Prognostic Influence of HSP-27 Expression in Malignant Fibrous Histiocytoma: A Clinicopathological and Immunohistochemical Study

Bernard Tétu, Bernard Lacasse, Henri-Louis Bouchard, Réal Lagacé, Jacques Huot, and Jacques Landry

Departments of Pathology [B. T., R. L.] and Orthopedics [B. L., H.-L. B.] and Centre de recherche en cancérologie [J. H., J. L.], l'Hôtel-Dieu de Quebec and Université Laval, Laval, Quebec, Canada

ABSTRACT

Malignant fibrous histiocytoma is a very aggressive sarcoma. After the tumor has disseminated, chemotherapy is of little influence on the course of the disease because of the resistance to most chemotherapy regimens. We evaluated by immunohistochemistry the prognostic influence of the expression of a member of the stress polypeptides family, the heat-shock protein of 27 KDa (HSP-27). HSP-27 was found to be associated with an aggressive behavior in breast carcinoma and was related to chemoresistance in cell cultures. Forty-three malignant fibrous histiocytomas with no evidence of metastases at the time of diagnosis and resected between 1974 and 1985 were retrieved from the files of the Pathology Department of L'Hôtel-Dieu de Québec hospital. The immunostaining was performed on Bouin-fixed, paraffin-embedded tissue. Regardless of the percentage of positive cells, HSP-27 was expressed in the cytoplasm of 25 (58.1%) cases. HSP-27 expression was associated with a more favorable prognosis, and a significant correlation was observed with overall survival ($P < 0.025$) and metastasis-free survival ($P < 0.05$). HSP-27 expression was found to be the strongest prognostic factor, and multivariate analysis revealed that it was independent of tumor size, necrosis, and histological subtype. However, in the 13 patients with recurrent disease who underwent chemotherapy, the antigenic expression did not help to predict the treatment response. HSP-27 expression is one of the rare prognostic markers in this tumor type.

INTRODUCTION

MFH is the most common sarcoma in adults. It is a highly malignant tumor, the rate of local recurrence being 44% and metastasis 42% (1). Recognized prognostic factors are limited; the most important are the stage, tumor size (2), histological subtype (1), and necrosis (2–4). Recently, cytometric and morphometric analyses proved to be prognostically relevant (5). In addition, contrary to other forms of sarcomas such as osteosarcoma and embryonal rhabdomyosarcoma, combination chemotherapy does little to help improve the course of the disease (6).

HSP-27 is a member of the small heat-shock protein family and is identical with the 24-kDa protein originally identified in human MCF-7 cells and human breast carcinomas (7, 8) and expressed in several hormone-sensitive organs (9) and human tumors (10, 11). The function of HSP-27 is unknown; however, elevated expression in cells has been correlated with increased cellular resistance to heat shock (12) and to a variety of cytotoxic agents, including some commonly used chemotherapeutic drugs (13). Recent reports suggest that the evaluation of the expression of HSP-27 might be of some prognostic value in breast carcinomas (14–16).

In the present study, we evaluated the expression of HSP-27 in MFH by immunohistochemistry in an attempt to find an additional prognostic marker in this tumor type. The influence of HSP-27 to predict response to salvage chemotherapy was also investigated.

MATERIALS AND METHODS

Population. From 1974 to 1985, 48 patients with MFH and no evidence of dissemination at the time of diagnosis were seen and treated at L'Hôtel-Dieu de Québec. Histopathological material from the original tumor was available in 43 cases, as well as metastatic tumor tissue in 5 instances. The medical charts of all patients were reviewed, and information regarding the age at diagnosis, sex, duration of symptoms before diagnosis, tumor size and location, local extension, therapy, and follow-up were noted.

Histopathology. The hematoxylin and eosin-stained sections from all 43 patients were reviewed by two of us (B. T., R. L.). All tumors were fixed in Bouin's fixative. The diagnosis was made by light microscopic examination, and the tumors were classified according to well-defined criteria (1). Additional electron microscopic study completed the investigation in most cases. In addition, the proportion of tumor necrosis was assessed in all cases using the threshold of 15%, as previously suggested (3, 4).

Immunohistochemistry. A representative paraffin block was selected for each tumor. The immunostaining was carried out using the avidin-biotin peroxidase method of Hsu et al. (17). Briefly, tissue sections were deparaffinized in xylene, rehydrated in graded alcohols, and soaked for 30 min in 1% hydrogen peroxide to block endogenous peroxidase. After washes in phosphate-buffered saline (PBS), slides were incubated for 2 h at room temperature with a polyclonal (rabbit) antibody directed against the human HSP-27 (Hu 27 antibody (12); dilution, 1/200). The immunoreaction was visualized by developing in 3-amin-9-ethylcarbazole and counterstained with Mayer's hematoxylin. The specificity of HSP-27 immunostaining was controlled by using MCF-7 cells which are known to stain positively. In addition, Chinese hamster 2.2 cells expressing high amounts of human HSP-27 following transfection with the human HSP-27 gene were compared to their parental 023 cells acting as negative controls. These results correlated with the expression detected by Western blot on the electrophoretic gel of the same cell cultures.

