CD90 Expression in Atypical Meningiomas and Meningioma Metastasis

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Key Words: CD90; Meningioma; Immunohistochemistry; Flow cytometry

ABSTRACT

Objectives: Meningiomas are slow-growing intracranial/ intraspinal tumors, with a wide range of histopathologic variants. The more aggressive atypical and malignant types can disseminate via the venous system, lymphatic, system, or cerebrospinal fluid, with the lung and pleura being the most common sites of extracranial metastases. A case of metastatic meningioma with high expression of CD90 was spotted during a review of flow cytometry data for lung malignancies. Therefore, we have analyzed CD90 expression in a series of meningioma metastases with their corresponding primary tumors and in a series of 92 primary meningioma tumors.

Methods: In addition to flow cytometry and immunohistochemical analysis of the case, a series of meningiomas and relative metastases has been evaluated for CD90 immunohistochemical expression. Furthermore, an immunohistochemical analysis has been conducted in a tissue microarray, including typical and atypical meningiomas.

Results: CD90 had high expression in three of four cases of metastases and in their corresponding primary atypical meningioma. In addition, CD90 was significantly expressed in atypical rather than in typical meningiomas (P = .003). However, the correlation of CD90 with patient survival reveals only a trend of statistical association with extracranial metastases.

Conclusions: CD90 is a biomarker overexpressed in atypical meningioma, with a potential role in metastatic switch of this tumor.

Meningiomas are slow-growing tumors accounting for 14% to 19% of all primary intracranial and intraspinal neoplasms.1 Different variants of meningioma have been recognized by the World Health Organization (WHO), with meningiothelial, fibrous, and transitional being the most common subtypes, classified as grade I tumors.1 Meningiomas with four or more mitotic figures per 10 high-power fields (hpf) or with the expression of three or more features such as hypercellularity, small cell change, necrosis, loss of pattern of growth, and cell pleomorphism, should be considered “atypical” (WHO grade II) tumors. As stated previously, brain-invasive meningiomas are now considered WHO grade II as well, even if otherwise benign appearing. Meningiomas with 20 or more mitotic figures per 10 hpf or lack of differentiation with a carcinomatous or sarcomatous-like appearance are anaplastic (malignant) WHO grade III tumors. Thus, grade I meningiomas are considered benign tumors, while grade II and III tumors are characterized by a more unfavorable clinical outcome. Other specific histologic subtypes have been associated with more aggressive behavior, including chordoid and clear cell meningiomas (WHO grade II tumors) and papillary and rhabdoid meningiomas (WHO grade III), irrespective of the presence of the histologic features related to atypical and anaplastic meningiomas mentioned above.1

The occurrence of distant metastasis, significantly worsening the prognosis, is generally a rare event, occurring in 5% of grade II and 30% of grade III tumors.2,3 Lung and pleura are the most frequent sites of meningioma metastasis (60%).4 The following organs show a lower frequency of meningioma metastasis: liver, long bones, vertebrae, ribs, mediastinum, and lymph nodes.5,6 The latency from diagnosis of meningioma to distant metastasis development is quite variable, ranging from
a few months to more than 20 years. The metastases and the malignant outcome of meningioma are not easily predictable, even if many biomarkers of an unfavorable outcome have been proposed. Although immunohistochemical analyses of cell proliferation markers, the Ki-67 and p53 labeling index, and CDKN2A deletion analysis seem to be useful tools for evaluating meningioma recurrence or metastasis risk, they are not completely validated.

During a flow cytometry analysis for the detection of cancer stem cells (CSCs) in lung malignancies, a case of metastatic meningioma with high expression of CD90 was identified. CD90 is a protein related to cancer stemness, which is associated with the aberrant activation of the self-renewal machinery that is normally restricted to stem cells, and conflicting results as a prognostic biomarker have been observed in different malignancies. In particular, in tumors of the central nervous system, CD90 has been observed in glioblastomas, where its loss is related to the inability to form neoplastic spheres. Moreover, it has been demonstrated that CD90-expressing cells are mainly clustered around the vessels, suggesting a potential CSC role in the generation of tumor vasculatures, a prerequisite for tumor progression.

