Immunohistochemical Expression of Osteopontin in Epithelioid Mesotheliomas and Reactive Mesothelial Proliferations

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Key Words: Osteopontin; Mesothelioma; Immunohistochemistry

Abstract

The morphologic distinction of epithelioid mesothelioma from a reactive mesothelial proliferation can be difficult. Recent studies have demonstrated that serum osteopontin levels are increased in patients with mesotheliomas. We sought to determine if osteopontin expression is diagnostically helpful in distinguishing epithelioid mesothelioma from reactive mesothelial proliferations. We studied 7 cases of epithelioid mesothelioma and 20 cases of reactive mesothelial proliferations by immunohistochemical analysis using standard technique. All 7 mesotheliomas and 19 of 20 reactive mesothelial proliferations showed osteopontin expression.

Osteopontin expression is not restricted to malignant mesothelial proliferations, and immunohistochemical analysis for osteopontin is not helpful in determining reactive vs malignant mesothelial proliferations. The reported usefulness of osteopontin as a serum tumor marker for mesothelioma may be due to differences in the amount or character of secreted protein in malignant mesothelioma compared with reactive mesothelial proliferations.

Malignant mesothelioma is a tumor of mesothelial cell origin, which lines serosal surfaces such as the pleural, peritoneal, pericardial, and testicular cavities. Pleural malignant mesotheliomas are strongly associated with previous asbestos exposure. The prognosis for pleural malignant mesotheliomas is poor, with a median survival of less than 12 months after diagnosis. Tissue pathology remains the “gold standard” of diagnosis of mesothelioma. The grave prognosis and medicolegal issues that are engendered by a diagnosis of malignant mesothelioma place pathologists in a critical and often difficult position as a clinical diagnostician. The differentiation of mesothelioma from carcinoma of nonmesothelial origin has been made somewhat easier during the last several years by the development and clinical characterization of immunohistochemical panels.

An equally problematic diagnostic dilemma is the differentiation of a malignant mesothelial process from a reactive one. Reactive mesothelial proliferations may show high cellularity, cytologic atypia, necrosis, papillary excrescences, and entrapment (“pseudoinvasion”; benign mesothelial cells entrapped within organizing pleuritis). Conversely, malignant mesotheliomas may appear bland. Ancillary studies are of little to no value in this scenario, and the distinction remains a clinicopathologic one, wherein the pathologic parameter is morphologic assessment by standard H&E light microscopy.

Osteopontin is a phosphoprotein with a variety of physiologic roles, including anchoring osteoclasts to bone mineral matrix, functioning as a normal component of elastic fibers of skin and aorta, and acting as a protein ligand of CD44. It has also been shown to be expressed in a variety of carcinomas, including lung, breast, and prostate. A recent study showed that patients with pleural malignant mesotheliomas...
had significantly higher osteopontin levels than those in a noncancer group with a history of asbestos exposure. Expression of osteopontin in pleural malignant mesotheliomas in this cohort was demonstrated by immunohistochemical staining, and lung parenchyma and adjacent normal pleura were reportedly negative.22

Given the recent findings linking increased serum osteopontin levels and malignant mesothelioma, we sought to determine if immunohistochemical osteopontin expression is diagnostically useful in distinguishing epithelioid mesothelioma from reactive mesothelial proliferations in recent material from our department’s case file. Differential expression would suggest that immunohistochemical analysis using the osteopontin antibody may be a useful ancillary study when the distinction is difficult by histomorphologic examination alone.

Materials and Methods

Approval for this research was granted by our local institutional review board. A total of 7 cases of epithelioid mesothelioma (2 autopsy cases, 5 surgical biopsy and resection specimens) and 20 cases of reactive or “atypical” mesothelial proliferations were studied. These cases were extracted from our departmental files using a retrospective SNOMED (Systematic Nomenclature of Medicine) terminology search in our laboratory information system (CERNER, Kansas City, MO). The diagnostic H&E-stained slides were reviewed from each case, and clinical follow-up was obtained in the cases of reactive or atypical mesothelial processes to ensure that none of the patients developed malignancy of the pleura up to the time of this study. Immunohistochemical studies were also reviewed for accuracy of interpretation in the cases of mesothelioma.

Immunohistochemical analysis was subsequently performed on formalin-fixed, paraffin-embedded tissue sections with antiosteopontin antibody (mouse, monoclonal, clone AKm2A1, Santa Cruz Biotechnology, Santa Cruz, CA). A citrate buffer microwave pretreatment was performed on unstained paraffin sections, and osteopontin antibody was applied at a 1:100 dilution using the DAKO Autostainer (DAKO, Carpinteria, CA). The detection system used was the DAKO EnVision Plus Polymer System. The chromogen used was DAB Plus (DakoCytomation, Carpinteria, CA). An epithelioid mesothelioma demonstrating robust staining with the osteopontin antibody was selected as a positive control sample for all runs, and negative control experiments were performed appropriately.

