Carbonic Anhydrase IX (CAIX) Does Not Differentiate Between Benign and Malignant Mesothelium

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ABSTRACT

Objectives: To examine carbonic anhydrase IX (CAIX), a marker of renal cell carcinoma that recently has been described in malignant effusions.

Methods: Pleural and peritoneal fluids with the following diagnoses—reactive (n = 23), carcinoma (n = 17), and “suspicious for mesothelioma” (n = 4)—were immunostained for CAIX, calretinin, Ber-EP4, and MOC31. A tissue microarray of epithelioid (n = 27) and sarcomatoid (n = 8) mesotheliomas and three cases of benign mesothelium were also immunostained for CAIX.

Results: Mesothelial cells in both reactive (18/23) and malignant effusions (18/21) were positive for CAIX (P > .05). In carcinomatous effusions, CAIX expression was restricted to the mesothelial cells. Agreement between CAIX and calretinin expression was present in 89% of cases. In tissues, CAIX was positive in 100% of benign and 91% of malignant mesothelium.

Conclusions: CAIX can be a useful ancillary marker for identifying mesothelial cells. There is no difference in CAIX expression between benign and malignant mesothelium. Caution should be exercised while evaluating for metastasis from renal cell carcinoma.

Carbonic anhydrase IX (CAIX) belongs to a family of zinc-containing metalloenzymes that catalyzes the interconversion of carbon dioxide and bicarbonate.1-3 The carbonic anhydrase family of enzymes has been involved in many physiologic reactions, including respiration, pH regulation, and electrolyte secretion. Furthermore, carbonic anhydrase inhibitors have been studied for their role in the management of pain and obesity, as well as topical applications for the management of glaucoma.1 CAIX is commonly used as one of the markers for renal cell carcinoma.4,5 A recent article noted that immunostaining for CAIX on pleural effusion samples can serve as a complementary tool in distinguishing carcinoma from reactive mesothelial cells.6 In addition, two studies noted CAIX expression in malignant pleural mesotheliomas and in metastatic renal cell carcinoma to the lung.2,7 Furthermore, Ramsey et al7 also observed CAIX immunostaining in one case of mesothelial hyperplasia of the pleura. This raises a couple of questions. First, is CAIX expressed in benign mesothelial cells? Second, can this immunostain reliably be used to distinguish carcinoma or mesothelioma from reactive mesothelial cells, particularly on cytology specimens?

In this study, we examined CAIX expression by immunohistochemistry on cell blocks from pleural and peritoneal fluids with either a benign or malignant (carcinoma and suspicious for mesothelioma) diagnosis. The aim was to determine if CAIX can help distinguish reactive mesothelial cells from malignant cells (epithelial or mesothelial). Furthermore, we also evaluated CAIX expression in tissue sections of mesothelioma, reactive pleurisy, and mesothelium-lined hernia sacs.
Materials and Methods

Case Selection and Cytology Review

Pleural and peritoneal fluids and pelvic washings with an established diagnosis of “reactive,” “carcinoma,” or “suspicious for mesothelioma” were retrieved from the pathology database (2010-2012) after obtaining institutional review board approval from the University of Chicago Hospital. Cases diagnosed as suspicious for mesothelioma on cytology had a confirmed diagnosis of mesothelioma on a concurrent or prior surgical specimen. Only samples with available cell blocks were used in the study. On clinical samples with adequate material, cell blocks were prepared by mixing the specimen centrifugate with an equal amount of a mixture containing 10% formalin solution and 95% ethyl alcohol solution (1:1 ratio). The solution was further centrifuged at 1,800 rpm for 10 minutes. The button of concentrated material was then wrapped in filter paper and processed into a cell block as per standard procedures. The cell blocks were subsequently cut into six 4-μm-thick sections, the first and last of which were stained with H&E stain, and the remaining unstained sections were saved for possible immunohistochemical/special stains if deemed necessary. The H&E-stained sections were re-reviewed by two participating pathologists (V.A. and T.A.) for confirmation of the primary diagnoses. The fluids were categorized into the categories described in Table 1 as per the pathologic and clinical diagnosis.

