Expression of Bone Morphogenetic Protein and its Receptors in Osteosarcoma and Malignant Fibrous Histiocytoma

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Background: Bone morphogenetic protein (BMP) activity has been found in cases of malignant fibrous histiocytoma (MFH) and osteosarcoma but only tumors in the latter category show evidence of ossification. The aim of this study was to try to understand this difference by examination of the distribution of BMP and its receptors (BMPR) for this bone inducing protein in these tumors.

Methods: Sections of 11 osteosarcoma and 10 MFH were analyzed immunohistochemically for BMP and BMPRs by use of the avidin–biotin peroxidase method.

Results: Nine out of 11 osteosarcoma cases (80.1%) showed positive staining for both BMP and BMPRs. Two cases of chondroblastic type osteosarcoma did not show any significant staining for BMP and BMPRs. In eight out of 10 MFH cases (80%) there was positive staining for BMP. No immunoreactivity for BMPRs was found in any case of MFH.

Conclusions: MFH does not express BMPRs and this may be the reason why MFH tumors do not ossify, even in the presence of BMP.

Key words: osteosarcoma – malignant fibrous histiocytoma – ossification – bone morphogenetic protein – bone morphogenetic protein receptor

INTRODUCTION

Bone morphogenetic proteins (BMPs) were originally identified as proteins that induce bone formation at extraskeletal sites (1). Other studies have shown that BMPs are multifunctional cytokines that regulate the growth, differentiation and apoptosis of various cell types (2). BMPs induce undifferentiated mesenchymal cells to differentiate through the chondrogenetic or osteogenetic pathway which results in ectopic bone formation (3). In a manner similar to other members of the TGF-β superfamily, BMPs mediate their effects by forming a complex of two different types of serine/threonine kinase receptors: type I and type II. These BMP receptors (BMPRs) are induced at physiological and pathological ossification sites and play a critical role in bone formation (4,5).

BMP has also been proved to be one of the significant factors in the prognosis of bone tumors (6,7). Detection of BMP in osteosarcoma and malignant fibrous histiocytoma (MFH) has been reported in several studies (6–10).

Ossification is a key feature found in osteosarcoma but absent in MFH, a fact which has made it possible to distinguish the two tumors. However, the routine histology of a less differentiated type of osteosarcoma (fibroblastic type) and MFH shows many similarities so it is difficult to diagnose these tumors differentially. In order to characterize MFH in more detail and to be able to differentiate it from osteosarcoma, we analyzed and compared 11 cases of osteosarcoma and 10 cases of MFH for the expression of BMP and BMPRs (BMPR-IA, BMP-RIB and BMPR-II) in both tumor groups by immunohistochemistry.

MATERIALS AND METHODS

Tissue Preparation

Eleven paraffin-embedded samples of osteosarcoma (four well-differentiated, three osteoblastic, two chondroblastic and two fibroblastic) and 10 samples of MFH (four MFH of bone and six MFH of soft tissue; eight pleomorphic type and two giant cell type) were obtained from Shinshu University Hospital Pathological Laboratory. Sections of 4 µm thickness were cut with a microtome and used for immunostaining. Hematoxylin and eosin stained sections of each test sample were prepared to confirm the original histological diagnosis. None of the diagnoses were changed.
ANTIBODY

Polyclonal goat anti-human BMP2/4 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used with the dilution of 10 µg/ml. Polyclonal rabbit antibodies for human BMPR-I (IA, IB) and BMPR-II were kindly provided by Dr K. Miyazono (Department of Biochemistry, Japanese Foundation for Cancer Research) and used at a dilution of 10 µg/ml. The specificity of antibodies for human BMPR-I (IA, IB) and BMPR-II was confirmed by methods reported previously (11).

IMMUNOHISTOCHEMICAL ANALYSIS

Immunostaining was performed by the avidin–biotin peroxidase method. After dewaxing with a graded ethanol series, sections were treated with ethanol containing 0.3% hydrogen peroxide for 30 min to block endogenous peroxidase. Sections selected for BMPR staining were treated with 0.1% trypsin–PBS (Nacalai Tesque, Kyoto, Japan) for 30 min at 37°C, while remaining sections were kept in PBS. After trypsin treatment all sections were washed with PBS + TritonX-100 (Eastman Kodak, Rochester, NY, USA) and incubated with normal serum (rabbit serum for BMP staining and goat serum for BMPRs) for 30 min at room temperature. The sections were incubated with primary antibodies for 2 h at room temperature followed by incubation with a biotinylated secondary antibody (Nichirei, Tokyo, Japan) for 15 min at room temperature. After washing with PBS, sections were incubated with avidin–biotin horseradish peroxidase for 15 min at room temperature. Color was developed by using 3,3-diaminobenzidine tetrachloride (Wako, Tokyo, Japan) for 15 min at room temperature. After washing with PBS, sections were treated with ethanol containing 0.3% hydrogen peroxide. The sections were counterstained with hematoxylin. Control sections were treated in the same manner but normal serum (1% bovine serum) was used instead of primary antibody. The sections were observed under a light microscope.

