

# Heterotopic ossification related to the use of recombinant human BMP-2 in osteonecrosis of femoral head

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## Abstract

Despite the wide use of recombinant human bone morphogenetic protein-2 (rhBMP-2) in bone defect, its application in treating osteonecrosis of femoral head (ONFH) is yet to be elucidated. The heterotopic ossification (HO) after rhBMP-2 usage in some orthopedic surgeries has been reported previously; however, only a few studies describe this complication in the treatment of ONFH.

The present study investigated whether the rhBMP-2 application would increase the risk of HO formation in selected ONFH patients with nonvascularized bone grafting surgery and enhance the surgical results of nonvascularized bone grafting as compared to patients who did not receive intraoperative rhBMP-2.

A retrospective analysis was performed on 94 patients (141 hips) who, with Association Research Circulation Osseous (ARCO) stages IIb, IIc, and IIIa ONFH, underwent nonvascularized bone grafting surgery. The first 46 patients (66 hips) received intraoperative rhBMP-2. The postoperative radiographic results (X-ray and CT scan) and Harris hip score (HHS) were reviewed in each patient to record the incidence of HO formation and evaluate the clinical efficacy of rhBMP-2, respectively.

HO formation frequently occurred in patients receiving intraoperative rhBMP-2 (8/66 hips) than those not receiving the protein (1/75 hips) ( $P = .02$ ). HHS improved from preoperatively at the final follow-up ( $P < .01$ ) in the BMP-positive group, with a survival rate of 83.3%. In the BMP-negative group, the HHS improved from preoperatively at the end of the follow-up ( $P < .01$ ), and the survival rate was 72.0%.

rhBMP-2 has osteoinductive property and might serve as an adjuvant therapy in the surgical treatment of ONFH. However, the incidence of HO formation might increase when used in high doses.

**Abbreviations:** ARCO = Association Research Circulation Osseous, BMP = bone morphogenetic protein, HHS = Harris hip score, HO = heterotopic ossification, IBG = impacted bone grafting, ONFH = osteonecrosis of femoral head.

**Keywords:** bone grafting, bone regeneration, heterotopic ossification, osteonecrosis of femoral head, recombinant human bone morphogenetic protein-2

## 1. Introduction

Bone morphogenetic proteins (BMPs), first described by Urist in 1965,<sup>[1]</sup> are members of the tumor growth factor-beta (TGF- $\beta$ ) superfamily. These proteins possess a significant osteoinductive property, thereby acting as potent inducers of bone formation by

promoting the differentiation of mesenchymal stem cells into osteoblasts.<sup>[1–3]</sup> Currently, 20 different BMPs have been identified and classified into several subgroups. Of these subgroups, recombinant human BMP-2 and BMP-7 (rhBMP-2 and rhBMP-7) are clinically approved by Food and Drug Administration (FDA) and commercially available for treatment purposes. rhBMP-2 is an autogenous bone graft substitute that has been widely used in the clinical practice of orthopedics. Indications for its use include spinal fusion in skeletally mature patients,<sup>[4,5]</sup> acute open tibial fractures,<sup>[6]</sup> nonunion fractures,<sup>[7]</sup> and specific bony oral maxillofacial defects.<sup>[8]</sup> Reportedly, the clinical results are satisfactory in bone healing after the use of rhBMP-2.<sup>[9]</sup> Thus, it was applied to the treatment of osteonecrosis of the femoral head (ONFH) gradually,<sup>[3,10]</sup> utilizing its potentialities in bone regeneration.

ONFH is an insidious and progressive disease that accompanies the apoptosis of osteocytes, frequently leading to femoral head collapse and subsequent osteoarthritis. The preservation of the femoral head by nonvascularized bone grafting is an attractive alternative treatment in patients without head collapse or with minimal collapse.<sup>[11,12]</sup> This surgical approach provides strong structural support for the femoral head. In addition, the bone grafts serve as a scaffold inducing bone reconstruction in combination with the osteoinductive material.<sup>[13,14]</sup> Several nonvascularized bone grafting techniques have been proposed, such as “Core Decompression tract,”<sup>[15]</sup> “Trap Door technique,”<sup>[16]</sup> and “Light Bulb procedure.”<sup>[17]</sup> In the present study,

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we used the “Light Bulb” method for our patients undergoing bone grafting. The surgical results may be enhanced by the addition of rhBMP-2.

