Utility of CD117 Immunoreactivity in Differentiating Metastatic Melanoma From Clear Cell Sarcoma

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Context.—Clear cell sarcoma is a malignant soft tissue tumor with melanocytic differentiation. Molecular methods are sometimes necessary to identify the unique t(12;22)(q13;q12) translocation and differentiate clear cell sarcoma from melanoma.

Objective.—To determine whether CD117 immunoreactivity may be useful in separating melanoma from clear cell sarcoma.

Design.—We identified 20 tumors listed in our surgical pathology files that were diagnosed as clear cell sarcoma or in which clear cell sarcoma was strongly considered. These were tested for the presence of the t(12;22) translocation by reverse transcriptase/polymerase chain reaction and sequencing from paraffin-embedded tissue. Tumors with a t(12;22) translocation were immunostained with an antibody to CD117 and compared with 16 similarly stained metastatic melanomas.

Results.—Twelve tumors from 9 patients demonstrated t(12;22). No metastatic melanomas demonstrated t(12;22). None of the 12 clear cell sarcomas showed membrane or cytoplasmic staining for CD117. Conversely, 10 (63%) of 16 metastatic melanomas were, at least focally, positive for CD117; this difference was significant (P < .001). Interestingly, 3 tumors in which clear cell sarcoma was initially considered as a diagnosis, but which lacked t(12;22), were also positive for CD117.

Conclusions.—Reverse transcriptase/polymerase chain reaction, performed on paraffin-embedded tissue, is a useful, rapid tool for identifying the presence of t(12;22) in clear cell sarcoma. The CD117 immunoreactivity may prove useful in the differential diagnosis of deep soft tissue or visceral lesions with melanocytic differentiation; positive staining results exclude clear cell sarcoma, but are compatible with metastatic melanoma.

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**MATERIALS AND METHODS**

**Selection of Cases**

Following approval of this study by the Institutional Committee for Human Research, we identified 20 tumors from 12 patients from the files of the Department of Pathology at the University of California, San Francisco, that were either diagnosed as clear cell sarcoma or for which clear cell sarcoma had been strongly considered at the time of diagnosis. The diagnosis was based on (1) evidence of melanocytic differentiation (based on light microscopy, immunophenotype, and ultrastructure), (2) location in deep soft tissue or visceræ, and (3) absence of a primary melanoma. Cytogenetic confirmation of the t(12;22) translocation was available for 1 patient (C9). Sixteen patients with metastatic (soft tissue, regional lymph node, visceral) melanoma were identified in which the diagnosis of clear cell sarcoma might be considered based on large tumor size, histology, immunophenotype, and location, but in which the diagnosis was excluded by a documented primary cutaneous melanoma. All slides were reviewed independently by 2 pathologists (J.J.G. and A.E.H.).

**RNA Preparation**

The RNA was prepared from formalin-fixed, paraffin-embedded tissue using the Optimum FFPE RNA Isolation Kit (Ambion, Austin, Tex) according to the manufacturer's directions. Briefly, blocks were trimmed of excess paraffin and 4 15-μm tissue sections from each block were deparaffinized, dried, digested in Proteinase K for 10 hours at 37°C, combined with RNA extraction buffer, and transferred to filter cartridges. Following washes, the
gel-purified and cloned into pCRII-TOPO (Invitrogen, Carlsbad, Calif), according to the manufacturer’s directions, for automated sequencing. Tumors that did not yield either a 246 or a 186 bp product (types I and II EWS-ATF1 fusions, respectively) were assayed for the type III translocation. Informative tumors were classified as clear cell sarcoma only if the presence of the EWS-ATF1 fusion transcript was confirmed by sequencing.

**Immunohistochemistry**

Immunohistochemical analysis was performed using previously published techniques. Briefly, 4-μm paraffin-embedded sections were deparaffinized, heated in Dako citrate buffer (DakoCytomation, Carpinteria, Calif) for 10 minutes each at 100% power and 20% power, respectively, in a 950-W microwave oven, blocked for endogenous peroxidase, avidin, and biotin, incubated with anti-CD117 (A4502, DakoCytomation), diluted 1:200 at 25°C for 30 minutes, then washed and developed using the LSAB kit (DakoCytomation). Gastrointestinal stromal tumors were used as positive controls for CD117 staining. Slides were scored by both J.J.G. and A.E.H., who were blinded to the diagnosis. Staining for CD117 was interpreted as positive if more than 10% of tumor cells showed strong, specific membrane and/or cytoplasmic staining.

**Statistics**

Tumors that were confirmed as clear cell sarcomas were compared with metastatic melanomas for the presence of CD117 staining using the χ² test. A P value <.05 was considered statistically significant.