In this view, CD90 seemed an interesting marker potentially related to a meningioma metastatic switch. Thus, we have also analyzed a couple CSC markers (CD133 and CD117) and CD90 immunohistochemical expression in four paired primary atypical meningiomas and corresponding metastases and 88 primary meningiomas to evaluate their expression in meningioma and their significance in relation to metastasis progression.

### Materials and Methods

#### Patients and Tissue Microarray Construction

Paraffin blocks and slides from the case reported were recovered from the Pathology Unit Biobank while a series of meningioma extracranial metastases and relative primary tumors were provided by archives of the Pathology Unit of Modena University Hospital and Santa Maria Nuova Hospital of Reggio Emilia. One type of tissue microarray (TMA) was built that included 88 primary meningiomas (M-TMAs). This study was approved by the Medical Ethics Committee of the INT Fondazione Pascale.

Following conventional protocols, we used a TMA device (TMA Galileo CK3500, Integrated System Engineering, Milan, Italy) to build the TMA block containing two different areas (1 mm) from each tumor. In particular, M-TMA included 41 typical meningiomas, 43 atypical meningiomas, and four anaplastic meningiomas with carcinomatous features. The pathologic details of neoplasms included in the TMAs are reported in Table 1 and Table 2.

#### Flow Cytometry Analysis

A specimen of lung meningioma metastasis was obtained from a male patient (case A; age, 68 years) undergoing tumor resection and enrolled into the study in October 2009 in the Division of Thoracic Surgery of the National Cancer Institute of Naples. The diagnosis was based on clinical and histologic parameters.

The lung meningioma specimen was ground, and the cell suspension was filtered through a 40-µm nylon mesh; the cells were then counted and washed in phosphate-buffered saline in 0.1% bovine serum albumin. At least 200,000 cells were incubated with 1 µg/mL of fluorescent-labeled monoclonal antibodies at 4°C for 30 minutes in a dark room. After washing steps, the labeled cells were analyzed by flow cytometry using the FACS ARIA II (Becton Dickinson, Mountain View, CA). The antibodies used were CD44 fluorescein isothiocyanate (FITC) conjugated (BD Pharmingen, Buccinasco, Milan, Italy), CD133/2PE mouse anti–human CD133 (Miltenyi Biotech, Auburn, CA), CD24 FITC conjugated (BD Pharmingen), CD326 phycoerythrin (PE) conjugated (BD Pharmingen), and mouse anti–human CD90 FITC conjugated (Biolegend, Milano, Italy).

Isotypes were used as controls. All data were analyzed using Diva software (Becton Dickinson).

### Table 1

#### Clinicopathologic Features

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CD90−</th>
<th>CD90+</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>18 (51.4)</td>
<td>17 (48.6)</td>
<td>.267</td>
</tr>
<tr>
<td>Female</td>
<td>36 (63.2)</td>
<td>21 (36.8)</td>
<td></td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>60.4 (16.7)</td>
<td>59.6 (15)</td>
<td></td>
</tr>
<tr>
<td>Tumor grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>31 (75.6)</td>
<td>10 (24.4)</td>
<td>.003</td>
</tr>
<tr>
<td>II</td>
<td>19 (40.4)</td>
<td>28 (59.6)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>4 (100)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Ki-67</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LPI</td>
<td>25 (61.0)</td>
<td>16 (39.0)</td>
<td>.669</td>
</tr>
<tr>
<td>HPI</td>
<td>29 (56.9)</td>
<td>22 (43.1)</td>
<td></td>
</tr>
</tbody>
</table>

HPI, high proliferative index; LPI, low proliferative index.

* Values are presented as number (%) unless otherwise indicated.

### Table 2

#### CD90 Expression in Primary Meningioma and Relative Metastasis

<table>
<thead>
<tr>
<th>CD90 Expression, %</th>
<th>Patient</th>
<th>Metastasis</th>
<th>Site</th>
<th>Sex</th>
<th>Age, y</th>
<th>MFS, mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>30</td>
<td>40</td>
<td>Lung</td>
<td>M</td>
<td>65</td>
<td>22</td>
</tr>
<tr>
<td>B</td>
<td>30</td>
<td>50</td>
<td>Bronchus</td>
<td>M</td>
<td>32</td>
<td>7</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>0</td>
<td>Liver</td>
<td>M</td>
<td>58</td>
<td>30</td>
</tr>
<tr>
<td>D</td>
<td>20</td>
<td>15</td>
<td>Lung</td>
<td>F</td>
<td>29</td>
<td>11</td>
</tr>
</tbody>
</table>