One of our previous studies has described the benefits of rhBMP-2 in the treatment of ONFH. It improves the speed and quality of bone repair inside the necrotic lesions of the femoral head.<sup>[18]</sup> However, rhBMP-2 is not safe and does exhibit some complications. Concentration is one of the key modulating factors for the effect of BMPs<sup>[19]</sup>; and hence, they are frequently used in an off-label manner. Nevertheless, the high doses of such material might trigger the undesirable complications such as heterotopic ossification (HO) formation and high risk of new cancer.<sup>[20]</sup> The complication of HO formation is associated with the physiological activity of BMPs that induces new bone formation both at ectopic and orthotopic sites.<sup>[21]</sup> Such complications are usually reported in cases with spinal fusion healing; however, little data are available from trials with respect to complications of rhBMP-2 usage in ONFH.

Therefore, we investigated the use of rhBMP-2 to determine whether it would increase the risk of HO formation in selected ONFH patients with nonvascularized bone grafting surgery and enhance the surgical results of nonvascularized bone grafting in compared to patients who did not receive the intraoperative rhBMP-2.

## 2. Patients and methods

A total of 101 patients (150 hips) who underwent debridement and impacted bone grafting (IBG) surgery for nontraumatic ONFH at China-Japan Friendship Hospital from January 2013 to December 2015 were analyzed retrospectively. Seven patients (9 hips) who were lost to follow-up (contact information changed or lost) were excluded; the remaining 94 patients (141 hips) were included in the study. All the operations were performed or supervised by experienced surgeons specializing in osteonecrosis, joint preserving, and reconstruction (Drs Sun and Li). According to the usage of rhBMP-2 in operation, the patients were divided into 2 groups. The 1st group (N=46, 66 hips) received the standard background surgery (debridement+IBG) in combination with rhBMP-2 application (classified as BMP-positive group), and the 2nd group (N=48, 75 hips) received the standard background surgery (debridement+IBG) alone (classified as BMP-negative group). The minimum period of follow-up in the BMP-positive and -negative groups was 12 (average, 18; range, 12–25) months and 13 (average, 16; range, 13–25) months, respectively. All the information was obtained from the medical records. The study was approved by our Institutional Review Board.

In this study, the causes of osteonecrosis were steroid intake, alcoholism, and idiopathy, following which, the patients with traumatic ONFH were excluded. All patients included in the analysis were aged 20 to 50 years. The patients with skeletal immaturity (<18-year-old or exhibiting open epiphysis) were also excluded as they were contraindicated to BMPs therapy according to FDA. Indication for this surgery was restricted primarily to Association Research Circulation Osseous (ARCO)<sup>[22]</sup> stages IIB, IIC, and IIIA ONFH. A preoperative MRI confirmed the diagnosis in all patients; several of them also underwent CT scanning and 2D reconstruction for both hips at the coronal and sagittal views. A significant difference was not observed in the general status (such as clinical course and historical treating) between the 2 groups of patients. The preoperative demographic characteristics of patients are listed in Table 1.

**Table 1**

**Preoperative characteristics of patients included in the study.**

Variables	Total group	BMP-positive	BMP-negative	P
Total number of patients	94	46	48	
Gender (male:female)	70:24	34:12	36:12	.904
Total hips	141	66	75	
ARCO stage				
IIB	28	12	16	.868
IIC	74	36	38	
IIIA	39	18	21	
Operated side				
Bilateral	47	20	27	.323
Left	30	18	12	
Right	17	8	9	
Preoperative HHS	69.0±6.0	69.7±6.4	68.5±5.7	.237

ARCO = Association Research Circulation Osseous, BMP = bone morphogenetic protein, HHS = Harris hip score.

