

Frequent immunoexpression of TGF- β 1, FGF-2 and BMP-2 in fibroblast-like cells in osteofibrous dysplasia

AKIO SAKAMOTO^{1,2}, YOSHINAO ODA², YUKIHIDE IWAMOTO¹ and MASAZUMI TSUNEYOSHI²

Departments of ¹Orthopaedic Surgery and ²Anatomic Pathology,
Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan

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Abstract. Osteofibrous dysplasia (OFD) and fibrous dysplasia (FD) are both benign bone lesions which comprise the proliferation of fibroblast-like cells with bone formation, and these fibroblast-like cells have the phenotype of osteoprogenitor cells. The roentgenograph of OFD shows a heterogeneous osteolytic lesion with surrounding osteosclerosis, whereas FD is typically characterized by a rather homogeneous osteolytic lesion, or 'ground-glass appearance', with a smaller amount of surrounding osteosclerosis. Growth factors of transforming growth factor- β 1 (TGF- β 1), fibroblast growth factor-2 (FGF-2) and bone morphogenetic protein-2 (BMP-2) modulate bone differentiation. Expression of these growth factors was examined in the fibroblast-like cells of 16 cases of OFD and 16 cases of FD, immunohistochemically. TGF- β 1 in fibroblast-like cells was frequently expressed both in the OFD (16/16) and the FD (15/16) cases. The frequency of FGF-2 (16/16) expression and BMP-2 (13/16) expression in the fibroblast-like cells of OFD was higher than that of those [FGF-2 (8/16) and BMP-2 (6/16)] in the fibroblast-like cells of FD, with a statistical significance. These results seem to suggest that fibroblast-like cells of OFD have greater bone-forming ability than those of FD, and may explain the roentgenographic difference between OFD and FD and a difference in the nature of fibroblast-like cells between these two types of lesions.

Introduction

Osteofibrous dysplasia (OFD) and fibrous dysplasia (FD) are both fibro-osseous lesions, and they have a different pathogenesis (1). OFD occurs almost exclusively in the tibia or fibula, and is characterized by an osteolytic lesion with surrounding osteosclerosis, whereas FD can occur in any

bone, and is characterized by an osteolytic lesion known as 'ground-glass appearance'. Surrounding osteosclerosis is not characteristic in FD. Histologically, both OFD and FD comprise a proliferation of fibroblast-like cells with bone formation. OFD typically has woven bone trabeculae with prominent osteoblastic rimming. On the other hand, FD has bone trabeculae with less osteoblastic rimming (2). It has been indicated that the fibroblast-like cells of FD share some phenotypic features with osteoprogenitor cells, as well as OFD (2,3). Clinically, OFD is a self-limiting lesion associated with bone formation, while being different from FD (4). Therefore, the bone-forming ability of fibroblast-like cells in OFD is suggested to be different from that in FD.

Osteoblast differentiation genes are regulated by the actions of systemic and local signaling factors (5). Among these factors, local growth factors regulating osteoblast differentiation include transforming growth factor- β (TGF), fibroblast growth factor (FGF) and bone morphogenetic protein (BMP). TGF- β 1 is the prototype of the TGF- β superfamily and it is an evolutionarily conserved family of structurally related dimeric cytokines (6). TGF- β has been implicated in the regulation of bone growth and turnover (7,8). FGF is a family of polypeptides that control the proliferation and differentiation of various cell types (9,10). FGF-2 is a potent mitogen for osteoprogenitor cells, and plays an important role in bone metabolism and in the regulation of osteoblastic cell proliferation and differentiation (11,12). BMPs, members of the TGF- β superfamily, play a pivotal role in the signaling network and are involved in nearly all processes associated with skeletal morphogenesis (13). BMPs have been shown to induce differentiation of multipotential mesenchymal cells (14,15), and to induce osteoblast differentiation in bone marrow stromal cells (16). The expression of BMP-2 modulates the differentiation of osteoblasts from osteoprogenitor cells (17,18).

In this study, we investigated the expression of growth factors of TGF- β 1, FGF-2 and BMP-2 immunohistochemically in fibroblast-like cells of OFD and FD to characterize their nature in terms of potent bone-forming ability.