MFS, metastasis-free survival.
**Immunohistochemistry**

Immunohistochemical analysis was performed using sections of M-TMA and whole sections of the four paired primary atypical meningiomas and respective metastases. All immunostaining techniques were performed using a previous step of a heat-induced antigen retrieval technique for all antibodies. Before incubation with the anti-CD90 antibody (clone EPR3132, 1:150 dilution; Abcam, Cambridge, England), anti-CD133 (clone AC 133, 1:150 dilution; Miltenyi Biotec), CD117 (clone A4502, 1:500 dilution; Dako, Glostrup, Denmark), and Ki-67 (clone 30-9; Ventana Medical Systems, Tucson, AZ), slides were heated in a pressure cooker for 3 minutes in a solution of EDTA buffer. Diagnosis of metastatic meningioma was further confirmed with immunohistochemical expression of vimentin (clone V9, 1:75 dilution; Novocastra, Newcastle upon Tyne, England), epithelial membrane antigen (EMA) (clone E29, 1:75 dilution; Dako), CD56 (clone 123C3; Ventana Medical Systems), and progesterone receptors (clone PgR 636, dilution 1:50; Dako). After incubation with the antibody, immunodetection was performed with avidin-biotin peroxidase complex kit reagents (MACH2; Biocare Medical, Concord, CA) with diaminobenzidine chromogen as the substrate in all detections and a negative external control have been included. As a positive control, a section of tonsil was used. The negative control was obtained by omitting the primary antibody. Positive cases for vimentin, EMA, CD56, and PgR were recorded when more than 50% of neoplastic cells were immunostained. Positive cases for CD90, CD133, and CD117 were recorded when more than 10% of neoplastic cells were immunostained.

The Ki-67 cutoff was stratified as follows: a value of more than 10% represents a high proliferative index, whereas a value of less than 10% indicates a low proliferative index. Briefly, distinct nuclear staining was recorded as positive. The Ki-67 (MIB-1 LI clone) index was defined as the percentage of immunostained cells divided by the total number of cells in the evaluated area. All counts were performed at a magnification of ×400 using an Olympus BXs1 Microscope (Olympus, Melville, NY). The count was performed in 10 hpf.

In each sample we evaluated the percentage of positive cancer cells by counting the number of positive cells over the total cancer cells in 10 nonoverlapping fields using ×400 magnification. We defined a scoring system to analyze tissue samples based on the percentage of positive cells.

Stained TMA sections were evaluated by two different pathologists (R.F. and A.D.) using uniform criteria. Discrepancies were resolved through simultaneous inspection and discussion of the results. Discrepancies between two cores from the same case were resolved in a joint analysis of the two cores.

Validation of the TMA section has been performed by comparing the immunohistochemical data from TMA sections and whole sections for routine diagnosis for EMA, vimentin, PgR, and Ki-67. A significant correspondence of these antigens in the sections has been observed.

**Statistical Analysis**

The median expression of CD90 (10%) was used as a cutoff score to classify cases as negative or positive for CD90 protein expression. The Pearson χ² test was used, where appropriate, to establish whether there were any relationships between the frequencies of different markers included in this study. Differences were considered significant for P values less than .05.

Disease-free survival (DFS) and metastasis-free survival (MFS) curves were calculated using the Kaplan-Meier method. Statistical significance of associations between individual variables and DFS/MFS was determined using the log-rank test. All statistical analyses were carried out using SPSS version 8.0 software (SPSS, Chicago, IL).

DFS was measured as the time from diagnosis to the occurrence of progression, while MFS was related only to extracranial metastases.

**Results**

**Cell Sorting of the Lung Meningioma Metastasis Sample With Regard to CSC Marker Expression**

Fluorescence-activated cell sorting was performed using a fresh sample of meningioma metastasis to the lung. We analyzed the expression of CD133, CD44, CD24, CD34, CD326, and CD90. The results showed that the mean percentage was 0.9% of CD133-positive cells, 12.1% of CD44-positive cells, 0.2% of CD24-positive cells, 13.0% of CD34-positive cells, and 0.1% of CD326-positive cells. CD90 expression detected by flow cytometry was 23.4%. The flow cytometry analysis with regard to CD90 expression is shown in Image 3.