Bone-grafting was performed using the “Light Bulb” procedure. The patient was anesthetized and placed in a lateral decubitus position. An incision approximately 5 to 6 cm was extended from the site over greater trochanter to the hip, in order to protect the blood supply to the femoral head. After splitting the fascia lata in the direction of the incision direction, the anterior gluteus medius could be found. A longitudinal incision was made in the capsule along the femoral head through the interval between the tensor fascia and the gluteus medius, and the head-neck junction was exposed. A nearly square bone window (length and width of 1.5 cm each and a depth of 0.5–1.0 cm) was made at the femoral head-neck junction using osteotomes. Subsequently, the necrotized lesion located at the upper and anterolateral side of the head was removed through the window using a curette and a high-speed drill. The procedure was monitored by C-arm fluoroscopy, and a minimum of 5 mm subchondral bone was retained. Multiple drilling was conducted utilizing a 3-mm drill bit in the sclerotic bone until fresh blood could be actively observed from the wound holes. The cavity was filled with an autologous cancellous bone that was harvested from the iliac external circumferential lamella mixed with the artificial bone (Shanghai Bio-lu Biomaterials Co., Ltd., Shanghai, China). These implants were compressed layer-by-layer, providing strong structural support to the head and preserving the natural hip geometry. After surgery, the originally excised bone plate was placed back and fixed to cover the bone window firmly. rhBMP-2 (4 mg, from Hangzhou Jiuyuan, China) was mixed with the implants for the patients in the 1st group. This product consisted of porous hydroxyapatite 1 to 5 mg, lecithin 15 to 30 mg, and medical gelatin 50 to 70 mg and was preserved at 4°C until implanted. Before the joint capsule was closed, the articular cavity was flushed with copious normal saline. The rhBMP-2 was added to the therapeutic regimen randomly rather than through a double-blind design.

Postoperatively, all patients followed a strict rehabilitation training program. In the initial 12 weeks, the patients were advised to keep the toe-touch weight bearing with 2 crutches. Approximately 50% weight-bearing with a cane or crutch in the opposite hand was allowed in the following 12 weeks. Full weight-bearing was tolerated at the beginning of the 7th month and heavy physical activity (such as running) up to 12 months postoperatively. All patients were followed up at 3 months, 6 months, 9 months, and 1 year and then at every 6 months for the following year by radiographical and clinical examinations.

In the radiological evaluation, 2 authors (LS, WS) independently assessed the radiographs (X-ray, anteroposterior, and frog lateral views) for the presence of HO and evaluated the degree of ectopic bone formation employing the Brooker grading system.<sup>[2,3]</sup> In addition, CT scan assessed the healing in the femoral head. The fragments of HO located on the anterolateral tissues but not on the other sites of the hip joint were specifically focused on because the bone grafts and rhBMP-2 sponge were implanted from the anterolateral side and the presence of any ectopic bone at the site would be potentially related to this product. Furthermore, flushing fluid and blood from the drilled sclerotic bone containing the solubilized agents of rhBMP-2 might seep out of the surgical site in the head-neck junction, leading to unnecessary ossification in the neighboring tissues. At each follow-up, the serial Harris hip score (HHS)<sup>[24]</sup> was determined as a part of the clinical evaluation, which included clinical success and the rate of survival. Clinical success was defined as excellent (>90) and good (80–90) HHS scores. The femoral head survival was defined as HHS scores >70 without revision surgery. We also recorded the wounds, skin problems, reoperation rates for removal of HO, and other complications.

The data were analyzed using SPSS Statistical Software version 19.0. The quantitative variables were represented as the mean  $\pm$  standard deviation (SD). Continuous correction chi-square test was used to assess the differences in HO formation and Pearson chi-square test for other qualitative variables. The between-group differences in the pre- and postoperative HHS were examined by an independent samples *t* test; whereas, the within-group changes were evaluated by a paired *t* test. In order to determine whether the age, gender, BMI, stage of ONFH, and use of rhBMP-2 predicted HO and the rate of survival of the femoral head, we performed binary logistic regression analysis. A multivariate logistic regression assessed the relation between postoperative HHS (excellent, good, and fair) and preoperative demographic characteristics of the patients.

