The expression and prognostic significance of bone morphogenetic protein-2 in patients with malignant fibrous histiocytoma

We investigated the rates of expression of bone morphogenetic protein-2 (BMP-2) in 29 adult patients with high-grade malignant fibrous histiocytoma of soft tissue, using the BMP-2-specific monoclonal antibody, AbH3b2/17, and found that they ranged from 1.9% to 78.9%. The survival at five years of the groups expressing high (≥30%) and low (<30%) levels of BMP-2 was 85.7% and 36.3%, respectively. Multivariable analysis showed that only BMP-2 had prognostic significance for continuous disease-free survival and for overall survival (p < 0.05). Our findings indicate that over-expression of BMP-2 in malignant fibrous histiocytoma of soft tissue is the most reliable prognostic indicator of the parameters assessed.

Malignant fibrous histiocytoma (MFH) is the most commonly diagnosed of all adult malignant mesenchymal neoplasms of soft tissue. It is characterised by a variety of histopathological features, aggressive local growth and frequent recurrence after surgical excision. The incidence is relatively high in the elderly and chemotherapy is of limited effectiveness. Radiotherapy does not prolong survival. MFH eventually metastasises to other organs, mainly the lungs, giving a poor clinical outcome. The maximal survival at five years is between 40% and 60%. Progress in the management of MFH has been minimal and its biological features remain unknown despite intensive investigation.

Studies have shown that bone morphogenetic protein-2 (BMP-2) is involved in the tumourogenic and metastatic potential of other types of malignant mesenchymal neoplasm. It is a physiologically active polypeptide of the transforming growth factor (TGF)-β superfamily and can induce formation of bone in ectopic extraskeletal sites in vivo by stimulating pluripotent mesenchymal cells to become osteoblasts. It also plays crucial roles in pleiotropic biological effects ranging from the regulation of early developmental processes of the mesoderm to organogenesis. Some malignant mesenchymal neoplasms express BMP-2 which is seen in 40% to 60% of osteosarcomas, 60% to 70% of liposarcomas, 30% to 40% of leiomyosarcomas, 40% of malignant schwannomas and in some of the MFH and dedifferentiated chondrosarcomas. It may affect biological processes such as tumourogenesis and metastatic potential in addition to the periosteal reaction of bone formation in tumours.

We have evaluated the hypothesis that expression of BMP-2 is related to the biological features of MFH in soft tissue, and that this reflects the clinical course of MFH as it does in other types of sarcoma.

Patients and methods
We studied 29 consecutive adults (15 men, 14 women) with a histologically confirmed diagnosis of MFH of soft tissue who had undergone treatment at the Department of Orthopaedic Surgery at Mie and Osaka University Hospitals between 1986 and 2002. At least two pathologists reassessed the lesions and reviewed the clinical, pathological, surgical and follow-up data. The median age of the patients at the time of diagnosis was 54 years (27 to 92). The maximal size of the tumour was more than 5 cm in 19 patients and less than 5 cm in the remainder. Thirteen were superficial and 16 were intramuscular; ten were found in the thigh, five in the buttock, three in the forearm, three in the shoulder, two in the upper arm, two in the lower leg and four in other sites. According to the Enneking surgical staging system for musculoskeletal sarcomas, 26 patients were in stage II B, and three were in stage III.

Before operation the diagnosis was confirmed histologically by open or needle biopsy. Thereafter, all patients were treated by operation, 23 by wide resection and six by marginal resection.
Eleven patients had chemotherapy alone, five had radiotherapy alone, eight had both chemotherapy and radiotherapy, and five had no further treatment. The median follow-up period was 53.5 months (6 to 180).

**Immunohistochemical staining for BMP-2.** Specimens were retrieved from all patients during surgical resection. Archival tumour blocks were fixed in 10% phosphate-buffered formaldehyde, and embedded in paraffin. Sections 4 µm thick were placed on silanised slides for histological haematoxylin and eosin and immunohistochemical staining using the avidin-biotin-peroxidase complex. We used the BMP-2 specific monoclonal antibody AbH3b2/17, synthesised by standard monoclonal antibody procedures in Chinese hamster ovary (CHO) cells using full-length recombinant human BMP-2 (GI Corporation, Cambridge, Massachusetts) as the immunogen.14

**Evaluation of BMP-2 staining rates.** All stained materials were examined with a microscope with x200 magnification. The area containing the strongest positive staining in the intracellular space was selected and the images entered into a personal computer using a CCD colour camera (Ikegami ICD-740, Tokyo, Japan). All the images were converted to 8-bits grey-scale images consisting of 320 x 200 pixels and their low-intensity areas were counted as the staining area using image-analysis software (NIH image, National Institute of Health; Bethesda, Maryland). The proportion of low-intensity area to total-image area was plotted using the Kaplan-Meier method and the outcomes of two-group comparisons were determined using the log-rank test. A p value below 0.05 was considered to be statistically significant. Multivariable Cox regression analysis was used to assess the correlations between these factors and the prognosis. The final results were expressed as hazard ratios, 95% confidence intervals (CI), and the p value.

