Diagnostic and Biological Implications of Mel-CAM Expression in Mesenchymal Neoplasms

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ABSTRACT
Mel-CAM (previously MUC18) is an integral membrane glycoprotein involved in heterophilic intercellular adhesions. Mel-CAM is expressed specifically in certain normal mesenchymal tissues, including smooth muscle, endothelium, and Schwann cells. As a member of the immunoglobulin supergene family of cell adhesion molecules (CAMs), Mel-CAM may play a pivotal role in the normal differentiation and functional activity of these tissues. To determine the distribution of Mel-CAM in mesenchymal neoplasms and to investigate its potential role as a factor in tumor progression, we evaluated a spectrum of mesenchymal neoplasms by immunohistochemistry using a Mel-CAM-specific polyclonal antibody on formalin-fixed tissues. Mel-CAM positivity was observed in 5 (100%) of 5 leiomyomas, 29 (91%) of 32 leiomyosarcomas, 5 (10%) of 5 hemangiomas, 5 (100%) of 5 angiosarcomas, 3 (100%) of 3 Kaposi's sarcomas, 8 (100%) of 8 schwannomas, 10 (100%) of 10 neurofibromas, 0 (0%) of 8 malignant peripheral nerve sheath tumors, 2 (15%) of 13 malignant fibrous histiocytomas, 0 (0%) of 8 fibrosarcomas, 0 (0%) of 7 synovial sarcomas, and 0 (0%) of 6 liposarcomas. These results show that Mel-CAM is expressed consistently in neoplasms of smooth muscle and vascular origin, and that immunostaining for Mel-CAM may serve as a useful adjunct in differentiating leiomyosarcomas, angiosarcomas, and Kaposi's sarcomas from other spindle cell neoplasms. Furthermore, the observation that Mel-CAM is expressed consistently in schwannomas and neurofibromas but not in malignant peripheral nerve sheath tumors implicates Mel-CAM as a potential modulator of malignant transformation in peripheral nerve tumors.

INTRODUCTION
CAMs are important in cell-cell and cell-matrix adhesion, mediating the complex interactions between the cell and its microenvironment (1). Thus, CAMs are thought to play important roles in modulating the differentiation and architectural stability of embryonic and adult tissues (2). Conversely, inappropriate or aberrant expression of CAMs may contribute to the progression of certain neoplasms, mediating, in part, such complex processes as invasive tumor growth and metastatic dissemination (1, 3–6). Indeed, alterations in expression and function of adhesion molecules have been identified now in a wide range of human cancers. The detection of cell type-specific adhesion molecules may be useful in diagnosing certain neoplasms and in predicting their biological behavior.

Mel-CAM, known previously as MUC18, is a newly recognized CAM belonging to the IgSf (7, 8). Nucleotide sequence analysis shows significant homology between Mel-CAM and other members of the IgSf involved in cell adhesion, including NCAM, BEN, ICAM-1, and DCC (9–12). Mel-CAM is an integral membrane glycoprotein with an apparent Mr of 113,000. It contains five immunoglobulin-like domains, and its cytoplasmic domain contains several protein kinase recognition motifs involved putatively in cell signaling (8). Mel-CAM may mediate heterotypic adhesion between cells, but the precise counterreceptor is not known currently (13). Cellular expression of Mel-CAM is regulated epigenetically by its microenvironment (14).

The observation that Mel-CAM is expressed in most malignant melanomas but not in normal epidermal melanocytes suggests that this molecule might serve as a marker of tumor progression (13, 15, 16). Mel-CAM, however, is not expressed in nonneoplastic epithelial tissues thus far evaluated; neither is it expressed in leukemias, lymphomas, or miscellaneous carcinomas arising from the colon and rectum, stomach, pancreas, biliary tract, kidney, endometrium, ovary, thyroid, major salivary glands, breast, and epidermis (13, 16). On the other hand, Mel-CAM is expressed in certain types of mesenchymal cells, including smooth muscle cells, endothelial cells, and Schwann cells; but little is known about its distribution in neoplasms arising from these tissues (13, 16, 17). In the present study, we describe the distribution of Mel-CAM expression in a spectrum of mesenchymal neoplasms. Given the tissue-specific expression of Mel-CAM, the detection of Mel-CAM in tissue samples may be potentially useful in recognizing certain mesenchymal neoplasms and in providing insight into the biological behavior of these neoplasms.

