH is diagnostic of malignancy in this context, failure to find loss of p16 does not rule out a mesothelioma.

BRCA1-associated protein 1 (BAP1) is a nuclear ubiquitin hydrolase that is believed to function as a tumor suppressor. It controls a number of functions including DNA repair and expression of genes related to cell cycle and cell proliferation; it can also induce cell death. Bott et al were the first to report BAP1 somatic mutations in mesotheliomas. At the same time, Testa et al described families with mesothelioma and germline mutations of BAP1. Further investigation of kindreds with germline mutations have shown an increased incidence of cutaneous and ocular melanomas, as well as benign melanocytic skin tumors, renal cell carcinomas, and probably other types of cancers as well.

The exact incidence of germline BAP1 mutation–associated mesotheliomas is uncertain, but it is probably on the order of 1% to 2% of cases.
BAP1 somatic mutations appear to be common in mesotheliomas, and, most important from the point of view of diagnosis, the presence of biallelic mutations in BAP1 determined by molecular analysis correlates with loss of immunohistochemical staining, while cells expressing at least 1 wild-type copy of BAP1 retain immunohistochemical staining. Thus far, there is considerable variability in the reported frequency of BAP1 protein loss but it appears that epithelial mesotheliomas lose BAP1 more frequently than do mixed or sarcomatous forms (Table 2), a point that needs to be kept in mind in deciding whether p16 FISH or BAP1 immunohistochemistry is more likely to be productive in a morphologically problematic case.

An added attraction of BAP1 immunohistochemistry is that stromal and inflammatory cells will always stain, thus providing a built-in control. Even in mesotheliomas arising in the setting of germline BAP1 mutation, nontumor cells will express a single wild-type copy and hence produce a positive immunohistochemistry result. To show loss of BAP1 immunoreactivity, both copies of BAP1 must be mutated, either by a combination of germline and somatic events as in BAP1 cancer syndrome, or by 2 somatic events in sporadic cancers.

There are relatively little data on BAP1 in benign reactions, but the data that exist are encouraging (Table 3). We found that none of 53 reactive mesothelial proliferations showed loss of BAP1 by immunohistochemistry. Similarly, none of 23 benign cases stained by the French Mesothelioma Panel had loss of BAP1. Cigognetti et al reported that 2 of 27 apparently benign reactions (7%) showed loss of BAP1, which at first glance would decrease the value of the test, but on follow-up these 2 cases turned out to be mesotheliomas, whereas 0 of 25 truly benign reactions had BAP1 loss. More data are obviously needed, but at least at this point, the specificity of BAP1 loss is 100%.

As is true of p16 FISH, the major drawback of BAP1 immunohistochemical staining relates to sensitivity (Table 2), so that a mesothelial proliferation that retains BAP1 expression may still be malignant. It is possible that a combined approach of both p16 FISH and BAP1 immunohistochemistry will at least partially solve this problem. We found, using a tissue microarray and taking all tumor morphologies and sites, that BAP1 was lost in 7 of 26 cases (27%), p16 in 14 of 27 (52%), and one or the other was lost in 14 of 24 (58%), only a very modest improvement. However, when the analysis was confined to pleural epithelial mesotheliomas, BAP1 was lost in 5 of 9 (56%), p16 in 7 of 12 (58%), and one or the other was lost in 7 of 9 (78%) cases.

Are we there yet? For BAP1 clearly more information is needed on staining of benign reactions, but the data that exist suggest that homozygous loss of p16 by FISH or loss of BAP1 by immunohistochemistry is never seen in benign reactions. The downside of both markers is that they are

### Table 1. Sensitivity of p16 Fluorescence In Situ Hybridization for Homozygous Deletions by Mesothelioma Type and Site and in Benign Reactions

<table>
<thead>
<tr>
<th>Source, y</th>
<th>Epithelial, No. (%)</th>
<th>Mixed, No. (%)</th>
<th>Sarcomatous, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illei et al, 2003</td>
<td>6/7 (86% not broken down by site)</td>
<td>0/19 (0)</td>
<td></td>
</tr>
<tr>
<td>Chiosea et al, 2008</td>
<td>35/52 (67)</td>
<td>5/10 (25)</td>
<td>0/40 (0)</td>
</tr>
<tr>
<td>Monaco et al, 2011</td>
<td>19/27 (70)</td>
<td>21/41 (51)</td>
<td>0/70 (0)</td>
</tr>
<tr>
<td>Krasinskas et al, 2010</td>
<td>Epithelial</td>
<td>9/25 (36)</td>
<td></td>
</tr>
<tr>
<td>Chung et al, 2010</td>
<td>Epithelial</td>
<td>19/42 (45)</td>
<td>0/11 (0)</td>
</tr>
<tr>
<td>Takeda et al, 2010</td>
<td>Epithelial</td>
<td>24/28 (86)</td>
<td></td>
</tr>
<tr>
<td>Wu et al, 2013</td>
<td>Epithelial</td>
<td>10/18 (56)</td>
<td>0/10 (“fibrous pleuritis,” 0%)</td>
</tr>
<tr>
<td>Hwang et al, 2014</td>
<td>Epithelial</td>
<td>5/11 (45)</td>
<td>1/7 (14)</td>
</tr>
<tr>
<td>Sheffield et al, 2015</td>
<td>Epithelial</td>
<td>7/12 (58)</td>
<td>9/19 (48)</td>
</tr>
<tr>
<td>Ito et al, 2015</td>
<td>Epithelial</td>
<td>6/7 (86)</td>
<td></td>
</tr>
<tr>
<td>Tochigi et al, 2013</td>
<td>Sarcomatous</td>
<td>26/32 (81)</td>
<td></td>
</tr>
<tr>
<td>Wu et al, 2013</td>
<td>Sarcomatous</td>
<td>22/22 (100)</td>
<td></td>
</tr>
<tr>
<td>Sheffield et al, 2015</td>
<td>Sarcomatous</td>
<td>4/8 (50)</td>
<td></td>
</tr>
</tbody>
</table>

a Empty cells indicate test not performed.
b All effusion cytology specimens.
c Assumed all epithelial.
d Includes effusion cytology specimens. Sixty-one tumors were epithelial, 3 mixed, 4 sarcomatous, but results by subtype were not broken out in the article.
e Specific site breakdown not provided, but 37 of 40 tumors (92%) were pleural.

Abbreviation: BAP1, BRCA1-associated protein 1.
before proceeding to BAP1 testing and/or p16 FISH.

Thus, it is crucial to confirm, using established immunohistochemical stains, that the process in question is mesothelial before proceeding to BAP1 testing and/or p16 FISH.

One important caveat must also be kept in mind. Loss of BAP1 staining or deletion of p16 by FISH is not specific to mesotheliomas, but can be seen in a variety of malignancies. Thus, it is crucial to confirm, using established immunohistochemical stains, that the process in question is mesothelial before proceeding to BAP1 testing and/or p16 FISH.

References


3. Henderson DW, Reid G, Kao SC, van Zandwijk N, Klebe S. Challenges and controversies in the diagnosis of mesothelioma: part 1, cytology-only diagnosis, efficiency and cost containment. BAP1 test be run first, if it is not lost, then p16 FISH should be tried. One important caveat must also be kept in mind. Loss of BAP1 staining or deletion of p16 by FISH is not specific to mesotheliomas, but can be seen in a variety of malignancies. Thus, it is crucial to confirm, using established immunohistochemical stains, that the process in question is mesothelial before proceeding to BAP1 testing and/or p16 FISH.

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