**Results**

Table I and II give the clinical details and the results in the high- and low-grade groups.

**Features of BMP-2 expression in MFH.** Histological staining with haematoxylin and eosin showed storiform-pleomorphic, myxoid, inflammatory and giant cell subtypes of MFH. The most common type was the storiform-pleomorphic form which was seen in 13 patients.

According to the morphological grading criteria proposed by the American Joint Committee on Cancer, our specimens were all grade 3 or more high-grade tumours.

Comparative immunohistochemical analysis detected heterogenous or focal AbH3b2/17 immunoreactivity (BMP-2 staining) in all 29 samples. Staining for AbH3b2/17 was predominantly localised in the cytoplasm of undif-
differentiated spindle-shaped cells. Figure 1 shows BMP-2 staining of pleomorphic and giant cells. BMP-2 staining was occasionally located in the cytoplasm of giant cells and histiocytelike cells, but never in the fibrous matrix.

BMP-2 was widely expressed and appeared to be random and independent of the histological subtype, cellularity and the state of mitosis of the tumour. The histological differences between BMP-2 positive and BMP-2 negative cells were not significant.

**Statistical analysis of BMP-2.** The staining rates for BMP-2 in the 29 specimens ranged from 1.9% to 78.5% (Fig. 2). Fourteen tumours were differentiated into the high-grade staining group (median 43.2%, range 30.1 to 78.5) and 15 into the low-grade group (median 16.5%, range 1.9 to 27.7) (Tables I and II).

The estimated survival rate at five years for all the 29 patients was 59.1% and the disease-free rate at five years was 40.3%. The overall five-year survival rates of the BMP-2 high- and low-grade staining groups were 85.7% and 36.1%, respectively, according to the Kaplan-Meier method. The five-year disease-free survival of the BMP-2 high-staining group was 65.7% and that of the low group 16.9% (Fig. 3). The estimated survival rate of the BMP-2 high-grade staining group was significantly higher than that