### 3. Results

#### 3.1. Radiological results

Patients receiving rhBMP-2 faced a higher risk (relative risk [RR], 9.0; 95% confidence interval [CI]: 1.2–70.8; *P* = .023) of developing HO than those not receiving this product, as assessed by the postoperative X-rays. Patients in the BMP-positive group showed a mean of 12.1% (8/66 hips) HO formation, which was greater than the 1.3% (1/75 hips) in the BMP-negative group (Table 2). A total of 7/9 confirmed HO cases occurred on the right hip and 2 on the left hip. In the BMP-positive group, the HO formation as class I was observed in 3 patients, class II in 3 (Fig. 1), class III in 1, and class IV in 1 (Fig. 2). In the BMP-negative group, the HO formation occurred on the right hip and was classified as class I. We found no association (*P* = .031) between the variables (age, gender, BMI, and stage of ONFH) other than the use of rhBMP-2 and the development of HO.

The assessment of bone healing by CT scan revealed 2 hips at ARCO stage IIb and 5 hips at stage IIc both progressed to stage III, with further femoral head collapse accompanied by painful limp. In the BMP-positive group, 4 hips at ARCO stage IIIa progressed to stage IV and were subjected to total hip arthroplasty (THA). In the BMP-negative group, 4 hips at ARCO stage IIb and 5 hips at stage IIc progressed to stage III, and 6 hips at stage IIIa progressed to stage IV at the end of follow-up. Any radiological changes were not observed throughout the follow-up period in the 6 hips; however, the functional score was

**Table 2**

**The specific conditions of the HO cases and clinical results of all patients.**

Variables	Total group	BMP-positive	BMP-negative	<i>P</i>
Radiographic results				
Cases of HO	9 (6.4%)	8 (12.1%)	1 (1.3%)	.023
Class I	4	3	1	
Class II	3	3	0	
Class III	1	1	0	
Class IV	1	1	0	
Gender (male:female)	5/4	5/3	0/1	
Age	33.8 $\pm$ 7.4	33.8 $\pm$ 7.8	34	
BMI	22.9 $\pm$ 2.5	22.9 $\pm$ 2.4	23.8	
ARCO stage				
IIa	1	1	0	
IIb	3	2	1	
IIIa	5	5	0	
Clinical results				
Excellent scores	68 (48.2%)	36 (54.5%)	32 (42.7%)	.382
Good scores	18 (12.8%)	8 (12.1%)	10 (13.3%)	
Fair scores	23 (16.3.3%)	11 (16.7%)	12 (16.0%)	
Postoperative HHS	82.9 $\pm$ 11.2	84.6 $\pm$ 10.9	81.4 $\pm$ 11.3	.091

Clinical success was defined as excellent and good HHS scores; survival rate was defined as excellent, good, and fair HHS scores. ARCO = Association Research Circulation Osseous. BMI = body mass index, BMP = bone morphogenetic protein, HHS = Harris hip score, HO = heterotopic ossification.