**Clinicopathologic Features of Patients With Meningioma**

The main clinicopathologic data of the patients with meningioma are reported in Table 1. In particular, of the four patients with distant metastases, three (75%) were men, with a mean age of 46 years. In all cases, the primary tumors were localized in cerebral meninges. The metastases were observed in the liver in one patient and in the lung in three patients, with a mean latency of 17.5 months (Table 2). In this series, 35 patients were male (38%), and the mean age was 60 years.
Image II Immunophenotype of case A meningioma specimens: (A) H&E morphology (×20), (B) H&E morphology (×40), (C) epithelial membrane antigen expression (×40), (D) vimentin expression (×40), and CD90 expression in (E) a primary meningioma lesion (×40) and (F) relative lung metastasis (×40).
Image 2 CD90 expression: (A) primary meningioma (x40) and (B) relative liver metastasis (x40), (C) primary meningioma (x40) and (D) relative lung metastasis (x40), and (E) primary meningioma (x40) and (F) relative bronchus metastasis (x40).
To determine the expression of CD90 and Ki-67 in meningiomas, we performed immunohistochemical and flow cytometry analyses. In a study involving 92 cases, CD90 was expressed in 38 (41.3%) of the cases, with a mostly cytoplasmic pattern. In atypical meningiomas, CD90 expression was significantly higher than in typical ones ($P = .003$).

Fifty-one (51.4%) of the 92 cases were recorded as high proliferative index (HPI) positive. In addition, 22 (43.1%) of the 51 HPI cases showed high CD90 expression. To further validate the concordance of expression, we performed CD90 immunohistochemistry expression on selected whole sections of meningioma samples and compared it with TMA cores.

**CD90 and Ki-67 Expression in Meningioma**

Morphology and immunophenotype related to meningioma and metastasis are represented in Image 1. Image 2 reports the high CD90 expression in two lung metastases and their own primary tumors, but no CD90 expression in a liver meningioma metastasis and its primary tumor (Image 2).

CD90 was expressed in 38 (41.3%) of 92 cases from our primary meningioma series, with a mostly cytoplasmic pattern. The positive cells were distributed mainly around the vessels. In particular, CD90 expression was significantly higher in the cases of atypical meningioma than in typical ones ($P = .003$).

Fifty-one (51.4%) of the 92 cases were recorded as HPI positive Image 4. In addition, 22 (43.1%) of the 51 HPI cases showed high CD90 expression. We performed CD90 immunohistochemistry expression also on selected whole sections of meningioma samples to show the concordance of expression with TMA cores Image 5.
Correlation of CD90 Expression With DFS and MFS in Patients With Meningioma

We calculated the MFS for meningioma samples included in the TMA. We did not discover a statistically significant trend between the expression of CD90 and the DFS (P = .142) in all relapse samples. Instead, we found a statistically significant trend between the expression of CD90 and MFS (P = .094) in meningioma extracranial metastases.

Discussion

The histopathologic criteria for malignancy and potential metastasis development in meningiomas remain at least partly uncertain. Thus, objective parameters have been proposed to better categorize meningioma to predict its aggressive behavior, especially in tumors grade II and higher. Specifically, immunohistochemical analysis of a nuclear protein related to cell proliferation, the Ki-67 and p53 labeling index, and molecular markers such as CDKN2A deletion seem to add useful information about the potential progression of these tumors. The Ki-67 label has been widely used, but no conclusive result as an independent prognostic factor in meningioma has been confirmed.

In this article, we show CD90 overexpression in one case of atypical meningioma lung metastasis, identified through flow cytometry and immunohistochemistry, that has led us to analyze a series of meningioma metastases and their respective primary tumors for evaluating CD90 and other CSC marker expression.