<table>
<thead>
<tr>
<th>Case</th>
<th>BMP (%)</th>
<th>Age (yrs)</th>
<th>Gender</th>
<th>Size of tumour (cm)</th>
<th>Depth</th>
<th>Subtype</th>
<th>Location</th>
<th>Resection</th>
<th>Chemo</th>
<th>Radiation</th>
<th>A/D*</th>
<th>CDF†</th>
<th>Follow-up (mths)</th>
<th>Outcome‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.9</td>
<td>74</td>
<td>M</td>
<td>≥5</td>
<td>S</td>
<td>Pleomorphic</td>
<td>Thigh</td>
<td>Wide</td>
<td>0</td>
<td>0</td>
<td>A</td>
<td>6</td>
<td>6.0</td>
<td>AWD</td>
</tr>
<tr>
<td>2</td>
<td>6.0</td>
<td>75</td>
<td>M</td>
<td>≥5</td>
<td>D</td>
<td>Storiform pleomorphic</td>
<td>Forearm</td>
<td>Wide</td>
<td>0</td>
<td>1</td>
<td>D</td>
<td>11</td>
<td>24.4</td>
<td>DOD</td>
</tr>
<tr>
<td>3</td>
<td>6.1</td>
<td>46</td>
<td>F</td>
<td>&lt;5</td>
<td>D</td>
<td>Storiform pleomorphic</td>
<td>Thigh</td>
<td>Wide</td>
<td>1</td>
<td>0</td>
<td>D</td>
<td>38</td>
<td>78.0</td>
<td>DOD</td>
</tr>
<tr>
<td>4</td>
<td>9.2</td>
<td>92</td>
<td>F</td>
<td>&lt;5</td>
<td>D</td>
<td>Storiform pleomorphic</td>
<td>Forearm</td>
<td>Marginal</td>
<td>0</td>
<td>0</td>
<td>D</td>
<td>20</td>
<td>29.2</td>
<td>DOD</td>
</tr>
<tr>
<td>5</td>
<td>11.6</td>
<td>27</td>
<td>M</td>
<td>≥5</td>
<td>D</td>
<td>Myxoid</td>
<td>Thigh</td>
<td>Marginal</td>
<td>1</td>
<td>0</td>
<td>D</td>
<td>0</td>
<td>7.7</td>
<td>DOD</td>
</tr>
<tr>
<td>6</td>
<td>12.9</td>
<td>62</td>
<td>F</td>
<td>≥5</td>
<td>D</td>
<td>Myxoid</td>
<td>Buttock</td>
<td>Wide</td>
<td>0</td>
<td>1</td>
<td>D</td>
<td>7</td>
<td>9.4</td>
<td>DOD</td>
</tr>
<tr>
<td>7</td>
<td>15.7</td>
<td>72</td>
<td>M</td>
<td>≥5</td>
<td>D</td>
<td>Myxoid</td>
<td>Knee</td>
<td>Wide</td>
<td>1</td>
<td>0</td>
<td>D</td>
<td>17</td>
<td>31.0</td>
<td>DOD</td>
</tr>
<tr>
<td>8</td>
<td>16.5</td>
<td>44</td>
<td>F</td>
<td>≥5</td>
<td>D</td>
<td>Myxoid</td>
<td>Thigh</td>
<td>Wide</td>
<td>1</td>
<td>0</td>
<td>D</td>
<td>24</td>
<td>40.0</td>
<td>DOD</td>
</tr>
<tr>
<td>9</td>
<td>16.8</td>
<td>50</td>
<td>M</td>
<td>&lt;5</td>
<td>S</td>
<td>Storiform pleomorphic</td>
<td>Abdominal wall</td>
<td>Wide</td>
<td>0</td>
<td>0</td>
<td>A</td>
<td>77</td>
<td>77.0</td>
<td>CDF</td>
</tr>
<tr>
<td>10</td>
<td>17.4</td>
<td>70</td>
<td>F</td>
<td>≥5</td>
<td>D</td>
<td>Storiform pleomorphic</td>
<td>Thigh</td>
<td>Wide</td>
<td>0</td>
<td>0</td>
<td>A</td>
<td>14</td>
<td>62.0</td>
<td>AWD</td>
</tr>
<tr>
<td>11</td>
<td>18.2</td>
<td>75</td>
<td>F</td>
<td>≥5</td>
<td>S</td>
<td>Storiform myxoid</td>
<td>Upperarm</td>
<td>Wide</td>
<td>0</td>
<td>0</td>
<td>A</td>
<td>9</td>
<td>9.0</td>
<td>AWD</td>
</tr>
<tr>
<td>12</td>
<td>22.1</td>
<td>76</td>
<td>M</td>
<td>≥5</td>
<td>D</td>
<td>Storiform pleomorphic</td>
<td>Thigh</td>
<td>Marginal</td>
<td>0</td>
<td>1</td>
<td>D</td>
<td>34</td>
<td>102.0</td>
<td>DOD</td>
</tr>
<tr>
<td>13</td>
<td>25.7</td>
<td>31</td>
<td>F</td>
<td>&lt;5</td>
<td>S</td>
<td>Inflammatory</td>
<td>Hand</td>
<td>Wide</td>
<td>1</td>
<td>1</td>
<td>D</td>
<td>5.3</td>
<td>51.0</td>
<td>DOD</td>
</tr>
<tr>
<td>14</td>
<td>27.3</td>
<td>59</td>
<td>M</td>
<td>≥5</td>
<td>D</td>
<td>Pleomorphic</td>
<td>Buttock</td>
<td>Wide</td>
<td>1</td>
<td>1</td>
<td>D</td>
<td>0</td>
<td>27.4</td>
<td>DOD</td>
</tr>
<tr>
<td>15</td>
<td>27.7</td>
<td>46</td>
<td>M</td>
<td>≥5</td>
<td>S</td>
<td>Storiform pleomorphic</td>
<td>Shoulder</td>
<td>Wide</td>
<td>1</td>
<td>0</td>
<td>D</td>
<td>99</td>
<td>118.0</td>
<td>DOD</td>
</tr>
</tbody>
</table>

* alive/dead  
† continuous disease free  
‡ AWD, alive with disease; DOD, died from disease

BMP-2 staining of pleomorphic (a) and giant cells (b). Staining is localised mainly in the cytoplasm of undifferentiated spindle-shaped cells (x 220).
of the low-grade group according to the log-rank test (p = 0.005 and 0.004; p < 0.05 for overall survival and continuously disease-free rates, respectively).