Materials and methods

Cases of OFD and FD. Sixteen cases of OFD and 16 cases of FD were collected from the histopathological files at the Department of Anatomic Pathology, Kyushu University. The age of the patients with OFD ranged from 3 to 31 years,

Correspondence to: Dr Akio Sakamoto, Department of Orthopaedic Surgery, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
E-mail: akio@med.kyushu-u.ac.jp

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Figure 1. Osteofibrous dysplasia. Roentgenograph of osteofibrous dysplasia shows an osteolytic lesion with surrounding osteosclerosis in the tibia (A). Woven bone trabeculae demonstrate osteoblastic rimming. Cellular proliferation of fibroblast-like cells can also be seen (B). Zonal phenomenon (C). Fibrous dysplasia. Roentgenograph of fibrous dysplasia shows an osteolytic lesion, with a ground glass appearance, in the femur (D). Cellular proliferation of fibroblast-like cells can also be seen. Note that the bone trabeculae do not demonstrate osteoblastic rimming (E). (H&E staining, original magnification: B, x170; C, x120; E, x150).

while that of patients with FD ranged from 9 to 45 years. The average age of the patients with OFD (16.4 years old) was lower than that of those with FD (27.5 years). The patients with OFD comprised 15 males and one female, and OFD occurred in the tibia or fibula without exception. In contrast, the patients with FD comprised 4 males and 12 females, and the bones affected by FD comprised the femur (8 cases), ileum (3 cases), facial bone (3 cases) and rib (2 cases).

Immunohistochemical staining. Immunohistochemical studies were performed using polyclonal antibodies against TGF- β 1 (sc-146: Santa Cruz Biotechnology, San Francisco, CA, USA), FGF-2 (sc-79: Santa Cruz Biotechnology) and BMP-2 (sc-6895: Santa Cruz Biotechnology). Histological sections of formalin-fixed, paraffin-embedded materials were deparaffinized and then the endogenous peroxidase was blocked. The sections were incubated with primary antibody at 4°C overnight, followed by staining with a streptavidin-biotin-peroxidase kit (Nichirei, Tokyo, Japan). The dilutions of primary antibodies were 1:100 for anti-TGF- β 1, 1:100 for anti-FGF-2 and 1:200 for anti-BMP-2. The sections were then finally reacted in a 3,3'-diaminobenzidine, peroxytrichloride substrate solution, counterstained with hematoxyline. As for anti-TGF- β 1 and anti-FGF-2, a microwave oven was used for the pretreatment. We evaluated the immunoreactivity of growth factors in the fibroblast-like cells of OFD and FD. When the fibroblast-like cells had diffuse, strong

immunoreactivity in >50% of cells, we interpreted the results as positive, whereas when the fibroblast-like cells were not stained or were only faintly stained, or else had strong but focal immunoreactivity in <50% of cells, the results were interpreted as negative.

Statistical analysis. Data regarding immunohistochemical expressions were analyzed by the Fisher's exact test, and a p-value of <0.05 was considered to indicate significant difference.

Results

The roentgenographs of OFD showed an osteosclerotic lesion with surrounding osteosclerosis in the tibia (Fig. 1A). Whereas, the roentgenograph of FD showed a homogeneous osteolytic lesion, or 'ground-glass appearance' with a smaller amount of surrounding osteosclerosis (Fig. 1D). Histologically, OFD was also composed of fibroblast-like spindle cells and bone trabeculae, but the bone trabeculae of OFD were rather irregular and surrounded by prominent osteoblastic rimming (Fig. 1B), whilst showing so-called 'zonal architecture' characterized by a central fibrous area with new bone formation radiating to the outer area of more mature anastomosing lamellar bone (Fig. 1C). On the other hand, FD was composed of fibroblast-like spindle cells and interspersed bone trabeculae, which had less osteoblastic rimming. These