Expression of osteopontin in malignant and reactive mesothelial cells was evaluated independently by 2 pathologists (D.T. and J.A.W.) using a semiquantitative scale denoting the extent of staining (percentage of the cells of interest that stain with osteopontin antibody). The scoring system applied was as follows: 0, no cells staining; 1, fewer than 25% of cells staining; 2, 25% to 50% of cells staining; and 3+, more than 50% of cells staining. For discrepancies, the cases were reexamined at a 2-headed scope, and the staining score was reached by consensus.

Results

The individual scoring of osteopontin expression and patient age and sex data are listed in Table 1. All 7 mesotheliomas and 19 of 20 reactive mesothelial proliferations showed cytoplasmic osteopontin expression Table 2. Positive osteopontin staining was observed in a granular cytoplasmic pattern without membrane or Golgi accentuation. Nuclear staining was not observed. A staining distribution of 2+ or greater was demonstrated by 7 (100%) of 7 mesotheliomas and 13 (65%) of 20 reactive mesothelial proliferations Table 3. In general, the intensity of the expression was subjectively greater in the malignant mesothelioma group Image 1 than in the reactive mesothelial group Image 2 and Image 3.

<table>
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<th>Table 1</th>
<th>Clinical Data and Osteopontin Expression Scores*</th>
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<td>Case No./Sex/Age (y) Date of Procedure Score</td>
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* The osteopontin scoring system was as follows: 0, no cells staining; 1+, <25% of cells staining; 2+, 25%-50% of cells staining; 3+, >50% of cells staining.

1 Mean age, 68.1 years; men, 71%.

2 Mean age, 45.0 years; males, 75%.
Discussion

Osteopontin expression has been observed in a variety of tumors, and recently it has been shown that significantly higher serum osteopontin levels are present in patients with mesothelioma compared with patients without mesothelioma with previous asbestos exposure.22 The implications of these findings are significant because they may provide a noninvasive means of surveillance for people with risk factors for mesothelioma. Likewise, if significant differences in the cellular expression of osteopontin exist between malignant and benign mesothelial proliferations, antiosteopontin immunohistochemical analysis may serve as an ancillary diagnostic tool in the differentiation of malignant from benign mesothelial proliferations in biopsy material.

In our study, we showed that osteopontin expression was not restricted to malignant mesothelial proliferations. All 7 mesotheliomas stained with osteopontin antibody, and all but 1 case of reactive mesothelial proliferation showed some proportion of staining. Subjectively, the staining intensity appeared to
be greater in the malignant mesothelial group than in the reactive mesothelial proliferation group. However, the presence of easily detectable expression of osteopontin in both groups does not support the use of this protein for determining reactive vs malignant mesothelial proliferations.

The reported usefulness of osteopontin as a serum tumor marker for mesothelioma may be due to differences in the amount or character of secreted protein in malignant mesothelioma compared with reactive mesothelial proliferations; however, a clear temporal relationship between the appearance of mesothelioma cancer cells and increasing levels of osteopontin has not been demonstrated.\textsuperscript{23} Osteopontin may represent another “tumor marker” that has limited diagnostic usefulness in select clinical settings, similar to proteins such as CA19-9 and α-fetoprotein (AFP). CA19-9 was initially a promising marker for pancreatic adenocarcinomas; however, the level was subsequently shown to be elevated in nonmalignant processes of the pancreas, including acute and chronic pancreatitis.\textsuperscript{24} Similarly, the serum AFP level is elevated in most hepatocellular carcinomas; however, the expression is quite variable and serum AFP elevations occur in noncancer conditions such as hepatitis and cirrhosis.\textsuperscript{25}

Our study has important limitations. First, we studied a relatively small number of cases, and it is possible a larger data set may show significant differences in the expression of osteopontin between reactive and malignant mesothelial processes. Second, we chose not to quantitate the intensity of staining. Subjective determination of staining intensity is problematic because there is significant interobserver variability, and intensity varies with technique. If real differences in protein expression exist between reactive and malignant mesothelial proliferations, other studies will be needed to address this. Third, our immunohistochemical techniques and materials differed from those used by Pass et al.\textsuperscript{22} We used a mouse monoclonal antibody from Santa Cruz Biotechnology, whereas Pass et al\textsuperscript{22} used a different clone supplied by Vector Laboratories (Burlingame, CA); the monoclonal antibody produced by Vector was no longer available at the time of our study. The 2 antibodies may recognize different epitopes of the osteopontin protein. In addition, our study used a different antigen-retrieval method, which may be more sensitive.\textsuperscript{26} Finally, we stained whole tissue sections, whereas Pass et al\textsuperscript{22} studied 2-mm cores of tissue. It is possible that some of our reactive mesothelial proliferation cases that showed partial staining for osteopontin would be interpreted as “negative” if smaller fragments of tissue were examined.

Although there may be some promise for the usefulness of serum osteopontin as a biomarker, use of the osteopontin antibody is not useful for distinguishing epithelioid mesotheliomas from reactive mesothelial proliferations.

References