Benign mesothelium-lined hernia sacs (n = 3) from recent surgical specimens and a mesothelioma tissue microarray (TMA) also were evaluated for CAIX expression. One to three 1-mm tissue replicate cores from epithelioid (n = 27) and sarcomatoid (n = 8) variants of mesothelioma were used for building the TMA as described previously.8

Immunohistochemistry

Sections from the cell blocks were immunostained for a panel of four immunohistochemical stains on the Dako autostainer (Dako, Carpinteria, CA) with appropriately reacting controls as per previously established laboratory protocols. The panel of immunohistochemical stains included CAIX (mouse monoclonal antibody, clone TH22, 1:100 dilution; Novocastra, Newcastle upon Tyne, England), calretinin (rabbit polyclonal antibody, 1:50 dilution; Invitrogen, Carlsbad, CA), Ber-EP4 (mouse monoclonal antibody, clone Ber-EP4, 1:80 dilution; Dako), and MOC31 (mouse monoclonal antibody, clone MOC31; Dako). Tissue sections were deparaffinized and rehydrated as per standard protocols before immunostaining.

Scoring

All immunohistochemical stains were evaluated for the presence or absence of staining. When staining was present, the type of cell reacting (ie, epithelial, mesothelial, or inflammatory cells), as well as the pattern of staining (ie, nuclear, cytoplasmic, or membranous), was noted. For all the fluid specimens, the percent staining scores between calretinin and CAIX were compared. Since the cell blocks were variably cellular and comprised a mixture of inflammatory and mesothelial cells, the percentage of mesothelial cells as estimated by the calretinin stain was considered 100%. This was further categorized as weak (1+), moderate (2+), or strong (3+) staining. The CAIX staining

<table>
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<tr>
<th>Cytology Diagnosis/Clinical Indication or Previous Diagnosis</th>
<th>Type of Fluid, No. of Cases</th>
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<tbody>
<tr>
<td></td>
<td>Pleural (n = 35)</td>
</tr>
<tr>
<td>Reactive&lt;br&gt;Medical liver disease</td>
<td>1</td>
</tr>
<tr>
<td>Medical kidney disease</td>
<td>1</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>4</td>
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<tr>
<td>History of epithelial malignancies</td>
<td>4</td>
</tr>
<tr>
<td>History of mesothelioma</td>
<td>1</td>
</tr>
<tr>
<td>History of hematologic malignancies</td>
<td>3</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>1</td>
</tr>
<tr>
<td>Malignant&lt;br&gt;Adenocarcinoma&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9</td>
</tr>
<tr>
<td>Poorly differentiated carcinoma</td>
<td>7</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Neuroendocrine carcinoma</td>
<td>1</td>
</tr>
<tr>
<td>Urothelial carcinoma</td>
<td>0</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>2</td>
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<sup>a</sup> For the “reactive” fluids, this included four pelvic washes, and for the “suspicious for mesothelioma” category, this included one pericardial fluid.

<sup>b</sup> In this study, adenocarcinoma category includes lung, breast, prostate, ovary, uterus, and thyroid as the primary sites.
performed on the subsequent section was then evaluated as a proportion of the calretinin-stained mesothelial cells. For example, if only 70% of the mesothelial cells appeared to be immunoreactive for CAIX, then that number was considered the percentage of CAIX staining cells. Like calretinin, the intensity of CAIX was also scored on a scale of 1 to 3. A semiquantitative composite score (CompScore) was obtained by multiplying the percentage of positive staining cells and the average intensity of staining. For the “malignant” fluids, the staining pattern of CAIX and MOC31/Ber-EP4 was visually compared without semiquantitative scoring. For the benign fluids, we did not expect to see any Ber-EP4/MOC31–positive cells.