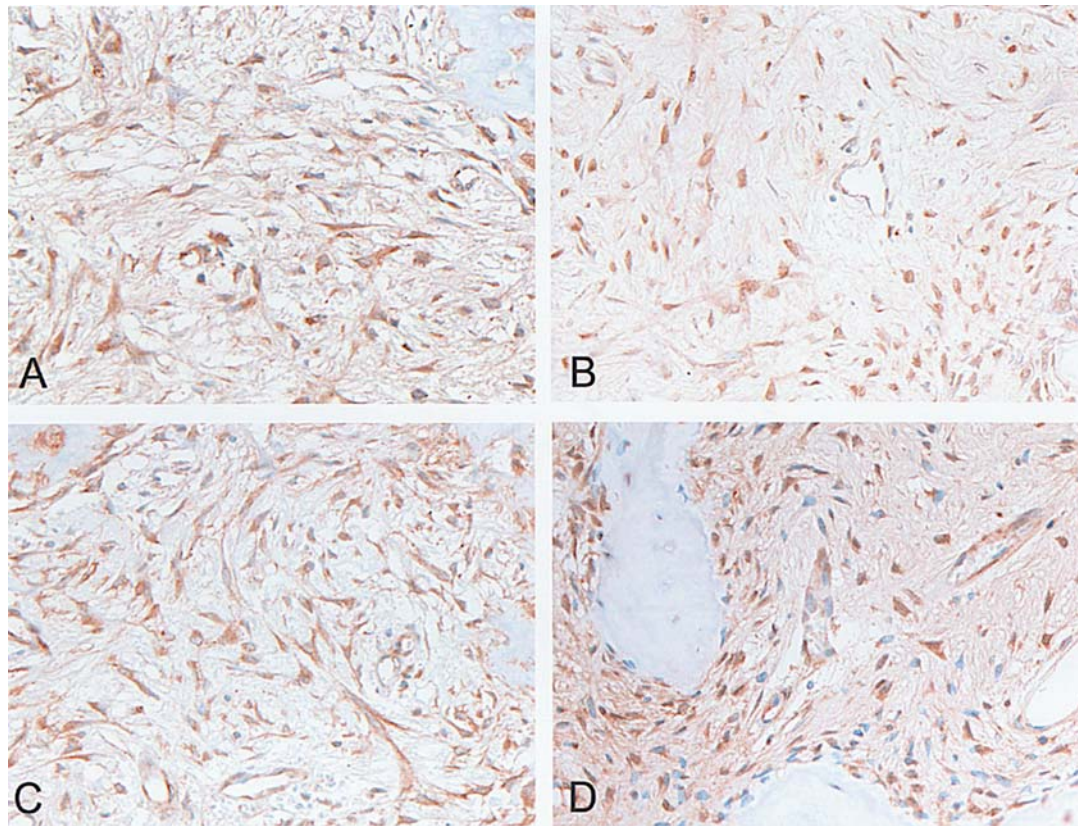


Figure 2. Expressions of TGF- β 1 (A), BMP-2 (B) and FGF-2 (C) can be seen in fibroblast-like cells of osteofibrous dysplasia. Expressions of TGF- β 1 can be seen in fibroblast-like cells of fibrous dysplasia (D) (Immunohistochemistry, original magnification: A-D, $\times 180$).

Table I. Immunohistochemical summary of fibroblast-like cells of osteofibrous dysplasia and fibrous dysplasia.

	FGF-2		TGF- β 1		BMP-2
OFD	16/16 (100%)	**	16/16 (100%)	*	13/16 (81%)
FD	8/16 (50%)		15/16 (94%)		6/16 (38%)

OFD, osteofibrous dysplasia; FD, fibrous dysplasia; FGF, fibroblast growth factor; TGF, transforming growth factor; BMP, bone morphogenetic protein. Note, positive cases/total cases: * $p < 0.05$, ** $p < 0.01$.

bone trabeculae were characteristically slender and curved (Fig. 1E). The expression of TGF- β 1 was seen in the fibroblast-like cells in all the OFD cases (16/16; 100%) (Fig. 2A), and in most of the FD cases (15/16; 94%) (Fig. 2D), whereas the expression of FGF-2 was seen in the fibroblast-like cells in all the OFD cases (16/16; 100%) (Fig. 2C), but in only half the FD cases (8/16; 50%), the frequency being significantly lower than that in OFD ($p < 0.01$). The expression of BMP-2 was frequently detected in the fibroblast-like cells of OFD (13/16; 81%) (Fig. 2B), but less frequently so in the fibroblast-like cells of FD (6/16; 38%) with a statistically significant difference ($p < 0.05$) (Table I).