The immunostaining was interpreted by one of us (B.T.) without knowledge of the clinical course of the disease. The number of positive cells was sorted into four categories ($O$, $<10$, $10–50$, $>50$%) and the staining intensity was evaluated on an arbitrary four-grade scale ($0$, $+$, $++$, $++++$).

Treatment Response. For patients with local recurrences and distant metastases, the objective response to salvage chemotherapy was evaluated by using well-defined criteria (18). A complete response represented a complete disappearance of this tumor, while a partial response was represented by a 50% decrease in tumor size. The response rate was based on radiological and/or pathological findings.

Statistical Analyses. Overall survival and disease-free survival curves according to Kaplan-Meier were obtained for the different factors recognized as prognostically relevant in the literature. Statistical differences between survival curves were calculated using the log-rank test. A Cox regression multivariate analysis was performed using the variables that were more significant by univariate analyses.
RESULTS

Clinical and Pathological Features. Patients age were 15–89 years of age (average, 57 years). There were 21 males and 22 females. Forty tumors arose from the soft tissues (thigh, 18 cases; lower leg, 10 cases; upper extremity, popliteal fossa, shoulder, and thorax, 2 cases each; foot, forearm, axilla and neck, 1 case each). Three tumors were of bone origin (tibia, fibula, pelvis).

Of the 43 tumors, 23 exceeded 5 cm in diameter. Most tumors (33 cases) were of the pleomorphic or giant cell type, while 10 were of the myxoid variant. Twenty-four tumors showed <15% necrosis.

The type of surgical approach varied greatly. The surgical margins were intralateral in 10 cases and marginal in 8. A wide resection was performed in 21 cases, and the surgical margin status was unknown in 4 instances.

Immunohistochemical Staining. Our immunohistochemical study was performed using Bouin-fixed material. However, the same antibody was also tested by us on formalin-fixed tissue in a variety of tumors and by others in a series of breast carcinomas (14). The staining pattern and intensity were comparable with either fixative.

The immunostaining for HSP-27 was positive (Fig. 1) in 25 cases (58.1%). There were >10% positive cells in 17 instances (39.5%), and staining intensity equal or greater than ++ was observed in 19 (44.1%) of the 43 cases. The staining was cytoplasmic and diffuse in all cases. Of the five metastases, two stained positively. The immunostaining was comparable to the original tumors except for one case. The tumor reacted with the antibody and gave rise to a metastasis with negative staining.

The correlation of the immunostaining with different morphological prognostic factors in MFH is shown in Table 1. No relationship was observed between the immunostaining and any of these factors, although a tendency was observed for HSP-27 expression to be associated with smaller tumors.

Survival and Treatment Response. The overall 5-year survival for patients with MFH was 48%. Of the 43 patients, 10 developed local recurrence, of which 2 received salvage chemotherapy. Twenty patients developed distant metastases (lung, 18 cases; bone, 3 cases; liver, 2 cases; brain, 1 case), and 11 underwent salvage chemotherapy. Varying chemotherapy regimens were used, and the number of courses ranged from 1–9. All patients received either doxorubicin, vincristine, or a combination of both drugs. Seven patients received additional dacarbazine, seven had cyclophosphamide, and two had methotrexate and actinomycin D. CCNU (Lomustine) and cisplatin were given to two different patients.

Parameters such as sex, duration of symptoms before diagnosis (> or <4 months), and completeness of surgery (large, intralateral, extralateral) did not influence significantly the overall survival by univariate analyses. The proportion of tumor necrosis was the only significant prognostic factor (P = 0.05), and a tendency only was observed for tumor size and histological subtypes to be prognostically relevant.

A significant relationship was observed between HSP-27 expression and overall survival (Fig. 2). The 5-year survival of patients with negative immunostaining was 28% as opposed to 62% for those with positive staining (P < 0.025). A similar relationship was observed with metastasis-free survival (P < 0.05) (Fig. 3). HSP-27 expression was thus associated with a more favorable outcome. A stratification based on the number of positive cells did not influence the significance of the relationship. By univariate analyses, HSP-27 was the strongest

![Fig. 1. Immunostaining of tumor sarcoma cells for HSP-27 (avidin-biotin peroxidase, X 160).](image)

![Fig. 2. Relationship between overall survival of patients with malignant fibrous histiocytoma and HSP-27 expression (P < 0.025).](image)
DISCUSSION

MFH is recognized as an aggressive tumor and is known to be resistant to most chemotherapeutic regimens (1, 6). However, the identification of a subgroup of less aggressive tumors is difficult because of the limited number of accepted prognostic factors. The stage, location, tumor size, histological subtypes (pleomorphic versus myxoid) (1, 2) and extent of tumor necrosis (3, 4) are regarded as the most significant factors. HSP-27 is a member of the heat-shock protein family and received a different designation in the literature. It had been reported to be a protein of 24 (9) and 28 kDa (8) or a stress-response protein (14). The transcription of HSP-27 was found to be regulated by estrogen stimulation (19) and thermal stress (10, 12).

