THE SIGNIFICANCES OF BONE MORPHOGENETIC PROTEIN (BMP-2) EXPRESSION FOR PREDICTING THE PROGNOSIS OF MALIGNANT FIBROUS HISTIOCYTOMA (MFH)

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Introduction
Malignant fibrous histiocytoma (MFH) is clinically most frequently diagnosed in adult’s soft tissue sarcoma. And not only in soft tissue but also rarely in the bone or organ parenchyma that’s occurrence has been reported. But because of treatments such as chemotherapies for MFH shows little effect so far, MFH’s prognosis has still not getting remarkable improvement. And it’s pivotal prognosis determining factor still remains unclear, too. While in osteosarcoma (OS), the relation between it’s prognosis and expression of bone morphogenetic protein-2 (BMP-2) has been studied in many researches. Some researchers reported that in proportion to increasing of it’s BMP-2 expression rate, it’s clinical courses are getting worse. Recently in MFH, BMP-2 expression has detected too. So we hypothesized that in BMP-2 detected in MFH, there are some correlation to it’s prognosis like OS.

Patients and method
- Patients and Specimens-
We researched for a series of 30 patients with primary MFH of soft tissue which were treated at Mie University Hospital and Osaka University Hospital, and these patient’s ages at the time to be diagnosed were ranged from 27 to 92 years old (median age were 52 years old). Eighteen patients were male and 12 patients were female. The location of MFH were thigh (10 patients), buttock (6), forearm (4), shoulder (3), upper arm (3), lower leg (2), and others (2). According to Enneking’s surgical-staging system for musculoskeletal sarcomas, 26 patients were in Stage IIB, and 4 patients were in Stage III. Open or needle biopsy has been done for histological confirming. All patients were treated with the radical operation (limb-salvage operation): wide resections were performed in 24 patients, marginal resections were in 4 patients and intralesional resections were in 2 patients. And randomized trial of adjuvant chemotherapy has been done for 20 patients. These patient’s follow-up periods were ranged from 6 to 156 months (Average months are 48.1). And Paraffin-embedded materials from MFH of soft tissue of all 30 patients were obtained during radical operation.

-Immunohistochemical staining for BMP-2-
These tumor tissues were fixed in 10% phosphate-buffered formaldehyde, these tissues were processed and embedded in paraffin routinely. And each tumor sections of four-micron thickness were cut and placed on silanized slides for immunohistochemical staining by using the avidin-biotinylated peroxidase complex method. We used the well-characterized BMP-2 specific monoclonal antibody (Abh3b2/17) synthesized from Chinese hamster ovary (CHO) which offered by GI corporation. The Abh3b2/17 reagent was made by standard monoclonal antibody procedures using full length of recombinant human BMP-2 as the immunogen (Fig 1.2.).

-Evaluation of staining of BMP expression-
All staining materials were examined with a microscope of 200 times magnifications. Positive cells of BMP-2 were calculated by the three independent observers. These staining levels were graded by the number of positive cells. Each staining rate was graded by average of them.

-Statistical analysis-
Survival estimates for each groups were calculated according to the Kaplan-Meier method and the outcome for each groups were compared by the generalized Wilcoxon test to evaluate the correlation of these BMP-2 expressions with the prognosis in these MFH of soft tissue.

Results
BMP-2 immunoreactivity was detected in all MFH of soft tissue, although BMP-positive samples showed heterogeneous or focal. The immunohistochemical staining of BMP-2 were localized predominantly in the cytoplasm of undifferentiated spindle shaped cells. And These staining rates were widely ranged from 1.9% to 78.5% (average 30.8%). So we determined each staining rates to divide into two grades as follows;

High grade staining group’s rate was more than 30.8% (≥30.8%) and Low grade staining group’s rate was less than 30.8% (<30.8%). Seventeen patients belonged to the high grade staining group (≥30.8%) and 13 patients to the low grade staining group (<30.8%). And researched each prognosis (5-years overall survival rate). The high grade staining group’s 5-years overall survival rate was 83.9%. On the other hand, in the low grade staining group’s 5-years overall survival rate was 29.5%. The survival time of each patients with the BMP-2 high grade staining group was significantly longer than that of those with the BMP-2 low grade staining group without any influences of operation and chemotherapies. (p=0.047, generalized Wilcoxon test).

Discussion and conclusion
In osteosarcoma, in proportion to increasing of it’s BMP-2 expression rate, it’s clinical courses are getting worse. But unexpectedly in MFH, the result of the relationship between BMP-2 expression rate and these prognosis in 30 patient’s MFH of soft tissue were completely opposite to OS without any influences of operation and chemotherapies.

Anyway, Unless the treatments such as operation and chemotherapy for MFH, the significant improvement in it’s prognosis could have not been seen so far, and it’s prognosis determining factors are still unknown. Our data shows that BMP expression rate in MFH of soft tissues is important factor to predict it’s clinical courses.

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