The TMA cores were scored on a 0 to 3 intensity scale for CAIX, and average intensity scores per case were computed. The hernia sacs were primarily evaluated for the presence or absence of staining.

Results

In fluid specimens and in tissue sections, mesothelial cells showed CAIX immunoreactivity in a continuous membranous pattern with minimal cytoplasmic staining Image 1. There was no difference in the percentage or intensity of staining between reactive (CompScore = 117) vs malignant fluids (CompScore = 142) (P = .49) Table 2. CAIX reactivity was noted in 18 of 23 reactive fluids and 18 of 21 “malignant” fluids, which included 17 carcinomatous effusions and four specimens categorized as suspicious for mesothelioma. CAIX immunoreactivity was noted only in the mesothelial cells. Inflammatory cells, macrophages, and epithelial cells were all negative for CAIX. Further examination of the adjoining CAIX-stained section showed that CAIX expression was restricted to the mesothelial cells with no immunoreactivity in the malignant epithelial cell clusters. The presence of epithelial cells in carcinomatous effusions was confirmed by MOC-31 and Ber-EP4 immunostaining. In the fluids categorized as suspicious for mesothelioma, the CAIX expression was noted to be stronger in the atypical/malignant mesothelial cells (Image 1) compared with CAIX expression of benign mesothelial cells. When scoring was dichotomized as present vs absent, CAIX was in concordance with calretinin in 89% of cases. However, when compared on a per-case basis, CAIX CompScores were much lower than those of calretinin Figure 1. The overall CompScores were low due to either a lower percentage of cells reacting or a lower intensity of CAIX expression.

Unlike the fluid specimens, CAIX expression in tissue sections of benign and malignant mesothelium was stronger and more uniform (Figure 1). Immunoreactivity was present in 26 of 27 epithelioid mesotheliomas and six of eight sarcomatoid mesotheliomas. There was no difference in the pattern or intensity of staining between epithelioid (mean, 2.68; 95% confidence interval [CI], 2.39-2.97) and sarcomatoid (mean, 1.78; 95% CI, 0.81-2.74) cases. All three hernia sacs showed uniform, strong CAIX reactivity of the overlying mesothelium. In contrast to the fluids, in tissue sections the staining of the benign mesothelium was comparable to that of the malignant mesothelium.

Table 2

<table>
<thead>
<tr>
<th>CAIX Staining, No. of Cases</th>
<th>CompScore, Mean (SD)</th>
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<tbody>
<tr>
<td>Reactive</td>
<td>5 18 117 (96)</td>
</tr>
<tr>
<td>Malignant</td>
<td>3 18 142 (117)</td>
</tr>
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CompScore, composite score.

| Figure 1 | Composite scores (CompScores) for calretinin and carbonic anhydrase IX (CAIX) in reactive (A) and malignant (B) effusions. Each line represents a single patient with corresponding calretinin and CAIX CompScores (see Materials and Methods for description of CompScore). In both kinds of effusions, in most cases, CompScore for CAIX is lower than that for calretinin.
Carbonic anhydrase IX (CAIX) immunoreactivity in cell blocks and tissue sections of benign and malignant mesothelium. Note the intense and strong membrane reactivity for CAIX in malignant mesothelium in effusions and in tissue microarray (B, C). The number of malignant mesothelial cells highlighted by CAIX roughly corresponds with calretinin staining (A). In contrast, scattered mesothelial cells stain for CAIX in benign effusions (D). Calretinin-stained benign effusion is not shown. However, the intensity and percentage of benign mesothelium staining for CAIX in the section from the hernia sac is nearly 100% (E). A–E, ×200.
Discussion