**RESULTS**

**RT-PCR and Sequencing Results**

The predicted RT-PCR and nested PCR products from the type I fusion between exon 8 of EWS and exon 4 of ATF1 are depicted schematically in Figure 1, A. The RT-PCR demonstrated the type I EWS-ATF1 fusion transcript in tumors from 9 patients (Figure 1, B and C). None of the tumors demonstrated the type II or III fusions (data not shown). When reverse transcriptase was omitted in simultaneous reactions, no specific amplified products were identified in either the first or subsequent nested PCR reactions (Figure 1, D and E). Tumors from 2 patients remained unclassified because EWS-ATF1 fusion transcripts could not be identified despite repeated attempts from multiple blocks and the presence of an internal control phosphoglucom kinase transcript (Figure 1, F). None of the melanomas tested demonstrated the EWS-ATF1 fusion transcripts. The presence of the type I fusion transcript was identified by sequencing of PCR products (Figure 1, G).

**Clinical Features**

A total of 9 patients (C1 through C9) were identified as having clear cell sarcoma, 3 of whom also had metastases (Table). The mean age of these patients was 44.8 years (range, 19–72 years), and 5 (56%) were women. The most common location for clear cell sarcoma was the lower extremity. The clear cell sarcomas ranged in size from 1.6 to 7.0 cm (average, 4.0 cm). Most tumors were located deep to the superficial fascia, involving tendon or skeletal muscle, but tumors from 3 patients (C3, C4, and C8) also had extension to the subcutaneous adipose tissue. The tumor of patient C9 was largely subcutaneous. The 2 pa-tients with groin involvement showed lymph node metastases, as well as larger deposits of tumor in adipose tissue without identifiable lymph nodes. For the patients with melanoma (M1 through M16), the mean age

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**Summary of Clinical and Immunohistochemical Data**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y/Sex</th>
<th>Site</th>
<th>Size</th>
<th>CD117</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>68/F</td>
<td>Pelvis</td>
<td>6.5</td>
<td>–</td>
</tr>
<tr>
<td>C2</td>
<td>29/F</td>
<td>Groin</td>
<td>4.0</td>
<td>–</td>
</tr>
<tr>
<td>C3</td>
<td>44/M</td>
<td>Foot</td>
<td>4.0</td>
<td>–</td>
</tr>
<tr>
<td>C4</td>
<td>47/F</td>
<td>Leg</td>
<td>7.0</td>
<td>–</td>
</tr>
<tr>
<td>C5</td>
<td>19/M</td>
<td>Foot</td>
<td>2.5</td>
<td>–</td>
</tr>
<tr>
<td>C6</td>
<td>72/F</td>
<td>Foot</td>
<td>1.6</td>
<td>–</td>
</tr>
<tr>
<td>C7</td>
<td>49/F</td>
<td>Hand</td>
<td>2.5</td>
<td>–</td>
</tr>
<tr>
<td>C8</td>
<td>50/M</td>
<td>Leg</td>
<td>7.0</td>
<td>–</td>
</tr>
<tr>
<td>C9</td>
<td>25/M</td>
<td>Axilla</td>
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<td>–</td>
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<tr>
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<td>Groin</td>
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<td>–</td>
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<tr>
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<tr>
<td>M3</td>
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<td>Groin</td>
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<td>+</td>
</tr>
<tr>
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<td>Lung</td>
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<td>M6</td>
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<td>Neck</td>
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<td>+</td>
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<td>M7</td>
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<td>0.8</td>
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<td>Adrenal</td>
<td>2.5</td>
<td>–</td>
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<tr>
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<td>+</td>
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<tr>
<td>U2</td>
<td>74/M</td>
<td>Calf</td>
<td>6.0</td>
<td>+</td>
</tr>
</tbody>
</table>

* A mass lesion was not identified.
was 63.9 years (range, 29–84 years), and 6 (38%) were women. The melanoma metastases ranged in size from 0.8 to 13.0 cm (average, 3.6 cm). Similar to the clear cell sarcoma, the melanomas in the groin or axilla (patients M1 through M4, and M12) were identified in lymph nodes and also as separate subcutaneous masses with extension into underlying fascia or skeletal muscle (patients M3, M4, and M12). The patients with unclassified tumors (U1 and U2) had primary lesions involving structures deep to the superficial fascia, and 1 of these patients (U1) had an axillary lymph node metastasis.

**Pathologic Features**

The tumors with a t(12;22) translocation showed relatively characteristic histologic features of clear cell sarcoma. Tumor cells were typically arranged in nests that were separated by delicate fibrous septa. The clear cell sarcoma tumors typically demonstrated uniform cytromorphology in a given case with polygonal or plump, spindled, clear-to-eosinophilic cytoplasm, vesicular chromatin, and prominent nucleoli. A subset of the metastatic melanomas showed similar cytromorphic features, although several melanomas also demonstrated larger epithelioid cells and more marked pleomorphism. All tumors (clear cell sarcoma and melanoma) were positive for S100 and HMB-45 (data not shown).