Discussion

TGF- β 1 regulates a broad range of biological processes, including cell proliferation, cell survival, cell differentiation, cell migration and production of extracellular matrix (6). TGF- β stimulates the recruitment and proliferation of osteoblast precursors, in addition to promoting the early stages of differentiation (6,19). On the other hand, it blocks the later phases of differentiation and mineralization (20,21). These later stages are regulated by other growth factors, such as the BMPs (22). BMPs, including BMP-2, are potent osteoblast differentiation factors, and induce the differentiation of multipotential mesenchymal cells in osteoblast precursor cells (13,22-25). BMP-2 has been reported to act in both an autocrine and paracrine fashion to stimulate osteoblastic cell differentiation and bone formation at the late stage of osteoblast differentiation (26). Based on all these findings, it has been stated that TGF- β stimulates the recruitment and proliferation of osteoblast precursors, whereas BMPs may induce the differentiation of osteoprogenitor cells into mature osteoblasts (19), thereby suggesting that TGF- β and BMP-2 may act in a sequential manner at different stages to promote bone marrow stromal cell differentiation towards the osteoblast phenotype (27). Fibroblast-like cells in OFD and FD share some phenotypic features with osteoprogenitor cells of normal osteogenic tissues (2,3). In the current study, the expression of TGF- β 1 in fibroblast-like cells was frequently seen both in the OFD and FD cases. The frequency of BMP-2 in the fibroblast-like cells of OFD was higher than that in FD, thereby

suggesting that fibroblast-like cells of OFD have greater bone-forming ability than those of FD, resulting in the osteosclerosis in OFD characterized in the roentgenographs.

FGF-2 is a potent mitogen for mesenchymal cells including osteoblasts (28), and it plays an important role in the regulation of osteoprogenitor cell proliferation and differentiation into mature osteoblasts (12). Osteoblast gene expression is regulated by FGF signaling during the course of osteogenesis (5). This effect is consistent with the *in vivo* situation where FGF-2 acts first on osteoblast precursor cell replication to increase osteoblast number and bone formation in endosteal bones (29). In bone marrow stromal cells of long bones, FGF-2 promotes cell growth which results in osteoblast differentiation and matrix mineralization (30-34). It would be of crucial importance to further identify the interactions between FGF and other growth factor-signaling pathways, such as BMPs, during osteogenesis (5). For instance, FGF-2 enhances the bone marrow stromal cell population that is responsive to BMP-2 (35,36). In the current study, the expression of FGF-2 in the fibroblast-like cells was seen in all the OFD cases, but only in half the FD cases. The FGF-2 expression in OFD may also suggest bone-forming ability in OFD. Moreover, the frequent expression of the growth factors, FGF-2, TGF- β 1 and BMP-2, seen in the OFD cases may reflect the fact that their role is essential in the development of OFD, unlike the case of FD.

The α subunit of signal-transducing G proteins ($G_s\alpha$) is ubiquitously expressed, and is required for receptor-stimulated cAMP generation and protein kinase A (PKA) activation in the PKA-signaling pathway (37). Mutation of $G_s\alpha$ at the Arg²⁰¹ codon results in activation of the PKA-signaling pathway, and is thought to be an underlying mechanism of FD development, in which the activation of the PKA-signaling pathway associated with an increased level of cAMP causes fibroblast-like cell proliferation (3,38-41). On the other hand, such mutations were not seen in a series of OFD cases (1). Furthermore, it has been suggested that there is the possible involvement of neurofibromin in the development of OFD, which is associated with the expression of Schwann cell markers in the fibroblast-like cells of OFD, whereas these molecules were not seen in a series of FD (42). These differences suggest a different pathogenesis between OFD and FD, even though OFD and FD sometimes share similar histopathological features. Differences in the signaling pathway between OFD and FD which result in the different expression pattern of growth factors seen in the current study needs to be furthered studied in the future.

In conclusion, the expression of TGF- β 1, FGF-2 and BMP-2 was frequently seen in the fibroblast-like cells of OFD cases, thus suggesting bone-forming ability in OFD. This could possibly explain the osteosclerotic features noted on roentgenographs in OFD. In contrast, FGF-2 and BMP-2 were expressed in about half of the FD cases, thus, suggesting a difference in nature between OFD and FD with regard to their development.

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