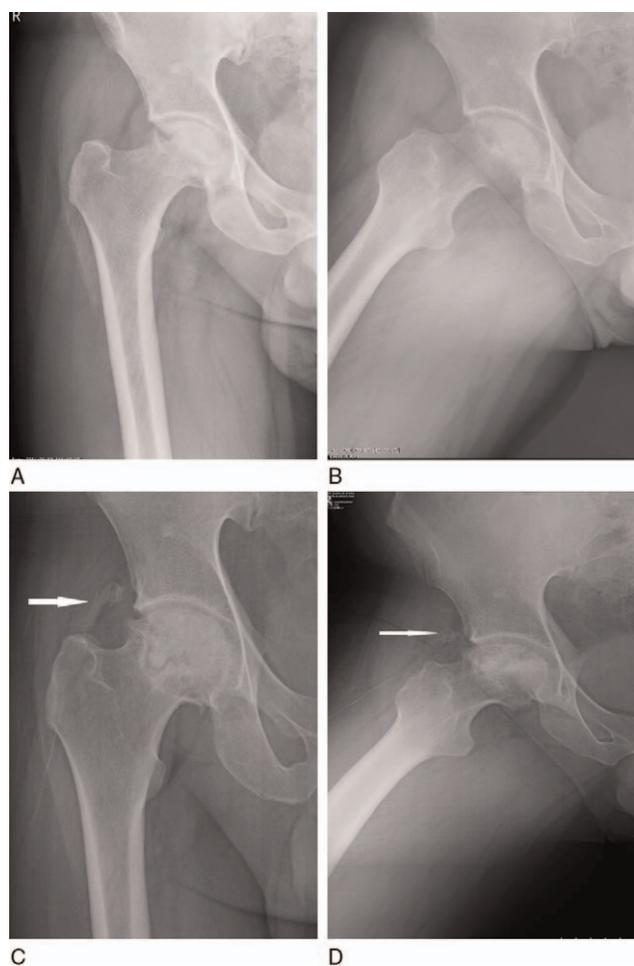
elevated. All the other hips in both groups of patients showed evident radiological signs of roundness and complete repair.

#### 3.2. Clinical results

The average postoperative HHS of both groups of patients was 82.9  $\pm$  11.2. In the BMP-positive group, clinical success was reported in 44 hips (66.7%): “excellent” and “good” scores in 36 and 8 hips, respectively, with substantial pain relief or functional improvement. Eleven hips had “fair” scores, and the survival rate of the femoral head was 83.3% (55/66 hips). The average postoperative HHS score was 84.6  $\pm$  10.9. In the BMP-negative group, clinical success was reported in 42 hips (56.0%): “excellent” and “good” scores in 32 and 10 hips, respectively. Twelve hips had “fair” scores, and the survival rate of the femoral head was 72.0% (54/75 hips). The average postoperative HHS score was 81.4  $\pm$  11.3.

Binary regression analysis suggested that the age, gender, BMI, and use of rhBMP-2 did not correlate with the survival rate of the femoral head (*P* > .05). However, it was associated with the stage of ONFH (*P* < .01). Thus, this surgical procedure could achieve a preferable rate of survival in patients with ARCO IIa and IIb ONFH over those with ARCO IIIa ONFH. The multiple regression analysis displayed similar results, that is, no association was found (*P* < .01) between the preoperative demographic characteristics (age, gender, BMI, and use of rhBMP-2), other than the stage of ONFH, and the postoperative HHS (excellent, good, and fair).

All the 7 patients in both groups diagnosed with class I and II HO formation showed neither serious pain symptoms nor severe functional impairment, obliterating the need for surgery for the removal of the extra bone. The patient with class III HO formation showed early pain, redness, and erythema around the hip joint at the 4th month after surgery, following which, minor limitations in the range of motion (ROM) of the hip joint were noted. These symptoms were resolved by aspirin administered orally for 3 months, without further deterioration in the ROM.



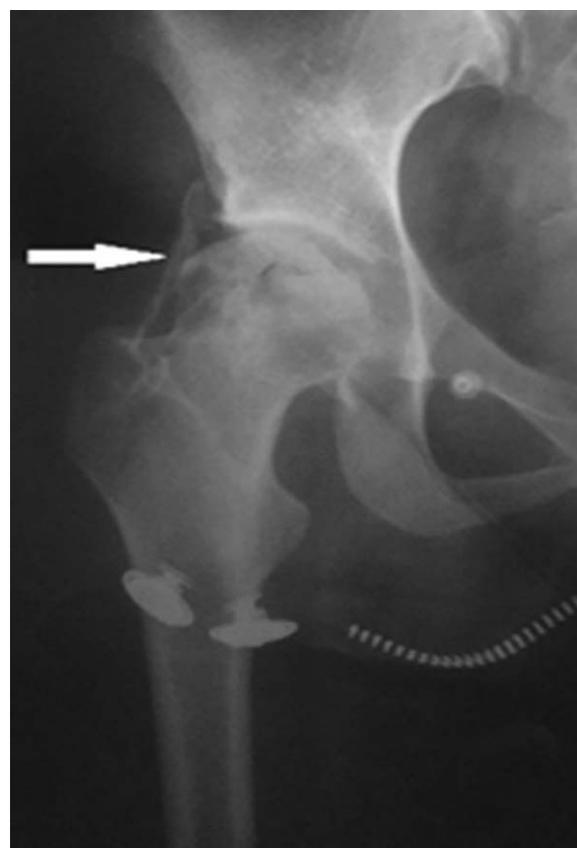
**Figure 1.** A 36-year-old male patient with ARCO stage IIIa osteonecrosis of the right femoral head. Graph A and B represent the preoperative anteroposterior and frog lateral X-ray images of the right hip, respectively. Graph C and D illustrate the postoperative anteroposterior and frog lateral X-ray images, respectively, at the 4th month after surgery, showing a lamellar fragment of HO (class II) in the anterolateral soft tissues of the hip joint (white arrow). ARCO= Association Research Circulation Osseous, HO=heterotopic ossification.