CAIX is one of the several downstream targets activated by hypoxia-inducible factor (HIF).\(^9\) The HIF1 pathway has been implicated in the upregulation of CAIX expression in both clear cell renal cell carcinoma and clear cell papillary renal cell carcinoma.\(^10\) In this regard, CAIX is commonly used as an ancillary immunostain to aid in the diagnosis of metastatic renal cell carcinoma.\(^4\) Recent studies\(^6,11\) showed that higher CAIX levels by immunocytochemical studies and/or by enzyme-linked immunosorbent assays are seen in malignant effusions in contrast to benign effusions. These studies suggest that benign-appearing, CAIX-immunoreactive, “mesothelial-like” cells seen on a Papanicolaou stain are likely to represent atypical/malignant cells. In contrast, in our study, CAIX immunoreactivity was observed in mesothelial cells from both benign and malignant effusions. The mesothelial nature of these cells was confirmed by direct comparison with a calretinin immunostain performed on an adjacent section. In contrast, carcinoma cells in malignant effusions and inflammatory cells were nonreactive for CAIX.

In general, the CAIX-stained cells corresponded with the calretinin-stained mesothelial cells, but the number of mesothelial cells highlighted by CAIX was fewer. Furthermore, the intensity of the staining for benign mesothelial cells was less than that for the atypical/malignant mesothelial cells. This phenomenon was observed only in the fluids and not in the tissue sections, where the benign mesothelium and malignant mesothelioma had similar staining patterns and intensity. We hypothesize that this could represent an artifact of cell block preparation and the use of alcohol in the CytoLyt (Cytyc, Boxborough, MA), which could potentially have a deleterious effect on certain immunomarkers, although the rest of the immunohistochemical stains in our study were not affected.\(^12,13\) We also speculate that atypical/malignant mesothelial cells have more rigid cell walls than do benign ones, which make them more resistant to centrifugation and cell block preparation, resulting in better membranous immunostaining for CAIX. We believe that this is a limitation of this study and can be adequately addressed by use of alternative fixatives or cell block preparation methods. The ideal method would be to stain the sections using a “double-stain” cocktail of CAIX and calretinin, thereby assessing the number of mesothelial cells that coexpress both markers. In concordance with our findings, Ramsey et al\(^7\) note that CAIX is a sensitive marker for mesothelial hyperplasia, adenomatoid tumors, and mesotheliomas.

A potential limitation of the present study is the lack of inclusion of effusion samples with a diagnosis of metastatic renal cell carcinoma. Small numbers and nonavailability of cell blocks precluded us from including this subset of specimens. However, sufficient literature supports the expression of CAIX in renal cell carcinoma. Presumably, CAIX expression is retained even in metastatic lesions of renal cell carcinoma.\(^7,14\) Considering that benign mesothelial cells express CAIX, there is a need to exercise caution in interpreting CAIX-positive cells in fluid specimens in patients with a history of renal cell carcinoma.

In case of TMAs, most of the mesotheliomas showed strong immunoreactivity for CAIX. Surprisingly, six of eight sarcomatoid variants showed strong staining in CAIX-positive cases, suggesting that including CAIX along with other mesothelioma markers\(^15,16\) can greatly improve the sensitivity in differentiating them from other spindle cell neoplasms of the lung and/or pleura. This is rather surprising considering the low sensitivity of many conventional mesothelial markers in sarcomatoid mesotheliomas. However, this finding might require further corroboration on additional larger case series.

In summary, CAIX can be used as an ancillary marker for identifying cells of mesothelial lineage regardless of whether they are benign or malignant. In tissue sections, both epithelioid and sarcomatoid variants showed similar immunoreactivity for CAIX. CAIX could therefore be potentially used in conjunction with other mesothelial markers for diagnosing sarcomatoid variants of mesothelioma since these are notoriously known for their inconsistent expression for calretinin or WT-1.\(^16\) Although there is no difference between CAIX expression in benign and malignant mesothelium in tissue sections, the reduced concordance with calretinin on fluid specimens suggests that CAIX cannot solely be used as a marker of mesothelial differentiation. In conclusion, benign mesothelial cells in effusion samples express CAIX. This could represent a serious pitfall if CAIX is used as a single marker to support the diagnosis of renal cell carcinoma in effusion specimens.

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References