**CD117 Immunohistochemistry Results**

More than 10% membrane and/or cytoplasmic staining with CD117 was regarded as positive. The CD117 positive staining was noted in 10 (63%) of the 16 metastatic melanomas (Figure 2, A and B). On the other hand, positive staining was not identified in any of the 12 tumors from the 9 patients with clear cell sarcoma (Figure 2, C through F), a difference that was statistically significant (P < .001). Interestingly, the unclassified tumors, previously diagnosed as clear cell sarcoma and which lacked the EWS-ATF1 transcript by RT-PCR, were also positive for CD117 (Figure 2, G and H).

**COMMENT**

Clear cell sarcoma is a rare sarcoma with melanocytic differentiation that typically involves the extremities of young adults. The clinical and histologic presentation of clear cell sarcoma may suggest the possibility of a metastatic melanoma. When a primary melanoma can be documented, the distinction can be readily made on clinical basis alone. However, in the absence of a known melanoma, a large malignant melanocytic soft tissue tumor may represent a metastasis from an occult melanoma or a primary clear cell sarcoma. Although the long-term survival of patients for both clear cell sarcoma and metastatic melanoma is poor, the distinction of these 2 entities may have therapeutic implications because primary soft tissue clear cell sarcoma may be amenable to cure by surgical excision and radiotherapy. Furthermore, differences in radiosensitivity related to the degree of pigmentation exist for melanoma cells and may also apply to clear cell sarcoma.

The reciprocal translocation, t(12;22)(q13;q12), that is unique to clear cell sarcoma may be demonstrated by using cytogenetics, fluorescent in situ hybridization, or RT-PCR and allows for the distinction between these entities. In our study, we verified the previously documented use of RT-PCR from paraffin-embedded tissue as a rapid and efficient means of confirming the diagnosis of clear cell sarcoma. Despite some limitations such as the inability to obtain sufficient RNA from archival specimens and the need for rigorous controls to minimize cross contamination, RT-PCR allows rapid analysis of large numbers of cases and can identify the specific breakpoints involved in a given fusion transcript.

In our study, the EWS-ATF1 fusion transcript was identified in 9 (75%) of 12 patients in whom the diagnosis of clear cell sarcoma was suspected. The DNA sequencing confirmed the type I EWS-ATF1 fusion transcript in each patient. We did not detect type II or III fusions in these patients. This finding is perhaps not surprising, given the relatively small number of cases and the rarity of the type II and III fusions. Type I fusions are reportedly present in 87% of clear cell sarcomas, and the prevalence may be slightly higher if tumors without any detectible fusion are excluded.

The immunohistochemical profiles of clear cell sarcoma and metastatic melanoma have historically been of little value in distinguishing these 2 entities. In the present study, 10 (63%) of 16 metastatic melanomas and none of the 12 cases of clear cell sarcoma were found to be positive for CD117. Recently, as part of a study of CD117 immunostaining in a large number of soft tissue tumors, CD117 was not identified in any of 10 cases of clear cell sarcoma.

In contrast, a prior study documented variable CD117 positivity in 7 (47%) of 15 cases of clear cell sarcoma, conflicting directly with our own results. As has already been suggested, the discrepancy may be explained by antibody choice and/or antigen retrieval methods.

In our experience, the Dako rabbit polyclonal antibody A4502 (DakoCytomation), even with gentle antigen retrieval as employed in the present study, is relatively devoid of background staining. Previous studies of melanomas have shown at least focal CD117 staining ranging from 5% to 72% of cases.

Again, the high variability of immunohistochemical results may be related to variations in antibody dilution, the use of antigen retrieval, and the threshold of a positive result. Furthermore, CD117 expression may correlate inversely with melanoma progression clinically and in vitro, and further contribute to the heterogeneity of CD117 results. Nevertheless, if conditions are strictly controlled, our methods demonstrate a significant distinction between clear cell sarcoma and metastatic melanoma using an immunohistochemical stain for CD117 and may serve as an adjunct to molecular techniques when the differential diagnosis includes these entities. Whether the lack of significant CD117 expression in clear cell sarcoma plays a role in the clinical behavior remains to be determined.

Three tumors from 2 patients did not demonstrate any
EWS-ATF1 fusion transcripts, despite the presence of detectable phosphoglucokinase RNA and analysis of multiple blocks. We chose to include these patients in our study, but categorized them as unclassified because we could not completely exclude either melanoma (metastatic from an occult primary) or clear cell sarcoma. Interestingly, these tumors were positive for CD117. We cannot exclude the possibility that the unclassified tumors represent clear cell sarcomas that lack EWS-ATF1 fusions. However, our results do suggest that CD117 immunoreactivity is present only in those malignant melanocytic lesions that lack a detectable EWS-ATF1 fusion and further support the potential utility of this marker.

References