The patient with class IV HO formation was subjected to THA due to further collapse, and the extra bone was removed simultaneously.

Significant complications, such as deep infection and other wound problems, were not observed in any patient who underwent this procedure. Three patients displayed lateral femoral cutaneous nerve lesion at the immediate follow-up (1 and 2 hips in the BMP-positive and negative group, respectively). The frequency of complications did not differ between the 2 groups of patients.

#### 4. Discussion

BMPs are paracrine and autocrine growth factors that are involved in multiple biological processes.<sup>[25–27]</sup> They were also shown to induce bone growth and cartilage formation *in vivo*<sup>[1]</sup> by initiating the differentiation of mesenchymal stem cells into chondroblasts and osteoblasts until woven bone is formed.<sup>[28,29]</sup> Related clinical studies and meta-analysis have demonstrated the efficiency of FDA-approved rhBMP-2 and -7 in inducing the repair of bone lesions, such as pseudoarthrosis,<sup>[30]</sup> arthrodesis,<sup>[31]</sup> and alveolar



**Figure 2.** A 45-year-old female patient with ARCO stage IIIa osteonecrosis of the right femoral head caused by large steroid intake owing to SLE. The figure represents the anteroposterior X-ray image of the right hip treated with impacted bone graft in combination with rhBMP-2 at 6 months after surgery, showing HO formation (white arrow). A bony bridge formed from the greater trochanter to the site above acetabulum (class IV). ARCO= Association Research Circulation Osseous, HO=heterotopic ossification, SLE=systemic lupus erythematosus.

bone.<sup>[32]</sup> Currently, the use of rhBMP-2 has been considered as potentially beneficial in improving the surgical outcomes in ONFH treatment.<sup>[21]</sup> Thus, we investigated whether the risk of HO formation would be increased when rhBMP-2 was used in combination with the bone grafting procedure in the treatment of ONFH and whether rhBMP-2 might improve the clinical efficacy of this procedure.

Nevertheless, our study exhibits some limitations. First, this retrospective study was restricted due to the small number of samples, short-term follow-up, and the limited number of HO cases. Accordingly, random grouping, experimental control, and prospective studies are essential to verify the complication of using rhBMP-2 in ONFH. Second, in this trial, the dose of rhBMP-2 was 4mg/hip, and we did not investigate the association between different doses of the protein and the risk of HO in the follow-up. For improved better clinical outcomes and reduced complications, the optimal dosage of rhBMP-2 will be the focus of our future research. Third, the surgical procedure utilized the autologous iliac bone and artificial bone as the void filler for enhanced structural integrity postremoval of the necrotized bone. In addition, the composited artificial bone substitute possesses adequate osteoinductivity and osteoconductivity and can also provide an osteoconductive scaffold for supporting the new bone and vascular ingrowth. Therefore, the

evaluation of the true isolated effect of rhBMP-2 in inducing the formation of new bone was challenging; however, we reported the clinical efficacy in ONFH. Moreover, a fixed ratio of artificial bone with the autogenous iliac bone was absent, and a number of mixed bone grafts were primarily based on the volume of the bone defect after the removal of the necrotic bone.