**Multivariable Cox regression analysis.** We used multivariable Cox regression analysis to assess the significance of high or low grades of staining of BMP, age (<54 years or ≥54 years), gender (male or female), size of the tumour (≥5 cm or <5 cm), depth (superficial or deep), resection (wide or marginal), and the presence or absence of chemotherapy and radiotherapy. BMP-2 showed prognostic significance in agreement with the univariate analysis according to continuous disease-free survival and overall survival (p = 0.016 and p = 0.010; p < 0.05). The estimated continuous disease-free survival was significantly higher in the no-radiotherapy group than in the radiotherapy group (p = 0.007; p < 0.05).

None of the other parameters showed statistical significance (Table III). We therefore concluded that only BMP-2 could predict the outcome of patients with MFH of soft tissue.

**Discussion**

MFH has a broad range of histological appearances and is divided into the following subtypes: storiform-pleomorphic, myxoid, giant-cell and inflammatory. However, these histological variants do not correlate with the prognosis which is consistently influenced by clinical factors such as the size, site and localisation of the tumour. A number of recent studies have suggested that aneuploidy, the nuclear shape, and heat shock protein 27 may provide prognostic information in high-grade MFH.

Some studies have suggested that BMP-2 activities may contribute to aggressive behaviour and influence the metastatic potential in some mesenchymal neoplasms. Yoshikawa et al found that expression of BMP-2 in the cytoplasm of osteosarcomas was most intense in the fibrohistiocytic type, detectable in the osteoblastic type and absent in the chondroblastic type. The overall survival rate at five years of patients with an osteosarcoma which produces BMP-2 is 42.9% and 63.1% if it does not. In our study the rate of expression of BMP-2 did not depend upon the histological subtype and a higher expression showed a better prognosis, the opposite to that seen in patients with osteosarcoma, although both neoplasms are derived from undifferentiated mesenchymal cells.

In general, BMP-2 is structurally related to transforming growth factor-β, which plays an essential role in modulation of the life cycle of the cell. After intracellular synthesis, secreted BMP-2 isoforms act through BMP-2-specific receptors on target-cell membranes. By intracellular signal transmission such as Smad, BMP-2 induces chemotaxis, which causes the proliferation and assembly of undifferen-
tiated mesenchymal cells for cell differentiation, the regulation of cell adhesion and cell-matrix interaction and the formation of extracellular matrix during early embryogenesis. Studies have also shown that BMP-2 may promote the selective differentiation of preosteoblasts and prechondroblasts into osteoblasts and chondroblasts. These processes are regulated by signals that stimulate specific transcriptional programmes which are required for osteogenesis and chondrogenesis. Besides osteochondrogenesis, BMP-2 also contributes to neural and cardiac development, suppresses the differentiation of skeletal muscle, and enhances the apoptosis of specific mature cells or arrests the cell cycle to modulate cell proliferation partially. Thus, BMP-2 is associated with the regulation of a variety of fates and functions of the cell and it regulates diverse aspects of vertebrate embryogenesis.

The BMP-2 which is synthesised in tumour cells may be secreted in the extracellular matrix where differentiation or proliferation of tumour cells is stimulated or modulated concomitantly with extracellular expression of BMP-2 around cells which do not express this protein. However, recent literature suggests that BMP-2 has antiproliferative effects against some tumours in vitro and in vivo, indicating that the multifunctional signals of activation of BMP-2 may be transmitted through several types of receptor. Differences in the expression of such receptors may affect the clinicopathological features and clinical course of tumours. For example, the expression of BMP receptors correlates with metastasis in osteosarcomas, suggesting that the BMP pathway participates in aggressiveness or progression of the tumour. Receptors for BMP are usually detected in other sarcomas as well as in osteosarcoma. No immunoreactivity for BMP receptors has been identified in MFH, which could explain the contradictory clinical courses of osteosarcoma and MFH.

At present we do not understand the mechanism which regulates the ratio of expression of BMP-2 in the various types of MFH. The activation of BMP-2 and BMP receptors remains puzzling and therefore further investigations should address the oncogenesis of MFH.

Our study has shown that the expression of BMP-2 is closely associated with the biological features of MFH and that it influences the clinical course of the tumour. Over-expression of BMP-2 in MFH of soft tissue is the most reliable prognostic indicator of the factors assessed in our study. Chemotherapy and radiotherapy are currently ineffective and the only viable strategy for MFH is surgical resection. The participation of BMP-2 activities in anti-proliferation or cell differentiation of the tumour may be critical in the determination of active chemotherapeutic regimes. Further analysis of BMP-2 may help to elucidate the molecular basis and pathogenesis of MFH.

References