RESULTS

Table 1 summarizes the data obtained from the immunohistochemical analysis of BMP and BMPRs in osteosarcoma and MFH.

BMP EXPRESSION

Nine of 11 (80.1%) cases of osteosarcoma showed positive staining for BMP. The immunoreactivity for BMP 2/4 varied with the different histological subtypes; all osteoblastic osteosarcoma cases were predominantly BMP positive in undifferentiated spindle-shaped cells (Fig. 1). An abundance of positive cells (more than 60%) was found in well-differentiated and fibroblastic osteosarcoma. Chondroblastic osteosarcoma did not exhibit any significant BMP staining.

In eight of 10 MFH samples, widespread immunostaining for BMP was observed in the undifferentiated spindle-shaped cells (Fig. 2). However, immunoreactivity was not observed in giant cell type MFH samples (two cases).

BMPR-IA, BMPR-IB AND BMPR-II EXPRESSION

Nine of 11 cases of osteosarcoma showed positive immunoreactivity for BMPRs (BMPR-IA, BMPR-IB and BMPR-II). All of the BMP receptor-positive sections of osteosarcoma also showed positive staining for BMP (Fig. 1). In contrast to osteosarcoma, immunoreactivity for BMPRs were not found in any of the MFH cases (Fig. 2).

DISCUSSION

BMPs play a fundamental role in morphogenetic events during development and fracture repair. BMPs mediate their effects by forming a complex with two different types of serine/threonine kinase receptors: type I and type II. These receptors are essential for BMP to exert its effects (4).

In this study, a high incidence of immunoreactivity for BMP was detected (9/11 in osteosarcoma, 8/10 in MFH), as was immunoreactivity for three receptors of two types (type-IA, type-IB and type-II) in osteosarcoma (9/11), but not in MFH (0/10). Frequent detection of BMP2/4 in both osteosarcoma (59%) and MFH (100%) was reported previously by Yoshikawa et al. (9). BMP expression is reported to be specific to these tumors (9). The results from the present study confirmed the findings in the previous reports. Immunoreactivity was not exhibited by any cells of BMP-positive tumors and the common morphology of BMP-positive cells in both tumors was spindle-shaped, probably at a less differentiated stage. Furthermore, BMP has been described as having prognostic and therapeutic significance in osteosarcoma (7,8,12), although its exact biological function in malignant tumors remains unknown. Since very few tumors do not express BMP, it was not possible to analyze the clinical significance of BMP and BMPR expression in this study.

Recently Guo et al. analyzed the expression of BMP and BMPR in various sarcomas, including 36 osteosarcomas and one MFH cell line, by RT-PCR (13). They reported that not only osteosarcoma but also MFH cell line expressed m-RNA of BMP and BMPRs; these results are different from our findings. The difference may come from the different methods and specimens used in the two studies.

Fibroblastic-type osteosarcomas and MFH have clinical, radiological and histological similarities, which make it difficult to differentiate between these tumors (14). Osteosarcoma is defined as a malignant tumor of mesenchymal origin with
bone or osteoid formation driven by the proliferating tumor cells (15) or by normal osteoblasts induced by tumor-derived BMP. MFH is also derived from multipotential primitive mesenchymal progenitor cells (16,17), but the tumor cells are devoid of bone-forming capacity despite BMP production as reported by Yoshikawa et al. (9) and confirmed in the present study. Possible explanations for the lack of bone formation in MFH despite the expression of BMP molecules may be derived from a defective molecular form of BMP produced by the tumor cells resulting in inactive BMP or defective signaling of BMP in MFH cells. The results of the present study demonstrate a lack of immunoreactivity of MFH cells for both BMPRs (type I and type II receptors), which are essential for BMP signaling. Based on the results presented here, the loss of the BMPRs on MFH cells seems to be the most likely reason for the lack of bone formation in BMP-producing MFH. However, other possibilities remain to be examined and confirmed in additional studies.

BMPRs were expressed with high incidence in osteosarcomas (9/11) and the expression was seen in all of the BMP-producing osteosarcomas. The co-expression of BMP and its receptors in all BMP-positive osteosarcomas and no expression of either BMP or BMPRs in two chondroblastic osteosarcomas is a similar pattern to that seen in normal bone. This may indicate that BMP is not able to induce BMPR expression in MFH by an autocrine or paracrine mechanism, as is thought to happen in normal tissue and BMP-producing osteosarcoma. However, further work is needed to determine the exact nature of this mechanism.

CONCLUSION

MFH tumors routinely produce immunoreactive BMP but do not express BMPRs, and this may be the reason why MFH tumor cells do not ossify, even in the presence of BMP. Positive immunohistochemical detection of BMP2/4 and the negative detection of BMPRs in tumor cells could be a useful indicator to identify MFH and discriminate it from the fibroblastic type of osteosarcoma.

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References