As shown in our study, the survival rate was higher in the patients who received rhBMP-2 during head-preserving surgery as compared to those without rhBMP-2 application (83.3% vs 72.0%). Although no statistical difference ( $P=.38$ ) was observed, the proportion of the patients with successful surgeries was improved. Of the various treatments available for ONFH, local debridement and IBG, introduced by Rosenwasser et al<sup>[17]</sup> and later modified by Mont et al,<sup>[12]</sup> is an appealing option for selected patients with ONFH. Several pieces of evidence demonstrated that BMPs exert distinct osteoinductive effect and can enhance the surgical outcomes.<sup>[10,12,33]</sup> When implanted into the necrotic area, highly concentrated rhBMP-2 would induce a rapid ossification in combination with artificial or allograft bone at the implanted site, thereby preventing further head collapse and ameliorating the compressive strength. The improvements in the mechanical functions were observed histologically in early animal experimental studies that investigated the application of BMPs in ONFH.<sup>[34–36]</sup> The cellular and therapeutic functions of BMPs were closely related to their downstream signaling pathways that were stimulated by binding to type I and type II serine-threonine kinase receptors.<sup>[27]</sup> These receptors transmit the extracellular signals to the nucleus through SMAD or SMAD-independent pathways, thereby modulating the activity of the transcription factors.<sup>[37,38]</sup>

Although the usage of BMPs in spinal fusion, fracture healing, and ONFH has been greatly accepted by surgeons, attention should be shifted to the clinically relevant complications, especially with large doses. Of these complications, HO formation is frequently reported in spinal fusion surgery when the spinal cord is exposed to rhBMP.<sup>[39–41]</sup> Boraiah et al<sup>[42]</sup> also reported a high risk of HO formation in treating the complex tibial plateau fractures using rhBMP-2. In the present study, our results using rhBMP-2 in combination with autologous cancellous and artificial bones were similar to those described in previous reports. The surgical site and approach might greatly impact the HO formation because all the surgeries in this study were performed through the anterolateral approach, and all the 9 confirmed HO cases were found in the anterolateral sites. Although the closed space was formed after the capsule closure, the postoperative BMP-2 fluid collection could still seep into the anterolateral tissues through the suture, which might be the putative cause of HO formation. The risks of HO formation might also be dose-related; a high concentration of BMP-2 is likely to induce the bone formation. Minimally invasive local application methods, low concentrations of BMP at the local site, and correctly placed BMP carriers might reduce the risk of ectopic bone formation. In this study, the amount of rhBMP-2 was 4 mg/hip, which was based on the pharmaceutical directions that recommended 4 to 6 mg dose for a single part; the dose could not exceed 6 mg. Moreover, this dosage was proved to be safe and effective in one of our previous studies.<sup>[18]</sup>

With respect to the carcinogenic effect of rhBMP-2, any cases of newly diagnosed tumors were not reported. The products with a high dose of BMP (rhBMP-2, 40 mg) have been associated with a high risk of cancer when used in clinical spine studies, despite limited evidence to date, indicating that BMPs are possibly carcinogenic to humans.<sup>[20,43]</sup> Furthermore, BMPs have been shown to regulate the differentiation of cancer stem cells.<sup>[44–46]</sup>

The effects of BMPs-mediated signaling in the formation of angiogenesis<sup>[47,48]</sup> and the maintenance of endothelial cells<sup>[49,50]</sup> is yet controversial. These findings have raised concerns since a large number of patients are currently exposed to high doses of rhBMP-2. Therefore, further analysis investigating the risk of cancer correlated to rhBMP-2 therapy is imperative.

In conclusion, the present retrospective analysis was conducted on patients who received intraoperative rhBMP-2 and had a high risk of HO formation during the treatment of ONFH. Although the patients in the rhBMP-2 group had a high rate of femoral head preservation and HHS scores, the statistical analysis showed no significant difference. Therefore, surgeons should weigh the risks and benefits of this product before using them in patients with ONFH.

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