MATERIALS AND METHODS

Tissue Samples. We evaluated 110 formalin-fixed and paraffin-embedded tissue samples for Mel-CAM immunoreac-

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2 The abbreviations used are: CAM, cell adhesion molecule; IgSf, immunoglobulin supergene family; MPNST, malignant peripheral nerve sheath tumor; MFH, malignant fibrous histiocytoma.

3 I. M. Shih, D. Speicher, T. L. Wang, and M. Herlyn. Mel-CAM is a novel Ca2+-independent cell adhesion molecule involved in cell-cell interactions, submitted for publication.
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Table 1 Mel-CAM immunoreactivity in various mesenchymal neoplasms

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Pattern of staining (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2+</td>
</tr>
<tr>
<td>Smooth muscle tumors</td>
<td></td>
</tr>
<tr>
<td>Leiomyoma (n = 5)</td>
<td>80</td>
</tr>
<tr>
<td>Leiomyosarcoma (n = 32)</td>
<td>72</td>
</tr>
<tr>
<td>Primary (n = 25)</td>
<td>69</td>
</tr>
<tr>
<td>Metastatic (n = 9)</td>
<td>78</td>
</tr>
<tr>
<td>Vascular tumors</td>
<td></td>
</tr>
<tr>
<td>Hemangioma (n = 5)</td>
<td>100</td>
</tr>
<tr>
<td>Angiosarcoma (n = 5)</td>
<td>100</td>
</tr>
<tr>
<td>Kaposis sarcoma (n = 3)</td>
<td>100</td>
</tr>
<tr>
<td>Peripheral nerve tumors</td>
<td></td>
</tr>
<tr>
<td>Schwannoma (n = 8)</td>
<td>75</td>
</tr>
<tr>
<td>Neurofibroma (n = 10)</td>
<td>50</td>
</tr>
<tr>
<td>MPNST (n = 8)</td>
<td>0</td>
</tr>
<tr>
<td>Fibrous/fibrohistiocytic tumors</td>
<td>0</td>
</tr>
<tr>
<td>MFH (n = 13)</td>
<td>0</td>
</tr>
<tr>
<td>Tumors of synovial tissue</td>
<td></td>
</tr>
<tr>
<td>Synovial sarcoma (n = 7)</td>
<td>0</td>
</tr>
<tr>
<td>Tumors of fat</td>
<td>0</td>
</tr>
</tbody>
</table>

*2+, strong and uniform staining; 1+, weak and focal staining; and 0, absent staining.

Immunohistochemical Staining. To generate a polyclonal antiserum specific for Mel-CAM, a recombinant, full-length Mel-CAM was produced by insect Sf cells infected with recombinant baculovirus. The full-length Mel-CAM (80 μg) was then inoculated into a rabbit. Immunized serum from the same rabbit was collected and used as negative control. To evaluate the specificity of Mel-CAM, the polyclonal antiserum was tested in conjunction with the well-established monoclonal Mel-CAM-specific antibody (monoclonal antibody A32) using a panel of human tissues. This previous comparison found that the polyclonal antibody and monoclonal antibody share identical immunostaining patterns and immunoblotting assays (13).

Five-μm sections were deparaffinized and incubated with the rabbit polyclonal Mel-CAM-specific antiserum (1:1000) using the avidin-biotin peroxidase method as described previously (14). Immunoreactivities were detected with 3,3′-diaminobenzidine chromagen followed by counterstaining with 0.1% hematoxylin. Positive controls consisted of normal vascular endothelia. The endothelium has been found to express Mel-CAM highly (13, 16). For negative controls, the primary antibody was substituted with either preimmunized rabbit serum or Mel-CAM-specific antiserum (1:1000) neutralized previously with an immunoaffinity-purified, soluble extracellular domain of Mel-CAM (final concentration, 50 μg/ml).