Recent reports suggested the influence of HSP-27 expression on the prognosis of breast cancer (14–16, 20). Western blot (14, 15, 20), Northern blot (14) and immunohistochemistry (14, 16) analyses have been used, and HSP-27 overexpression was usually regarded as a marker of poor prognosis (14, 15, 20).

In our study, HSP-27 was associated with a better prognosis in MFH. No relationship was observed with either the tumor size, histological subtype, or necrosis. This apparent inconsistency with results obtained in breast cancer is intriguing. It is clear, however, that the prognostic significance of the marker was not influenced by chemotherapy since none of our patients received any additional systemic treatment following surgery, unless they developed metastases. Thus, in sarcoma, HSP-27 expression might reflect a better degree of differentiation and may represent an intrinsic prognostic factor.

Preliminary studies of cell cultures suggest a relationship between HSP-27 overexpression and resistance to doxorubicin, Vinca alkaloids, colchicine, and dactinomycin (13). In the literature on breast carcinoma, a number of patients received additional chemotherapy and hormone therapy (14, 16). In one study, HSP-27 expression was associated with a poorer prognosis in node-positive patients, but the proportion of patients who received adjuvant therapy is not stated (14). In another study of patients with disseminated disease and who underwent palliative chemotherapy or hormone therapy (16), contrary to results obtained from cell cultures, HSP-27 expression was associated with a better response to treatment. In our study, the influence of HSP-27 expression on chemotherapy response was investigated in 13 patients who developed distant metastases and received salvage chemotherapy. The tumors of patients with disseminated MFH and expressing HSP-27 were not more resistant to chemotherapy than HSP-27-negative tumors. There was no relation with either immediate chemotherapy response of the tumor or survival following the beginning of chemotherapy. On the contrary, 3 of 5 patients with HSP-27 expression showed complete response or partial response, while only 1 of 5 without staining responded. However, the chemotherapeutic regimens were very different for each patient.

We conclude that HSP-27 represents an important prognostic factor in MFH and is independent of tumor size, histological subtype, and necrosis. Its influence on resistance to chemotherapy should be further investigated in a prospective study with patients receiving standardized treatments.

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Fig. 3. Relationship between metastasis-free survival of patients with malignant fibrous histiocytoma and HSP-27 expression (P < 0.05).

Table 2 Relationship between the immunostaining of the primary tumor for HSP-27 and the outcome of 13 patients with recurrence who underwent salvage chemotherapy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Follow-up postchemotherapy (mo)</th>
<th>Status at last follow-up</th>
<th>HSP-27 immunostaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>71</td>
<td>45</td>
<td>AWD*</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>49</td>
<td>30</td>
<td>AWD</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>29</td>
<td>AWD</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td>22</td>
<td>AWD</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>42</td>
<td>16</td>
<td>AWD</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>44</td>
<td>7</td>
<td>AWD</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>26</td>
<td>0</td>
<td>AWD</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>55</td>
<td>59</td>
<td>DOC</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>55</td>
<td>18</td>
<td>DOD</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>62</td>
<td>8</td>
<td>DOD</td>
<td>–</td>
</tr>
<tr>
<td>11</td>
<td>21</td>
<td>6</td>
<td>DOD</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>57</td>
<td>5</td>
<td>DOD</td>
<td>–</td>
</tr>
<tr>
<td>13</td>
<td>60</td>
<td>1</td>
<td>DOD</td>
<td>–</td>
</tr>
</tbody>
</table>

* AWD, alive with disease; DOD, dead of disease; DOC, dead of other cause.

Table 3 Relationship between HSP-27 immunostaining and response to salvage chemotherapy in 13 patients with recurrent tumor

<table>
<thead>
<tr>
<th>HSP-27 staining</th>
<th>No. of cases</th>
<th>Response</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>6</td>
<td>None</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Positive</td>
<td>7</td>
<td>Partial</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

prognostic factor. By multivariate analysis, it was independent of tumor size, necrosis, and histological subtype.

Table 2 shows the outcome of the 13 (30.2%) patients who were treated with salvage chemotherapy. Seven were alive after the completion of this study, and 6 were dead. Four of the 7 tumors of patients who survived >1 year during chemotherapy expressed HSP-27; 2 of the 4 who died of their disease within 1 year stained for this antiserum. Table 3 shows the relationship between HSP-27 immunostaining and the objective response to salvage chemotherapy. One of the 5 tumors with negative staining and whose response to chemotherapy could be evaluated revealed a partial response. Three of 5 tumors with positive staining responded.
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REFERENCES


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