CD90 is a 25- to 37-kDa cell surface glycoprotein expressed in several types of cells, including fibroblasts, blood stem cells, endothelial cells, and adult neurons. Its role has not been further investigated. It seems to be involved in immunologic activity, particularly in T-cell activation, and in nonimmunologic activity, such as cell reorganization and signaling, apoptosis, cell adhesion and migration, and neurite outgrowth modulation. CD90 is also an important biomarker of human hepatic stem/progenitor cells and seems to play a significant role in carcinogenesis. It behaves as a tumor suppressor gene in ovarian cancer and nasopharyngeal carcinoma (NPC). In an ovarian cancer cell line, CD90 is able to upregulate thrombospondin 1 and fibronectin, preventing tumor angiogenesis and metastasis. In NPC, CD90 is generally scantly expressed, and CD90 transfection inhibits the formation of cancer colonies and invasive activities of NPC cells. In contrast with these data, in other cancer histotypes, CD90 is potentially associated with a poor prognosis. Indeed, CD90 loss is significantly related to poor survival rates in patients with neuroblastoma. In addition, CD90-positive cells derived from hepatocellular carcinoma (HCC) cell lines, tumor specimens, and blood samples display tumorigenic capacity, suggesting that CD90 could be considered a CSC biomarker. In a series of 59 patients with HCC, CD90 seemed to be significantly related to poor prognosis. It has been recently demonstrated that CD90-positive gastric cancer cells possess a higher ability to initiate tumors in vivo. In addition, it has been found that ERBB2 expression correlates with higher levels of CD90 expression in high-tumorigenic gastric primary tumor models. Finally, trastuzumab treatment reduces CD90-positive cells in
these tumor masses and suppresses tumor growth when combined with traditional chemotherapy.\textsuperscript{33}

In our study, CD90 overexpression was found in three of four cases of primary atypical meningioma and their respective metastases. In addition, we evaluated CD90 in a series of primary meningiomas, observing a significant association between its expression and atypical histotypes with respect to typical and anaplastic ones. In anaplastic meningiomas, the lack of expression of CD90 is perhaps conditioned by the complete loss of meningoeendothelial differentiation by the tumor cells, with all four analyzed cases having divergent differentiation. Moreover, we did not find significant relationships between the expression of CD90 and Ki-67. Other CSC markers, such as CD133 and CD117, did not provide relevant information for this tumor.

To define the prognostic value of this marker, we also correlated CD90 expression with DFS and MFS in 49 patients with meningioma. No significant association was obtained with all recurrences, only a trend of statistical correlation with extracranial metastases. Therefore, although we cannot define CD90 as a prognostic marker, its overexpression could be used to potentially identify atypical meningiomas with a more aggressive behavior. Its expression could be interpreted as being related to cancer stemness, since CSCs are responsible for tumor growth and differentiation of heterogeneous cell populations within tumors.\textsuperscript{32} In particular, in nervous tissue tumors, CD90 has been demonstrated only in gliomas, particularly in glioblastomas, with an in vitro experiment demonstrating that the loss of CD90 parallels the loss of the ability to form neoplastic spheres, constituted by CD90-positive cells with or without CD133 expression. This has not been observed in meningioma, which is a mesodermal-derived cell tumor.\textsuperscript{12} In addition, the authors have demonstrated in a TMA series of glioblastomas that CD90-positive neoplastic cells were clustered around the vessels, suggesting CD90 not only as a potential prognostic marker for high-grade gliomas but also as a marker for glioma CSCs, residing within endothelial niches, with a potential critical role in the generation of tumor vasculatures via differentiation into endothelial cells.\textsuperscript{12} Also in our series, CD90-positive meningioma cells clustered around vessel spaces, and no cells were positive for CD133, as demonstrated in a part of glioma CSCs. It has to be noted that a unique profile of CSCs does not exist, but CSC biomarkers are characterized by tumor tissue specificity, even varying from one histotype to another in the same organ.\textsuperscript{11} Thus, aggressive CD90-positive meningiomas in our series could also be attributed to the neoplastic stemness, but these data should be investigated further.

In the literature, some scientific data have been published on stemness in meningioma.\textsuperscript{11,34} In particular, Rath et al\textsuperscript{11} describe a tumor-initiating cell population derived from an atypical meningioma, revealing CD133, CD44, and CD166 surface marker expression and the ability to self-renewal and differentiate into mature nervous cell (neuronal and astrocytic) lineages.

In conclusion, CD90 appears frequently to be expressed in meningioma metastases and their own primary tumors, and it is significantly more expressed in atypical rather than in typical meningioma. However, since there is no direct correlation between its expression and outcome in patients with meningioma, we can only speculate about the role of this marker in the metastatic switch of this tumor. Further studies are warranted to clarify the role of CSCs in meningioma development and progression and to define the CSC markers in this neoplasm, including CD90.

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