The pattern of staining was scored on a three-tier scale. The distribution and intensity of staining were quantified as uniform and strong (2+), focal and weak (1+), or entirely absent (0).

RESULTS

Smooth Muscle Tumors. The results of the immunohistochemical detection of Mel-CAM are summarized in Table 1. Mel-CAM reactivity was characterized by diffuse cytoplasmic and/or membranous staining. Mel-CAM immunoreactivity was observed in the vast majority of smooth muscle tumors. All 5 cases (100%) of leiomyomas demonstrated generalized and intense staining. Among the leiomyosarcomas, immunoreactivity was present in 29 (91%) of 32 cases. In 23 (72%) of the leiomyosarcomas, the pattern of staining was strong and diffuse, whereas in 6 cases (19%), the staining was weak and focal. In those cases of leiomyosarcoma showing focal staining, the immunoreactivity was confined to areas of the tumor characterized by cohesive spindle cell histology (Fig. 1, A and B). Conversely, areas of the tumor formed by discohesive and pleomorphic tumor cells were not immunoreactive (Fig. 2). Aside from the correlation between Mel-CAM immunoreactivity and a cohesive spindle cell morphology, histological grade had no influence on the frequency of Mel-CAM reactivity. Likewise, anatomic location had no influence on Mel-CAM reactivity, and metastatic leiomyosarcomas were as likely to exhibit Mel-CAM reactivity as were primary leiomyosarcomas.

Vascular Tumors. Mel-CAM immunoreactivity was also observed consistently in tumors of vascular origin. Strong and diffuse immunoreactivity was observed in all 5 (100%) hemangiomas (Fig. 3A), in all 3 (100%) Kaposi’s sarcomas, and in all 5 (100%) angiosarcomas (Fig. 3B). In all of the vascular neoplasms evaluated, Mel-CAM staining was strong and diffuse.

Neurogenic Tumors. Mel-CAM immunoreactivity was also observed in all of the benign peripheral neural tumors, which included 8 (100%) schwannomas (Fig. 4A) and 10 (100%) neurofibromas (Fig. 4B). Most of the schwannomas were strongly and diffusely immunoreactive. Immunoreactivity in the neurofibromas was more heterogeneous and tended to vary inversely with tumor cellularity. In striking contrast to the benign peripheral neural tumors, Mel-CAM immunoreactivity was not observed in any of the 8 (0%) MPNSTs (Fig. 4C). Indeed, of the specimens from patients with malignant transformation of plexiform neurofibromas, positive Mel-CAM staining was observed in the benign plexiform neurofibromas but not in the areas showing malignant degeneration.

Other Mesenchymal Tumors. Among the other types of mesenchymal neoplasms evaluated, only 2 (15%) of 13 MFHs were found to be immunoreactive for Mel-CAM. The pattern of
staining in these 2 MFHs was weak and focal. Interestingly, the focal areas of Mel-CAM immunoreactivity were also found to be immunoreactive for smooth muscle actin. Thus, in retrospect, these MFHs showing Mel-CAM reactivity may be classified best as leiomyosarcomas with pleomorphic (i.e., discohesive, nonspindled) histologies. Mel-CAM immunoreactivity was not observed in any of the synovial sarcomas [0 (0%) of 7], fibrosarcomas [0 (0%) of 8], or liposarcomas [0 (0%) of 6].

**Positive and Negative Controls.** Mel-CAM immunoreactivity was also observed in various nonneoplastic tissues. In all cases evaluated, strong Mel-CAM positivity was observed in normal endothelium, vascular smooth muscle, and Schwann cells. This positivity served as an internal control (Figs. 2 and 4, arrows). In contrast, staining was not observed when the Mel-CAM antibody was preneutralized with the soluble extracellular domain of Mel-CAM (C; hematoxylin counterstain).

**Effects of Therapeutic Intervention.** To determine whether the loss of Mel-CAM expression observed in some of the smooth muscle neoplasms and all of the MPNSTs could be attributed to therapy, we reviewed the medical histories on this selected group of patients. Radiation therapy and/or chemotherapy did not influence Mel-CAM expression. Moderate or strong Mel-CAM staining was observed in all leiomyosarcomas removed from the patients who received radiation therapy (3 patients), chemotherapy (2 patients), or a combination of radiation and chemotherapy (1 patient) prior to surgical resection of their neoplasms. The 3 leiomyosarcomas that did not express Mel-CAM were from the larger group of 23 patients who did not receive prior therapy, but the differences in the treated and nontreated groups were not significant. Likewise, the loss of Mel-CAM expression in the MPNSTs could not be attributed to treatment effect. Of this group of 8 patients, only 1 received treatment (chemotherapy) prior to surgical resection.

**DISCUSSION**

CAMs play a fundamental role in maintaining normal tissue architecture by modulating the interactions between a cell and its microenvironment (1). Conversely, the disruption of these interactions may play a role in the progression of some neoplasms. Indeed, the importance of these CAMs in modulating local tissue invasion and metastatic dissemination has been implicated along various lines. Molecular studies have shown
that some genetic mutations seen in human cancers actually reduce the expression of certain CAMs (11, 18). In vitro and in vivo studies have shown that experimental modulation of cell adhesion can promote tumor cell growth and invasion (19, 20). Finally, immunohistochemical analysis of human tumors has documented aberrant expression of CAMs in a variety of neoplasms (1). For example, overexpression of the CAM CD44 is noted in invasive gastric carcinomas, and this overexpression is associated with a poorer prognosis (21); integrins are expressed differentially in normal, hyperplastic, and neoplastic prostate and breast epithelia (22, 23); and loss of cadherin expression is associated with a more malignant phenotype for a variety of carcinomas (24, 25).

The IgSf is comprised of a number of transmembrane molecules that have in common a 70–110 segment of amino acids forming a β-pleated sheet, the immunoglobulin structural homology unit (26). Within the IgSf are a group of molecules believed to be important in cell adhesion, including CEA, ICAM-1, VCAM-1, NCAM, PECAM-1, and the DCC gene product. Like other families of CAMs, aberrant expression of these molecules also have been implicated in tumor progression. Loss of NCAM expression, for example, is associated with contact-independent growth of transformed mouse fibroblast cell lines (27). Furthermore, the DCC gene, encoding for an NCAM-like IgSf molecule, is altered in colorectal adenomas and invasive carcinomas frequently (11). Mel-CAM is a recently recognized CAM belonging to the IgSf. The findings that Mel-CAM is expressed in melanomas but not in nonneoplastic melanocytes and that this expression correlates with metastatic potential suggest the potential role of Mel-CAM as a determinant of tumor progression (15, 16).

In nonneoplastic mesenchymal tissues, the expression of
Mel-CAM is limited to smooth muscle, endothelium, and Schwann cells, but it is not known whether altered expression of Mel-CAM contributes to the cellular disohesion, stromal infiltration, and metastatic dissemination characterizing their malignant counterparts (16, 17). In this study, we evaluated the expression of Mel-CAM in a variety of types and grades of mesenchymal neoplasms using a polyclonal antibody on paraffin-embedded and formalin-fixed tumor samples.

We found that the expression of Mel-CAM in mesenchymal tissues is lineage specific. Among the smooth muscle neoplasms that we evaluated, Mel-CAM immunoreactivity was observed in all leiomyomas and the majority of leiomyosarcomas. Immunoreactivity correlated generally with a cohesive and spindle cell morphology (Fig. 1). The three leiomyosarcomas that were not immunoreactive for Mel-CAM consisted predominantly of pleomorphic cells growing in a haphazard fashion (Fig. 2). These observations suggest that Mel-CAM expression may contribute to the fascicular growth pattern characterizing leiomyosarcomas, and that loss of this expression may give rise to an undifferentiated phenotype. Mel-CAM reactivity was also observed consistently in tumors of vascular origin (Fig. 3).

Strong and generalized immunoreactivity was observed in all vascular tumors analyzed, ranging from benign hemangiomas to malignant angiosarcomas and Kaposi’s sarcomas. These findings are consistent with those of Kuzu et al. (18), who used monoclonal antibodies on fresh-frozen tissues to demonstrate that Mel-CAM (i.e., MUC-18) is expressed in most vascular tumors consistently and strongly.

Genetic sequencing data have provided some insight into the molecular mechanisms underlying the tissue specificity of Mel-CAM expression. The promoter region of Mel-CAM contains regulatory sequences that are shared with smooth muscle actin genes, including a CArG box and a c-myc-binding site (8). This finding suggests that the Mel-CAM gene can be activated transcriptionally by determinants that also regulate the expression of smooth muscle actin, and it enables one to predict that normal and neoplastic smooth muscle also will express Mel-CAM. Although we did not attempt to correlate Mel-CAM expression and smooth muscle actin expression for all tumors, we did evaluate the two MFHs that showed focal Mel-CAM positivity. Notably, immunohistochemical staining of these tu-
mors showed an identical distribution of staining for Mel-CAM and smooth muscle actin.

The tissue specificity of Mel-CAM expression for vascular and smooth muscle neoplasms suggests a potential role of this marker as an adjunct in the diagnosis of these neoplasms. We found that Mel-CAM is a highly sensitive marker of malignant vascular and smooth muscle sarcomas but is not expressed in other neoplasms that enter the difficult differential diagnosis of malignant spindle cell sarcomas commonly. Specifically, Mel-CAM immunoreactivity was not detected in any of the MPNSTs, fibrosarcomas, synovial sarcomas, or liposarcomas, and it was detected only focally in a small proportion of the MFHs. Given the finding of focal actin positivity, these two MFHs might be classified best as poorly differentiated leiomyosarcomas (28).

The immunohistochemical detection of Mel-CAM also may provide insight into biological mechanisms that may be important in the progression of certain neoplasms. To address these issues further, we evaluated the differential expression of Mel-CAM in benign mesenchymal tumors and their malignant counterparts. Up-regulated expression of adhesion molecules has been shown to be important in the progression of some tumors. Mel-CAM itself serves as a prime example of the potential importance of up-regulated expression of a CAM in tumor progression: Mel-CAM is expressed in melanomas but not in nonneoplastic melanocytes, and this expression correlates with metastatic potential (13, 15, 16). Although intriguing, the role of up-regulated expression of Mel-CAM could not be addressed adequately for tumors of vascular and smooth muscle origin. Because Mel-CAM is expressed strongly in nonneoplastic smooth muscle and vascular endothelium, it is difficult to know whether Mel-CAM expression is involved in the progression of smooth muscle or vascular tumors. The ability to distinguish between normal and up-regulated expression of Mel-CAM was beyond the limits of our immunohistochemical approach.

Down-regulation of Mel-CAM expression may play a role in the malignant transformation of benign peripheral neural tumors. All of the schwannomas and neurofibromas that we evaluated were strongly and diffusely Mel-CAM positive, whereas none of the MPNSTs demonstrated Mel-CAM positivity. True loss of Mel-CAM staining is underscored by the fact that all MPNSTs that we evaluated arose in association with Mel-CAM-positive plexiform neurofibromas in patients with neurofibromatosis. In some of these cases, the plexiform neurofibromas were separated from the MPNSTs by transition zones showing increased cellularity, with only scattered individual cells demonstrating Mel-CAM positivity. On the basis of our immunohistochemical approach, it is not clear whether the loss of Mel-CAM expression is regulated at the transcriptional, translational, or posttranslational level.

This study used a polyclonal antibody specific for Mel-CAM to evaluate the distribution of this newly recognized CAM in a wide spectrum of mesenchymal neoplasms. We found that Mel-CAM has a limited and specific pattern of expression among mesenchymal neoplasms. Mel-CAM is expressed consistently among neoplasms of smooth muscle, vascular, and neural origin, but not in neoplasms derived from other mesenchymal tissues. At least for neoplasms of smooth muscle lineage, this specificity in part may reflect common regulatory sequences within gene promoters. Mel-CAM immunoreactivity may serve as a useful diagnostic marker in differentiating leiomyosarcomas, angiosarcomas, and Kaposi’s sarcomas from other spindle cell tumors. Furthermore, the loss of Mel-CAM expression in peripheral nerve sheath tumors that have undergone malignant transformation suggests a potential role for Mel-CAM as a modulator of progression for certain mesenchymal neoplasms.

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